Advance your HPLC capabilities

安捷伦Poroshell 120
液相色谱柱应用文集

The Measure of Confidence

Agilent Technologies

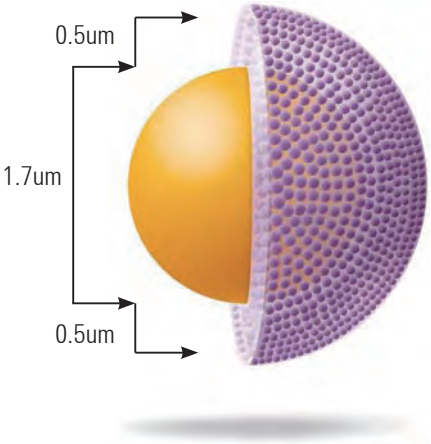
Poroshell 120

前言

在当今液相色谱分析中，如何在现有分析的基础上，实现更快更有效的分析是一大热点也是大势所趋。本文集旨在展示如何在您现有的仪器上，使用Agilent Poroshell 120，实现快速高效的分离。通篇文集显示，只需稍加改动，即可将常规5 μm 柱上的方法转换到Agilent Poroshell 120上，为您节省大量实验时间和成本。

Poroshell 120 是什么?

与亚二微米全多孔填料不同，Poroshell 120 填料采用安捷伦公司专利的制造工艺，在实心核外包上表面多孔层。



Poroshell 120 填料总粒径2.7 μm，由1.7 μm 直径的实心核和0.5 μm 厚的多孔外层组成。因此您将获得与亚二微米全多孔色谱柱相媲美的性能，但是2.7 μm 填料色谱柱的压力要比亚二微米填料低40~50%。低的反压可以帮助您在常规液相上实现快速高效分离提供很大程度的可行性。

本文集中，有不少应用是在普通的耐400bar的仪器上开发的，大家一致的体会是使用这样的色谱柱可以最大化实验室现有仪器的性能。同时，文集中整理了不同行业的应用。如：在恭普生药物分析中，采用Poroshell 120色谱柱，不仅符合美国药典的要求，而且分析速度最终提高4.5倍!同时还减少了77% 的溶剂用量。在讨论实验结果的同时，文集有些文章也分享了方法学上的细节，要确保得到最好的分析结果，让Poroshell 120充分发挥完美性能，都应注意到数据采集速率、流通池体积、柱外体积等的正确搭配。

本文集主要整理了安捷伦公司及相关不同行业的用户使用Poroshell 120色谱柱的应用文章，行业应用包括：食品安全、环境分析、药物分析、化工分析和生物分析等热门领域；同时还有部份的理论探讨文章，从理论的角度深入挖掘Poroshell 120的优势及性能。希望该文集的出版，能帮助大家更好地了解Poroshell 120表面多孔层色谱柱的特点，并在日后工作中能充分利用以实现快速高效的实验室分析工作。

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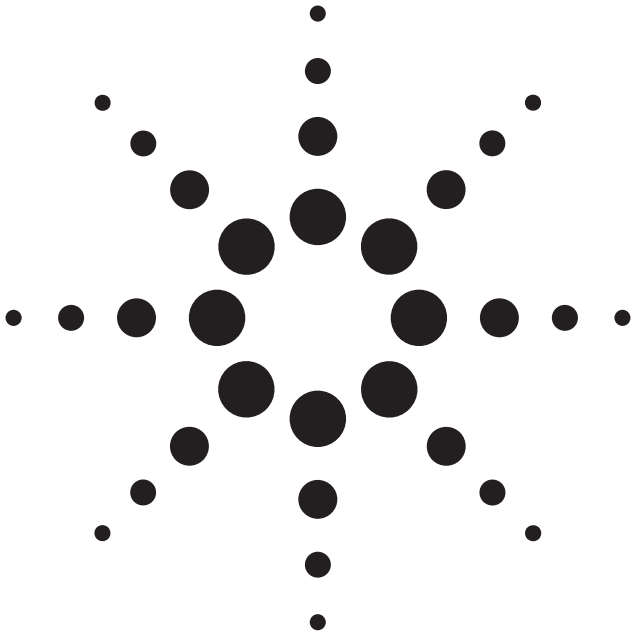

药物分析

采用Poroshell 120色谱柱根据药典方法快速分析头孢类抗生素

应用领域

药物分析

关键词

HPLC；Poroshell 120；头孢泊肟酯，头孢呋辛酯片，头孢哌酮舒巴坦

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摘要

国家药典对头孢类抗生素的分析方法中使用的常规液相色谱仪和反相C18液相分析柱，分析时间长，且有机试剂耗用量大，因此，快速液相色谱仪和快速液相分析柱应运而生[5-6]，使得分析时间和试剂耗量大大降低。但针对目前基层实验室常规液相色谱仪的普及，与快速液相分析柱的不兼容性，使得快速分析检测很难实现。安捷伦公司最新推出的Poroshell 120系列表面多孔层色谱柱，由于其具有低反压，高柱效的特点，从而真正实现在常规液相色谱仪上进行快速分析的可操作性。本文使用Poroshell 120色谱柱，并采用常规液相色谱仪，对头孢泊肟酯，头孢呋辛酯片，头孢哌酮钠舒巴坦钠几种药物的检测方法加以改进，分析时间和试剂耗量均降低了1/4。以下是实验结果，仅供参考。

1实验部分

1头孢泊肟酯分析

原方法：

色 谱 柱： Discovery C18 5u 4.6*250mm NO.104563-02

色谱条件

柱 流 量 : 1.000 ml/min

停止时间 : 20.00 min

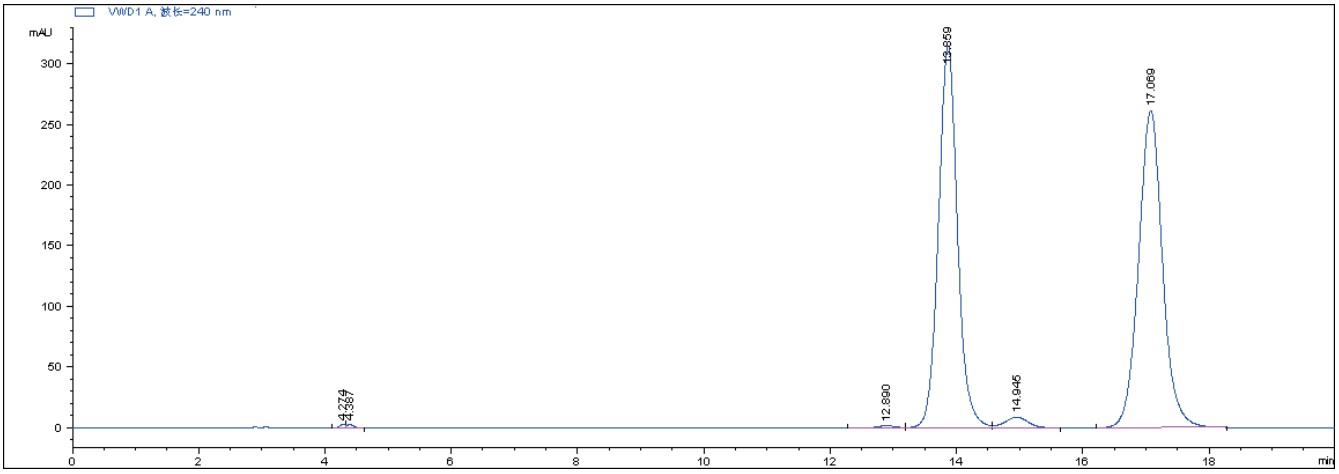
流 动 相 ： 甲醇：水=50:50

检测波长 : 240 nm

进样体积 ： 20μL

柱 温 ： 30℃

分析谱图：



快速分析方法

色 谱 柱：Poroshell 120 EC--C18 4.6mm * 2.7um * 75mm, NO.USCFT01067

色谱条件

柱 流 量: 1.000 ml/min

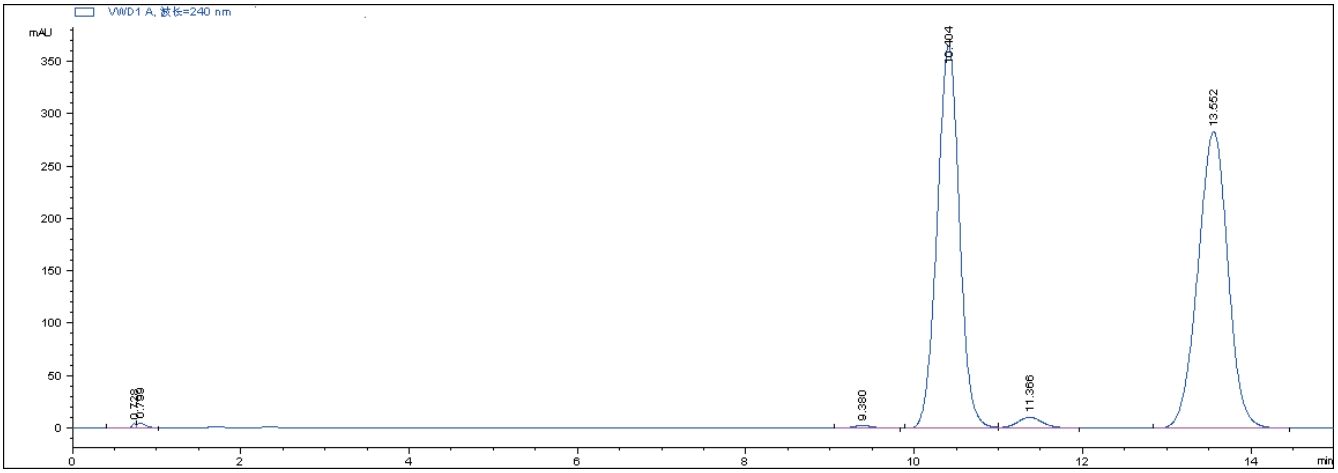
流 动 相：甲醇：水=45:55

检测波长: 240 nm

进样体积：10μL

柱　温：30℃

分析谱图



色 谱 柱：Poroshell 120 EC-C18 4.6mm * 2.7um * 50mm, NO.USCFU02894

色谱条件

柱 流 量: 1.000 ml/min

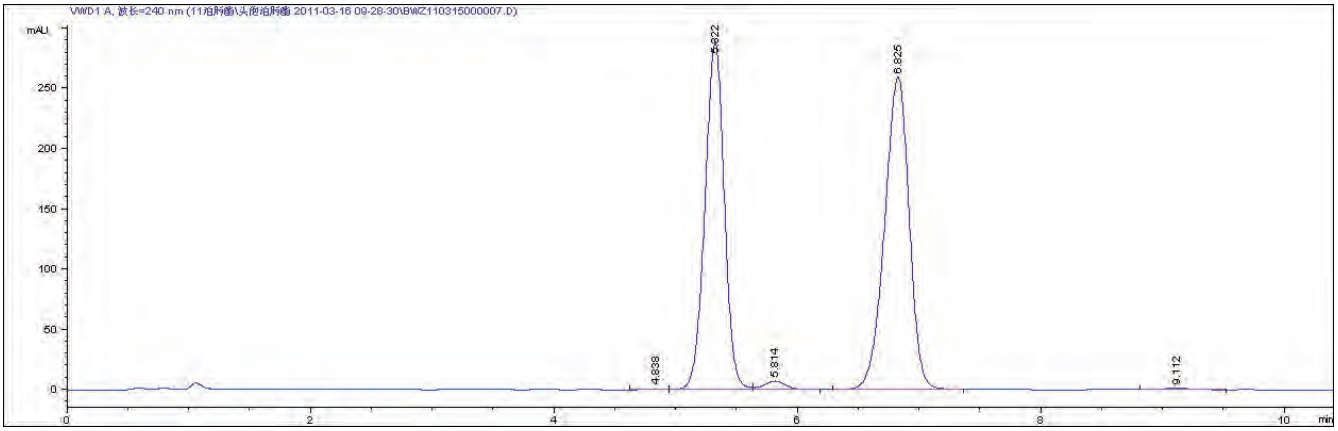
流 动 相：甲醇：水=47:53

检测波长: 240 nm

进样体积：10μL

柱　温：30℃

分析谱图



2头孢呋辛酯

原方法：

色谱柱：Hypersil BDS C18 5u 4.6*250mm NO.0734265

色谱条件

柱 流 量 : 1.000 ml/min

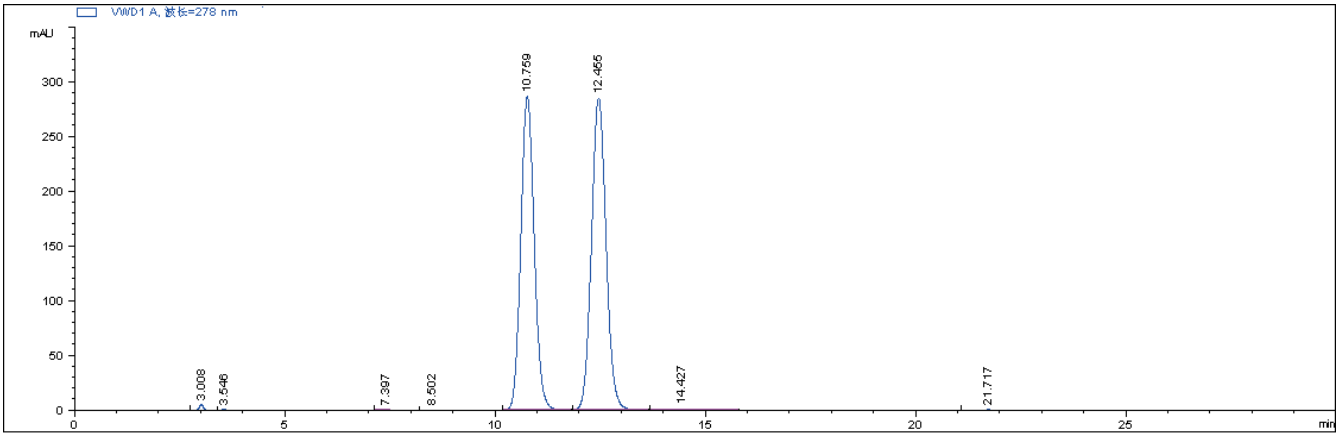
流 动 相 ： 0.2mol/l磷酸二氢铵溶液：甲醇=620:380

检测波长: 278 nm

进样体积： 20μL

柱 温： 30℃

分析谱图（头孢呋辛酯A，B异构体）



快速分析方法

色谱柱：Proshell 120 EC--C18 4.6mm * 2.7um * 100mm, NO.USCFS01004

色谱条件

柱 流 量 : 1.000 ml/min

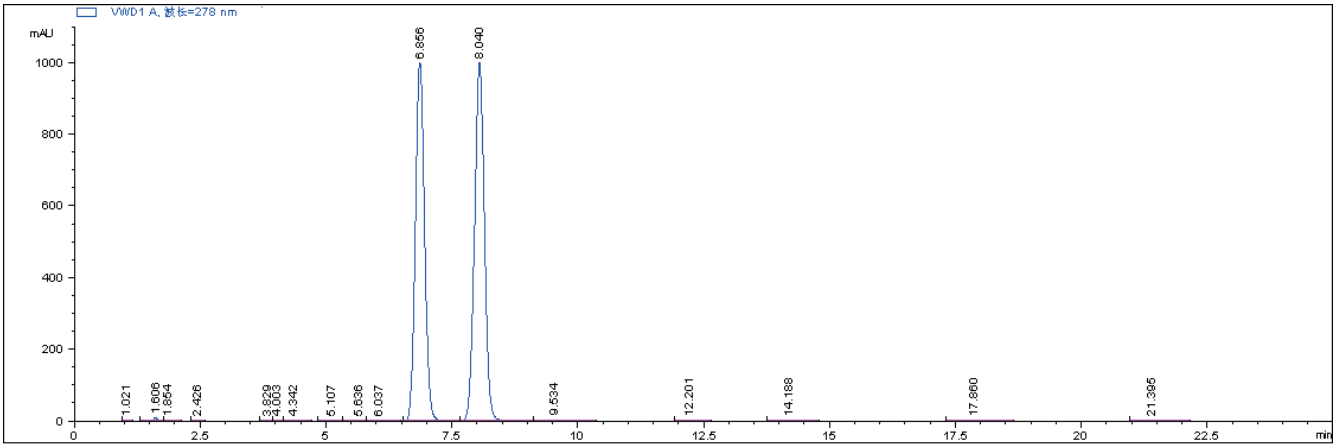
流 动 相 ： 0.2mol/l磷酸二氢铵溶液：甲醇=620:380

检测波长: 278 nm

进样体积： 20μL

柱 温： 30℃

分析谱图（头孢呋辛酯A，B异构体）



3小结

由实验结果可见,在系统适应性试验满足中国药典2010版的前提下分析头孢泊肟酯，使用常规液相色谱柱运行一个样品需要20min，而使用Poroshell 120柱仅需15min，节约了1/4时间，从而在进行头孢泊肟酯干混悬剂含量均匀度测定时大大缩短了分析时间。Proshell 120柱在分析头孢呋辛酯时同样在保证分离度不变的同时达到了快速分离。

采用Poroshell 120色谱柱快速高效分析甜叶菊中的瑞鲍迪苷、甜菊糖苷

应用领域

药物分析

关键词

HPLC；Poroshell 120；瑞鲍迪苷A、瑞鲍迪苷C、甜菊糖苷

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摘要

随着对健康的日益关注，人们正努力寻求一种安全、天然、健康、有效的“代糖”作为蔗糖的替代品。甜菊糖苷是从甜叶菊中提取、分离的一种天然甜味剂，安全性好，甜度高，正越来越被大家认识和接受。很多国内外生产厂家瞄准了这个产业，并积极寻找瑞鲍迪苷A和总甜叶菊苷符合一定要求的甜叶菊叶子。目前，国家标准仅有对甜菊糖苷提取物的分析方法，该方法采用氨基柱分离，而对于原料：甜叶菊叶子，由于基质复杂，如果样品不做任何前处理净化，采用此法进行分离就不甚理想。也有一些文献报道采用的是一般的C18柱，但是对瑞鲍迪苷A和甜菊糖苷的分离度达不到定量的要求。安捷伦公司最新推出的Poroshell 120系列表面多孔层色谱柱，由于其具有低反压，高柱效的特点：相当于超高效液相色谱柱的柱效，从而真正实现了在常规液相色谱仪上得到超高效液相色谱的分离效果。本文使用Poroshell 120色谱柱，并采用1200液相色谱仪，对瑞鲍迪苷A、瑞鲍迪苷C、甜菊糖苷的GB检测方法加以改进，不仅分离度得到提高，同时还缩短了检测时间降低了分析成本并有效地提高了实验室工作效率。

1 试验材料与方法

1.1 仪器与试剂

1200高效液相色谱仪(Agilent公司)；乙腈、甲醇(HPLC级,美国默克公司)；水由SG净化系统制得；瑞鲍迪苷A标准品，甜菊糖苷混合标准品（成都曼思特生物科技有限公司)。

1.2 色谱条件

色谱仪条件：Poroshell 120 EC-C18柱(4.6 mm i. d. × 150 mm, 2.7 μm)；柱温40℃；流速1.2 mL /min；0.1%磷酸溶液-乙腈（70：30，v/v）；紫外检测器：波长210nm；进样体积10 μL。

1.3 试验样品

甜叶菊，样品为甜叶菊供应商提供。

1.4 试验方法

1.4.1标准储备液的配制

分别准确称取10mg瑞鲍迪苷A、甜菊糖苷混合标准品于两个10ml容量瓶中，加适量水溶解，定容，使均成为1000mg/L的标准储备液，4°C 避光条件下保存。

1.4.2 混合标准工作液

分别吸取不同体积的瑞鲍迪苷A储备液，用水配制成浓度分别为50 μ g/mL、100 μ g/mL、250 μ g/mL、500 μ g/mL、1000 μ g/mL的混合标准工作液。

1.4.3 样品处理

准确称取10.00g甜叶菊样品于250 mL的锥形瓶,加入150毫升水，在60DC水浴中提取3个小时，期间，每30 min摇晃一次。取出，用纱布过滤，在5000rpm下离心15分钟，测量Brix，并将其用水稀释至Brix0.2-0.3左右，经0.45 μ m的滤膜,待测。

1.4.4 测定

按1.2操作进行，以保留时间定性，外标法定量。

2 结果与讨论

2.1 色谱条件的选择

Poroshell 120柱（4.6 mm i. d. × 150 mm, 2.7 μ m）作为分析柱，用0.1%磷酸溶液，比较了乙腈和甲醇分别作为有机相对分离效率的影响。实验发现，以30%乙腈作为流动相分离瑞鲍迪苷、甜菊糖苷时，柱压低且瑞鲍迪苷A和甜菊糖苷可实现完全分离；而甲醇作为流动相，不仅柱压相对较高，且瑞鲍迪苷A和甜菊糖苷的分离效率较乙腈的差。故选用0.1%磷酸溶液-乙腈（70：30，v/v），一次分析仅需8min。这样，不仅瑞鲍迪苷A和甜菊糖苷的分离度得到提高并缩短了分析时间（GB8270-1999至少需要35分钟），大大地提高了实验室的工作效率，且节省了对有机溶剂的使用。色谱图如下：

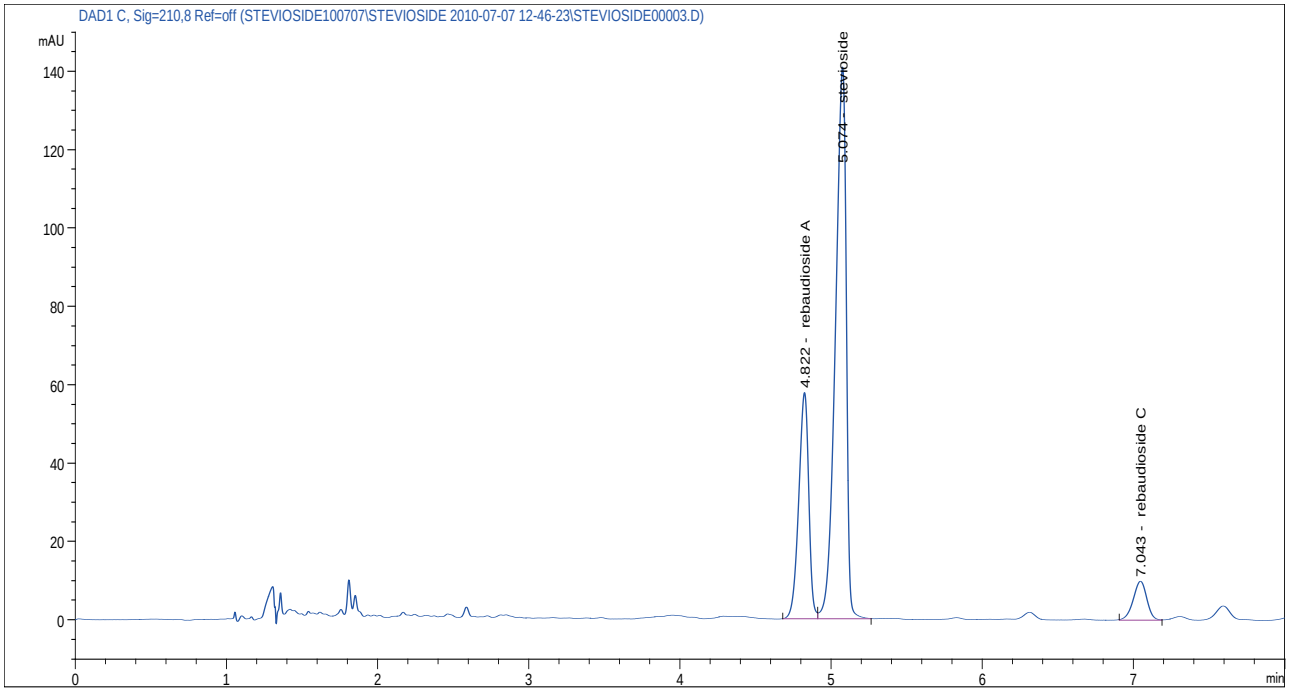

图1 瑞鲍迪苷A、C、甜菊糖苷混合标准色谱图（浓度之和1000 μ g/mL）

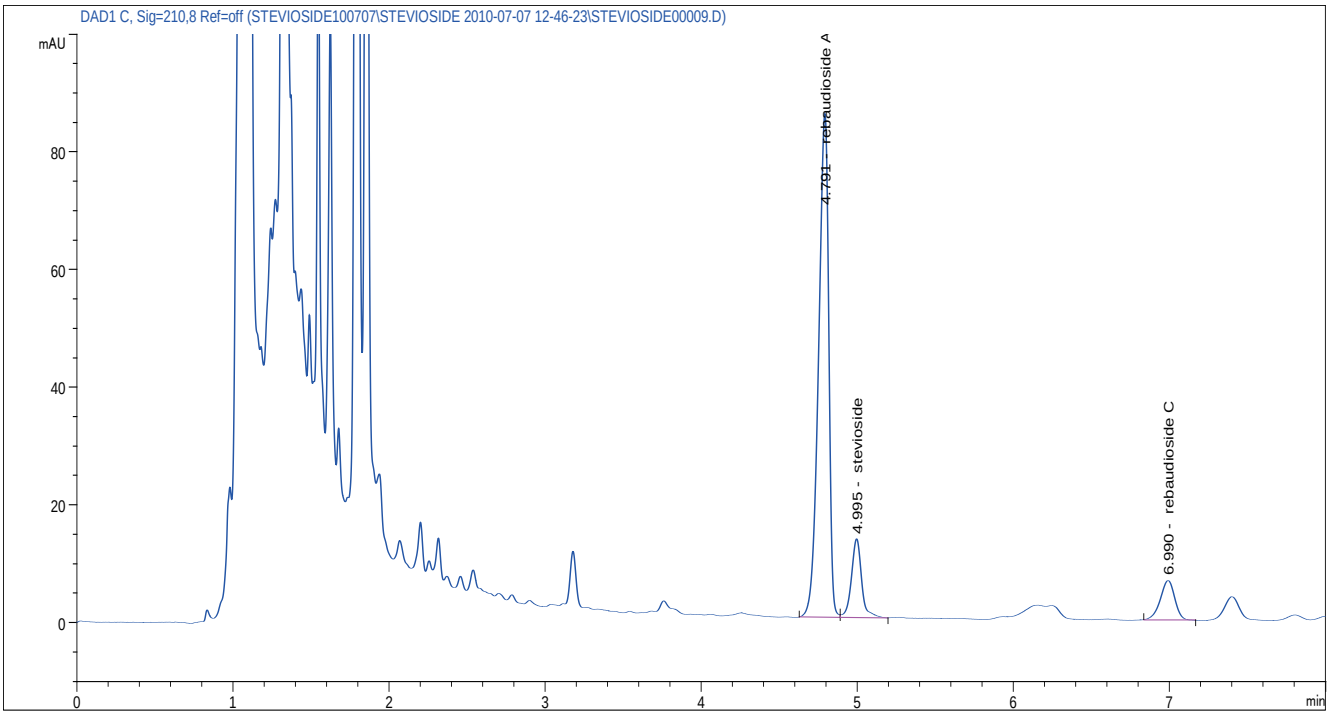

图2 甜叶菊中瑞鲍迪苷A、C、甜菊糖苷样品色谱图

2.2 使用传统色谱柱的液相色谱图（混合标准品）：

* 前三个谱图的液相条件和前面所述基本一致，仅流速有所变化，后面会说明

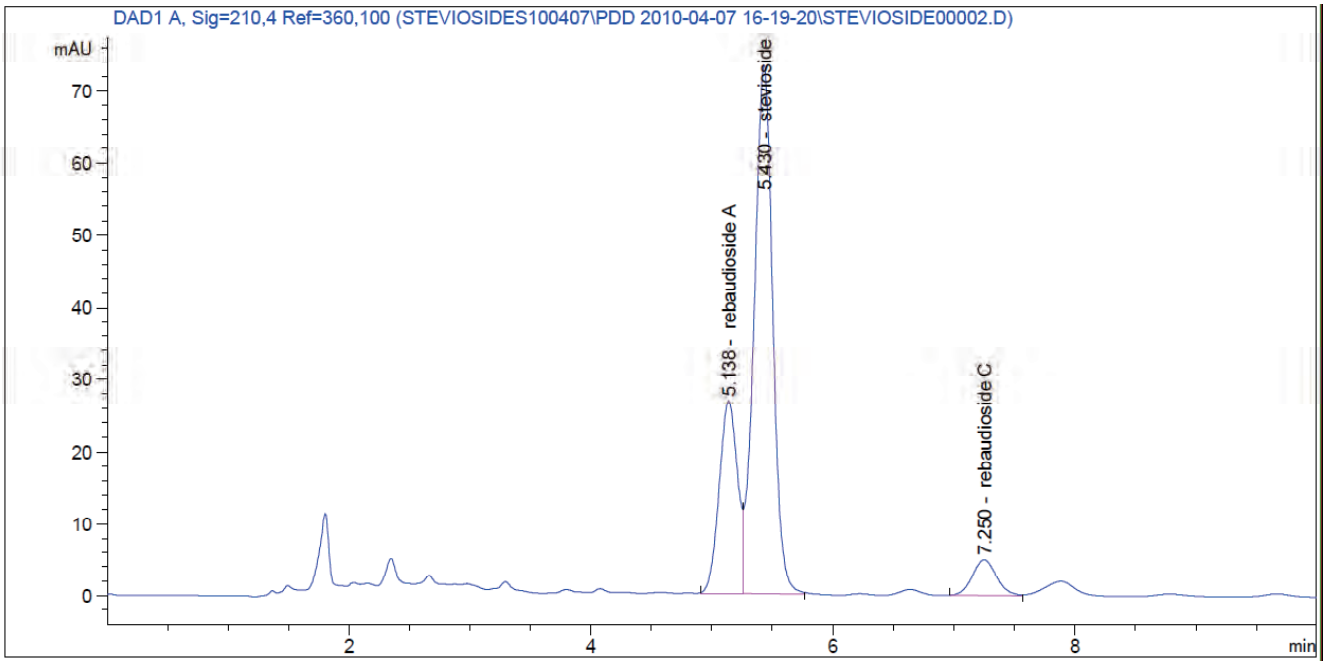

图3 SB-C18, 4.6*150mm, 5um

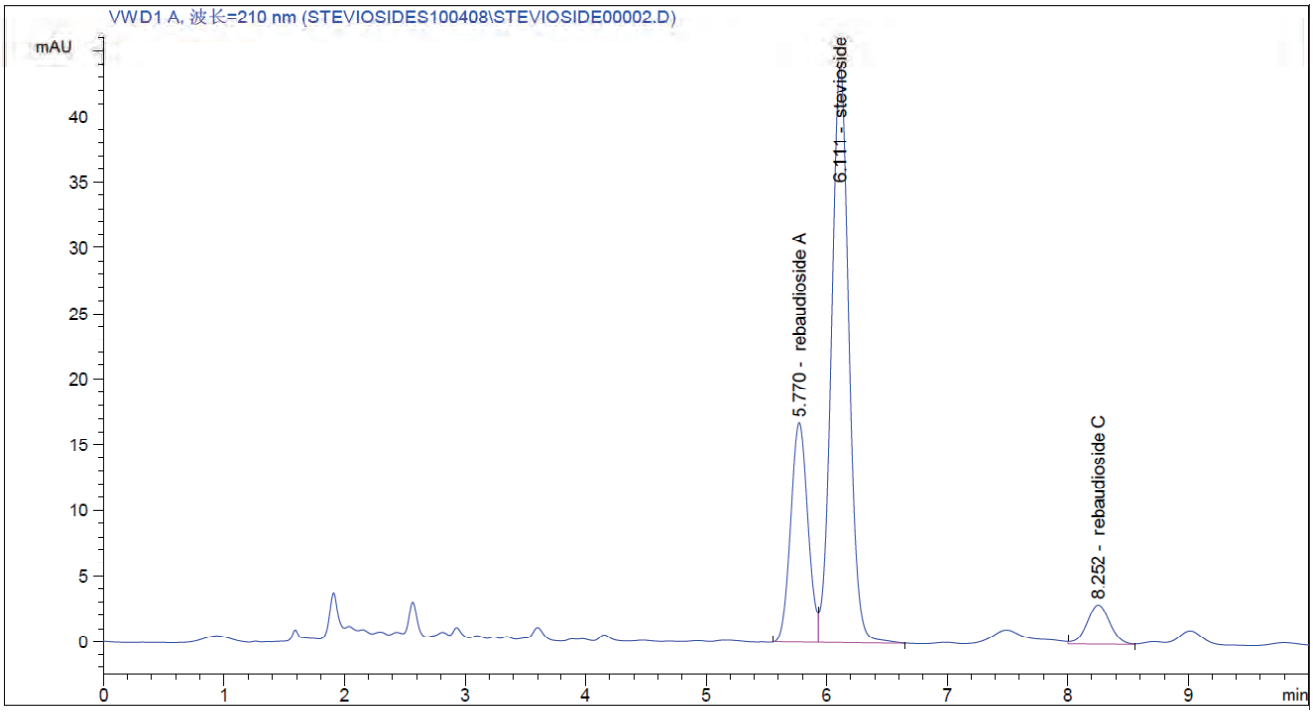

图4　Eclipse Plus C18, 4.6*250mm, 5um

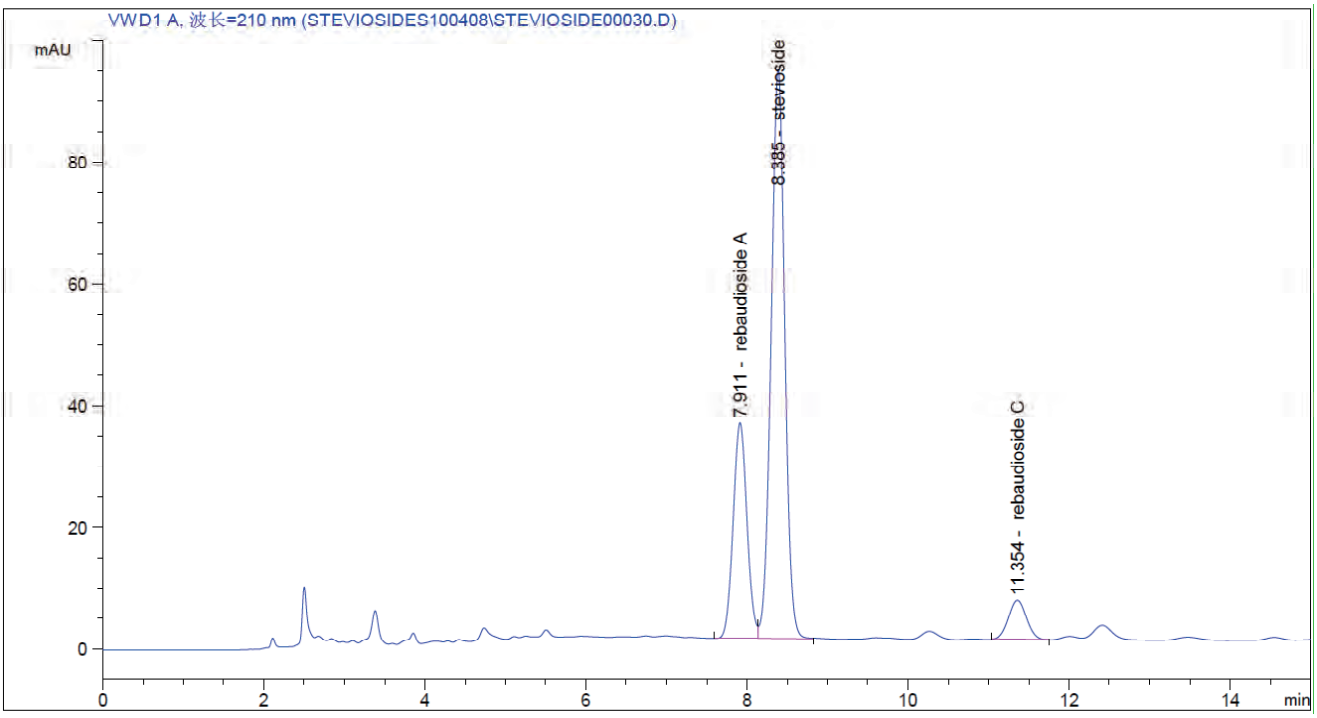

图5 SB-C18, 4.6*150mm, 5um 串联 Eclipse Plus C18, 4.6*250mm, 5um

* 以下色谱图中的流动相为乙腈-水（80：20，v/v），色谱柱是NH2柱，

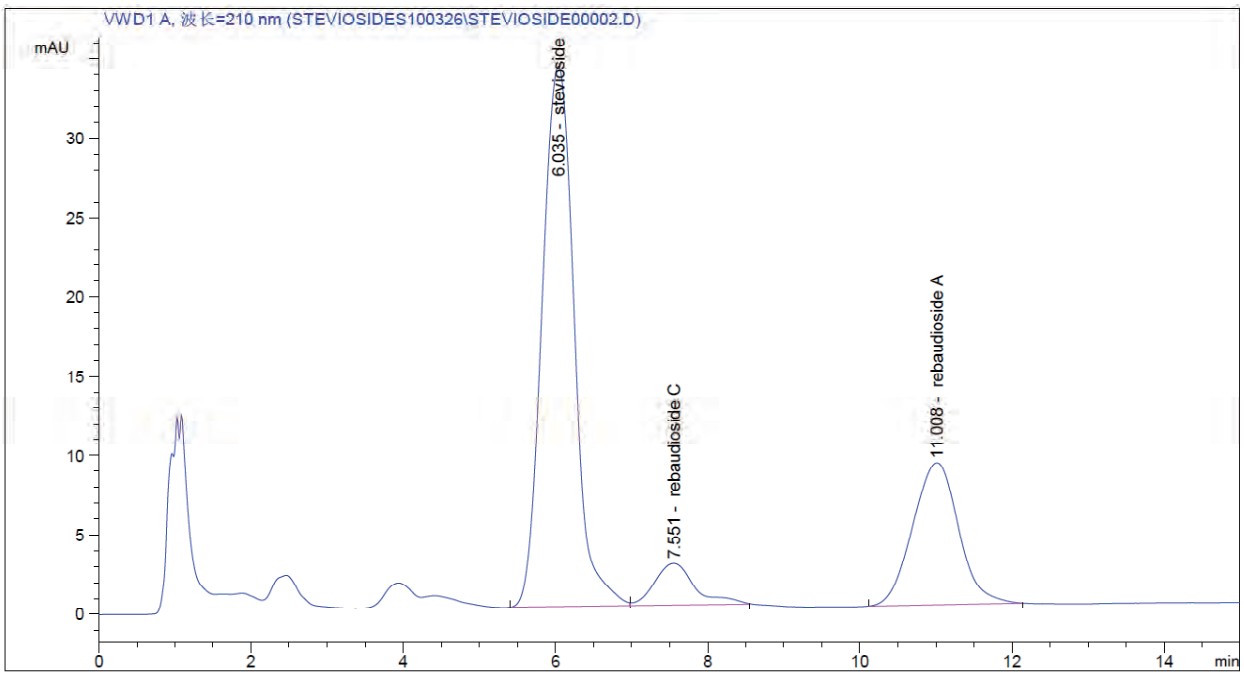

图6 NH2, 4.6*150mm, 5um

2.3 柱效、分离度比较

色谱柱名称	流速 (ml/min)	瑞鲍迪苷A塔板数	甜菊糖苷塔板数	瑞鲍迪苷C塔板数	瑞鲍迪苷A和甜菊糖苷的分离度
SB-C18 4.6*150mm, 5um	1.0	4627	6251	6399	1.01
Eclipse Plus C18 4.6*250mm, 5um	1.5	7892	8945	9080	1.31
SB-C18 4.6*150mm,5um串联Eclipse Plus C18 4.6*250mm, 5um	1.8	10328	12080	12374	1.54
Poroshell 120 EC-C18 4.6*150mm, 2.7um	1.2	25186	25436	30448	2.06
NH2 4.6*150mm, 5um	1.4	1012	1150	1611	1.84

一般来说，液相色谱的分离度需要达到1.5以上才能用于准确定量，原先使用普通5um C18柱时，需要两根串联才能勉强达到此分离度。使用Poroshell 120后，分离度超过了2.0，实现了完全分离，柱效也比使用5um C18柱和NH2柱时有了很大的提高。与此同时，液相流速和分析时间却下降了，大大节省了有机溶剂的耗费和实验的时间。

3 小结

本方法采用Poroshell 120，在1200液相色谱仪上测定甜叶菊中的瑞鲍迪苷A、C和甜菊糖苷。该方法的分离度好、柱效高、分析迅捷、试剂消耗少，结果令人满意，并无需对提取的样品进行净化，很适合于对甜叶菊叶子的甜叶菊糖苷成分分析并定量，从而帮助甜菊糖苷生产企业挑选出合格的甜叶菊叶子供应商。

采用Poroshell 120色谱柱快速分析西洋参中的人参皂苷Rg 1、人参皂苷Rb1和人参皂苷Re

应用领域

药物分析

关键词

HPLC；Poroshell 120；人参皂苷Rg 1、人参皂苷Rb1和人参皂苷Re

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摘要

西洋参主产于美国、加拿大，自上个世纪七十年代起我国开始引种，目前我国为第三大西洋参生产国。西洋参具有补气养阴，清热生津的功效，可用于气虚阴亏，内热，咳喘痰血，虚热烦倦，消渴，口燥咽干。随着生活水平的不断提高，人们的自我保健意识不断增强，西洋参作为保健品的应用越来越广泛。国家标准对其中人参皂苷的含量制定了相应的液相检测方法，但规定的方法中使用的常规液相色谱仪和液相分析柱，分析时间长，有机试剂耗用量大，因此，快速液相色谱仪和快速液相分析柱应运而生，使得分析时间和试剂耗量大大降低。但针对目前基层实验室常规液相色谱仪的普及，与快速液相分析柱的不兼容性，使得快速分析检测很难实现。安捷伦公司最新推出的Poroshell 120系列表面多孔层色谱柱，由于其具有低反压，高柱效的特点，从而真正实现在常规液相色谱仪上进行快速分析的可操作性。本文使用Poroshell 120色谱柱，并采用常规液相色谱仪，对人参皂苷Rg 1、人参皂苷Rb1和人参皂苷Re同时进行检测，与普通色谱柱相比，分析时间和试剂耗量均降低了50%，一次分析虽然仍需55min，但降低了近1小时。与目前实验室用于人参皂苷类成分效果最好的色谱柱（Agilent Zorbax SB-Aq 4.6* 250mm, 5um）相比分析时间和试剂消耗量亦降低了40%，其优势还是非常明显的。

1 试验材料与方法

1.1 仪器与试剂

岛津10AVp高效液相色谱仪(日本岛津公司)；乙腈、甲醇(HPLC级)；水由Milli-Q Academic净化系统制得；人参皂苷Rg 1、人参皂苷Rb1和人参皂苷Re对照品 (中国药品生物制品检定所)。

1.2 色谱条件

色谱仪条件：Poroshell 120 SB-C18柱(4.6 mm i. d. ×100 mm, 2.7 μm)；柱温30℃；流速1.0 mL /min；以乙腈为流动相A，以0.1%磷酸溶液为流动相B，按小表中的规定进行梯度洗脱；检测波长为203nm；进样体积10 μL。

时间（分钟）	流动相A（%）	流动相B（%）
0～20	17.8	82.2
20～40	17.8➔40	82.2➔60
40～55	40➔60	60➔40

1.3 试验样品

西洋参（市购）。

1.4 试验方法

1.4.1对照品溶液的配制

精密称取人参皂苷Rg 1、人参皂苷Re和人参皂苷Rb1对照品适量，加甲醇溶解并制成每1mL分别含0.03、0.2、1mg/mL的对照品溶液。

1.4.2 样品溶液

取样品，粉碎，精密称取细粉0.5g，精密加入甲醇50mL，称定重量，加热回流1.5小时，放冷，用甲醇补足减失的重量，摇匀，滤过。精密量取续滤液25mL，蒸干，残渣加水饱和的正丁醇50mL，分次转移至分液漏斗中，加氨试液振摇提取2次，每次5mL，弃去氨液，正丁醇液用正丁醇饱和的水洗涤2次，每次10mL，弃去水液，正丁醇液蒸干，残渣加甲醇溶解并转移至10mL量瓶中，定容，经0.45μm的滤膜，待测。

1.4.3 测定

按1.2操作进行，以保留时间定性，外标法定量。

2 结果与讨论

2.1 色谱条件的选择

以Poroshell 120柱（4.6 mm i. d. ×100 mm, 2.7μm）作为分析柱，以乙腈-0.1%磷酸溶液为流动相进行HPLC梯度条件的优化。实验发现，人参皂苷Rg 1、人参皂苷Re和人参皂苷Rb1 3种成分均能实现基线分离，一次分析从原来的110分钟缩短至55分钟，大大提高了工作效率，而且节省了有机溶剂。

2.2 谱图

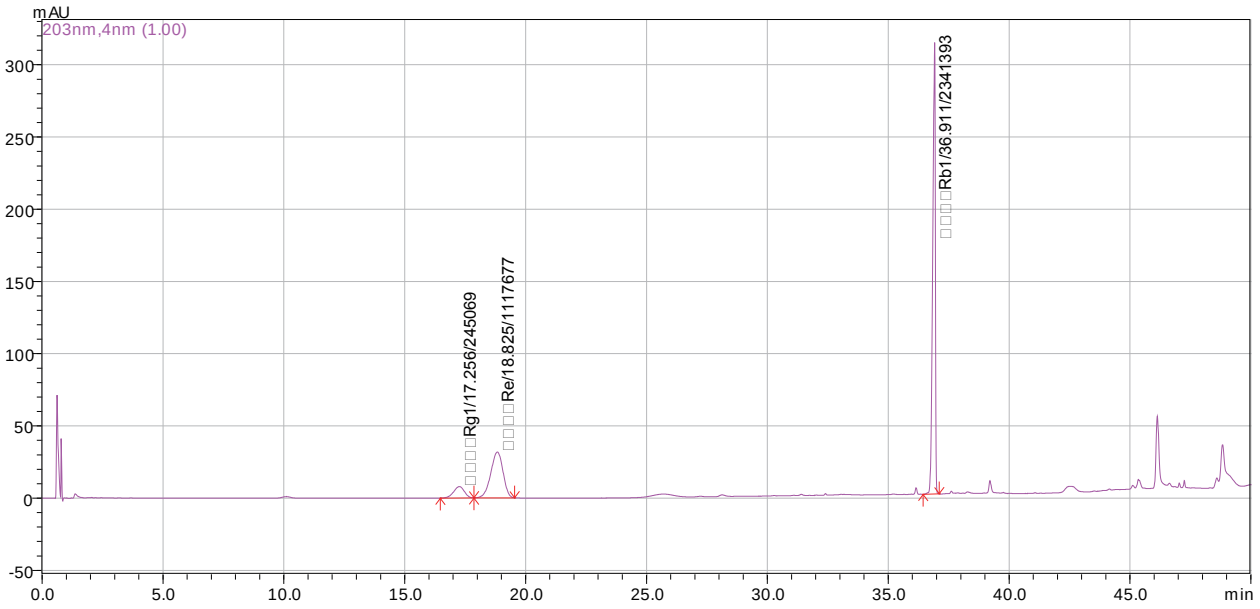

图1 对照品色谱图

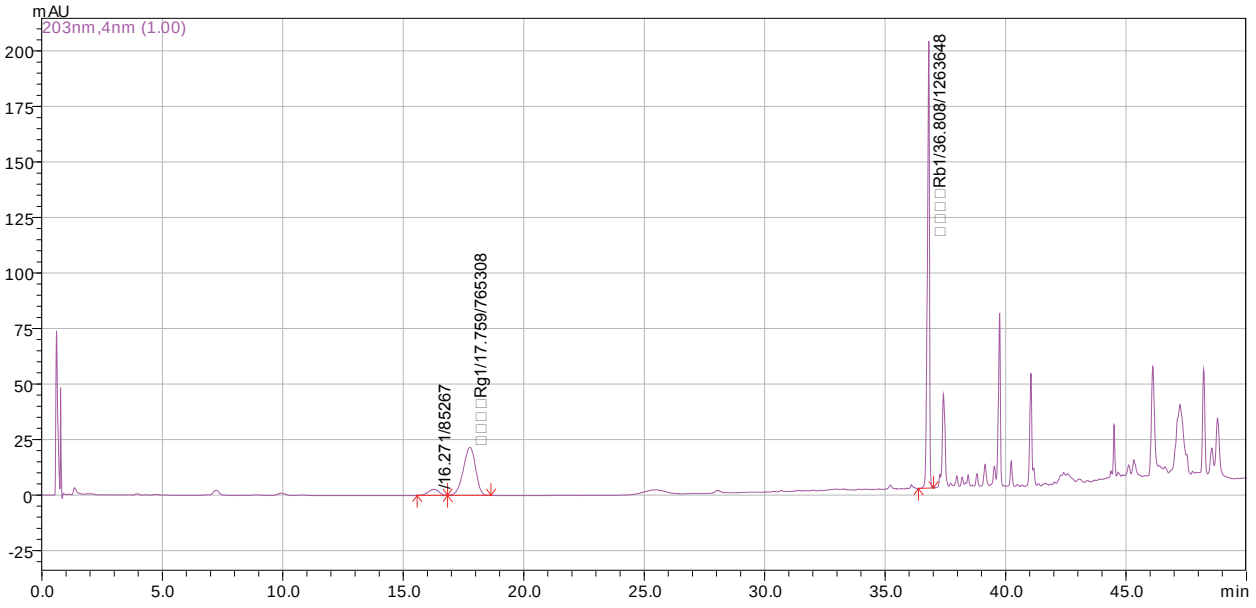

图2 样品色谱图

2.3 使用传统色谱柱的结果

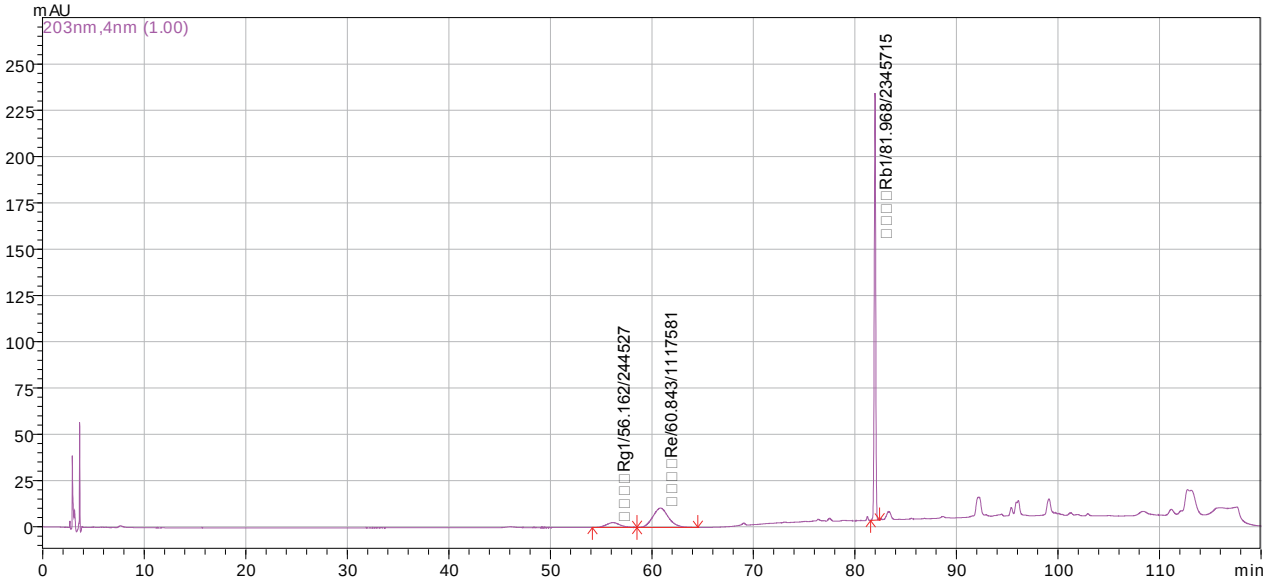

图3 对照品溶液色谱图

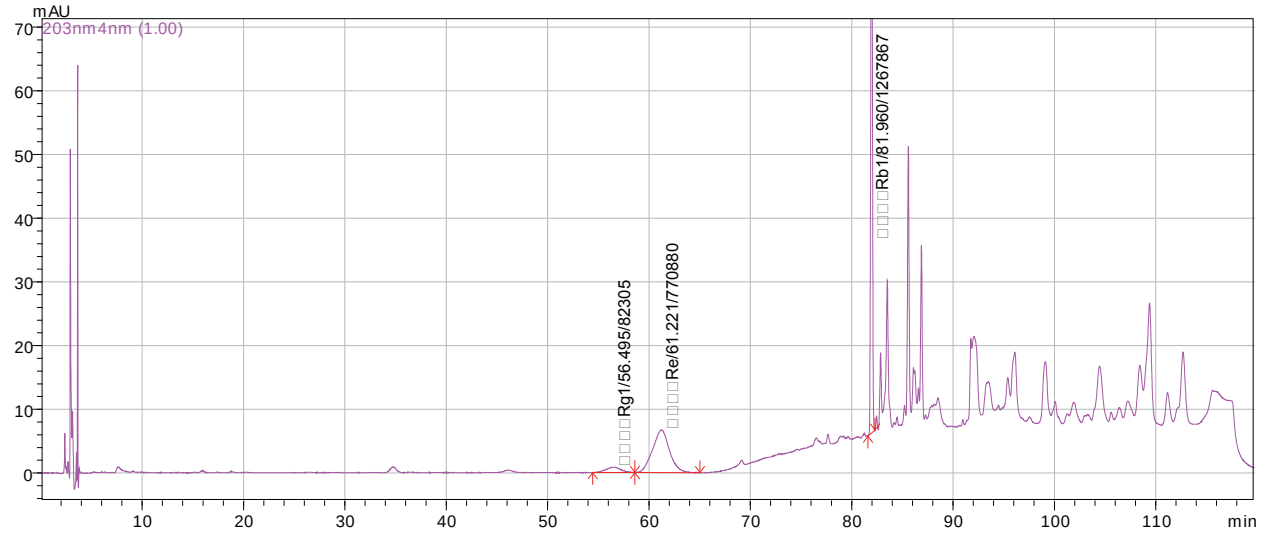

图4 样品色谱图

色谱条件：

岛津10Avp高效液相色谱仪(日本岛津公司)，检测波长203nm，标准流通池
色谱柱：Phenomenex Gemmni C18 4.6* 250mm, 5um
流动相：乙腈为流动相A，0.1%磷酸溶液为流动相B；0～60min A19.5%；60～90minA19.5➔55%；90～110min A 55➔60%
流速：1mL/min
柱温：30℃
进样量：10uL

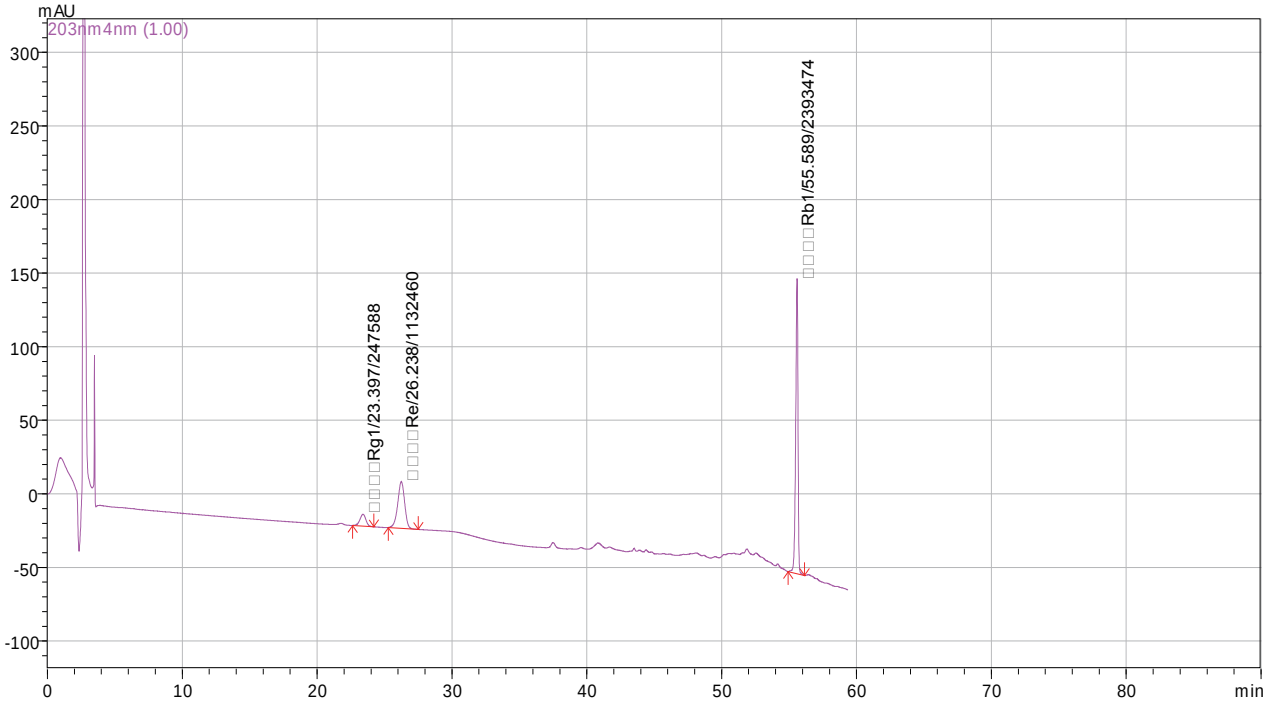

图5 对照品溶液色谱图

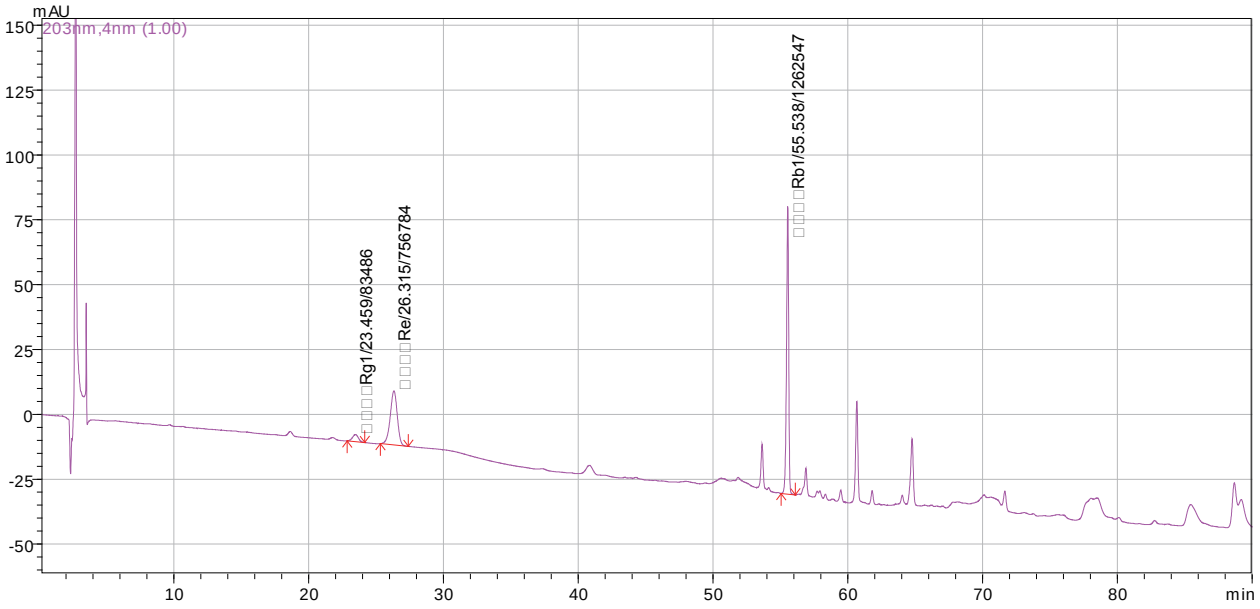

图6 样品色谱图

色谱条件：

岛津10Avp高效液相色谱仪(日本岛津公司)，检测波长203nm，标准流通池

色谱柱：Agilent Zorbax SB-Aq 4.6* 250mm, 5um

流动相：乙腈为流动相A，0.1%磷酸溶液为流动相B；0～25min A 20%；25～60min A 20➔40%；60～90min A 40➔60%

流速：1mL/min

柱温：30℃

进样量：10uL

3 小结

本法采用Poroshell120，在基层普遍使用的常规液相色谱仪上测定人参皂苷Rg 1、人参皂苷Rb1和人参皂苷Re 3种皂苷，与普通色谱柱相比，分析时间和试剂耗量均降低了50%，一次分析虽然仍需55min，但降低了近1小时。与目前实验室用于人参皂苷类成分效果最好的色谱柱（Agilent Zorbax SB-Aq 4.6* 250mm, 5um）相比分析时间和试剂消耗量亦降低了40%，其优势非常明显的。该方法的分析速度迅捷、试剂消耗少，符合批量样品的检测要求，结果令人满意，很适合于基层检测工作的开展。

采用Poroshell 120色谱柱快速分析银杏叶片中的银杏内酯A、B、C和白果内酯

应用领域

药物分析

关键词

HPLC；Poroshell 120；银杏内酯A、银杏内酯B、银杏内酯C、白果内酯

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摘要

近年来，银杏叶提取物对心脑血管疾病的独特疗效引起了众多学者的研究兴趣，银杏叶片为银杏叶提取物制成的片剂，具有活血化瘀的功效，可用于治疗血瘀型胸痹及血瘀型轻度脑动脉硬化引起的眩晕，冠心病、心绞痛等疾病。中国药典对其中的萜类内酯含量制定了相应的液相检测方法，但规定的方法中使用的常规液相色谱仪和液相分析柱，分析时间长，有机试剂耗用量大，因此，快速液相色谱仪和快速液相分析柱应运而生，使得分析时间和试剂耗量大大降低。但针对目前基层实验室常规液相色谱仪的普及，与快速液相分析柱的不兼容性，使得快速分析检测很难实现。安捷伦公司最新推出的Poroshell 120系列表面多孔层色谱柱，由于其具有低反压，高柱效的特点，从而真正实现在常规液相色谱仪上进行快速分析的可操作性。本文使用Poroshell 120色谱柱，并采用常规液相色谱仪，对银杏内酯A、银杏内酯B、银杏内酯C和白果内酯同时进行检测，分析时间和试剂耗量均降低了3/4，一次分析仅需6min。

1 试验材料与方法

1.1 仪器与试剂

岛津10Avp高效液相色谱仪(日本岛津公司)；甲醇、四氢呋喃(HPLC级)；水由Milli-Q Academic净化系统制得；银杏内酯A、银杏内酯B、银杏内酯C和白果内酯对照品 (中国药品生物制品检定所)。

1.2 色谱条件

色谱仪条件：Poroshell 120 SB-C18柱(4.6 mm i. d. ×100 mm, 2.7 μm)；柱温30℃；流速1.0 mL /min；流动相：水-甲醇-四氢呋喃（75：20：10）；ELSD检测器(Alltech 2000)；进样体10 μL。

1.3 试验样品

银杏叶片（市购）。

1.4 试验方法

1.4.1对照品溶液的配制

精密称取银杏内酯A、银杏内酯B、银杏内酯C和白果内酯对照品适量，加丙酮溶解并制成每1mL分别含0.3、0.2、0.2、0.4mg/mL的对照品溶液。

1.4.2 样品溶液

取样品，研细，精密称取1.5g，加甲醇50mL，称定重量，超声提取20 min,放置冷却后,用甲醇补足减失的重量，摇匀，滤过。精密量取续滤液20mL，蒸干，残渣加水10mL，微热使溶散，加2%盐酸溶液2滴，用乙酸乙酯振摇提取4次（15mL、10mL、10mL、10mL），合并提取液，用5%醋酸钠溶液20mL洗涤，分取醋酸钠液，用乙酸乙酯10mL洗涤，合并乙酸乙酯提取液和洗液，用水洗涤2次，每次20mL，合并水液，用乙酸乙酯10mL洗涤，合并乙酸乙酯液，蒸干，残渣用甲醇溶解并转移至5mL量瓶中，定容，经0.45μm的滤膜,待测。

1.4.3 测定

按1.2操作进行，以保留时间定性，外标法定量。

2 结果与讨论

2.1 色谱条件的选择

以Poroshell 120柱（4.6 mm i. d. ×100 mm, 2.7 μm）作为分析柱，以水-甲醇-四氢呋喃为流动相进行HPLC条件的优化。实验发现，银杏内酯A、银杏内酯B、银杏内酯C和白果内酯4种成分均能实现很好的分离，一次分析从原来的30分钟缩短至6min，不仅提高了工作效率，而且节省了有机溶剂。

2.2 谱图

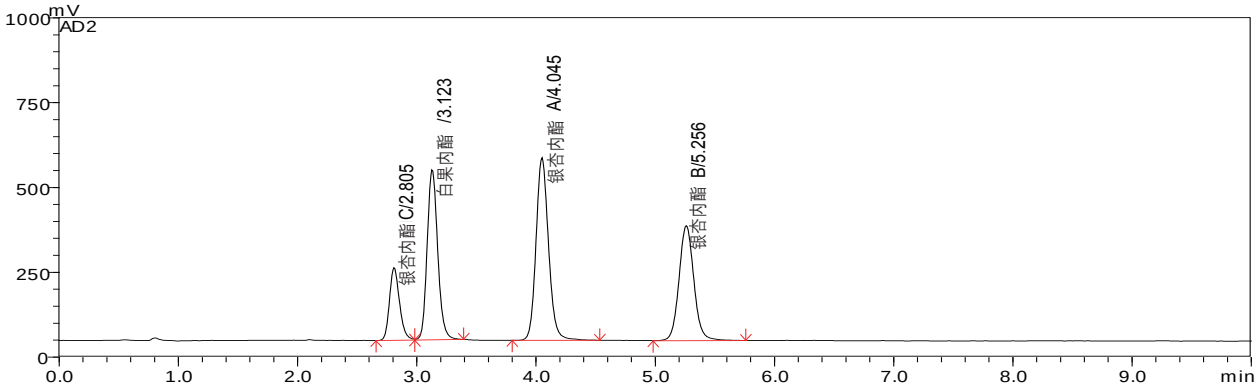

图1 对照品色谱图

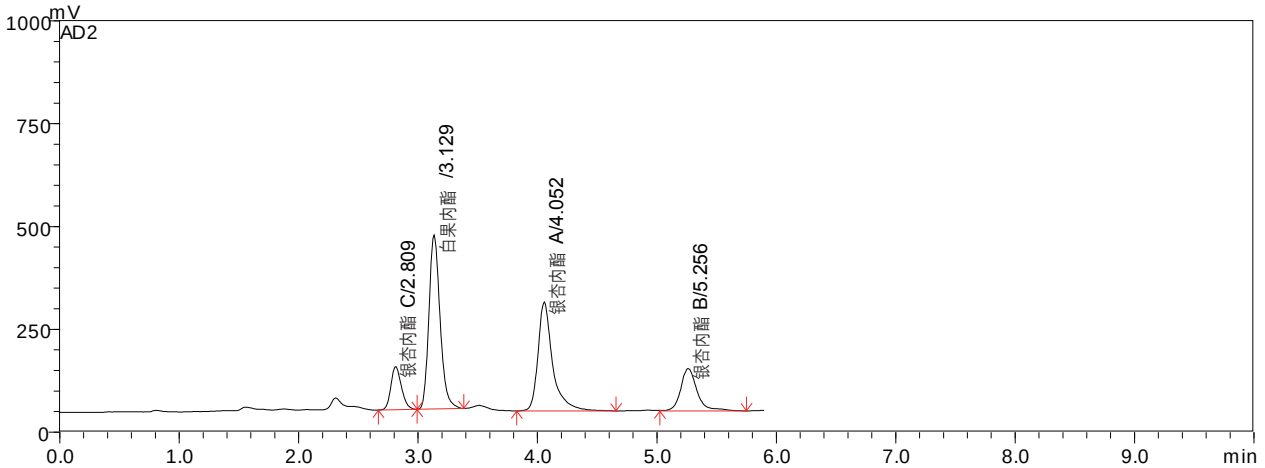

图2 样品色谱图

2.3 使用传统色谱柱的结果

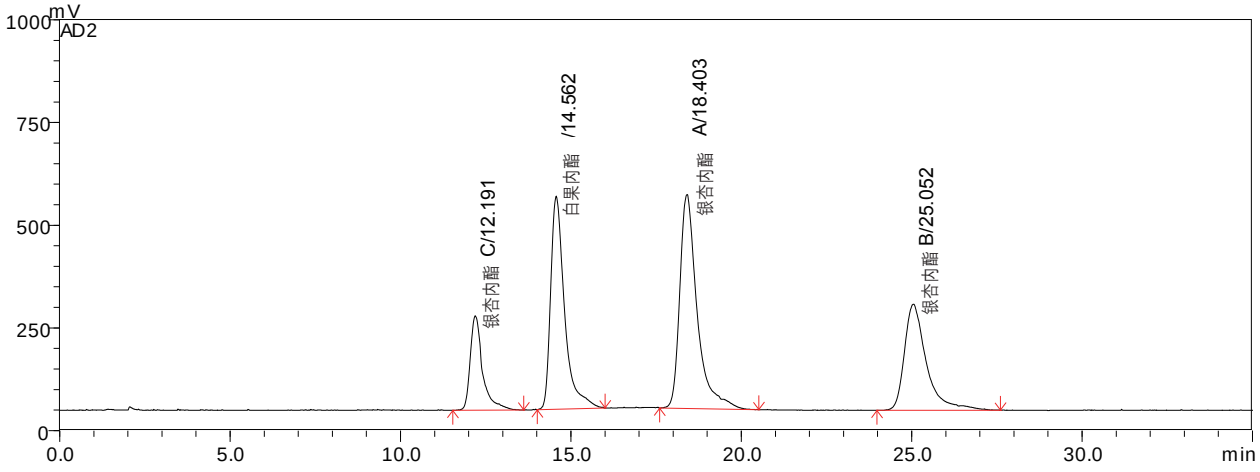

图3 对照品溶液色谱图

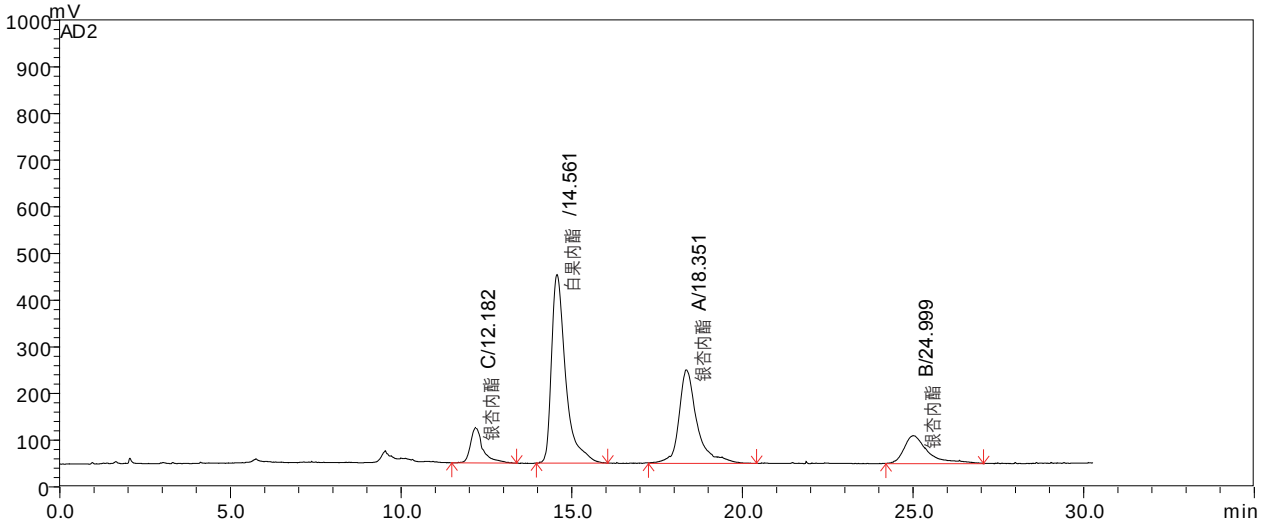

图4 样品色谱图

色谱条件：

岛津10Avp高效液相色谱仪(日本岛津公司)，ELSD检测器，标准流通池

色谱柱：Agela Venusil MP C18 4.6*150mm, 5um

流动相：水-甲醇-四氢呋喃（75：20：10）

流速：1mL/min

柱温：30℃

进样量：10uL

3 小结

本法采用Poroshell120，在基层普遍使用的常规液相色谱仪上测定银杏内酯A、银杏内酯B、银杏内酯C和白果内酯4种萜类内酯。该方法的分析速度迅捷、试剂消耗少，符合批量样品的检测要求，结果令人满意，很适合于基层检测工作的开展。

采用Poroshell 120色谱柱快速高效分离柴胡遵循2010版中国药典方法

应用领域

药物分析

关键词

HPLC；Poroshell 120；柴胡; 2010版中国药典

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摘要

柴胡 (Bupleurum chinense DC.) 别名：地薰、芏胡、山菜、菇草、柴草，。《本草图经》载：“（柴胡）今关、陕、江湖间，近道皆有之，以银州者为胜。二月生苗，甚香，茎青紫，叶似竹叶稍紫……七月开黄花……根赤色，似前胡而强。芦头有赤毛如鼠尾，独窠长者好。二月八月采根。”2010版中国药典[1]规定采用液相色谱法对北柴胡进行含量测定，对照品为柴胡皂苷a的柱效不低于10,000。本文分别采用了传统5 um的液相色谱柱与安捷伦最新推出的Poroshell 120表面多孔层液相色谱柱，遵循中国药典方法，对柴胡进行了分离分析。

1 试验材料与方法

1.1 仪器与试剂

1200SL高效液相色谱仪(Agilent公司)；1100高效液相色谱仪(Agilent公司)；乙腈(HPLC级, Dikma)；水由Milli-Q净化系统制得；柴胡及柴胡皂苷a、柴胡皂苷d对照品均由中国药品生物制品检定所提供。

1.2 色谱条件

Poroshell 120色谱条件：色谱柱：Agilent Poroshell EC-C18，100 mm × 4.6 mm，2.7 µm；
进样量：5 µl；检测波长：210nm；柱温：25˚C；流速：1 ml/min; 1200 SL液相色谱仪；
传统色谱柱方法：色谱柱：Agilent Zorbax SB-C18， 250 mm × 4.6mm，5 µ m；进样量：20 µ l；检测波长：210nm；柱温：25˚C；流速：1ml/min; 1100液相色谱仪。

1.3试验方法

1.3.1对照品溶液的配制

柴胡皂苷a和柴胡皂苷d混合对照溶液为含 柴胡皂苷a ：0.53mg/mL的溶液；含柴胡皂苷d为0.41mg/mL的溶液。

1.3.2 供试品溶液的制备

取本品粉末（过四号筛）约0.5g，精密称定，置具塞锥形瓶中，加入含5%浓氨试液的甲醇溶液

25ml，密塞，30℃水温超声处理（功率200W，频率40KHz）30分钟，滤过，用甲醇20ml分两次洗涤容器和药渣，洗液与滤液合并，回收溶剂至干。残渣加甲醇溶解并转移至5ml量瓶中，加甲醇稀释至刻度，摇匀，即得。

2 结果与讨论

2.1 色谱条件的选择

2010版药典方法规定了十八烷基硅烷键合硅胶为填充剂。以乙腈为流动相A, 以水为流动相B，按药典中的梯度方法：

时间(分钟)	流动相A（%）	流动相B（%）
0~55	25~90	75~10
50~55	90	10

使用该梯度方法时，在Agilent Zorbax SB-C18， 250 mm × 4.6mm，5 μ m色谱柱上遵照该梯度条件可以实现较好的分离，为了保证色谱柱有更好的使用寿命，在梯度运行到55min后将有机相比例进一步调高，提高洗脱强度，以减少污染物的残留，梯度见表1。结果见谱图1a

表1 Agilent Zorbax SB-C18， 250 mm × 4.6mm，5 μ m分析柴胡梯度方法

时间 (min)	0	50	55	56	61
A%	25	90	90	25	25

当使用Poroshell 120时，由于2.7 um的表面多孔层填料与1.8 um的全多孔填料柱效相当，因此，这里我们可以将2.7 um的表面多孔层填料近似为1.8 um的全多孔填料，并以此进行方法的线性转换。即当柱内径相同的情况下，梯度按柱长的比例成正比进行转换，。结果见谱图1 b，从谱图可以看出，使用了表面多孔层的色谱柱后，在保证分离度的前提下，分析时间实现了很大的节省，显著地提高了常规检测的工作效率，梯度见表2。

表2 Poroshell EC-C18，100 mm × 4.6 mm，2.7 μm分析柴胡梯度方法

时间 (min)	0	20	22	23
A%	25	90	90	25

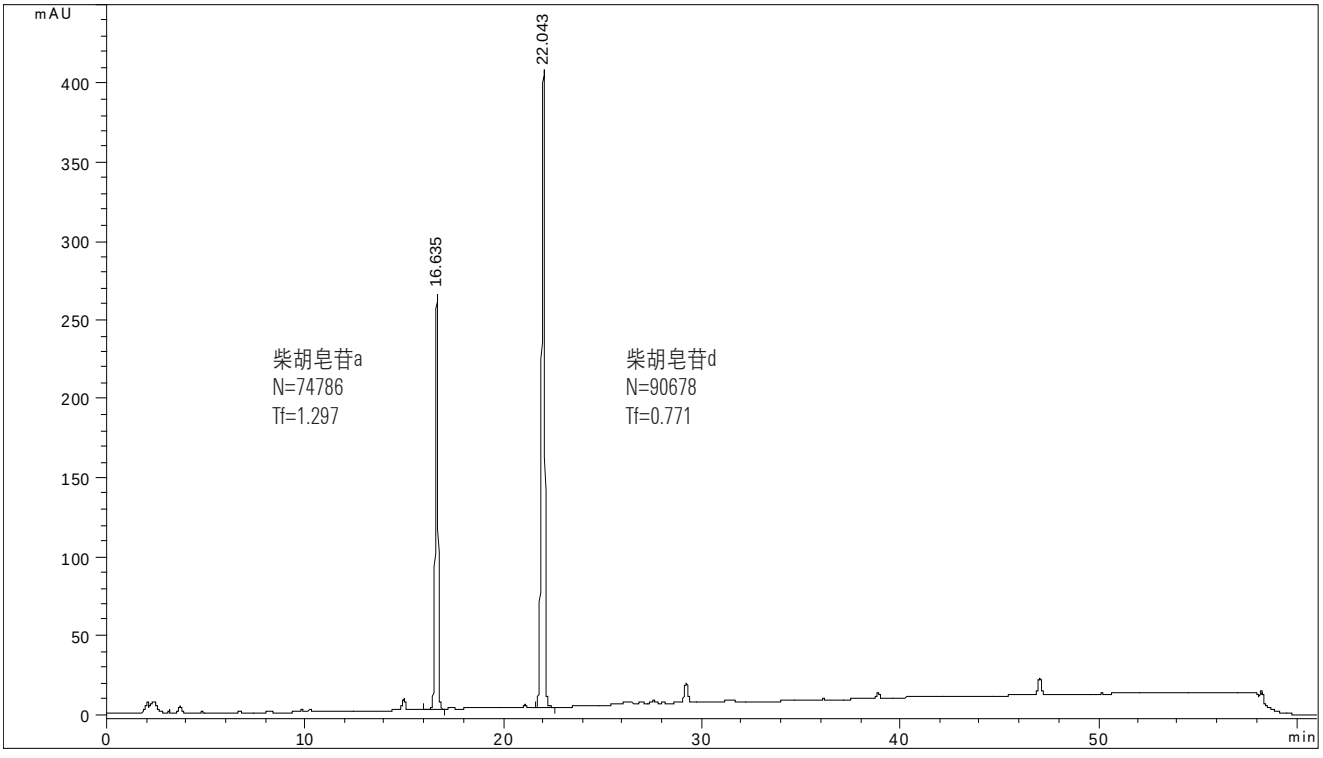

图1a Agilent Zorbax SB-C18， 250 mm × 4.6mm，5 μ m分析柴胡中的对照品柴胡皂苷a、d

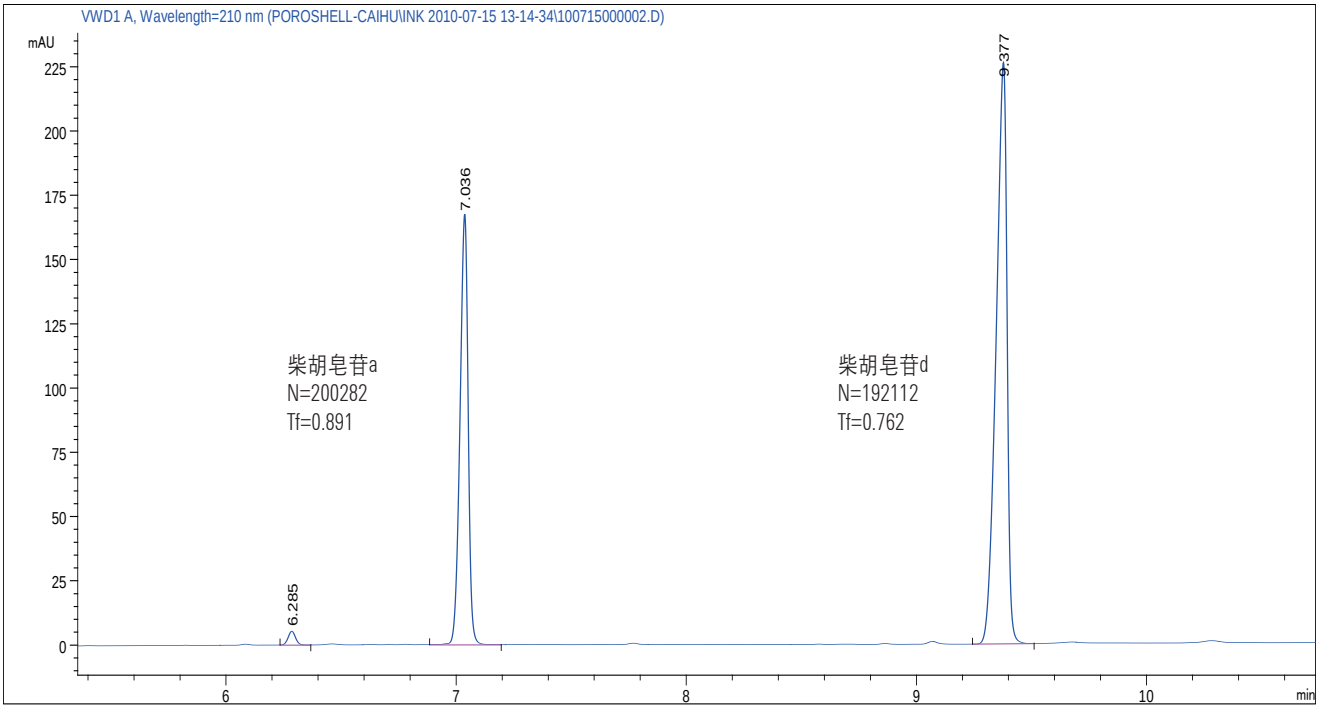

图1b Agilent Poroshell EC-C18，100 mm × 4.6 mm，2.7 μm分析柴胡中的对照品柴胡皂苷a、d

2.2 实际样品分析谱图

图2a和图2b的分别为采用传统规格的色谱柱与表面多孔层Poroshell 120液相色谱柱对供试品的分析结果。

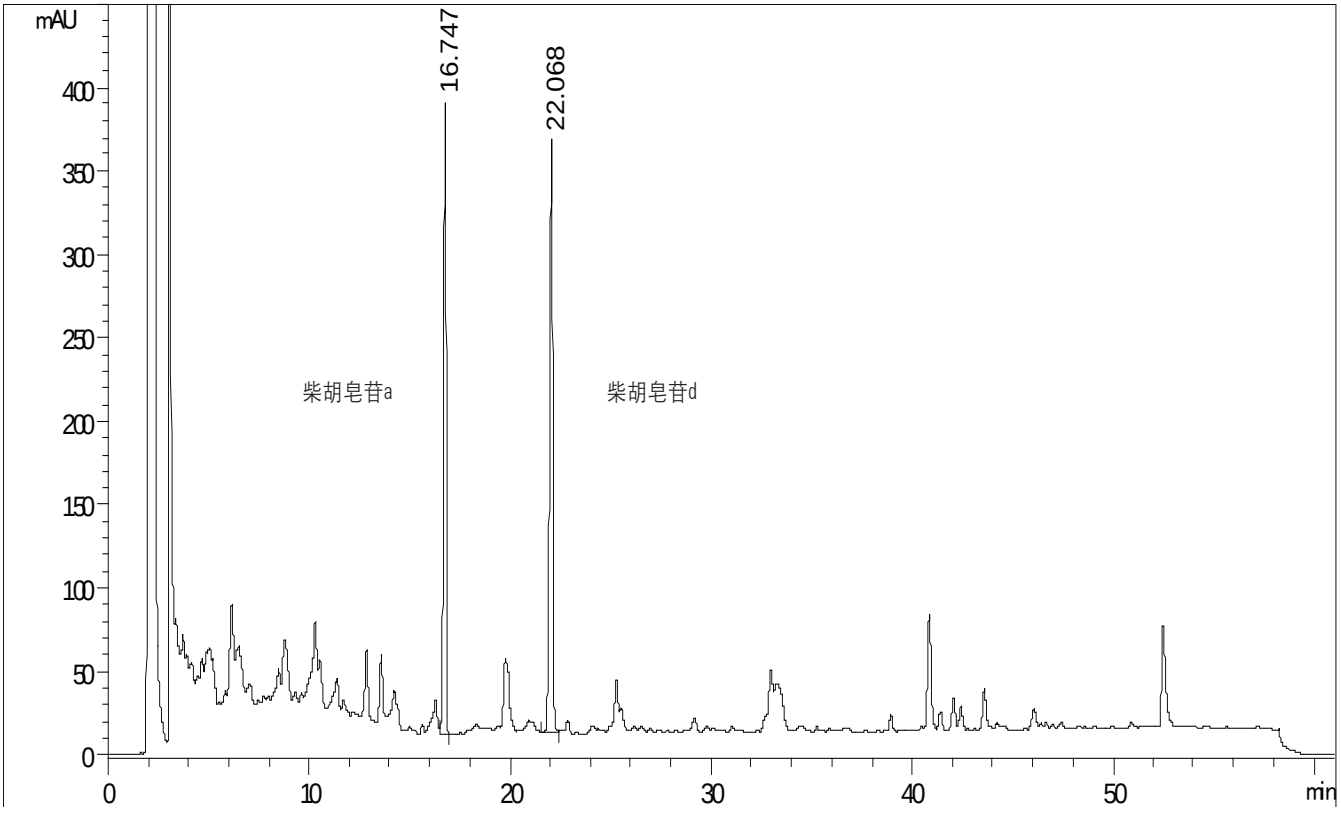

图2a Agilent Zorbax SB-C18， 250 mm × 4.6mm，5 μ m分析柴胡供试品

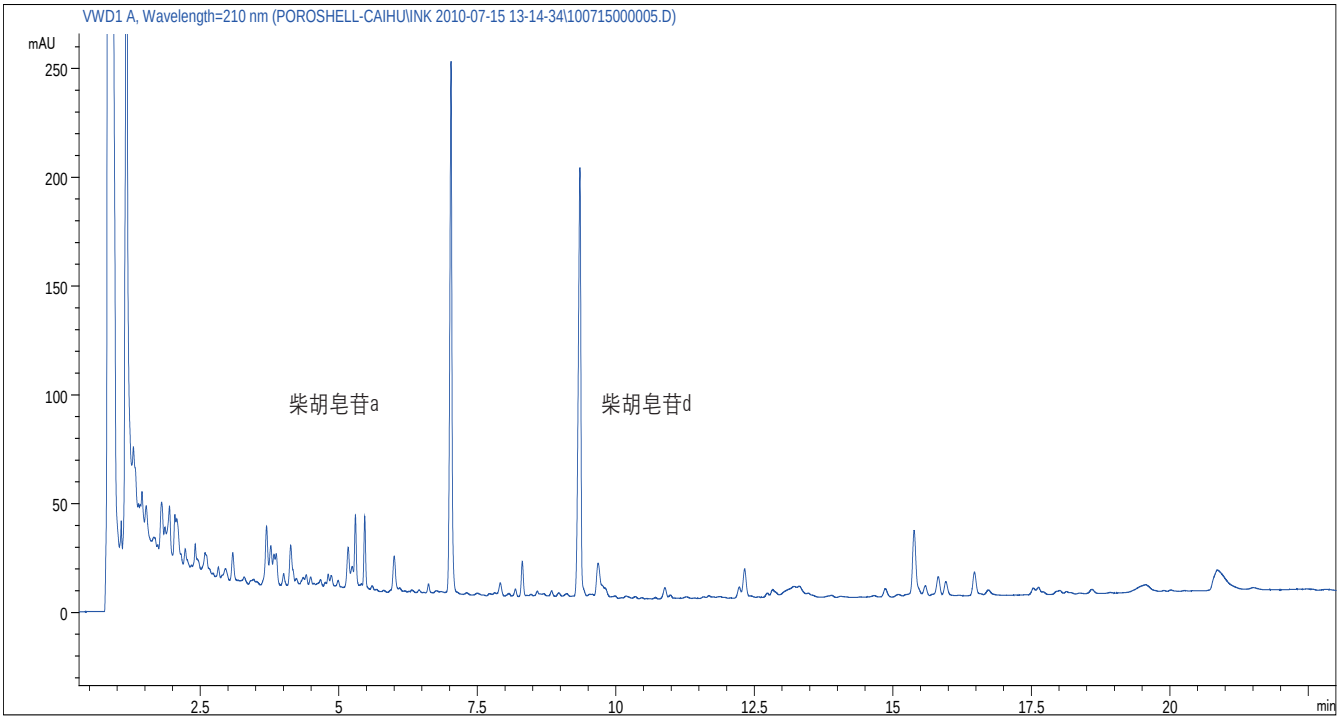

图2b Agilent Poroshell EC-C18，100 mm × 4.6 mm，2.7 μm分析柴胡供试品

3 小结

本法采用Poroshell120 EC-C18,参考2010版药典方法分离柴胡，从实验结果可以看出，与传统的5um粒径的Agilent Zorbax SB-C18相比，可以将柴胡的分离从原来的61分钟减少到23分钟。在提高分离效率的同时，从对照品峰的柱效来看，表面多孔层的Poroshell 120表现出了更好的柱效。而且，在使用Poroshell 120色谱柱时，整个方法压力最高只达230bar，完全可以与常规液相相兼容，实现高效快速的分离分析。

[参考文献]

[1]中国药典2010版，一部，263，柴胡。

采用Poroshell 120色谱柱快速高效分析甘草遵照2010版中国药典方法

应用领域

药物分析

关键词

HPLC；Poroshell 120；甘草; 2010版中国药典一部

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摘要

甘草(Radix Glycyrrhiza)别名甜草根或蜜草，是一种补益中药。根和根茎主要含三萜皂甙，甘草叶则富含黄酮类化合物。药典中液相色谱方法主要考察了甘草根及根茎部位中的甘草苷、甘草酸铵等活性成份。本文遵照2010版药典方法一部中的甘草方法，对甘草对照药材及相关的对照品进行了液相方法的建立，并同时采用了传统色谱柱及表面多孔层快速液相色谱柱对甘草进行分析。

1 试验材料与方法

1.1 仪器与试剂

1200SL高效液相色谱仪(Agilent公司)；1100高效液相色谱仪(Agilent公司)；乙腈(HPLC级, Dikma)；水由Milli-Q净化系统制得；磷酸(优级纯，北京化学试剂厂); 甘草及甘草苷、甘草酸铵对照品均由中国药品生物制品检定所提供。

1.2 色谱条件

Poroshell 120色谱条件：Agilent Poroshell SB-C18，150 × 3.0 mm，2.7 µm；进样量：2.5 µl；检测波长：237nm；柱温：60˚C；流速：0.4 ml/min；流动相： A: 0.05%磷酸、B: 乙腈;1200 SL液相色谱仪；

传统色谱柱方法：色谱柱：Agilent Zorbax SB-C18， 250 mm × 4.6mm，5 µ m；进样量：10 µ l；检测波长：237nm；柱温：25˚C；流速：0.4 ml/min; 流动相： A: 0.05%磷酸、B: 乙腈；1100液相色谱仪。

1.3试验方法

1.3.1对照品溶液的配制

取甘草苷对照品（1mg/mL）、甘草酸铵对照品（1.04mg/mL）溶液适量，加70%乙醇制成每1ml各含0.02mg（20.8ug）、0.2mg的溶液，即得。

1.3.2 供试品溶液的制备

取本品粉末（过三号筛）0.2g，精密称定，置具塞锥形瓶中，精密加入70%乙醇100ml，密塞，称定重量，超声处理（功率250W，频率40kHz）30分钟，取出，放冷，再称定重量，用70%乙醇补足减失

的重量，摇匀，滤过，取续滤液，即得。

2 结果与讨论

2.1 色谱条件的选择

2010版药典中甘草【含量测定】项下，要求使用十八烷基硅烷键合硅胶为填充剂的色谱柱，以乙腈(A)和0.05%磷酸溶液(B)作流动相，对甘草苷峰理论板数计算不得低于5000。按药典中的梯度方法：

时间(分钟)	流动相A（%）	流动相B（%）
0~8	19	81
8~35	19~50	81~50
35~36	50~100	50~0
36~40	100~19	0~81

实验中发现，如果遵照药典方法，甘草对照药材供试品谱图中，甘草酸与紧随其后的杂质峰无法实现分离，影响了含量测定的结果。因此，对梯度进行了一定的修改，在Agilent Zorbax SB-C18， 250 mm × 4.6mm，5 μ m，梯度见表1。对照品结果见谱图1a.

表1 Agilent Zorbax SB-C18， 250 mm × 4.6mm，5 μ m分析甘草梯度方法

时间 (min)	0	8	60	70	75	80
A%	19	19	35	40	0	19

当使用Poroshell 120时，由于使用的柱长为150mm。即当柱内径相同的情况下，梯度按柱长的比例成正比进行转换，梯度见表2。

表2 Poroshell SB-C18，100 mm × 3.0 mm，2.7 μm分析甘草梯度方法

时间 (min)	0	4.8	18	30	35	38
A%	21	21	35	40	100	19

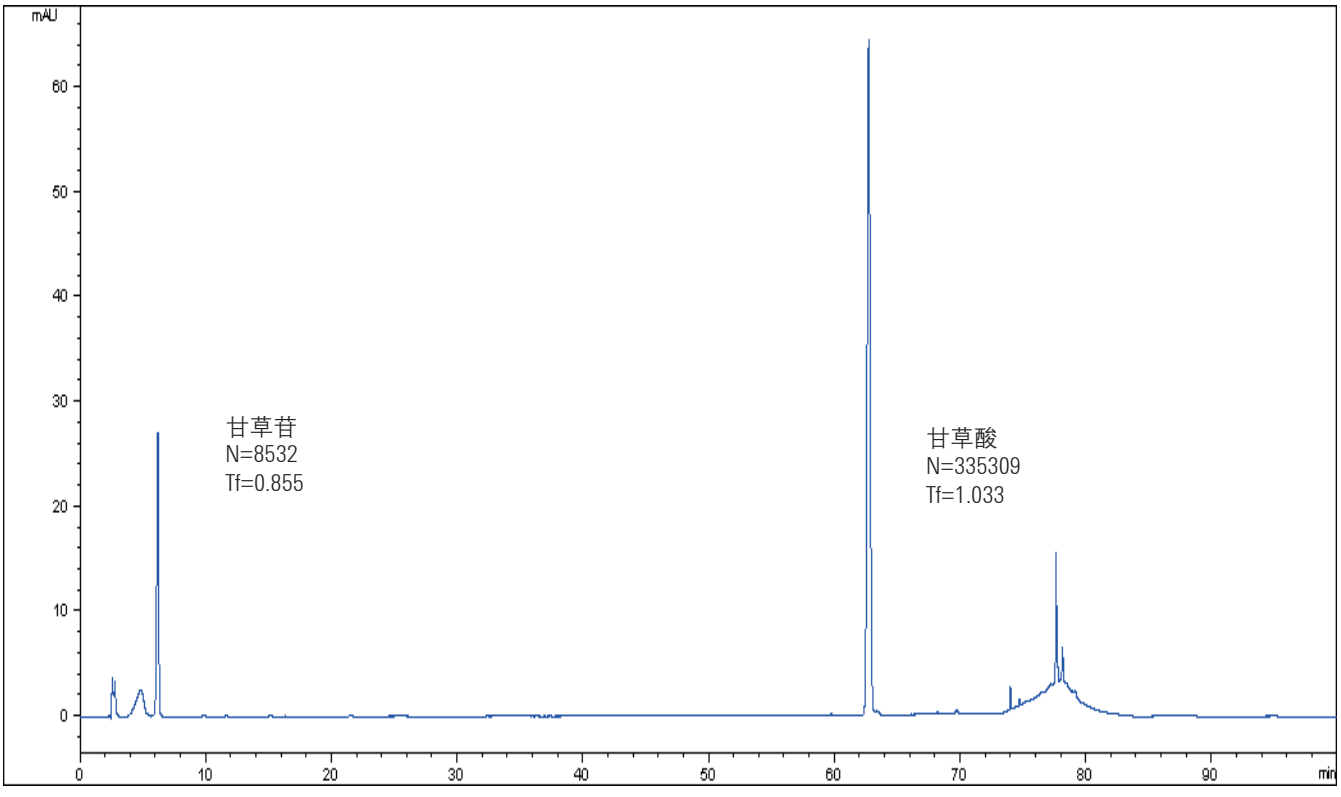

图1a Agilent Zorbax SB-C18， 250 mm × 4.6mm，5 μ m分析甘草中的对照品甘草苷和甘草酸

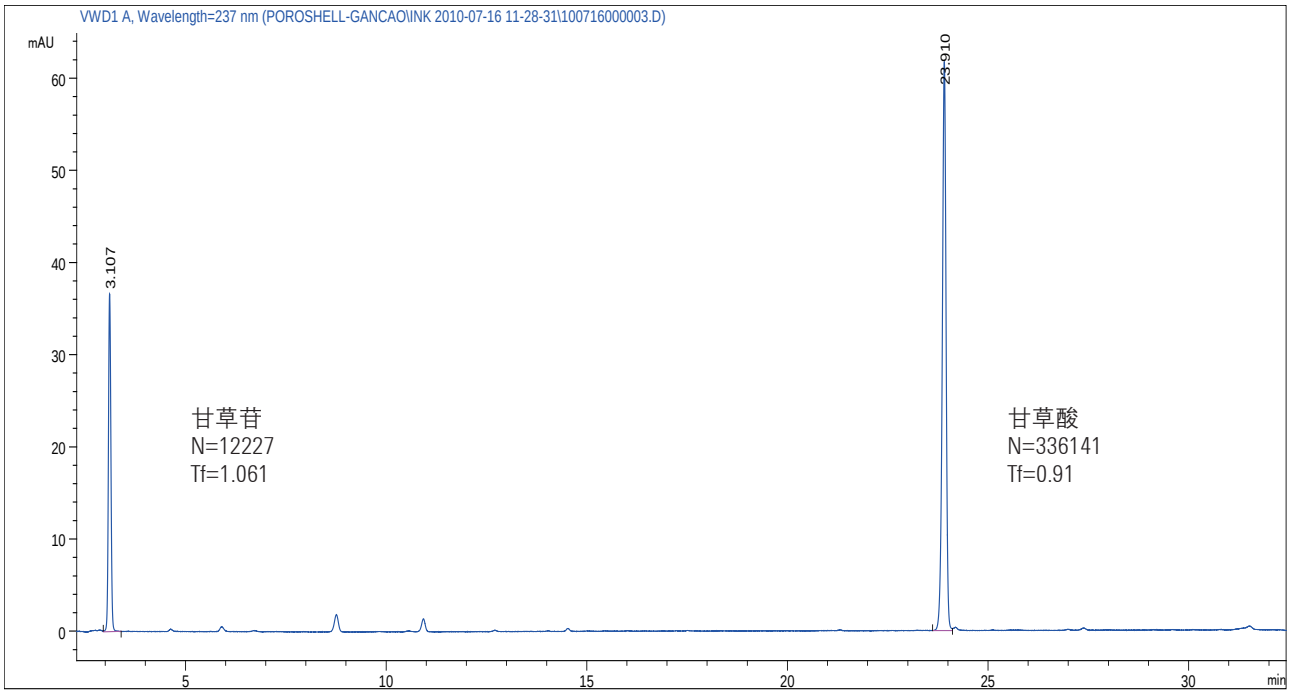

图1b Agilent Poroshell SB-C18，150 mm × 3.0 mm，2.7 µm分析甘草中的甘草苷和甘草酸

从以上结果可以看出，Poroshell 120 SB-C18柱效更高，对甘草酸旁的杂质分离所得的分离度更好。

2.2 实际样品分析谱图

图2a和图2b的分别为采用传统规格的色谱柱与表面多孔层Poroshell 120液相色谱柱对供试品的分析结果。

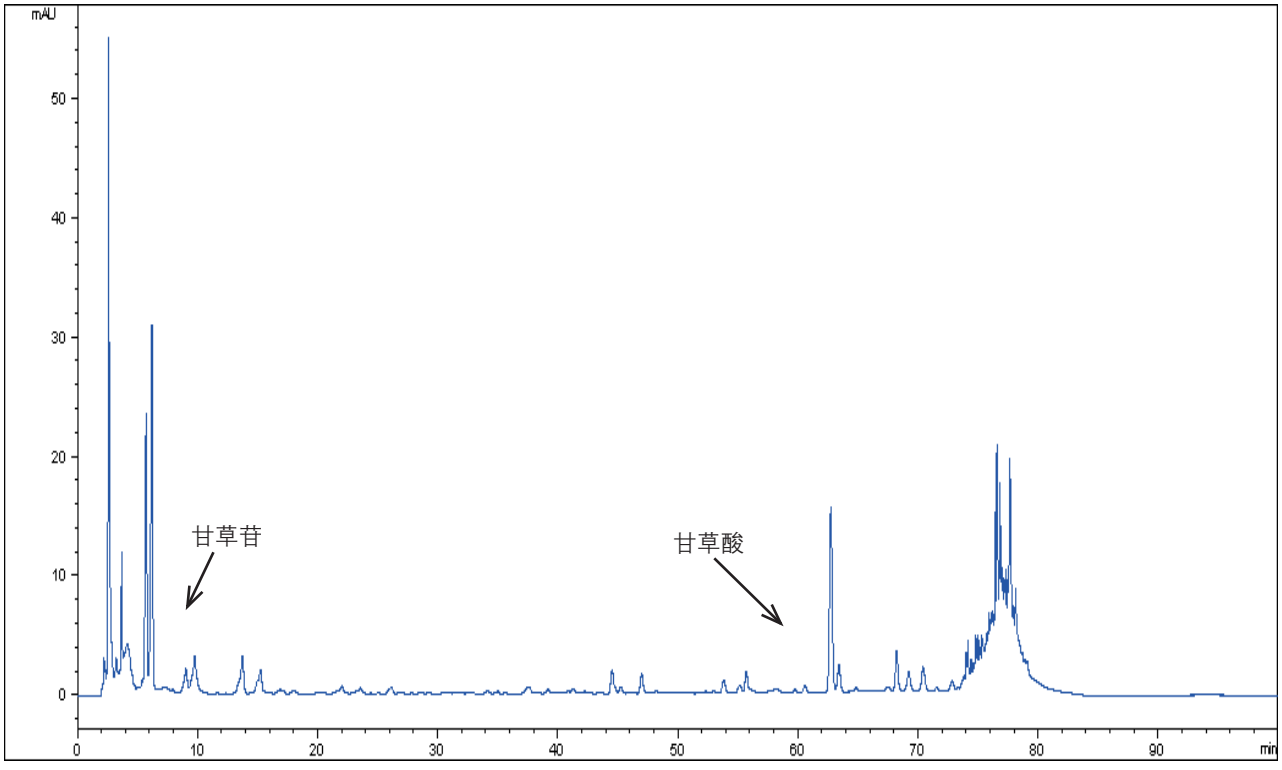

图2a Agilent Zorbax SB-C18， 250 mm × 4.6mm，5 µ m分析甘草供试品

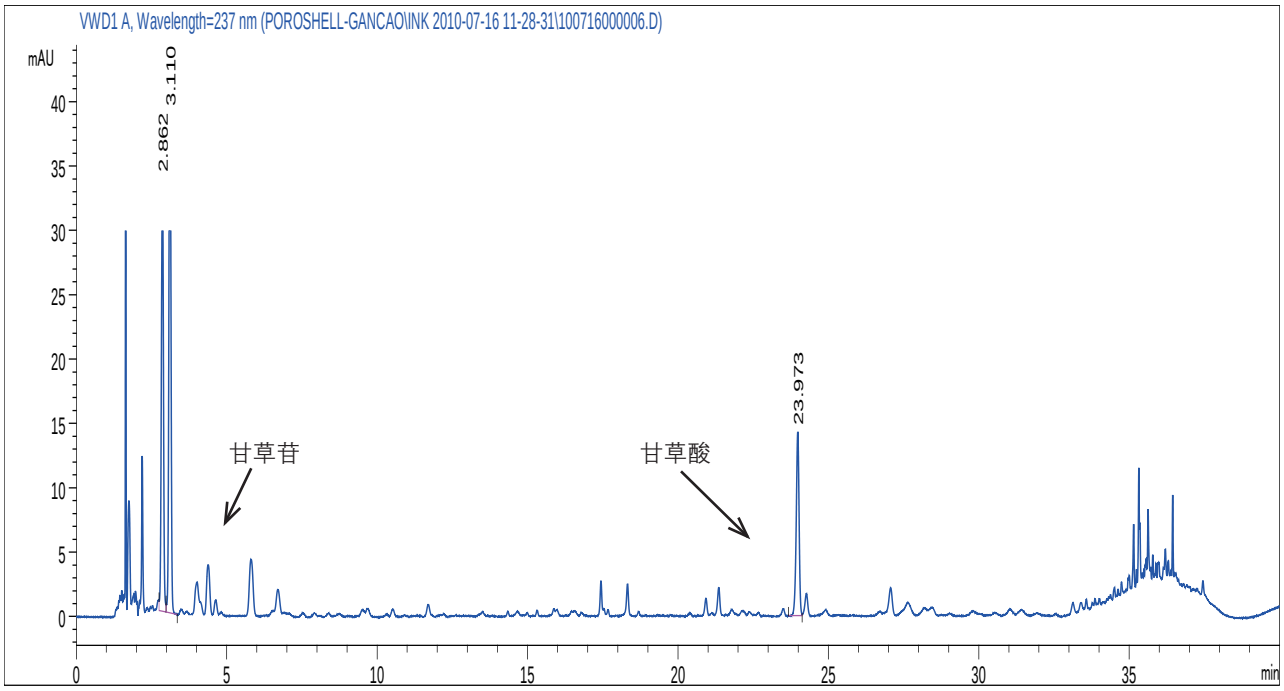

图2b Agilent Poroshell SB-C18，150 mm × 3.0 mm，2.7 µm分析甘草供试品

组分名称 （对照药材色谱图）	保留时间 (min)	峰面积 (mAU*s)	塔板数 (N)	分离度 (Rs)	拖尾因子 (USP Tf)
甘草苷	3.11	207.3	9024	1.89	1.01
甘草酸铵	23.973	95.2	321628	16.95	0.90

3 小结

本法主要采用Poroshell120 SB-C18,参考2010版药典方法分离甘草，为从实验结果可以看出，与传统的5um粒径的Agilent Zorbax SB-C18相比，不仅分析时间从原来的80分钟减少到38分钟，同时柱效有时显的提高，主峰旁的杂质峰分离度更好。而且，在使用Poroshell 120色谱柱时，整个方法压力最高只达140 bar，完全可以与常规液相相兼容，实现高效快速的分离分析。

[参考文献]

[1]中国药典2010版，一部，80，甘草。

采用Poroshell 120色谱柱快速高效分析豨莶草遵循2010版中国药典方法

应用领域

药物分析

关键词

HPLC；Poroshell 120；豨莶草; 2010版中国药典一部

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摘要

豨莶草(Glandularstalk St.Paulswort Herb)，豨莶草出自《唐本草》：“豨莶，叶似酸浆而狭长，花黄白色，田野皆识之。”别名为粘金强子、粘为扎、珠草、棉苍狼。性寒，味辛、苦。可祛风湿，利关节，解毒。本品化学成份复杂，药典中主要以奇壬醇进行含量测定。本文同时采用了传统5 um粒径的全多孔色谱柱与安捷伦最新推出的Poroshell 120表面多孔层色谱柱对　莶草供试品及对照品进行了分析，建立了适合于常规液相色谱仪的普通及高效快速方法。

1 试验材料与方法

1.1 仪器与试剂

1200SL高效液相色谱仪(Agilent公司)；1100高效液相色谱仪(Agilent公司)；甲醇(HPLC级, Dikma)；水由Milli-Q净化系统制得；豨莶草及奇壬醇对照品均由中国药品生物制品检定所提供。

1.2 色谱条件

Poroshell 120色谱条件：Agilent Poroshell EC-C18，100 × 4.6 mm，2.7 μm；进样量：4 μl；检测波长：215 nm；柱温：35 ˚C；流速：1 ml/min；流动相： A:乙腈、B: 水; 所使用液相仪器：1200 SL液相色谱仪；

传统色谱柱方法：色谱柱：Agilent Zorbax SB-C18， 250 mm × 4.6mm，5 μ m；进样量：10 μ l；检测波长：215 nm；柱温：35˚C；流速：1 ml/min; 流动相： A:乙腈、B: 水; 所使用液相色谱仪为1100液相色谱仪。

1.3试验方法

1.3.1对照品溶液的配制

奇壬醇1.48mg/ml浓标对照溶液用甲醇稀释为奇壬醇19.98μg/mL的溶液。

1.3.2 供试品溶液的制备

取本品粉末（过三号筛）0.9609g，精密称定，置具塞锥形瓶中，精密加入甲醇50ml，密塞，称定重量，加热回流5小时，放冷，再称定重量，用甲醇补足减失的重量，摇匀，滤过，取续滤液，即

得。

2 结果与讨论

2.1 色谱条件的选择

2010版药典中豨莶草【含量测定】项下，要求使用十八烷基硅烷键合硅胶为填充剂的色谱柱，以乙腈(A)和0水作流动相，对奇壬醇对照品理论板数计算不得低于5000。药典中的梯度方法：

时间(分钟)	流动相A（%）	流动相B（%）
0~5	5~24	95~76
5~30	24	76

原始方法中给出的梯度最终的有机相比例不够高，以致于有部份强保留物质仍在色谱柱上保留。为延长色谱柱寿命及提高方法重现性，将梯度表中最终的有机相比例调至100%。而且考虑到奇壬醇前的杂质峰在升高柱温的情况下可以显著提高分离度，故最终将柱温设置为35oC。具体梯度见表一。

表1 Agilent Zorbax SB-C18， 250 mm × 4.6mm，5 μ m分析豨莶草梯度方法

时间 (min)	0	5	40	43	48	49
A%	5	23	23	100	100	5

当使用Poroshell 120时，由于使用的柱长为100mm。梯度时间进行了适当的调整，如表2所示。

表2 Poroshell EC-C18，100 mm × 4.6 mm，2.7 μm分析豨莶草梯度方法

时间 (min)	0	2	16	17	19	20
A%	5	20	20	100	100	5

下列谱图为对照品谱图。

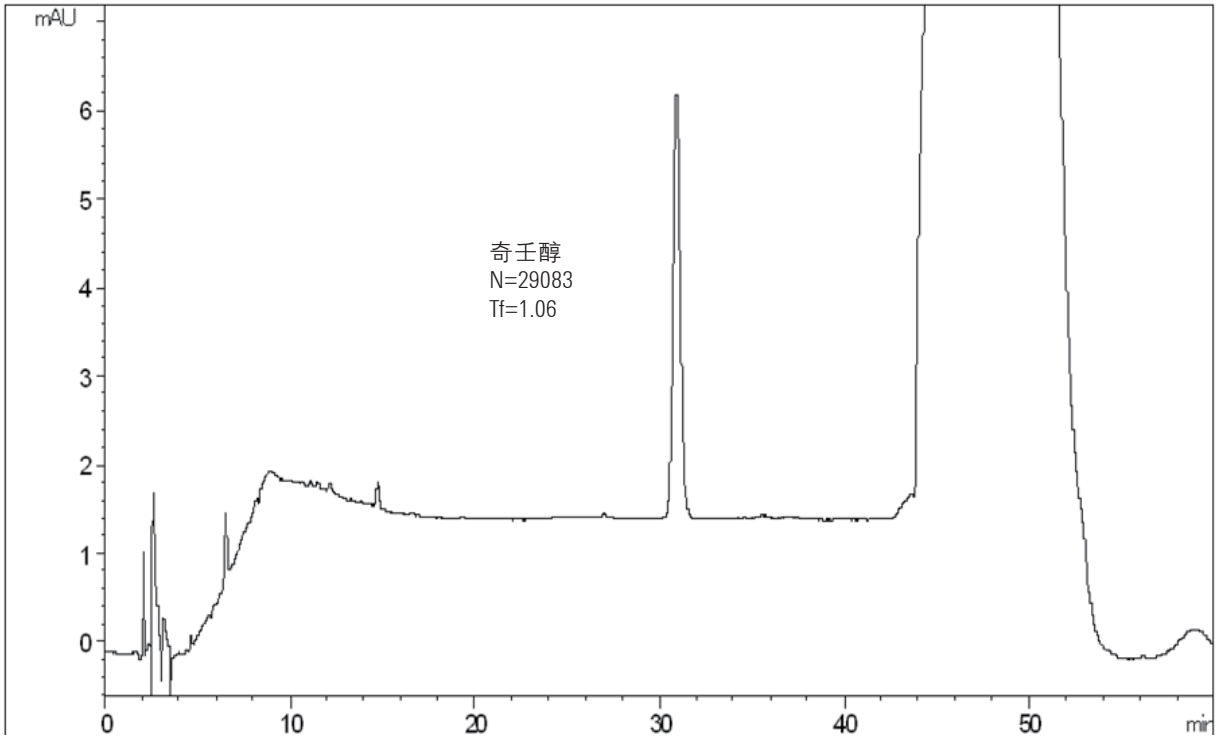

图1a Agilent Zorbax SB-C18， 250 mm × 4.6mm，5 μ m分析奇壬醇

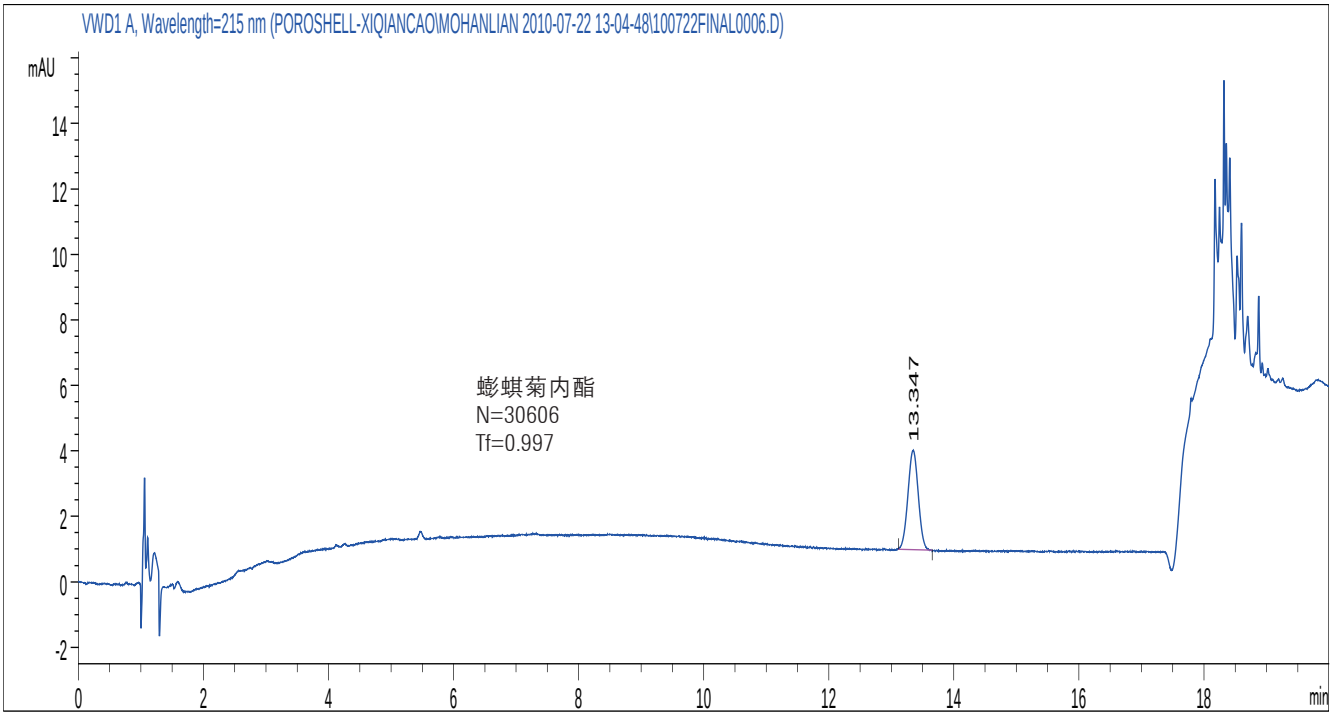

图1b Agilent Poroshell EC-C18，100 mm × 4.6 mm，2.7 μm分析奇壬醇

从以上结果可以看出，Poroshell 120 Plus-C18不仅分离时间显著缩短，且柱效更好。

2.2 实际样品分析谱图

图2a和图2b的分别为采用传统规格的色谱柱与表面多孔层Poroshell 120液相色谱柱对供试品的分析结果。

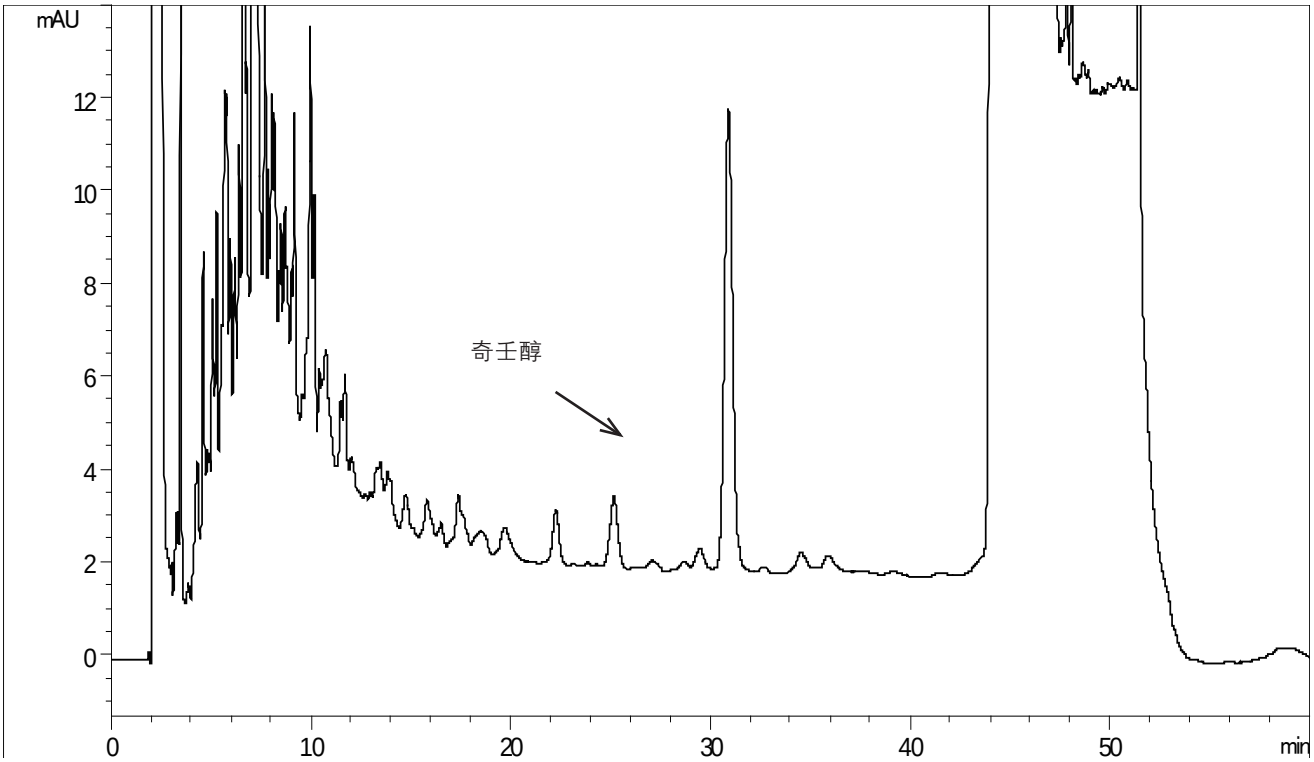

图2a Agilent Zorbax SB-C18， 250 mm × 4.6mm，5 μ m分析豨莶草供试品

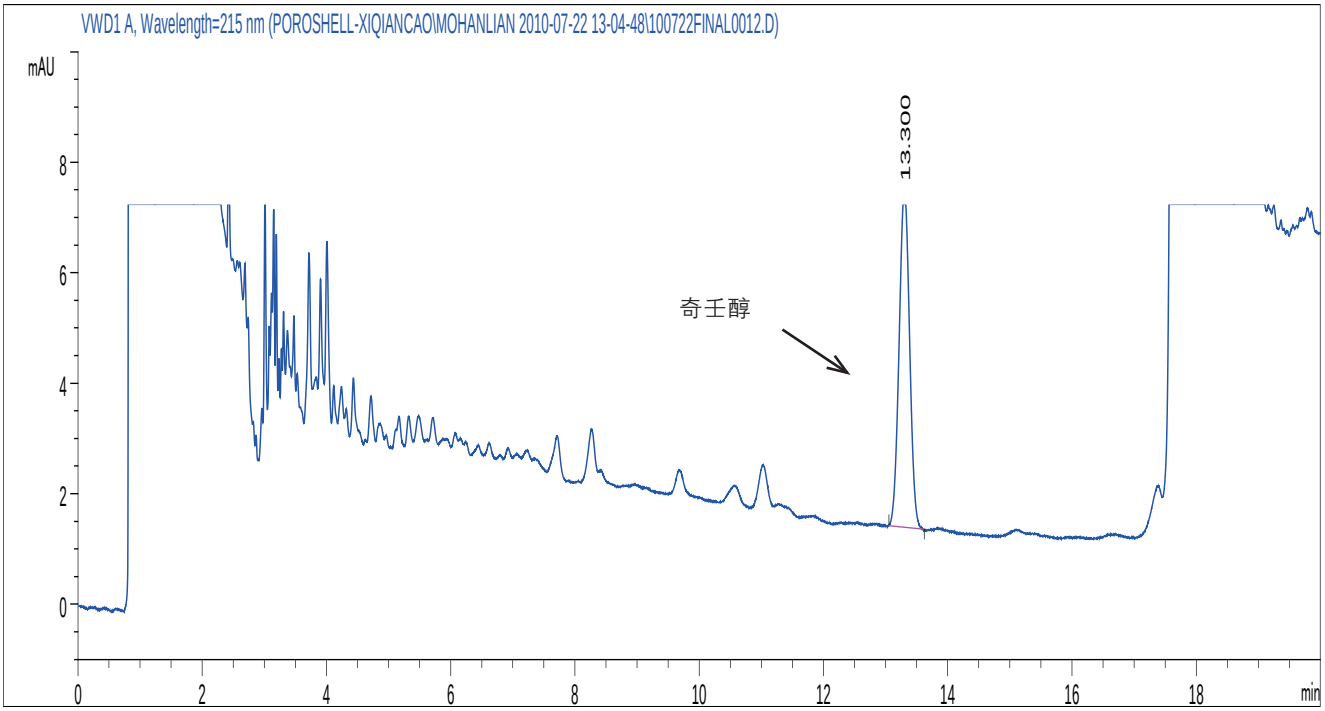

图2b Agilent Poroshell EC-C18，100 mm × 4.6 mm，2.7 µm分析豨莶草供试品

组分名称 （对照药材色谱图）	保留时间 (min)	峰面积 (mAU*s)	塔板数 (N)	分离度 (Rs)	拖尾因子 (USP Tf)
奇壬醇	13.30	71.7	29587	/	1.004

3 小结

本法主要采用Poroshell120 EC-C18,参考2010版药典方法分离豨莶草，为从实验结果可以看出，与传统的5um粒径的Agilent Zorbax SB-C18相比，不仅分析时间从原来的49分钟缩短至20分钟，即每针溶剂消耗节省29 mL，同时柱效有时显的提高，从供试品主峰奇壬醇与前面杂质峰的分离度可以看出，Poroshell 120 提供更好的分离度。而且，在使用Poroshell 120色谱柱时，整个方法压力最高只达140 bar，完全可以与常规液相相兼容，实现高效快速的分离分析。

[参考文献]

[1]中国药典2010版，一部，345，豨莶草。

采用Poroshell 120色谱柱快速高效分析墨旱莲遵循2010版中国药典方法

应用领域

药物分析

关键词

HPLC；Poroshell 120；墨旱莲; 2010版中国药典一部

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摘要

墨旱莲(Ecliptae Herba)，别名为旱莲草，黑墨草等，为菊科植物鳢肠(Eclipta prostrasta L.)的地上部分。具有滋补肝肾，凉血止血的功效。全草主含皂苷1.32% 和烟碱（约0.08%），叶含蟛蜞菊内酯(Wedelolac-tone)、去甲基蟛蜞菊内酯。去甲基蟛蜞菊内酯-7-葡萄糖甙。药典10版【含量测定】项下，要求使用高效液相色谱方法进行分离，主要观察的化合物为蟛蜞菊内酯。本文同时采用了传统规格5 um的色谱柱以及表面多孔层的Poroshell 120色谱柱对墨旱莲及其相关对照品进行了分析，建立了适合于常规液相色谱仪的常规及高效快速分析方法。

1 试验材料与方法

1.1 仪器与试剂

1200SL高效液相色谱仪(Agilent公司)；1100高效液相色谱仪(Agilent公司)；甲醇(HPLC级, Dikma)；水由Milli-Q净化系统制得；醋酸(色谱纯，迪马试剂); 墨旱莲及蟛蜞菊内酯对照品均由中国药品生物制品检定所提供。

1.2 色谱条件

Poroshell 120色谱条件：Agilent Poroshell EC-C18，100 × 4.6 mm，2.7 µm；进样量：4 µl；检测波长：351 nm；柱温：40 ˚C；流速：1 ml/min；流动相： A:甲醇、B: 0.5%醋酸水溶液; 所使用液相仪器：1200 SL液相色谱仪；

传统色谱柱方法：色谱柱：Agilent Zorbax Plus-C18， 250 mm × 4.6mm，5 µ m；进样量：10 µ l；检测波长：351 nm；柱温：40˚C；流速：1 ml/min; 流动相： A:甲醇、B: 0.5%醋酸溶液; 所使用液相色谱仪为1100液相色谱仪。

1.3试验方法

1.3.1对照品溶液的配制

取蟛蜞菊内脂浓标对照溶液（0.424mg/ml）用70%乙醇水溶液稀释为蟛蜞菊内脂：10.76µg/ml的溶液。

1.3.2 供试品溶液的制备

精密称定本品粉末（过三号筛）1.0017g，置具塞锥形瓶中，精密加入70%乙醇50ml，密塞，称定重量，加热回流1小时，放冷，再称定重量，用70%乙醇补足减失的重量，摇匀，滤过，取续滤液，即得。

2 结果与讨论

2.1 色谱条件的选择

2010版药典中墨旱莲【含量测定】项下，要求使用十八烷基硅烷键合硅胶为填充剂的色谱柱，以甲醇(A)和0.5%醋酸水溶液作流动相，对蟛蜞菊内酯理论板数计算不得低于6000。药典中的梯度方法：

时间(分钟)	流动相A（%）	流动相B（%）
0~10	35~59	65~41
10~20	59	41

原始方法中给出的梯度时间只包括对照品出峰的时间，实际操作中，因为供试品中在20Wv钟后仍有不少杂质，因此，建议在梯度表中增加清洗色谱柱的步聚，以提高色谱柱的使用寿命及方法的重现性，如表一。

表1 Agilent Zorbax Plus-C18， 250 mm × 4.6mm，5 μ m分析墨旱莲梯度方法

时间 (min)	0	10	20	21	26	27
A%	35	59	59	95	95	35

当使用Poroshell 120时，由于使用的柱长为100mm。梯度时间进行了适当的调整，如表2所示。

表2 Poroshell EC-C18，100 mm × 4.6 mm，2.7 μm分析墨旱莲梯度方法

时间 (min)	0	4	8	9	11	12
B%	35	50	50	100	100	35

下列谱图为对照品谱图。

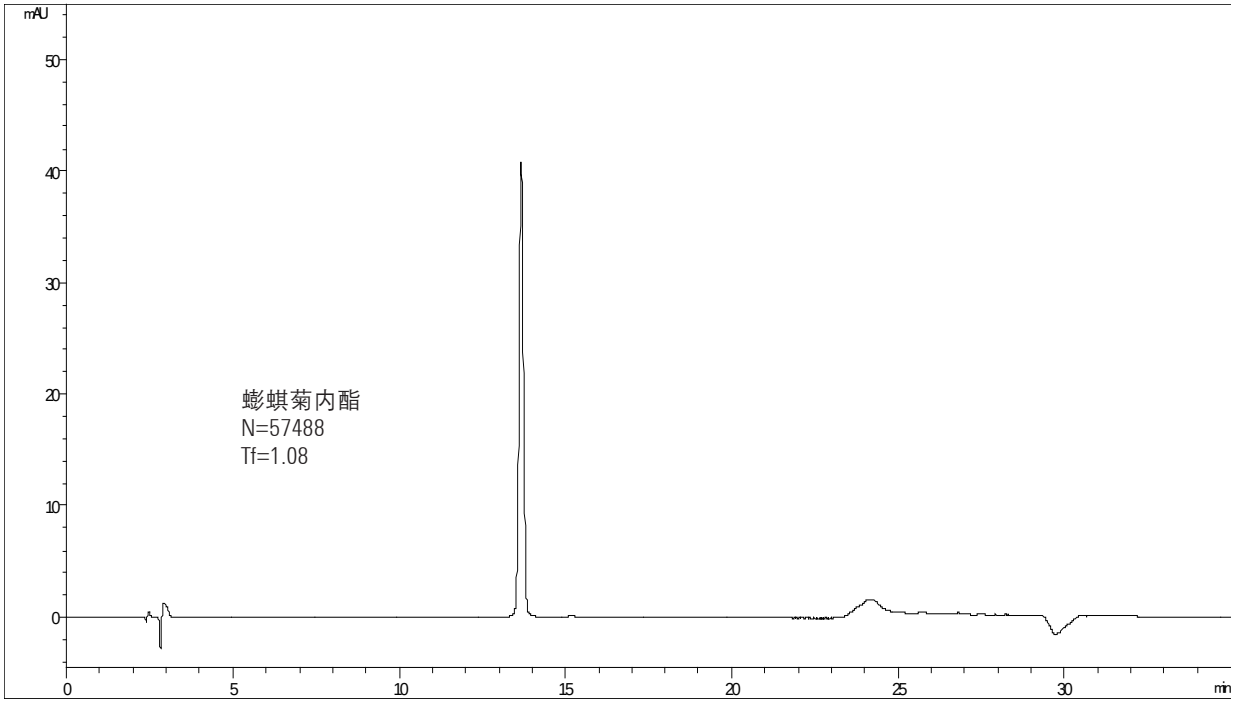

图1a Agilent Zorbax Plus-C18， 250 mm × 4.6mm，5 μ m分析蟛蜞菊内酯

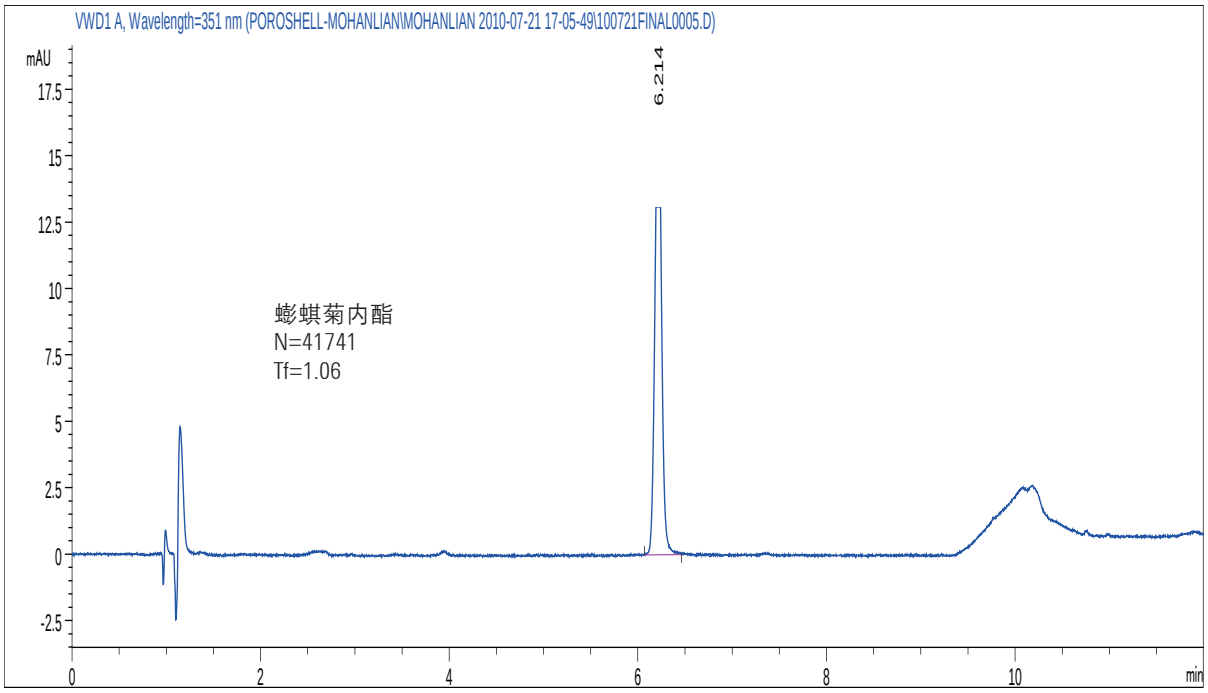

图1b Agilent Poroshell EC-C18，100 mm × 4.6 mm，2.7 μm分析蟎蜱菊内酯

从以上结果可以看出，Poroshell 120 Plus-C18不仅分离时间显著缩短，且柱效更好。

2.2 实际样品分析谱图

图2a和图2b的分别为采用传统规格的色谱柱与表面多孔层Poroshell 120液相色谱柱对供试品的分析结果。

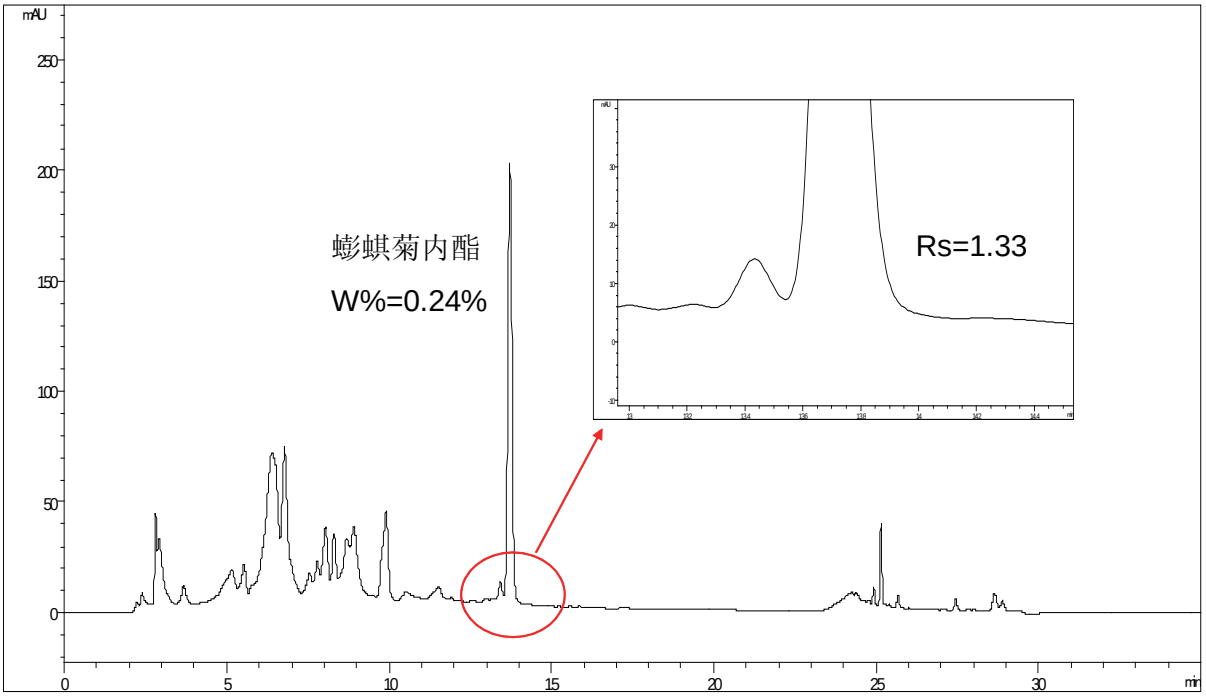

图2a Agilent Zorbax Plus-C18， 250 mm × 4.6mm，5 μ m分析墨旱莲供试品

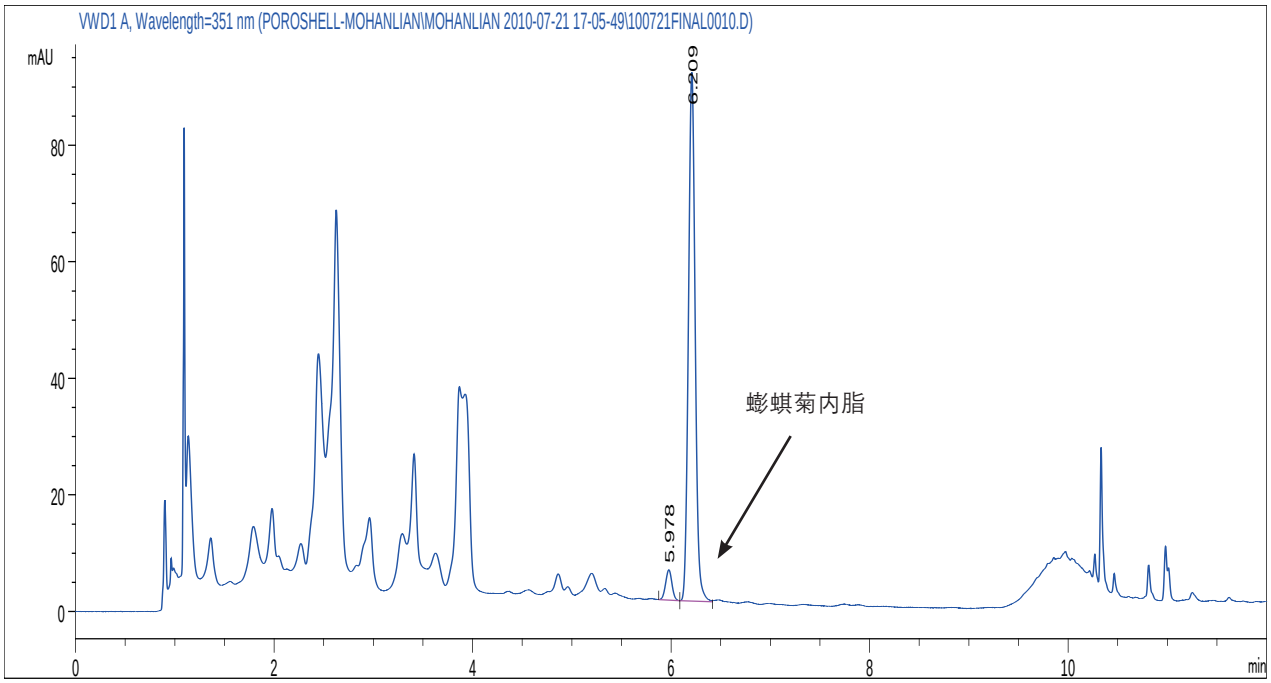

图2b Agilent Poroshell EC-C18，100 mm × 4.6 mm，2.7 μm分析墨旱莲供试品

组分名称 （对照药材色谱图）	保留时间 (min)	峰面积 (mAU*s)	塔板数 (N)	分离度 (Rs)	拖尾因子 (USP Tf)
蟛蜞菊内脂	6.209	427.4	42395	1.94	1.06

3 小结

本法主要采用Poroshell120 EC-C18,参考2010版药典方法分离墨旱莲，为从实验结果可以看出，与传统的5um粒径的Agilent Zorbax SB-C18相比，不仅分析时间节省了15分钟，即每针溶剂消耗节省15 mL，同时柱效有时显的提高，从供试品主峰蟛蜞菊内酯与前面杂质峰的分离度可以看出，Poroshell 120 提供更好的分离度。而且，在使用Poroshell 120色谱柱时，整个方法压力最高只达140 bar，完全可以与常规液相相兼容，实现高效快速的分离分析。

[参考文献]

[1]中国药典2010版，一部，351，墨旱莲。

采用Poroshell 120色谱柱快速高效分析关黄柏遵循2010版中国药典方法

应用领域

药物分析

关键词

HPLC；Poroshell 120；关黄柏; 2010版中国药典一部

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摘要

关黄柏(Amur Cork-tree Bark)别名黄柏，为芸香科植物黄蘗(Phellodendron amurense Rupr.)的树皮。性寒味苦，具有清热燥湿，泻火除蒸，解毒疗疮等功效。关黄柏含有多种生物碱，主要为小檗碱，含量为0.6%~2.5%，以及少量的巴马汀、药根碱、黄柏碱等多种生物碱。2010版药典一部要求采用液相色谱法对关黄柏进行含量测定。本文使用了Poroshell 120表面多层的液相色谱柱在遵循药典方法要求下，高效快速分离了关黄柏，且整个方法在普通液相色谱仪上具有很好的兼容性。

1 试验材料与方法

1.1 仪器与试剂

1200SL高效液相色谱仪(Agilent公司)；1100高效液相色谱仪(Agilent公司)；乙腈(HPLC级, Dikma)；水由Milli-Q净化系统制得；磷酸(分析纯，北京化学试剂厂); 磷酸二氢钠(北京益利精细化学品有限公司，分析纯)，关黄柏及盐酸小檗碱、盐酸巴马汀对照品均由中国药品生物制品检定所提供。

1.2 色谱条件

Poroshell 120色谱条件：Agilent Poroshell EC-C18，100 × 4.6 mm，2.7 µm；进样量：4 µl；检测波长：345 nm；柱温：25 ˚C；流速：1 ml/min；流动相： A:乙腈、B: 0.1%磷酸溶液(加入磷酸二氢钠使其达到0.02 mol/L的浓度); 所使用液相仪器：1200 SL液相色谱仪；

传统色谱柱方法：色谱柱：Agilent Zorbax SB-C18， 250 mm × 4.6mm，5 µ m；进样量：10 µ l；检测波长：345 nm；柱温：28˚C；流速：1 ml/min; 流动相： A:乙腈、B: 0.1%磷酸溶液(加入磷酸二氢钠使其达到0.02 mol/L的浓度); 所使用液相色谱仪为1100液相色谱仪。

1.3试验方法

1.3.1对照品溶液的配制

盐酸巴马汀和盐酸小檗碱浓标对照溶液用60%乙醇水溶液稀释为盐酸巴马汀：50.31ug/mL的溶液；盐酸小檗碱稀释为50.625ug/mL的溶液。

1.3.2 供试品溶液的制备

精密称取关黄柏对照药材粉末0.2000g，精密称定，置具塞锥形瓶中，精密加入60%乙醇50ml，密塞，称定重量，超声处理（功率250W，频率40kHz）45分钟，取出，放冷，再称定重量，用60%乙醇补足减失的重量，摇匀，滤过，取续滤液，即得。

2 结果与讨论

2.1 色谱条件的选择

2010版药典中关黄柏【含量测定】项下，要求使用十八烷基硅烷键合硅胶为填充剂的色谱柱，以乙腈(A)和0.05%磷酸溶液(B)作流动相，对盐酸小檗碱理论板数计算不得低于4000。药典中的梯度方法：

时间(分钟)	流动相A（%）	流动相B（%）
0~20	25	75
20~40	25~65	75~35
40~45	65~90	35~10
45~50	90	10
50~65	25	75

实验中发现，供试品中大部份主分在20分钟前基本上出完，因此，方法中将梯度方法直接从20min时的25%直接升至90%，如表一。

表1 Agilent Zorbax SB-C18， 250 mm × 4.6mm，5 μ m分析关黄柏梯度方法

时间 (min)	0	20	25	35	36	42
A%	25	25	90	90	25	25

当使用Poroshell 120时，由于使用的柱长为100mm。梯度时间进行了适当的调整，如表2所示。

表2 Poroshell EC-C18，100 mm × 4.6 mm，2.7 μm分析关黄柏梯度方法

时间 (min)	0	4	8	9	10	11
B%	25	25	55	90	90	25

下列谱图为对照品谱图。

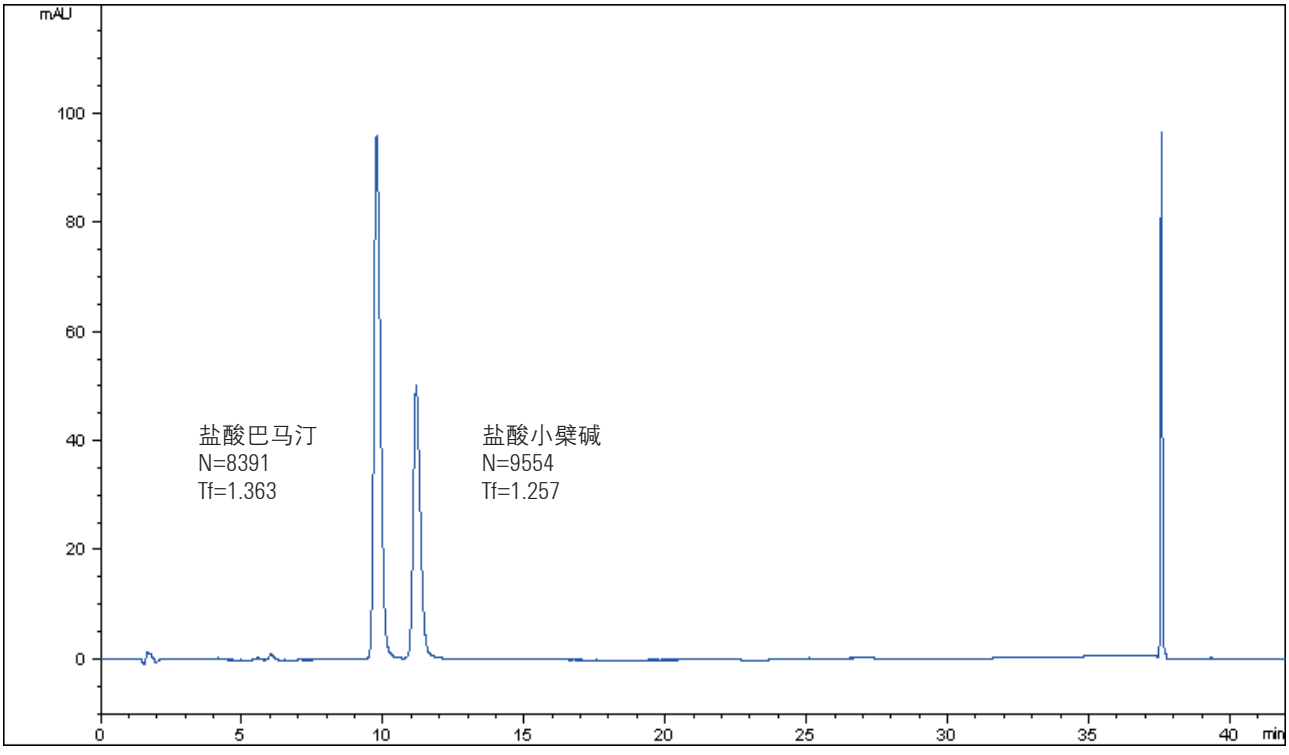

图1a Agilent Zorbax SB-C18， 250 mm × 4.6mm，5 μ m分析关黄柏中的盐酸巴马汀和盐酸小檗碱

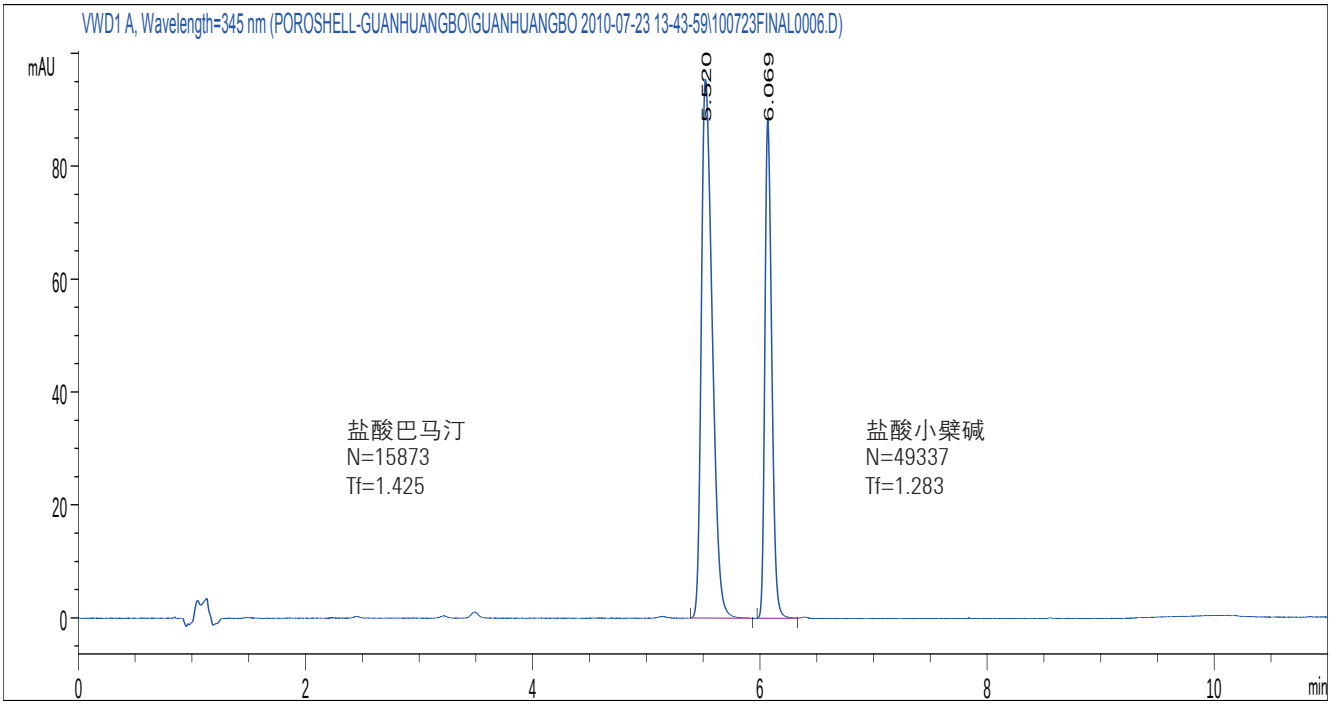

图1b Agilent Poroshell EC-C18，100 mm × 4.6 mm，2.7 µm分析关黄柏中的盐酸巴马汀和盐酸小檗碱

从以上结果可以看出，Poroshell 120 SB-C18柱效更高，而且分离时间显著缩短。

2.2 实际样品分析谱图

图2a和图2b的分别为采用传统规格的色谱柱与表面多孔层Poroshell 120液相色谱柱对供试品的分析结果。

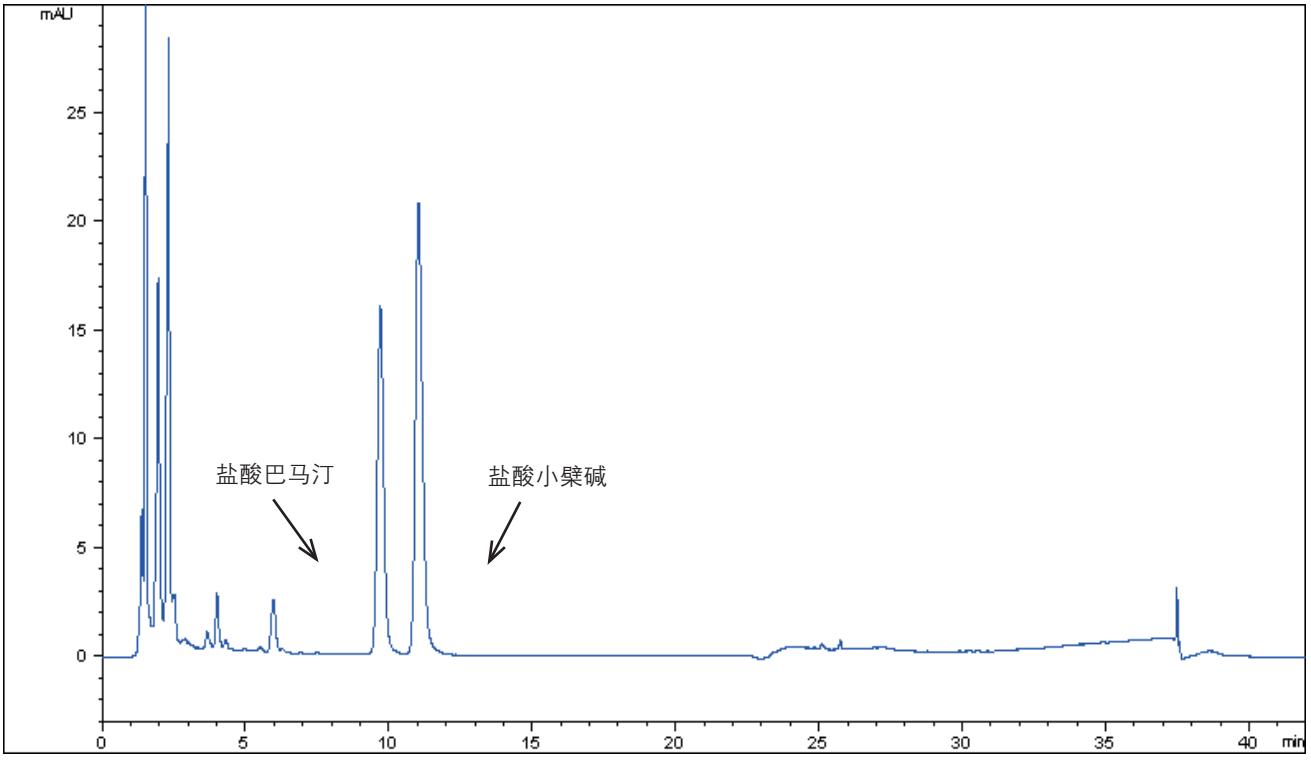

图2a Agilent Zorbax SB-C18， 250 mm × 4.6mm，5 µ m分析关黄柏供试品

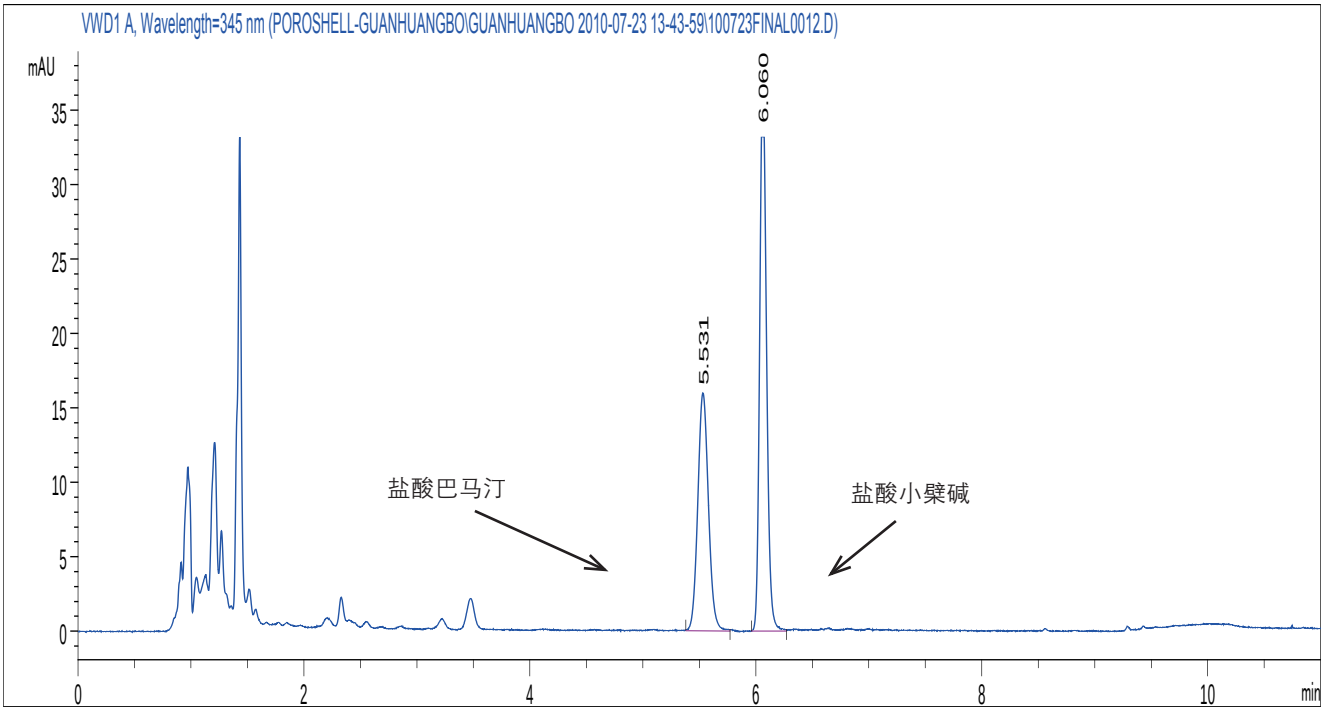

图2b Agilent Poroshell EC-C18，100 mm × 4.6 mm，2.7 μm分析关黄柏供试品

组分名称 （对照药材色谱图）	保留时间 (min)	峰面积 (mAU*s)	塔板数 (N)	分离度 (Rs)	拖尾因子 (USP Tf)
盐酸巴马汀	5.531	104.6	17544	/	1.087
盐酸小檗碱	6.060	155.1	51114	3.86	1.186

3 小结

本法主要采用Poroshell120 EC-C18,参考2010版药典方法分离关黄柏，为从实验结果可以看出，与传统的5um粒径的Agilent Zorbax SB-C18相比，不仅分析时间从原来的42分钟减少到13分钟，同时柱效有时显的提高。而且，在使用Poroshell 120色谱柱时，整个方法压力最高只达140 bar，完全可以与常规液相相兼容，实现高效快速的分离分析，且实验中供试品的含量测定测得值与传统液相方法完全一致。

[参考文献]

[1]中国药典2010版，一部，137，关黄柏。

采用Poroshell 120色谱柱快速分析菊花中的主要成分

应用领域

药物分析

关键词

HPLC；Poroshell 120；菊花

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摘要

菊花是传统的中药材之一，也是很多人日常喜爱的茶品。著名的《本草纲目》中就对菊花的药效有着详细的记载：性甘，微寒，具有散风热，平肝明目之功效。现代医学也证实：菊花具有降血压、扩张冠状动脉和抑菌等效果。长期饮用能增加人体钙质，调节心肌功能，降低胆固醇，对肝火旺及长时间用眼产生的双眼干涩也有较好的疗效。菊花中黄酮类成分量较高，其中木犀草苷临床和药理作用明显，另外，菊花中的3，5—0双咖啡酰基奎宁酸具有抗呼吸道病毒活性，因此两者均可作为菊花质量控制的指标。中国药典中对于菊花药材中主要有效成分如绿原酸，木犀草苷和3,5--O-双咖啡酰基奎宁酸的液相色谱分析方法作了相关的规定[1]，但规定的方法中使用的常规液相色谱仪和液相分析柱，分析时间长，且有机试剂耗用量大。如何对这类中草药复杂体系进行快速有效的分离分析成为缩短分析时间，提高工作效率并降低分析成本的关键。安捷伦公司最新推出的Poroshell 120系列表面多孔层色谱柱，由于其具有低反压，高柱效的特点，从而实现在常规液相色谱仪上进行快速分析的可操作性。本文使用Poroshell 120色谱柱，并采用1290UHPLC液相色谱仪，对菊花中绿原酸，木犀草苷和3,5--O-双咖啡酰基奎宁酸的同时检测方法加以改进，对原有体系中存在的位置异构体进行了有效分离。

1 试验材料与方法

1.1 仪器与试剂

1290UHPLC超高效液相色谱仪(Agilent公司)；甲醇、乙腈(HPLC级,美国Sigma-Aldrich公司)；去离子水由Milli-Q Academic净化系统制得；绿原酸，木犀草苷和3,5--O-双咖啡酰基奎宁酸标准品储备液均来自中国药品与生物制品检定所，浓度分别为35 μg/mL（绿原酸）、25 μg/mL（木犀草苷）和80 μg/mL（3,5--O-双咖啡酰基奎宁酸）。

1.2 色谱条件

初始色谱条件（依据中国药典2010版1部页方法转换）：

Zorbax Plus-C18柱 (2.1 × 100 mm, 1.8 μ m)；柱温25℃；流速0.4 mL /min；流动相A：0.1%磷酸溶液；流动相B：乙腈；二极管阵列检测器：波长348nm；进样体积0.8 μ L。

时间(min)	0	2.2	6	8	8.1	9	9.1
B%	10	18	20	20	100	100	10

优化色谱条件

Poroshell 120 EC-C18柱（3.0x150mm，2.7 μ m），柱温30℃；流速0.8 mL /min；流动相A：0.1%磷酸溶液；流动相B：甲醇:乙腈3:2；二极管阵列检测器：波长348nm；进样体积1.6 μ L。

时间(min)	0	2	8	8.5	9.5	9.6
B%	10	25	27	100	100	10

1.3 药材对照品

菊花药材对照品粉末来自中国药品与生物制品检定所。

1.4 试验方法

1.4.1 混合对照品制备

分别吸取不同体积的标准品储备液，加70%甲醇制成每1ml 含绿原酸35μg，木犀草苷25μg，3,5-O-双咖啡酰基奎宁酸80μg的混合溶液，即得（10℃以下保存）。

1.4.2 供试品制备

取菊花药材对照品粉末（过一号筛）约0.25g，精密称定，置具塞锥形瓶中，精密加入70%甲醇25 ml，密塞，称定重量，超声处理（功率300W，频率45kHz）40分钟，放冷，再称定重量，用70%甲醇补足减失的重量，摇匀，滤过，取续滤液，即得。.

1.4.3 测定

按1.2操作进行，以保留时间定性，外标法定量。

2 结果与讨论

2.1 初始色谱条件获得的结果

药典初始条件并不能有效地分离3,5-O-双咖啡酰基奎宁酸及其异构体。如下图1所示，在:色谱图局部放大之后可以看到包峰的现象。由于杂质与3,5-O双咖啡酰基奎宁酸的峰保留时间十分接近，因此考虑如何从两个方面改善分离度：1、改变选择性；2、提高柱效。

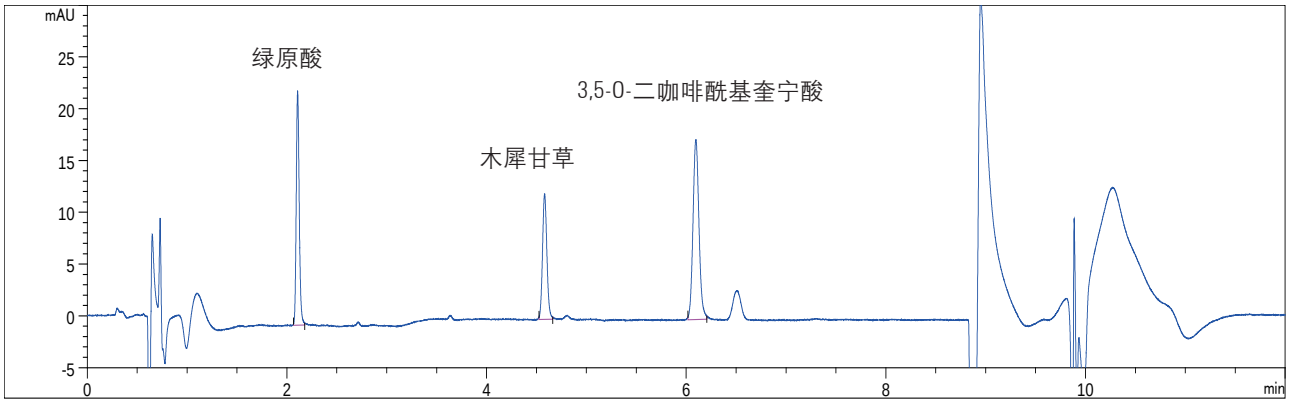

图1a 对照品溶液色谱图，色谱柱：Zorbax Plus-C18柱 (2.1 × 100 mm, 1.8 μ m)

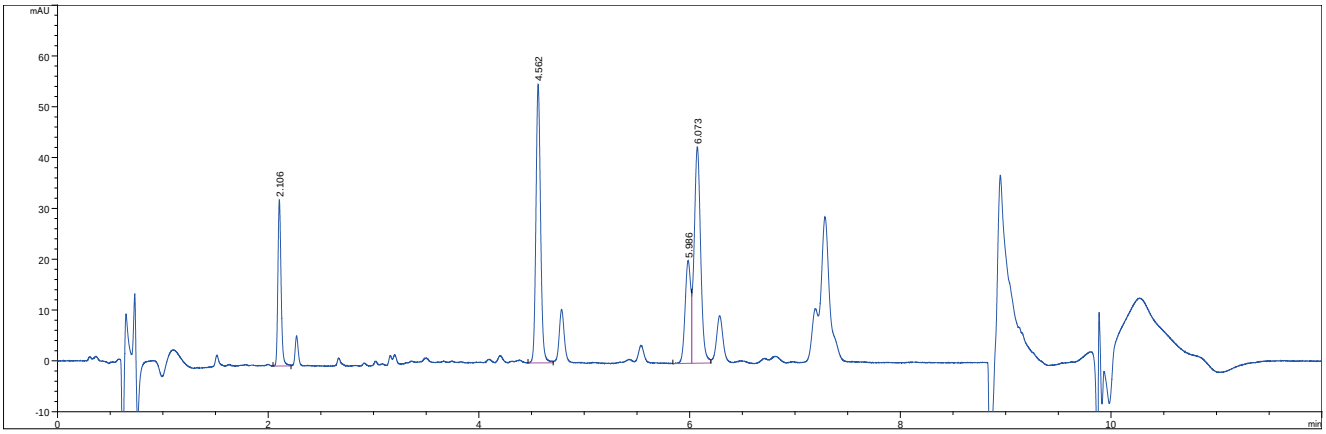

图1b 菊花对照药材提取溶液色谱图，色谱柱：Zorbax Plus-C18柱 (2.1 × 100 mm, 1.8 μ m)

2.2 优化色谱条件获得的结果

换用一根较长的Poroshell色谱柱，规格3.0x150, 2.7 μ m，将有机相变为甲醇/乙腈=3:2的混合溶剂以改变选择性，就能够将难分离的异构体基线分离，如图2：

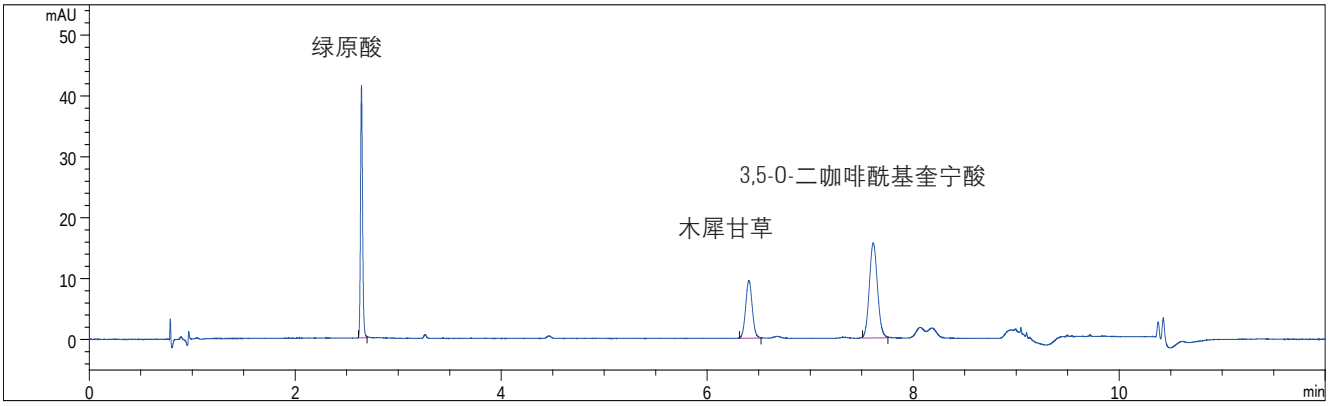

图2a 优化后的对照品色谱图，色谱柱：Poroshell 120 EC-C18柱（3.0x150mm，2.7 μ m）

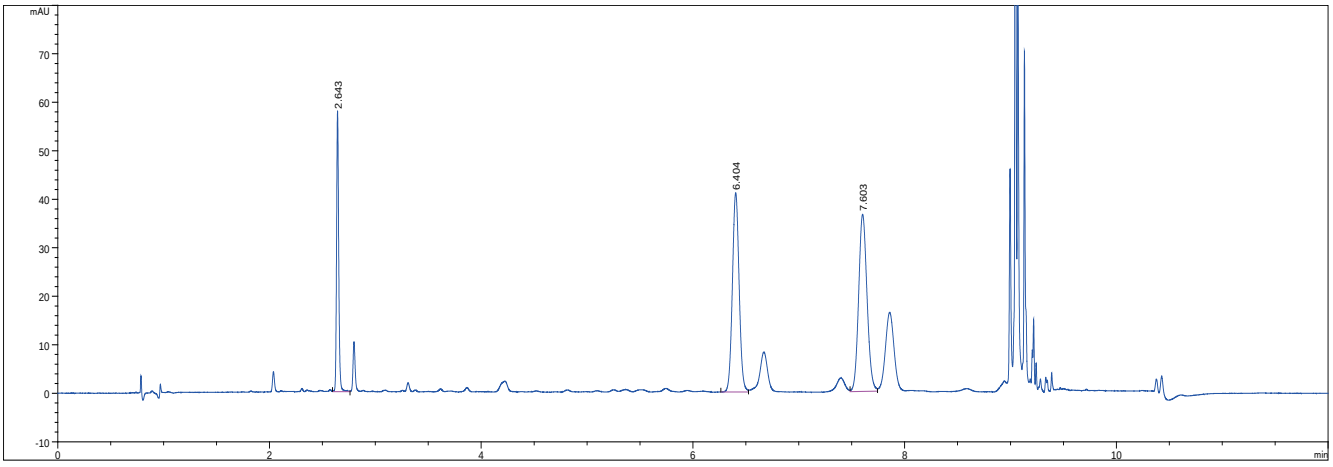

图2b 优化后的菊花药材提取溶液色谱图，色谱柱：Poroshell 120 EC-C18柱（3.0x150mm，2.7 μ m）

3 小结

本法采用Poroshell120 EC-C18色谱柱，在较低的压力（515bar）下通过改变流动相溶剂种类就能实现对菊花样品中难分离物质对的有效分离。采用本文的方法完全能够在常规液相色谱（如Agilent 1260HPLC， 600bar最大耐压）上实现快速分析。分离时间为12分钟，较药典中原始方法的约60分钟缩短80%，并且具有明显优越的分离效果。

[参考文献]

[1] 2010版中国药典第一部，菊花

用Poroshell 120色谱柱快速分析中国药典人参样品

应用领域

药物分析

关键词

HPLC；Poroshell 120；人参皂苷Rg 1、人参皂苷Rb1和人参皂苷Re

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摘要

人参是中国药典收录的品种，2010年版药典仍沿用前版的梯度分析方法测定其中的人参皂苷Rg1,Re和Rb1的含量，且整个梯度运行时间超过100分钟【1】。本文研究了用Agilent 新开发的表面多孔型硅胶为基质的反相C18色谱柱用于中国药典人参的分析。新色谱柱可以使原方法整个梯度时间按比例缩短，以减少分析时间，节约溶剂的用量，最短的分析时间可以缩短至约10分钟。

1实验部分

1.1 仪器和试剂

仪器： Agilent 1200SL 液相色谱仪（耐600bar），配二元泵，自动进样器和DAD检测器

色谱柱： Agilent ZORBAX Stable Bond（4.6x150mm，5um）；

Agilent ZORBAX Plus C18（4.6x150mm，5um）；

Agilent Poroshell 120 EC C18 (3.0x100mm, 2.7um)；

Agilent Poroshell 120 EC C18 (3.0x50mm, 2.7um)

Agilent Poroshell 120 SB C18 (3.0x50mm, 2.7um)

流速：不同色谱柱规格流速见各自谱图所示

流动相：A, 水; B,乙腈

原始梯度:

时间 (min)	0	35	55	70	100
%B	19	19	29	29	40

检测波长： 203 nm

进样体积：见具体谱图所示

试剂： 乙腈，磷酸 色谱级； 超纯水

试样：人参粉

1.2 样品制备

取本品粉末约1克，置索氏提取器中，加三氯甲烷适量，加热回流3小时，弃去三氯甲烷液，滤渣挥干溶剂，连同滤纸筒移入100mL锥形瓶中，精密加入水饱和正丁醇50mL，密塞，放置过夜，超声处理30分钟，滤过弃去初滤液，精密量取续滤液25mL，蒸干，残渣加甲醇溶解，定容至5mL，摇匀，用0.45 μm的再生纤维素膜过滤，吸取一定量注入液相色谱仪分析。

2实验结果和讨论

2010版中国药典人参含量测定方法项要求色谱柱为C18柱，梯度方法见实验部分，我们先用常用规格的色谱柱4.6x150mm, 5um对人参样品进行分析，选用Agilent两款常用色谱柱ZORBAX Plus C18和ZORBAX SB C18，色谱条件完全同药典。图1分别为两种不同键合相C18分离人参样品的色谱图。人参皂苷Rg1和Re都达到基线分离，且Rg1的理论板数大于药典规定的6000。两款不同键合相的C18柱都能满足药典规定的三种皂苷的含量测定，但分析时间长，加上梯度后平衡时间大概需要110分钟。

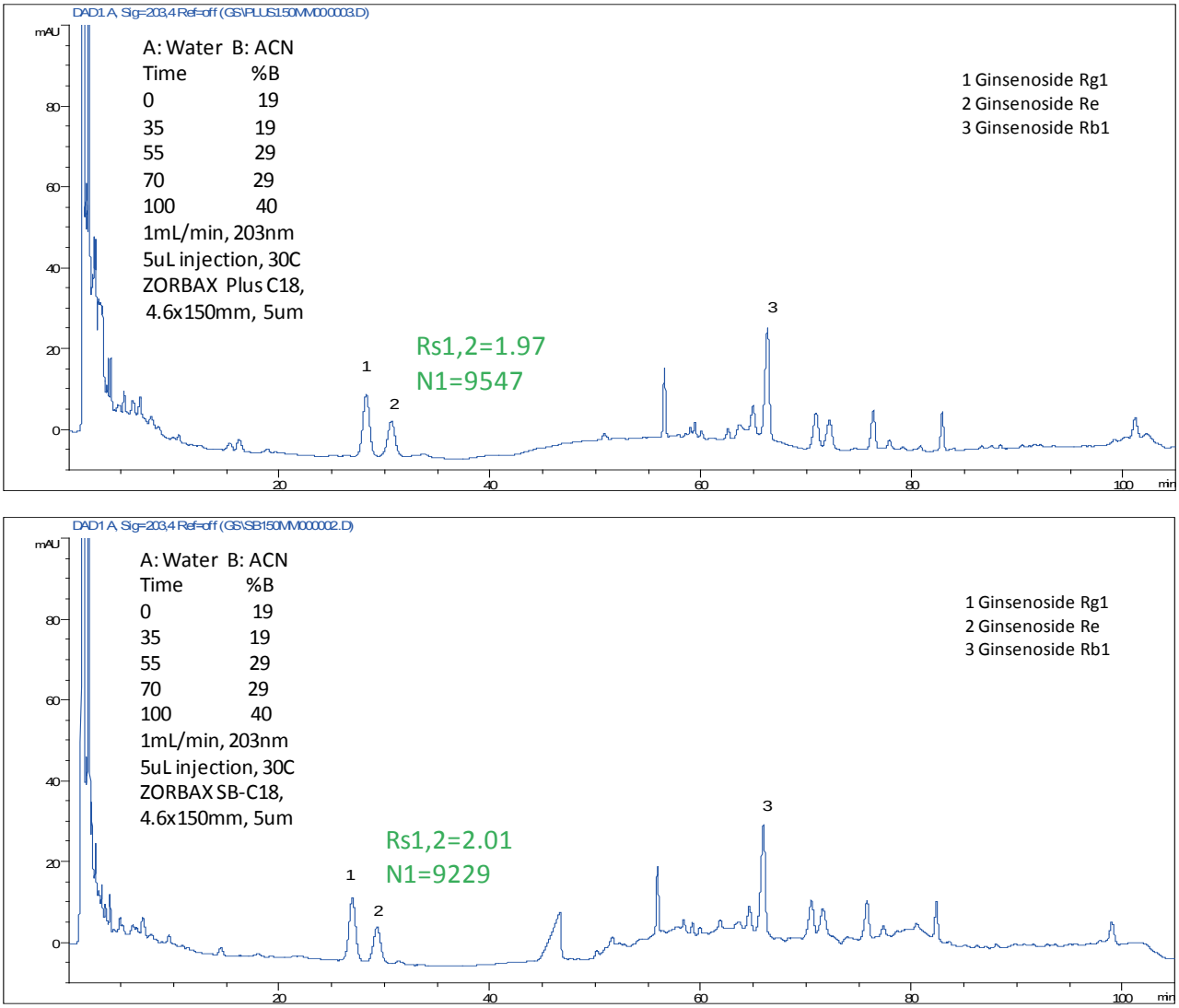


图1 用ZORBAX Plus C18, 4.6x150mm, 5um和ZORBAX SB C18, 4.6x150mm, 5um分析人参样品的色谱图

把原方法转移到Agilent新开发的多孔层填料的色谱柱Poroshell 120 EC-C18，3.0x100mm ,2.7um上来，按照线流速一致的规则，流速与色谱柱内径的平方成正比，因此原1ml/min的流速相当于在3.0内径上的0.425ml/min。同时梯度时间按照色谱柱长度成比例缩短，按新计算的梯度对人参进行分析后见图2色谱图及方法参数。图中数据表明Rg1和Re的分离度和Rg1的理论板数都优于原方法，在同样的线流速条件下，分析时间按柱长缩短了1/3，为70分钟，压力只为174bar，方法可以在普通仪器上实现。

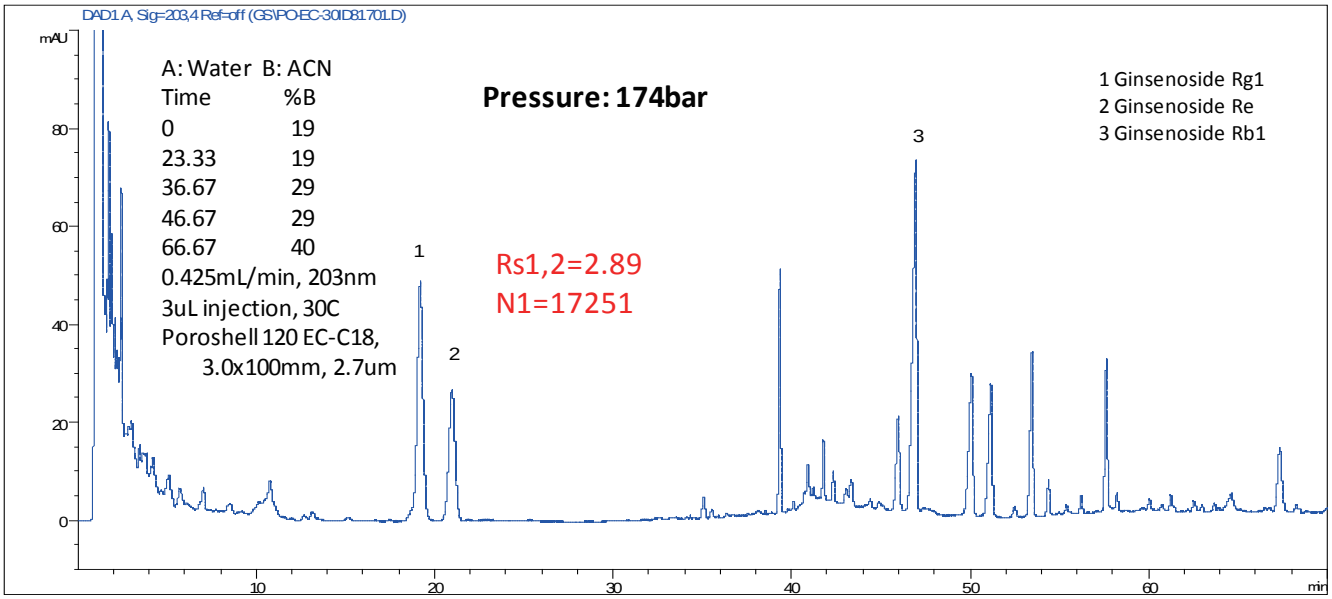
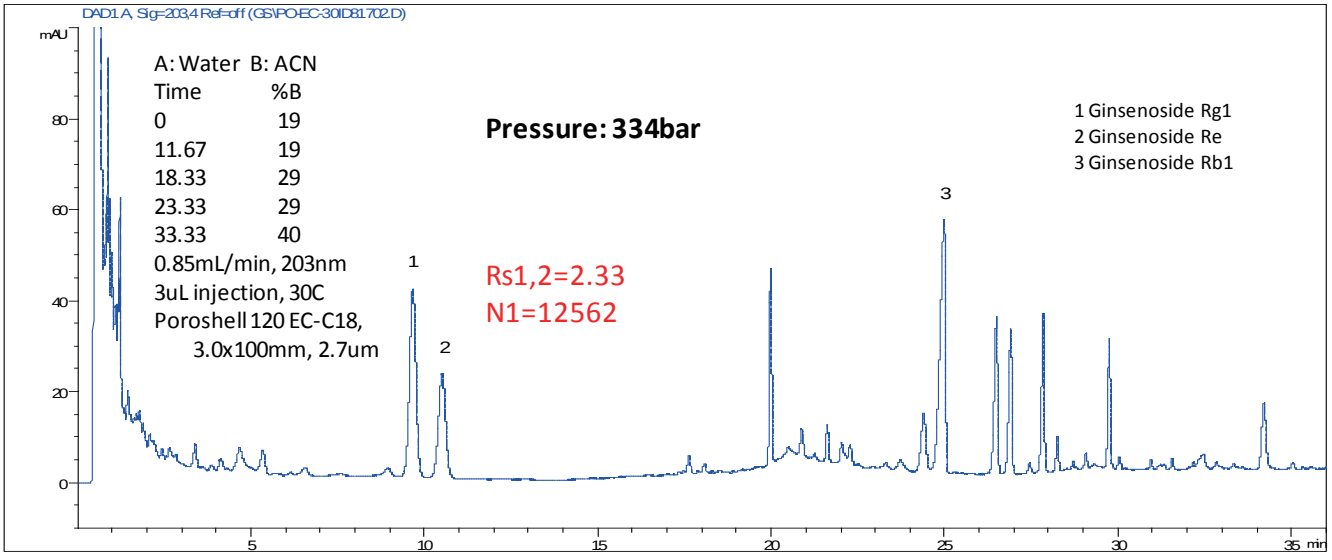


图2 用Poroshell 120 EC-C18,3.0x100mm, 2.7um色谱柱分析人参样品的HPLC谱图。

2.7um Poroshell 120填料具有和亚二微米的色谱填料类似的Van-Deemter曲线，在高流速下柱效下降较慢，分离度基本保持不变，因此可以通过加大流速来进一步提高分析速度，减少分析时间。图3两张色谱图分别在2倍和3倍原始流速下获得的分离，人参皂苷Rg1和Re分离度仍然保持2.0以上，且Rg1理论板数超过10000，高于药典标准。当3倍流速时，操作压力高于普通仪器400bar的上限，而低于色谱柱的最大耐压600bar，这时应使用耐高压的仪器。



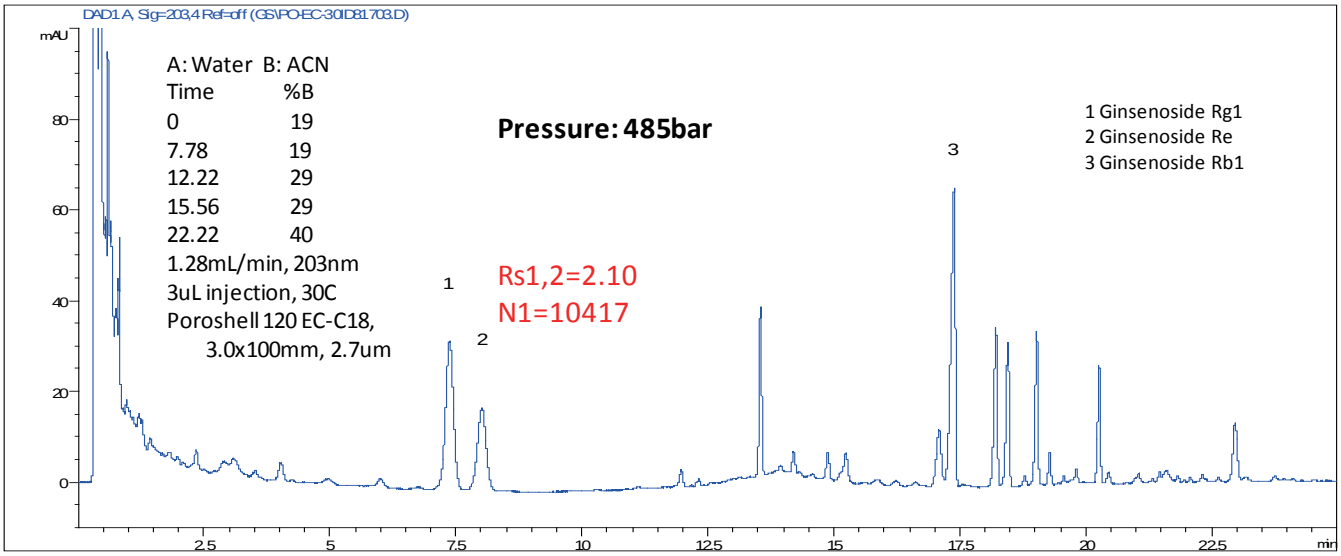


图3 用Poroshell 120 EC-C18,3.0x100mm, 2.7um色谱柱在较高流速下的人参分析色谱图。

下图中，把不同流速下的色谱图和原方法的色谱图叠加起来，能更清楚的看出用新的Poroshell 120色谱柱能大大缩短分析时间，从原来的105min缩短至25min。

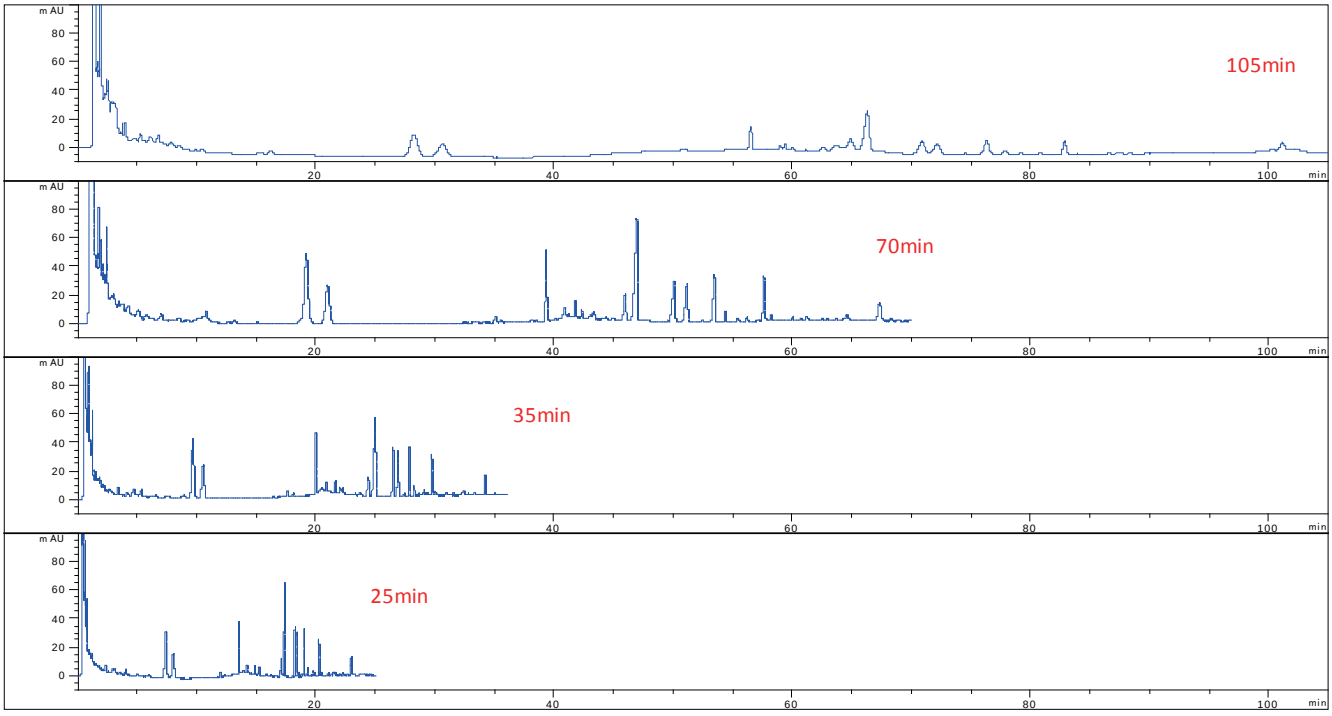


图4 人参样品用ZORBAX Plus C18,4.6x150mm，5um和不同流速下用Poroshell 120 EC-C18,3.0x100mm，2.7um色谱柱分析的图谱比较

用100mm，2.7um规格的Poroshell色谱柱能完全满足药典的要求，并能实现快速分离。在Rg1和Re的分离度足够大的前提下，可以把方法继续转换到更短的50mm长色谱柱上，实验所用的是3.0x50mm的色谱柱，当流速设定在0.5ml/min时，分析时间需要23分钟，Rg1和Re的分离度接近2.0，Rg1的柱效高于药典规定。当流速增加一倍时，整个分析可以在11分钟左右完成，只是初始方法的1/10，这时可以看到Rg1和Re的分离度和柱效有所下降，但仍符合分离的要求和药典关于Rg1最低柱效的要求。但需要注意的是本实验是在Agilent 1200SL，600bar的标准配置的仪器上开发的，因操作压力低于400bar，理论上方法可以转移到耐400bar的系统上，但由于400bar系统的柱外体积以及系统延迟体积大于本实验系统，可能需要进行必要的系统和色谱条件的优化来达到理想的分离。

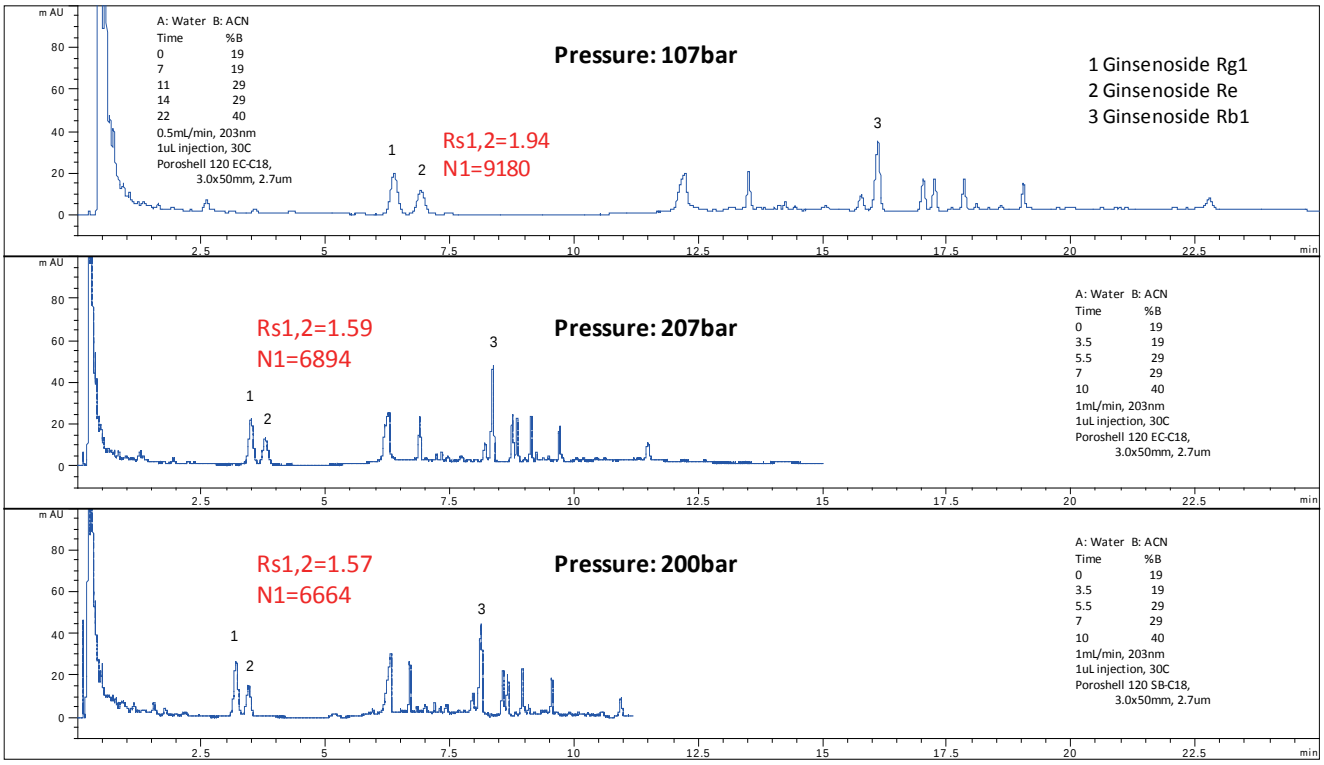


图5 人参样品用Poroshell 120 EC-C18,3.0x50mm，2.7um和SB-C18, 2.7um色谱柱分析的图谱

3 小结

Agilent 新开发的Poroshell 120色谱柱能用于2010版中国药典人参的分析，且使用不同长度的色谱柱能不同程度缩短分析时间，在加快流速的情况下能进一步缩短分析时间；即使在50mm长的色谱柱上，人参皂苷的分离度和柱效也能满足药典规定；2.7um填料的Porosell 120色谱柱在保证柱效的同时，压力只有亚二微米填料色谱柱的60%，在很多条件下可以用于常规耐400bar的仪器。

[参考文献]

[1]《中国药典2010年版一部》人参含量测定项下。

新型Agilent Poroshell 120液相色谱柱用于中国药典2010版复方丹参滴丸的分析

应用领域

药物分析

关键词

作者

傅荣杰

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摘要

复方丹参滴丸是中国药典品种，由丹参，三七和冰片等药物组成，主要作用是活血化瘀、理气止痛，用于胸中憋闷、心绞痛。2010年新版药典收录了复方丹参滴丸指纹图谱的质量控制方法，方法条件中描述了用Waters UPLC 和Waters HSS T3 色谱柱。 本文研究了使用Agilent 新开发的表面多孔层的反相C18色谱柱进行复方丹参滴丸指纹图谱方法的开发。新型的色谱柱填料的颗粒组成为具有一个1.7 um 直径的实心球体，表面涂覆一层0.5um厚的多孔硅胶，这种结构使得被分析物质的分子的扩散路径大大缩短（只有0.5um），从而加快分析速度，且柱效与亚二微米填料相近，但柱压下降约40%-50%。

1实验部分

1.1 仪器和试剂

仪器： Agilent 1200SL 液相色谱仪，配有自动进样器和DAD检测器

色谱柱： Agilent Poroshell 120 EC C18 (3.0x100mm, 2.7um);
Agilent Poroshell 120 SB C18 (4.6x100mm, 2.7um);
Waters HSS T3 (2.1x100,1.8um)

流速： 根据不同色谱柱内径调整体积流速，以保持相同的线速度（0.4 ml/min 用于 2.1 mm 内径色谱柱, 0.8 mL/min 用于 3.0 mm 内径色谱柱, 1 mL/min用于4.6 mm内径色谱柱）

流动相：A： 0.02% 磷酸溶液; B： 含0.02%磷酸的乙腈/水 (80/20)混合溶液

梯度:

时间 (min)	0	1.6	1.8	8.0	8.4	10
%B	9	22	26	39	9	9

检测波长： 280 nm

进样体积：根据不同柱体积适当调整（2 µL 于 2.1 和3.0 mm内径的色谱柱， 4.0 µL 用于4.6 mm内径的色谱柱）

试剂：乙腈，磷酸（色谱级）；Milli Q超纯水

试样：复方丹参滴丸 （天津天士力制药股份有限公司生产）

1.2 样品制备

取滴丸10丸用水溶解，并定容至10mL。用0.45 μm的再生纤维素膜过滤，去一定量注入色谱仪分析。

2实验结果和讨论

图1比较了不同色谱柱获得的色谱图，上图是用药典推荐的Waters HSS T3按药典方法获得的色谱图，下图是在Agilent新的色谱柱Poroshell 120 EC C18上重复药典的方法获得的色谱图。新方法和原方法比较分析时间短，且8个主峰都能达到基线分离，在新色谱柱上获得了更对称的峰型，由图可见，前两个色谱峰的拖尾因子明显小于原色谱柱。值得注意的是最大的操作压力从原方法的503bar降低到了287bar，这使得原先只能在超高压液相上应用的药典方法也可以在低于400bar的常规仪器上使用。

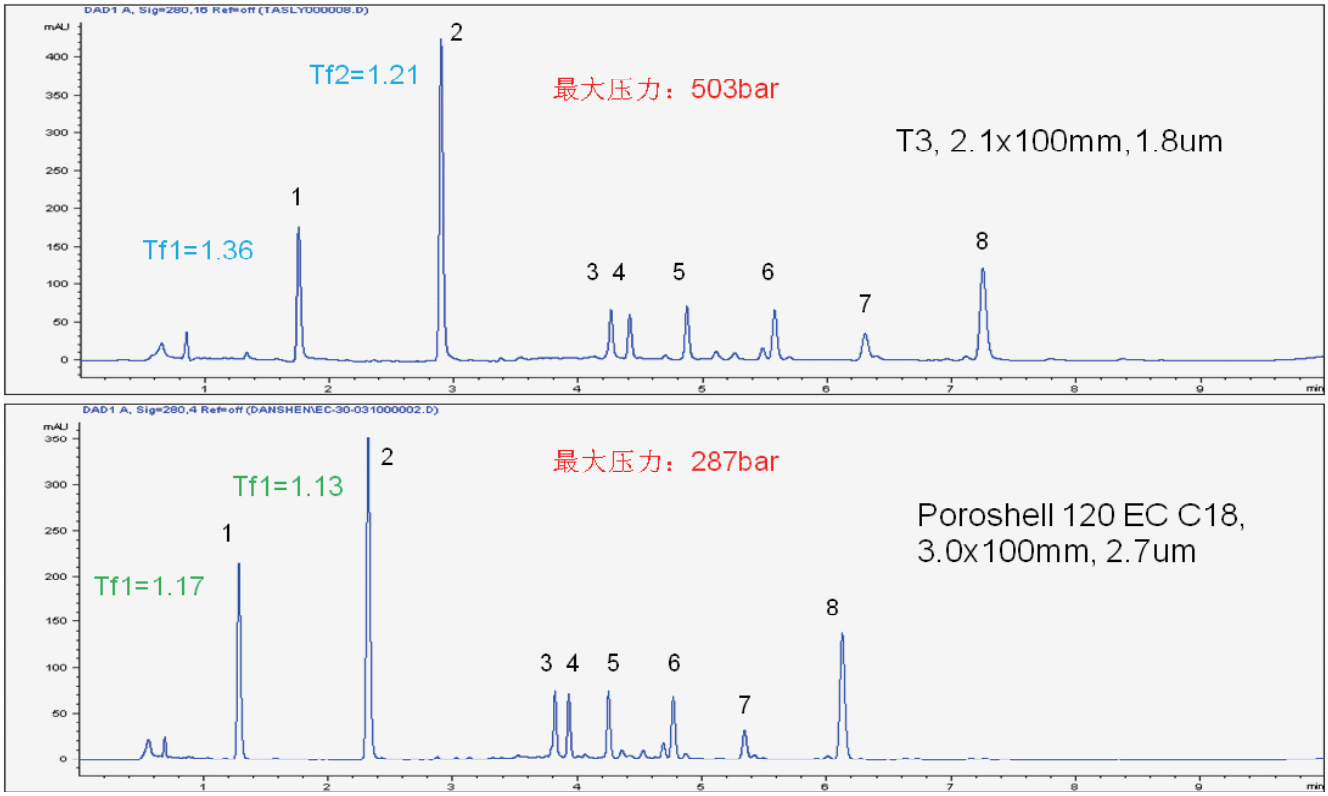

图1. Agilent Poroshell 120 EC C18和Waters HSS T3色谱柱分析复方丹参滴丸的色谱图比较

图2 显示在不同的流速下得到的色谱图，可以在较高的流速条件下获得更快的分离，但不损失分离度，这也是Poroshell类型的填料的另一突出的优点。

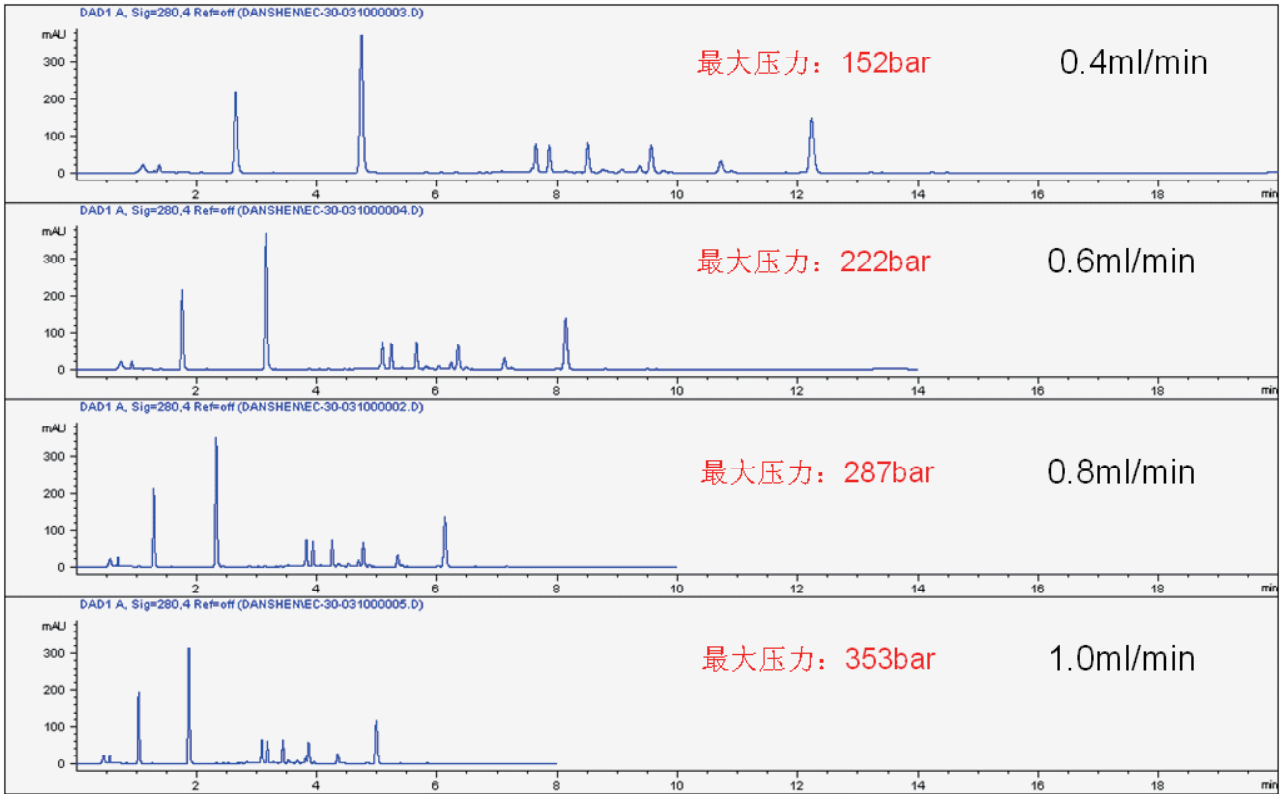

图2 不同流速下的色谱图

3结论

Agilent 120 可用于复方丹参滴丸指纹图谱的分析，能获得原UPLC方法相似的图谱，且能在较低的压力下实现，特别适用于常规的液相色谱仪实现快速分析。

参考文献

1.复方丹参滴丸，中国药典2010年版：906

Fast Analysis of Cefepime and Related Impurities on Poroshell 120 EC-C18

Application Note

Pharmaceuticals

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Introduction

Cephalosporins are the most frequently prescribed class of antibiotics. They are structurally and pharmacologically related to the penicillins. Like the penicillins, cephalosporins have a beta-lactam ring structure that interferes with synthesis of the bacterial cell wall which means that they kill bacteria. Cephalosporin compounds were first isolated from cultures of Cephalosporium acremonium in 1948 by Italian scientist Giuseppe Brotzu. The first commercial product, Cephalothin was launched by Eli Lily in 1964.

Cephalosporins are bactericidal agents and have the same mode of action as other beta-lactam antibiotics (such as penicillins). All bacterial cells have a cell wall that protects them. Cephalosporins disrupt the synthesis of the peptidoglycan layer of bacterial cell walls, which causes the walls to break down and eventually the bacteria die. Cephalosporins are beta-lactam compounds in which the beta-lactam ring is fused to a 6-membered dihydrothiazine ring, thus forming the cephem nucleus. Modifications to the side chain modifications of the ring structure can improve antibacterial and pharmacokinetic activity. Based on their spectrum of activity, cephalosporins can be broadly categorized into four generations.

First generation cephalosporins are predominantly active against gram-positive bacteria, and successive generations have increased activity against gram-negative bacteria (often with reduced activity against gram-positive organisms). Gram-negative bacteria have a unique outer membrane that prevents many drugs from penetrating them, making gram-negative bacteria generally more resistant to antibiotics than are gram-positive bacteria [1].

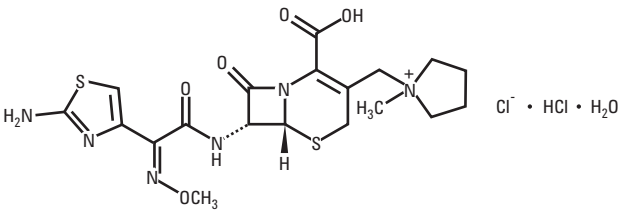

Figure 1. Structure of Cefepime.

Cefepime, fourth generation cephalosporin, is a broad spectrum antibiotic with improved activity against Gram-negative bacteria over other commercially available cephalosporin drugs. The structure of Cefepime is shown in Figure 1.

Despite extensive research on this class of drugs, quantitative analysis and purity assays remain problematic [2]. The EP and USP have published methods determine cefepime and the related compounds [3,4]. These methods use a phosphate/acetonitrile eluent at 1.0 mL/min on a 4.6 × 250 mm, 5 µm column. In this work, an Agilent Poroshell 120 EC-C18 column, 4.6 × 75 mm, 5 µm will be used to quickly analyze the Cefepime related compounds while meeting the requirements for both USP and EP methods. The shorter column rate used with this column saves time solvent due to less frequent eluent preparation, and produces less waste compared to the original EP and USP methods. Moreover, using a shorter column allows quick evaluation of allowable adjustments to methods resulting in better performance in less time.

Experimental

An Agilent 1200 Series Rapid Resolution LC (RRLC) system with an Agilent:

- G1312B Binary Pump SL Gradient times vary depending on column dimensions and flow rate, (Table 1).
- G1367C Automatic Liquid Sampler (ALS) SL, injection volumes are dependent upon specific method parameters, see Table 1.
- G1316B Thermostated Column Compartment (TCC) SL with temperature controlled at 25 °C.
- G1367C Diode Array Detector, set to 254 nm as described in the method.

Two Agilent columns were used in this work:

- Agilent ZORBAX Eclipse Plus C18, 4.6 × 250 mm, 5 µm, p/n 959990-902
- Agilent Poroshell 120 EC-C18, 4.6 × 75 mm, 2.7 µm, p/n 697975-902

In addition, two 4.6 × 250 mm, 5 µm C18 competitive columns were also examined, and are designated C1 and C2.

Acetonitrile used was Burdick and Jackson ACS/HPLC Certified solvent, purchased from Honeywell. Monobasic Potassium Phosphate ACS/USP Grade purchased from VWR. Water used was produced on site using a Millipore Milli-Q system,18 M filtered to 0.2 µm. 0.45 µm Regenerated Cellulose Filter media (Agilent Technologies) was used for buffer filtration. USP Cefepime Hydrochloride and USP Cefepime Hydrochloride System Suitability RS was purchased from United State Pharmacopeia. Sample and mobile phase preparation are made following directions from the USP and EP. [3,4].

Mobile Phase Preparation

This method uses a gradient composed of a monobasic potassium phosphate buffer mixed with an amount of acetonitrile. The buffer is prepared by dissolving 0.68 g of monobasic potassium phosphate in 1000 mL of water. This buffer is adjusted with potassium hydroxide or phosphoric acid to a pH of 5.0, filtered through 0.45 µm filter media (regenerated cellulose was used in our lab) and degassed ultrasonically. The initial USP method uses a 9:1 ratio of buffer to acetonitrile for Mobile Phase A. All samples are subsequently prepared from this mobile phase. In the course of method adjustments, varied ratio's of "Mobile Phase A" are prepared. Mobile Phase B is prepared from the monobasic potassium phosphate buffer mixed with a 1:1 ratio v/v acetonitrile. The European Pharmacopeia (EP) method is similar but specifies the concentration of Potassium Hydroxide or Phosphoric acid used to adjust the solution pH, (0.05 M) and specifies that the mobile phase pH is adjusted before the addition of acetonitrile.

Assay Preparation

About 70 mg of Cefepime Hydrochloride, should be accurately weighed and transferred, to a 50-mL volumetric flask, dissolved in and diluted with Mobile Phase A to volume. This solution should be sonicated for approximately 30 minutes. The system suitablility sample is prepared at 7 mg/5 mL in Mobile Phase A. Sonication is important as impurity B is reluctantly soluble. NOTE: These solutions should be used immediately, or stored in a refrigerator and injected within 12 hours.

Results and Discussion

Chromatographic conditions as described in the USP and EP

were followed. In both cases an L1 (C18 column) is 4.6 × 250 mm, 5 µm is specified. The gradient program shown in Table 2. The liquid chromatograph uses an initial isocratic hold for 10 min of 100% mobile phase A increasing to 50% over the next 20 min. An isocratic hold at 50% A is maintained for 5 min, after which the solvent re-equilibrates to the initial 100% composition A. The total run time is 36 min.

In the original USP and EP methods a 4.6 × 250 mm, 5 µm L1 column is specified. Three different columns are shown in Figure 2. The method specifies a gradient and with recovery time approximately 45 min are required for each sample. Speeding up this method through adjustments presents a good opportunity for improving the method.

The chromatographic and performance requirements of the method are listed in the USP method. These are summarized below [1].

- 4.6 mm × 250 mm column, L1 column (C18)
- N of the analyte not less than 4000 plates.
- The resolution, R, between cefepime and cefepime related compound A is not less than five.
- The resolution, R between cefepime and cefepime related compound B is not less than 10.
- The capacity factor, k' of cefepime, is more than 0.6.
- Column efficiency is not less than 4000 theoretical plates.
- The tailing factor is not more than 1.5.

For the purpose of identification, the relative retention times are about 1.0 for cefepime, 2.7 for cefepime related compound A, and about 4.3 for cefepime related compound B.

The USP updated chapter <621> presents recommendations on how much a method can be modified such that the changes are considered an adjustment. [5] Table 1 summarizes these modifications. In previous work, modification of particle size and column dimensions have been demonstrated. [6,7]. In addition, new changes to these compendial methods are proposed that could allow linear velocity to remain constant as particle size decreases, thus increasing the flow rate beyond the present ± 50% [8]. In this case, we also look at the advantages of modifying the mobile phase composition on a substantially smaller column.

Table 1. Allowable Method Modifications under USP Chapter 621

Column length	± 70%
Column internal diameter	± 25%
Column material particle size	Reduction of up to 50%, no increase
Flow rate	± 50%
Injection volume	Changes are allowed as long as system suitability testing (SST) criteria are met
Column temperature	± 10%
pH of mobile phase	± 0.2
UV wavelength	No change outside manufacturer specifications
Concentration of salts in buffer	± 10%
Composition of mobile phase (adjustment of the minor component is allowed ± 30% or ± 10% absolute whichever is smaller (as discussed in the USP)	

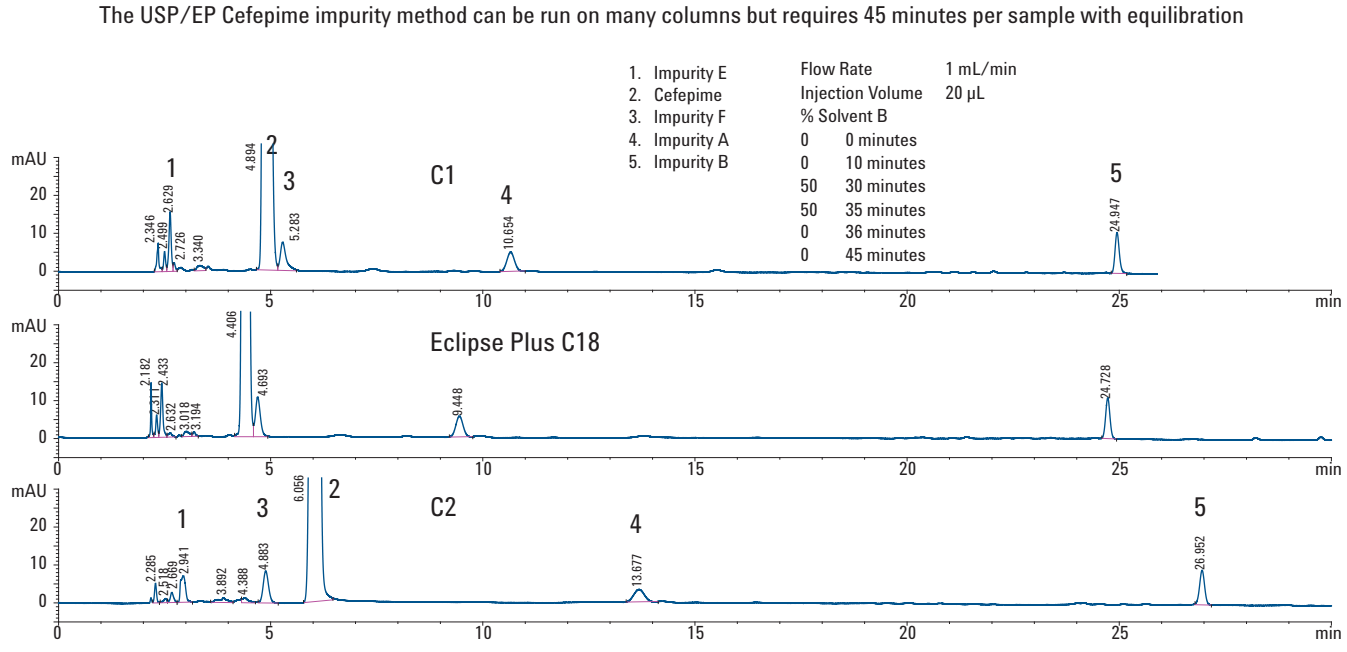

Figure 2. Original Method on 3 different columns, approximately 45 minutes per analysis.

Gradient Conditions were scaled according to the formula:

When a constant k* value is to be maintained, the equation above can be reduced into the following equation to calculate new gradient times:

$t_{g2} = (t_{g1}d_2^2L_2F_1)/(d_1^2L_1F_2)$

t_{g1} and t_{g2} are the original and new gradient times

d_1 and d_2 are the original and new column id's

L_1 and L_2 are the original and new column lengths

F_1 and F_2 are the original and new flow rates

For this work several allowed modifications were made. First the particle size is changed from 5 µm to 2.7 µm. This change yields an increase in efficiency as well as an increase in pressure. Because the column is 70% shorter the increase in pressure is minimized. An advantage of an Agilent Poroshell 120 is the narrow particle size distribution because of this, a 2 µm frit can be used, the same size used on 5 and 3.5 µm columns. This means that no additional care must be made in preparing samples than was used in the original method. Columns with particle sizes of 3, 2.5, and of course sub 2 µm use smaller size frit to retain the packing material in the column and as such are more apt to clogging [9].

The second modification made is a change from 250 mm length to 75 mm. This 70% reduction in length is allowed and can easily lead to higher throughput of samples if the performance of the column allows such a change.

Table 2. Table of Gradients

Inj. Volume % Solvent B	4.6 × 250 mm 5 µm, 1 mL/min, 20 µL	4.6 × 75 mm 2.7 µm, 1 mL/min, 6 µL	4.6 × 75 mm 2.7 µm, 1.5 mL/min, 6 µL	4.6 × 75 mm 2.7 µm, 2 mL/min, 6 µL
0	0 min	0.0 min	0.0 min	0.0 min
0	10 min	3.0 min	2.0 min	1.5 min
50	30 min	10.0 min	6.67 min	5.0 min
50	35 min	10.5 min	7.0 min	5.25 min
0	36 min	10.8 min	7.2 min	5.4 min
0	45 min	13.5 min	10 min	6.75 min

The minor component in the mobile phase is the Acetonitrile at 10%. Under USP modification rules an allowed change of ± 30% or ± 10% absolute whichever is smaller means that the acetonitrile can be reduced to 7% or increased to 13%. Under EP rules this change is still 7 to 13%. In this work, several concentrations of acetonitrile can be quickly calculated using this dramatically shorter column. It is important to note that the chromatographic solvents are varied in this method but

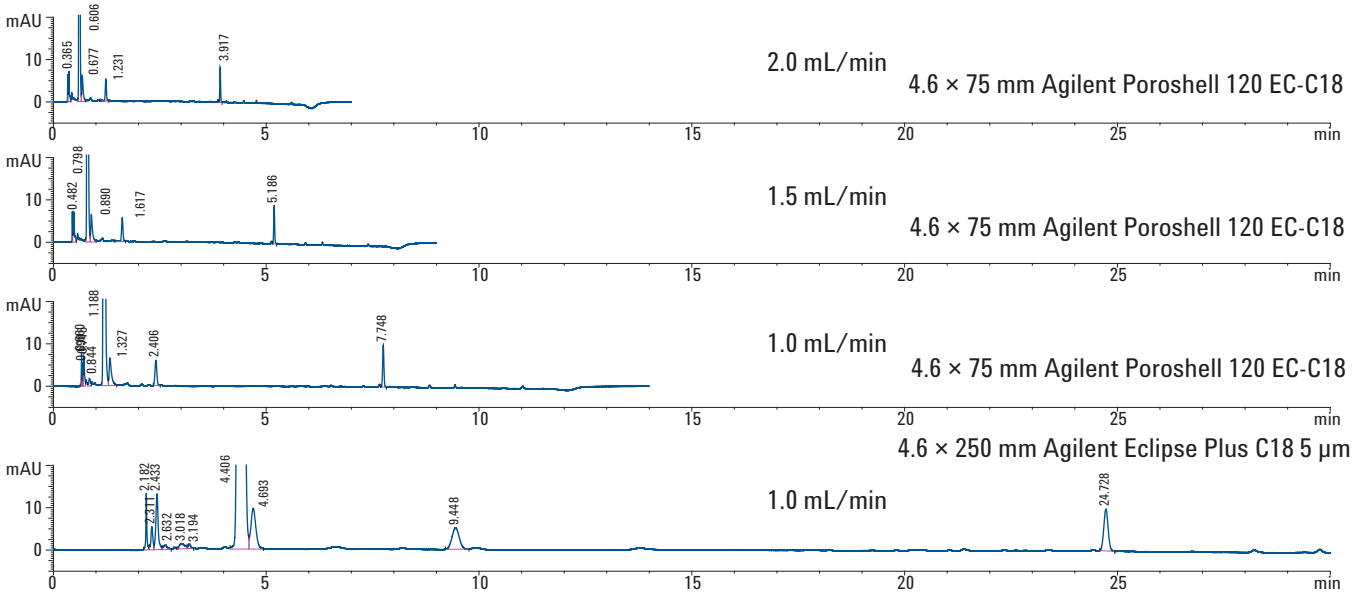

Figure 3. Totally Porous Agilent Eclipse Plus C18 column and Agilent Poroshell 120 EC-C18 column.

the sample preparation is left intact. It was noticed during the course of this work that Impurity B is less soluble than other components, and without sufficient ultra-sonication as specified in the method, this compound is not dissolved.

In Figure 4, the flow rate is increased to 2 mL/min to allow even faster method evaluation.

As can be seen, the initial acetonitrile is varied from 11% to 8%, within compendial guidelines for adjustment. At 10% the resolution of the cefepime and impurity B is 2, but by decreasing the initial organic modifier content to 8% the resolution between these peaks is increased to 4.9. It becomes evident that decreasing the initial concentration beyond 7% could potentially lead to additional peak resolution, but modifications outside these ranges are considered changes and require re-validation.

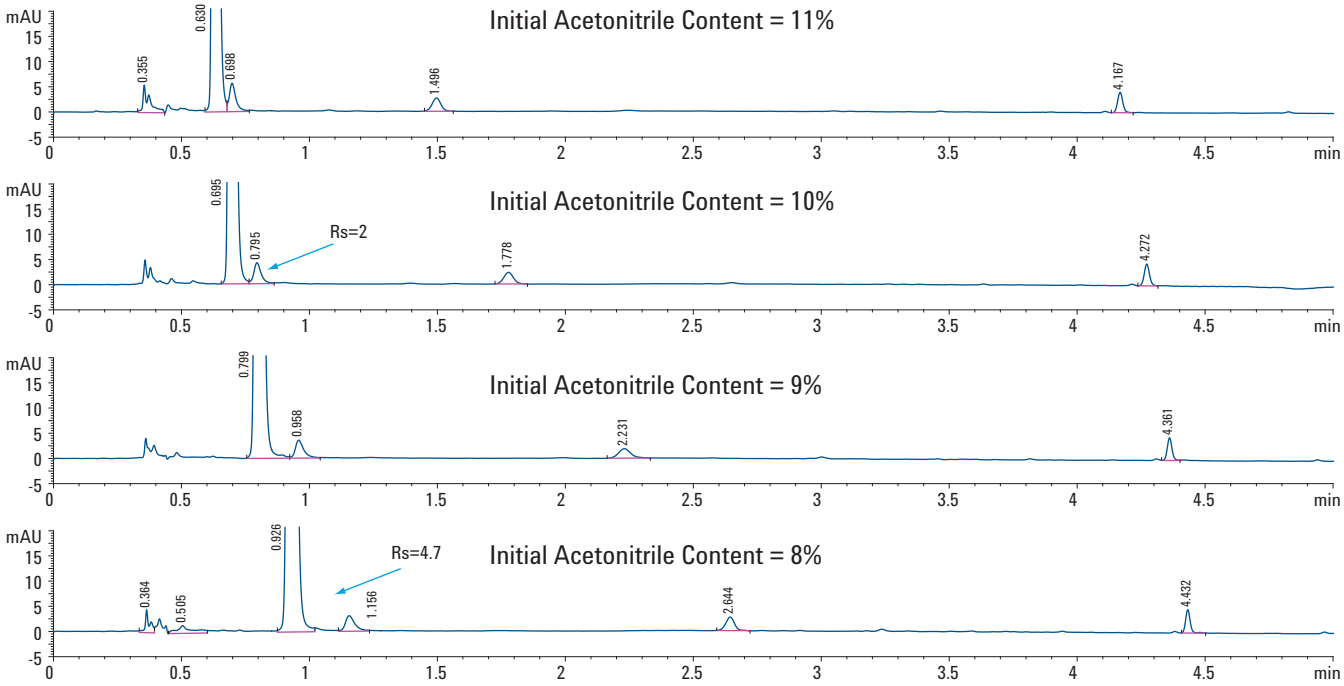

Figure 4. Modification of organic content to improve resolution. Fast method development with Agilent Poroshell 120 EC-C18.

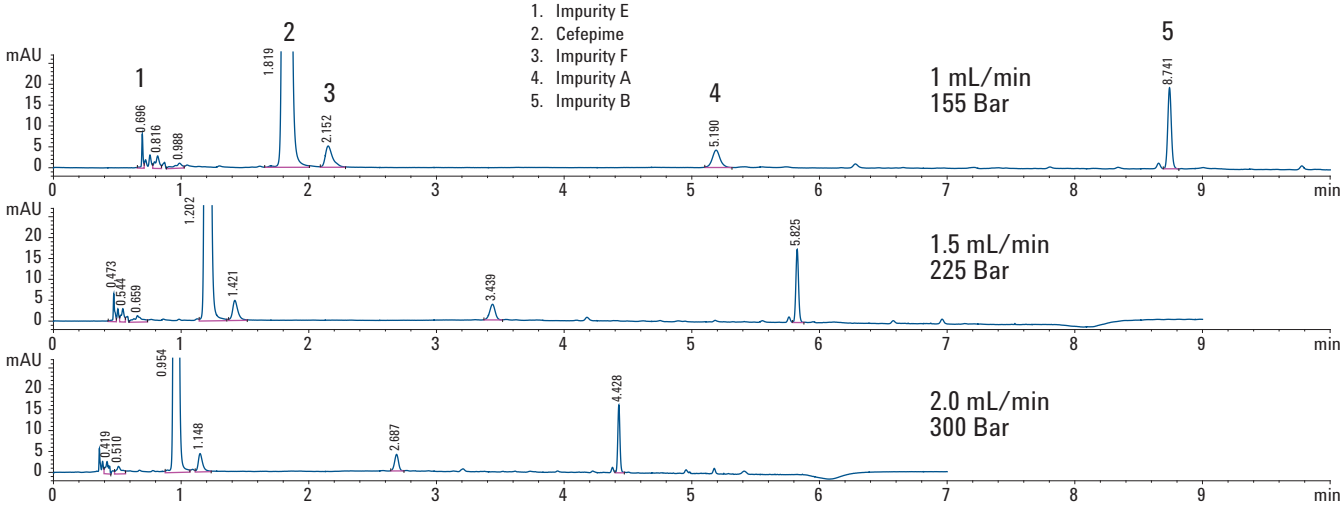

Figure 5. Final optimization of USP cefepime impurity Method, 8% initial acetonitrile at varied flow rates, using Agilent Poroshell 120 EC-C18 4.6 × 75 mm column.

Table 3.
Requirement: Agilent Poroshell 120 EC-C18 4.6×75 mm, 2.7 µm

	1 mL/min 156 bar	1.5 mL/min 221 bar	2 mL/min 300 bar
Tf<1.5	1.28	1.28	1.30
N>4000	18288	11993	7250
k'>0.6	1.4	1.4	1.4
Rs C/a	5.9	5.9	5.9
Rs C/b	10.7	10.7	10.7

Modifications outside these ranges are considered changes and require re-validation. If the analyst chooses to use a shorter column, such as a 4.6 mm × 50 mm, the same analysis could be accomplished in potentially 20% of the time. Further only 20% of the solvent would be used. However, this would require a complete revalidation. In cases such as assay methods, it might be easier to justify revalidation of a method, but impurity methods, are run less frequently. Tables 2 and 3 indicate that this analysis could easily be reduced in time from 36 minutes to 7.2 minutes without any need for new equipment, with an 80% reduction in analysis time. This would allow a lab to assay an incoming raw material within two hours of receipt instead of within 24 hours of receipt.

Conclusion

Laboratories performing compendia analysis with 250 mm fully-porous LC columns can benefit from the increased speed, resolution, and sensitivity that superficially porous, Poroshell 120 columns provide without having to replace existing instrumentation. The 75 mm column length is within the allowed modification range of USP and EP guidelines. Faster analysis times resulting in higher throughput and greater productivity can be achieved with Poroshell 120 columns. Method adjustments to these compendia methods with shorter length columns and the smaller 2.7 µm particle size provide these improved results.

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Fast Analysis of Illicit Drug Residues on Currency using Agilent Poroshell 120

Application Note

Forensics and Toxicology

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Abstract

Illicit drugs, like cocaine, are frequently found on US currency. While a more interesting perception might be that all bills were used to inhale the drug, the truth is much more mundane. Drug trafficking is thought to be the initial source of drug residues on a small percentage of bills, and because these compounds are fine powders, they are easily transferable from one surface to another. As money is processed through counting machines and automated teller machines (ATM), small amounts of drugs are readily transferred. An Agilent application note (Agilent Publication Number 5990-4254EN) details an application kit for the screening of 25 compounds considered in forensic and toxicology analyses using an Agilent 1200 Series LC system with an Agilent 6410 Triple Quadrupole LC/MS. In this work, an Agilent Poroshell 120 EC-C18 column is used to analyze 25 compounds found in the Agilent LC/MS Toxicology Test Mixture (Agilent p/n 5190-0470). This ammonium formate/acetonitrile gradient analysis is scaled using faster flow rates to shorten analysis time and exploit the low back pressure of this superficially porous column. Calibration curves for each of the 25 compounds are generated, and as a demonstration of the method a $1 bill was extracted into methanol, analyzed and quantified.

Introduction

The interest in superficially porous particles has led to discussions of method transfer from larger 5-µm totally porous particles, as well as from sub-2-µm totally porous particles. The high efficiency of superficially porous particles is similar to sub-2-µm totally porous particles. This is due to short mass transfer distance and substantially narrower particle size distribution.

The benefit of transferring from larger particle columns is very significant time savings, because the superficially porous particles are optimally run at faster flow rates (usually double) and are able to achieve similar resolution with a much shorter column length [1-2]. Because analysts will likely change column length and flow rate when transferring from larger totally porous particles to superficially porous columns, calculations must be performed to proportionally scale a gradient method and preserve the chromatographic selectivity (Equation 1).

Equation 1

$$t_2 = \frac{t_1 \bullet d_2^2 \bullet L_2 \bullet F_1}{d_1^2 \bullet L_1 \bullet F_2}$$

Where:

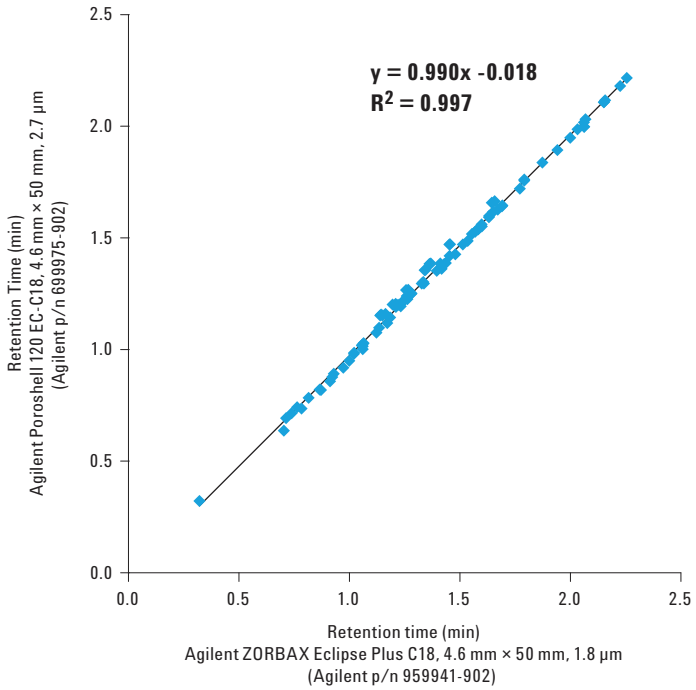
- *t_1 and t_2 are the original and new gradient times (min)*
- *d_1 and d_2 are the original and new column internal diameters (mm)*
- *L_1 and L_2 are the original and new column lengths (mm)*
- *F_1 and F_2 are the original and new flow rates (mL/min)*

In some cases, it may be useful to take advantage of the lower back pressure associated with superficially porous columns as compared to totally porous sub-2-µm columns. Depending upon operating conditions, the back pressure can be up to 50% less. This can give analysts the freedom to increase flow rates for higher throughput, or to increase column length to enhance resolution without exceeding the system pressure limits. Adjustments to flow rate and/or column length will require gradient scaling (Equation 1).

Method transfer can be especially easy, when columns like the superficially porous Agilent Poroshell 120 EC-C18 and totally porous Agilent ZORBAX Eclipse Plus C18 are manufactured to have similar bonding chemistries and use similar retention mechanisms. Figure 1 shows the similar retention of 90 compounds on Poroshell 120 EC-C18 and Eclipse Plus C18 columns using a generic gradient analysis with a variety of compounds from different chemical classifications. The high correlation coefficient (R^2) indicates a high degree of similarity between the interactions involved in the separation on the two C18 columns, while the slope ≈1 implies similar interaction strengths [3-4]. However, while many compounds give similar selectivity, it cannot be guaranteed that every application will transfer without adjustment.

This application note shows how a Poroshell 120 column can be used in a complex analysis, previously performed on a 1.8 µm column. This separation was demonstrated on Eclipse Plus in a previous Agilent application note (Publication Number 5990-4254EN) [5]. A 25-component LC/MS Toxicology Test Mixture (Agilent p/n 5190-0470) is used to illustrate the interchangeability between the two columns. Calibration curves for each of the 25 compounds on Poroshell 120 are constructed. A $1 bill is extracted in methanol to show significant presence of cocaine, as well as noticeable quantities of oxycodone, methamphetamine, PCP and THC. Trace amounts of several more illicit and prescription drugs can be detected also. Drug trafficking is assumed to be the cause for their initial presence on US currency, while ATM's and counting machines are likely the cause of their widespread presence [6]. Additionally, this gradient analysis is transferred to a Poroshell 120 SB-C18 column, which shows some selectivity differences; however it can be run at higher temperatures to allow for even faster flow rates and analysis times. Agilent Poroshell 120 columns are availabe with two different C18 phases in order to change selectivity and still have a C18 column choice. Flow rates were increased to reach 400 and 600 bar to show performance achievable on both conventional HPLC's and newer UHPLC's.

Agilent Poroshell 120 EC-C18 has Very Similar Selectivity to Agilent ZORBAX Eclipse Plus C18



Mobile phase:	A: 10 mM ammonium formate, pH 3 B: Acetonitrile
Gradient:	5% B at t_0 ramp to 95% B in 2 min, hold 95% B for 1 min
Flow rate:	2 mL/min
Sample:	1 µL of 1 mg/mL standard in H_2O

Furazolidone	Biphenyl	Acetanilide	DL phenylalanine	Oxybutynin chloride 1
Chloramphenicol	Acenaphthene	Fenoprofen	Doxepin hydrochloride	Diphenhydramine
Pyrimethamine	Methoxy naphthalene	Catechol	Ephedrine hydrochloride	Diflunisal
Sulfaquinoxaline	Anisole	Phenol	Loperamide	Nisoldipine
Sulfamonomethoxine	Dimethoxy benzene	Resorcial	Procaine hydrochloride	Diclofenac sodium salt
Nimopidin	Corticosterone	Hydroquinone	Fenoprofen calcium salt hydrate	Hydrocortisone
Sulfadimethoxine	Alpha hydroxyprogesterone	4 nitro phenol	Erythromycin	4 hydroxybenzoic acid
Sulfamethoxazole	Porgesterone	O cresol	Econazole nitrate	Procainamide hydrochloride
Sulfachloropyridazine	Alpha hydroxyprogesterone	P cresol	Gemfibrozil	Lidocaine
Sulfamethoxypyridazine	Prednisolone	3,4 dimethyl phenol	Beta estradiol	Terfenadine
Sulfamethizole	Mestranol	2,3 dimethyl phenol	Metoprolol	Terfenaine
Sulfamethazine	Deoxycorticosterone	2 nitro phenol	Prednisone	Chlortetracycline hydrochloride
Sulfamerazine	Progesterone	2,4 dimethyl phenol	Protriptyline	Chlorpheniramine maleate salt
Sulfathiazole	Chlorphenamine	2,5 dimethyl phenol	2-hydroxyhippuric acid	Chloramphenicol
Sulfadiazine	Berberine	1 napthol	Hydroxyisophthalic acid	Buspirone hydrochloride
Benzaldehyde	Impramithue	Imipramine hydrochloride	Flufenamic acid	Benzocaine
Iodobenzene	Norethindrone	D methionine	Pramoxine hydrochloride	Antipyrine
Phenanthrene	Phenacetin	3,4 dihydroxy-l-phenyl alanine	Naproxen	Acetylsalicyclic acid

Figure 1. Scatter plot of retention time of 90 compounds on Agilent Poroshell 120 EC-C18 versus Agilent Eclipse Plus C18.

Experimental

An Agilent 1200 Series Rapid Resolution LC (RRLC) system with an Agilent 6410 Triple Quadrupole LC/MS system was used for this work:

- G1312B Binary Pump SL with mobile phase A: 5 mM ammonium formate with 0.01% formic acid, and B: acetonitrile with 0.01% formic acid. Gradient was 10% B at t_0, ramp to 15% B, ramp to 50% B, then ramp to 95% B and hold 95% B. Gradient times vary depending on column dimensions and flow rate (Table 1).
- G1367C Automatic Liquid Sampler (ALS) SL. Injection volume was 1.0 µL.
- G1316B Thermostated Column Compartment (TCC) SL with temperature set to 60 °C or 90 °C (on Poroshell 120 SB-C18 only).
- G6410A Triple Quadrupole LC/MS: electrospray AP-ESI, drying gas temperature and flow: 350 °C, 12 L/min, nebulizer gas pressure: 30 psi, capillary voltage: 2000 V, in dMRM mode, transitions found in Table 2.
- MassHunter versions B.02.01, B.02.00 and B.03.01 were used for data acquisition, qualitative and quantitative analyses respectively.

Three Agilent columns were used in this work:

- Agilent Poroshell 120 EC-C18, 2.1 mm × 100 mm, 2.7 µm (p/n 695775-902)
- Agilent Poroshell 120 SB-C18, 2.1 mm × 100 mm, 2.7 µm (p/n 685775-902)
- Agilent ZORBAX RRHT Eclipse Plus C18, 2.1 mm × 100 mm, 1.8 µm (p/n 959764-902)

The compounds of interest are shown in Table 2, with their respective retention times on Poroshell 120 EC-C18 at 0.5 mL/min, and their qualitative and quantitative MRM transitions. Sample is a 1 µg/mL standard in methanol purchased from Agilent Technologies (LC/MS Toxicology Test Mixture, Agilent p/n 5190-0470). Serial dilutions in methanol were prepared for the calibration standards. The $1 bill sample was extracted in 7 mL of methanol and ultrasonicated for 30 min. Additionally, acetonitrile, formic acid and ammonium formate were purchased from Sigma Aldrich (Bellefont, PA). Methanol was purchased from Honeywell, Burdick and Jackson (Muskegon, MI). Water used was 18 M-Ω Milli-Q water (Bedford, MA).

Table 1. HPLC Method Parameters for Various Columns and Conditions

Gradient and method parameters	2.1 × 100 mm 1.8-µm Agilent ZORBAX Eclipse Plus C18	2.1 × 100 mm 2.7-µm Agilent Poroshell 120 EC-C18	2.1 × 100 mm 2.7-µm Agilent Poroshell 120 EC-C18	2.1 × 100 mm 2.7-µm Agilent Poroshell 120 EC-C18	2.1 × 100 mm 2.7-µm Agilent Poroshell 120 SB-C18	2.1 × 100 mm 2.7-µm Agilent Poroshell 120 SB-C18	2.1 × 100 mm 2.7-µm Agilent Poroshell 120 SB-C18
Flow rate (mL/min)	0.5	0.5	0.7	1.0	0.5	0.9	1.4
10% B (min)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15% B (min)	0.50	0.50	0.36	0.25	0.50	0.28	0.18
50% B (min)	3.00	3.00	2.14	1.50	3.00	1.67	1.07
95% B (min)	4.00	4.00	2.86	2.00	4.00	2.22	1.43
95% B (min)	6.00	6.00	4.29	3.00	6.00	3.33	2.14
Stop time (min)	6.00	6.00	4.29	3.00	6.00	3.33	2.14
Post run time (min)	2.00	2.00	1.43	1.00	2.00	1.11	0.71
Overall cycle time (min)	8.00	8.00	5.71	4.00	8.00	4.44	2.86
TCC temperature (°C)	60	60	60	60	90	90	90
Injection volume (µL)	1.0	1.0	1.0	1.0	1.0	1.0	1.0
System pressure (bar)	375	280	385	550	195	370	595

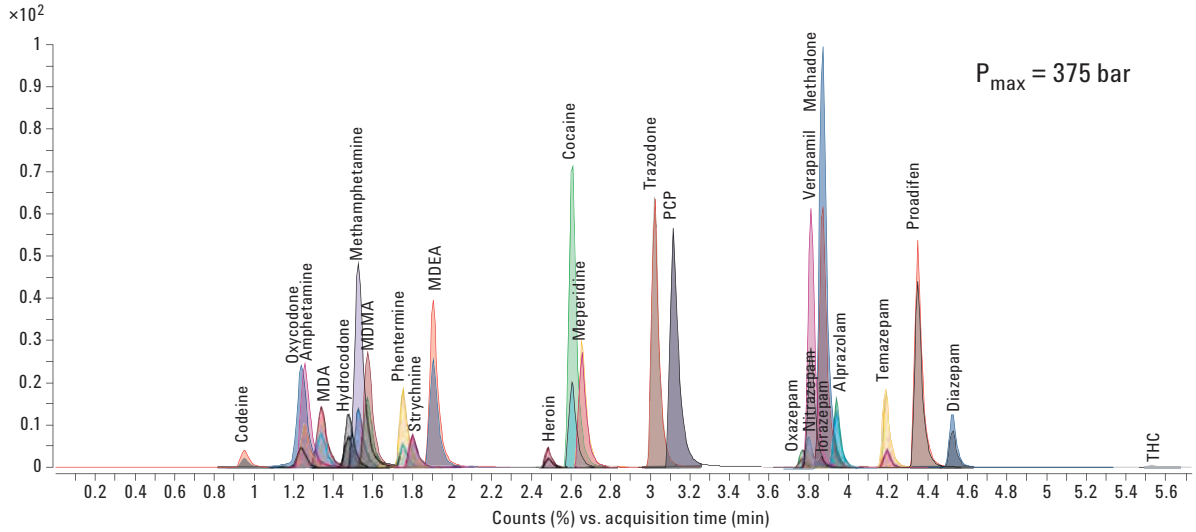
Table 2. MRM Transitions for 25 Compounds in Toxicology Test Mixture

Compound name	Precursor ion	Fragmentor voltage	Product ion 1	Collision energy 1	Product ion 2	Collision energy 2	Retention time (min)	Delta retention time
Codeine	300.2	158	165.1	45	58.1	29	0.89	0.4
Oxycodone	316.2	143	298.1	17	256.1	25	1.14	0.4
Amphetamine	136.1	66	119.1	5	91	17	1.19	0.4
MDA	180.1	61	163	5	105	21	1.25	0.4
Hydrocodone	300.2	159	199	29	128	65	1.34	0.4
Methamphetamine	150.1	92	119	5	91	17	1.43	0.4
MDMA	194.1	97	163	9	105	25	1.46	0.4
Strychnine	335.2	195	184	41	156	53	1.66	0.4
Phentermine	150	66	133	5	91	25	1.66	0.4
MDEA	208.1	107	163	9	105	25	1.8	0.4
Heroin	370.2	149	268.1	37	165	61	2.4	0.4
Cocaine	304.2	138	182.1	17	77	61	2.52	0.4
Meperidine	248.2	128	220.1	21	174.1	17	2.59	0.4
Trazodone	372.2	159	176	25	148	37	2.95	0.4
PCP	244.2	86	91	41	86.1	9	3.05	0.4
Oxazepam	287	150	269	12	241	20	3.66	0.4
Nitrazepam	282.1	148	236.1	25	180	41	3.66	0.4
Verapamil	455.3	158	165	37	150	45	3.75	0.4
Lorazepam	321	102	275	21	194	49	3.75	0.4
Methadone	310.2	112	265.1	9	105	29	3.83	0.4
Alprazolam	309.1	179	281	25	205	49	3.84	0.4
Temazepam	301.1	117	255.1	29	177	45	4.05	0.4
Proadifen	354.2	153	167	29	91.1	45	4.33	0.4
Diazepam	285.1	169	193	45	154	25	4.41	0.4
THC	315.2	150	193.2	20	123.3	30	5.4	0.4

Results and Discussion

Figure 2 shows the original method developed by P. Stone on an Agilent ZORBAX Eclipse Plus C18 2.1 mm × 100 mm, 1.8 µm column. This analysis is accomplished in 6 min with a 2-min post run time at 375 bar. Figure 3 shows the same method with an Agilent Poroshell 120 EC-C18 2.1 mm × 100 mm, 2.7 µm column. Analysis and post run time are identical to the Eclipse Plus method, while the system back pressure is reduced to 280 bar. While there are slight variations between elution patterns in Figures 2 and 3, overall selectivity is very similar, as would be predicted by Figure 1.

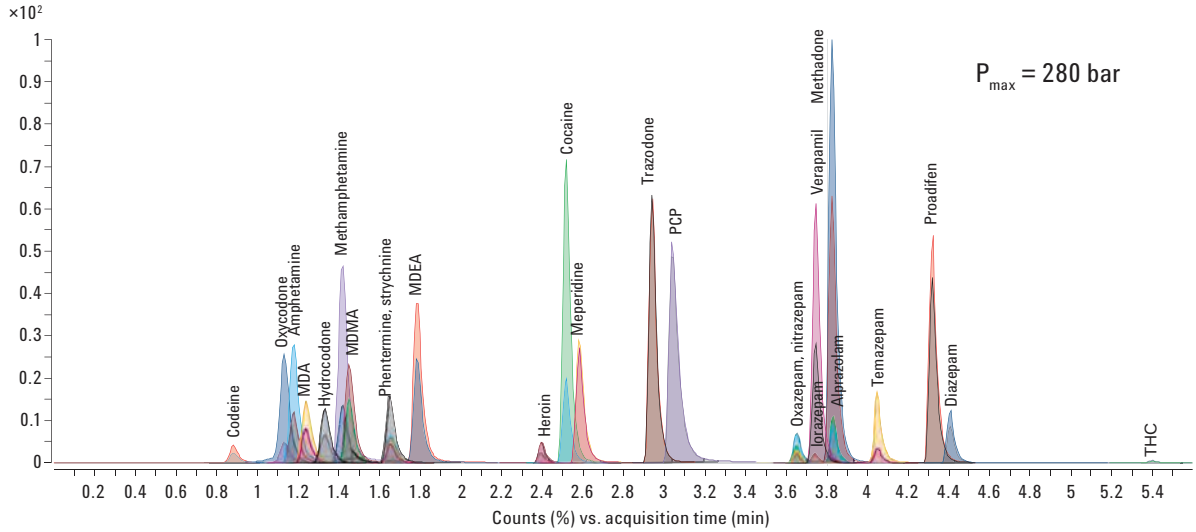
Original Toxicology Method on Agilent ZORBAX Eclipse Plus C18 2.1 mm × 100 mm, 1.8 µm (Agilent p/n 959764-902)



A: 5 mM ammonium formate w/ 0.01% formic acid (1 L water + 0.3153 g ammonium formate + 0.1 mL formic acid),
B: acetonitrile w/ 0.01% formic acid (1 L acetonitrile + 0.1 mL formic acid); 0.5 mL/min; 10% B at t_0 , ramp to 15% B in 0.5 min,
ramp to 50% B in 2.5 min, ramp to 95% B in 1 min, hold 95% B for 2 min; stop time 6 min, post run 2 min;
Sample: injector program: draw 5 µL water, draw 1 µL LC/MS Toxicology Test Mixture (p/n 5190-0470), inject; TCC = 60 °C
MS Source: electrospray AP-ESI, drying gas temperature and flow: 350 °C, 12 L/min, nebulizer gas pressure: 30 psi, capillary voltage: 2000V;
MS Acquisition: dynamic MRM (see Table 2 for MRM transitions), positive ionization polarity

Figure 2. Agilent LC/MS Toxicology Test Mixture (Agilent p/n 5190-0470) analyzed on Agilent ZORBAX Eclipse Plus C18 via an Agilent 1200 Series LC system with detection by an Agilent 6410 Triple Quadrupole LC/MS.

Original Toxicology Method on Agilent Poroshell 120 EC-C18 2.1 mm × 100 mm, 2.7 µm (Agilent p/n 695775-902)



A: 5 mM ammonium formate w/ 0.01% formic acid (1 L water + 0.3153 g ammonium formate + 0.1 mL formic acid),
B: acetonitrile w/ 0.01% formic acid (1 L acetonitrile + 0.1 mL formic acid); 0.5 mL/min; 10% B at t_0 , ramp to 15% B in 0.5 min,
ramp to 50% B in 2.5 min, ramp to 95% B in 1 min, hold 95% B for 2 min; stop time 6 min, post run 2 min;
Sample: injector program: draw 5 µL water, draw 1 µL LC/MS Toxicology Test Mixture (p/n 5190-0470), inject; TCC = 60 °C
MS Source: electrospray AP-ESI, drying gas temperature and flow: 350 °C, 12 L/min, nebulizer gas pressure: 30 psi, capillary voltage: 2000V;
MS Acquisition: dynamic MRM (see Table 2 for MRM transitions), positive ionization polarity

Figure 3. Agilent LC/MS Toxicology Test Mixture (Agilent p/n 5190-0470) analyzed on Agilent Poroshell 120 EC-C18 via an Agilent 1200 Series LC system with detection by an Agilent 6410 Triple Quadrupole LC/MS.

Table 3 shows calibration data for all 25 compounds found in the Agilent LC/MS Toxicology Test Mixture on Poroshell 120. All compounds exhibit strong linear correlations, with R^2 > 0.9979. Calibration data was used to quantify a methanol-extracted US $1 bill sample; chromatographic and quantitative results are shown in Figure 4. A significant amount of cocaine was found on the dollar bill. Oxycodone, methamphetamine, PCP and THC were also detected. Smaller quantities of amphetamine, hydrocodone, MDMA, heroin, methadone and diazepam were also found. Quantities of these substances on US currency are consistent with previous findings [6-8].

Table 3. Calibration Data for 25 Toxicology Compounds on Poroshell 120

Compound name	Linear calibration curve	Correlation coefficient, R^2
Codeine	y = 25.4023 × + 3.1628	0.99990276
Oxycodone	y = 138.9535 × – 0.6269	0.99938632
Amphetamine	y = 196.3425 × + 50.1606	0.99987385
MDA	y = 121.2945 × + 180.2165	0.99945701
Hydrocodone	y = 72.1351 × – 8.1010	0.99964622
Methamphetamine	y = 286.7936 × + 429.4970	0.99789141
MDMA	y = 121.4217 × – 55.0435	0.99874569
Phentermine	y = 110.8083 × – 65.1028	0.99914972
Strychnine	y = 39.3465 × – 9.5339	0.99964358
MDEA	y = 200.4804 × – 14.2886	0.99980092
Heroin	y = 18.2969 × + 0.4442	0.99987634
Cocaine	y = 295.8654 × – 5.6261	0.99963342
Meperidine	y = 145.0367 × + 17.2273	0.99986118
Trazodone	y = 286.1986 × – 12.4408	0.99969366
PCP	y = 287.4395 × – 24.8090	0.99989199
Oxazepam	y = 14.7883 × – 0.4919	0.99900677
Nitrazepam	y = 49.1750 × + 69.2747	0.99876656
Verapamil	y = 273.3001 × + 17.3890	0.99986678
Lorazepam	y = 11.2911 × + 6.0687	0.99896851
Methadone	y = 439.7238 × – 6.7890	0.9997511
Alprazolam	y = 80.2721 × + 18.5435	0.99969734
Temazepam	y = 70.9899 × + 15.5246	0.99976598
Proadifen	y = 243.9474 × – 13.0696	0.99990655
Diazepam	y = 68.9622 × + 26.0608	0.99948978
THC	y = 3.1838 × – 2.7072	0.99801611

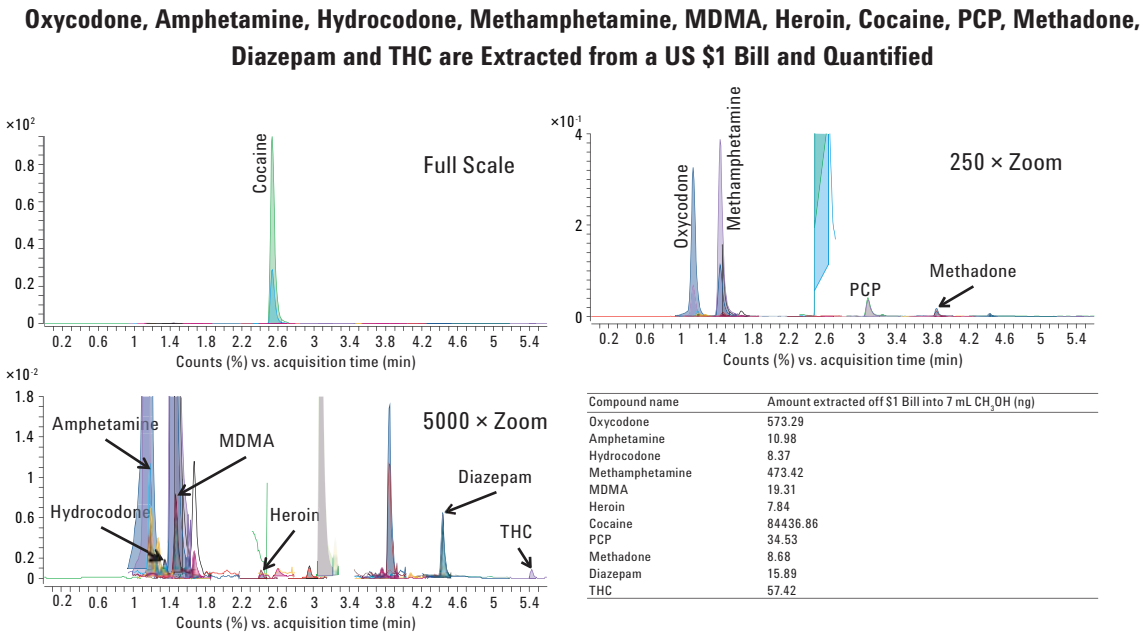


Figure 4. Chromatographic and quantitative results from a random US $1 bill sample extracted with 7 mL of methanol and ultrasonicated for 30 minutes.

Due to the low system back pressure generated with the Poroshell 120 column, the flow rate can be increased from 0.5 mL/min to 0.7 mL/min without exceeding 400 bar for use on a standard HPLC, or it can be increased to 1 mL/min without exceeding 600 bar for use on a UHPLC, as shown in Figure 5. The increased flow rate may be desirable when high throughput is important and when a UHPLC is available for use. Overall cycle time can be decreased by 2.3 minutes while keeping pressure below 400 bar, or by 4 minutes while keeping pressure below 600 bar (a 50% reduction in cycle time). Increasing the flow rate to this degree does cause some loss in resolution, but with MS detection this is not critical.

Significant Time Savings are Possible by Increasing Flow Rate with Agilent Poroshell 120 EC-C18 to LC System Pressure Limits, whether 400 or 600 bar

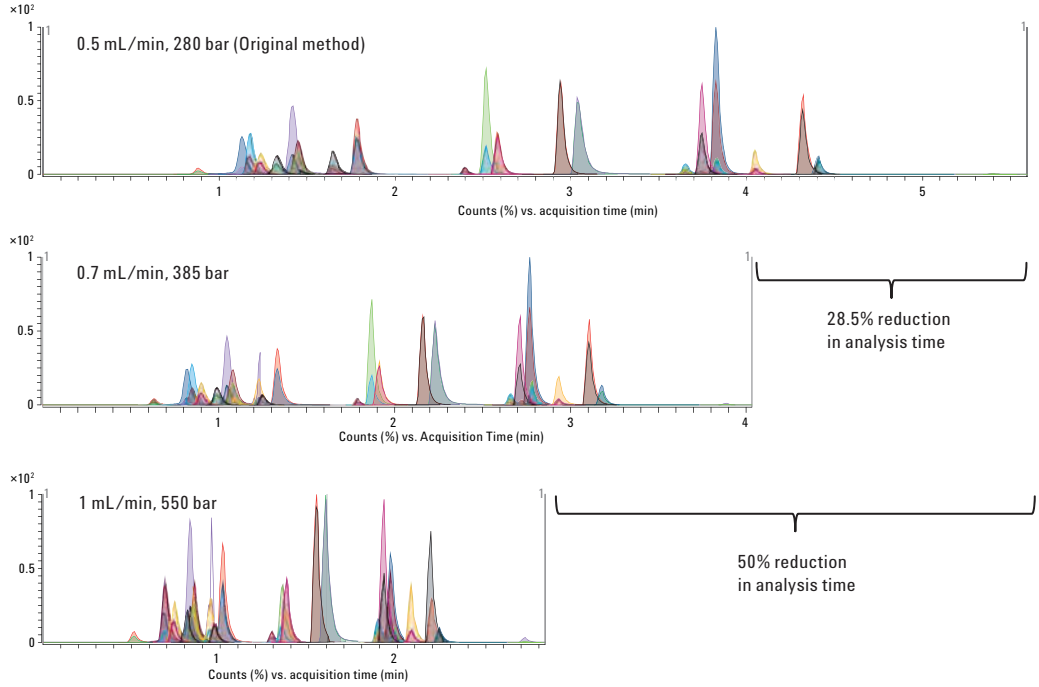


Figure 5. Overlay of Agilent Poroshell 120 EC-C18 toxicology analysis showing time savings by increasing flow rate to reach a 400 or 600 bar system limit.

Flow rate can be further increased by elevating temperature, thereby reducing mobile phase viscosity. The original method however was run at 60 °C, which is the maximum operating temperature for both Eclipse Plus C18 and Poroshell 120 EC-C18. In order to perform this analysis at a higher temperature, the column must be replaced with a Poroshell 120 SB-C18, which has a maximum operating temperature of 90 °C. Figure 6 shows the fast chromatography possible with Poroshell 120 SB-C18. With a 600 bar system pressure limit, it is possible to reduce run time by 64.3%, however this comes at the cost of reduced resolution. For an analysis as complex as this toxicology method, this loss of resolution and significant coelution will cost the analysts a reduction in data points across all peaks, therefore reducing the quality of the results. A simple solution may be to increae column length. A slight increase in column length from 100 mm to 150 mm will increase the resolution of all compounds. While the longer column cannot be run at quite as fast flow rates the analyst can still glean significant time savings by running it at its respective highest flow rate without exceeding system limitations.

Very Significant Time Savings are Possible by Increasing Temperature and Flow Rate with Agilent Poroshell 120 SB-C18 to LC System Pressure Limits, whether 400 or 600 bar

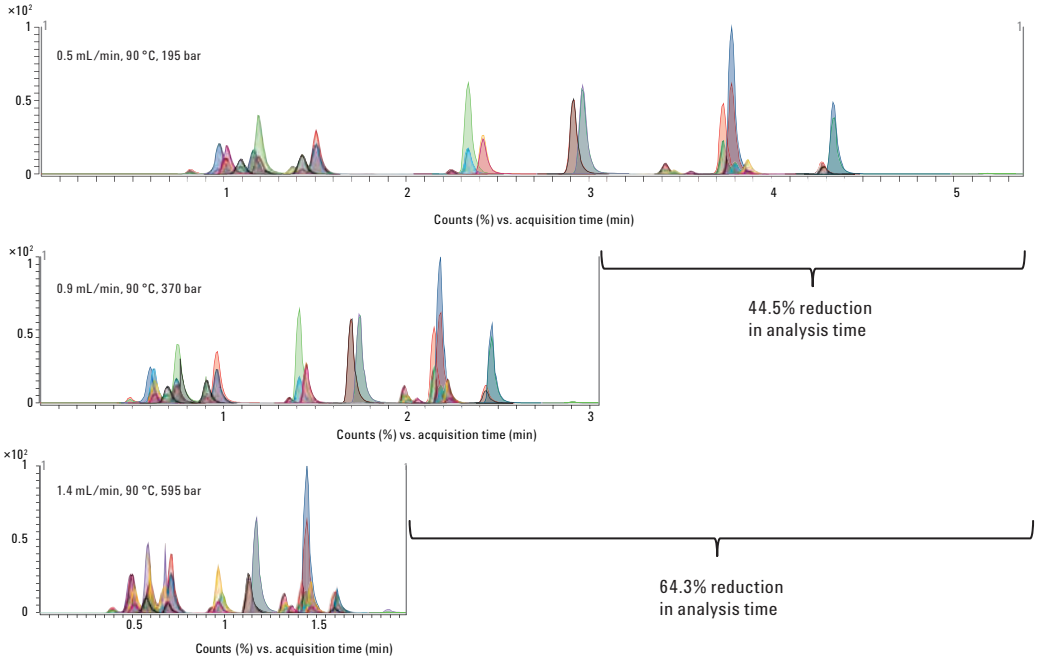


Figure 6. Overlay of Agilent Poroshell 120 SB-C18 toxicology analysis showing time savings by increasing temperature and flow rate to reach a 400 or 600 bar system limit.

Conclusion

A complex analysis of 25 toxicology compounds, that was originally performed on an Agilent ZORBAX Eclipse Plus C18 column, was easily carried out on a superficially porous Agilent Poroshell 120 EC-C18 column with high quality results and substantial time savings. Other complex analyses can likely be transferred from 1.8-µm Eclipse Plus C18 to Poroshell 120 EC-C18 of the same dimensions without method modification, due to very similar selectivity and efficiency. The lower back pressure of Poroshell 120's 2.7-µm particles can be exploited for productivity gains; faster flow rates may be used to shorten analysis time without exceeding system pressure limits for 400 bar HPLC's or higher pressure UHPLC's. This method was used to detect and quantify several drugs of abuse found on a $1 bill, including: cocaine, oxycodone, methamphetamine, PCP and THC.

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Profiling medications for decorative or aquarium fish using the Agilent 1290 Infinity LC system and Agilent ZORBAX Poroshell 120 2.7 µm columns

Application Note

Consumer Products

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Abstract

The Agilent 1290 Infinity LC system has significant capabilities for a wide range of HPLC and UHPLC applications. It exhibits a broader power range (for example, the combination of pressure and flow capabilities), and the flexibility to operate a wide range of column dimensions and particle sizes than any other commercially available system. Advanced optical design in the diode array detector allows a wide dynamic range and high sensitivity, both of which are critical in the monitoring of small impurities in fine chemicals.

The combined benefits are demonstrated by a separation of primary components and related impurities including sulfa drugs, nitrofurans and malachite green found in samples of fish medications. A broad range of products for treating ailments in decorative or pet fish are available. Many of these medications are banned or restricted for use in edible fish. If present in edible fish, the levels would be very low or undetectable by HPLC with UV based detection. These examples show a few medications and detail the rapid method development used to establish a rapid MS-compatible separation environment. Many fish medications advertise the use of "pharmaceutical quality" ingredients, and may imply pharmaceutical quality manufacturing and quality control procedures. When profiling these products one should expect to see very low levels of related impurities, consistent with the goals of pharmaceutical quality manufacturing

The high pressure capability of the system allows the use of methanol, and acetonitrile, to explore the selectivity of the two solvents. At 1 mL/min, using a simple 3 minute gradient and a 3.0 mm x 50 mm Poroshell 120 column, the analysis time is only less than 5 minutes including the late eluting phthalate artifact. The separation of the main components of a medicated powder with acetonitrile and methanol is shown in Figure 1, and the extraction of a medicated feed is shown in Figure 2.

The speed, resolution and flexibility of the system are further demonstrated by a separation of a sulfa standard mix using solvent, gradient and temperature optimization with a 100 mm length Poroshell 120 column (see Figure 3).

After further optimization of the sulfa mix using methanol with the elevated temperature, all of the samples were run with the final method configuration, as shown in Figure 4.

Configuration

- Agilent 1290 Infinity Binary Pump with Integrated Vacuum Degasser (G4220A)
- Agilent 1290 Infinity Autosampler (G4226A)
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)
- Agilent 1200 Diode Array Detector (G1315C)

Conclusion

Taking advantage of flexible solvent and column selection features, and high pressure capability, of the system allows one to use highly efficient columns to rapidly develop separations with remarkable resolution while conserving solvent over the use of 4.6 mm id columns.

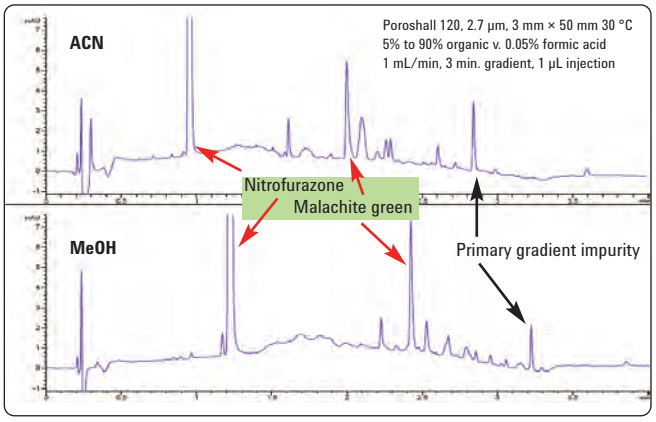

Figure 1
"Super Ick" medicated powder.

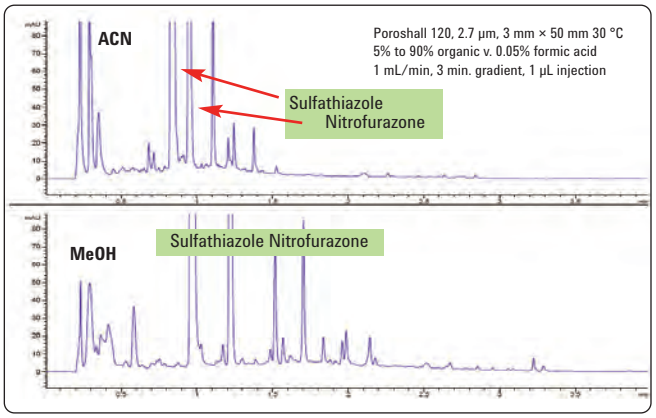

Figure 2
Separation of "Jungle" medicated fish food after methanol/water/formic acid extraction.

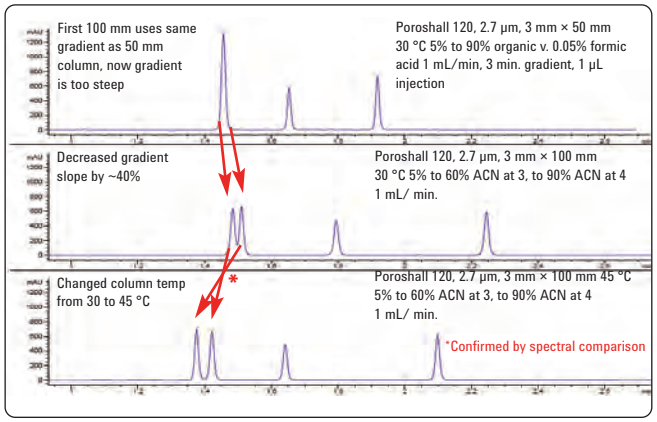

Figure 3
Sulfa standard mixture (Agilent p/n 59987-20033).

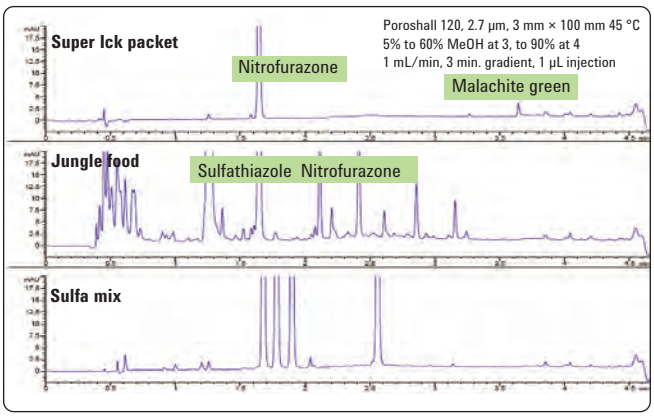

Figure 4
Final method configuration.

使用 Agilent 1100 系列液相色谱配合安捷伦 Poroshell 120 EC-C18 色谱柱对磺胺类药物进行快速分析

Application

Food

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Abstract

本文实验把 Agilent 1100 系列液相色谱上原本采用 4.6×250 mm，5 µm 色谱柱分离 10 种磺胺类药物的方法转移至安捷伦 Poroshell 120 EC-C18 色谱柱。文中还给出了方法转换的简单指导原则。新的分离方法将分析时间从 30 min 缩短到 8 min，且由于两种色谱柱均采用 2 µm 滤头，样品的制备方法无需更改。尽管压力相比原方法有所增加，但仍然低于 400 bar，可以很容易地转换到几乎任何 HPLC 系统上。本文实验采用未经过滤的蛋白碎裂的牛奶样品（使用磺胺二甲嘧啶作为加标物），结果显示色谱柱使用寿命超过 1000 次进样。

Introduction

Sulfonamides were the first chemical substances systematically used to treat and prevent bacterial infections in humans. Sulfonamides are bacteriostatic drugs; they work by inhibiting the growth and multiplication of bacteria without killing them. Currently, their most common use in humans is treating urinary tract infections. Other newer drugs are used to fight infections, such as beta-lactam antibiotics. Today, sulfa drugs are commonly used as antibiotics in veterinary applications; primarily, they are fed to animals to prevent infection. In addition, they are also used to fight disease in honey bees. Residues of these antibiotics are quite often found in honey samples and are of concern to consumers around the world due to toxic or allergic reactions. [1,2]

The Agilent Poroshell 120 EC-C18, 2.7-µm columns have similar performance to sub-2-µm completely porous materials, but since they use 2-µm column frits similar to those found on 5-µm columns, they require no additional sample preparation. This allows a more seamless method transfer.

In this work, a gradient method is transferred and optimized from a 4.6 × 250 mm, 5-µm column to a 4.6 × 100 mm Agilent Poroshell 120 EC-C18 column. Gradient time was decreased from 30 min to 8 min. Time can be further reduced to less than 4 min using a 4.6 × 50 mm column, with some loss of resolution.

Experimental

An Agilent 1100 Series HPLC modified for Rapid Resolution LC components was used for this work. This system consisted of a G1312A Binary Pump, capable of delivering up to 400 bar pressure; a G1316A Thermostatted Column Compartment (TCC), a G1376A High Performance Autosampler, a G1315B Diode Array Detector equipped with a semimicro flow cell with a 3-mm path length. When using the Poroshell 120 column all tubing was changed to 0.12 mm id tubing of the shortest lengths; the needle seat was changed to a 0.12 mm id part; and the detector data collection rate was increased to the fastest setting (40 Hz) in accordance with recommendations for optimization shown in previous documents and presentations. [3, 4] Data was collected using Agilent ChemStation version A.10.02. Columns used in this work include Agilent ZORBAX Eclipse Plus C18 4.6 × 250 mm, 5-µm (p/n 959990-902) and Agilent Poroshell 120 EC-C18 4.6 × 100 mm columns (695975-902).

The following sulfa drugs were purchased from Sigma Aldrich: sulfadiazine, sulfathiazole, sulfapyridine, sulfamerazine, sulfamethazine, sulfamethazole, sulfamethoxypyridazine, sulfachloropyridazine, sulfamethoxazole, sulfadimethoxine. These compounds were prepared in 50/50 v/v acetonitrile:water at 1 mg/mL and then mixed in equal amounts to produce a solution of 0.1 mg/mL each.

The milk sample used was Similac. Similac is a commercial infant formula developed to be similar to breast milk. The composition of this material is strictly controlled by the manufacturer, so its protein composition is uniform, which is unlike that of milk (Abbott Laboratories Columbus Ohio). The most commonly used infant formulas contain purified cow's milk whey and casein as a protein source, a blend of vegetable oils as a fat source, lactose as a carbohydrate source, a vitamin-mineral mix, and other ingredients.

A 2-mL aliquot of Similac spiked with sulfadimethoxine was added to 18 mL of 200:800:1 solution of water:acetonitrile:formic acid and shaken vigorously for 1 min. The solution was then allowed to stand for 3 min and an aliquot was transferred to a centrifuge tube. The tubes were centrifuged at 5000 rpm for 5 min. The solution was transferred to an auto sampler vial without further filtration.

Discussion

Faster analyses with small particle columns have been demonstrated for several years. However, unlike 5 or 3.5-µm columns, smaller frits are required to keep the packing material in the column. A 2-µm frit is used to hold in particles of 5 and 3.5 micron size. For particles that are 3 µm or smaller, frits of 1 or 0.5 µm are required. A totally porous particle will have a particle size distribution that is 25% greater than a superficially porous particle. A narrow particle size distribution material such as Poroshell 120 can be held in the column using a 2 µm frit. If the frit size on the new column is smaller than that on the older column, more care must be taken with sample preparation when transferring the method, to prevent clogging the frit with a dirty sample. [5] By keeping the column frit at a larger size no additional sample cleanup is required when transferring a method from a 5 or 3.5 µm column to a Poroshell 120 column.

The initial method shown in Figure 1 demonstrates a ten compound separation using a 4.6 × 250 mm, 5 µm column and a 30-min gradient. The separation yields baseline resolution on most compounds, and a minimum resolution of 1.7 on one peak pair. This separation may not have received further optimization due to the lengthy experiments required. The method is transferred to a 2.7 µm superficially porous column in several short steps:

1. Transfer of the gradient (gradient of the original method is proportionally shortened to the column length to maintain the original separation, preserving k'). Since the original method time was 30 min and the original column was 250 mm, using a 100 mm column at the same flow rate shortens the gradient time by 100/250 or 0.4. The injection volume is also decreased by the same amount. [4,6,7] This is shown in Figure 2.

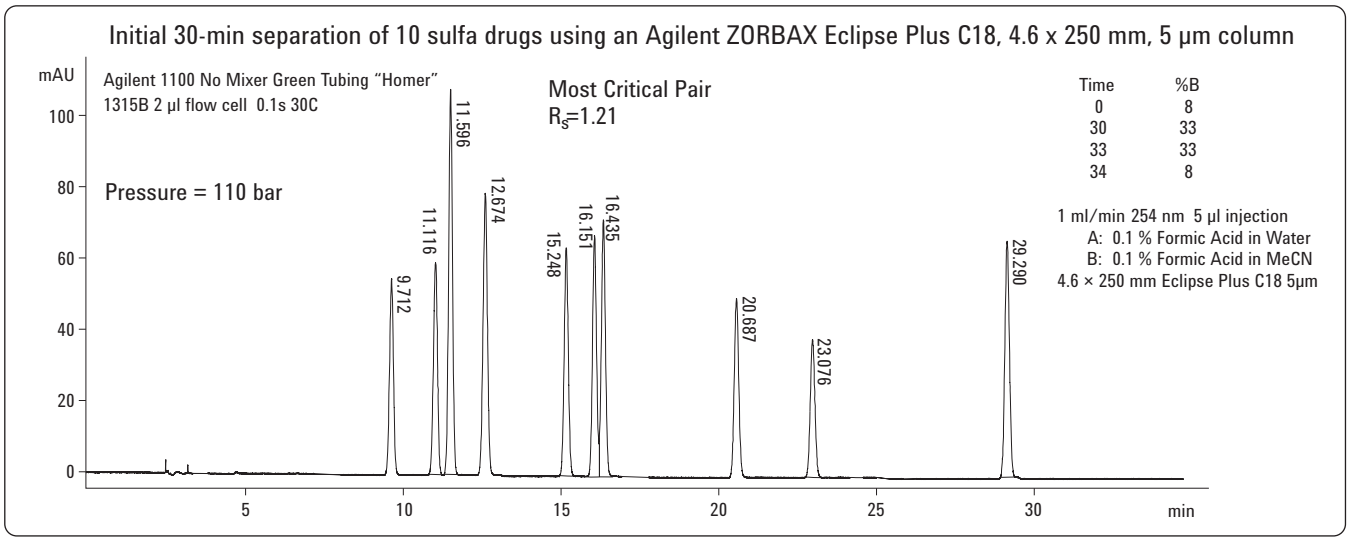

Figure 1. A separation of 10 sulfa drugs on an Agilent ZORBAX Eclipse Plus C18 4.6 × 250 mm, 5 µm in 30 min using a formic acid/acetonitrile gradient.

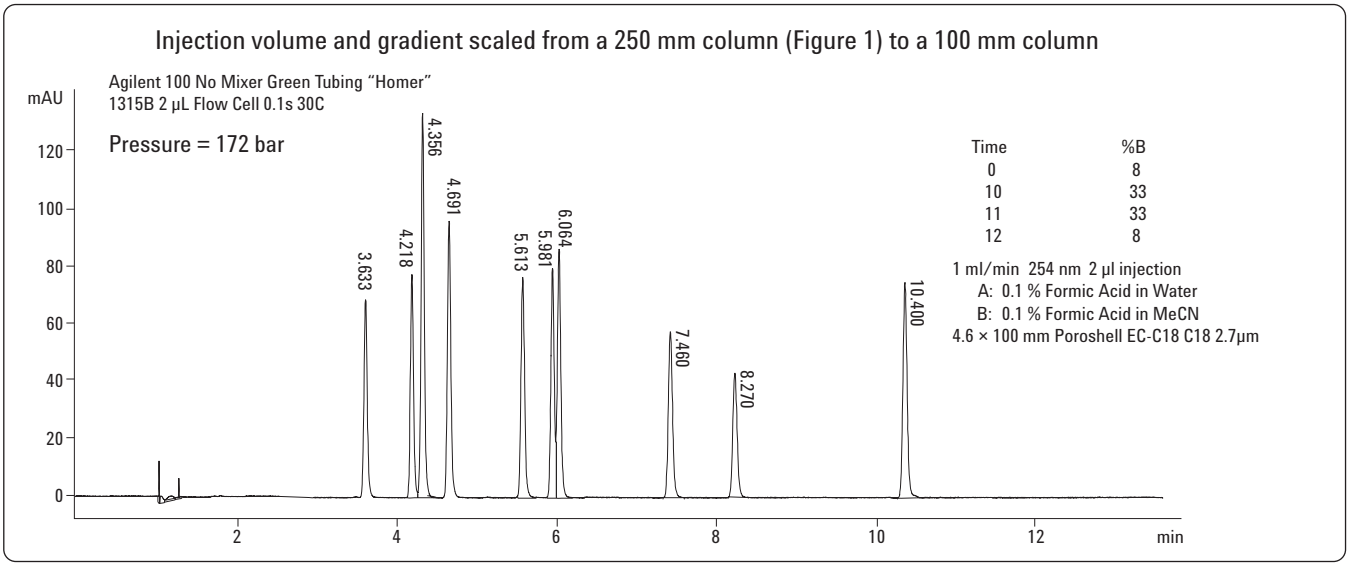

Figure 2. A separation of 10 sulfa drugs on an Agilent Poroshell 120 EC-C18 4.6 × 100 mm, 2.7 µm column in 12 min using a formic acid/acetonitrile gradient.

2. Flow rate is increased, but the number of column volumes in the separation is maintained, as seen in Figure 3. k' is preserved in a separation by keeping gradient steepness (G_s) constant on similar columns. Using the formula, shown as Equation 1.

Equation 1. $G_s = (V_m/F)(\Delta\%B/t_G)$ [7],

and rearranging the formula to

Equation 2. $(F)(t_G) = ((\Delta\%B)(V_m) / G_s)$

where

F is flow rate,
t_G is gradient time,
V_m is column volume,
Δ%B is the change in solvent strength.

We find that we can shorten gradient time by increasing flow rate. So our initial separation of 8 to 33% B at 1 mL/min over 10 min is converted to 8 to 33 % at 1.5 mL/min over 6.7 min and finally 8 to 33 % B over 5 min at 2 ml/min. (1 mL/min × 10 min = 1.5 mL/min × 6.7 min = 2 mL/min × 5 min) following the formula below:

Equation 3. $$t_2 = t_1 \times \frac{F_1}{F_2}$$

where

t_2 is time of new gradient
t_1 is time of old gradient
F_2 is new flow rate
F_1 is old flow rate

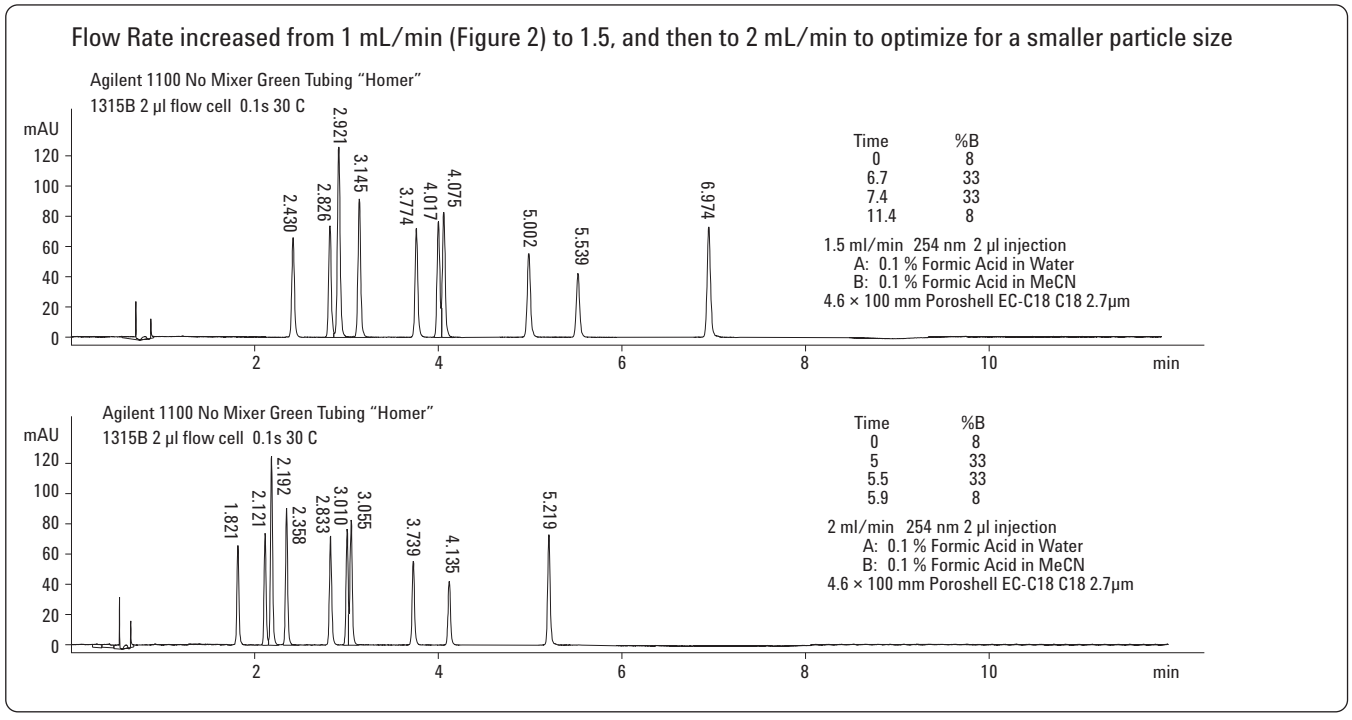

Figure 3. A separation of 10 sulfa drugs on an Agilent Poroshell 120 EC-C18 4.6 × 100 mm, 2.7 µm column showing the flow rate and gradient scaled from 1 mL/min (Figure 2), to 1.5 mL/min, and finally to 2 mL/min, resulting in a 6 min separation using a formic acid/acetonitrile gradient.

3. Optimization of the gradient. Since the experiment can be run in a fraction of the time required in the original experiment, several quick gradient changes are made leading to an even faster separation or perhaps better resolution. This is accomplished in less than 1 hr. Some of this work could have been performed on the longer column, but due to the longer analysis times, analysts would need to spend 4 to 8 hr to accomplish this step. Figure 4 demonstrates the steps taken to optimize selectivity on the Poroshell 120 column.

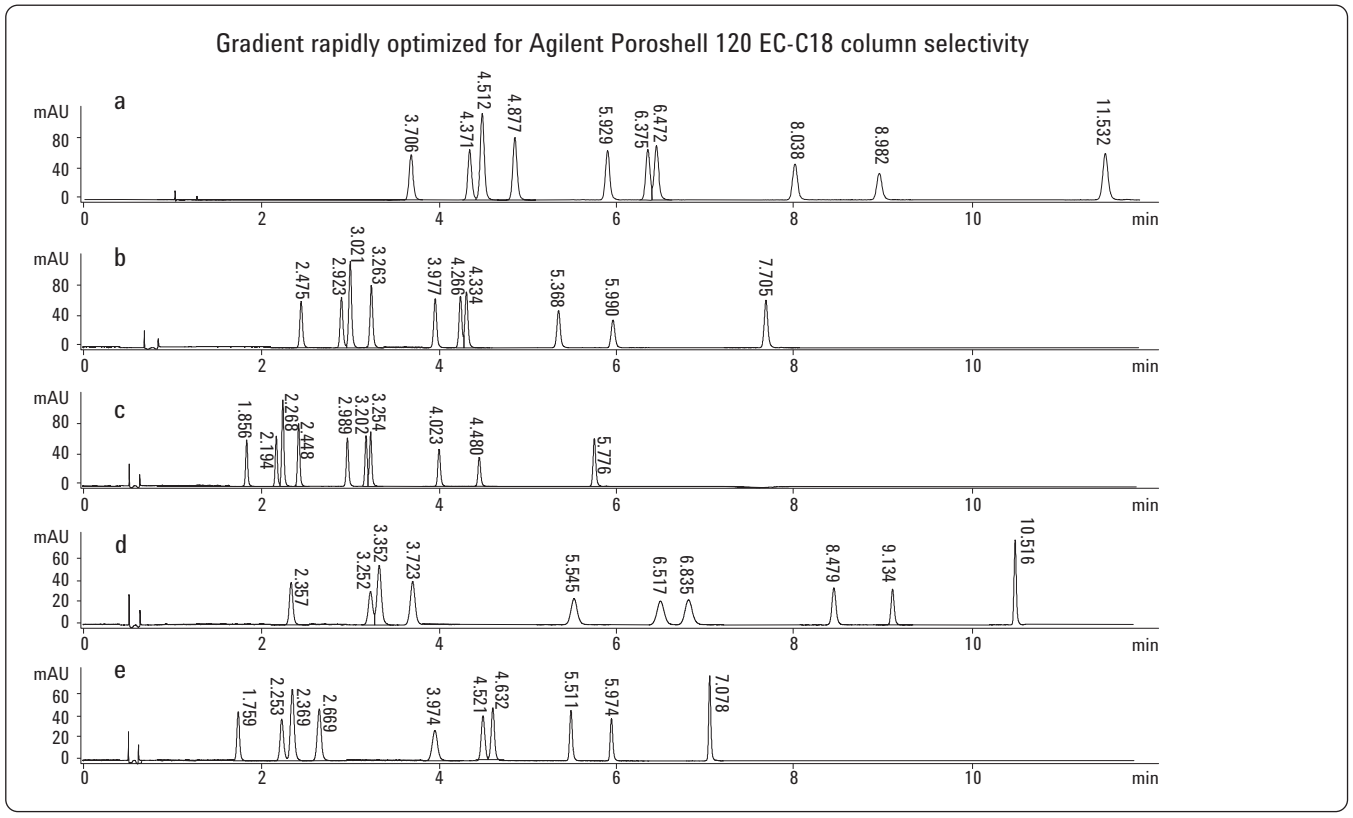

Figure 4. Optimizing a separation of 10 sulfa drugs on a 2.7 µm Agilent Poroshell 120 EC-C18 column, by adjusting the formic acid/acetonitrile gradient. (a) Original Scaled Chromatogram from 250 mm column, at 1 ml/min.(also shown in Figure 2) (b) Chromatogram scaled to faster flow rate of 1.5 ml/min shortening analysis time by 33 %. (also shown in Figure 3)(c) Chromatogram scaled to faster flow rate of 2 ml/min shortening analysis time by 50 %. (d) Adjusting gradient program to improve selectivity of peak pair 6,7. (e) Adjusting gradient program to improve selectivity of peak pair 2,3.

4. Complete optimization of the Agilent 1100 LC system. With small columns, additional performance gains can be made with attention to instrument configuration. The most important parameter is detector speed. In this case, the detector is set to maximum collection rate (40 Hz). Other important factors include: minimizing the tubing, changing the flow cell to smaller volume to reduce peak broadening, and changing the injector seat to a smaller volume. The initial 250-mm method is overlaid with the new 100-mm method in Figure 5. These chromatograms show the significant time savings, with the last peak on the new, faster method eluting before the first peak on the older 5-µm method.

Finally, a sample of milk simulant, Similac, spiked with sulfadimethazine is extracted by precipitation of proteins with acidified acetonitrile. The sample is shaken and allowed to stand for 5 minutes before being decanted. As can be seen in the scatter plot, (Figure 6), no change in the pressure is noted over the period of testing. In addition, the efficiency remains nearly constant. The analysis of this unfiltered protein precipitated milk sample demonstrates the ruggedness of the Agilent Poroshell 120 EC-C18 column.

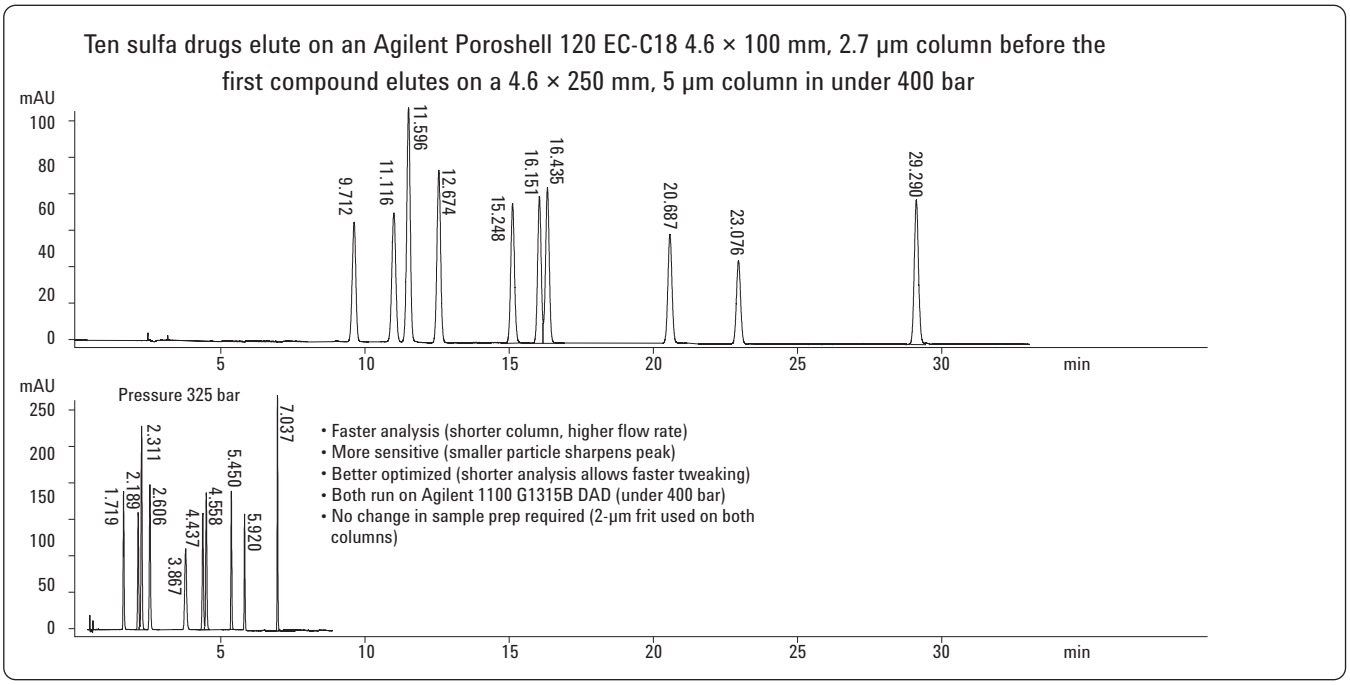

Figure 5. A separation of 10 sulfa drugs scaled from an Agilent ZORBAX Eclipse Plus C18 4.6 × 250 mm, 5 µm column to an Agilent Poroshell 120 EC-C18 4.6 × 100 mm, 2.7 µm column showing analysis time decreased from 30 min to 8 min using a formic acid/acetonitrile gradient.

Conclusions

This work has shown that an existing method with a 4.6 × 250 mm, 5 µm C18 column, can be easily adapted to a faster, but similar, method using an Agilent Poroshell 120 EC-C18 4.6 × 100 mm column. This method can still be used with a traditional HPLC system, as the maximum pressure is well below 400 bar. Additionally, sample preparation can stay the same, since both columns use 2-µm frits. The time and solvent savings gleaned from this method transfer may allow analysts to further optimize their method parameters, an option that may not have been reasonable with a significantly longer analysis time.

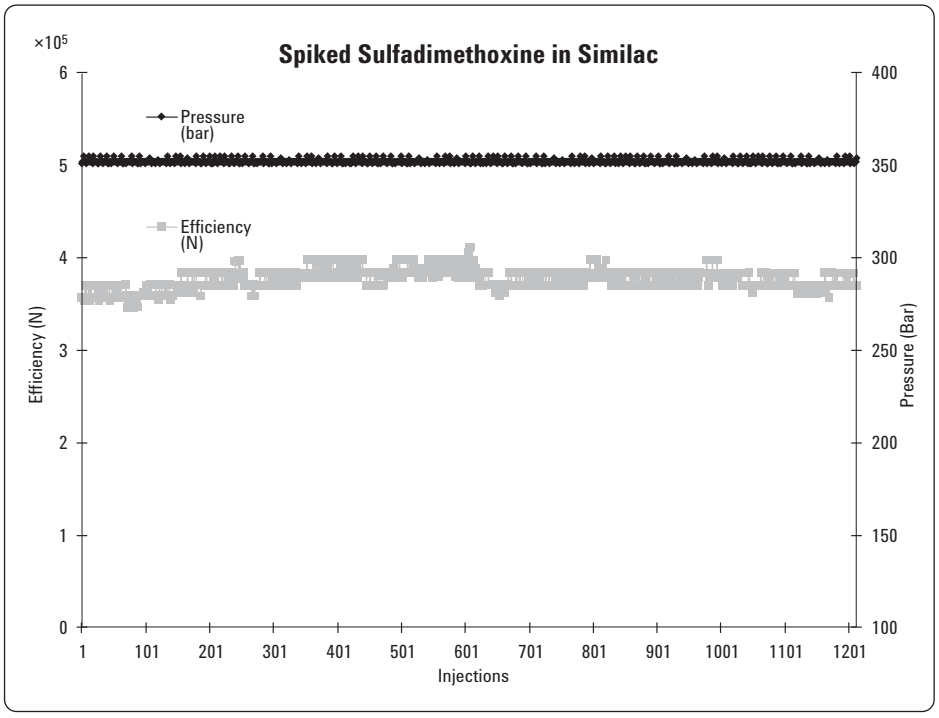

Figure 6. Scatter plot showing the Agilent Poroshell 120 100-mm column (Sulfadimethoxine enduring over 1000 injections of precipitated Similac, efficiency of sulfa drug plotted, no increase in pressure detected.)

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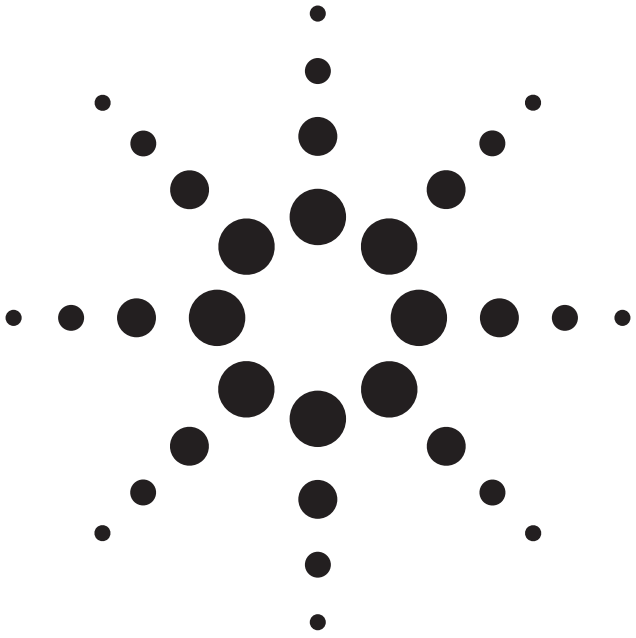

食品安全

采用Poroshell 120色谱柱快速分析饮料中的4种添加剂

应用领域

食品安全

关键词

表面多孔层柱；HPLC；Poroshell 120

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摘要

安赛蜜、糖精钠、苯甲酸、山梨酸是食品生产过程中常用的食品添加剂，但部分食品中超标现象十分严重，对人体健康造成很大的潜在危害。国家食品卫生标准对这几项指标有明确的限量[1]，并制定了相应的液相检测方法[2-4]，但规定的方法中使用的常规液相色谱仪和液相分析柱，分析时间长，且有机试剂耗用量大，因此，快速液相色谱仪和快速液相分析柱应运而生[5-6]，使得分析时间和试剂耗量大大降低。但针对目前基层实验室常规液相色谱仪的普及，与快速液相分析柱的不兼容性，使得快速分析检测很难实现。安捷伦公司最新推出的Poroshell 120系列表面多孔层色谱柱，由于其具有低反压，高柱效的特点，从而真正实现在常规液相色谱仪上进行快速分析的可操作性。本文使用Poroshell 120色谱柱，并采用常规液相色谱仪，对安赛蜜、糖精钠、苯甲酸、山梨酸的同时检测方法加以改进，分析时间和试剂耗量均降低了2/3，一次分析仅需5min。现将实验结果报告如下，以供参考。

1 试验材料与方法

1.1 仪器与试剂

1200高效液相色谱仪(Agilent公司)；甲醇、乙腈(HPLC级,美国天地公司)；水由Milli-Q Academic净化系统制得；苯甲酸，山梨酸，安塞蜜，糖精钠标准品浓度均为1000 μg/mL (农业部环境保护科研监测所)。

1.2 色谱条件

色谱仪条件：Poroshell 120 SB-C18柱(4.6 mm i. d. × 100 mm, 2.7 μm)；柱温40℃；流速1.0 mL /min；流动相A：5%乙酸铵溶液(0．02 mol/L)；流动相B：95%乙腈；紫外检测器：波长230nm；进样体积2 μL。

1.3 试验样品

含乳饮料，果蔬汁饮料，样品均为市场采购。

1.4 试验方法

1.4.1标准储备液的配制

准确移取适量上述标准溶液并用甲醇定容至25mL，使成为10mg/L的标准储备液，4℃避光条件下保存。

1.4.2 混合标准工作液

分别吸取不同体积的储备液，用水：乙腈溶液（95：5）配制成浓度分别为0.20μg/mL、0.50μg/mL、1.00μg/mL、2.00μg/mL、5.00μg/mL、10.00μg/mL的混合标准工作液。

1.4.3 样品处理

1.4.3.1 低蛋白含量样品　准确称取10.0g液体样品于50 mL的容量瓶,加入30毫升水超声波提取30 min,放置冷却后,用水定容至刻度,摇匀。提取溶液用定性滤纸过滤后,经0.45μm的滤膜,待测。

1.4.3.2 高蛋白含量样品　准确称取10.0g液体样品于50 mL的容量瓶,加入30毫升水超声波提取30 min,放置冷却后,加入2.5毫升乙酸铅溶液（1.00mol/L）和2.5mL亚铁氰化钾溶液(0.25mol/L)，用水定容至刻度,摇匀。提取溶液用定性滤纸过滤后,经0.45μm的滤膜,待测。

1.4.4 测定

按1.2操作进行，以保留时间定性，外标法定量。

2 结果与讨论

2.1 色谱条件的选择

以Poroshell 120EC-C18柱（4.6 mm i. d. ×100 mm, 2.7μm）作为分析柱，以0.02 mg/L乙酸铵溶液-甲醇和0.02 mg/L乙酸铵溶液-乙腈为流动相进行HPLC条件的优化。实验发现，以乙腈作为流动相分析安赛蜜等5种添加剂时柱压低，安赛蜜和苯甲酸可实现基本分离，而甲醇作为流动相，柱压相对较高，安赛蜜和苯甲酸分离效果较差。故选用0.02 mg/L乙酸铵溶液-乙腈（95：5，v/v），一次分析仅需5min。这样，每个样品从原来的分析时间15分钟缩短至5min，不仅提高了工作效率，而且节省了有机溶剂。

2.2 方法的线性范围和检出限

混合标准工作液的测定按照1.4的操作步骤，以添加剂的峰面积为纵坐标，含量为横坐标，绘制标准曲线。本方法中14种添加剂的线性范围为0.02～0.5μg/mL，回归方程和最低检出限（信噪比为3：1）结果见表1。标准工作溶液的色谱图见图1。

2.3 方法的加标回收率和精密度

取上述4种空白样品10.00g，分别添加混合标准储备液，使得样品中4种添加剂的浓度设置0.005g/kg、0.02 g/kg两个水平，按样品前处理操作，方法的加标回收率和精密度结果见表1，样品色谱图见图2。

表1 4种食品添加剂的回归方程、回收率、精密度和检出限(n=3)

农药名称	回归方程	平均回收率（%）	精密度（RSD%）	最低检出限（mg/kg）
安赛蜜	Y=6.2738X-0.9521　0.9995	99.5	3.15	0.002
苯甲酸	Y=7.0295X-0.9103　0.9991	97.2	4.25	0.002
山梨酸	Y=10.6401X-0.8024 0.9997	95.7	4.01	0.002
糖精钠	Y=4.4489X-0.7102　0.9995	95.9	4.89	0.005

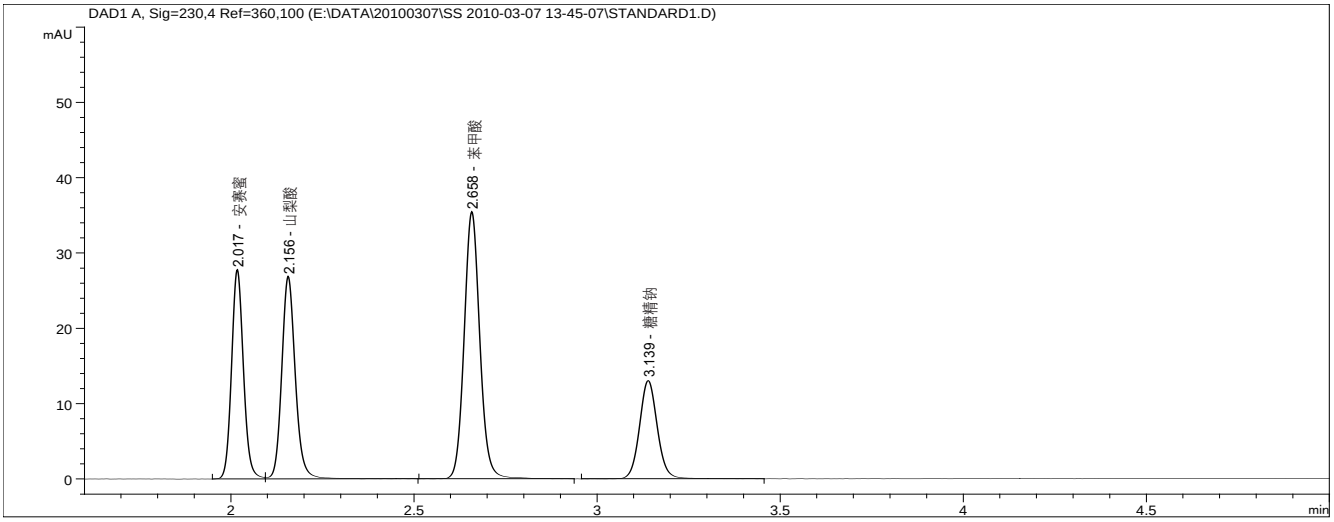

图1 4种食品添加剂标准色谱图（浓度均为10μg/mL）

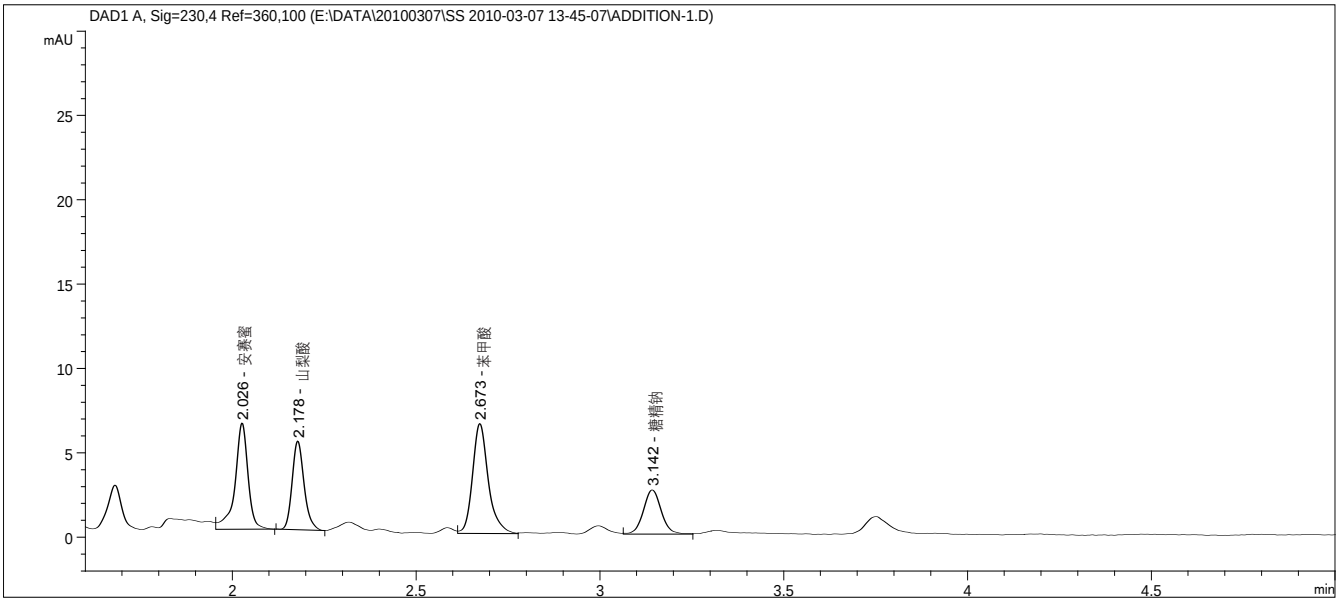

图2 果汁饮料中4种食品添加剂加标样品色谱图（加标浓度均为0.02 mg/kg）

2.4 使用传统色谱柱的结果

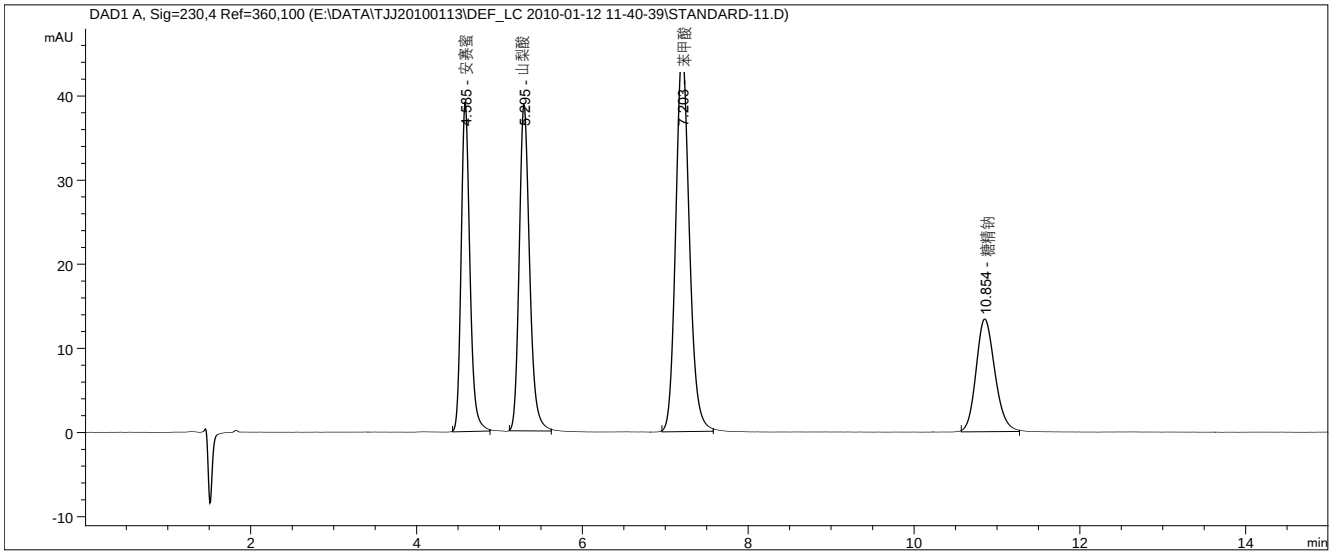

图3 4种食品添加剂在传统色谱柱上的标准色谱图（浓度均为10μg/mL）

色谱条件：

1200液相，DAD检测器，标准流通池

色谱柱：Zobax SB-C18 4.6*150mm, 5um

流动相：5%甲醇/95乙酸铵溶液（0.02mol/L）

压力：90bar

流速：1mL/min

柱温：20℃

进样量：10uL，浓度10ppm

3 小结

本法采用Poroshell120，在基层普遍使用的常规液相色谱仪上测定安赛蜜、糖精钠、苯甲酸和山梨酸4种食品添加剂。该方法的分析速度迅捷、试剂消耗少、最低检出限、回收率和重复性均符合批量样品的检测要求，结果令人满意，很适合于基层提高检测工作速度的开展。

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采用Poroshell 120色谱柱同时快速高效分析食品中的苯甲酸、山梨酸、脱氢乙酸、糖精钠

应用领域

食品安全

关键词

Poroshell 120 EC-C18色谱柱；苯甲酸、山梨酸、脱氢乙酸、糖精钠；高效液相色谱法；

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摘要

建立采用Poroshell 120 EC-C18 （4.6×100mm ，2.7μm）色谱柱快速高效分析食品中的苯甲酸、山梨酸、脱氢乙酸、糖精钠的方法。流动相为MeOH：0.02mol/LNH4Ac（10：90），流速：1.0mL/min，柱温：40℃，波长：230nm,6min完成分析。四种组份的平均回收率为88.8%～102.0%，相对标准偏差为0.52%～1.74%，本方法苯甲酸、山梨酸、脱氢乙酸、糖精钠的最低检出限分别为：0.5、0.5、2.0、1.0mg/kg。采用Poroshell 120色谱柱反相高效液相色谱法测定食品中的苯甲酸、山梨酸、糖精钠、脱氢乙酸，灵敏度高，回收率和重现性良好，快速简便，结果准确可靠，节约试剂，提高工作效率。

近年来，由于消费者对食品的安全性日益关注，特别是防腐剂[苯甲酸(BA)、山梨酸(SA)、脱氢乙酸(DHA)]和人工合成甜味剂[糖精钠(SS)]的使用越来越受到人们的重视[1-2]。由于防腐剂、甜味剂都有一定的毒性，过量摄入会对人体带来伤害，还可引发癌症[3]。由于这几种防腐剂和甜味剂允许同时添加在许多食品中，在我国食品添加剂使用卫生标准(GB2760—2007)中对这些添加剂的使用范围和最大使用限量均有明确规定。由于这几种防腐剂和甜味剂允许同时添加在许多食品中，因此同时快速检测成为必要，而现行国标方法GB/T23495-2009和GB/T23377-2009分别测定BA、SA、SS和DHA，如果沿用国家标准分开检测，不但增加了工作成本，而且降低了工作效率。我们在文献[1-6]的基础上加以改进，探讨了用高效液相色谱法同时对BA、SA、DHA和SS进行检测，同时比较了Poroshell 120色谱柱和普通色谱柱的区别，结果表明采用Poroshell 120色谱柱同时检测食品中的BA、SA、DHA和SS更快速，更高效。

1 试验材料与方法

1.1 仪器与试剂

Waters 600E型高效液相色谱仪、Waters 2996型二极管阵列检测器（美国 Waters公司），AL204电子天平（瑞士），PT-310电子天平，超声波清洗器，KDC-1042低速台式离心机，Anke TGL-16B高速离

心机。

Poroshell 120 EC-C18（4.6×100 mm 2.7μm）色谱柱（美国 Agilent公司）。

苯甲酸标准溶液[GBW(E)100006]、 山梨酸标准溶液[GBW(E)100007] 、糖精钠标准溶液[GBW(E)100007]三种标准溶液浓度均为1.00mg/mL（国家标准物质 中国计量科学研究院）；脱氢乙酸：（98% 美国 ACROS公司）；甲醇（色谱纯）；乙酸铵、硫酸锌、氢氧化钠、石油醚30～60（分析纯）；水为超纯水。

1.2 样本来源

酱腌菜制品60件，糕点90件，样品均为市场采购。

1.3 测定方法

1.3.1 色谱条件

色谱柱：ZORBAX Poroshell 120 EC-C18 （4.6×100 mm 2.7μm）

流动相：MeOH：0.02mol/LNH4Ac (10：90) 柱温：40℃

流 速：1.0 mL /min 检测器：λ：230nm

进样量：10μL 根据保留时间定性，外标法定量。

1.3.2标准储备液的配制

1.00mg/mL DHA标准溶液：准确称取0.1000g DHA标准品用10mL 20mg/mL氢氧化钠溶液溶解，至置于100mL容量瓶中，用水定容至刻度（4℃保存6个月）。

200μg/mL BA、SS、SA和DHA 混合标准溶液：分别准确吸取10.00 mL浓度均为1.00mg/mLBA、SS、SA和DHA标准溶液置于50mL容量瓶中，用水定容至刻度（4℃保存3个月）。

1.3.3标准溶液系列的配制

将200μg/mLBA、SS、SA和DHA混合标准溶液逐级配制成浓度分别为1.00、5.00、10.00、20.00、50.00μg/mL的标准溶液系列（临用现配）。

1.3.4样品处理

参照国标方法GB/T23495-2009和GB/T23377-2009，以及参考文献[1-6]，含有二氧化碳的液体样品需超声脱气；蛋白质含量高的样品需沉淀蛋白质（用氢氧化钠溶液和硫酸锌溶液或者用亚铁氰化钾溶液和乙酸锌溶液）；脂肪含量高的样品需脱脂（用石油醚或者正己烷）。将样品粉碎混匀，称取5g（精确至0.01g）样品于50 mL的比色管中，加入约30mL水，用氢氧化钠溶液调pH直至7～8，加水定容至刻度，超声提取10min。3000r/min,离心10min，取上清液经0.45μm的滤膜过滤后待进样。

1.3.5测定结果

标准系列溶液与样品制备液按照1.3.1的色谱条件进行检测，BA、SS、SA和DHA与样品中其它共存物质分离良好，测定不受干扰。试样中BA、SS、SA和DHA的含量按式（1）进行计算

$$X=\frac{A\times V\times 1000}{m\times 1000\times 1000} \qquad \cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots (1)$$

式中：

X—试样中BA、SS、SA和DHA的含量，单位为克每千克（g/kg）；

A—制备液测定的含量，单位为微克每毫升（μg/mL）；

V—试样稀释总体积，单位为毫升（mL）；

m—试样质量，单位为克（g）。

计算结果保留两位有效数字。

2 结果

2.1 色谱图 在1.3.1色谱条件下BA、SS、SA和DHA的最大吸收波长分别为223、210、254、230nm，其中DHA还有个次吸收峰波长为292 nm，保留时间分别为2.4、2.8、3.4、3.7min，各组份均基线分离，与共存的其他物质分离良好，其混合标准溶液的色谱图如图l示。

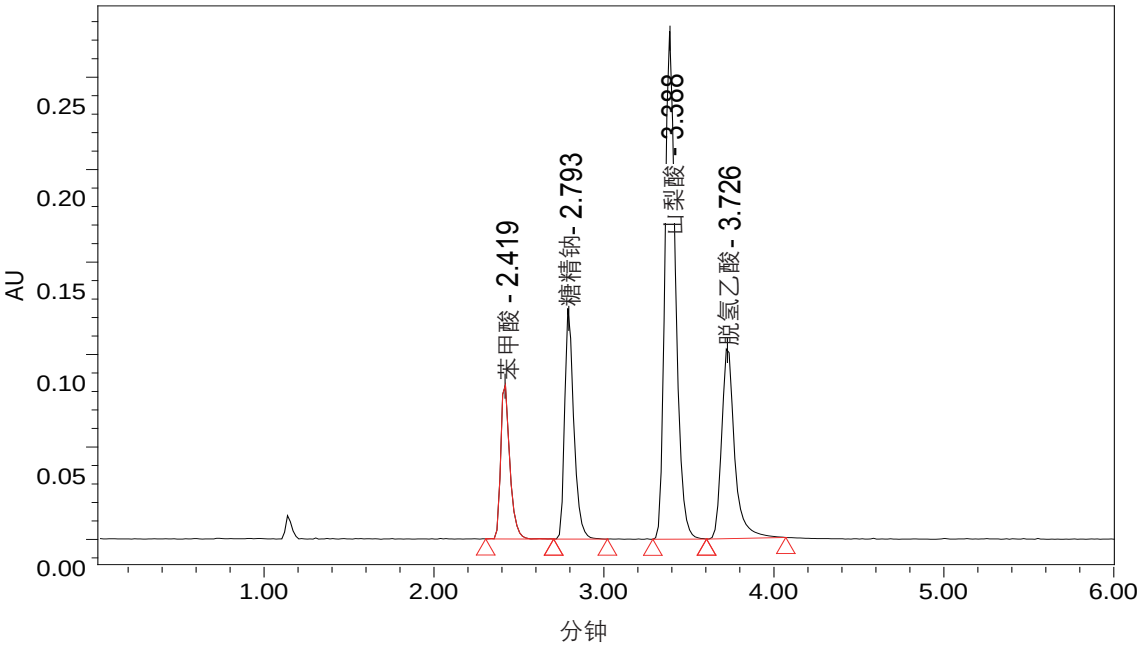

图1 10 μg/mLBA、SS、SA和DHA混标溶液色谱图（色谱柱1）

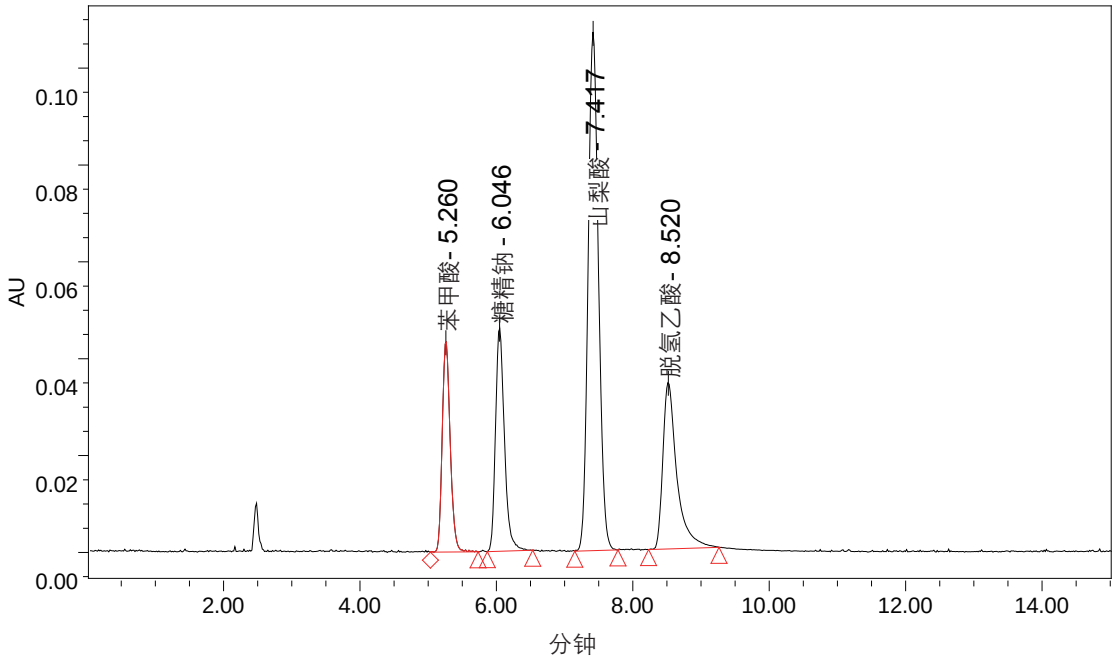

图2 10 μg/mLBA、SS、SA和DHA混标溶液色谱图（色谱柱2）

2.2 工作曲线与检测限

BA、SS、SA和DHA的回归方程分别为：Y=3.88e+004X-1.86e+004、Y=4.64e+004X+4.94e+003、Y=1.21e+005X+1.97e+003、 Y=5.77e+004X-1.89e+004， γ 均为0.999，在1～50μg/mL浓度范围内与峰面积呈良好的线性关系，将标准溶液逐级稀释至0.05、0.1、0.2μg/mL进样量为10μL时，BA、SA（0.05μg/mL），SS（0.1μg/mL），DHA（0.2μg/mL），峰高大于3倍噪音信号，即BA、SS、SA和DHA仪器最低检出量为：0.5、1.0、0.5、2.0ng，该方法BA、SS、SA和DHA最低检出限为0.5、1.0、0.5、2.0 mg/kg。

2.3 加标回收率与精密度

分别称取5.00g同一混合均匀的样品共八份，其中两份本底，六份分别准确加入5.00mL200μg/mLBA、SS、SA和DHA混合标准储备液做加标回收，按照1.3的方法操作，分别定容至50mL容量瓶,制备液相当于各组分均加标20μg/mL，测定空白、加标样品制备液浓度，计算加标回收率。样品本底为苯甲酸未检出、脱氢乙酸均未检出；山梨酸为50.27μg/mL，糖精钠为11.13μg/mL，BA、SS、SA和DHA的加标平均回收率依次为：100.3%、102.0%、99.6%、88.8%，相对标准偏差依次为：0.52%、0.93%、1.74%、0.99%（n=6），保留时间的相对标准偏差依次为：0.50%、0.54%、0.41%、0.63%（n=6），用BA、SS、SA和DHA标准溶液作外标定量，结果准确可靠，重现性良好。

2.4 样品中BA、SS、SA和DHA的检测结果

我国食品添加剂使用卫生标准(GB2760—2007)规定，酱腌菜中BA、SS、SA和DHA最大使用量分别为：0.5g/kg、0.15 g/kg 、0.5 g/kg、0.3 g/kg；月饼和糕点中BA、SS、SA和DHA最大使用量分别为：不得检出、0.15 g/kg、1.0 g/kg、0.5 g/kg。按照1．3的方法测定了60件酱腌菜、90件糕点中的BA、SS、SA和DHA，其中酱腌菜中的BA检出15件，结果为0.091～6.2g/kg，SS检出5件，结果为0.076～0.26 g/kg，SA 检出7件，结果为 0.013～3.2 g/kg，DHA检出8件，结果为0.027～0.48g/kg; 糕点中的BA均未检出，SS均未检出，SA检出7件，结果为0.024～0.62g/kg，DHA检出17件，结果为0.026～0.58g/kg。其它样品中的BA、SS、SA和DHA均为未检出，即BA<0.5mg/kg 、SA<0.5mg/kg、SS<1.0mg/kg 、DHA<2.0mg/kg。

3 讨论

3.1 现行国标方法GB/T23495-2009和GB/T23377-2009分别测定BA、SA、 SS和DHA，使用本方法可同时测定BA、SS、SA和DHA，而且比国标方法大大缩短分析时间，提高了灵敏度和工作效率，节约实验耗材。

3.2柱温提高到40℃，有利于提高灵敏度，缩短分析时间。BA、SS、SA和DHA各组份的最大吸收波长分别为223、210、254、230nm，选择230nm测定各组份可得到满意结果，适合于基层单位具有普通液相色谱仪检测工作的开展。

3.3 色谱柱1：Poroshell 120 EC-C18 （4.6 × 100 mm,2.7 μ m），色谱柱2：XDB-C18 (4.6 × 250 mm,5 μ m)，两只色谱柱均为美国Agilent公司提供，在1.3条件下，使用色谱柱1、2，BA、SS、SA和DHA各组份均能基线分离，由图1和图2可以看出，色谱柱1比色谱柱2灵敏度更高。其它色谱条件相同，色谱柱1比色谱柱2保留时间明显缩短。6分钟即可完成分析。因此，色谱柱1不仅有较高的灵敏度，而且还提高了工作效率，节约试剂。

4 小结

本法采用Poroshell 120色谱柱同时检测食品中的苯甲酸、山梨酸、脱氢乙酸、糖精钠更快速，更高效。因此，符合批量样品的检测要求，结果令人满意，适合于基层检测工作的开展。

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Profiling capsaicinoids in spicy foods and food products using the Agilent 1290 Infinity LC system and Agilent ZORBAX Poroshell 120 2.7 µm columns with UV/Vis diode array and fluorescence detection

Application Note

Food

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Abstract

The Agilent 1290 Infinity LC system has significant capabilities for a wide range of HPLC and UHPLC applications. It exhibits a broader power range (for example, the combination of pressure and flow capabilities), and the flexibility to operate a wide range of column dimensions and particle sizes than any other commercially available system. Advanced optical design in the diode array detector allows a wide dynamic range and high sensitivity, both of which are critical in the monitoring of natural and synthetic food flavorings.

The combined benefits are demonstrated by a separation of primary capsaicinoid components found in chili pods, sauces and spices. Capsaicin is the primary component recognized as the "hot" component of chili peppers, members of the capsicum family. The capsaicinoid class is comprised of eight or more compounds that variably contribute a heat component to flavor. Capsaicin or synthetic capsaicin (nonivamide) has also been used in topical creams and applications to relieve arthritis, itching, neuropathy and other ailments. The core structure is phenolic and primary variation is found in the hydrophobic alkyl chain.

The high pressure capability of the system allows the use of methanol, and acetonitrile, to explore the selectivity of the two solvents. Various column configurations, including porous 1.8 µm and superficially porous 2.7 µm materials, were evaluated. The structure of capsaicin is shown in Figure 1.

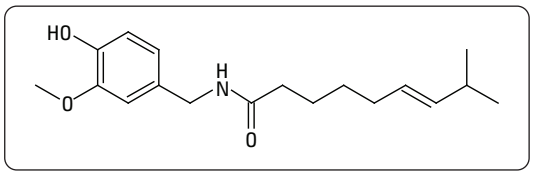


Figure 1
The structure of capsaicin ((E)-N- (4-hydroxy-3-methoxybenzyl) - 8-methylnon-6-enamide).

The distinctive spectrum of capsaicin allows one to use the diode array detector to interrogate peaks in capsaicinoid regions to determine if the compound is likely to be an active component. The UV ratio for 229 nm/280 nm is nominally 2.4 (Figure 2).

After optimization of the standard mixture, a number of extracted samples were chromatographed (Figure 3).

Cayenne and other pure chili spices and fresh pods were easily analyzed with UV detection. Low level samples such as American paprika and complex blends such as chili and curry powders were more likely to show interferences. The solution to this problem was the Agilent 1200 Series fluorescence detector (G1321A) as shown in Figure 4. The remarkable selectivity and sensitivity of the FLD minimized or eliminated non-capsaicinoid peaks from the analyte region.

Configuration

- Agilent 1290 Infinity Binary Pump with Integrated Vacuum Degasser (G4220A)
- Agilent 1290 Infinity Autosampler (G4226A)
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)
- Agilent 1200 Series Diode Array Detector (G1315C)

Conclusion

The flexible solvent and column selection features, and high pressure capability, of the system allows one to use highly efficient columns to rapidly develop separations with remarkable resolution while conserving solvent over the use of 4.6 mm id columns. The added selectivity of fluorescence detection provides solutions to unexpected interferences that would otherwise have required redevelopment of the separation method.

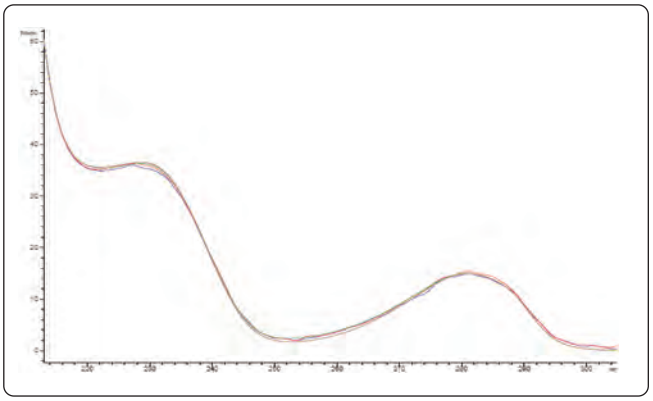


Figure 2
Overlaid extracted UV spectra of capsaicinoid compounds in chili.

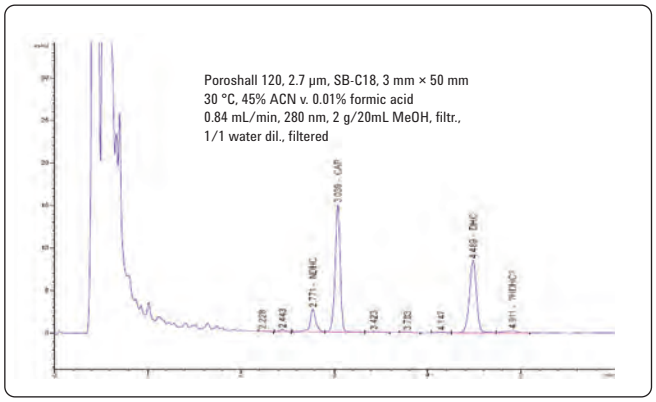
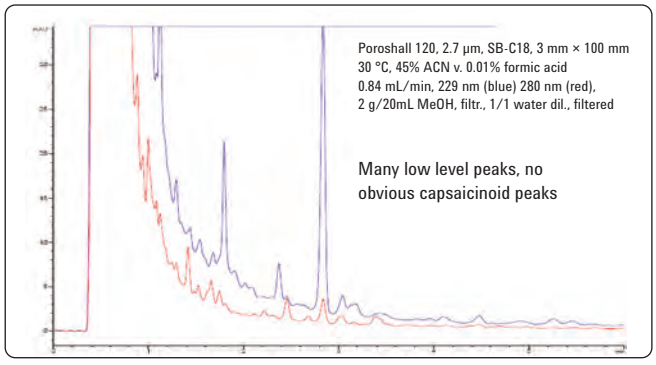


Figure 3
Separation of cayenne dried ground spice (CAP- capsaicin, NDHC – nordihydrocapsaicin, DHC – dihydrocapsaicin).



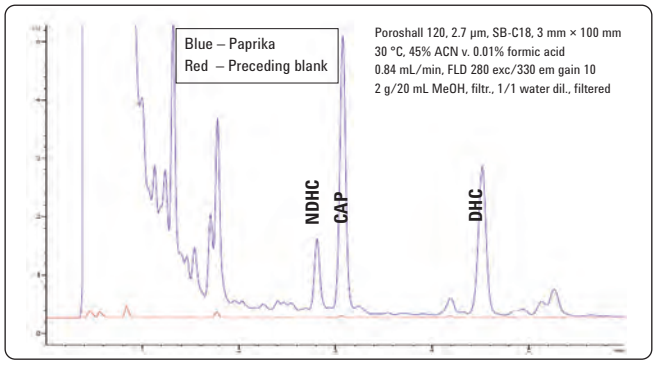


Figure 4
Comparison of UV and fluorescence signals for American Paprika extract (two frames).

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Rapid Tea Analysis on Poroshell 120 SB-C18 with LC/MS

Application Note

Food and Beverage

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Abstract

An analysis of ten compounds (9 catechins + caffeine) commonly found in green tea demonstrates similar selectivity on Agilent ZORBAX SB-C18 and Agilent Poroshell 120 SB-C18. The 1.4 min gradient analysis on Poroshell 120 generates linear calibration curves for all ten compounds through LC/MS. Several bottled and brewed tea samples are quantified and compared. An unfiltered, undiluted brewed green tea sample demonstrates a lifetime of more than 1500 injections on the Poroshell 120 column with a dirty sample at high pressure.

Introduction

Polyphenolic compounds reduce the risk of heart disease, prevent cancer, and combat other illnesses. A popular source of polyphenols is fresh tea leaves or green tea, which contain high levels of catechins. Catechins affect the color and flavor of tea, contributing to the characteristic bitterness associated with tea [1]. Of the catechins found in tea, epigallocatechin gallate (EGCG) is given attention, as it is the most abundant of the polyphenolic compounds found in tea extract [2].

While all teas originate from the same plant, Camellia sinensis, different processing methods produce varied teas. The amount of fermentation (oxidation) that a tea leaf undergoes following harvesting dictates what type of tea the leaf becomes. Tea leaves begin to wilt and oxidize quickly, if not dried soon after harvesting. During this process, the leaves darken as chlorophyll breaks down and tannins are released. The oxidation process is stopped at a controlled time by heating the leaves and deactivating the enzymes responsible for breaking down chlorophyll. Black tea is fully oxidized, oolong tea is semi-oxidized and green tea is un-oxidized [3,4]. Because oxidation lowers the catechin levels, green tea provides the highest quantity of catechin antioxidants per serving, while black tea delivers the least.

In this application note, an HPLC method for catechins in tea developed by Yoshida et al [5] on an Agilent ZORBAX SB-C18 column is transferred to a similar dimension Agilent Poroshell 120 SB-C18 column to demonstrate similar selectivity. The method is optimized for LC/MS. Calibrations curves are generated and tea samples, both bottled and brewed, are analyzed for comparison. Additionally, a lifetime study using an undiluted, unfiltered brewed green tea sample demonstrates the benefits of the Poroshell column's large 2-µm frits with dirty samples.

While a tea analysis via HPLC is not novel, this method shows Poroshell 120's effectiveness in analyzing other natural product samples. The Poroshell 120 column is shown to separate a group of 10 closely related compounds, including four epimer pairs using a representative sample that is affordable and easily obtainable.

Experimental

An Agilent 1200 Series Rapid Resolution LC (RRLC) system with an Agilent 6410 Triple Quadrupole Mass Spectrometer (QQQ) was used for this work:

- G1312B Binary Pump SL with mobile phase A: various modifiers in H_2O (0.1% H_3PO_4, 0.2% HCOOH, 0.2% CH_3COOH, 0.02% CF_3COOH, 10 mM CH_3COONH_4 pH 3.6-5.6, and 10 mM $HCOONH_4$ pH 3-4.5), and B: CH_3CN. Gradient was 10% B at t_0, ramp to 15% B, and then ramp to 27% B. Gradient times vary depending on column dimensions and flow rate. See Table 1.
- G1367C Automatic Liquid Sampler (ALS) SL, injection volumes are dependent upon specific method parameters. See Table 1.
- G1316B Thermostated Column Compartment (TCC) SL with temperature controlled at 40 °C.
- G6410A QQQ Mass Spectrometer with MS Source: electrospray AP-ESI, drying gas temperature and flow: 350 °C, 10 L/min, nebulizer gas pressure: 50 psi, capillary voltage: ± 3500 V, in SIM mode, *m/z* values shown in Figure 1. Catechins are monitored in negative mode, while caffeine is monitored in positive mode.
- MassHunter versions B.02.01, B.02.00 and B.03.01 were used for data acquisition, qualitative and quantitative analyses respectively.

Table 1. Various Method Parameters for Catechin Analysis

	Agilent ZORBAX SB-C18, 4.6 × 150 mm, 5 µm (p/n 883975-902)	**Agilent ZORBAX RRHT SB-C18, 4.6 × 50 mm, 1.8 µm (p/n 827975-902)**	**Agilent Poroshell 120 SB-C18, 4.6 × 50 mm, 2.7 µm (p/n 689975-902)**	**Agilent ZORBAX RRHT SB-C18, 2.1 × 50 mm, 1.8 µm (p/n 827700-902)**	**Agilent Poroshell 120 SB-C18, 2.1 × 50 mm, 2.7 µm (p/n 689775-902)**	**Agilent Poroshell 120 SB-C18, 2.1 × 50 mm, 2.7 µm (p/n 689775-902)**	**Agilent Poroshell 120 SB-C18, 2.1 × 50 mm, 2.7 µm (p/n 689775-902)**	**Agilent Poroshell 120 SB-C18, 2.1 × 100 mm, 2.7 µm (p/n 685775-902)**
Flow rate (mL/min)	1.00	1.00	1.00	1.00	1.00	1.25	1.50	0.83
Mobile phase A	0.1% H_3PO_4 in H_2O	0.1% H_3PO_4 in H_2O	0.1% H_3PO_4 in H_2O	0.1% H_3PO_4 in H_2O	Various additives in H_2O	0.2% CH_3COOH in H_2O	0.2% CH_3COOH in H_2O	0.2% HCOOH in H_2O
Mobile phase B	CH_3CN	CH_3CN	CH_3CN	CH_3CN	CH_3CN	CH_3CN	CH_3CN	0.2% HCOOH in CH_3CN
10% B	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15% B	7.50	2.50	2.50	0.50	0.50	0.42	0.36	1.25
27% B	15.00	5.00	5.00	1.00	1.00	0.83	0.71	2.50
Stop time (min)	15.00	5.00	5.00	1.40	1.40	1.20	0.95	4.00 (includes re-equilibration)
Post run time (min)	10.00	3.00	3.00	1.00	1.00	0.80	0.60	n/a
Overall cycle time (min)	25.00	8.00	8.00	2.40	2.40	2.00	1.55	4.00
TCC temperature (°C)	40	40	40	40	40	40	40	40
Injection volume (µL)	15.0	5.0	5.0	1.0	1.0 (LC/UV), 1.5 (LC/MS)	1.5	1.5	2.0
Sample concentration (mg/mL)	0.03	0.03	0.03	0.03	0.03 (LC/UV), 0.003 (LC/MS)	0.003	0.003	n/a
System pressure (bar)	84	169	117	575	380 (LC/UV), 425 (LC/MS)	505	585	540

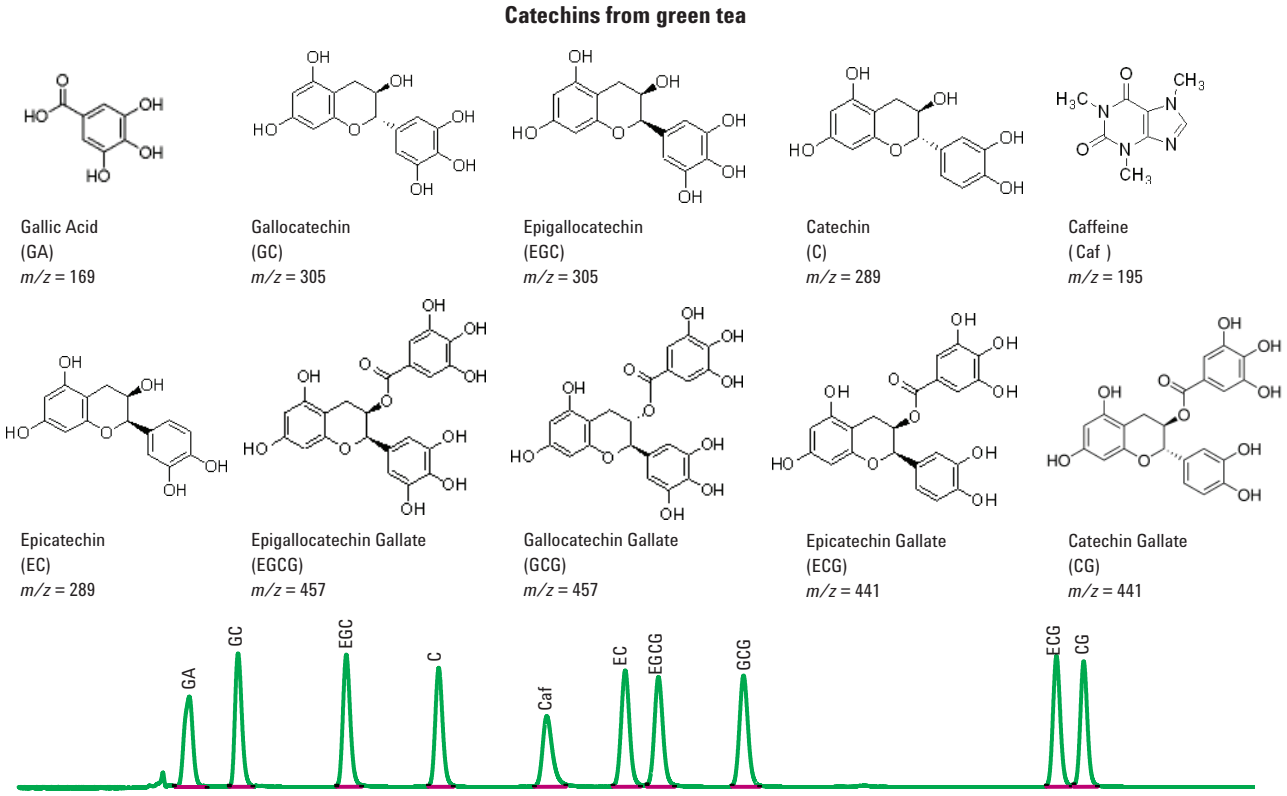


Figure 1. Compounds of interest, with elution order are shown on an Agilent ZORBAX SB-C18 with H_3PO_4 mobile phase. (Note: Selectivity may change slightly in subsequent chromatograms, but elution order remains constant.)

Six Agilent columns were used in this work:

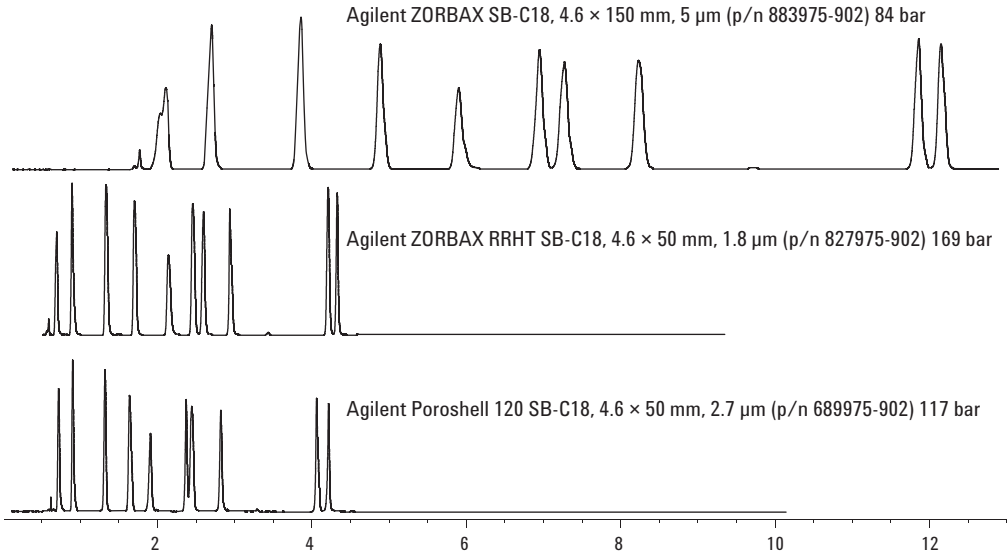
- Agilent ZORBAX SB-C18, 4.6 × 150 mm, 5 µm
  p/n 883975-902
- Agilent ZORBAX RRHT SB-C18, 4.6 × 50 mm, 1.8 µm
  p/n 827975-902
- Agilent Poroshell 120 SB-C18, 4.6 × 50 mm, 2.7 µm
  p/n 689975-902
- Agilent ZORBAX RRHT SB-C18, 2.1 × 50 mm, 1.8 µm
  p/n 827700-902
- Agilent Poroshell 120 SB-C18, 2.1 × 50 mm, 2.7 µm
  p/n 689775-902
- Agilent Poroshell 120 SB-C18, 2.1 × 100 mm, 2.7 µm
  p/n 685775-902

The compounds of interest are shown in Figure 1, with a chromatogram illustrating elution order. All analytes were purchased as dry powders from Sigma Aldrich (Bellefont, PA). Individual standards of gallic acid, epigallocatechin, catechin, caffeine, and epigallocatechin gallate were each prepared in H_2O at 1 mg/mL. Individual standards of gallocatechin, epicatechin, gallocatechin gallate, epicatechin gallate, and catechin gallate were each prepared in CH_3CN/H_2O (1:1) at 0.5 mg/mL. A composite sample was prepared by mixing 1 part each of the 1 mg/mL standards and 2 parts each of the 0.5 mg/mL standards, yielding 0.03 mg/mL of each analyte. Dilutions of this composite sample were prepared as necessary with H_2O. Tea samples were purchased locally, with the exception of bottled sample A, which was shipped from a colleague in Japan. Tea samples for quantitation (both bottled and brewed) were diluted 1:10 with H_2O prior to injection. The brewed green tea sample used for the lifetime study was not diluted or filtered prior to injection. Additionally, acetonitrile, phosphoric acid, formic acid, acetic acid, trifluoroacetic acid, ammonium acetate, and ammonium formate were also purchased from Sigma Aldrich (Bellefont, PA). Water used was 18 M-Ω Milli-Q water (Bedford, MA).

Results and Discussion

Previous work by T. Yoshida et al [5] shows a catechin analysis on an Agilent ZORBAX SB-C18, 4.6 × 150-mm, 5-µm column in 15 min scaled to an Agilent ZORBAX Rapid Resolution High Throughput SB-C18, 4.6 × 50-mm, 1.8-µm column in 5 min. Added to this work is an Agilent Poroshell 120 SB-C18 column for comparison. Figure 2 shows the time saved using shorter HPLC columns with smaller particle sizes, while maintaining resolution.

The method is scaled further to a 2.1 × 50 mm column in just over 1 min. The smaller column id allows the same analysis to run with lower flow rates, which are more suitable for MS work. The selectivity between the ZORBAX SB-C18 and Poroshell 120 SB-C18 is similar to allow for easy method transfer, as shown in Figure 3. System back pressure is a noticeable difference between the 1.8-µm ZORBAX column and the 2.7-µm Poroshell column. The larger superficially porous particles in the Poroshell column generate significantly less pressure than the smaller totally porous particles in the ZORBAX column. The Poroshell particles achieve similar performance due to a short mass transfer distance through the porous shell and substantially narrower particle size distribution as compared to the totally porous sub-2 µm material. In this case, the difference in pressure is enough to dictate whether a 400 or 600 bar instrument can be used.



A	0.1% H_3PO_4 in H_2O
B	CH_3CN
1 mL/min	
40 °C	
Sig = 210,4 nm, Ref = Off	
2-µL, 3-mm micro flow cell (p/n G1315-60024)	
Sample:	0.03 mg/mL each of GA, GC, EGC, C, Caf, EC, EGCG, GCG, ECG, CG in H_2O/CH_3CN

4.6 × 150 mm

Time (min)	0.00	7.50	15.00
%B	10	15	27

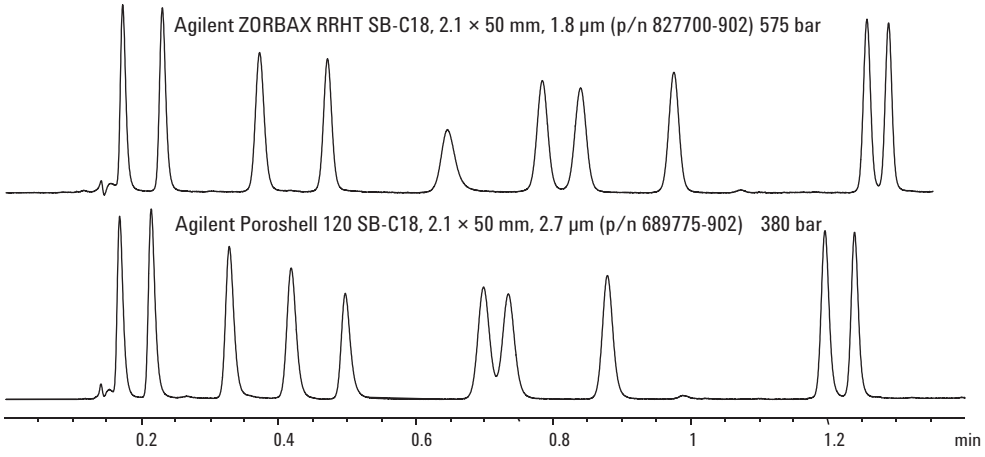
15 µL injection

4.6 × 50 mm

Time (min)	0.00	2.50	5.00
%B	10	15	27

5 µL injection

Figure 2. Original 150-mm, 5-µm catechin method scaled to an Agilent ZORBAX SB-C18, 50-mm, 1.8-µm and to a 50-mm, superficially porous Agilent Poroshell 120 SB-C18, 2.7-µm.



A	0.1% H_3PO_4 in H_2O
B	CH_3CN
1 mL/min	

Time (min)	0.00	0.50	1.00
%B	10	15	27

40 °C	
Sig = 210,4 nm, Ref = Off	
2-µL, 3-mm micro flow cell (p/n G1315-60024)	
Sample:	1 µL injection of 0.03 mg/mL each of GA, GC, EGC, C, Caf, EC, EGCG, GCG, ECG, CG in H_2O/CH_3CN

Figure 3. Catechin analysis is transferred to 2.1-mm id columns for use with LC/MS, analysis time is further reduced by maintaining 1 mL/min flow rate from original 4.6 × 150-mm method and scaling gradient times according to column volume.

The 2.1 mm id columns are suited for use with LC/MS due to the lower flow rates used. The original phosphoric acid mobile phase, however, is not compatible with the LC/MS system. Figure 4 shows several MS-friendly mobile phases that were screened for use with this catechin analysis. In addition to the results shown in Figure 4, a selection of 10 mM ammonium formate buffers were also screened from pH 3-4.5, the resulting chromatograms were nearly identical to the ammonium acetate data. Overall, the selectivity remained constant throughout this screening process. Consequently, the optimal mobile phase was selected based on signal strength of the analytes. All chromatograms in Figure 4 are shown on the same scale. Significant ion suppression is present with the buffers prepared from ammonium salts, as well as with the trifluoroacetic acid mobile phase. The two best contenders were formic acid and acetic acid, with acetic acid producing a slightly more intense signal for all compounds. It should be noted that the negative scans are shown as representative chromatograms in Figure 4, as the positive scans appeared to be less effected by ion suppression; however, the positive scans for caffeine still followed the same pattern.

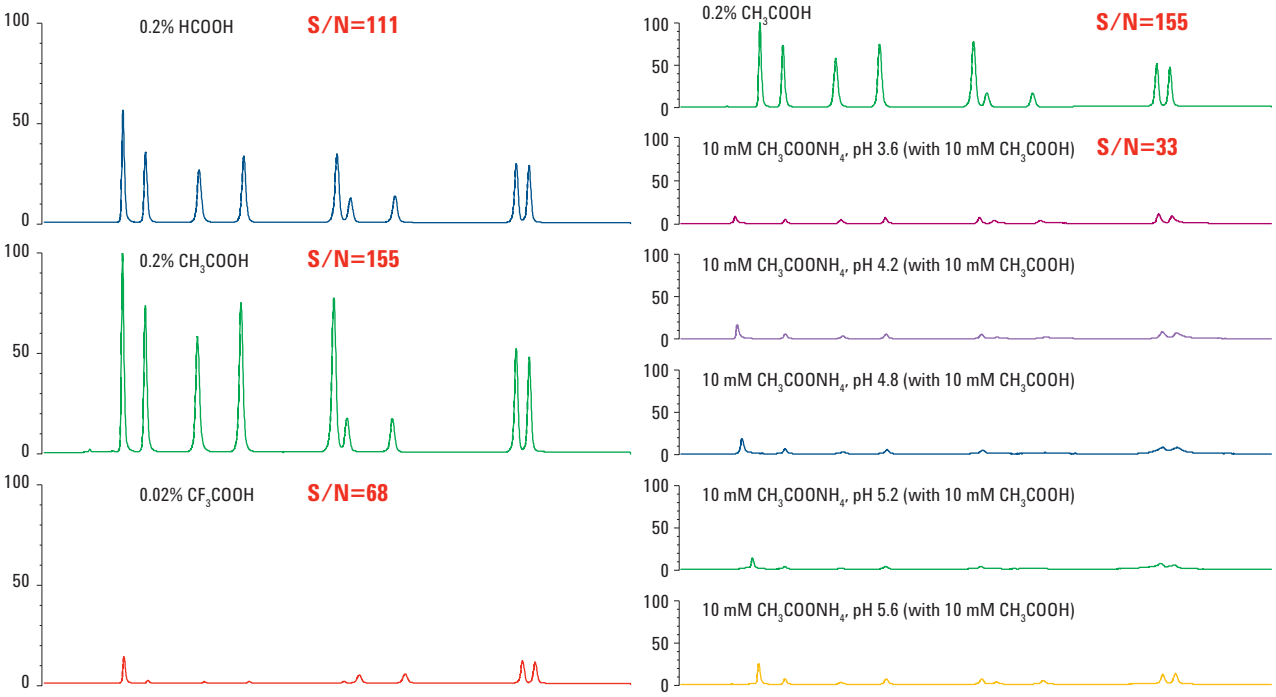


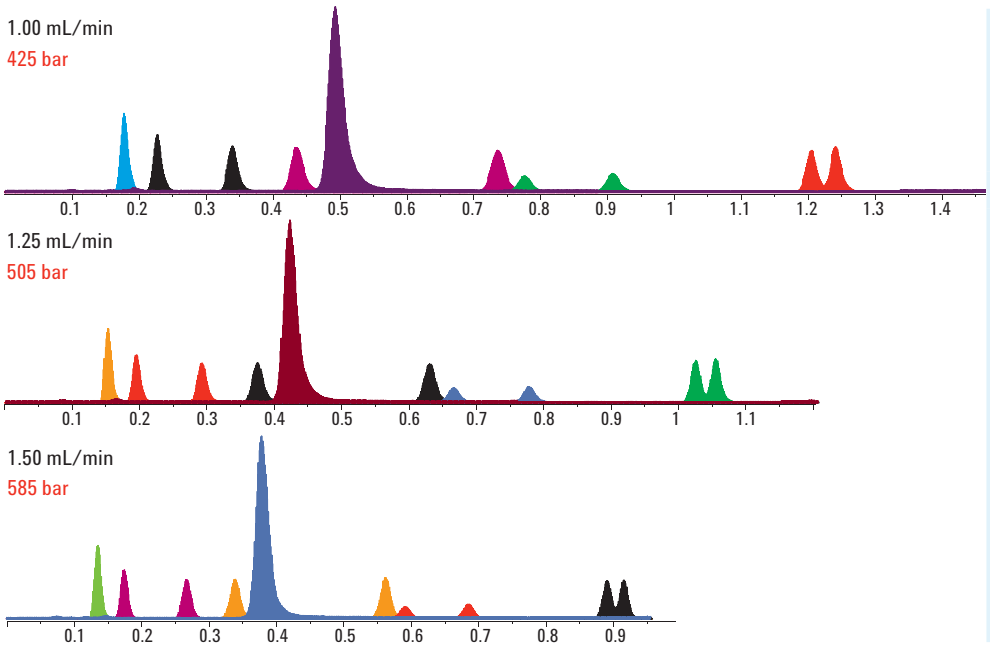
Figure 4. Various MS-friendly mobile phases are screened in order to find a replacement for the H_3PO_4 used in the original LC/UV method (Note: Positive SIM chromatograms of caffeine are not shown, because it is significantly less affected by ion suppression as compared to the catechins.)

EIC overlays in Figure 5 show that because of the lower back pressure generated by the Agilent Poroshell column, more rapid analyses are possible in under 600 bar. A 15 minute method that started out on a 150-mm column, can be reduced to less than 1 minute analysis time on a 50-mm Poroshell 120 column, while preserving the selectivity of the original method. Comparing Figures 3 and 5 shows that the same method (1 mL/min) run on the same column (Agilent Poroshell 120 SB-C18, 2.1 × 50-mm, 2.7-µm) generated notably different back pressures. The difference in back pressure is primarily due to a long piece of small, 0.12 mm, id transfer tubing used to connect the HPLC to the MS. Larger id transfer tubing was not considered for this application as band broadening is likely to occur and would reduce the resolution of this finely tuned, rapid analysis.

Calibration curves for each of the 10 compounds of interest were constructed with a minimum of six points (maximum of 10), while each standard was run in triplicate. Linear regression and correlation coefficient data are shown in Table 2 for all 10 analytes. All curves exhibit a high degree of linearity up to a maximum analyzed amount of 10 ng on column (Poroshell 120 SB-C18, 2.1 × 50 mm). All tea samples were diluted 1:10 with water prior to injection in attempt to not exceed the highest concentration calibration standard. Only one compound, EGCG, in the brewed green tea sample exceeded the maximum concentration after the 1:10 dilution; the concentration of EGCG was extrapolated from the linear regression equation found in Table 2.

Table 2. Calibration data for Catechins and Caffeine; Minimum Six Point Calibration Curve with all Standards Run in Triplicate

	Linear regression line	Correlation coefficient, R^2
Gallic acid	y = 0.466 x	0.995
Gallocatechin	y = 0.407 x	0.996
Epigallocatechin	y = 0.355 x	0.996
Catechin	y = 0.601 x	0.996
Caffeine	y = 3.439 x	0.995
Epicatechin	y = 0.638 x	0.995
Epigallocatechin gallate	y = 0.153 x	0.998
Gallocatechin gallate	y = 0.183 x	0.996
Epicatechin gallate	y = 0.396 x	0.998
Catechin gallate	y = 0.371 x	0.996



A	0.2% CH_3COOH in H_2O		
B	CH_3CN		
40 °C			
Column	Agilent Poroshell 120 SB-C18, 2.1 × 50 mm, 2.7 µm		
Source	350 °C, 10 L/min, 50 psi, -/+3500 V		
Acquisition	SIM- (169, 305, 289, 457, 441), SIM+ (195)		
Sample	1.5 µL injection of 0.003 mg/mL each of GA, GC, EGC, C, Caf, EC, EGCG, GCG, ECG, CG in H_2O/CH_3CN		
1.00 mL/min			
Time (min)	0.00	0.50	1.00
%B	10	15	27
1.25 mL/min			
Time (min)	0.00	0.42	0.83
%B	10	15	27
1.50 mL/min			
Time (min)	0.00	0.36	0.71
%B	10	15	27

Figure 5. Catechin analysis is further sped up by increasing flow rate and scaling the gradient according to column volume.

A selection of bottled teas was analyzed, ingredient lists and country of origin for each tea sample are shown in Table 3, and quantitative results are shown in Figure 6. Tea sample A is a Japanese tea that has been stored unopened at room temperature for approximately 3 years. Only gallic acid and caffeine were found in sample A; it is likely that additional catechins were present in this tea originally, but have degraded over time. Bottled tea samples B and D are different brands of Japanese green teas. Both report the same ingredients on their respective labels, and contain approximately the same level of all catechins analyzed in this method. Tea sample C is also a green tea, with the same ingredients reported as B and D, however it is a Taiwanese tea. Compared to the two Japanese green teas, sample C shows a higher concentration of epicatechin gallate and catechin gallate, but a lower concentration of the other eight analytes. Bottled tea samples E and F are Japanese tea blends, both of which contain some amount of green tea according to their labels. Tea E lists barley as its main ingredient, consequently the caffeine and catechin concentrations are all substantially less than the other tea samples. Tea F is an oolong tea blend, which shows a slightly different composition than the green teas. Compared to the two pure Japanese green tea samples B and D, the oolong blend contains more gallic acid and caffeine, similar amounts of epicatechin gallate and catechin gallate, but lower concentrations of the remaining catechin compounds.

Table 3. Ingredient Lists and Country of Origin for Bottled Tea Samples

Bottled tea sample	Country of origin	Ingredients
A	Japan	(unknown)
B	Japan	purified water, green tea, ascorbic acid
C	Taiwan	mineral water, green tea, vitamin C, natural flavor
D	Japan	water, green tea, ascorbic acid
E	Japan	pearl barley, brown rice, sprouted rice, green tea, barley, houttuynia cordata, chickory, quinoa, angelica keiskei, vitamin C
F	Japan	oolong tea, puaru tea, green tea, brown tea, chickory, soybean, sesame, vitamin C

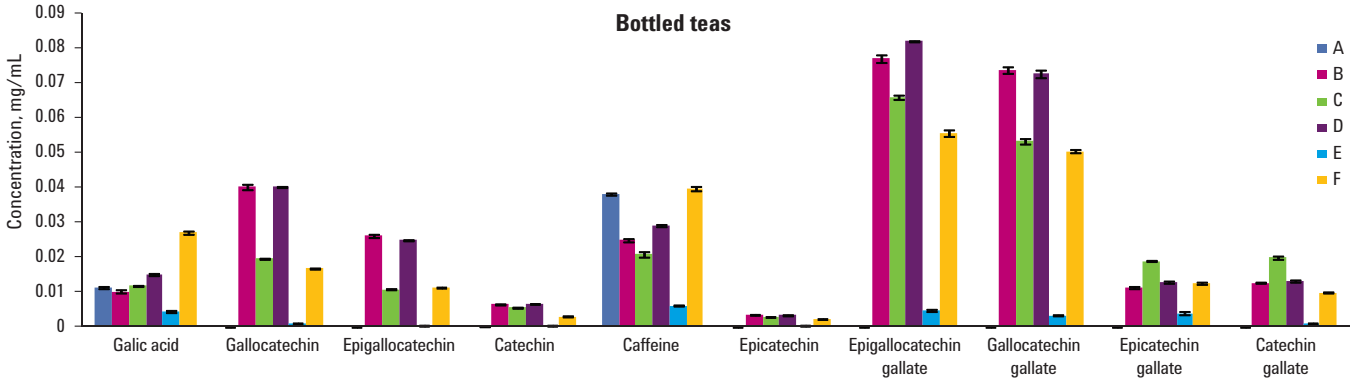


Figure 6. Six bottled tea samples analyzed; 3 green teas (2 Japanese [B,D], 1 Taiwanese [C]), 1 barley tea blend (E), 1 oolong tea blend (F), and 1 unknown (A).

For comparison to the bottled tea samples shown in Figure 6, Figures 7 and 8 show freshly brewed green and black tea samples respectively. Both brewed tea samples show peak concentrations of most compounds when the tea bag is allowed to steep for six to 10 minutes. After this optimal steep time, compounds begin to degrade in both cases. Most notably is epigallocatehin gallate, which degrades by more than 50% of the maximum concentration in 60 minutes. Also interesting regarding epigallocatechin gallate is how much more concentrated it is with the brewed green tea sample than with the bottled green tea samples.

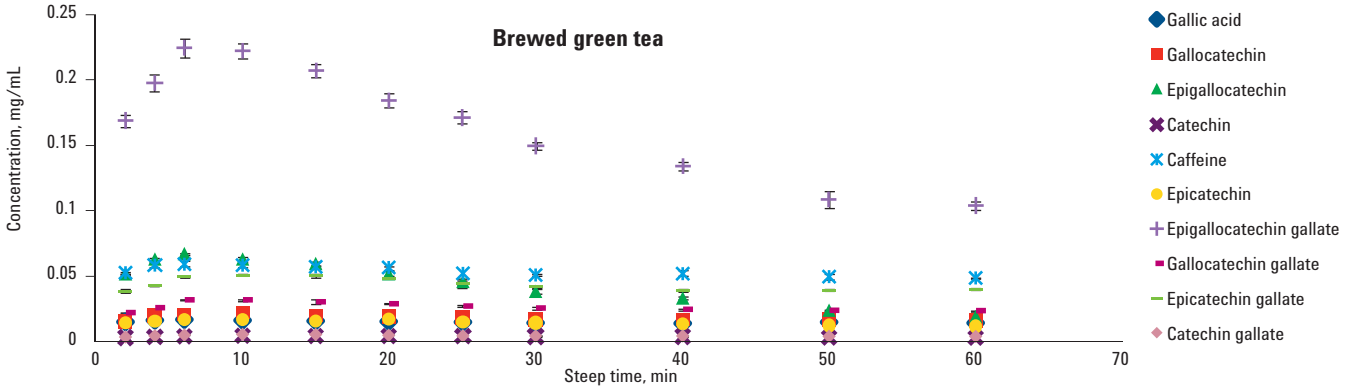


Figure 7. Freshly brewed green tea sample; 1 commercial tea bag steeped in 6 oz initially boiling water, with samples taken over time.

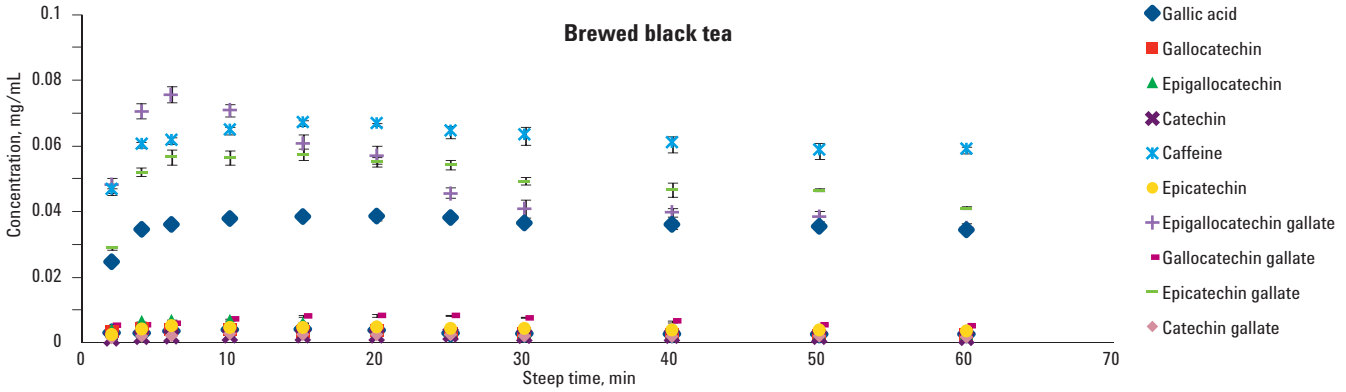


Figure 8. Freshly brewed black tea sample; 1 commercial tea bag steeped in 6 oz initially boiling water, with samples taken over time.

Figure 9 shows a lifetime study of more than 1500 injections of a dirty sample at high pressure (550 bar) without gaining pressure or increasing peak width. The green tea sample was brewed from a commercial tea bag in 6 oz of initially boiling water for six minutes, and then injected directly into the HPLC without any filtration or dilution. The sample was replaced twice daily, as compound degradation was prevalent for the catechins (caffeine was relatively stable). The 2-µm frit found in the Agilent Poroshell 120 SB-C18 (2.1 × 100 mm, 2.7 µm) column is ideal for dirty samples, as it resists plugging more than the 0.5-µm frit found in sub-2 µm columns.

Conclusion

An existing HPLC method for the analysis of catechins in green tea was successfully transferred from totally porous Agilent ZORBAX SB-C18, 1.8-µm to superficially porous Agilent Poroshell 120 SB-C18, 2.7-µm. The selectivity of the two columns is similar enough that no method adjustments were necessary to maintain the 10 compound separation. Highly linear calibration curves were constructed for all compounds, and various bottled and freshly brewed teas were quantified and compared for catechin content. The larger 2-µm frit in the Poroshell 120 column was also exploited in a lifetime test, showing more than 1500 injections of a dirty sample at high pressure without negative effects on chromatography.

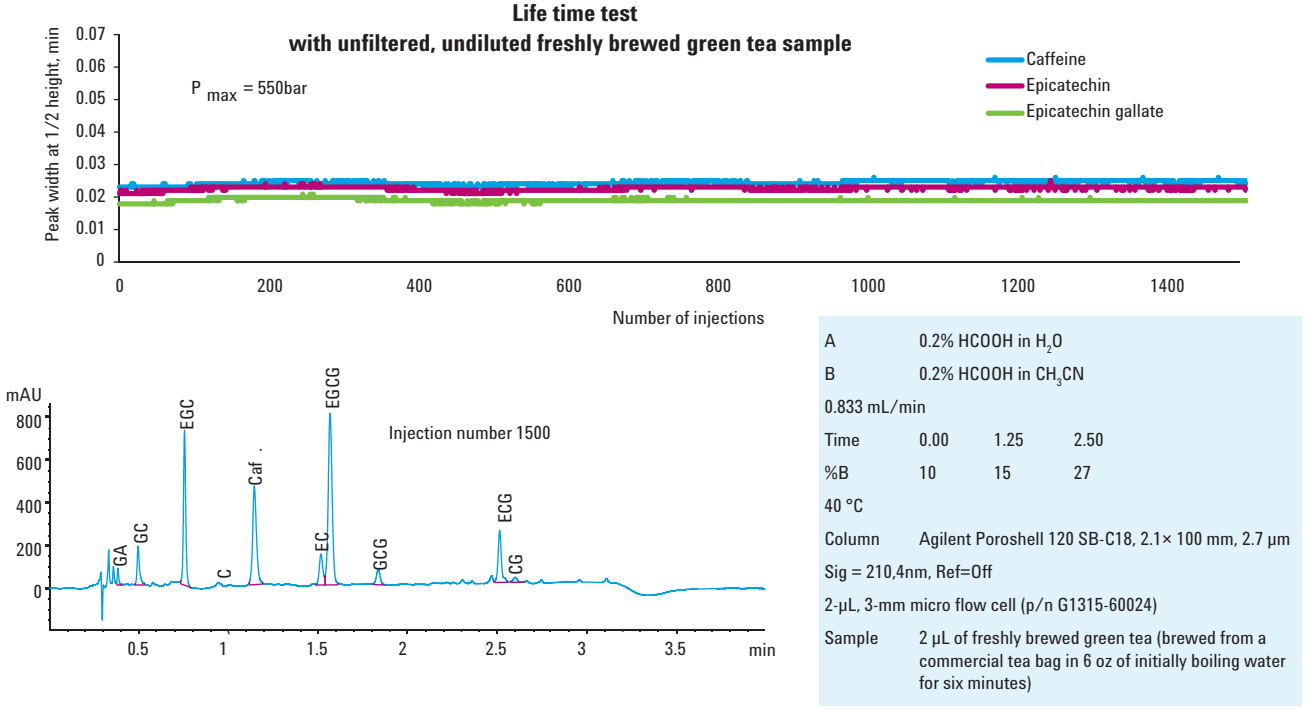


Figure 9. Lifetime study of 1500 injections of an unfiltered, undiluted, freshly brewed green tea sample showing no peak broadening or increase in pressure.

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使用安捷伦 Poroshell 120 EC-C18 表面多孔色谱柱对食品和饮料添加剂进行快速低压分析

Application Note

Food and Beverage

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Abstract

本文实验通过安捷伦 Poroshell 120 EC-C18 色谱柱（3.0 mm×100 mm，2.7 µm），使用乙酸铵/乙腈梯度洗脱，对 11 种非营养型食品和饮料添加剂进行了分析，耗时不到 3 分钟。此分析的最大压力低于 400 bar，因此本方法适用于标准的 HPLC 仪器。安捷伦 Poroshell 120 EC-C18 色谱柱的选择性和效率与安捷伦 ZORBAX Eclipse Plus C18 色谱柱（3.0 mm×100 mm，1.8 µm 和 4.6 mm×250 mm，5 µm）类似。虽然 1.8 µm 安捷伦 ZORBAX Eclipse Plus 色谱柱获得预期分离效果所用的时间与安捷伦 Poroshell 120 相同，但它的最大压力超过了 400 bar，因此必须采用高压 HPLC。反之，5 µm 安捷伦 ZORBAX Eclipse Plus 色谱柱可以在低于 400 bar 的压力下进行同一分析，但需花费相当长的时间才能完成分离。本应用摘要阐明了在 HPLC 系统上，将使用安捷伦 ZORBAX Eclipse Plus C18 色谱柱的旧式方法转移到新型安捷伦 Poroshell 120 EC-C18 色谱柱上，在不改变效率和选择性的条件下还能够节省大量时间。

Introduction

Conventional 400-bar HPLC systems are found in many laboratories. With the introduction of UHPLC, there is a desire to move towards sub-2-µm particles. HPLC columns packed with sub-2-µm particles provide superior resolution and decreased analysis time over traditional 5-µm particles. However this also increases system back pressure. For analysts that wish to translate their current methods to a sub-2-µm method but cannot afford a high pressure LC, columns packed with superficially porous particles may be the answer. Columns like the Agilent Poroshell 120 column offer resolution and speed similar to columns packed with 1.8-µm particles, without generating high back pressure.

The high efficiency of Agilent Poroshell 120 particles is similar to sub-2-µm totally porous particles. This is due to short mass transfer distance and substantially narrower particle size distribution. The larger 2.7-µm Agilent Poroshell 120 particles generate very low back pressure, about 40% to 60% of the back pressure generated by sub-2 µm totally porous particles. This allows the columns to run faster flow rates without exceeding HPLC pressures. Agilent Poroshell 120 columns with 2-µm frits are more forgiving with dirty samples than 1.8-µm columns, providing a more seamless method transfer from traditional 5-µm columns [1-3].

This application note demonstrates the transfer of established methods using longer columns packed with 5-µm particles to a UHPLC-like method using superficially porous particles packed in shorter columns, while keeping the system back pressure below 400 bar. The benefits are a decrease in sample and mobile phase consumption, significant time savings, and the selectivity and efficiency of a sub-2-µm analysis on virtually any HPLC system.

A group of 11 non-nutritive food and beverage additives is used to demonstrate this method translation. These compounds include preservatives, artificial sweeteners, an energy supplement and a flavoring agent. While these compounds are not harmful in appropriate amounts, they can cause sensitization and allergic reactions after excessive exposure [4]. Therefore, detection and quantification of these additive compounds are important.

Experimental

An Agilent 1200 Rapid Resolution LC (RRLC) system was used for this work:

- G1312B Binary Pump SL with mobile phase A: 20 mM ammonium acetate (pH 4.80) and B: acetonitrile. Gradient was 14% B at t_0, ramp to 52% B. Gradient times vary depending on column dimensions and flow rate, see Table 1.
- G1367C Automatic Liquid Sampler (ALS) SL. Injection volume was 11.8 and 2.0 µL for the 4.6 mm × 250 mm and 3.0 mm × 100 mm columns respectively.
- G1316B Thermostatted Column Compartment (TCC) SL with temperature set to 30 °C.
- G1315C Diode Array Detector (DAD) SL with the signal set to 230, 4 nm and reference set to 360, 100 nm, using a G1315-60024 micro flow cell (3-mm path, 2-µL volume).
- ChemStation version B.04.01 (491) was used to control the HPLC and process the data.

Table 1. HPLC Method Parameters for Various Columns

Column	Flow rate (mL/min)	Gradient time (min)	Stop time (min)	Post run time (min)
Agilent ZORBAX Eclipse Plus C18 4.6 mm × 250 mm, 5.0 µm	1.000	12.00	13.10	7.00
Agilent ZORBAX Eclipse Plus C18 3.0 mm × 100 mm, 3.5 µm	0.638	3.00	3.50	2.00
Agilent ZORBAX Eclipse Plus C18 3.0 mm × 100 mm, 1.8 µm	0.851	2.10	2.60	1.80
Agilent Poroshell 120 EC-C18 3.0 mm × 100 mm, 2.7 µm	0.851	2.10	2.60	1.80

Four Agilent columns were used in this work:

- Agilent Poroshell 120 EC-C18, 3.0 mm × 100 mm, 2.7 µm

  (p/n 695975-302)

- Agilent ZORBAX Eclipse Plus C18, 3.0 mm × 100 mm, 1.8 µm

  (p/n 959964-302)

- Agilent ZORBAX Eclipse Plus C18, 3.0 mm × 100 mm, 3.5 µm

  (p/n 959961-302)

- Agilent ZORBAX Eclipse Plus C18, 4.6 mm × 250 mm, 5 µm

  (p/n 959990-902)

The compounds of interest are shown in Figure 1, with their respective structure, pKa value and additive function. Compounds were dissolved in water at 1 mg/mL. Equal aliquots were combined to produce a mixed sample. Compounds were purchased from Sigma Aldrich (Bellefonte, PA). Additionally, acetonitrile and ammonium acetate were purchased from Sigma Aldrich. Water used was 18 MΩ Milli-Q water (Bedford, MA).

Results and Discussion

Figure 2 shows the separation of 11 food and beverage additives on a traditional Agilent ZORBAX Eclipse Plus C18, 4.6 mm × 250 mm, 5 µm column in just over 13 minutes. Figure 3 shows the same separation scaled to an Agilent ZORBAX Eclipse Plus C18, 3.0 mm × 100 mm, 3.5 µm with a

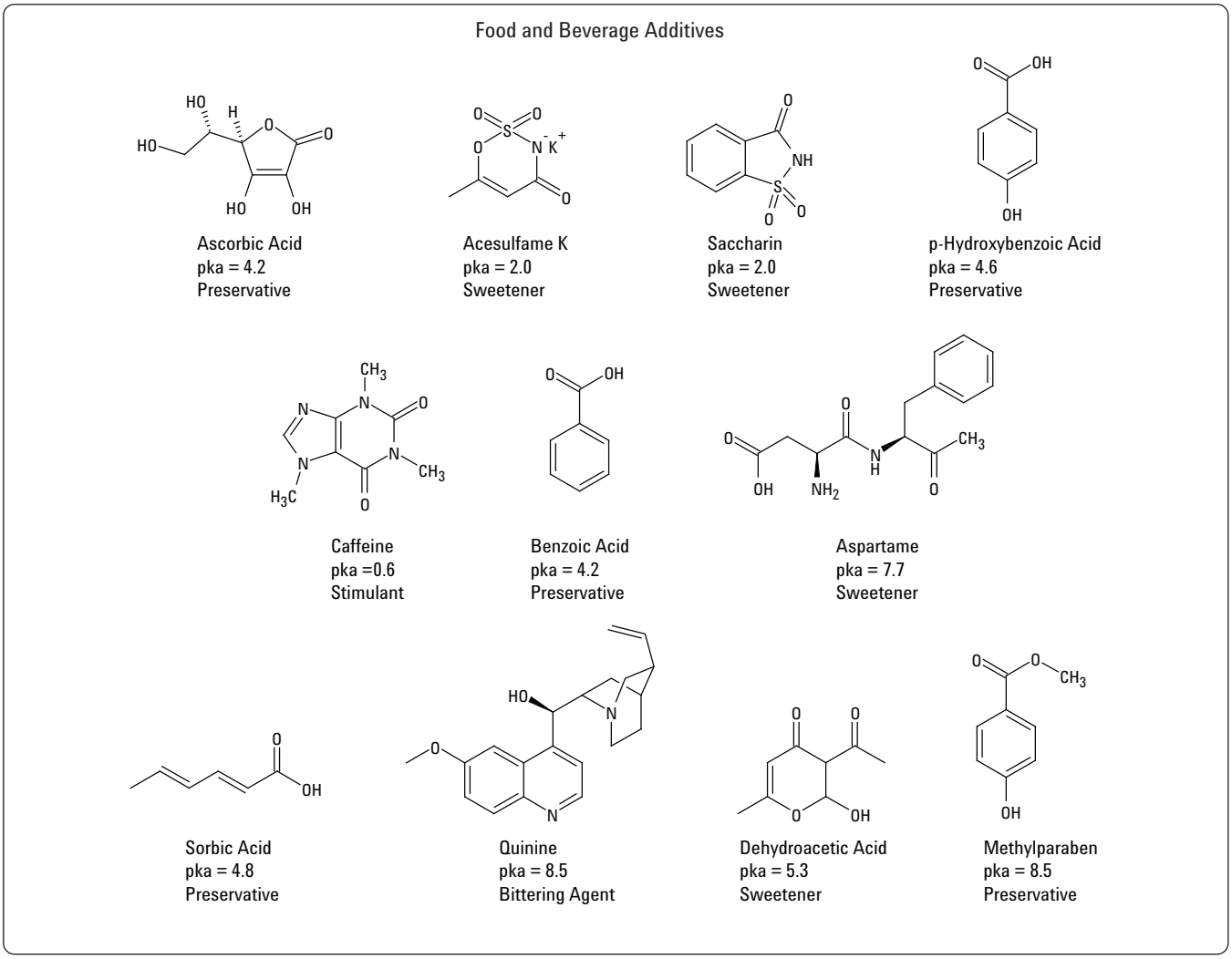

Figure 1. Compounds of interest.

significantly shorter analysis time of 3.5 minutes. The flow rate for this analysis was adjusted to compensate for the smaller internal diameter and for the smaller particle size. In this case, the 3.5-µm column does not resolve two peak pairs: benzoic acid/aspartame and dehydroacetic acid/methylparaben. Figure 4 shows the same separation, scaled to an Agilent ZORBAX Eclipse Plus C18, 3.0 mm × 100 mm, 1.8 µm column. Flow rate for the 1.8 µm column was adjusted to optimize the smaller sub-2-µm particles. All compounds were well resolved in just over 2.5 minutes. This faster analysis can enhance laboratory productivity, and lower the mobile phase and solvent consumption. A smaller sample volume is required for the smaller 3.0 mm × 100 mm column, thus yielding sample preservation. The benefits of this sub-2-µm separation, however are at the cost of higher back pressure (483 bar), resulting in the need for a high pressure LC system. Figure 5 shows the same separation on a superficially porous Agilent Poroshell 120 EC-C18, 3.0 mm × 100 mm, 2.7-µm with the same shortened analysis time as the 1.8-µm column. This separation has the added benefit of lower back pressure (356 bar). The more than 100-bar difference in pressure is very significant, because it determines if a high pressure HPLC system is needed or if a traditional system (400 bar maximum) is sufficient for this analysis.

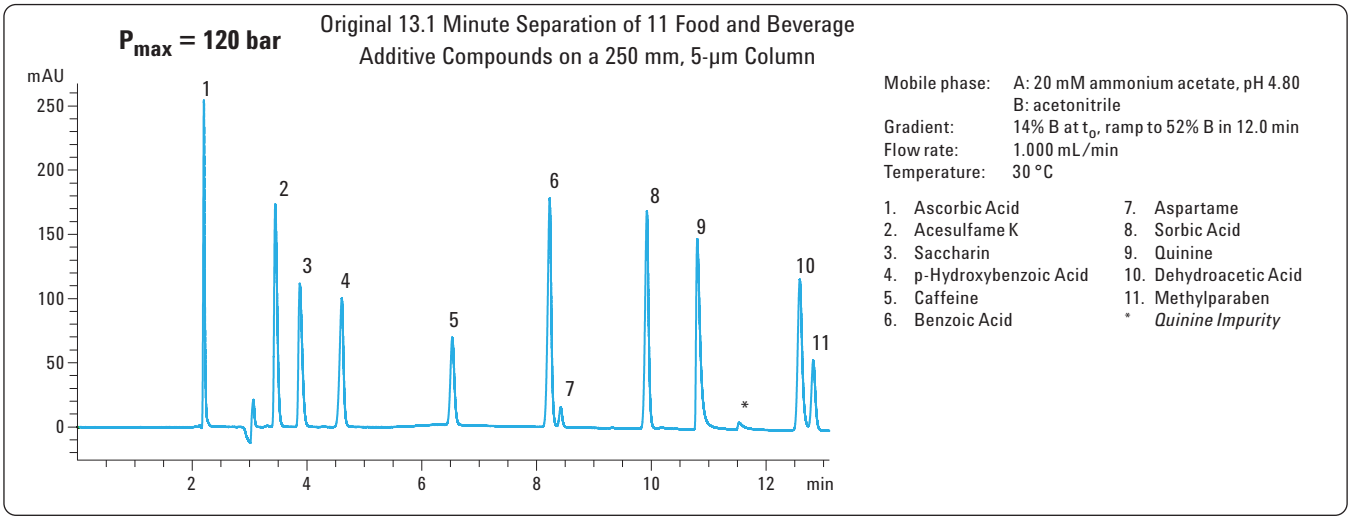

Figure 2. Separation of 11 food and beverage additives on a Agilent ZORBAX Eclipse Plus C18, 4.6 mm × 250 mm, 5-µm column using an ammonium acetate/acetonitrile gradient.

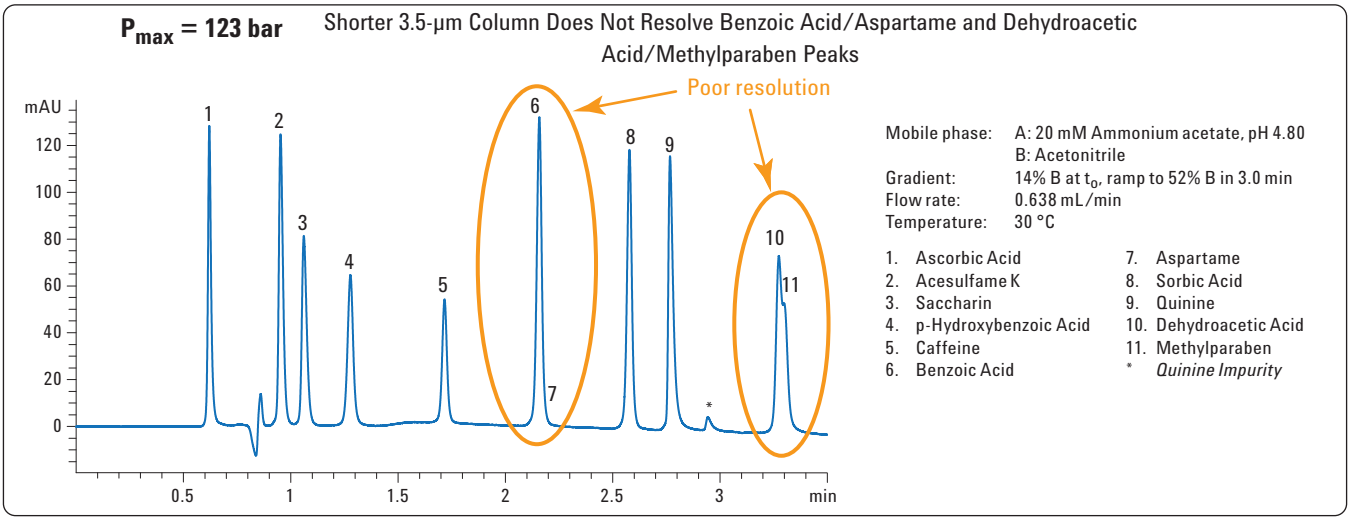

Figure 3. Separation of 11 food and beverage additives on a Agilent ZORBAX Eclipse Plus C18, 3.0 mm × 100 mm, 3.5-µm column using an ammonium acetate/acetonitrile gradient.

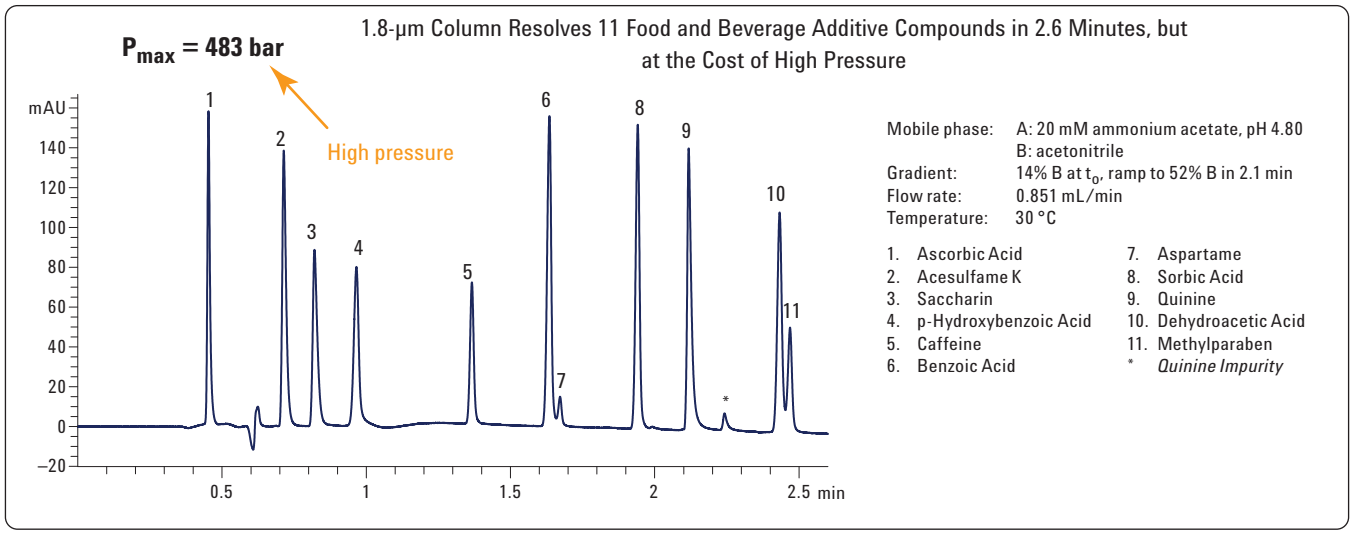

Figure 4. Separation of 11 food and beverage additives on a Agilent ZORBAX Eclipse Plus C18, 3.0 mm × 100 mm, 1.8 µm column using an ammonium acetate/acetonitrile gradient.

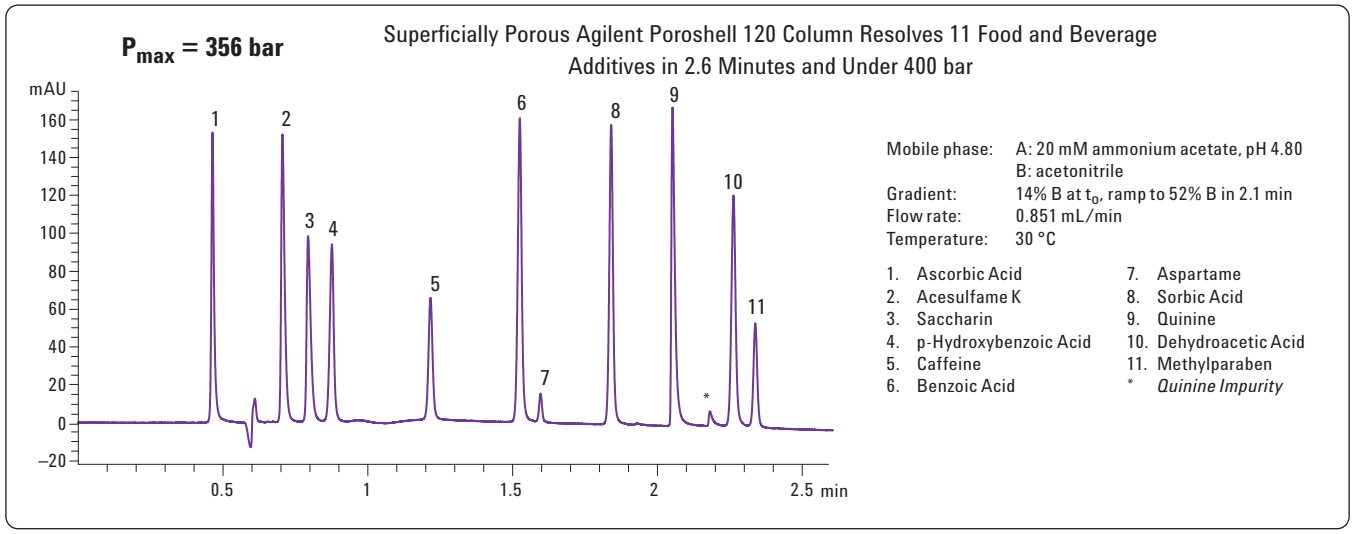

Figure 5. Separation of 11 food and beverage additives on a Agilent Poroshell 120 EC-C18, 3.0 mm × 100 mm, 2.7 µm superficially porous column using an ammonium acetate/acetonitrile gradient.

Figure 6 shows an overlay of the original 5-µm method compared to the new Agilent Poroshell 120 method. Analysis time is reduced from 13.1 to 2.6 min, with the post run time reduced from 7 to 1.8 min. Solvent and mobile phase consumption are reduced by more than 80%. Resolution of the critical pair (dehydroacetic acid and methylparaben) improved from 1.79 to 3.01 on the Agilent Poroshell 120 method, compared to the longer 5-µm Agilent ZORBAX Eclipse Plus method. Note in Figure 6, that the last peak on Agilent Poroshell 120 elutes at approximately the same time as the first peak on the 5-µm Agilent ZORBAX Eclipse Plus.

Four consumer product samples are successfully analyzed in less than 2.6 minutes and under 400 bar (Figure 7). The energy drink contains caffeine, benzoic acid and sorbic acid. The diet soda has saccharin, caffeine, benzoic acid and aspartame. Mouthwash includes saccharin and benzoic acid. The sugar-free chewing gum contains acesulfame k and aspartame.

When high throughput is important and HPLC system limits allow, the flow rate can be increased with little loss in chromatographic quality, as shown in Figure 8. The flow rate on the Agilent Poroshell 120, 3.0 mm × 100 mm column can be increased from 0.851 to 1.489 mL/min to further reduce run time by 40% in under 600 bar. This achieves little loss in resolution of the critical pair and has minimal effects on conditional peak capacity (n_c) [5]. The result is a baseline separation of 11 compounds in under 600 bar back pressure with an analysis time of 1.5 minutes.

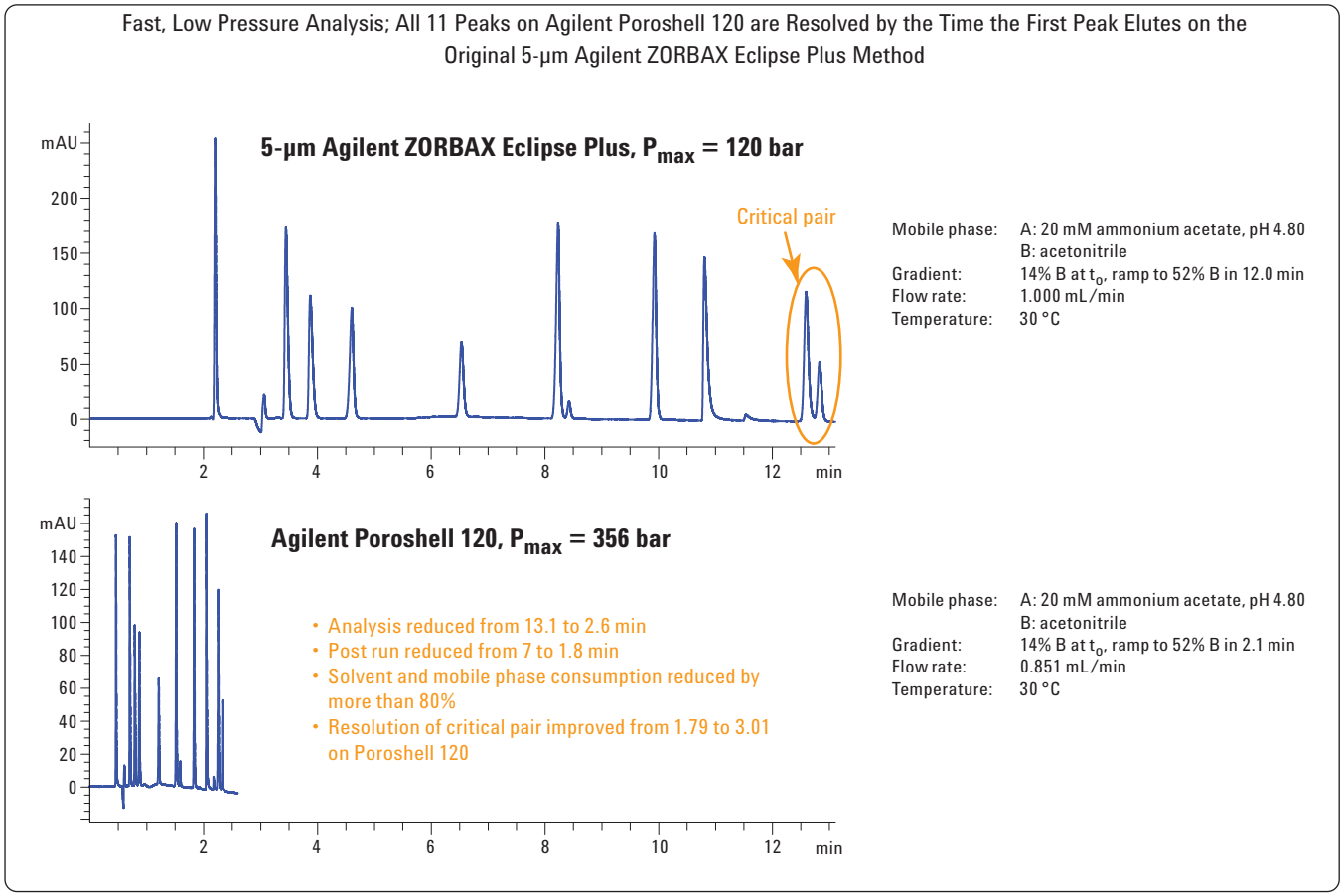

Figure 6. An overlay of the original Agilent Eclipse Plus 5-µm method and new Agilent Poroshell 120 method.

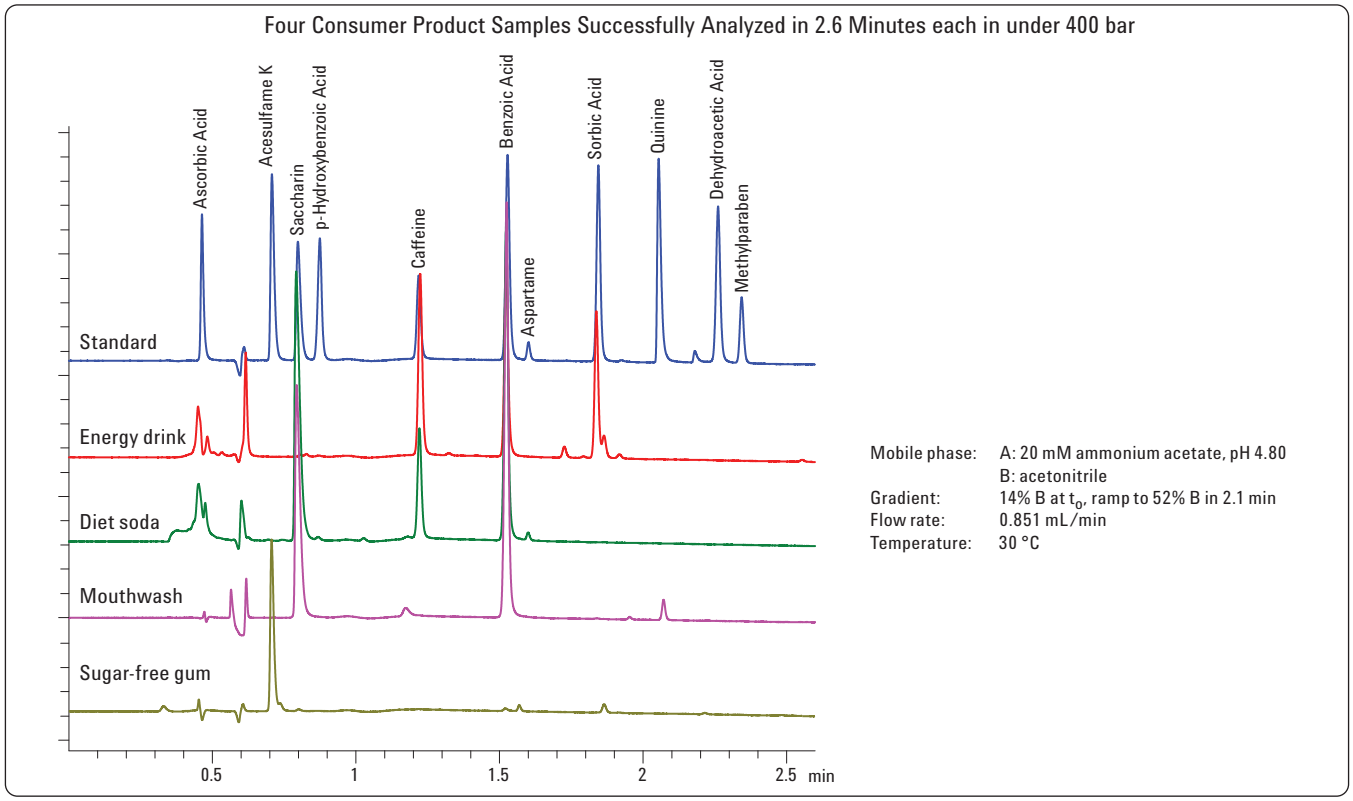

Figure 7. Four consumer product samples analyzed on a Agilent Poroshell 120 EC-C18, 3.0 mm × 100 mm, 2.7 µm superficially porous column.

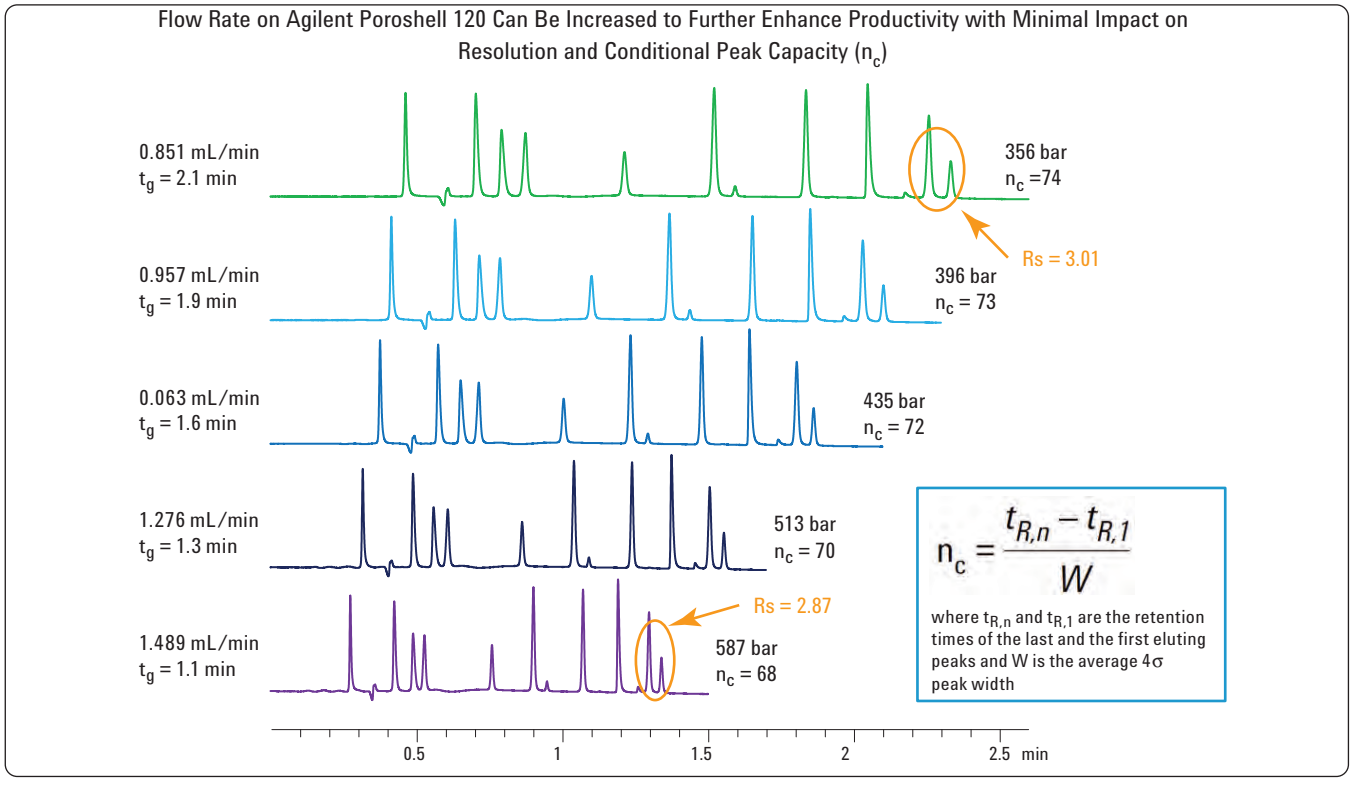

Figure 8. Overlay showing effects on chromatographic quality with increased flow rate on Agilent Poroshell 120.

Conclusion

HPLC columns packed with superficially porous particles offer many advantages over columns packed with conventional, fully porous particles. The superficially porous 2.7-µm Agilent Poroshell 120 EC-C18 column offers similar efficiency and selectivity to the 1.8-µm Agilent ZORBAX Eclipse Plus C18 column, without the high back pressure. While larger 5-µm particles packed in longer columns can yield similar efficiency without high back pressure, they result in a significantly longer analysis time. Due to the similar selectivity between Poroshell 120 EC-C18 and Eclipse Plus C18, methods can easily be transferred from older Eclipse Plus C18 columns to new Poroshell 120 EC-C18 columns. This achieves lower back pressure for older 400 bar HPLC systems, shorter run times, and solvent savings.

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采用配备安捷伦 Poroshell 120 LC 色谱柱的 Agilent 1290 Infinity LC 检测虾中的激素（通过安捷伦 QuEChERS 进行样品制备）

Application Note

Food

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Abstract

本研究开发了一种使用配备有安捷伦 Poroshell 120 EC-C18 色谱柱的 Agilent 1290 Infinity LC 测定虾中激素的方法。同时还开发并优化了一种采用 QuEChERS 的样品制备方法，用以提取虾中的 13 种激素。结果表明，使用 QuEChERS 处理样品，再通过安捷伦 Poroshell 120 EC-C18 色谱柱（3.0 mm×100 mm，2.7 µm）进行 HPLC 分析适用于虾中激素类化合物的测定。13 个目标化合物完全分离，色谱图中没有显示其它干扰。方法回收率在 91.6 % 到 107.2 % 之间，相对标准偏差（RSD）在 0.15 % 到 3.5 % 之间。

Introduction

There are currently several hormones and hormone-like agents with marked ability to improve the rate of growth and efficiency of feed intake for animals. In some countries hormones are a common food additive and controlled use of certain compounds is even considered safe. However, longterm consumption of glucocorticoids can lead to hyperglycemia, osteoporosis, birth defects, and immune function decline. Other hormones such as estrogen, androgen, and progesterone are carcinogenic and can lead to breast cancer, ovarian cancer and cell carcinoma. Some other countries therefore strictly prohibit their application, especially the use of estrogens. A previous application note showed a new method for detecting hormones in crucian carp fish meat using an Agilent SampliQ OPT SPE cartridge for sample preparation and an Agilent ZORBAX Eclipse Plus C18 column (4.6 mm × 250 mm, 5 µm) for HPLC analysis [1].

Although the current QuEChERS methodology has been designed for removing matrix interferences in food products of plant origin, such as polar organic acids, sugars, and lipids, it also has potential for other food matrices such as meat or seafood. Based upon the chemical properties of the compounds of interest and food matrices, some modifications of the original method might be necessary to obtain accurate and precise results. The purpose of this work is to extend the QuEChERS methodology to veterinary drug residues in seafood. Agilent QuEChERS EN buffered extraction kits (p/n 5982-5650) and dispersive-SPE 15 mL kits for drug residues in meat (p/n 5982-4956) were used for the analysis of 13 hormones (Table 1) in shrimp. The method was validated in terms of recovery and reproducibility.

A newly developed column, the Agilent Poroshell 120 EC-C18 that is packed with 2.7 µm superficially porous materials was used to separate these 13 common hormones. The new column has almost the same efficiency as sub-2-µm totally porous materials and can be used to provide a fast and high resolution analysis.

Table 1. Hormones Used in this Study

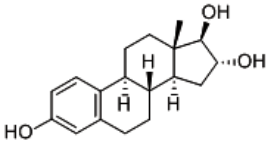
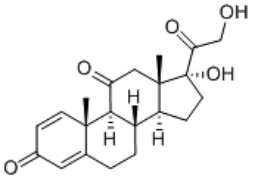
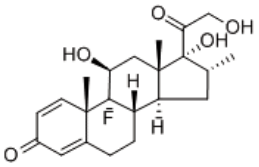
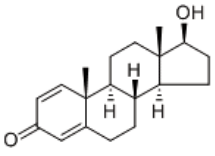
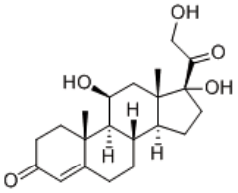
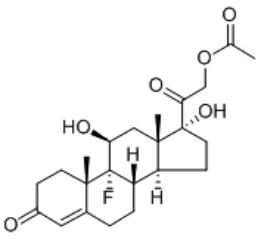
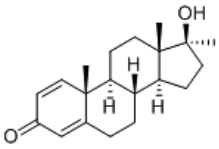
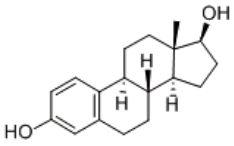
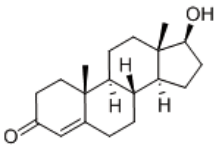
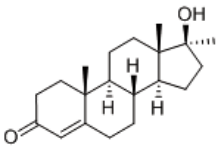
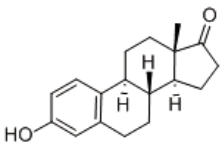
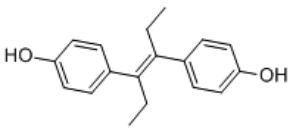
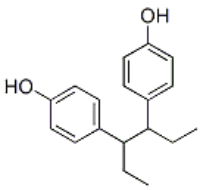
No.	Compound	CAS No.	Structure
1	Estriol	50-27-1	
2	Prednisone	53-03-2	
3	Dexamethasone	50-02-2	
4	Boldenone	846-48-0	
5	Hydrocortisone	50-23-7	

Table 1. Hormones Used in this Study (continued)

6	Fludrocortisone acetate	514-36-3	
7	Metandienone	72-63-9	
8	Estradiol	50-28-2	
9	Testosterone	58-22-0	
10	Methyltestosterone	58-18-4	
11	Estrone	53-16-7	
12	Diethylstilbestrol	56-53-1	
13	Hexestrol	84-16-2	

Experimental

Reagents and Chemicals

All reagents and solvents were HPLC or analytical grade. Hormone standards were purchased from NICPBP (National Institute for the Control of Pharmaceutical and Biological Products). Shrimp was purchased from a local market.

Stock solutions (1 mg/mL) were prepared in methanol and kept in the freezer (–20 °C). Working solutions were prepared using the stock solution diluted with methanol. The working solutions should be prepared every week and stored below 4 °C.

Equipment and Materials

Agilent QuEChERS EN Extraction kits, p/n 5982-5650, and Agilent QuEChERS dispersive-SPE kits for Drug Residues in Meat, 15 mL, p/n 5982-4956 (Agilent Technologies Inc., DE, USA). Flying Pigeon Centrifuge (Anting Science Instrument, Shanghai, P.R.China).

HPLC Method

Instrument	Agilent 1290 Infinity LC with DAD detector	
Column	Agilent Poroshell 120 EC-C18 3.0 x 100 mm 2.7 µm (p/n 695975-302)	
Flow rate	0.8 mL/min	
Injection volume	10 µL	
Column temperature	30 °C	
Detection wavelength	230 nm	
Mobile phase	Water-acetonitrile gradient	
Time (minutes)	% Water	% Acetonitrile
0	80	20
6	50	50
8	10	90

Sample Preparation

The sample preparation procedure includes sample homogenization, extraction/partitioning, and dispersive-SPE cleanup.

The shrimp purchased from a local grocery store, was washed and chopped into small pieces. The chopped shrimp was homogenized thoroughly with a food grinder and stored at 20 °C. A 5-g (±0.05 g) sample of homogenized shrimp was weighed in a 50 mL centrifuge tube. The tubes were centrifuged for 30 s to move the sample from the inside tube wall to the bottom of the tube. Samples were then fortified with appropriate QC spiking solutions when necessary. After vortexing the sample for 30 s, 5 mL of water were added. Tubes were then vortexed for 10 s to mix. A 10 mL volume of ACN was added to each tube. Tubes were capped and shaken by hand for 30 s. An Agilent QuEChERS EN extraction salt packet (p/n 5982-5650) was added to each tube. Sample tubes were capped tightly and shaken vigorously for 1 min. Tubes were centrifuged at 4,000 rpm for 5 min at 4 °C.

A 6 mL aliquot of the upper ACN layer was transferred into an Agilent QuEChERS dispersive-SPE 15 mL tube for drug residues in meat (p/n 5982-4956). This 15 mL dispersive-SPE tube contained 150 mg of C18 and 900 mg of anhydrous MgSO4. The tubes were tightly capped and vortexed for 1 min. The 15 mL tubes were centrifuged at 13,000 rpm for 3 min at 4 °C. A 4-mL volume of extract was transferred into another tube and dried by N_2 flow at 35 °C. Samples were reconstituted into 2 mL of 1:4 ACN/H_2O. After vortexing and sonicating for 10 min, the sample was filtered through a 0.22 µm Cellulose Acetate Spin Filter (p/n 5185-5990). The clear, filtered sample was transferred into an autosampler vial. The samples were capped and vortexed thoroughly in preparation for HPLC analysis.

Results and Discussion

Separation

Figure 1 shows the standard hormone mixture separated on a Poroshell 120 EC-C18 column. All 13 compounds were well separated in 8 minutes in a 20% acetonitrile solution. Each compound was present at a 1 ppm level and excellent sensitivity was achieved at this level. To study the system suitability for the shrimp sample, the hormone mixture was spiked into the blank sample after the QuEChERS extraction method. The gradient method was adjusted so that the target compounds were separated from the matrix. Figure 2 shows the chromatograms of blank sample and 0.5 ppm spiked into the blank sample. The analysis time was extended to 10 minutes to allow matrix and nonrelevant sample components to be eluted. This is an excellent overall analysis time for this sample matrix and is a result of achieving high resolution quickly on the Poroshell 120 EC-C18 column.

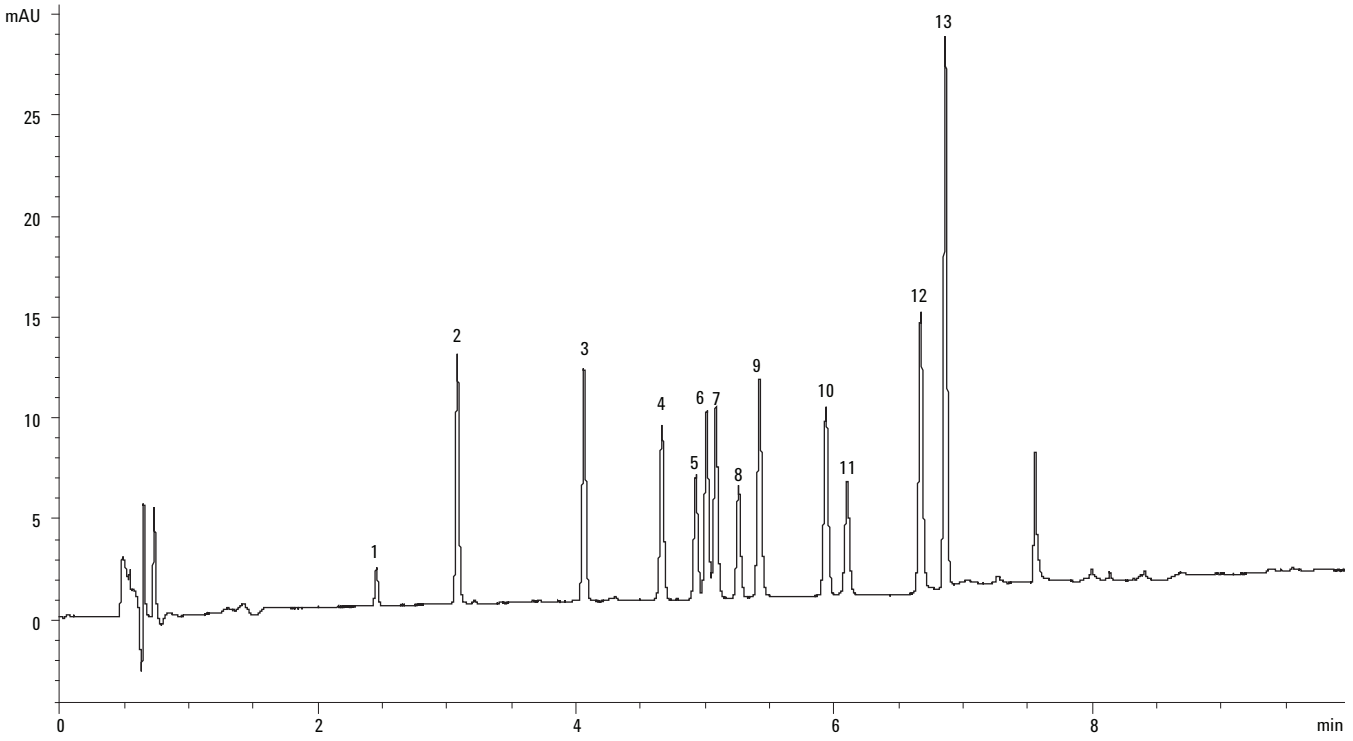


Figure 1. Chromatogram of 1 ppm standard compounds in 20% acetonitrile solution on the Agilent Poroshell 120 EC-C18, 3.0 x 100 mm, 2.7 µm column.

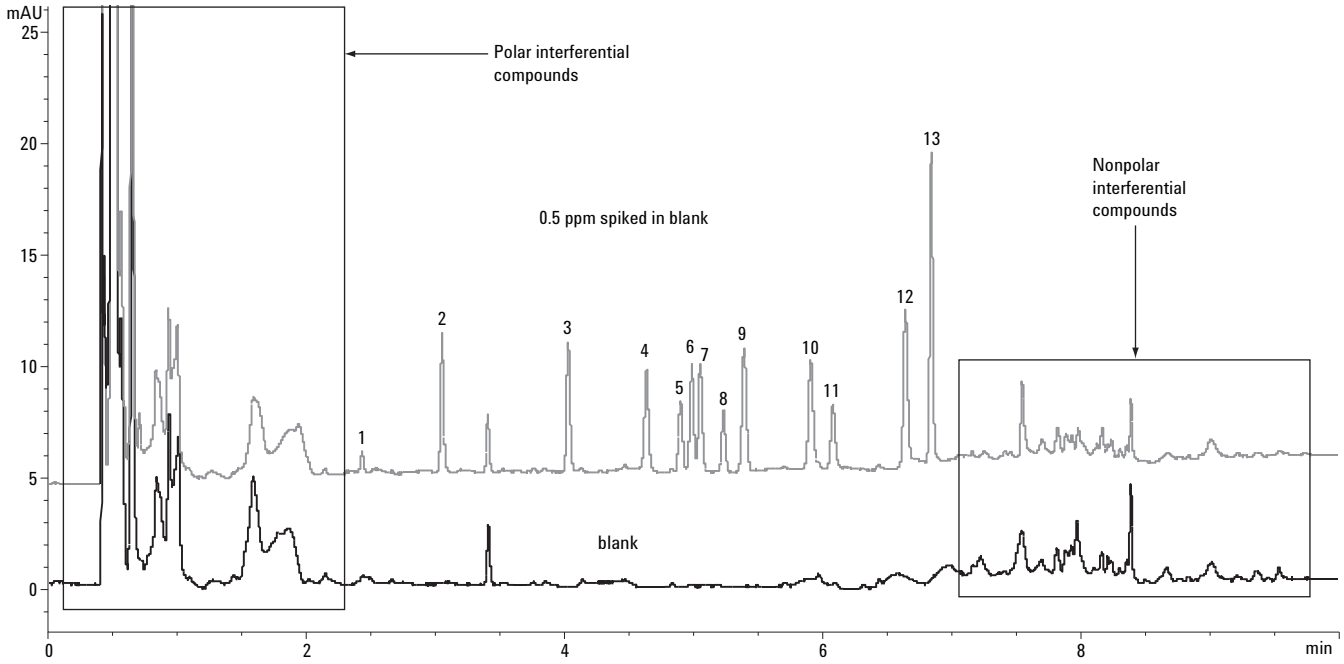


Figure 2. Chromatograms of blank sample and 0.5 ppm spiked in blank sample.

Linearity, Limits of Detection

The stock solution was diluted into 0.5 ppm, 1 ppm, 2 ppm, 5 ppm and 10 ppm standard solutions with blank solution as the calibration standard and they were analyzed by HPLC to make a calibration curve. Linear regressions were calculated for the hormones based on the peak areas and the solution concentrations. Limits of detection (LOD) were calculated with a signal-to-noise ratio of 3 based on the data at 0.5 ppm concentration. The linearity and LOD are shown in Table 2. The data showed good linearity in the range of 0.5–10 mg/kg.

Table 2. Linearity and LOD of Hormones by HPLC

No.	Compound	Regression equation	Correlation coefficient	LOD (mg/kg)
1	Estriol	Y = 3.331x+0.306	0.9997	0.22
2	Prednisone	Y = 22.182x+0.0212	0.9999	0.023
3	Dexamethasone	Y = 22.622x+0.0523	0.9999	0.0086
4	Boldenone	Y = 18.829x+1.955	0.9998	0.031
5	Hydrocortisone	Y = 14.085x+0.0388	0.9999	0.044
6	Fludrocortisone acetate	Y = 21.152x+0.0591	0.9999	0.029
7	Metandienone	Y = 22.052x+0.0325	0.9999	0.022
8	Estradiol	Y = 12.377x+0.0108	0.9999	0.039
9	Testosterone	Y = 25.370x+0.0748	0.9999	0.019
10	Methyltestosterone	Y = 23.149x+0.651	0.9999	0.033
11	Estrone	Y = 13.641x+0.690	0.9999	0.057
12	Diethylstilbestrol	Y = 31.526x+0.347	0.9999	0.023
13	Hexestrol	Y = 51.174x+0.224	0.9999	0.012

Note: x- Concentration (ng/uL); y- Area

Recovery and Repeatability

The precision of the method was determined in terms of the recovery of spiked hormone standards in homogenized shrimp at 0.5, and 10 mg/kg. The analysis was repeated six times for each level. The chromatograms of the blank and the spiked standard (0.5 mg/kg and 10 mg/kg) samples are shown in Figure 3. The data in Table 3 demonstrate excellent recovery and reproducibility for the QuEChERS method developed for hormone determination in shrimp.

Table 3. Recoveries and RSDs of Hormones in Shrimp

Compound	Spiked level (mg/kg)	Recovery (%)	RSD (n = 6, %)
Estriol	0.5	107.2	3.5
	10	98.2	0.98
Prednisone	0.5	97.6	2.3
	10	101.7	0.58
Dexamethasone	0.5	101.8	0.96
	10	96.1	1.2
Boldenone	0.5	98.9	1.5
	10	96.2	1.8
Hydrocortisone	0.5	103.5	1.5
	10	92.3	0.23
Fludrocortisone acetate	0.5	104.3	1.9
	10	91.8	0.17
Metandienone	0.5	100.0	1.4
	10	95.6	0.25
Estradiol	0.5	99.4	1.3
	10	97.8	0.54
Testosterone	0.5	98.0	0.85
	10	98.2	0.15
Methyltestosterone	0.5	97.1	0.99
	10	92.1	0.63
Estrone	0.5	103.4	1.2
	10	92.5	0.68
Diethylstilbestrol	0.5	100.9	1.9
	10	97.3	0.79
Hexestrol	0.5	98.5	1.6
	10	91.6	0.81

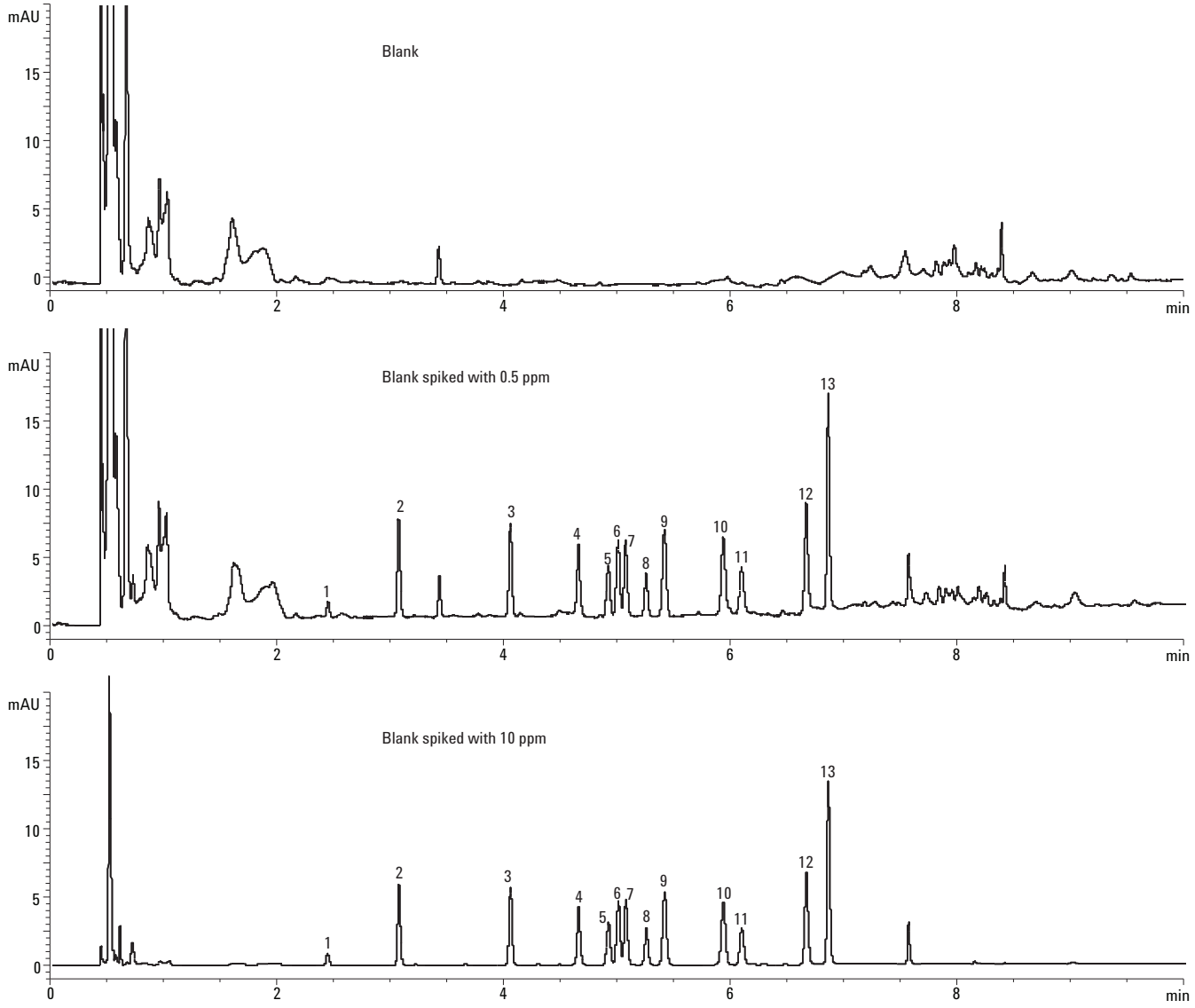


Figure 3. Chromatograms of shrimp sample blank and shrimp sample spiked with 0.5 ppm and 10 ppm standard mixture.

Conclusions

The Agilent QuEChERS Buffered Extraction EN kit and the Agilent dispersive-SPE kit for drug residues in meat provide a simple, fast and effective method for the purification of hormones in shrimp. Compared to the other sample pretreatment methods, such as LLE and SPE, the QuEChERS method is easier to handle, faster, labor-saving, and cheaper. The recovery and reproducibility, based on matrix spiked standards, were acceptable for multiresidue hormone determination in shrimp. The Agilent 1290 Infinity LC with the Agilent Poroshell 120 column resolved the 13 compounds in 10 min and all the compounds were well separated from the matrix. The method developed is suitable for the determination of hormones in shrimp at low mg/kg levels.

Reference

1. Chen-Hao Zhai and Yun Zou, "Determination of Hormones in Fish (Carassius Carassius) by SampliQ OPT Solid Phase Extraction with High Performance Liquid Chromatography" Agilent Technologies publication, 5990-3845EN (2009).

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将现有肉类抗生素分析方法转换和优化为使用 MS/MS 检测的安捷伦 Poroshell 120 EC-C18 色谱柱分析方法

Application Note

Food

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Abstract

本文的实验通过采用紫外检测的一般梯度方法，针对肉中抗生素分析的快速方法优化评估流动相的选择，最终目的是开发出与质谱仪兼容的方法。评估内容包括 4 种缓冲溶液和 2 种有机溶剂的选择。实验将可获得最佳分离效果的流动相组合转移到安捷伦 Poroshell 120 EC-C18 色谱柱（2.1 mm×100 mm，2.7 µm），并进行优化。梯度时间从 45 min 缩短到 12 min。如果使用 3 mm×50 mm 色谱柱，再牺牲一定的分离度，此时间可进一步缩短。在 Agilent 1200 系列快速分离液相色谱与 Agilent 6410 三重串联四极杆 LC/MS 系统上对该方法进行了验证。

Table 1. Method Parameters for Various Column Dimensions

	4.6 × 50 mm Poroshell 120 EC-C18	3.0 × 50 mM Poroshell 120 EC-C18	2.1 × 100 mM Poroshell 120 EC-C18
Mobile Phase	A: Buffer, varies B: Organic, varies	A: 10 mm ammonium formate pH 3.8 B: Acetonitrile	A: 10 mm ammonium formate pH 3.8 B: Acetonitrile
Gradient	10-40% B	10-40% B	10-40% B
Gradient Time	12 min	12 min	12 min
Flow Rate	2 mL/min	0.85 mL/min	0.42 mL/min
Injection Volume	0.5 µL	5 µL	2.5 µL or 10 µL
Sample	0.1 mg/mL antibiotics	1 µg/mL antibiotics	1 µg/mL or 10 ng/mL antibiotics
TCC Temperature	30 °C	30 °C	30 °C
Detector	DAD: Sig = 270, 4 nm; Ref = 360, 100 nm	MS/MS: See Table 2	MS/MS: See Table 2

Introduction

Administration of antibiotics is a common practice in chicken, pork, beef and fish farming. Many domestic cattle receive various antibiotics in their feed for the prevention and control of disease caused by fungi and bacteria. Many countries regulate acceptable residue levels of these compounds in agricultural and animal products. In this work, an older method is transferred from a 5 µm, 250 mm column to a new superficially porous column to increase the speed of the analysis and change the method of detection from UV to MS/MS. An increase in throughput of 5 to 10 times is demonstrated, while minimally impacting sample preparation. Since the analysis time is shortened dramatically, time is available for optimization of mobile phase selectivity (pH, buffer types and organic modifier).

Transition methods can be developed by modifying an existing method or starting fresh. In this case, the objective was to develop a new MS-compatible separation from an existing UV separation. Consequently, a change in the mobile phase was required because 0.7 % phosphoric acid is not a desirable solvent for MS detection. A generic screening method using 0.1 % formic acid was investigated, but additional MS-compatible solvent systems were also evaluated. In this work a method is developed by first screening different mobile phase combinations using a short Agilent Poroshell 120 column using UV detection, then transferring that method to an Agilent 6410 triple quadrupole LC/MS System. A major advantage of the Agilent Poroshell 120 EC-C18 is that it uses the same 2 µm frit as the original 5 um column, negating the need for sample preparation method development.

Agilent Poroshell 120 EC-C18 4.6 mm × 50 mm, 2.7 µm columns have similar performance to 1.8-µm totally porous Agilent ZORBAX Eclipse Plus C18 columns, but since they use 2-µm column frits similar to those found on 5-µm columns, they require no additional sample preparation. This allows for a more seamless method transfer. While some previous work demonstrates the use of Agilent Poroshell 120 columns on older Agilent 1100 systems, they are ideally used on more modern systems such as the Agilent 1200 or 1260 series UHPLC's.

Table 2. MRM Transitions for Antibiotic Compounds.

Compound name	Precursor ion	Product ion	Fragmentor voltage	Collision energy
Sulfamerazine	265	172	100	25
Sulfamerazine	265	108	100	25
Thiamphenicol	338	308	140	10
Thiamphenicol	338	118	140	50
Sulfamethazine	279	124	100	25
Sulfamethazine	279	108	100	30
Furazolidone	226	137	140	25
Furazolidone	226	122	140	25
Sulfamonomethoxine	281	126	100	25
Sulfamonomethoxine	281	108	100	25
Oxolinic acid	262	160	100	40
Oxolinic acid	262	130	100	45
Pyrimethamine	249	198	140	45
Pyrimethamine	249	128	140	60
Sulfadimethoxine	311	156	140	25
Sulfadimethoxine	311	108	140	55
Sulfaquinoxaline	301	129	100	50
Sulfaquinoxaline	301	108	100	40
Difurazone	361	222	100	15
Difurazone	361	154	100	45

Experimental

Method development is based upon the use of a generic gradient. Using a short 4.6 mm × 50 mm Poroshell 120 EC-C18, 2.7 µm column, several different mobile phases can be quickly evaluated. The generic gradient is run at 2.0 mL/min, starts at 10% and proceeds to 40% organic over 12 min. This gradient is later transferred to 2.1 mm × 100 mm and 3 mm × 50 mm columns by changing the gradient according to Equation 1. The three gradients used are listed in Table 1 with MRM transitions shown in Table 2. MS-compatible mobile phases consisting of volatile buffer components such as formic acid, ammonium formate buffer and ammonium acetate buffer are used.

An Agilent 1200 Rapid Resolution LC (RRLC) system was used for this work:

- G1312B Binary Pump SL.
- G1367C Automatic Liquid Sampler (ALS) SL.
- G1316C Agilent 1290 Infinity Thermostatted Column Compartment (TCC) SL.
- G1315C Agilent Diode Array Detector (DAD) SL using a G1315-60024 micro flow cell (3-mm path, 2-µL volume).
- G6410 Agilent Triple Quadrupole LC/MS System with Electrospray (ESI).
- ChemStation version B.04.01 was used to control the HPLC and process the data. Agilent MassHunter Version 2.0 was also used to control the Agilent 6410 Triple Quadrupole LC/MS System, the Agilent 1200 Rapid Resolution LC (RRLC), and to analyze the data.

Three Agilent Poroshell 120 EC-C18 columns were used in this work:

- 4.6 mm × 50 mm, 2.7 µm p/n 699975-902
- 3.0 mm × 50 mm, 2.7 µm p/n 699975-302
- 2.1 mm × 100 mm, 2.7 µm p/n 695775-902

The compounds of interest are shown in Figure 1, with their respective structures. Compounds were dissolved in water at 1 mg/mL. Equal aliquots were combined to produce a mixed sample. Compounds were purchased from Sigma Aldrich (Bellefonte, PA). Additionally, methanol, acetonitrile, ammonium formate, ammonium acetate, formic acid, and glacial acetic acid were purchased from Sigma Aldrich. Water used was 18 M-Ω Milli-Q water (Bedford, MA).

Buffers used in this work were prepared by dissolving an appropriate amount of the ammonium salt to produce a 10 mM solution, adding 950 mL water and titrating the solution with either formic acid (for the ammonium formate buffers) or glacial acetic acid (for the ammonium acetate buffers). The buffer solutions were then brought to a 1 L volume.

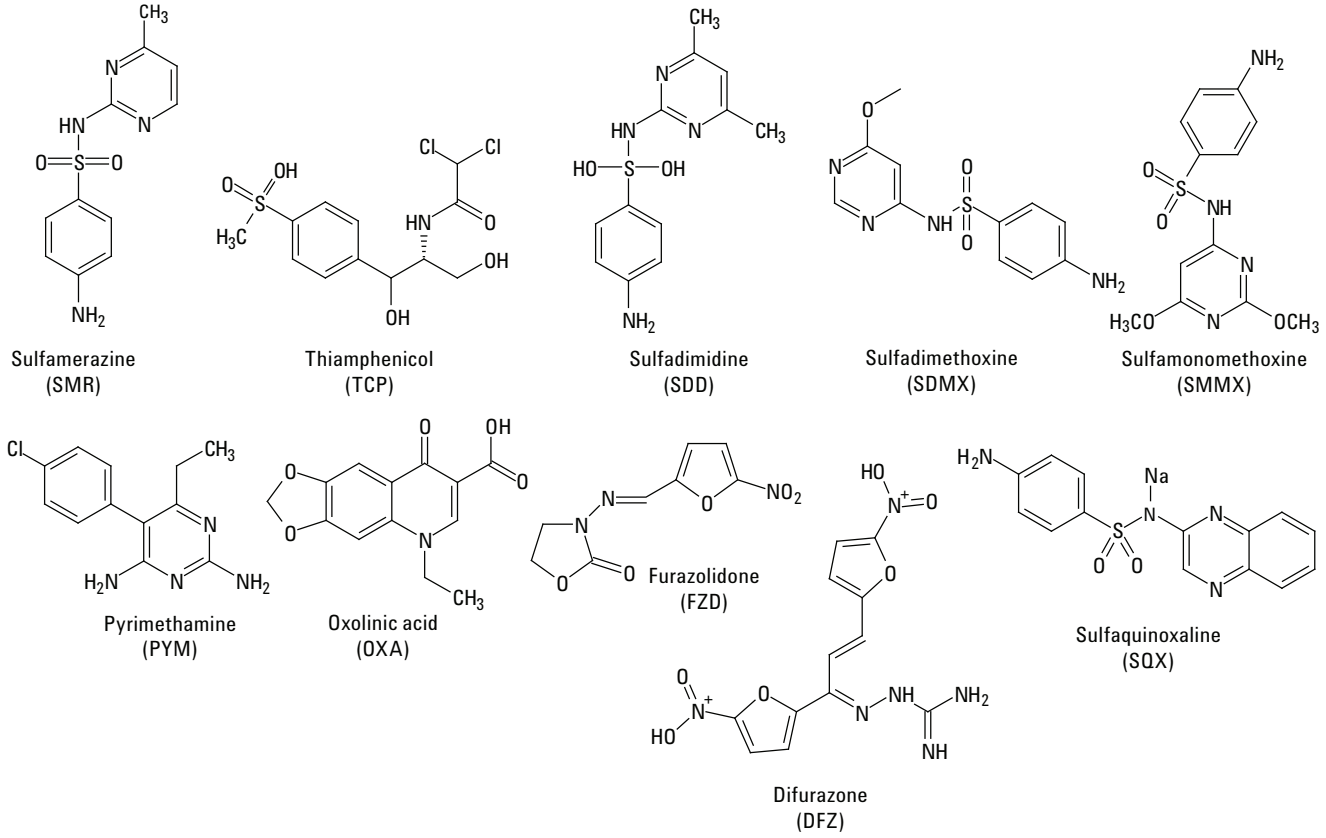


Figure 1. Compounds of interest.

Results and Discussion

The original method published in 2002 by Kumagai and Onigbinde provides an effective method for the analysis of antibiotics in meat using UV detection. As seen in Figure 2, the method separates the analytes in approximately 45 min. However the nonvolatile phosphoric acid in the mobile phase is not compatible with MS detection.

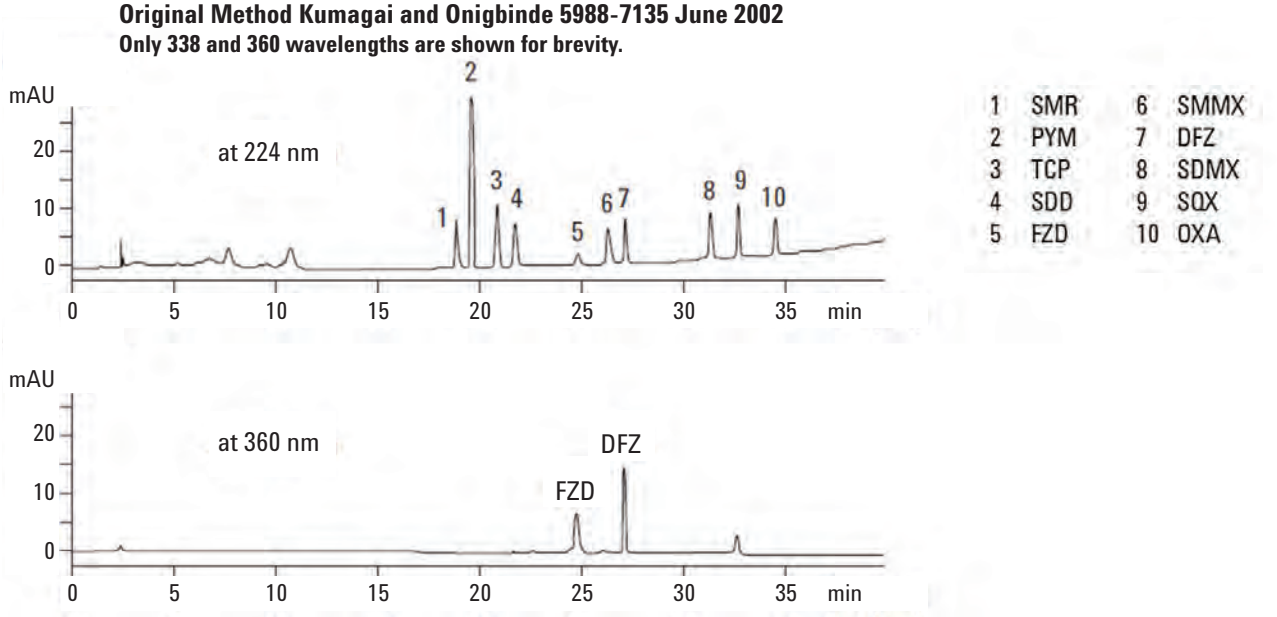
In many cases, simple scaling of a method will allow for a fast method transfer. In this case, however, a change in the mobile phase was required for LC/MS compatibility. The use of short Poroshell 120 EC-18 4.6 mm × 50 mm, 2.7 µm columns for assessing mobile phase changes has several advantages. One advantage is that they allow quick separations without sacrificing resolving power. In addition, since they are used at 2 mL/min with a generic gradient, the solvent is rapidly purged through the system. This ensures that the solvent screening experiment can be quickly performed by changing solvent bottles, with no concerns about residual solvents in the HPLC pump or the degasser. These columns can be used for LC/MS but typically smaller diameter columns such as 3.0 or 2.1 mm columns are used.

As discussed in reference 5, once a separation has been optimized according to selectivity and retention index, it is possible to further improve the chromatography by varying column length, particle size and flow rate. However the k* value must be maintained, while varying these column conditions so as not to lose selectivity.

Equation 1: $k^* = (t_g F)/(d/2)^2 L(\Delta\%B)$

Where:

t_g is the gradient time
F is the flow rate
L is the column length
d is the column internal diameter
Δ%B is the change in organic content across the gradient segment



Instrument:	Agilent 1100 Series HPLC
Column:	250 mm × 4 mm id, RP-18 Purospher, 5 µm, p/n 79925PU-584
Mobile phase:	A = 0.7% Phosphoric acid, B = CH_3CN
Gradient:	0.0 min 5% B; 10.0 min 5% B; 40.0 min 65% B; 45.0 min 65% B; Post Time 7.0 min 5% B
Flow rate:	1.0 mL/min
Temperature:	40 °C
Injection volume:	20 µL
Diode array detector:	A-338/10 nm, reference wavelength off B-264/8 nm, reference wavelength off C-360/8 nm, reference wavelength off

Figure 2. Original method produces excellent results on a 250 mm column with UV detection.

As illustrated in Figure 3, generic gradients using methanol or acetonitrile are used to separate the compounds of interest. The gradients using methanol generate 50% higher pressure (300 bar instead of 200 bar). While this is not critical when using a 50 mm column, this does become more important as the length of the column is increased to 100 or 150 mm.

With methanol, the last compound elutes later due to the lower solvent strength. Formic acid, while a convenient mobile phase additive, produces less optimal results than 10 mM ammonium formate buffer (pH =3), particularly for pyrimethamine. In addition to peak shape improvements, elution order changes also occur most notably with pyrimethamine.

Many selectivity improvements and changes can be produced by choice of pH or organic modifier. As noted earlier, the peak shape of many basic compounds are improved when using methanol, however Poroshell 120 EC-C18 yields excellent peak shape for all compounds in this study. By adjusting the pH even slightly, both the elution order and peak spacing can be changed. This is most evident in Figure 3, where methanol and pH act to dramatically change the elution order. For the compounds in this study the best mobile phase combination is found at pH 3.8, ammonium formate with acetonitrile.

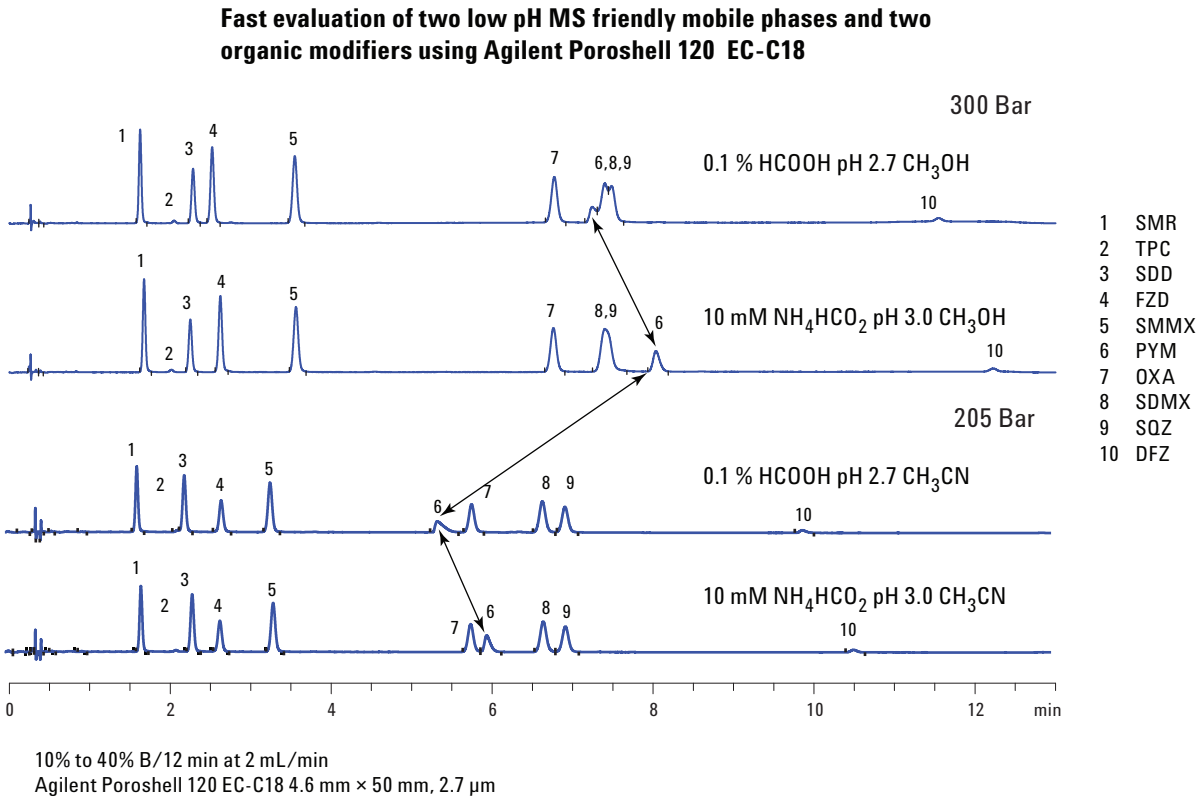
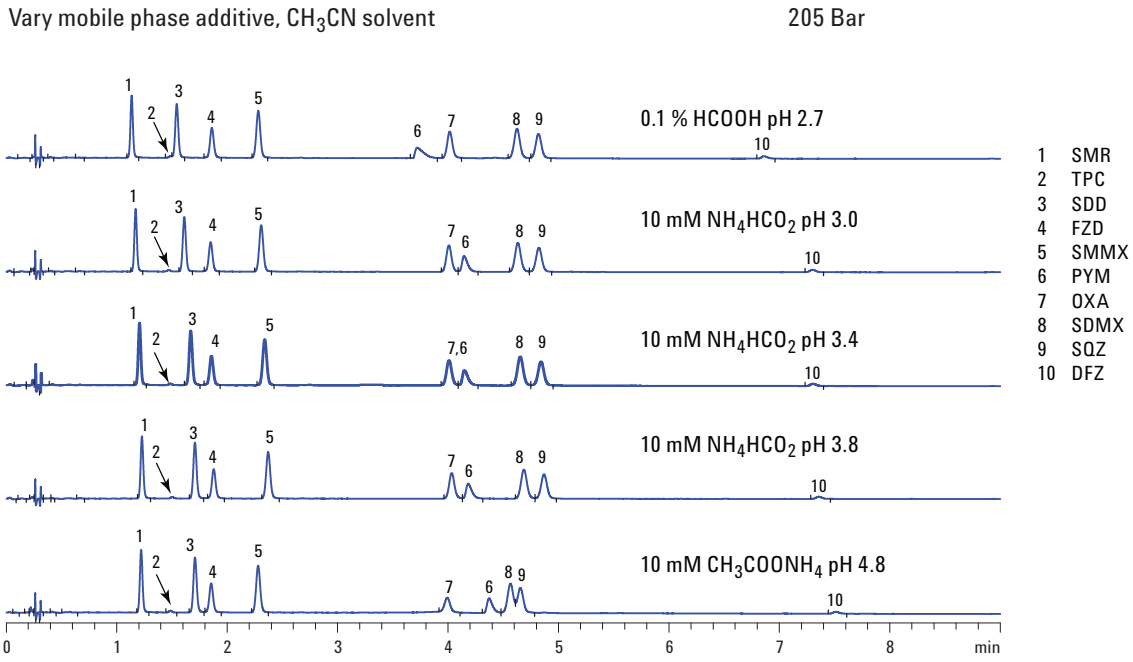


Figure 3. Comparison of chromatographic conditions: buffer, 0.1 % formic acid, CH_3OH, CH_3CN.

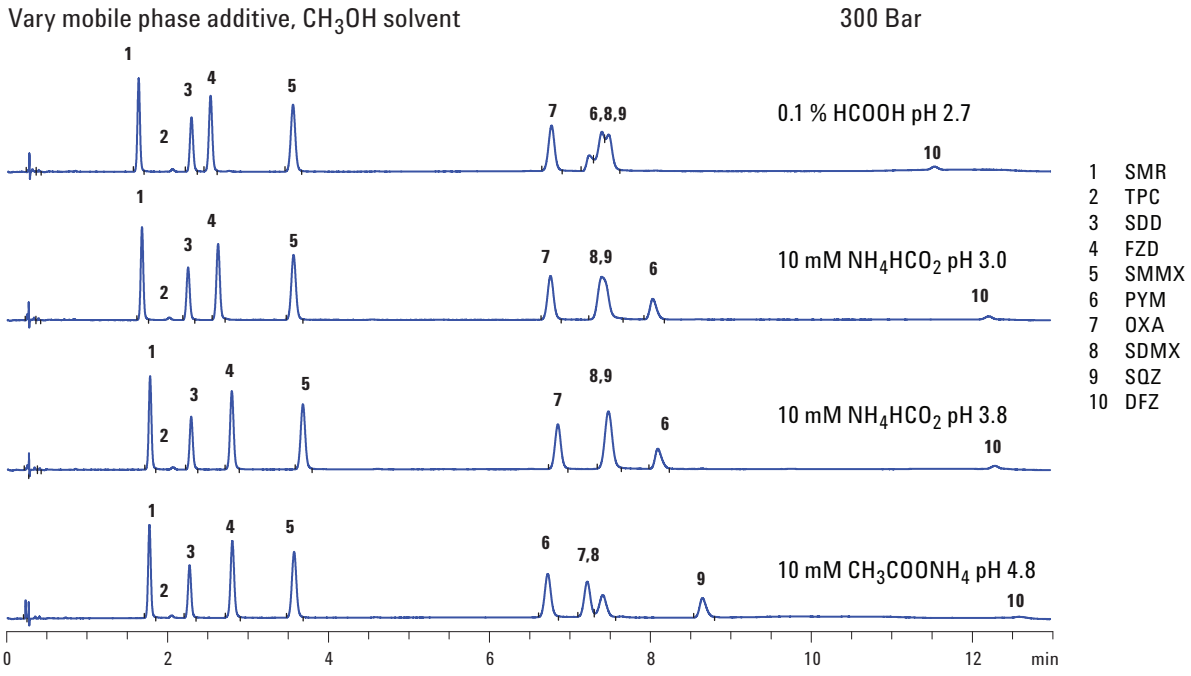
Acetonitrile with ammonium formate buffer yields excellent peak shape and selectivity with pH 3.8 being optimal for these analytes



10% to 40% B/12 min at 2 mL/min
Agilent Poroshell 120 EC-C18 4.6 mm × 50 mm, 2.7 µm

Figure 4. Comparison of buffers with CH_3CN.

Methanol with ammonium acetate buffer yields excellent peak shape with pH 4.8 being optimal for these analytes



10% to 40% B/12 min at 2 mL/min
Agilent Poroshell 120 EC-C18 4.6 mm × 50 mm, 2.7 µm

Figure 5. Comparison of buffers with CH_3OH.

Figure 6 illustrates a total ion chromatogram based on the scouting work shown in Figures 3, 4 and 5. Conditions were scaled according to Equation 1 for the 3.0 mm × 50 mm column. This easy change demonstrates that the 3 mm column can be easily used for both conventional UV and more sensitive MS. In addition, a 2.1 mm × 100 mm column is also used with the same gradient with only the flow rate changed. If the gradient had been exactly scaled, the analysis time would have been twice as long, but as illustrated, the resolution is adequate. Figure 7 shows an MRM chromatogram of the antibiotic mixture. The compounds are sufficiently separated even with a large sample volume injected on-column. Conditions are listed in Tables 1 and 2.

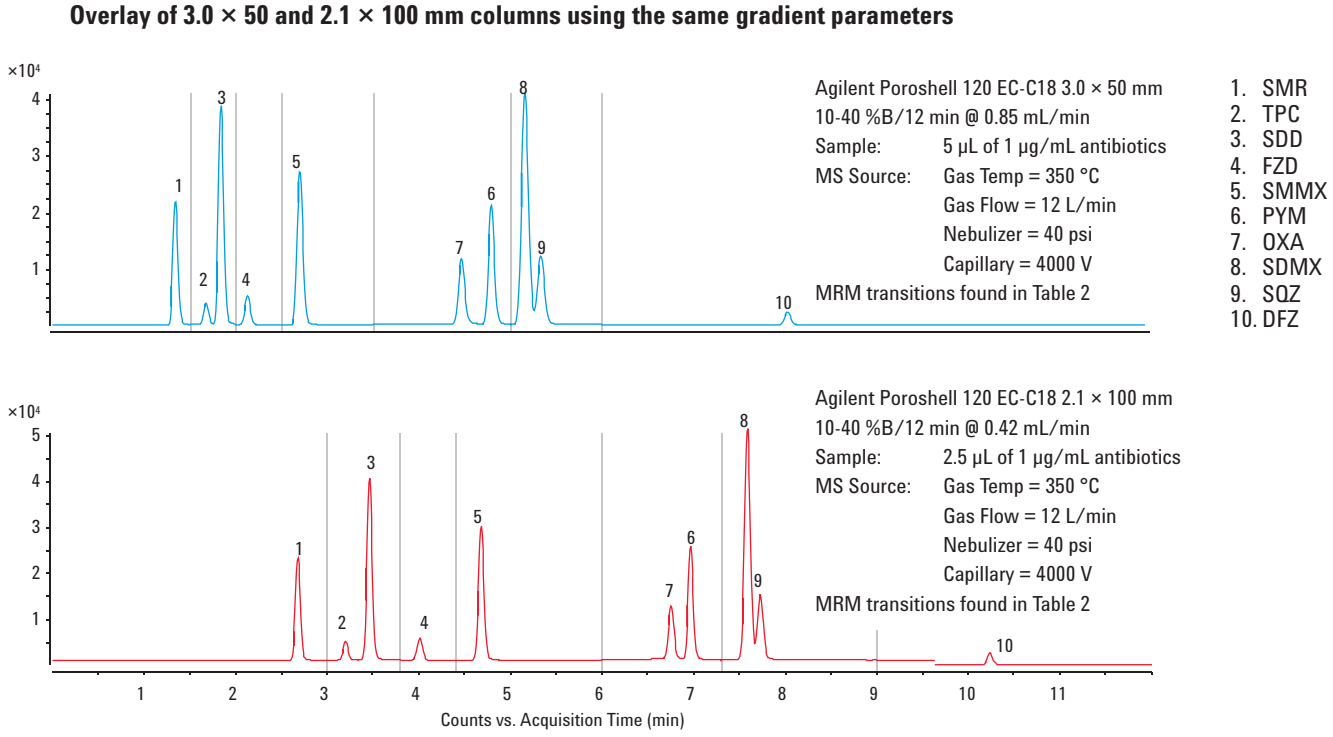
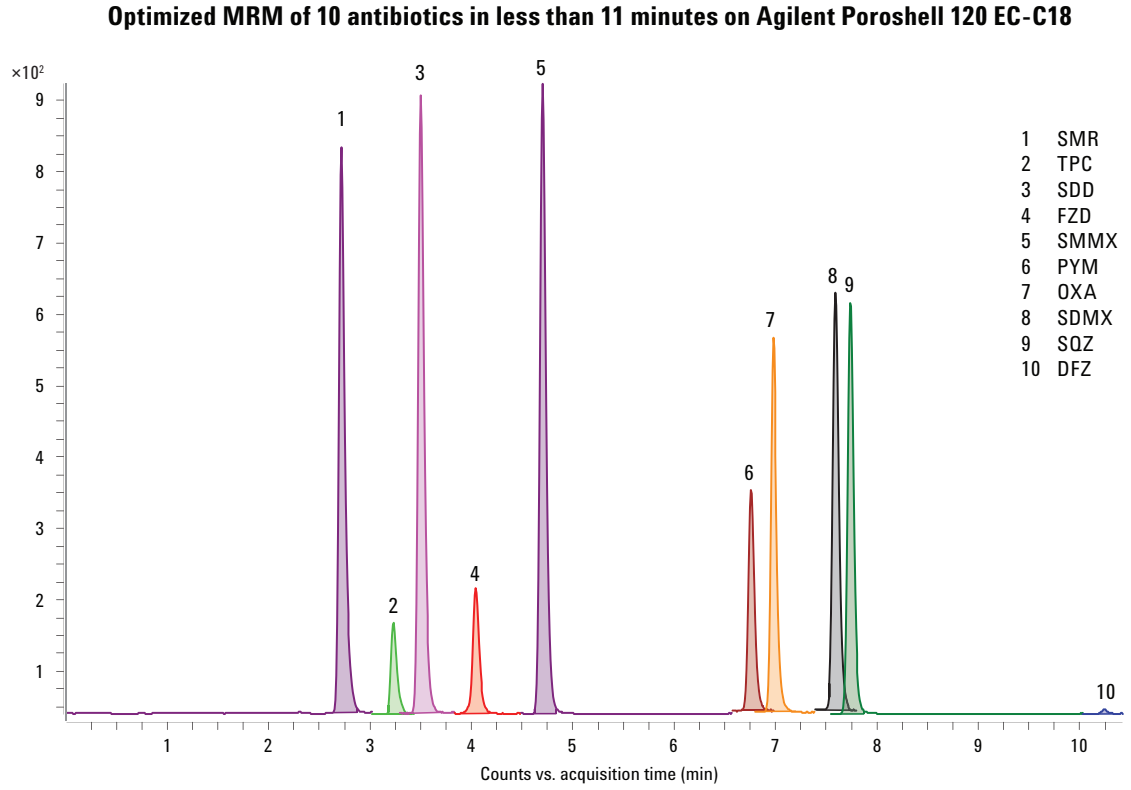


Figure 6. Total ion chromatograms of antibiotic mixture on 3 × 50 mm, and 2.1 × 100 mm Agilent Poroshell 120 EC-C18 columns.



Agilent Poroshell 120 EC-C18 2.1 mm × 100 mm, 2.7 µm
10% CH_3CN at t_0, ramp to 40% CH_3CN in 12 min (buffer 10 mM NH_4HCO_2 pH 3.8 adjusted with concentrated formic acid), 0.42 mL/min
Sample: 10 uL of 10 ng/mL antibiotics
using dynamic MRM mode on MS/MS

Figure 7. Dynamic MRM of antibiotic mixture on Agilent Poroshell 120.

Conclusions

This work shows that in method migration, modern colums and fast liquid chromatographs make it easier to start fresh. Using a generic gradient on short columns, 10 mobile phase combinations are quickly evaluated. Following basic scaling equations, a method can easily be transferred to a column of another dimension. By optimizing the mobile phase using a UV detector, the method is partially developed on an instrument that may be commonly used in a lab rather than the more expensive and possibly less available instrument that the method will be transferred to.

Poroshell 120 columns are good to use for LC/MS of complex samples at low pressure. Regardless of the analytical power of the triple quadrupole mass spectrometer, a better separation simplifies data analysis, which may shorten cycle time. Baseline separated compounds also allow the mass spectrometer to maximize dwell time for a given peak to yield more accurate and reproducible results. This ensures the best possible quantitation. Additionally, less chance of ion suppression is possible caused by coeluting compounds.

Several additional factors are also demonstrated. Optimal conditions for this mixture are found using the fast scouting method in acetonitrile ammonium formate buffer pH 3.8 (8 min). The analysis also works in methanol with pH 4.8, ammonium acetate (13 min). This could easily be shortened by changing the gradient to elute the last peak more quickly. For example, ramp organic more quickly at the end with a second step; however this would increase pressure further. The use of a "true buffer" such as 10 mM ammonium formate provides better peak shape for bases than a buffering solution such as 0.1 % formic acid at similar pH. The method as shown is chromatographically optimized and work is in progress to optimize detection conditions.

References

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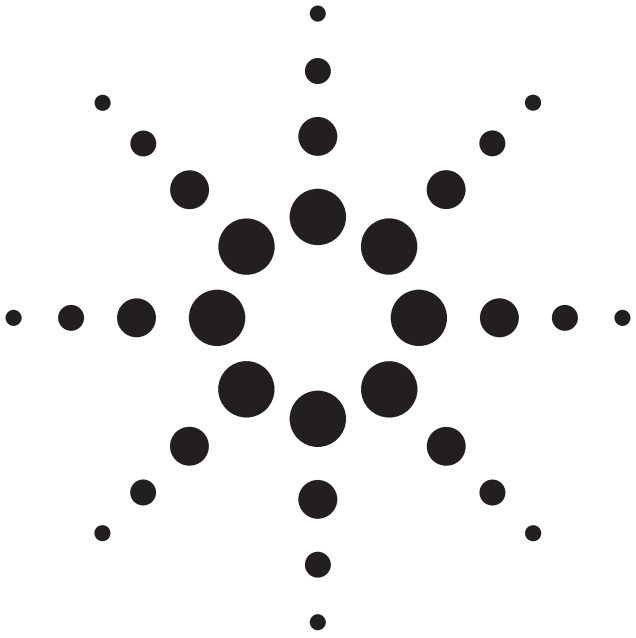

化工分析

采用Poroshell 120色谱柱分析纺织品中禁用芳香胺化合物

应用领域

化工分析

关键词

高效液相色谱，Poroshell 120色谱柱，联苯胺，4-氨基联苯，3-二甲氧基联苯胺

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摘要

禁用偶氮染料检测是所有生态纺织检验中开展最早和最广泛得到认可和重视的一个项目，我国2006年开始实施的《纺织品安全技术规范》中明确要求对此项目进行检测。GB/T 17592-2006使用高效液相色谱法对这些物质进行检测时，需要的分析时间比较长，且有机试剂耗用量大，因此，快速液相色谱仪和快速液相分析柱应运而生没事的分析时间和试剂耗用量大大降低。但针对目前基层实验室常规液相色谱仪的普及，与快速液相分析柱的不兼容性，使得快速分析检测很难实现。安捷伦公司最新推出的Poroshell 120系列表面多孔层色谱柱，由于其具有低反压，高柱效的特点，从而真正实现在常规液相色谱仪上进行快速分析的可操作性。本文使用Poroshell 120色谱柱，并采用常规液相色谱仪，对纺织品禁用偶氮染料当中联苯胺，4-氨基联苯，3，3-二甲氧基联苯胺的检测方法加以改进，分析时间和实际耗用量均降低了2/3，现将实验结果报告如下，以供参考。

1 试验材料与方法

1.1 仪器与试剂

Agilent 1200高效液相色谱仪，HPLC级甲醇，Milli-Q制得得超纯水，联苯胺，4-氨基联苯，3，3-二甲氧基联苯胺标准品。

1.2色谱条件

Poroshell 120 EC-C18柱（4.6mm*100mm,2.7μm）；柱温40℃；流速1.0~1.3mL、min；流动相A：甲醇，流动相B:水；紫外检测波长：240nm, 280nm, 305nm；进样体积5μL。

1.3 试验样品

经印染加工的纺织产品

1.4 试验方法

1.4.1 标准储备液的配制

准确称取适量联苯胺，4-氨基联苯，3，3-二甲氧基联苯胺标准品，并用甲醇定容25mL,使成为600mg/L的标准储备液，4℃冷藏保存。

1.4.2 混合标准工作液

分别吸取1mL储备液，用甲醇定容置10mL。分别移取不同体积用甲醇稀释配成浓度分别为3mg/L、4mg/L、6mg/L、12mg/L、30mg/L、60mg/L的混合标准工作液。

1.4.3 样品处理

称取1g样品（精确至0.01g)于反应管中，加16mL0.06mol/L,pH=6.0的柠檬酸盐缓冲盐溶液，70℃恒温水浴震荡30min，加入3mL200mg/mL的连二亚硫酸钠水溶液，70℃恒温水浴震荡30min。将反应液全部倒入硅藻土提取柱中，用4X20mL乙醚洗涤提取，提取液浓缩至1mL，氮吹至近干。准确移取1mL甲醇至圆底烧瓶，洗涤烧瓶壁，静置，移取洗涤液至分析样品瓶中，待HPLC分析测试用。

1.4.4 测定

按1.2操作进行，以保留时间定性，外标法定量。

2 结果与讨论

2.1 色谱条件的选择

以Waters C18柱（4.6mm*250mm,5μm）作为分析柱，以1.0-1.3ml/min0.575g/L磷酸二氢胺+0.7g/L磷酸氢二钠+100mL甲醇-甲醇和甲醇-水作为流动相进行HPLC条件的优化。实验发现，以甲醇-水作为流动相梯度洗脱分析联苯胺，4-氨基联苯，3，3-二甲氧基联苯胺可实现完全分离，分析时间缩短为45min，比标准方法缩短了25min，而且甲醇-磷酸盐作为流动相，柱压相对较高，需要配制流动相比较麻烦。以同样的洗脱方法选用Poroshell 120柱（4.6mm*100mm,2.7μm）分析联苯胺， 4-氨基联苯，3，3-二甲氧基联苯胺，分析时间只需12min。故选用Poroshell 120柱，甲醇-水作为流动相操作简单，大大提高工作效率的同时节省了有机溶剂。

2.2 快速分析方法

色谱条件：

Agilent 1200液相,DAD检测器，标准流通池

色谱柱：Poroshell 120(4.6mm*100mm,2.7μm)

柱温：40℃

进样量：5μL

流动相：

时间（min）	流动相A：甲醇（%）	流动相B：水（%）	流速（ml/min）
0.00	35	65	1.000
2.00	40	60	1.000
7.00	75	25	1.300
9.00	35	65	1.300
10.00	35	65	1.000

分析谱图：

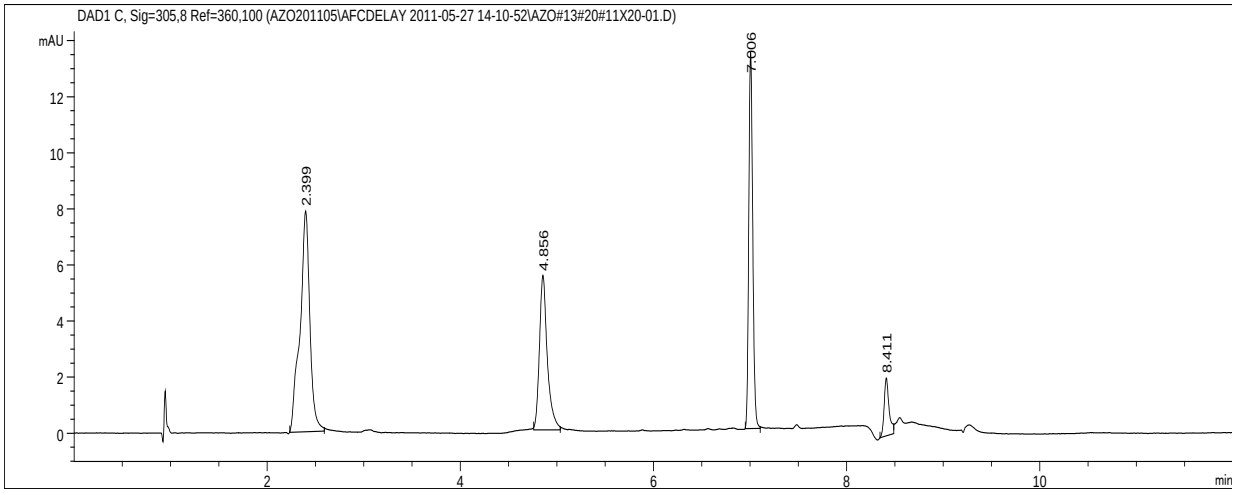

图1 联苯胺，4-氨基联苯，3，3-二甲氧基联苯胺Poroshell 120色谱图

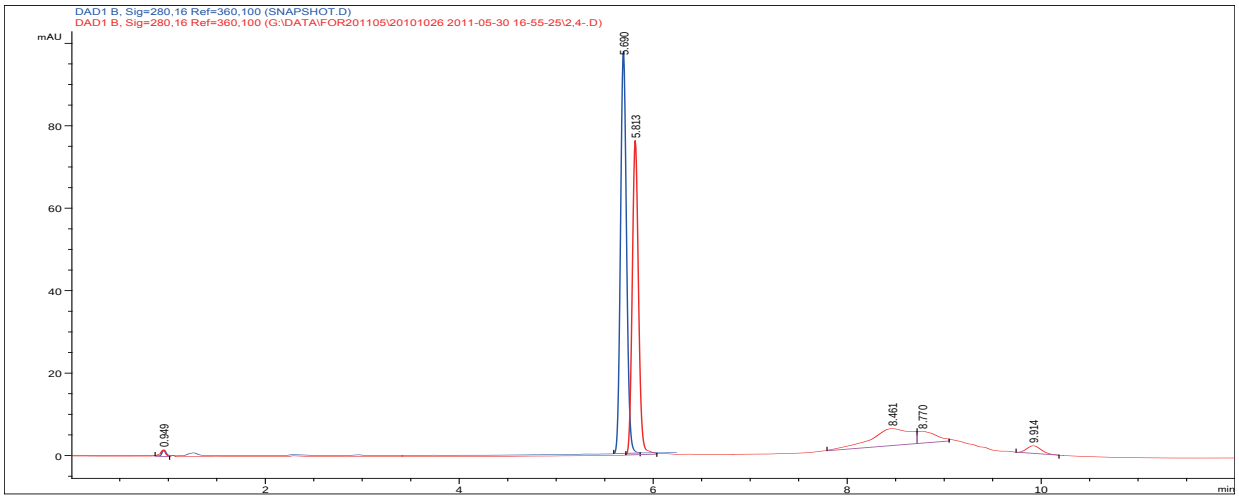

图2 24-二甲基苯胺，2，6-二甲基苯胺Poroshell 120色谱图

2.3 使用传统色谱柱的结果

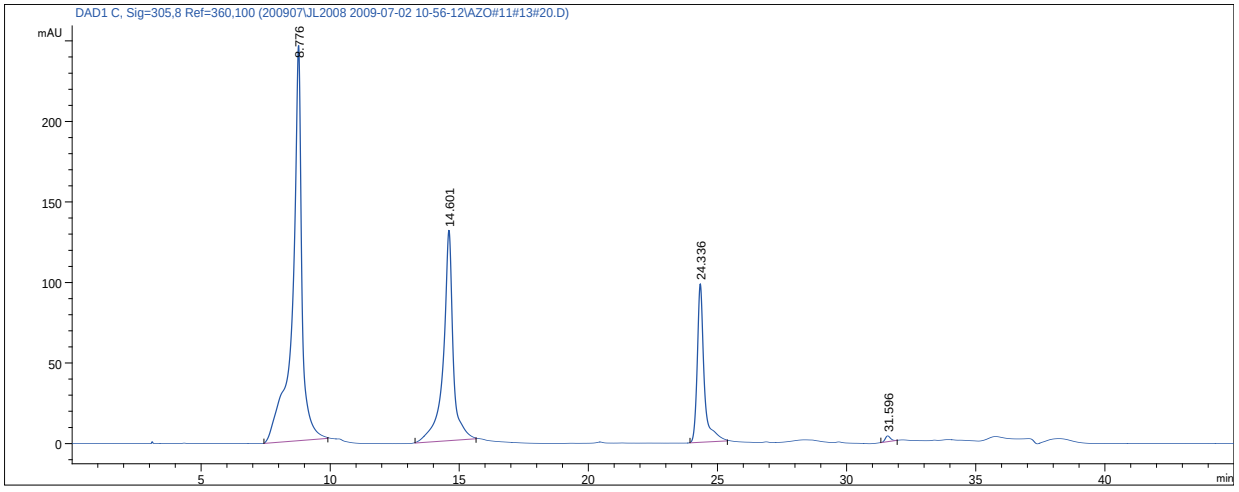

图3 联苯胺，4-氨基联苯，3，3-二甲氧基联苯胺Waters C18色谱图

色谱条件：

Agilent 1200液相,DAD检测器，标准流通池

色谱柱：Waters C18(4.6mm*250mm,5 μ m)

柱温：40℃

进样量：20 μ L

流动相：

时间（min）	流动相A：甲醇（%）	流动相B：水（%）	流速（ml/min）
0.00	35	65	1.000
11.00	40	60	1.300
35.00	75	25	1.300
40.00	35	65	1.000

3小结

由分析结果可见，使用常规液相色谱柱，在分析禁用芳香胺化合物时运行一个样品需要45min,而使用Poroshell 120柱仅需12min即可满足分析要求，节约了2/3时间，并确保2，4-二甲基苯胺，2，6-二甲基苯胺异构体基本分离度，在完全不影响分离度的前提下，大大节约了分析时间和试剂。

使用安捷伦 Poroshell 120 EC-C18 色谱柱对染发剂进行快速分析

Application Note

Cosmetics

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Abstract

该方法是为在 Agilent 1200 SL 系列 LC 系统中使用安捷伦 Poroshell 120 EC-C18 色谱柱分离染发剂中常见的 17 种成分而开发的。所有成分可在 10 分钟内完成分离。对两种商业染发剂进行了分析，以测试本方法对复杂样品的处理能力。

Introduction

Hair dyes are used by people all over the world. Commonly used hair dyes contain modified aromatic aniline and phenolic compounds that may cause allergic reactions or cancer. Due to these potentially harmful effects, the amounts of these compounds are restricted in many countries.

Methods for the quantitative measurement of these compounds in hair dyes include GC, GC/MS, LC and LC/MS [1]. HPLC methods are popular because they can analyze compounds that are not thermally stable, but are strongly polar with low volatility.

The Agilent Poroshell 120 EC-C18, 2.7 µm columns are packed with superficially porous materials. These columns have almost the same efficiency as that for sub-2-µm totally porous materials and provide similar fast and high resolution analyses. For this application, a gradient method was developed on an Agilent Poroshell 120 EC-C18, 3.0 × 100 mm, 2.7 µm column. In this study, two commercial samples were analyzed and the dye compounds detected. Within 10 minutes, 17 compounds were well separated from other components in the hair dyes.

Experimental

The Agilent 1200 Series SL LC System includes a binary pump, a thermostatted column compartment (TCC), a high performance autosampler and a diode array detector (DAD). The column used in the application is an Agilent Poroshell 120 EC-C18 3.0 × 100 mm, 2.7 µm (p/n 695975-302).

The following compounds were separated: p-phenylenediamine, p-aminophenol, 3-aminophenol, 1,3-benzenediol, 2-methylresorcinol, 2-chloro-1,4-diamino-benzol-sulfat, 2-nitro-1,4-penylenediamine, 5-amino-o-cresol, 2-amino-5-methylphenol, 4-amino-3-nitophenol, o-phenylene-diamine, 6-hydroxyindole, 4,n,n-diethy-2-methyl-p-phenylene-diamine, 1,5-dihydroxy-naphthalin 2,7-dihydroxy-naphthalin, n-phenyle-1,4-phenylene-diamine, 1-naphthol, o-aminophenol.

Each compound was prepared in methanol at 10 mg/mL, mixed together, and diluted down to a level of 0.05 mg/mL each with 2 g/L sodium hydrogen sulfite solution.

The two samples were purchased locally and designated A and B. A 0.5-g amount of each sample was extracted using 10 mL of acetonitrile, then placed in an ultrasonic bath for 10 minutes. The solution was then filtered through a 0.2-µm regenerated cellulose filter. The filtered solution was transferred to an auto sampler vial for HPLC analysis.

Discussion

Chromatographers use sub-2-µm particle columns for fast HPLC analysis because they achieve excellent results. A UHPLC with a pressure limit of greater than 400 bar is often used for fast, high resolution analysis with sub-2-µm particle columns. However, Agilent Poroshell 120 columns have 2.7-µm superficially porous particles that provide similar efficiency to the sub-2-µm columns but at 40–50% less back pressure, at below 400 bar. This makes them suitable for HPLC instruments. These are achievable results because superficially porous particles have shorter diffusion paths and a much tighter particle size distribution than totally porous particles. These two particle features mean the small, superficially porous particles generate high efficiency, similar to the efficiency of sub-2-µm columns.

Figure 1 shows the separation of 17 potential hair dye standard components on an Agilent Poroshell 120 EC-C18 column in a 10-min gradient. Reasonable resolution is achieved between all the sample components as well as some minor impurities present in the sample. It is likely that the impurities were oxidative degradation products of the major compounds that were not sufficiently stabilized by the addition of the sodium hydrogen sulfite solution.

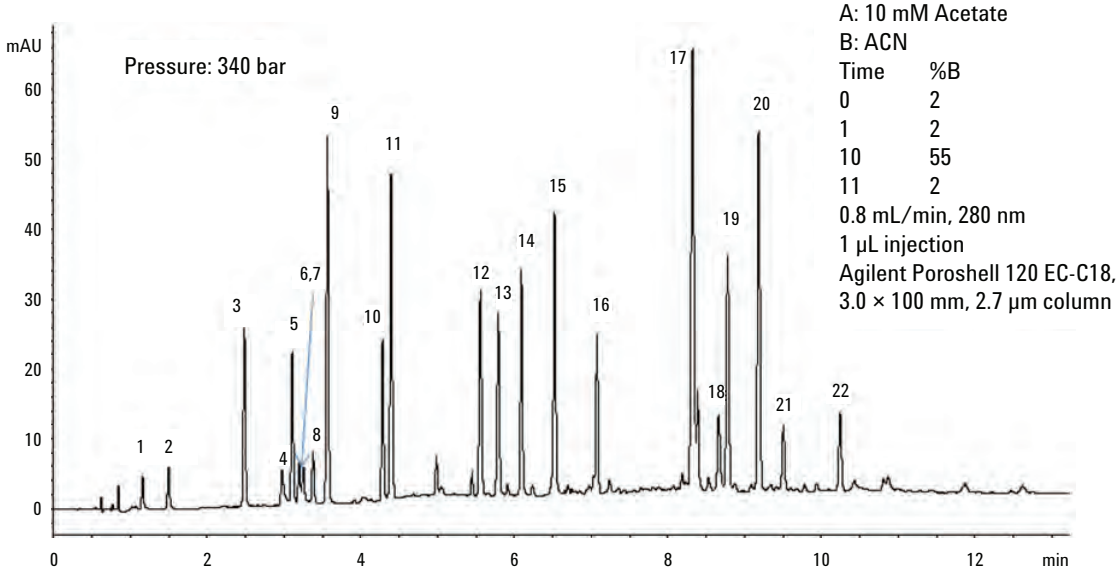

Figure 1. Standards chromatogram using Agilent Poroshell 120 EC-C18, 2.7 µm column. Standards: 1. p-phenylenediamine 2. p-aminophenol 3. 3-aminophenol 4. o-phenylenediamine 5. 1,3-benzenediol 6.unknown 7. 2-chloro-1,4-diamino-benzol-sulfat 8. 2-methylresorcinol 9. 2-nitro-1,4-penylenediamine 10. 5-amino-o-cresol 11. 4-amino-3-nitophenol 12. 6-hydroxyindole 13. 1,5-dihydroxy-naphthalin 14. 2,7-dihydroxy-naphthalin 15. unknown 16. unknown 17. n-phenyle-1,4-phenylene-diamine 18. 2-amino-5-methylphenol 19. 1-naphthol 20. o-aminophenol 21. unknown 22. unknown.

A 3.0 mm internal diameter column was used for this separation at a flow rate of 0.8 mL/min, which is almost twice the normal flow rate of the 0.4–0.5 mL/min typically used with this column ID. This produced a fast separation with no compromise in performance, due to the fast diffusion in the particles. Many may relate this to the flat region of the Van-Deemter curve at high flow rates – the "C" term. The Poroshell 120 2.7 µm particles have a Van-Deemter curve similar to columns with 1.8 µm particles. The performance of the Poroshell column does not decrease at high flow rate, compared to that of columns with 1.8 µm packing, but has lower pressure (340 bar), which is compatible with that of the 400-bar instruments. An example Van Deemter curve is shown in Figure 3.

Figure 2 shows an overlay of the chromatograms for Samples A and B and the standards. Only compound 12 (6-hydroxyindole) was found in Sample A and compounds 1 (p-phenylenediamine), 3 (3-aminophenol) and 5 (1,3-benzenediol) were detected in Sample B. Different components are used in hair dyes produced by different manufacturers. The amounts of detected compounds can be measured given the concentration of standards.

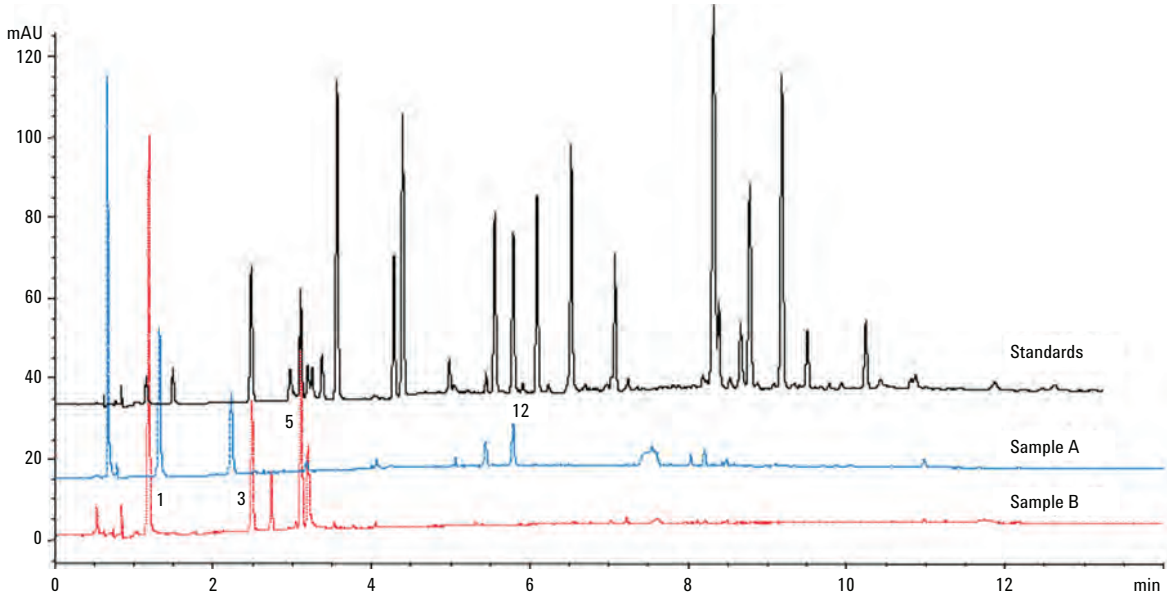

Figure 2. Chromatogram overlay for samples and standard components.

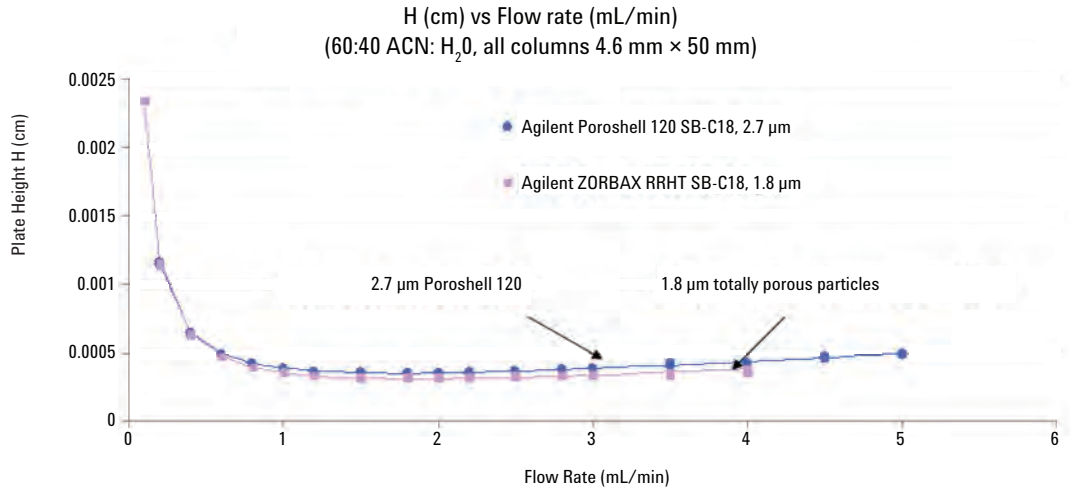

Figure 3. Van Deemter curve of Agilent Poroshell 120 column versus totally porous 1.8 µm particle columns.

Conclusion

The method developed for the separation of hair dye components on the new Agilent Poroshell 120 EC-C18 column provides good resolution and is suitable for fast screening of these compounds. The superficially porous 2.7 µm particle columns provide similar performance to the totally porous sub-2-µm columns but with lower pressure. Due to the low pressure, a 400-bar instrument can run this method. A higher flow rate allows faster separations on a UHPLC, up to the 600-bar pressure limit of the column.

Reference

1. "Determination of 22 components in hair dyes by high performance liquid chromatography," Chinese Journal of Chromatography, 26(5): 554-558.

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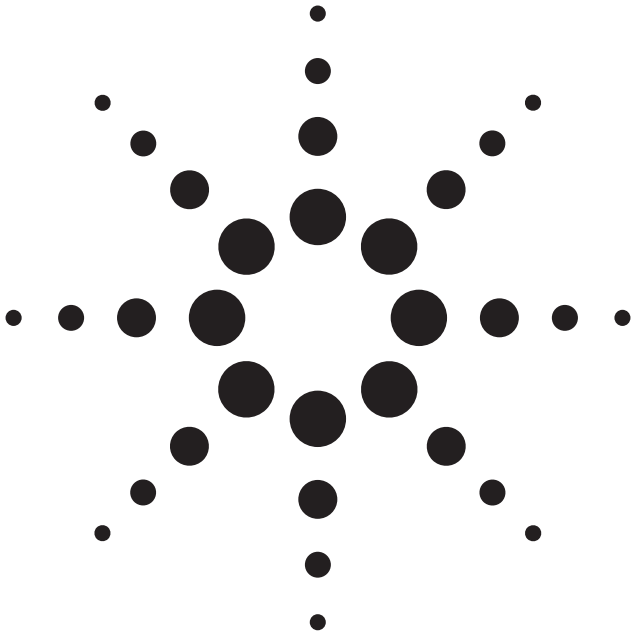

环境分析

采用Poroshell 120色谱柱快速分析24种偶氮化合物

应用领域

环境分析

关键词

HPLC；Poroshell 120；偶氮染料

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摘要

偶氮染料是纺织品印染工业最常用的一类合成染料，其分子结构上有两个互相以双键连接的氮原子，故称为偶氮；常用于天然或合成纤维的印花或染色；也可用于油漆、塑料和橡胶等的着色。在特殊条件下，它能分解产生20多种致癌芳香胺，经过活化作用改变人体的DNA结构引起病变和诱发癌症。因此，生活中的大部份日用品，如纺织品、鞋、皮革等都要求对部份偶氮染料进行检测。本文主要探讨的是欧盟官方所公布的EN14362标准[1,2]中的22种偶氮染料加上两种内标化合物，总共24种偶氮的液相分离方法，即采用了安捷伦最新推出的Poroshell 120 EC-C18色谱柱在240 nm吸收处高效分离24种偶氮染料，基本实现基线分离。

1 试验材料与方法

1.1 仪器与试剂

1290高效液相色谱仪(Agilent公司)；甲醇(HPLC级,Dikam)；水由Milli-Q净化系统制得；磷酸二氢铵(北京化学试剂厂，优级纯)，磷酸氢二钠(北京化学试剂厂，优级纯)，磷酸(DikmaPure, 99.5%)。目标化合物纯度及相关信息见表1，所有试剂均购自百灵威化学技术有限公司，纯度均在98%以上。样品浓度12 ug/mL。

表1 偶氮染料化合物列表

编号	英文名	中文名	CAS号
1	1,4-phenylendiamine	对苯二胺	106-50-3
2	4-methoxy-m-phenylenediamine	2,4-二氨基苯甲醚	615-05-4
3	4-methyl-m-phenylenediamine	2，4-二氨基甲苯	95-80-7
4	Aniline	苯胺	62-53-3
5	benzidine	联苯胺	92-87-5
6	o-anisidine 2-methoxyaniline	2-甲氧基苯胺	90-04-0
7	4,4'-oxydianiline	4,4′–二氨基二苯醚	101-80-4
8	o-toluidine 2-aminotoluene	邻甲苯胺	95-53-4
9	4-chloroaniline	4-氯苯胺	106-47-8
10	5-nitro-o-toluidine	5-硝基邻甲苯胺	99-55-8
11	4,4'-methylenedianiline 4,4'-diaminodiphenylmethane	4，4-二氨基二苯甲烷	101-77-9
12	6-methoxy-m-toluidine p-cresidine	2-甲氧基-5-甲基苯氨 对克力西丁	120-71-8
13	2,6-dimethylaniline	2,6-二甲基苯胺	87-62-7
14	2,4-dimethylaniline	2,4-二甲基苯胺	95-68-1
15	3,3'-dimethoxybenzidine o-dianisidine	3,3′-二甲氧基联苯胺	119-90-4
16	3,3'-dimethylbenzidine 4,4'-bi-o-toluidine	3,3′-二甲基联苯胺	119-93-7
17	4,4'-thiodianiline	4,4′–二氨基二苯硫醚	139-65-1
18	2-naphthylamine	2-萘胺	91-59-8
19	4-chloro-o-toluidine	4-氯邻甲苯胺	95-69-2
20	2,4,5-trimethylaniline	2，4，5-三甲基苯胺	137-17-7
21	4,4'-methylenedi-o-toluidine	2，4-二甲基苯胺	838-88-0
22	biphenyl-4-ylamine 4-aminobiphenylxenylamine	4-氨基联苯乙胺	92-67-1
23	3,3'-dichlorobenzidine 3,3'-dichlorobiphenyl-4,4'-ylenediamine	3,3-二氯联苯胺	91-94-1
24	4-aminoazobenzene	4-氨基偶氮苯	60-09-3
25	4,4'-methylene-bis-(2-chloro-aniline) 2,2'-dichloro-4.4′-methylene-dianiline	2,2'-二氯-4.4′-亚甲基-双苯胺	101-14-4
26	o-aminoazotoluene 4-amino-2',3-dimethylazobenzene 4-o-tolylazo-o-toluidine	邻氨基偶氮甲苯	97-56-3

1.2 色谱条件

色谱柱：Poroshell 120 EC-C18柱(3.0 mm i. d. ×150 mm, 2.7 μ m)；柱温：40℃；流速：0.8 mL /min；流动相A配制如下：0.575 g磷酸二氢铵，0.7 g磷酸氢二钠溶于900 mL水后加100mL甲醇充分混合后，用磷酸调节pH=6.9 流动相B为甲醇，检测波长： 240nm; 280nm; 305 nm，无参比。 进样体积： 1 uL。

1.3 样品的配制

单标及混合标样的配制准确称取偶氮各固体标样，并用甲醇及流动相以10:90混合定容，使之成为12 ug/mL的标准储备液，4℃避光条件下保存。

2 结果与讨论

2.1 色谱柱及色谱条件的选择

实验过程中，分别尝试了Poroshell 120 EC-C18及Poroshell 120 SB-C18两种不同键合相的色谱柱，发现EC-C18比SB-C18选择性更合适，故最终选择Poroshell 120 EC-C18色谱柱，规格为3.0 mm i. d. ×150 mm, 2.7 μ m。经过梯度及流速的优化，最终所选择的流速为0.8 mL/min；在偶氮的分离中，4，4-二氨基二苯甲烷、2-甲氧基-5-甲基苯氨、2,6-二甲基苯胺、2,4-二甲基苯胺、3,3′-二甲氧基联苯胺、3,3′-二甲基联苯胺这六个化合物是24种偶氮分离的难点，实验过程中发现，该六个化合物对梯度的优化极其敏感，但同时峰的扩散也严重影响后四个化合物的分离度，因此，将流速设置在0.8 mL/min（3.0mm内径的色谱柱），可以显著减小峰的扩散，并结合梯度的优化，可以将该六个化合物实现较理想的分离(见图1)。最终采用的梯度表如下：

Time	B%
0	14
4.5	29
8	29
9	50
10.5	65
12	90
14	100
15	14

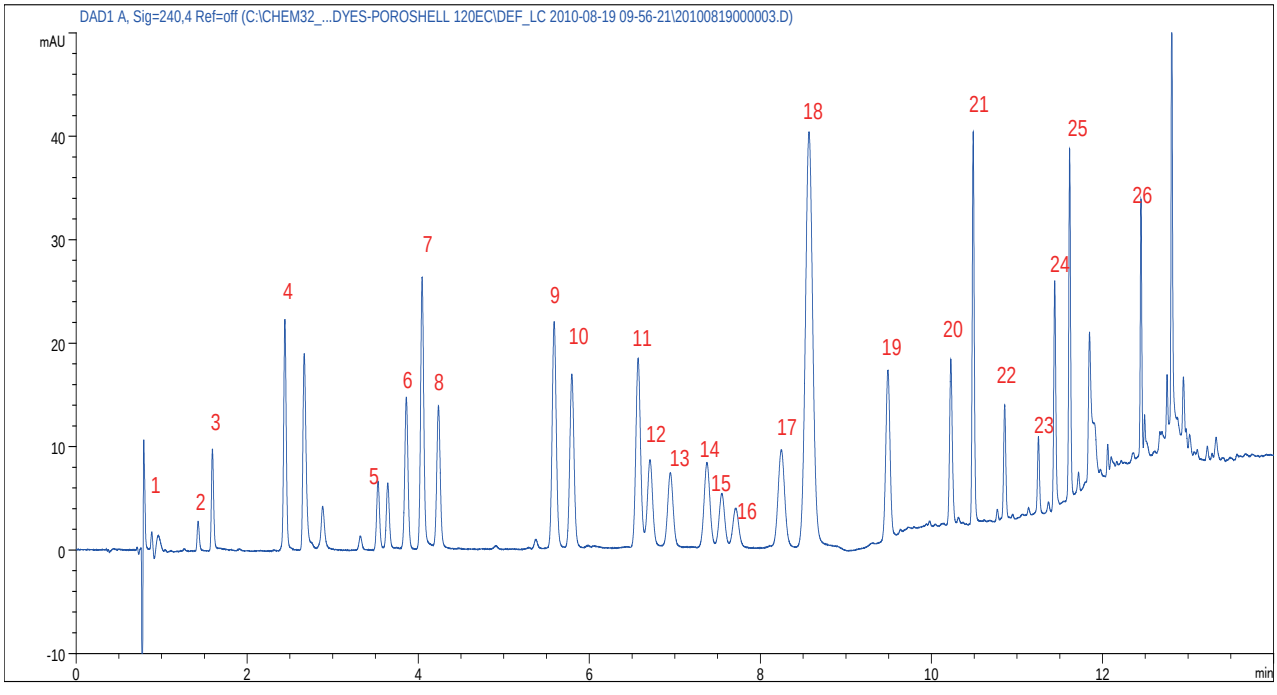

图1 24种偶氮的标准色谱图（浓度均为12 μ g/mL）

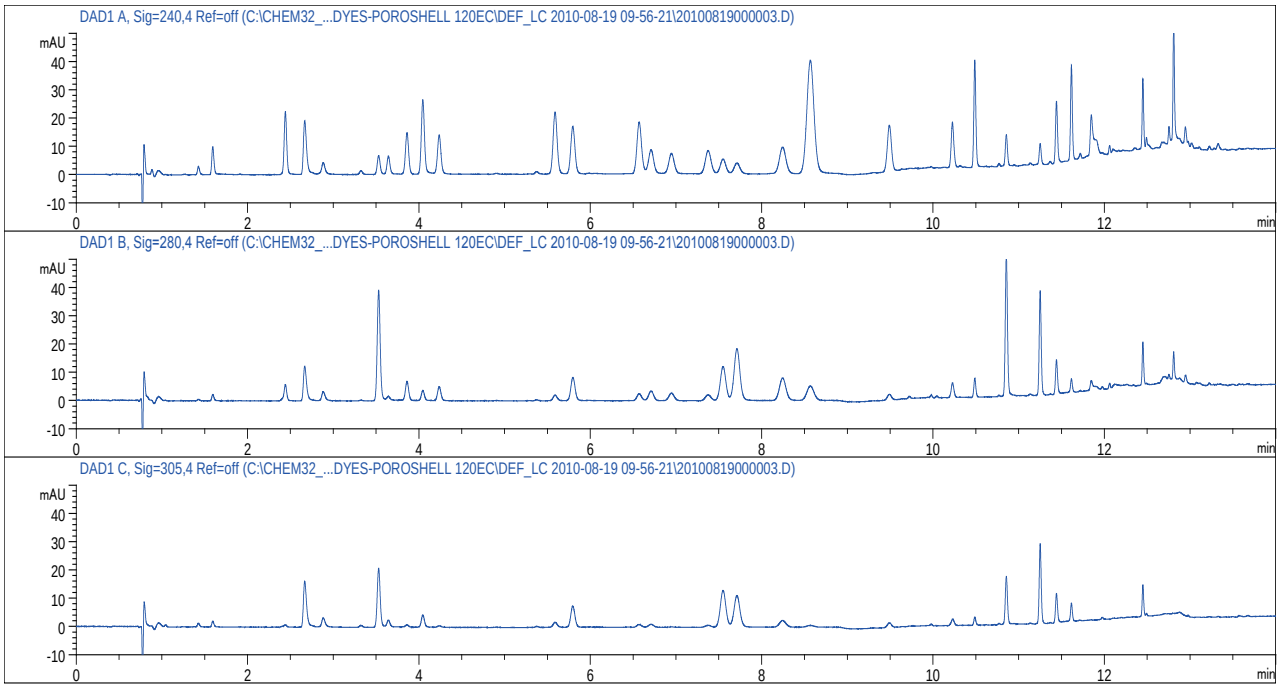

图2 不同波长(240 nm, 280 nm和305 nm)波长下的紫外吸收谱图

2.2 方法的兼容性及重复性讨论

本文采用Poroshell120 EC-C18 (3.0mm, 150mm, 2.7 um)，该色谱柱为表面多孔层柱，其2.7 um粒径填料的柱效可以与1.8 um的全多孔柱相媲美，因此，采用了150 mm长的色谱柱，其相当于常规5 um色谱柱 150mm长柱效的3倍，这也是本实验可实现高分离度的原因之一。同时，由于填料粒径较1.8 um大将近 1um，因此，在实现高分离度的同时，色谱柱的反压却比同样规格的1.8 um全多孔柱降低了近40%。实验中，压力最高为580bar，起始及结束的压力为487bar，使得该实验可以在当今任何一台能耐受600bar的UHPLC上重现。以下为实验过程中六针进样的叠加谱图，其中考察了重新配置流动相、连续进样及隔天进样的保留时间重现性)，从谱图的叠加可以看出，保留时间及峰面积重现性优异。

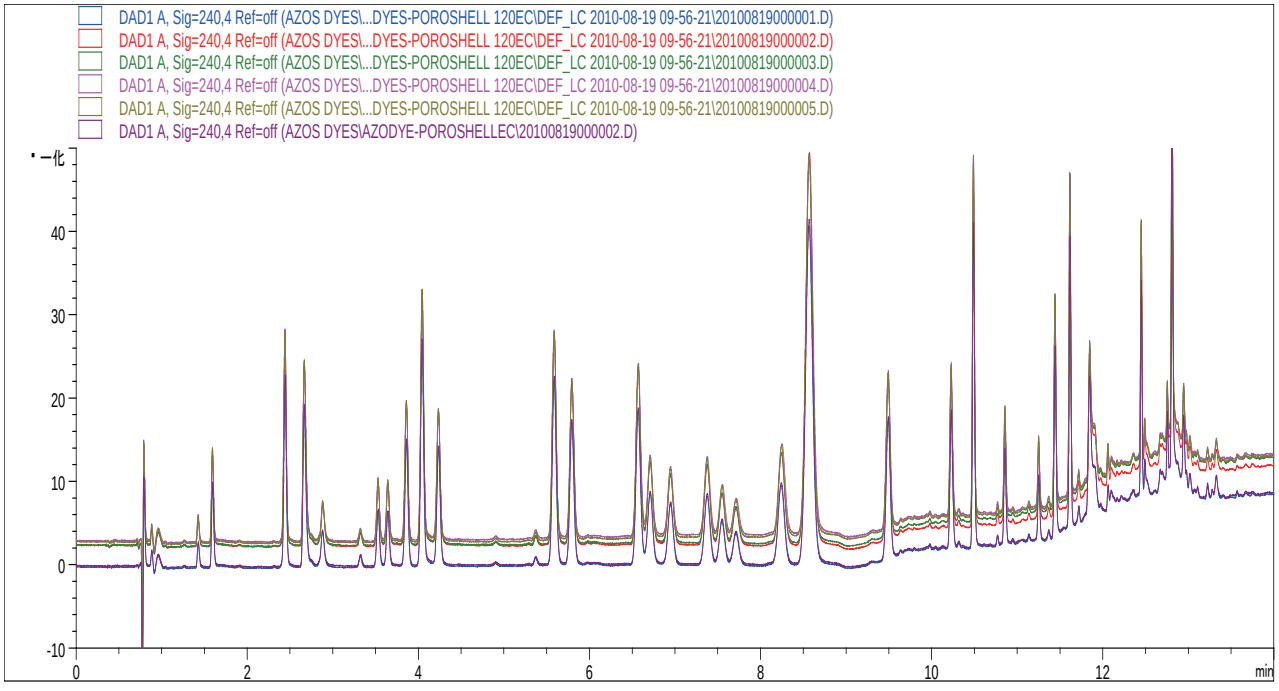

图2 六针谱图的叠加图

3 小结

采用Poroshell 120 EC-C18, 3.0mm, 150mm, 2.7 um色谱柱高效分离24种偶氮染料，对大部份的目标物实现了基线分离，该方法配合耐压600bar的UHPLC液相系统，具有优异的方法重现性。

[参考文献]

[1] EN 14362-1-2003 纺织品 从偶氨着色剂衍化的某些芳族胺的测定方法.
[2] EN 14362-2-2003 纺织品 从偶氨着色剂衍化的某些芳族胺的测定方法.

Trace analysis of explosives in soil samples using the Agilent 1290 Infinity LC System equipped with an Agilent Max-Light 60-mm cartridge cell

Application Note

Environmental

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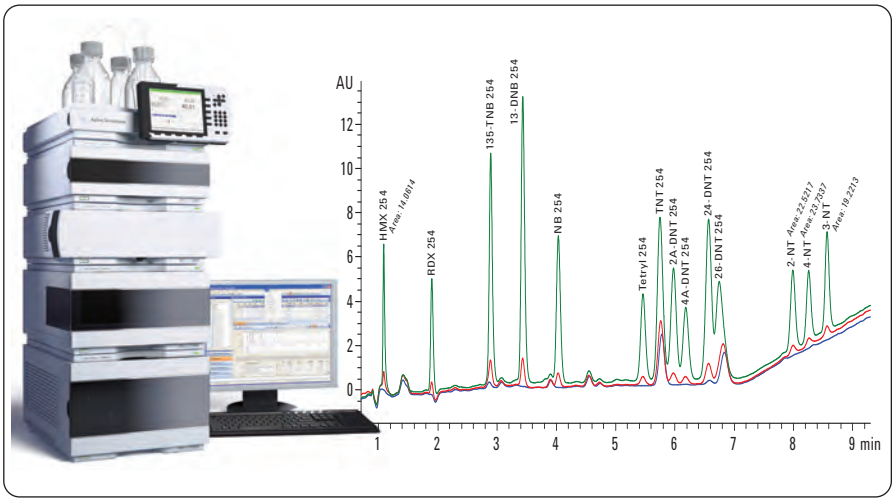

Abstract

This Application Note describes how the Agilent 1290 Infinity LC system equipped with the Agilent Max-Light 60 mm Diode Array Detector cartridge cell was used for residue analyses of nitroaromatic explosives in soil. Excellent sensitivity can be achieved with detection limits below 1 µg/L for standard solutions. Chromatographic selectivity for the structurally related compounds was obtained using an Agilent Poroshell 120 EC-C18 column operated at 44 °C. The developed method is validated and applied to a set of soil samples.

Introduction

Explosive residues are found in groundwater, sediment, and soil that have been contaminated by military or terrorist activities and civil activities such as mining and construction.
Trinitrotoluene (TNT) and its metabolites and hexogen (RDX) are the most commonly used explosives[1,2]. Bacteria in the soil transform TNT to the toxic and mutagenic metabolites 2-amino-4,6-dinitrotoluene (2A-DNT) and 4-amino-2,6-dinitrotoluene (4A-DNT).

The United States Environmental Protection Agency has published EPA Method 8330 for the analysis of nitroaromatics and nitramines[3,4]. The structures of the 14 compounds studied in this work are shown in Table 1. The determination of explosives in environmental samples is a challenging task. The inherent limited thermal stability of some of the explosives makes them unsuitable for GC analysis. Consequently, LC is the method of choice for these compounds. LC/MS using atmospheric pressure chemical ionization (APCI) is applicable for explosive residue analysis[2] because the sensitivity for certain explosives is as low as 1 to 10 ng/kg or ng/L. However, for some compounds the sensitivity is not within standards and the instrumental cost is high.

Explosive residues can be detected at relatively low levels with UV or DAD detectors. One of the disadvantages of using UV-based detection compared to mass selective detection is the lack of selectivity. This can be problematic because some of the compounds are structurally very similar, which makes it difficult to separate them chromatographically. The EPA Method 8330, therefore, recommends the use of two columns. A C18 phase is used as the primary column while an additional analysis on a CN phase is required for confirmation purposes. Coupling columns in series produces a combination of both stationary phases in the same analyses[5]. However, sample matrixes can vary significantly and produce interferences during sample analyses. Nevertheless, LC with UV detection (EPA 8330) is still the method of choice for this analysis.

This Application Note shows the advantages of using state-of-the-art equipment combined with a sensitive detector for the analysis of all explosives, especially those in soil samples. The analyses were performed using the Agilent Poroshell 120 column.

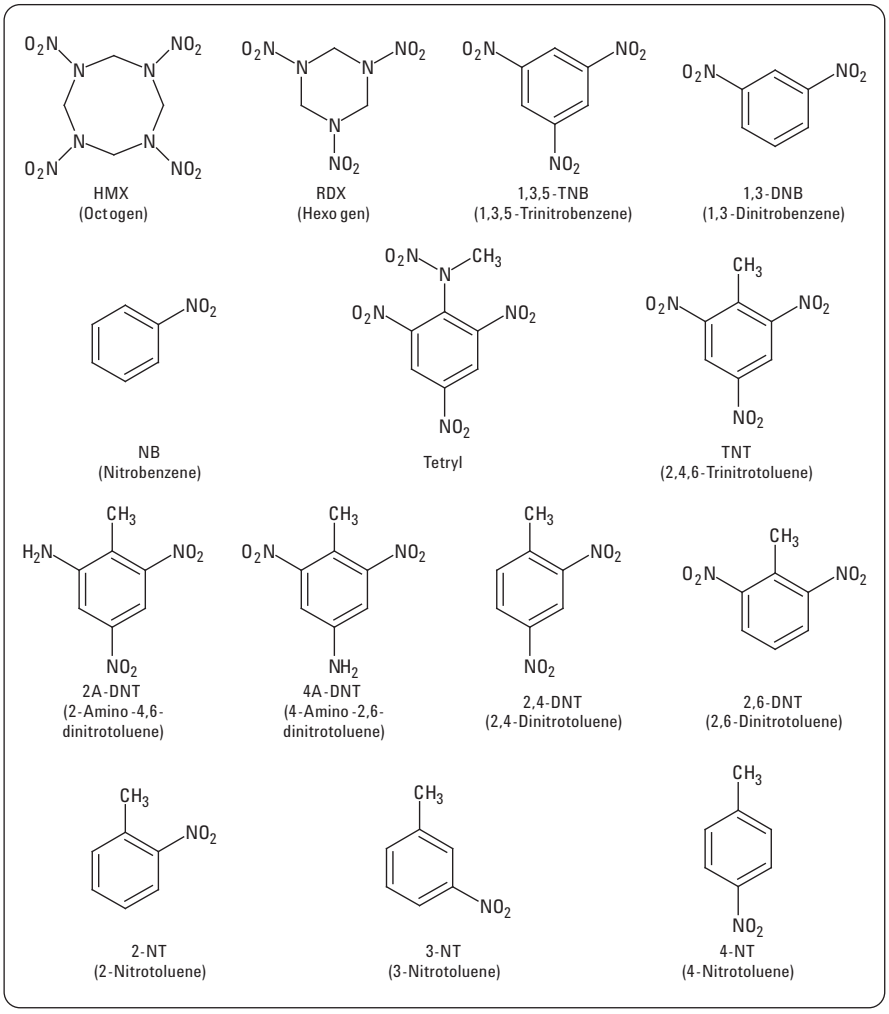

Table 1
Compounds listed in US EPA Method 8330.

Experimental

Chemicals and solutions

The explosive standard mixtures were purchased from Dr. Ehrenstorfer (Augsburg, Germany). The mixtures were prepared as 100 ng/mL of each in acetonitrile (Table 2).

All dilutions and sample solutions were acidified with 0.1% (v/v) formic acid to prevent degradation of tetryl. The final solvent for injection was a mixture of 0.1% formic acid in water/acetonitrile 75/25 (v/v).

Sample preparation

Dry soil samples were extracted as follows:

1. Weigh 2 g of soil in a 15 mL centrifuge tube.
2. Spike with explosives (optional).
3. Add 5 mL 0.1% formic acid in acetonitrile.
4. Shake for 10 s.
5. Ultrasonicate at room temperature for 15 min.
6. Store in refrigerator for 2 h.
7. Ultrasonocate at room temperature for 15 min.
8. Centrifuge.
9. Dilute 1 mL of the liquid with 3 mL of 0.1% formic acid in water.
10. Mix and filter through membrane filter (regenerated cellu lose, 0.45 µm).

Nitroaromate-Explosive Mix 1 (08330100)	Nitroaromate-Explosive Mix 2 (08330200)
1,3-DNB	2A-DNT
2,4-DNT	4A-DNT
RDX	2,6-DNT
NB	2-NT
HMX	3-NT
1,3,5-TNB	4-NT
TNT	Tetryl

Table 2
Sample solutions.

Chromatographic conditions

Method parameters:

Column	Agilent Poroshell 120 EC-C18, 100 mm x 2.1 mm, 2.7 µm (p/n 695775-902)	
Mobile phase	A=0.01% (v/v) formic acid in water B=methanol	
Flow rate	0.55 mL/min	
Gradient	0–2.5 min 2.5–6.5 min 6.5–11.5 min 11.5–13 min	20% to 28% B 28% to 30% B 30% to 70%B 20% B
Column temperature	44 °C	
Injection	20 µL, with needle wash (flushport, 5 s, water/methanol 1/1)	
Sample temperature	15 °C	
DAD	Signal A: 254/8 nm (quantification) Signal B: 234/8 nm (qualifier wavelength for confirmation) Peakwidth > 0.025 min	

Equipment

An Agilent 1290 Infinity LC system with the following configuration was used:

- 1290 Infinity Binary Pump with integrated vacuum degasser (G4220A)
- 1290 Infinity Standard Autosampler (G4226A)
- 1290 Infinity Thermostat (G1330B)
- 1290 Infinity Thermostatted Column Compartment (G1316C)
- 1290 Infinity Diode Array Detector (G4212A)
- Max-Light Cartridge High Sensitivity Cell (60 mm optical path length) (G4212-60007)
- Max-Light Cartridge Standard Cell (10 mm optical path length) (G4212-60008)

Results and discussion

Method optimization

The separation of the 14 compounds in a reasonable time is not straightforward because of structural similarities. Consequently, the choice of the stationary and mobile phase is important. The final chromatographic method is based on the method described in Technical Overview 5990-5552EN[6], but a gradient is used instead of the original isocratic method to analyze the soil matrix. A shallow gradient is applied for separation and elution of the explosives and a column rinsing step is incorporated at the end of the run. The total analysis time including column re-equilibration is 13 min.

Control of the column temperature is crucial for the separation of explosives 6 to 11[3,4,7]. A temperature of 44°C was best to achieve the desired selectivity. A small amount of formic acid was added to mobile phase A to ensure the stability of tetryl. The addition of the acid does not affect the retention of the investigated compounds but has an effect on the baseline shape during the gradient. Therefore, a low concentration of 0.01% (v/v) of formic acid was finally selected to obtain a relatively flat baseline.

Since extraction of the soil samples is carried out with acetonitrile, it is impossible to inject large volumes of the extract without sacrificing chromatographic efficiency and peak shape. The injection volume should be kept below 2 µL under the given analytical conditions. When larger volumes must be injected to increase sensitivity the acetonitrile is diluted with a weaker solvent, such as water. Diluting the samples three times with 0.1% formic acid in water permits the use of injection volumes of 20 µL and higher. For a 20 µL-injection of a diluted sample, the amount loaded on the column is increased 2.5 times compared to an injection of undiluted samples and solutions. An example of the influence of sample solvent and injection volume is shown in Figure 1.

Efforts were made to further concentrate the extract by evaporation under nitrogen and reconstitution in a smaller volume of injection solvent. Unfortunately, some of the more volatile explosives (such as nitrobenzene and nitrotoluenes) were lost during this step.

Method validation

The detection limit with a 20-µL injection was found to be approximately 0.5 ppb (µg/L) in the injected solution when the 60 mm flow cell was installed. This means that the LOD in soil samples should be around 5 ppb (µg/kg). The Max-Light Cartridge high

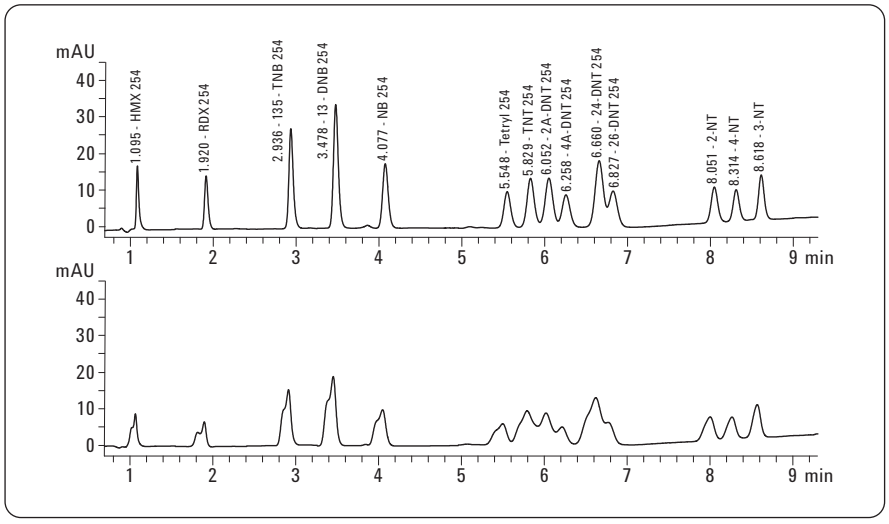

Figure 1
Comparison of an analysis of a standard solution in acetonitrile (500 ng/mL, 5 µL injected) – bottom chromatogram and in 0.1% formic acid in water/acetonitrile (125 ng/mL, 20 µL injected) – top chromatogram. In both analyses, the amount of each explosive on-column was 2.5 ng.

sensitivity flow cell has a significantly longer optical path length (60 mm) compared to the standard flow cell (10 mm) and should in theory improve the sensitivity by a factor of 6. The performance of both flow cells was compared and the results are summarized in Table 3. The signal-to-noise ratio is increased by a factor of approximately 4.5 for injection of a 5 ppb standard solution while the resolution remains unaffected. Consequently, the sensitivity will be about 5 times higher with the high sensitivity cell. This is confirmed in Table 3 when comparing the data for the analysis of a 5 ppb standard solution on a 10 mm flow cell and a 1 ppb standard solution on a 60 mm flow cell. With the standard 10 mm flow cell installed, several of the test compounds could not even be detected at the 1 ppb level. The influence of the increased path length on the signal for a 1 ppb standard solution is shown in Figure 2.

The repeatability of the method was investigated at three different levels. Standard solutions with a concentration of 1, 10, and 100 ppb were each injected eight consecutive times and the RSD was calculated. A calibration curve was constructed by single injections of the following standard solutions: 1, 2.5, 5, 10, 25, 50, 100, 250, and 500 ppb. The results are summarized in Table 4.

	10 mm, 5 ppb		60 mm, 5 ppb		60 mm, 1 ppb	
	S/N ratio	Resolution	S/N ratio	Resolution	S/N ratio	Resolution
Tetryl	4.5		19.5		3.5	
TNT	5.2	1.74	23.4	1.81	4.0	1.65
2A-DNT	5.5	1.30	24.3	1.36	4.7	1.32
4A-DNT	3.8	1.17	16.5	1.16	3.2	1.05
24-DNT	7.0	2.15	32.3	2.16	5.7	2.05
26-DNT	4.3	0.77	20.1	0.84	7.0	0.85

Table 3
Comparison of performance of the Agilent Max-Light 10 mm Cartridge and 60 mm flow cells.

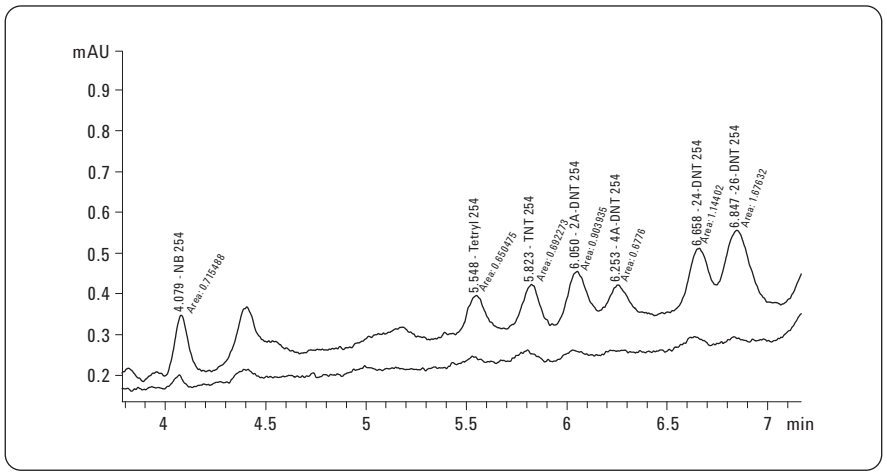

Figure 2
Detail of analysis of a 1 ppb standard solution with Max-Light Cartridge Standard Flow Cell (10 mm optical path length) – bottom chromatogram and with Max-Light Cartridge High Sensitivity Flow Cell (60 mm optical path length) – top chromatogram.

Compound	Repeatability (n=8), %RSD				Linearity (1-500 ppb) R^2
	Retention time 10 ppb	Area 1 ppb	Area 10 ppb	Area 100 ppb	
HMX	0.09	5.94	1.68	1.05	0.9994
RDX	0.11	5.96	1.91	0.88	0.9999
135-TNB	0.10	10.84	1.49	0.46	0.9995
13DNB	0.08	3.71	1.04	0.96	0.9995
NB	0.07	9.05	2.00	1.58	0.9996
Tetryl	0.09	10.85	1.63	1.64	0.9988
TNT	0.10	9.07	1.65	1.29	0.9992
2A-DNT	0.08	6.78	1.55	0.83	0.9994
4A-DNT	0.08	3.51	2.29	1.43	0.9992
24-DNT	0.08	6.78	1.84	1.17	0.9992
26-DNT	0.08	7.65	2.18	1.20	0.9996
2-NT	0.03	13.88	4.18	1.17	0.9997
4-NT	0.03	12.15	3.15	1.48	0.9998
3-NT	0.02	4.62	3.05	1.19	0.9995

Table 4
Method performance.

Sample analyses

Three different soil samples are analyzed before and after spiking with the explosives at the 50 and 500 ppb (µg/kg soil) level. After sample preparation and analysis, the result is compared to the injection of a 5 and 50 ppb (ng/mL) standard solution. The recovery for the spiked samples is calculated after the subtraction of peaks present in the non-spiked samples. The data are shown in Table 5. For most samples the recovery is within reasonable limits (for example, between 80 and 120%).

Some values, however, such as the 50 µg/kg spikes of TNT and 26-DNT are significantly higher than expected with interferences of the sample matrix. In the developed method, the DAD signal at 234 nm was additionally stored for confirmational purposes and the calibration was performed with this wavelength as well. When the detected peak corresponds with the respective explosive the calculated concentrations should be very similar for both wavelengths. This was not the case for the detected peaks in the soil samples in this study. Therefore it was concluded that these peaks are interferences of another nature.

This demonstrates the well known lack of selectivity of UV-based detectors compared to mass selective detection for very low concentrations of explosives. However, a DAD detector remains the first choice for expected concentrations in the range of approximately 100 µg/kg. An example of an analysis of nonspiked and spiked soil samples is shown in Figure 3.

	Spike 50 µg/kg			Spike 500 µg/kg		
Compound	**Soil 1**	**Soil 2**	**Soil 3**	**Soil 1**	**Soil 2**	**Soil 3**
HMX	80.7	72.0	69.2	91.9	95.3	90.3
RDX	106.8	95.0	94.2	90.0	89.6	89.6
135-TNB	79.5	100.2	104.5	93.3	99.2	100.5
13DNB	129.6	139.0	152.6	96.9	100.4	100.9
NB	104.8	110.7	107.0	104.3	111.2	108.3
Tetryl	95.1	95.4	94.7	92.7	94.2	96.6
TNT	137.5	248.9	191.1	100.6	97.6	93.1
2A-DNT	101.7	96.6	97.9	93.2	95.0	96.9
4A-DNT	102.4	91.5	96.5	90.5	93.1	95.3
24-DNT	121.0	89.9	88.8	98.3	96.7	97.9
26-DNT	157.7	230.5	202.0	99.3	93.8	93.6
2-NT	83.0	91.2	107.8	108.3	118.0	105.0
4-NT	115.7	103.2	97.2	108.8	104.9	99.1
3-NT	98.5	106.7	87.2	105.0	108.1	99.7

Table 5
Recovery of explosives in spiked soil samples (values are in % recovery).

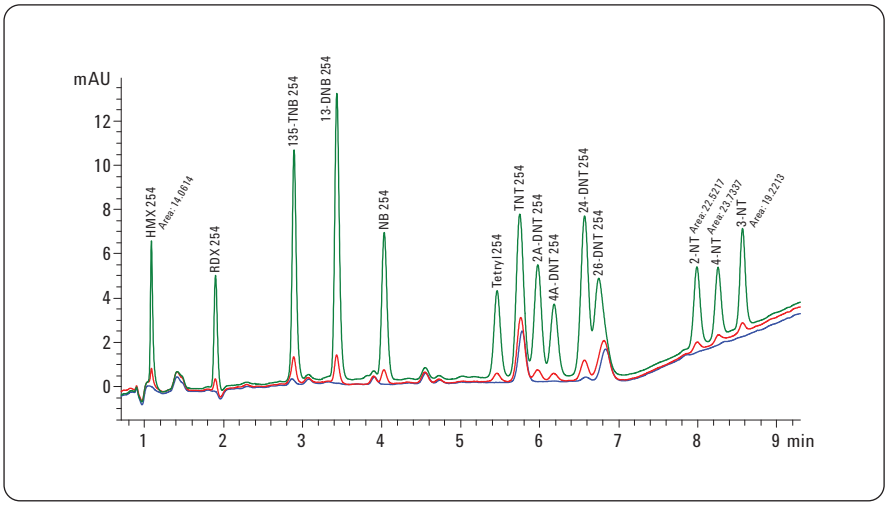

Figure 3
Overlay of a soil sample and spiked soil samples (50 and 500 µg/kg).

Conclusion

The developed method allows detection of the U.S. EPA 8330 explosives at sub-ng/mL levels in standard solutions. When applying the method to soil samples this corresponds to a detection limit of approximatly 5 ppb (µg/kg). Reaching these low levels is possible because the sensitivity of the new Agilent 1290 Infinity DAD in combination with a Max-Light Cartridge High Sensitivity Flow Cell. The results for repeatability and linearity in standard solutions and recovery in spiked soil samples demonstrate the applicability of this approach for routine analysis. Additionally the Agilent Poroshell 120 EC-C18 column proved its significance for supplying the necessary selectivity to separate these structurally similar compounds in a relatively short analysis time.

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使用安捷伦 Poroshell 120 EC-C18 色谱柱快速分析环境中的酚类

Application Note

Environmental

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Abstract

本文实验将最初在 4.6×100 mm，5 µm 色谱柱上开发的 9 种酚类化合物分离方法转换到了安捷伦 Poroshell 120 EC-C18 4.6×100 mm，2.7 µm 色谱柱。通过调节梯度和流速，维持恒定的保留指数以确定被评估色谱柱的最佳流速。本文还提供了方法转换的简单指导原则。新的分离方法将分析时间从 20 分钟缩短到 3 分钟以内，并且峰容量更高，由于两种色谱柱均采用 2 µm 滤头，因此样品制备方法无需更改。进一步将该方法扩展到 50 mm 色谱柱，得到的分离度略低于 100 mm 色谱柱，但只需 2 分钟的分析时间就可获得同原方法相当的峰容量。尽管压力相比原方法有所增加，但仍然低于 400 bar，可以很容易地转换到任何 HPLC 系统上。

Introduction

The presence of phenols in the environment is caused by their generation from many industrial processes. These processes include the manufacture of phenolic resins, antioxidants, pesticides and the combustion of wood, coal and petroleum. Some phenols are germicidal and are used in formulating disinfectants, and other phenols are formed during natural processes. When phenolic compounds are discharged into the environment they can present a serious hazard by contaminating water because they cause devastation to the majority of the aquatic organisms and induce bioaccumulation in the food chain at trace levels. Others possess estrogenic or endocrine disrupting activity. Frequently, these compounds find their way into the environment as water pollution. The analytical determination of phenol and substituted phenols is necessary because of their toxicity, persistency, and widespread use in industry. Many phenols are on the priority pollutant list.[1]

Agilent Poroshell 120 EC-C18, 2.7 µm columns perform similarly to sub-2-µm totally porous materials, but since they use 2-µm column frits like those found on 5-µm columns, they require no additional sample preparation. This allows a more seamless method transfer to new Poroshell 120, from established methods using 5-µm columns.[2,3,4]

In this work, a gradient method is transferred and optimized from a 4.6 × 100 mm, 5-µm column to an Agilent Poroshell 120 EC-C18, 4.6 × 100 mm column. Gradient time was decreased from 20 minutes to 3 minutes, and a higher peak capacity resulted. Time can be further reduced using a 4.6 × 50 mm column, while maintaining the peak capacity of the original method.

Experimental

An Agilent 1200 Rapid Resolution liquid chromatography (RRLC) system was used for this work:

- G1312B Agilent Binary Pump SL with mobile phase A: 0.1 % Formic Acid in water and B: Acetonitrile. The gradient started at 5% B, held at that concentration; then ramped to 40 % B, held at that concentration, and then re-equilibrated to the initial condition. Gradient times vary depending on column dimensions and flow rate (Tables 1 and 2). The system is configured with the pulse damper and standard mixer installed.
- G1367C Agilent Automatic Liquid Sampler (ALS) SL. Injection volume was 20 µL and 10 µL for the 4.6 × 100 mm and 4.6 × 50 mm columns respectively.
- G1316B Agilent Thermostatted Column Compartment (TCC) SL with temperature set to 35 °C.
- G1315C Agilent Diode Array Detector (DAD) SL with the signal set to 270, 4 nm and reference set to 360, 100 nm, using a G1315-60024 micro flow cell (3-mm path, 2-µL volume).
- Agilent ChemStation version B.04.01 was used to control the HPLC and process the data.

Five Agilent columns were used in this work:

- Agilent Poroshell 120 EC-C18,4.6 × 100 mm, 2.7 µm – Agilent p/n: 695975-902
- Agilent Poroshell 120 EC-C18, 4.6 × 50 mm, 2.7 µm
  - Agilent p/n: 699975-902
- Agilent ZORBAX Eclipse Plus C18, 4.6 × 100 mm, 1.8 µm
  - Agilent p/n: 959964-302
- Agilent ZORBAX Eclipse Plus C18, 4.6 × 100 mm, 3.5 µm
  - Agilent p/n: 959961-902
- Agilent ZORBAX Eclipse Plus C18, 4.6 × 100 mm, 5 µm
  - Agilent p/n: 959996-902

The compounds of interest are shown in Figure 1, with their respective structures. Compounds were dissolved in water at 1 mg/mL. Equal aliquots were combined to produce a mixed sample. Thiourea was used as a void marker in all samples to determine t_0.

The following compounds were purchased from Sigma Aldrich: hydroquinone, resorcinol, phenol, 4-nitrophenol, p-cresol, o-cresol, 2,3 dimethylphenol, 2,5 dimethylphenol, 1-napthol. Formic acid was purchased from Sigma Aldrich (Bellefonte, PA). Acetonitrile was purchased from Honeywell, Burdick and Jackson High Purity, (Muskegon, MI). Water used was 18 M-Ω Milli-Q water (Bedford, MA).

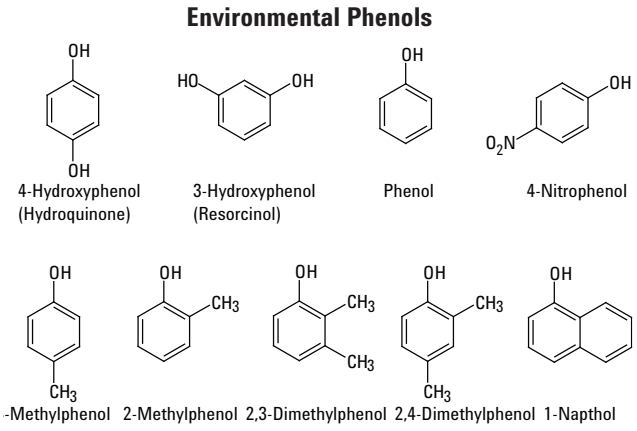

Figure 1. Compounds of interest.

Results and Discussion

The original work shows an excellent separation of phenols commonly identified in drinking and surface water. The objective in this work was to chromatographically improve the method; either by increasing the peak capacity of the analysis or substantially shortening the chromatography. A change in the acidic modifier from acetic acid to formic acid is made. The use of formic acid instead of acetic acid lowers the pH of the mobile phase slightly and can allow cleaner baselines. As discussed in reference 5, once a separation has been optimized (selectivity and retention index), it is possible to further improve the chromatography by varying column length, particle size and flow rate. However the k* value must be maintained, while varying these column conditions so as not to lose selectivity while gaining peak capacity.

Equation 1: $k^* = (t_g F)/(d/2)^2 L(\Delta\%B)$

Where:

t_g is the gradient time,
F is the flow rate
L is the column length
d is the column diameter
Δ%B is the change in organic content across the gradient segment

As shown in a previous note, the initial gradient is scaled keeping column volumes constant, and preserving method selectivity. In this case, the flow rate is varied between 0.5 mL/min and 3.5 mL/min at 0.5 mL/min intervals. Using Equation 1 as a guideline, the conditions listed in Table 1 are developed. These conditions were calculated manually but could just as easily have been calculated using the Agilent 1200 Series Rapid Resolution LC Method Translator and Cost Savings Calculator [6]. These conditions are the basis of the chromatographic programs used for the 100 mm columns in this study. As can be seen, all steps in the program are proportionately shortened as the flow rate is increased. In the resulting chromatographs in Figures 2a and 2b, elution order remains similar for the scaled chromatograms of both the 5 µm Agilent ZORBAX Eclipse Plus C18 and the 2.7 µm Agilent Poroshell 120 EC-C18 columns. In addition to these columns, the same experiments are performed on 3.5 µm Agilent ZORBAX Eclipse Plus C18 and 1.8 µm Agilent ZORBAX Eclipse Plus C18 columns with the resulting chromatograms depicted in Figures 3a and 3b respectively. The pressure generated using the 4.6 × 100 mm, 1.8-µm column approaches 550 bar at 2 mL/min. Therefore, no further experiments are considered. In all cases, the separation elution order is maintained. The peak capacity of each chromatogram is calculated using Equation 2. Peak capacity in the simplest terms, is the number of peaks of a given width that can fit between the first and last peaks.

Table 1. Gradient Program Used with 4.6 × 100 mm Columns

%B	Time (min)						
5	4	2	1.33	1	0.8	0.67	0.34
40	34	17	11.33	8.5	6.8	5.67	2.84
40	40	20	13.33	10	8	6.67	3.34
5	42	21	14	10.5	8.4	7	3.5
5	50	25	16.67	12.5	10	8.34	4.17
Flow rate (mL/min)	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**	**3.5**

Similar selectivity allows convenient method transfer between Agilent ZORBAX Eclipse Plus C18 and Agilent Poroshell 120 EC-C18 column

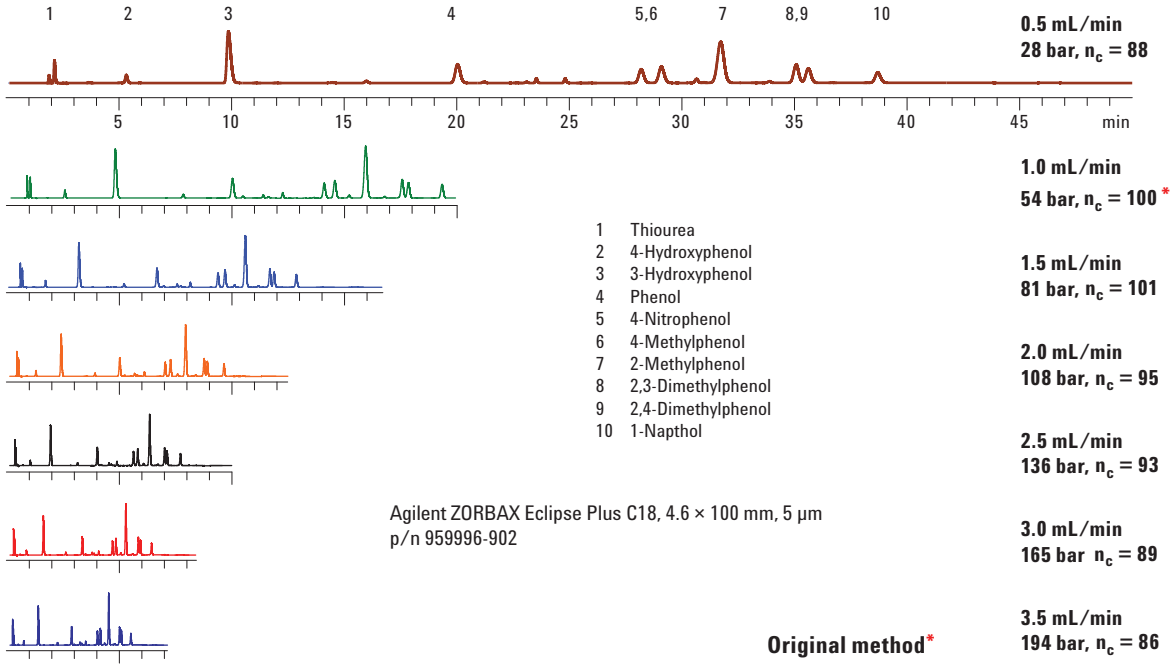

Figure 2a. Scaled chromatography using Agilent ZORBAX Eclipse Plus C18, 4.6 × 100 mm, 5 µm column.

Similar selectivity allows convenient method transfer between Agilent ZORBAX Eclipse Plus C18 and Agilent Poroshell 120 EC-C18 column

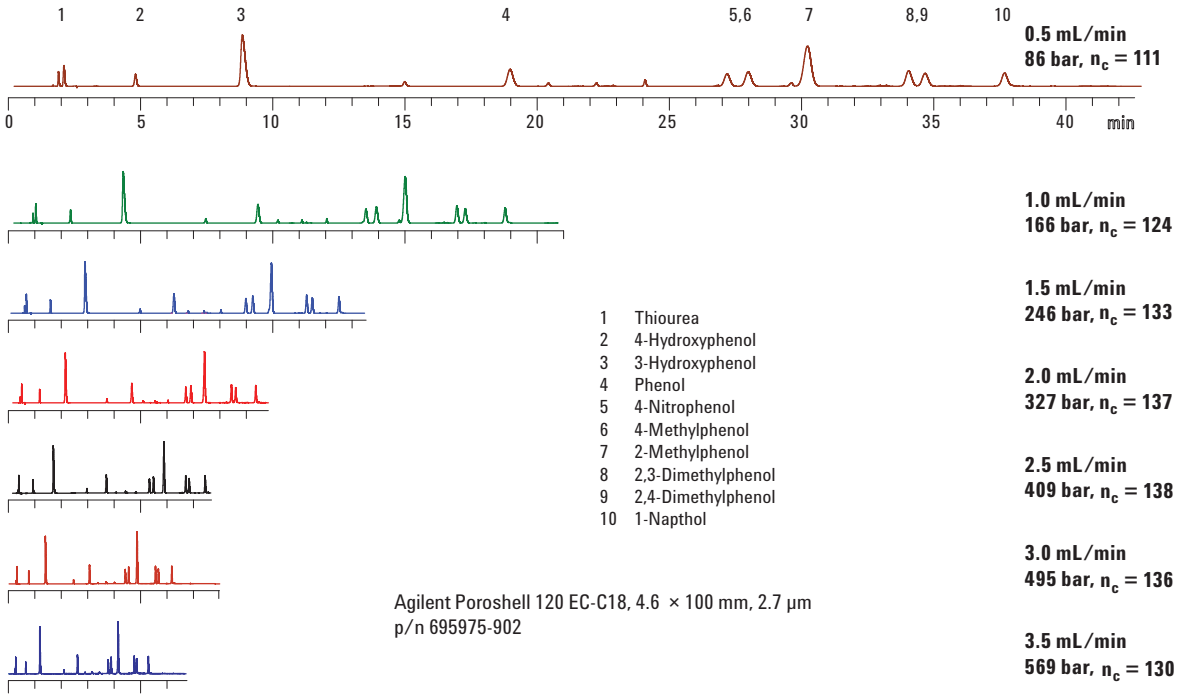

Figure 2b. Scaled chromatography using Agilent Poroshell 120 EC-C18, 4.6 × 100 mm, 2.7 µm column.

Similar selectivity continues along the Agilent ZORBAX Eclipse Plus C18 family and Agilent Poroshell 120 EC-C18 column

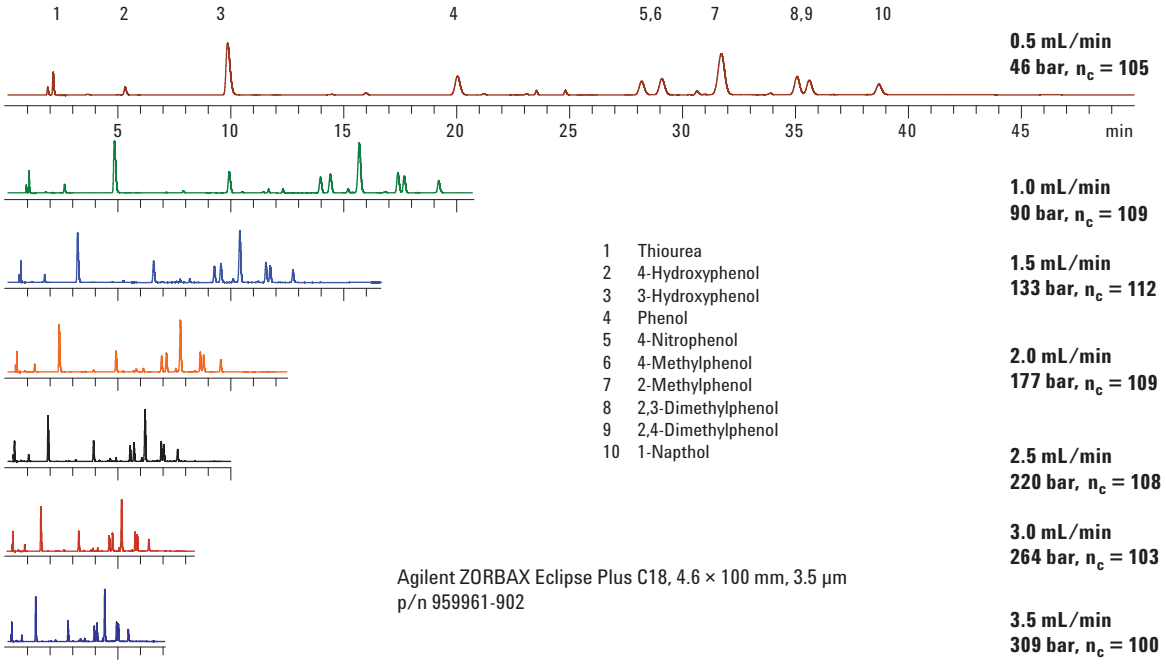

Figure 3a. Scaled chromatography using, Agilent ZORBAX Eclipse Plus C18, 4.6 × 100 mm, 3.5 µm column.

Similar selectivity continues along the Agilent ZORBAX Eclipse Plus C18 family and Agilent Poroshell 120 EC-C18 column

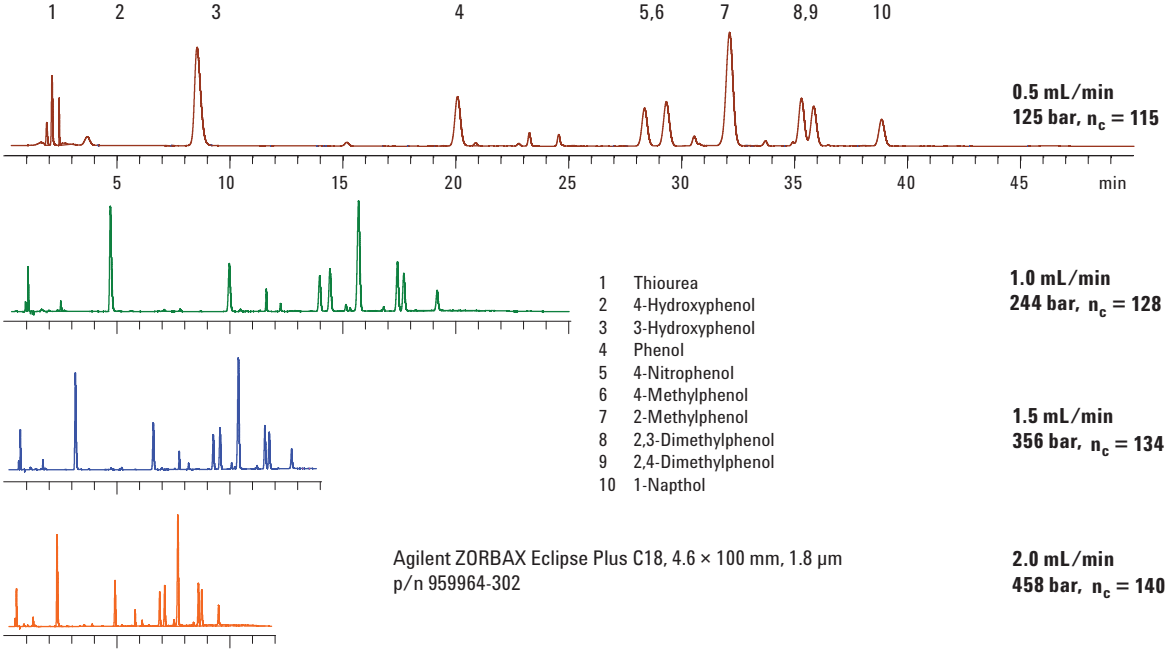

Figure 3b. Scaled chromatography using, Agilent ZORBAX Eclipse Plus C18, 4.6 × 100 mm, 1.8 µm column.

Equation 2:

Conditional Peak Capacity $n_c = (t_{R,n} - t_{R,1})/w$

Where:

$t_{R,n}$ and $t_{R,1}$: Retention times of the last and first eluting peaks

w is the 4σ peak width

$w = (W_{½} /2.35) \times 4$

$W_{½}$ = peak width at half height

Peak capacity for each of the chromatograms shown in Figures 2 a and b and 3 a and b are plotted in Figure 4. The highest peak capacity is found for the 1.8 µm Agilent ZORBAX Eclipse Plus C18 column at 2 mL/min. It is likely that the peak capacity would have been higher at faster flow rates. The 100 mm Agilent Poroshell 120 EC-C18 column generates the next highest peak capacity between 2 and 3 mL/min. Figure 5 indicates that a faster separation is possible with only a slight loss of peak capacity. The 3.5 µm column has an optimal peak capacity at approximately 1.5 mL/min, while the 5 µm column has an optimal peak capacity, between 1 and 1.5 mL/min. In general, with totally porous columns of the same dimension, larger particle columns yield lower peak capacities at lower optimal flow rates. The chromatographic scaling experiment is also carried out using a 50 mm Agilent Poroshell 120 EC-C18 column, with the gradient conditions summarized in Table 2, and the results depicted in Figure 5. A comparison between the peak capacities of 50 mm and 100 mm Agilent

Peak Capacity of Agilent Poroshell 120 Column is similar to 1.8 µm column of same size and higher than 3.5 or 5 µm columns of similar size, Peak capacity of totally porous particles is inversely proportional to their size.

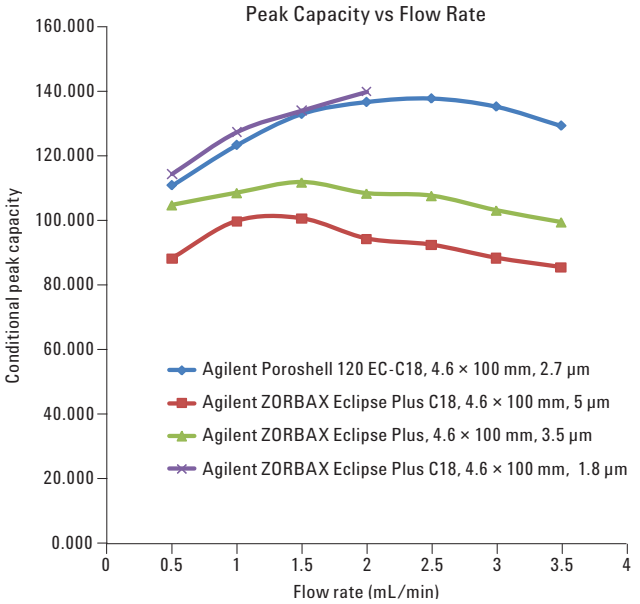

Figure 4. Peak capacity of Agilent Poroshell 120 EC-C18 compared to totally porous Agilent ZORBAX Eclipse Plus C18 columns of different particle sizes at varied flow rates.

Agilent Poroshell 120 EC-C18, 4.6 x 50 mm column can provide peak capacity of original method at 182 bar in 3 minutes instead of 20 minutes

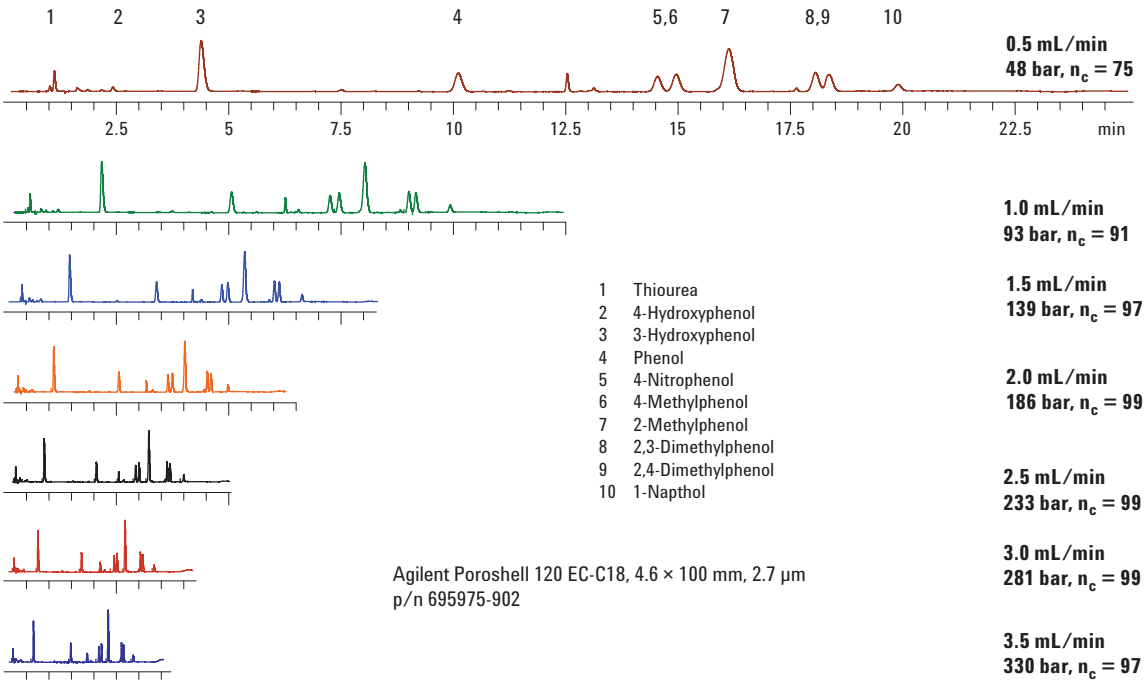

Figure 5. Scaled Chromatography using Agilent Poroshell 120 EC-C18, 4.6 × 50 mm, 2.7 µm column.

Table 2. Gradient Program Used With 4.6 × 50 mm Columns

%B	Time (min)						
5	4	2	1.33	1	0.8	0.67	0.34
40	34	17	11.33	8.5	6.8	5.67	2.84
40	40	20	13.33	10	8	6.67	3.34
5	42	21	14	10.5	8.4	7	3.5
5	50	25	16.67	12.5	10	8.34	4.17
Flow rate (mL/min)	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**	**3.5**

Poroshell 120 EC-C18 columns is shown in Figure 6. The optimal peak capacity of the 50 mm Agilent Poroshell 120 EC-C18 column is found to be between 2 and 3 mL/min, at 3 min. The last peak is eluted at 2 min with a peak capacity equivalent to the peak capacity of the original 20-min 4.6 × 100 mm, 5 µm method.

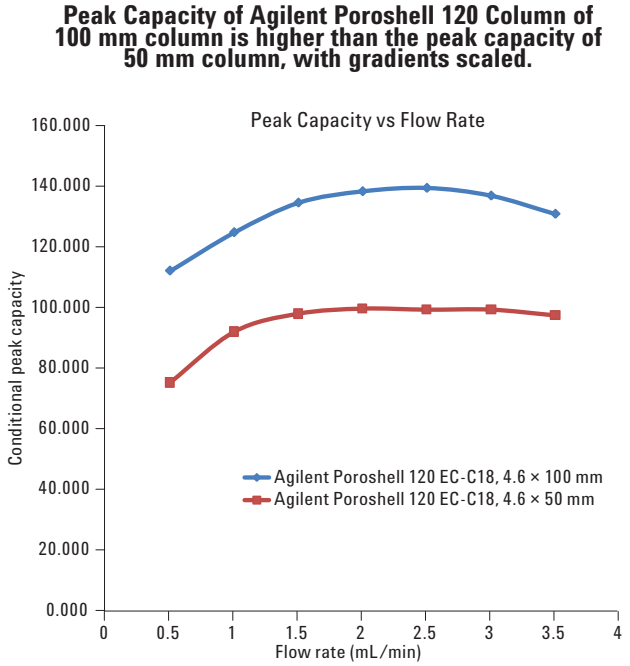

Figure 6. Peak Capacity of 50 and 100 mm Poroshell 120 EC-C18 at varied flow rates.

Conclusion

HPLC columns packed with superficially porous particles offer many advantages over columns packed with conventional, fully porous particles. The superficially porous 2.7-µm Agilent Poroshell 120 EC-C18 offers similar efficiency and selectivity to the 1.8-µm Agilent ZORBAX Eclipse Plus C18 column, without the high back pressure.

While columns packed with larger 5-µm particles can yield excellent separations, many separations can be improved by implementing a column change and appropriate gradient scaling. Due to the similar selectivity between Agilent Poroshell 120 EC-C18 and Agilent ZORBAX Eclipse Plus C18 columns, methods can easily be transferred from older Agilent ZORBAX Eclipse Plus C18 columns to new Agilent Poroshell 120 EC-C18 to decrease run time, improve throughput and increase peak capacity.

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硝基化合物和爆炸物的分离、纯化和色谱分析

Technical Overview

Introduction

爆炸物以及结构相关的硝基化合物是一类通常具有毒性的化合物。因此，EPA 和其它环保机构均对其进行了监控。由于这些化合物能够从土壤转移到河流中，因此本文实验针对安捷伦 SampliQ DVB 固相萃取（SPE）管分离和纯化水中这类化合物的效果进行了评估。同时，还评估了安捷伦 PoroShell 120 EC-C18 色谱柱对这些化合物的色谱分离效果。

A dilute aqueous solution of EPA Method 8330 Calibration Mix A and B standards (14 explosive and nitro compounds) was applied to Agilent's SampliQ DVB SPE tubes for isolation and sample cleanup. Samples were eluted with acetonitrile (ACN). The eluent was diluted to 30% acetonitrile/water and analyzed on Agilent's Poroshell 120 EC-C18 column.

An Agilent SampliQ DVB SPE tube (3 mL tube, 60 mg, p/n 5982-3136) was used for isolation and concentration of the sample (1 µg/mL) from the 2% acetonitrile/water solution. Twelve milliliters of the sample were applied to the SampliQ DVB SPE tube as illustrated in Figure 2. The combined acetonitrile effluent was diluted to 30% acetonitrile/water, and analyzed at 210 nm and 254 nm using external standards on Agilent's Poroshell 120 EC-C18 column (3.0 mm × 100 mm, 2.7 µm, p/n 695975-302).

Recoveries for the 14 compounds ranged from 90% to 105%. The RSDs were equal to or less than 1.2% for three replicates. The results presented here show that Agilent's SampliQ DVB SPE tubes, coupled with Agilent's Poroshell 120 EC-C18 column is an excellent combination for the isolation and analysis of these compounds.

Study Purpose and Methodology

The purpose of the study was to evaluate the effectiveness of Agilent's SampliQ DVB SPE tubes for the isolation and purification of nitro-containing compounds and explosives (Figure 1). Given the similarity of many of the structures, this sample provides an excellent opportunity to evaluate the separation on Agilent's Poroshell 120 EC-C18 line of columns.

An aliquot of 14 standards in acetonitrile (50 µg/mL each) was diluted to provide a 2% acetonitrile/water solution of standards (1 µg/mL each std). This solution was used as the sample for application to the SampliQ DVB SPE tube.

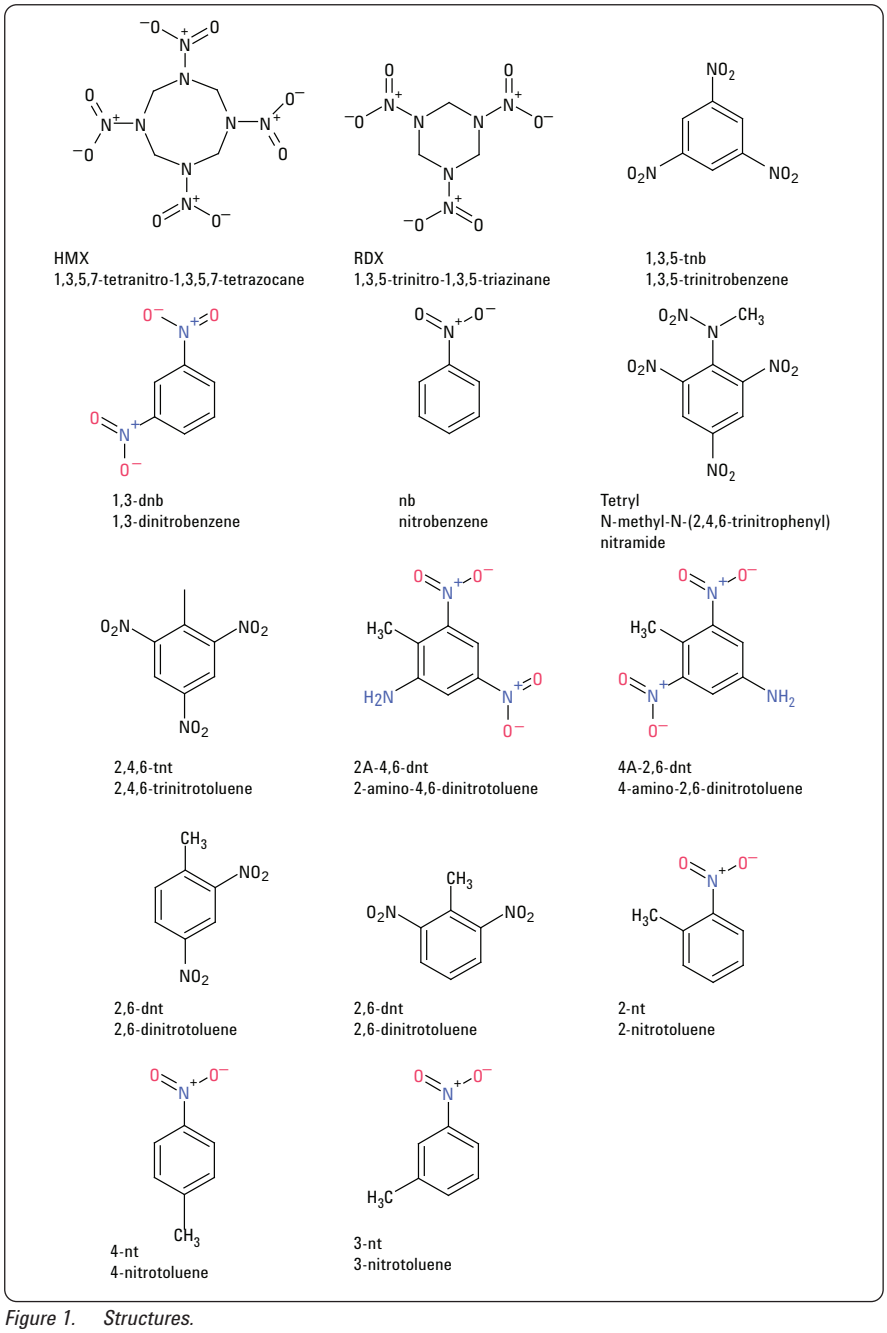

Figure 1. Structures.

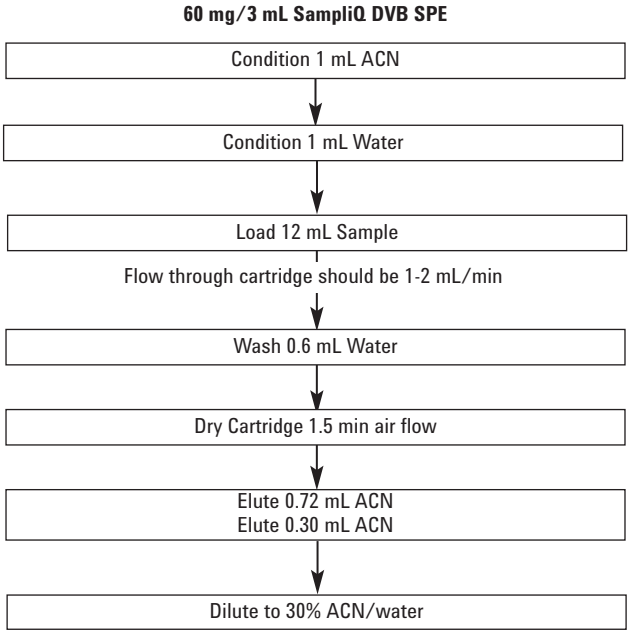

Figure 2. Sample cleanup scheme using Agilent SampliQ DVB SPE

Results and Discussion

The average recoveries of triplicate samples ranged from 90% to 105% (Table 1). The highest RSDs for the three replicate recoveries was 1.2%.

Table 1. Recovery Data from Agilent's Polymer SampliQ DVB SPE Tubes

Number	Compound ID	Sample % Recovery	Replicate %RSD (n = 3)	Full name
1	HMX	104	0.8	1,3,5,7-tetranitro-1,3,5,7-tetrazocane
2	RDX	103	0.6	1,3,5-trinitro-1,3,5-triazinane
3	1,3,5-tnb	105	0.6	1,3,5-trinitrobenzene
4	1,3-dnb	103	0.7	1,3-dinitrobenzene
5	nb	101	1.2	Nitrobenzene
6	Tetryl	90	1.2	N-methyl-N-(2,4,6-trinitrophenyl) nitramide
7	2,4,6-tnt	103	0.6	2,4,6-trinitrotoluene
8	2A-4,6-dnt	103	0.6	2-amino-4,6-dinitrotoluene
9	4A-2,6-dnt	101	0.6	4-amino-2,6-dinitrotoluene
10	2,4-dnt	102	0.6	2,4-dinitrotoluene
11	2,6-dnt	102	0.7	2,6-dinitrotoluene
12	2-nt	98	1.2	2-nitrotoluene
13	4-nt	98	1.0	4-nitrotoluene
14	3-nt	97	1.2	3-nitrotoluene

These compounds are a mixture of the EPA Method 8330 Calibration Mix A and B.

Excellent separation of the compounds was achieved with the Agilent Poroshell 120 column as illustrated in Figure 3.

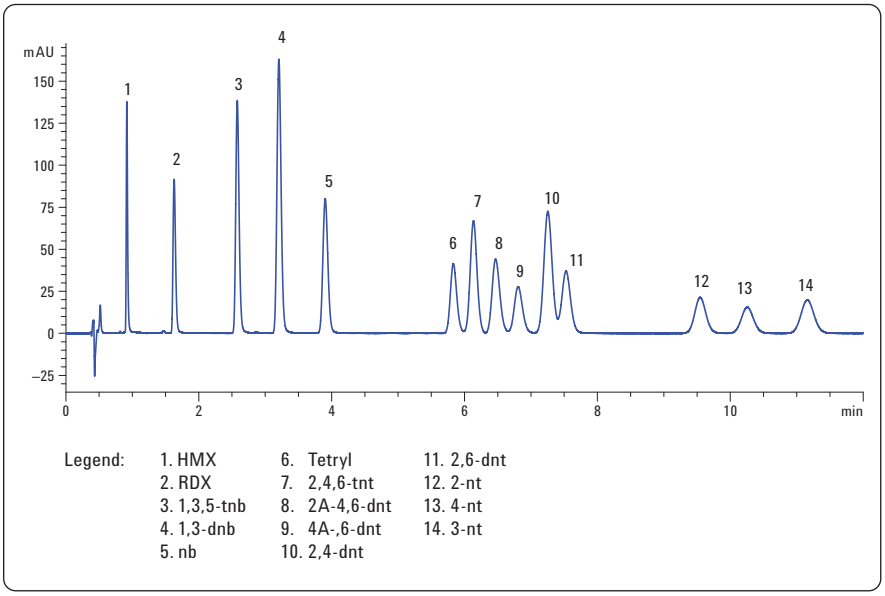

Figure 3. Representative standard chromatogram, 5 µg/mL, 254 nm.

HPLC Analysis

Column:	Agilent Poroshell 120 EC-C18, 3.0 mm × 100 mm, 2.7 µm (Agilent p/n 695975-302)
Mobile phase:	25% MeOH/water (isocratic)
Flow rate:	1.0 mL/min
Detection DAD:	254, 210 nm
Column temperature:	44 °C
Injection volume:	30 µL
Flow cell:	10 mm, 13 µL

Conclusion

The use of Agilent's SampliQ DVB SPE tubes provided high recoveries with excellent reproducibility. This, coupled with the separation achieved with Agilent's Poroshell 120 column, provides a complete package for the analysis of nitro compounds and explosives. One would expect this procedure to be applicable to additional nitro compounds and explosives.

Agilent SPE part numbers

Description	Part number
SampliQ DVB SPE tube, 3 mL tube, 60 mg	5982-3136
Poroshell 120 EC-C18, 3.0 mm × 100 mm, 2.7 µm	695975-302

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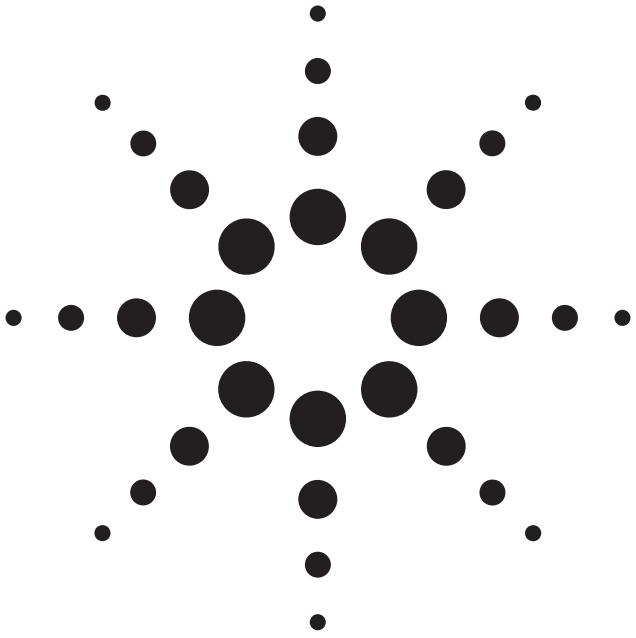

生物分析

采用Poroshell 120色谱柱快速分析重组单克隆抗体肽图

应用领域

生物分析

关键词

HPLC；Poroshell 120； 重组单克隆抗体

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摘要

肽图是用于对蛋白质药物一级结构进行鉴定以及检测蛋白质产品批间一致性的常规检测方法。一般进行肽图谱鉴别时往往需要时间较长，如重组人胰岛素肽图测定在中国药典、美国药典、欧洲药典中均采用反相HPLC法，检测时间长达60~86min不等；本公司的重组单克隆抗体肽图的检测时间也长达52分钟。目前的方法中使用的常规液相色谱仪和液相分析柱，分析时间长，且有机试剂耗用量大，快速液相色谱仪和快速液相分析柱的应运而生，使得分析时间和试剂耗量大大降低。但针对目前基层实验室常规液相色谱仪的普及，与快速液相分析柱的不兼容性，使得快速分析检测很难实现。安捷伦公司最新推出的Poroshell 120系列表面多孔层色谱柱，由于其具有低反压，高柱效的特点，从而真正实现在常规液相色谱仪上进行快速分析的可操作性。本文使用Poroshell 120色谱柱，并采用常规液相色谱仪，对重组单克隆抗体肽图检测方法加以改进，分析时间和试剂耗量均降低了1/2以上，一次分析仅需26分钟；色谱峰的理论塔板数及分离度也优于常规检测方法。现将实验结果报告如下，以供参考。

1 试验材料与方法

1.1 仪器与试剂

1200高效液相色谱仪(Agilent公司)；

乙腈(HPLC级,SIGMA公司)；

超纯水（Milli-Q级）；

单克隆抗体浓度均为2.5mg/mL (公司自制)。

1.2 色谱条件

色谱仪条件：Poroshell 120 SB-C18柱(4.6 mm i. d. × 100 mm, 2.7 μ m)；柱温60˚C；流速1.0 mL /min；流动相A：0.1%三氟乙酸水溶液；流动相B：80%乙腈水溶液（每升80%乙腈水溶液加入0.85ml三氟乙酸）；紫外检测器：波长214nm；进样体积20 μ L。流动相梯度如下表所示。

编号	时间/分钟	流速mL/min	流动相 A%	流动相 B%
1	0.0	1.000	100.0	0.0
2	1.0	1.000	100.0	0.0
3	31.0	1.000	50	50
4	32.0	1.000	100	0.0
5	40.0	1.000	100	0.0

1.3 试验样品

重组单克隆抗体（2.5mg/ml）。

1.4 试验方法

1.4.1流动相配制

流动相A：取1ml TFA 加入1000ml 超纯水中，0.22μm水相滤膜过滤。

流动相B：取800ml 乙腈与200ml 超纯水混合后加入0.85ml TFA，0.22μm有机相滤膜 过滤。

1.4.2 重组单克隆抗体酶切

1.4.2.1 使用移液器（20~200μl）取200μl蛋白样品于1.5ml EP管中，加入192μl 超纯水、50μl 10%NH_4HCO_3、3.5μl DTT（100mg/ml）,于水浴锅中50℃水浴15分钟。

1.4.2.2 到时间后取出EP管冷却至室温，加入4.5μl IAA，混匀后避光反应15min。加入胰蛋白酶-TPCK 25μl，混匀后37℃水浴4小时。补加酶25μl，混匀后37℃水浴16小时。

1.4.2.3 将酶切后的蛋白样品于100℃水浴15分钟，用离心机以13000g离心10min，取上清用于检测。

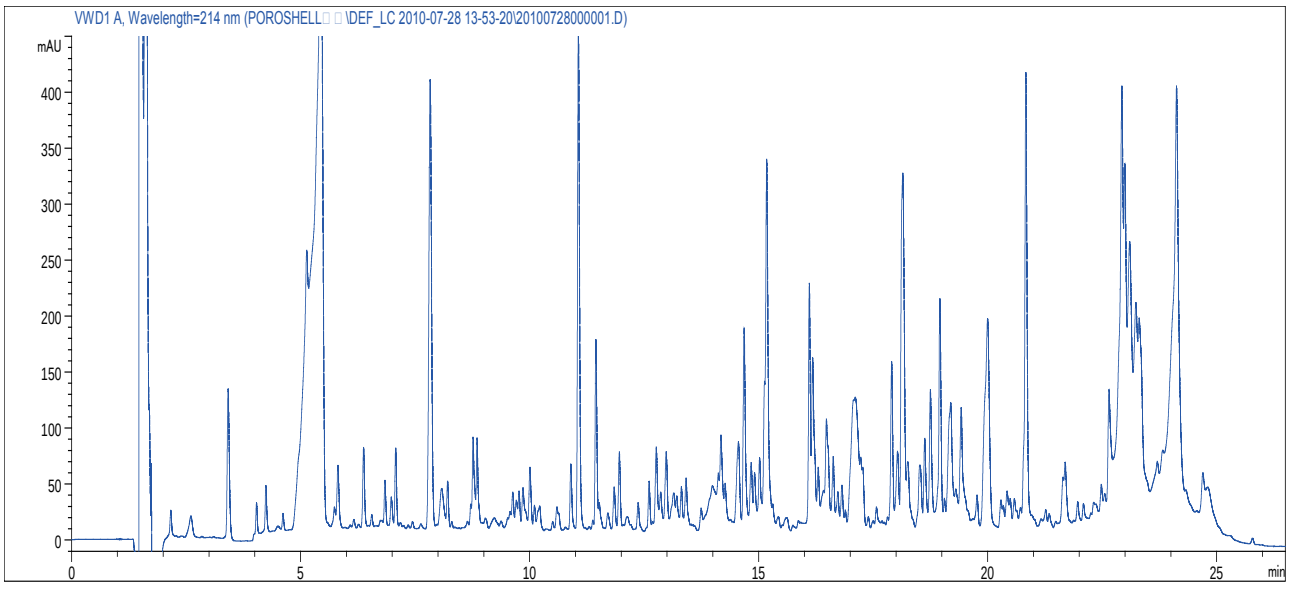
1.4.3 测定

按照1.2 所列的色谱条件进行检测。

2 结果与讨论

2.1 色谱条件的选择

以Poroshell 120柱（4.6 mm i. d. ×150 mm, 2.7μm）作为分析柱，乙腈/水/三氟乙酸为流动相进行HPLC条件的优化。实验发现，较高的柱温（60℃）有助于提高方法的分离度以及分辨率；流动相B中加入较少的TFA（0.85ml/L）有助于梯度基线的水平；此外，通过不断缩短梯度时间以达到缩短分析时间的目的。故选定了1.2的色谱条件，一次分析样品仅需26min。这样，每个样品从原来的分析时间52分钟缩短至26min，不仅提高了工作效率，而且节省了有机溶剂。



2.4 使用传统色谱柱的结果

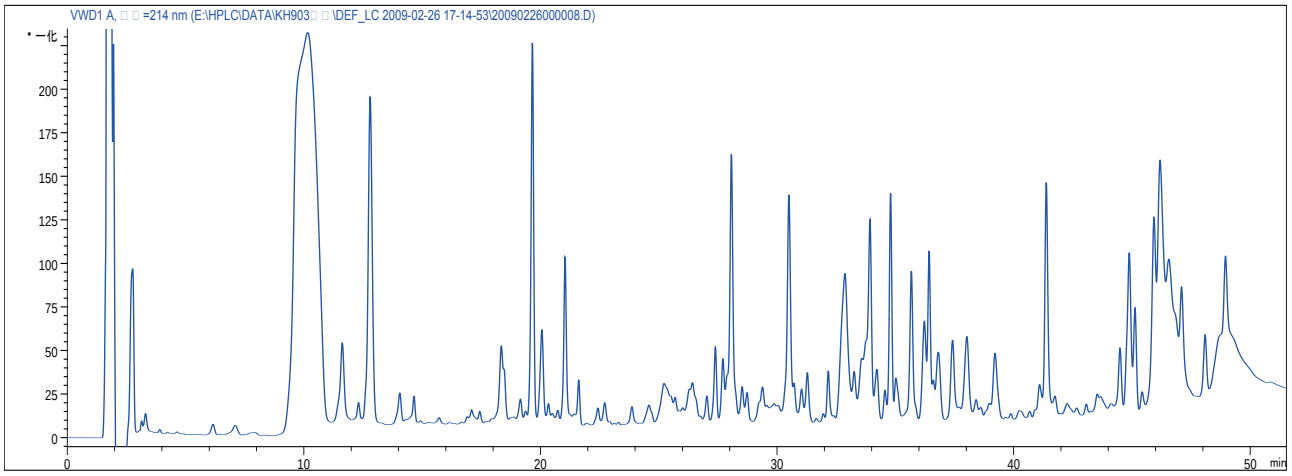
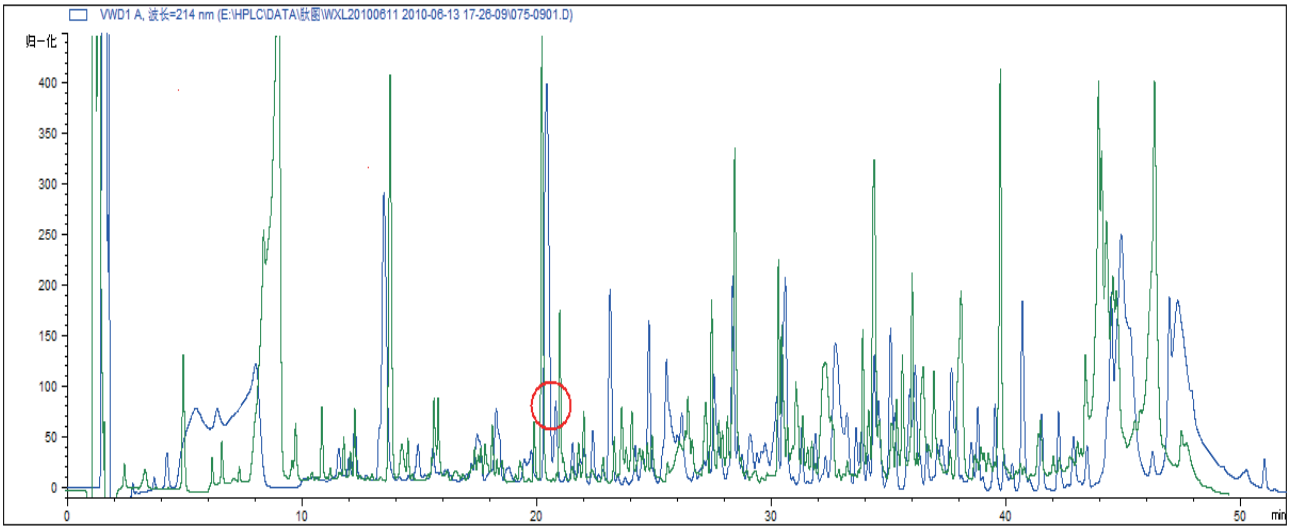


图2 重组单克隆抗体标准色谱图（浓度为1mg/mL）

2.5 Poroshell 120柱检测结果与传统方法结果色谱图比较



保留时间 [min]	K′	峰面积 [MAU*S]	峰高 [MAU]	对称因子	峰宽 [min]	塔板数	分离度	选择性
11.064	-	1389.23596	449.46976	0.70	0.0465	314220	-	-
11.446	-	563.41101	170.52788	0.51	0.0421	409852	5.07	1.03

保留时间 [min]	K′	峰面积 [MAU*S]	峰高 [MAU]	对称因子	峰宽 [min]	塔板数	分离度	选择性
20.431	-	4977.20703	382.30249	1.05	0.2135	50711	-	-
20.816	-	498.06061	67.61501	1.10	0.1201	166366	1.35	1.02

绿色为Poroshell 120柱检测结果,蓝色为传统方法检测结果.取其中两个相邻的肽段色谱峰比较分离度及理论塔板数, Poroshell 120色谱柱均优于传统方法.

传统方法检测色谱条件：

1200液相，MWD检测器，标准流通池

色谱柱：SunFire C18 4.6*150mm, 5um

流动相A：0.1% TFA

流动相B：0.85ml TFA+200ml H2O+800ml 乙腈

压力：120bar

流速：1mL/min

柱温：60°C

进样量：50uL，浓度1mg/ml

梯度条件:

编号	时间/分钟	流速mL/min	流动相 A%	流动相 B%
1	0.00	1.000	100.0	0.0
2	5	1.000	100.0	0.0
3	75	1.000	30.0	70.0
4	75.01	1.000	0.00	100.0
5	85.0	1.000	0.00	100.0
6	85.01	1.000	100.0	0.0
7	95.0	1.000	100.0	0.0

3 小结

本法采用Poroshell120，在基层普遍使用的常规液相色谱仪上测定重组单克隆抗体肽图。该方法的分析速度更快、试剂消耗少、灵敏度更高、回收率和重复性均符合批量样品的检测要求，结果令人满意，很适合于基层检测蛋白类产品肽图工作的开展。

[参考文献]

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Agilent Technologies

Peptide Mapping of a Monoclonal Antibody Using Poroshell 120

Susanne C. Moyer, Agilent Technologies, Inc.

Protein-based pharmaceutical drugs, such as fusion proteins and monoclonal antibodies (mAb), are playing an increasing role in modern therapeutics. Analytical characterization of protein therapeutics is very complex and time consuming. One type of analysis that is undertaken to determine the primary structure of the protein is peptide mapping. In peptide mapping analysis, it is imperative that 100% sequence coverage be achieved and that modifications to the molecule are identified. Typical LC–MS peptide mapping protocols can have LC gradients in excess of 100 min. Obviously, such lengthy run times adversely affect throughput capabilities and methods for reducing these run times while achieving analytical goals are needed. The Agilent Poroshell 120-SB C18 column was used for the LC–MS analysis of a tryptic digest of a monoclonal antibody standard to demonstrate a greatly reduced analysis time of 20 min while achieving 100% sequence coverage of both the heavy and the light chains.

Experimental Conditions

A 3.0 × 150 mm Agilent Poroshell 120 SB-C18 column (PN 683975-302) was used with an Agilent 1200 LC and 6520 QTOF for peptide mapping experiments. Mobile phase A: 0.1% formic acid in water and mobile phase B: 0.1% formic acid in acetonitrile. The flow rate of the column was 0.3 mL/min and the column temperature was maintained at 40 °C. The LC gradient was: 0–3 min 2%B, 3–13 min 2%–45%B, 13–15 min 45%–65%B, 15–15.1 min 65%–90%B, 15.1–17 min 90%B, 17–18 min 90%–2%B, stop time 20 min.

Results and Discussion

Complex tryptic digests of mAbs employ shallow gradients, often stepped, to separate the many peptide fragments into discreet peaks. Often tryptic maps take 2 h to complete using 2.1 × 250 mm, 5 µm particle-sized columns, significantly affecting laboratory productivity. Sample degradation also may occur

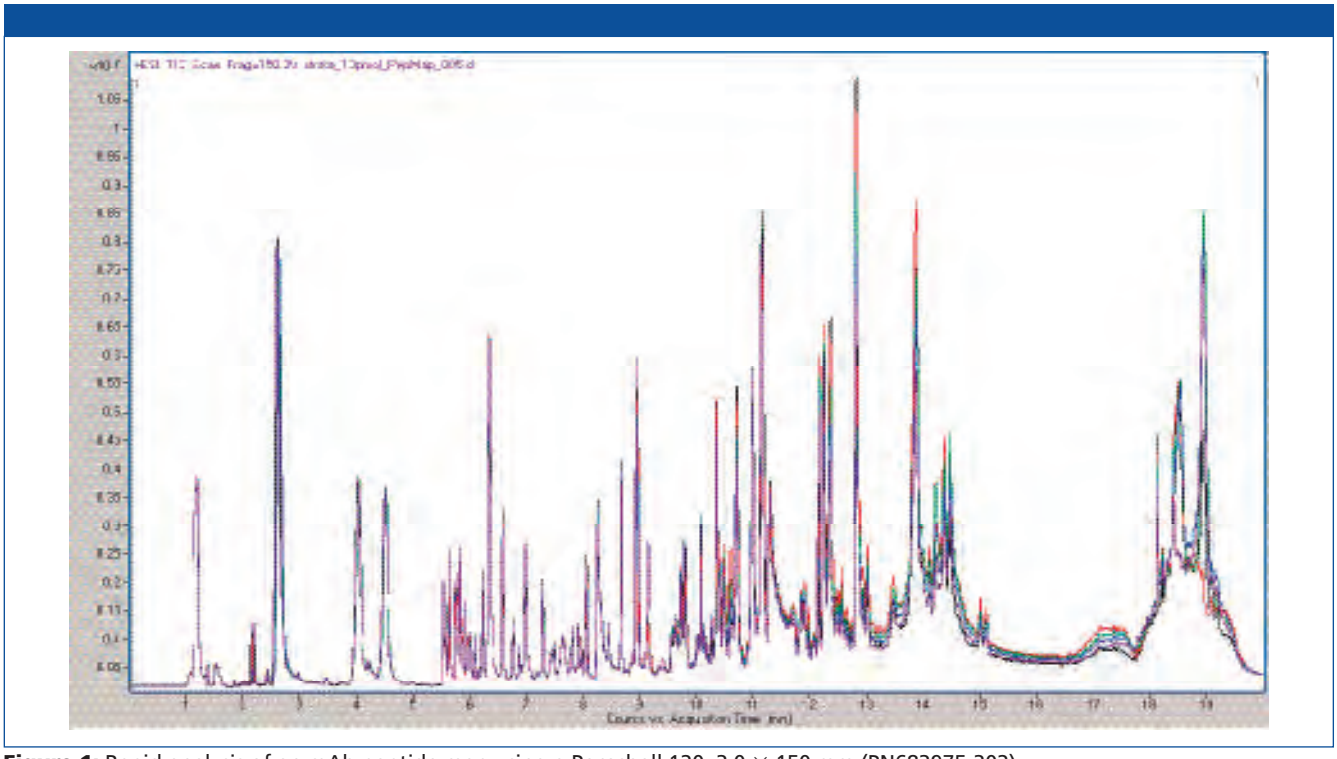


Figure 1: Rapid analysis of an mAb peptide map using a Poroshell 120, 3.0 × 150 mm (PN683975-302).

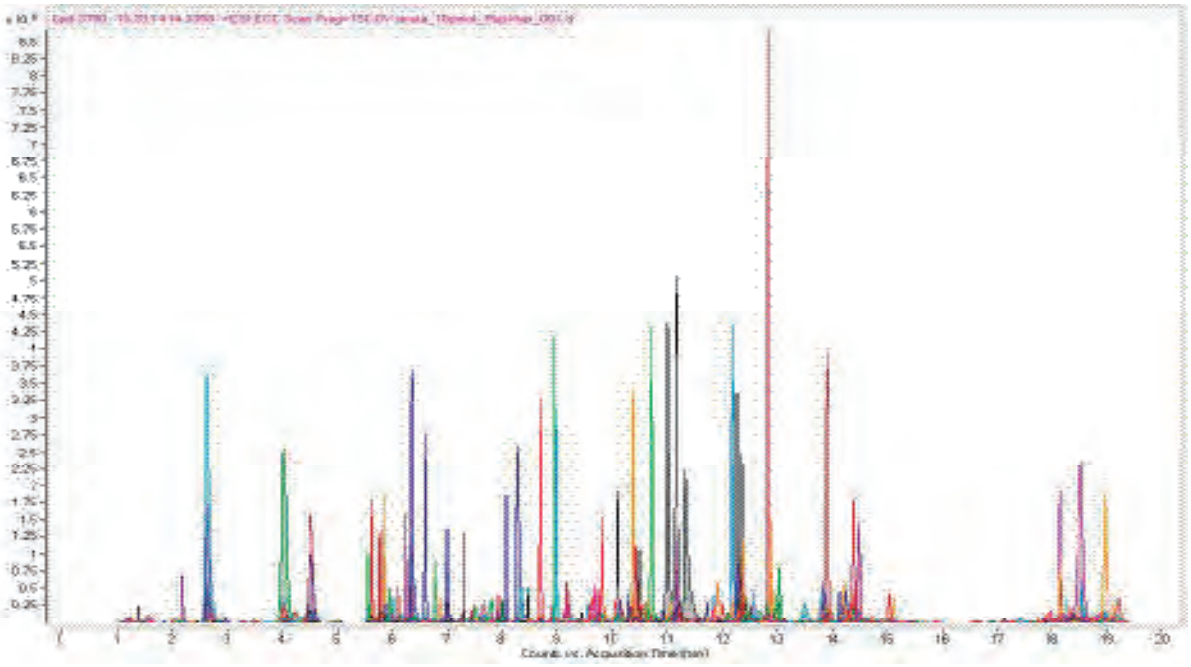


Figure 2: 100% sequence coverage achieved of mAb with many unique compounds identified using MassHunter.

between long injection cycles so any reduction in analysis time is welcome.

One way to achieve shorter analysis times is to use Poroshell 120 columns. These columns contain 2.7 µm superficially porous particles that provide almost equivalent efficiency to UHPLC (sub-two micron totally porous) columns. Thus, more efficient Poroshell 120 particles packed in a shorter column length can provide similar chromatographic results to their longer 5 µm predecessors in a fraction of the time.

A tryptic digestion of a standard 10 pmol mAb was injected five times onto a Poroshell 120 column. Total analysis time including gradient re-equilibration was only 20 min. Figure 1 shows the five overlays of the total ion chromatograms (TIC). Good retention time, reproducibility, and peak-to-peak resolution indicate a stable instrument, column, and mobile phase gradient and re-equilibration, and is the first indicator of a robust method for validation. Also, Poroshell 120 columns have larger frit pores compared to smaller sub-two micron column frits, to resist plugging from "dirty" or complex samples such as protein hydrolysates.

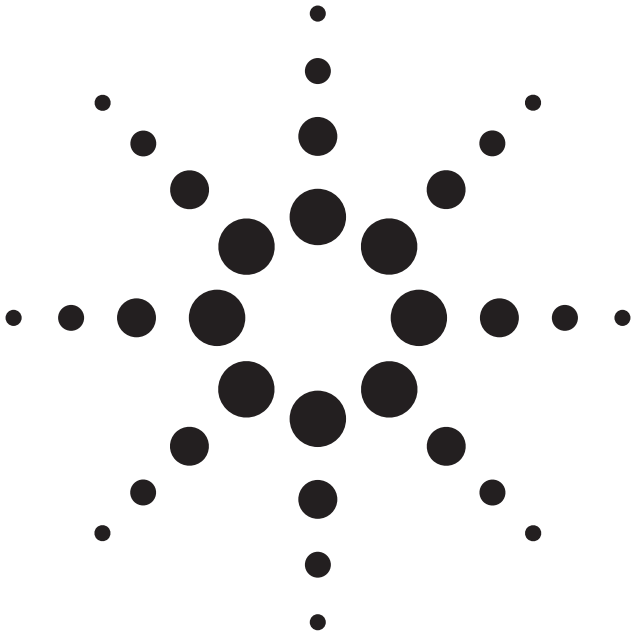
The faster analysis gained from Poroshell 120 did not compromise the chromatographic map of the mAb. Figure 2 shows the results from Agilent MassHunter Molecular Feature Extractor (MFE). MFE is an algorithm that uses the LC retention time and the QTOF accurate mass to extract unique compounds from the data set. These MFE compound results are then matched back to the heavy chain and light chain protein sequences from the mAb standard. This 20 min Poroshell-120 method resulted in 100% sequence coverage for both the heavy and light chains of the mAb.

Conclusion

The Poroshell 120 SB-C18 column can greatly reduce the analysis time of an LC–MS peptide mapping experiment, while achieving high-performance and highly reproducible peptide separations. The combination of highly efficient 2.7 µm superficially porous particles and relatively large pored frits make Poroshell 120 ideal for analysis if complex samples such as mAb digests.

References

(1) L.W. Dick Jr. et al., *J. Chromatogr. B*, **877** 230–236, (2009).



理论探讨

Maximizing efficiency using Agilent Poroshell 120 columns

100000 plates in less than 5 min using coupled column technology

Application Note

Food, Environmental, Chemical, Pharmaceutical

Author

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Abstract

Columns based on superficially porous technologies are an alternative to sub-2-µm particle based columns. The combination of these columns with the Agilent 1290 Infinity LC system produces high efficiency separations. Agilent Poroshell 120 columns offer:

- Lower back pressure
- Highest efficiency
- Comparable volume capacity

Introduction

Recently, sub-2-µm particle columns have gained a lot of interest, due to their high efficiency. They can be used at higher flow rates than those evaluated by the van Deemter equation. The loss in efficiency at higher flow rates is minor in comparison to the efficiency at the optimum flow rate. Run times and cycle times can be shortened and results obtained faster.

The drawback of these columns is that significantly higher back pressures are obtained, due to the small particle sizes. In many cases, especially for long sub-2-µm columns, the LC instrumentation must allow back pressures of >400 bar.

The superficially porous particle technology offers an alternative for very high resolution analyses[1], because these columns show significantly less back pressure. The efficiency of these columns, compared to that of sub-2-µm particle columns is slightly lower. It is possible to obtain very high plate counts by coupling columns, due to less back pressure.

This Application Note demonstrates that the coupling of three long Agilent Poroshell 120 columns results in extremely high efficiencies. It is also demonstrated that the back pressure can be kept below 400 bar, unless special LC equipment is available. In that case higher flow rates are possible to save analysis and equilibration time. Finally, a comparison was made between one 2.7 µm porous shell column and one sub-2-µm particle size column.

Experimental

Equipment

An Agilent 1290 Infinity LC system equipped with a binary pump, autosampler, thermostatted column compartment and diode-array detector with a 10-mm path length cell was used for the experiments.

An Agilent ZORBAX Rapid Resolution HT 4.6 mm × 150 mm, 1.8 µm column and an Agilent Poroshell 120, 4.6 mm × 150 mm,
2.7 µm column were used. These columns can be used up to 600 bar.

The ChemStation software revision B.04.02 was used.

Results and discussion

Potential benefits of superficially porous columns

Superficially porous column technology is based on particles with a solid core and a superficially porous shell. These particles consist of a 1.7-µm solid core with a 0.5-µm porous silica shell. In total, the particle size is about 2.7 µm. The 2.7 µm superficially porous particles provide 40–50% lower back pressure and 80–90% of the efficiency of a sub-2-µm totally porous particle. The superficially porous particles have a narrower particle size distribution than a totally porous particle. This results in a more homogeneous column and reduces diffusion in the column. At the same time the small particle and the porous shell allow for lower resistance to mass transfer. The result is higher flow rates without efficiency loss.[1,2]

Configuring the system

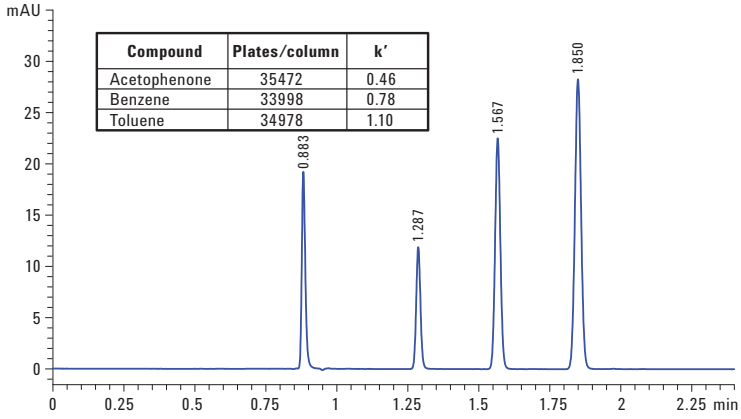
The following experiments evaluated the performance of the Agilent Poroshell 120 columns. The internal diameter was 4.6 mm and the column length 150 mm for all columns used.

- Evaluation of the plate number of a single column at 1.5 mL/min
- Evaluation of the plate number for three coupled columns at 1.5 mL/min
- Evaluation of the plate number for three coupled columns at higher flow rates
- Precision of retention times using isocratic and gradient conditions
- Comparison of a porous shell versus a sub-2-µm particle column

Column efficiency (plate number) is typically measured using isocratic conditions. For a symmetrical peak use the following equation to calculate the plate number (N):

$$N = 5.54\ (RT/W)^2$$

where RT is the retention time and W the peak width at half height.



Compound	Plates/column	k'
Acetophenone	35472	0.46
Benzene	33998	0.78
Toluene	34978	1.10

Chromatographic conditions:

Column:	Agilent Poroshell 120 SB-C18 150 mm × 4.6 mm, 2.7 µm
Sample:	Thiourea, Acetophenone, Benzene, Toluene
Mobile phase:	Water: ACN = 30:70
Flow rate:	1.5 mL/min
Injection volume:	1 µL
Column temperature:	50 °C
Detector:	DAD 254 nm/10, Ref 360/100 nm, 20 Hz, standard cell

Figure 1
Chromatogram to evaluate N for the Agilent Poroshell 120 150 mm × 4.6 mm column.

Evaluation of plate numbers for single column

The following compounds were used to evaluate the plate number for a single column: uracil, acetophenone, benzene and toluene.

The resulting chromatogram and evaluated plate numbers are shown in Figure 1.

The result was approximately 35000 plates/column for toluene under the chromatographic conditions specified.

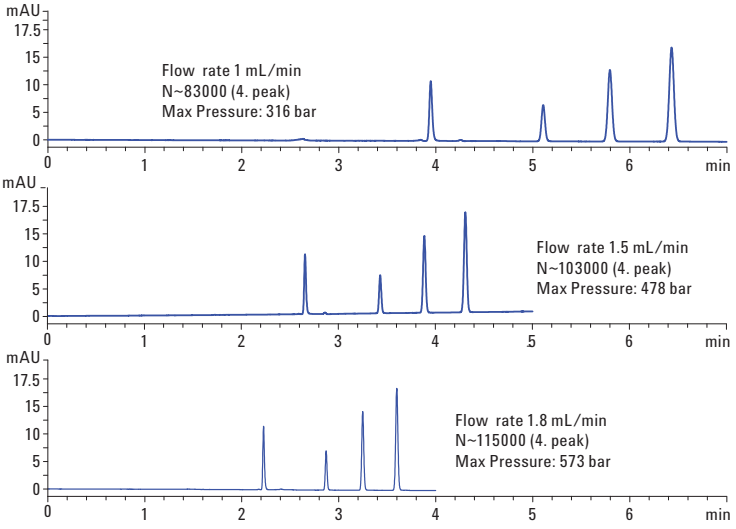
Evaluation of plate numbers for three coupled columns

The plate number for one column is approximately 35000 plates. The expectation is that three columns deliver a plate number of 105000 plates. Column coupling was done using stainless steel capillaries, 90 mm × 0.12 mm. Plate numbers were evaluated for different flow rates.

The resulting chromatograms are shown in Figure 2. If a 400-bar LC system is used, about 80000 plates can be obtained at 1 mL/min flow rate. However, higher flow rates and efficiencies can be obtained with this LC system, which allows pressures up to 1200 bars.

At 1.5 mL/min flow rate the obtained plate number of approximately 103000 plates is close to the expected value.

The best result for toluene with approximately 115000 plates was obtained at 1.8 mL/min with a retention time < 5 min (Table 1).



Chromatographic conditions:

Sample:	Thiourea, Acetophenone, Benzene, Toluene
Column:	Three coupled Agilent Poroshell 120 SB-C18, 150 mm × 4.6 mm, 2.7 µm columns
Mobile phase:	Water: ACN = 20:80
Flow rate:	1, 1.5, 1.8 mL/min
Injection volume:	1 µL
Column temperature:	60 °C
Detector:	DAD 254 nm/10, Ref 360/100 nm, 20 Hz, standard cell

Figure 2
Two chromatograms to evaluate N for three coupled Agilent Poroshell 120 150 mm × 4.6 mm columns at different flow rates.

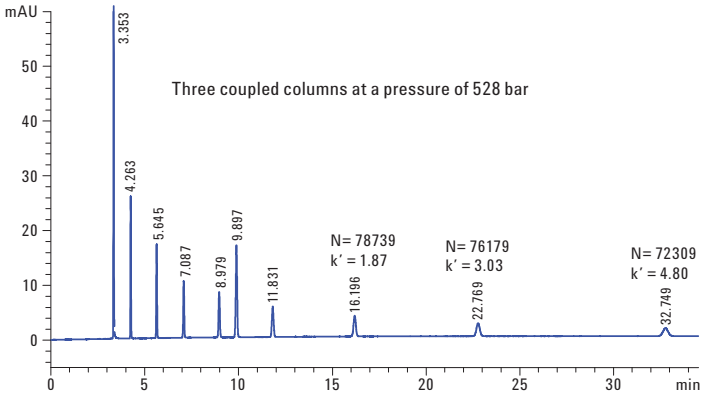
Compound	Plates	k'
Acetophenone	114120	0.29
Benzene	109931	0.46
Toluene	114800	0.62

Table 1
Plate numbers at 1.8 mL/min flow rate.

For higher k' values good results are obtained using three coupled columns. A flow rate of 1.2 mL/min was used. (Figure 3)

Precision of retention times using isocratic conditions

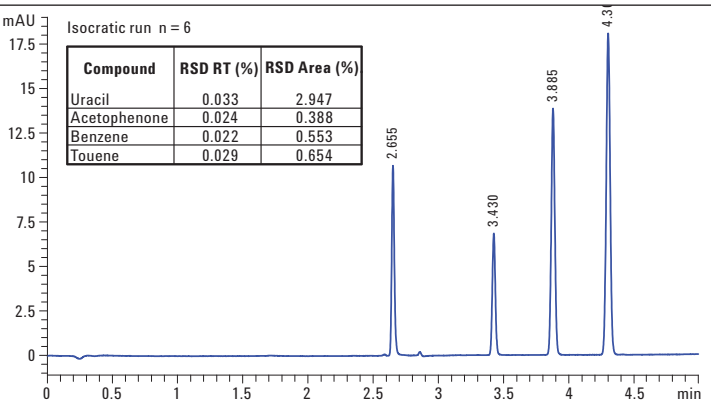
Precision for isocratic conditions at 1.5 mL/min was evaluated and results are shown in Figure 4 together with an overlay of six consecutive runs. The precision of retention times is < 0.034% RSD, and the precision for areas is < 0.66% RSD, except for uracil.



Chromatographic conditions:

Sample:	Thiourea+Test sample: Set of nine compounds, 100 ng/uL each, dissolved in water/ACN (65/35) 1. Acetanilide, 2. Acetophenone, 3. Propiophenone, 4. Butyrophenone (200 ng/µL), 5. Benzophenone, 6. Valerophenone, 7. Hexanophenone, 8. Heptanophenone, 9. Octanophenone
Column:	Three coupled Agilent Poroshell 120 SB-C18, 150 mm × 4.6 mm, 2.7 µm columns
Mobile phase:	ACN/Water 60/40
Column temperature:	60 °C
Flow rate:	1.2 mL/min
Detector:	DAD 254 nm/10 nm, ref 360/100 nm, 20 Hz, Standard cell

Figure 3
Plate numbers at higher k' values for three coupled columns at 528 bar and 1.2 mL/min flow rate.



Chromatographic conditions:

Sample:	Uracil, Acetophenone, Benzene, Toluene
Column:	Three coupled Agilent Poroshell 120 SB-C18 150 mm × 4.6 mm, 2.7 µm columns
Mobile phase:	Water: ACN = 20:80
Flow rate:	1.5, mL/min
Injection volume:	1 µL
Column temperature:	60 °C
Detector:	DAD 254 nm/10, Ref 360/100 nm, 20 Hz, standard cell

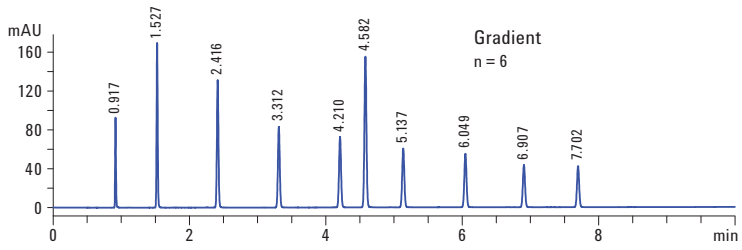
Figure 4
Overlay of six consecutive runs using isocratic conditions and precision data for retention times and areas.

Precision for retention times and areas using gradient conditions

The precision for gradient analysis was evaluated using a gradient from 35 to 95% in 10 min. The results and the overlay of six consecutive runs are shown in Figure 5.

Excellent precision was achieved for retention times of all compounds (RSD < 0.04%), except for Thiourea (Figure 5).

The RSDs for the areas of all compound peaks were less than 0.38% for a 1-µL injection.



Peak	RSD Retention time (%)	RSD Area (%)
Thiourea	0.092	0.372
1	0.020	0.238
2	0.038	0.255
3	0.033	0.211
4	0.029	0.186
5	0.027	0.227
6	0.023	0.194
7	0.018	0.183
8	0.017	0.251
9	0.017	0.167

Chromatographic conditions:

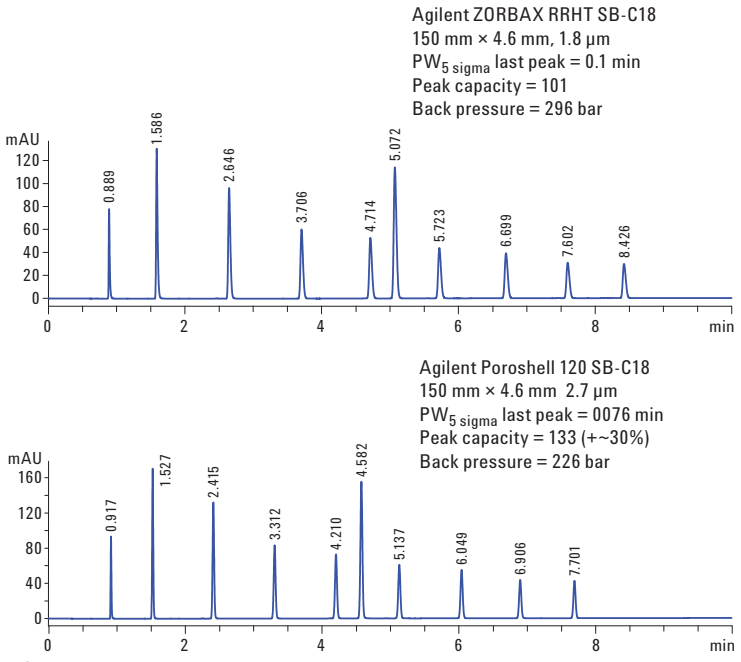
Sample:	Thiourea + Test sample: Set of nine compounds, 100 ng/µL each, dissolved in water/ACN (65/35) 1. Acetanilide, 2. Acetophenone, 3. Propiophenone, 4. Butyrophenone (200 ng/µL), Benzophenone, 6. Valerophenone, 7. Hexanophenone, 8. Heptanophenone, 9. Octanophenone
Column:	Agilent Poroshell 120 SB-C18, 150 mm × 4.6 mm, 2.7 µm
Mobile phase:	Water and ACN
Gradient:	At 0 min 35% ACN, at 10 min 95% ACN
Flow rate:	1.5 mL/min
Injection volume:	1 µL
Column temperature:	60 °C
Detector:	DAD 245/10 nm, Ref 400/100 nm, 20 Hz, standard cell

Figure 5
Overlay of 10 consecutive gradient runs and precision data for retention times and areas.

Comparison of the peak capacity of a porous shell column versus a sub-2-µm particle column

To illustrate the difference between porous shell and sub-2-µm columns, two 150 mm × 4.6 mm id columns were compared analyzing a set of 10 compounds (Figure 6).

The Agilent Poroshell 120 column shows shorter elution times, and smaller peak width, which results in a higher peak capacity for the porous shell column. The Agilent Poroshell 120 column shows 133 peaks with a higher peak capacity than the sub-2-µm column with a peak capacity of 101 peaks. This shows 30% higher efficiency for the Agilent Poroshell 120 column compared to the sub-2-µm column for the conditions used.



Chromatographic conditions:

Sample:	Thiourea + Test sample: Set of nine compounds, 100 ng/µL each, dissolved in water/ACN (65/35) 1. Acetanilide, 2. Acetophenone, 3. Propiophenone, 4. Butyrophenone (200ng/µL), Benzophenone, 6. Valerophenone, 7. Hexanophenone, 8. Heptanophenone, 9. Octanophenone
Column:	Agilent ZORBAX RRHT SB-C18, 150 mm × 4.6 mm, 1.8 µm, Agilent Poroshell 120 SB-C18, 150 mm × 4.6 mm, 2.7 µm
Mobile phase:	Water and ACN
Gradient:	0 min 35% ACN, 10 min 95% ACN
Flow rate:	1.5 mL/min
Injection volume:	1 µL
Column temperature:	60 °C
Detector:	DAD 245/10 nm, Ref 400/100 nm, 20 Hz, standard cell

Figure 6
Chromatograms of a "Phenone" mix analyzed on porous shell and sub-2-µm particle columns.

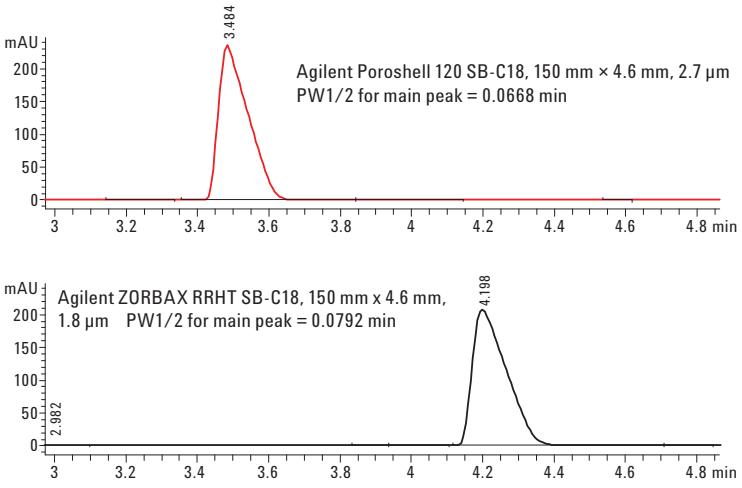
Comparison of volume capacity

To test whether porous shell columns have the same or lower volume capacity than column packed with 1.8 µm particles, a highly concentrated sample was injected. The injection volume was 10 µL and the concentration was approximately 20 µg in 10 µL (Figure 7).

No significant differences were observed for the main peak using the selected conditions. The peak width for the Poroshell 120 column was somewhat lower because in this case the peak eluted earlier. The peak width is typically smaller.

Comparison of signal-to-noise

Impurities in a pharmaceutical drug were analyzed to evaluate the signal-to-noise ratio. The impurities were present in a 0.02–0.03 percentage range. The chromatographic conditions are listed in Figure 7.



Chromatographic conditions:

Test Sample:	Tramadol 2,022 mL/mL containing impurities,
Column:	Agilent Poroshell 120 SB-C18, 150 mm × 4.6 mm, 2.7 µm Agilent ZORBAX RRHT SB-C18, 150 mm × 4.6 mm, 1.8 µm
Pump:	
Solvent A:	Water + 0.2 % TFA and Solvent B: ACN + 0.16% TFA
Gradient:	17 to 45% B in 5 min, Stop Time 7 min, Post time 3 min
Flow rate:	1.5 mL/min
Autosampler	
Injection volume:	10 µL
Wash time:	10 s
Thermostatted Column Compartment	
Temperature:	30 °C
DAD:	1290 270/10 nm, Ref. 360/100 nm, 20 Hz, Standard flow cell of 10 mm path length

Figure 7
Capacity comparison of porous shell and sub-2-µm columns; Injection volume 10 µL = 20 µg.

Figure 8 shows an overlay of a section of the complete chromatograms. The red trace represents the Poroshell 120 chromatogram and the black trace represents the sub-2-µm chromatogram.

In Table 2, the signal-to-noise calculations for both columns are combined. Impurity 1 and 2 were analyzed on the Poroshell 120 column and on the sub-2-µm column.

Conclusion

Porous shell columns represent a real alternative to sub-2-µm columns. The lower back pressure allows flow rates of 1 mL/min for a 4.6 mm x 150 mm, 2.7 µm column without exceeding the 400 bar limit. In this case, 35000 plates are achievable or more than 235000 plates/meter.

Column coupling of three 4.6 mm × 150 mm columns result in a plate number of 100000 plates in under 5 min without exceeding the 600 bar limit.

Agilent Poroshell 120 columns show excellent precision data for isocratic and gradient analysis.

Typically for Agilent Poroshell 120 columns shorter elution times than that of the similar sub 2-µm banded phase columns can be expected if the same chromatographic conditions are applied. The shorter elution times result in smaller peak widths and consequently higher peak capacities.

References

1. J. M. Cunliffe, T. D. Maloney, "Fused-core particle technology as an alternative to sub-2-µm particles to achieve high separation efficiency with low back pressure", *J. Sep. Sci.* 2007, 30, 3104-3109

2. F. Griiti, A. Cavazzini, N. Marchetti, G. Guiochon," Comparison between the efficiencies of columns packed with fully and partially porous C18-bonded silica materials", *Journal of Chromatography A*, 1157, 289-303, 2007

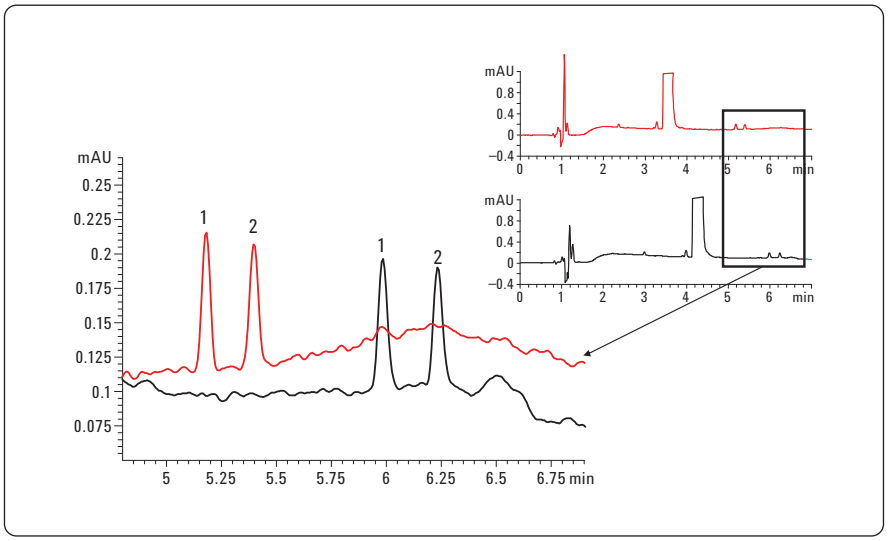


Figure 8
Comparison signal-to-noise ratio, red represents the porous shell column and black trace represents the 1.8 µm particle column. Modifier TFA was used.

Peak	Poroshell 120 S/N	1.8 µm S/N
1	14	13.6
2	12.8	12

Table 2
Comparison of signal-to-noise ratios for porous shell and 1.8 µm particle columns.

表面多孔高效液相色谱柱和柱超载

Technical Overview

Introduction

新型的表面多孔颗粒高效液相色谱柱是一类强大的分析工具，能够在高效液相色谱的压力范围内实现高效率、高分离度的快速分析。安捷伦 Poroshell 120 色谱柱由 2.7 µm 的表面多孔颗粒填充，可实现相当于亚 2 µm 颗粒填充色谱柱 80% 至 90% 的性能，而反压却比后者低将近 50%，因此此类色谱柱可适用于所有的 HPLC 和 UHPLC 系统。

这些表面多孔颗粒填充的色谱柱在分析分离许多小分子时，能够表现出卓越的效率和性能。此外，色谱柱必须能够适应典型的样品载量，才能成为方法开发和常规应用的主要分析工具。只有满足此条件，在该色谱柱上进行的分离才能具有足够的灵敏度。许多行业领域都非常关注样品载量，因为这关系到确定样品中的杂质所需要进行的研究数量。在许多情况下，通常采用较大的进样体积使低含量杂质的检测峰更为明显。这种情况下，主要组分的峰形必须足够尖，以免由于谱带变宽、漂移或拖尾而干扰小峰的定量。

Superficially porous particle columns have a solid core and a porous shell. The Poroshell 120 particle, for example, has a 1.7 µm solid core and a 0.5 µm porous shell as shown in Figure 1.

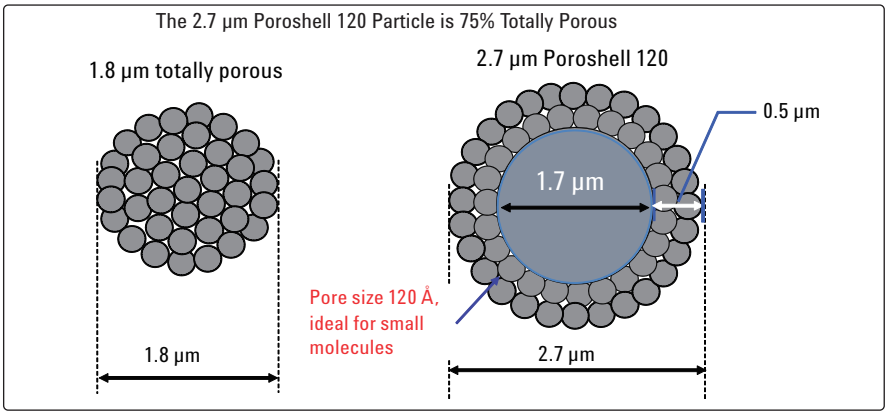


Figure 1. Diagram comparing a superficially porous Poroshell 120 particle to a totally porous 1.8-µm particle.

The total particle size is 2.7 µm. Based on this structure the 2.7 µm particle is 75% totally porous and only 25% of it is the solid core. With so much of the particle totally porous, sample loading on the column falls into a standard analytical range. And the sample loading compares well to that achieved on a totally porous sub 2-µm column.

The following illustrates calculations of Volume Total, and Volume Core:

$$V = \frac{4}{3} pi^{*} r^{3}$$

r_{core} = radius of the core = 0.85 µm

r_{total} = radius of the whole particle = 1.35 µm

Volume total = 10.31 μm^3 Volume Core = 2.57 μm^3

Volume % core = (2.57 μm^3/10.31 μm^3) × 100% = 25%

Volume Porous Shell = 100% – (volume % of core) = 100% – 25% = 75% totally porous

A series of loading experiments was performed to compare the loading capacity of the Poroshell 120 superficially porous particle column to another superficially porous particle column, as well as to both 1.8 µm and 3.5 µm totally porous particle columns (both Agilent ZORBAX Eclipse Plus C18's).

The experiments were designed to evaluate mass loading, which is the amount of material on the column with each injection. In mass overloading, the peak shape will start to change as the loading of the column increases. When the column is overloaded the peak shape will no longer be symmetrical. One typical change is for the peak to broaden with the increasing mass load and have a sharp peak front shifted earlier along with a sloping tail. This change in peak shape can be seen visually in a chromatogram, but that level of distortion clearly indicates sample overloading.

At a more analytical level we can measure the peak width at half-height, and when the peak width doubles we recognize the peak as being overloaded. Peak width is not the only parameter that can be measured, but it is one good choice. Efficiency loss is another parameter that can easily be measured.

The actual loading of acidic and basic compounds can vary based on the nature of these ionizable compounds. Therefore, the experiments included both acidic and basic compounds. A neutral compound was also analyzed, but in general neutral compounds have better sample loading and are of less interest to most chromatographers. The experiments were designed to start at low, analytical sample loads and increase the sample loading until the columns were clearly overloaded.

Peak width at half-height served as a primary measure of the sample overloading and the parameter used for comparison to the loading on the other columns. The first compound evaluated was an acidic compound, benzoic acid. The mobile phase was 25 mM NaH_2PO_4 at a pH of 3, which should typically result in good peak shape for benzoic acid, with pKa = 4.2. The sample loading started out at 0.082 µg on column and ended at 123 µg on column for 3 mm × 100 mm columns. For each injection a number of parameters were measured, including peak width at half-height. A plot of the peak width vs. sample load of benzoic acid is shown in Figure 2.

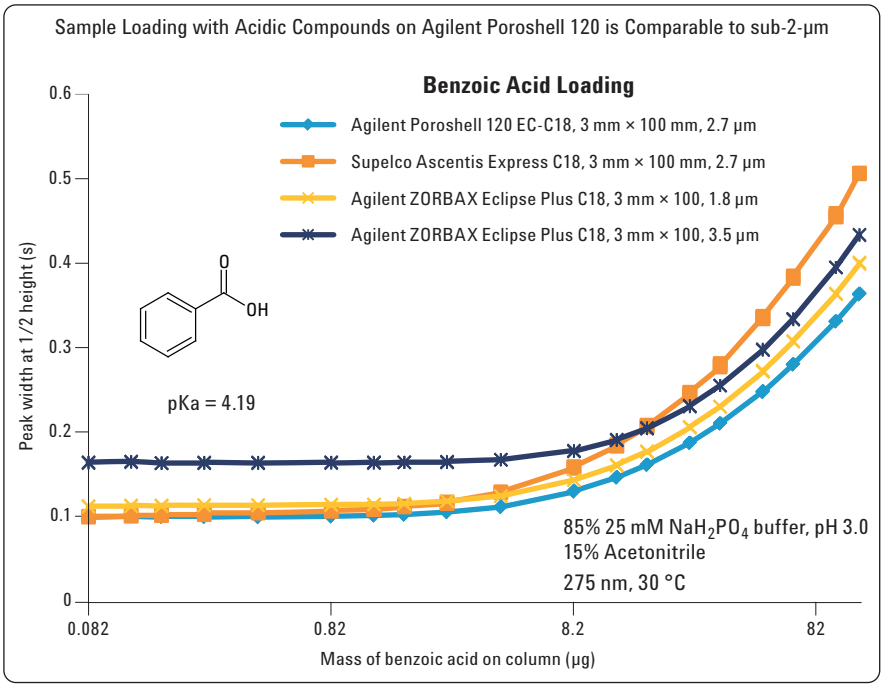


Figure 2. Benzoic acid loading on 3 mm × 100 mm columns.

A number of observations can be made from this plot. The plot shows that the superficially porous Poroshell 120 and 1.8 µm totally porous Eclipse Plus columns have roughly the same loading ability. Through a range of reasonable sample loads, each column maintains peak width at a given value. The flat region of the curves shows that each of the superficially porous and the 1.8 µm totally porous columns yields similar peak width for benzoic acid at pH 3. The only outlier in this plot is the 3.5 µm totally porous Eclipse Plus column, which produces notably wider peaks at low sample loads. This could compromise the resolution of small impurities. The chromatograms are shown in Figure 3.

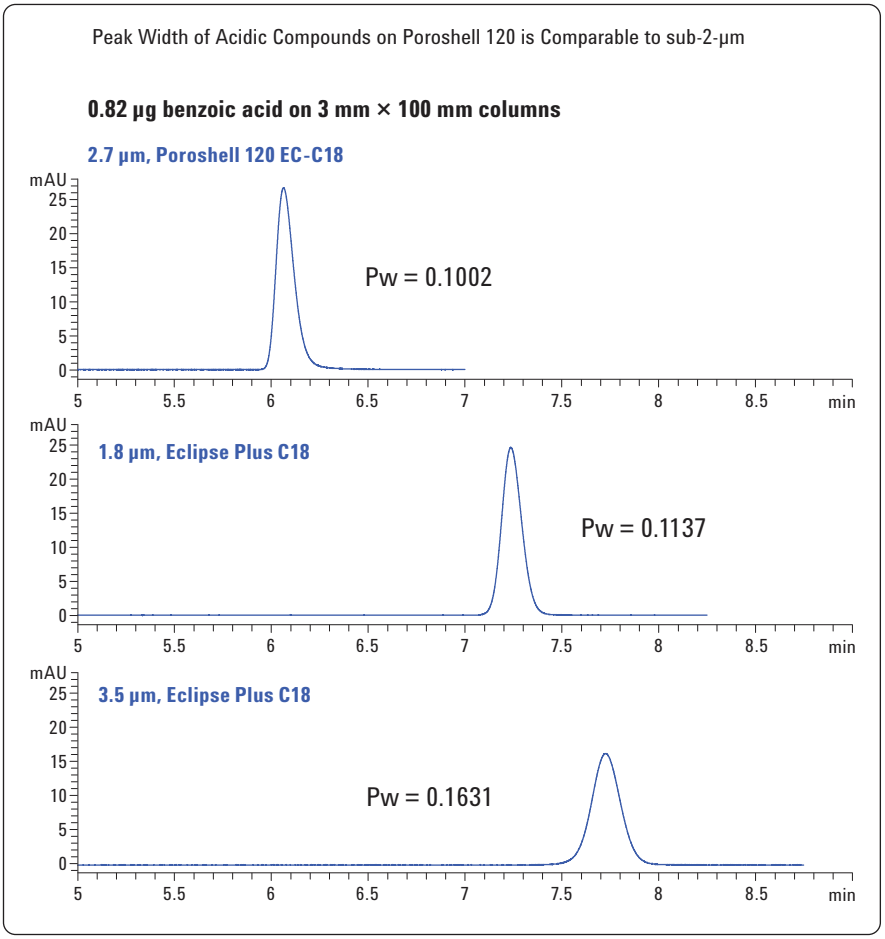


Figure 3. Normal peak width comparison of 2.7, 1.8 and 3.5 µm particles with benzoic acid on 3 mm × 100 mm columns.

Overloading of the sample is depicted when each curve in Figure 2 begins to trend upward. This can be seen at roughly the same amount of benzoic acid on-column for each of the superficially porous and sub-2-µm columns. Again, the 3.5 µm column stands out, because it starts with a much higher peak width, and the peak width doubles at approximately twice the concentration of the other columns. While this appears to be an advantage, the broader peak widths on the 3.5 µm column will limit resolution.

Overloading is noted by a change in peak width, peak shape and retention time on all these columns, as can be seen in Figure 4. For both Poroshell 120 and 1.8-µm Eclipse Plus columns, low sample load peak widths are roughly the same. They begin to increase as overloading occurs; for the two columns in Figure 4, overload occurs at about the same sample load of benzoic acid. In this experiment, overloading is determined when peak width at half-height doubles. Also notable is the inward shift in retention time and increase in tailing.

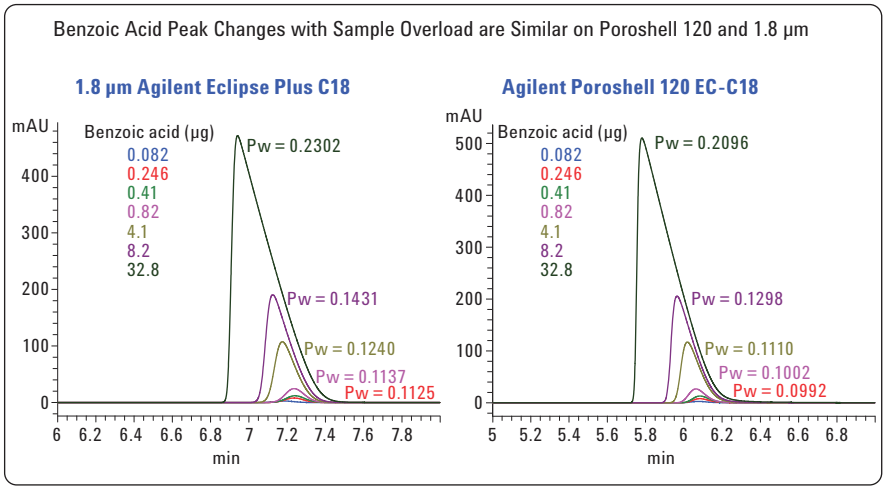


Figure 4. Overlay showing benzoic acid peak shape change with sample overload on 3 mm × 100 mm columns.

Figure 5 shows another loading curve with a basic compound, nortriptyline. This series of data was also collected with a 25 mM NaH_2PO_4 pH 3 mobile phase, which should produce good peak shape for nortriptyline with pKa=9.7. Sample loading ranged from 0.008 µg to 4 µg on 3 mm × 100 mm columns. For each injection, peak width at half-height was monitored and plotted in Figure 5 to determine the point of overload.

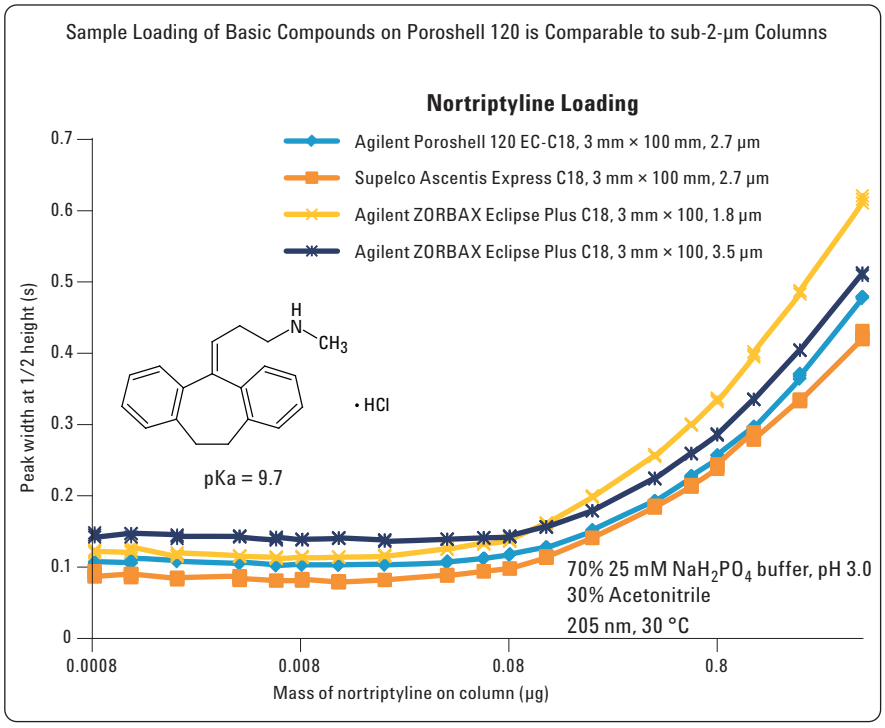


Figure 5. Nortriptyline loading on 3 mm × 100 mm columns.

As in the acidic compound plot, this nortriptyline plot shows similar loading for superficially porous particle columns and sub-2 µm totally porous columns. The most different column, as would be expected, is the 3.5-µm totally porous column because of the much broader peak width on this larger particle size. For the 3.5-µm Eclipse Plus column, the peak width doubles at a higher load as compared to the superficially porous and totally porous sub-2 µm columns because of the broader starting peak width. Each of the superficially porous columns and the 1.8 µm totally porous column start with similar peak width measurements, and display characteristics indicative of overload at nearly the same mass load on-column. Normal peak widths for 2.7 µm superficially porous, 1.8 and 3.5 µm totally porous columns are shown in Figure 6.

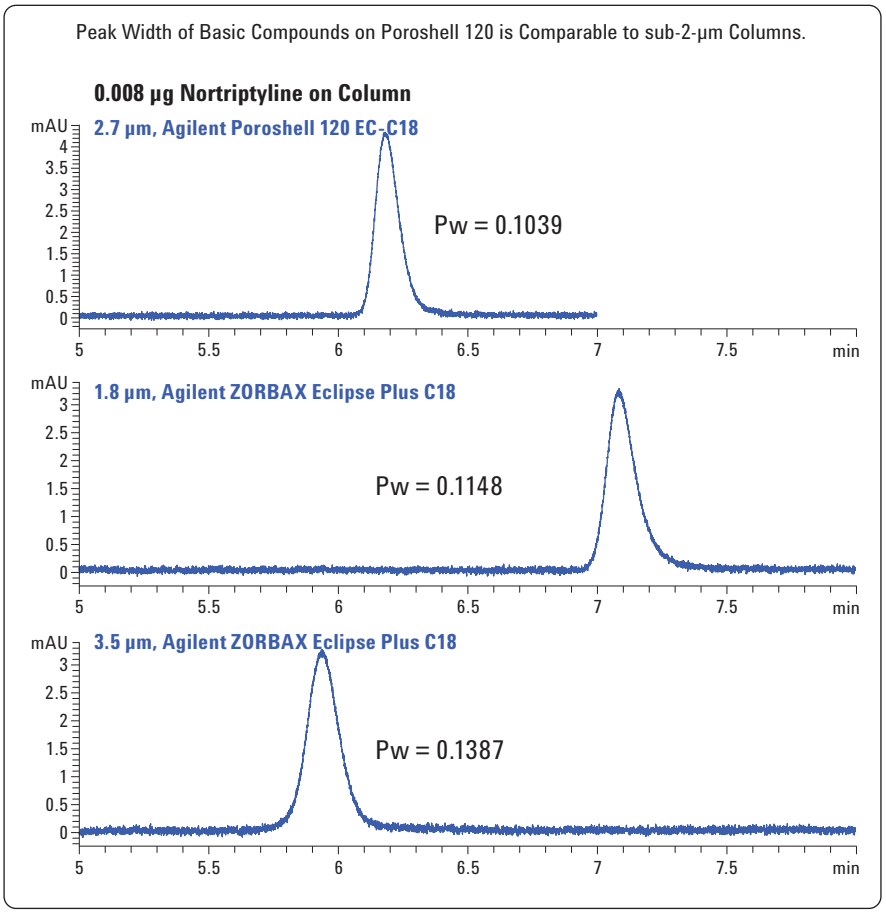


Figure 6. Normal peak width comparison of 2.7, 1.8 and 3.5 um particles with nortriptyline on 3 mm × 100 mm columns.

The trend in peak width, shape and retention time as nortriptyline load increases is similar to that of benzoic acid. Peaks shift inward, tail more and grow wider as sample is overloaded onto the column for both Poroshell 120 and 1.8-µm Eclipse Plus columns, shown in Figure 7. Most notable, again, is the similar mass load of nortriptyline for which peak width doubles on the superficially porous and totally porous sub-2-µm columns. This indicates very similar sample loading capabilities for these two columns.

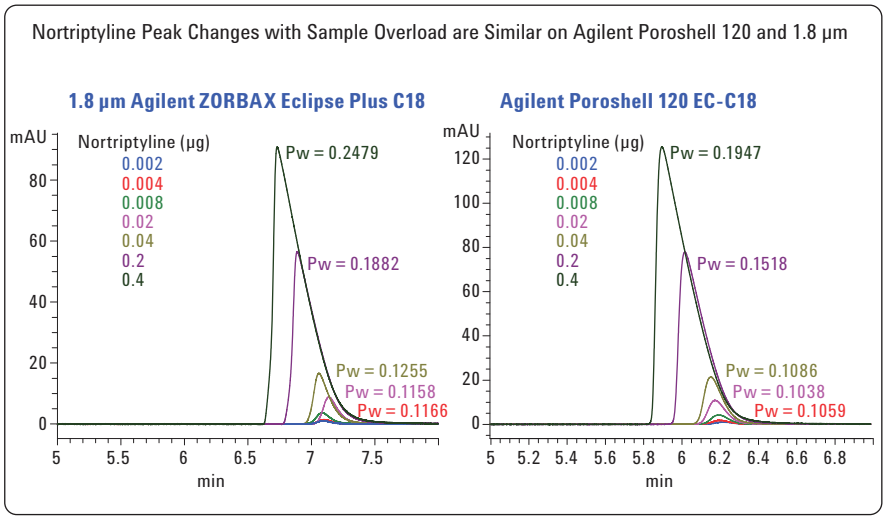


Figure 7. Overlay showing nortriptyline peak shape change with sample overload on 3 mm × 100 mm columns.

Valerophenone was used as the probe for a neutral compound loading study. Data was collected using a neutral mobile phase of water/acetonitrile, producing sharp, narrow peaks for valerophenone at low sample mass loading. Masses ranging from 0.2 to 300 µg of valerophenone were loaded onto 3.0 mm × 100 mm columns. Peak width at half-height was measured and plotted against sample load, as shown in Figure 8. Trends are similar to those found in both the acidic and basic compound loading studies.

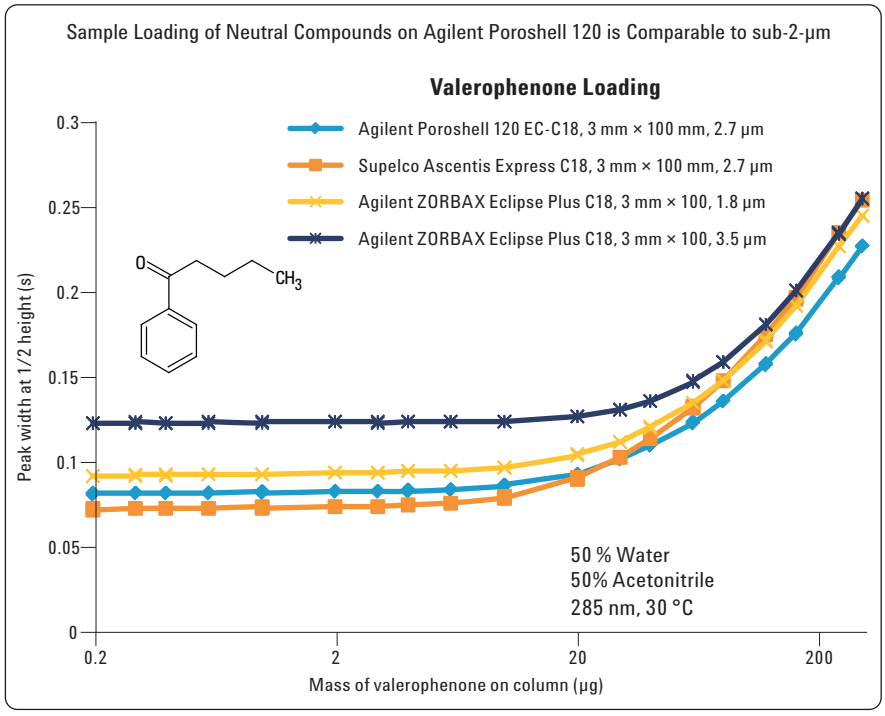


Figure 8. Valerophenone loading on 3 mm × 100 mm columns.

The totally porous 1.8 µm and superficially porous columns perform similarly with valerophenone loading, while the 3.5 µm totally porous column produces wider peaks (Figure 9). This allows for nearly twice the sample load on-column before the peak width at half-height doubles, but potentially compromises resolution more, due to the broader peaks.

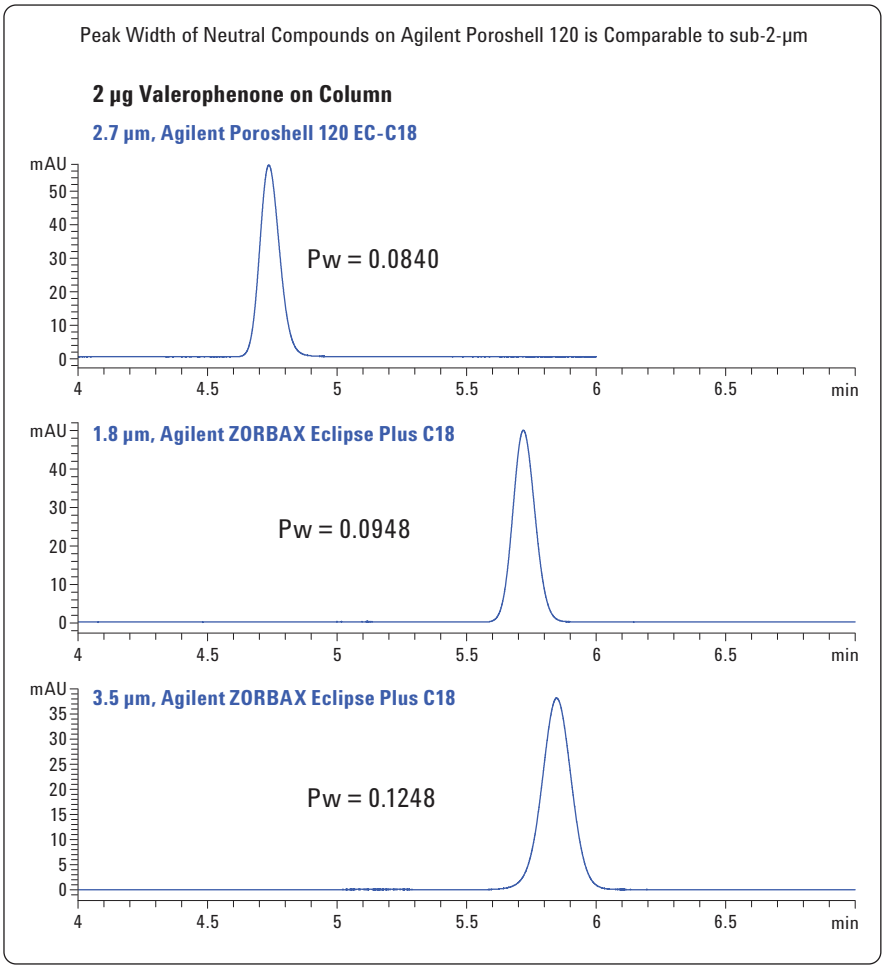


Figure 9. Normal peak width comparison of 2.7, 1.8 and 3.5 µm particles with valerophenone on 3 mm × 100 mm columns.

The chromatographic indications of sample overload for the neutral compound, valerophenone, are similar to the benzoic acid and nortriptyline examples provided earlier. Peak width increases as the sample mass approaches overload, while retention time shifts inward and tailing increases. Figure 10 shows the chromatographic changes that are evident of overload, highlighting the similarities between the superficially porous Poroshell 120 and totally porous 1.8-µm Eclipse Plus loading capacities.

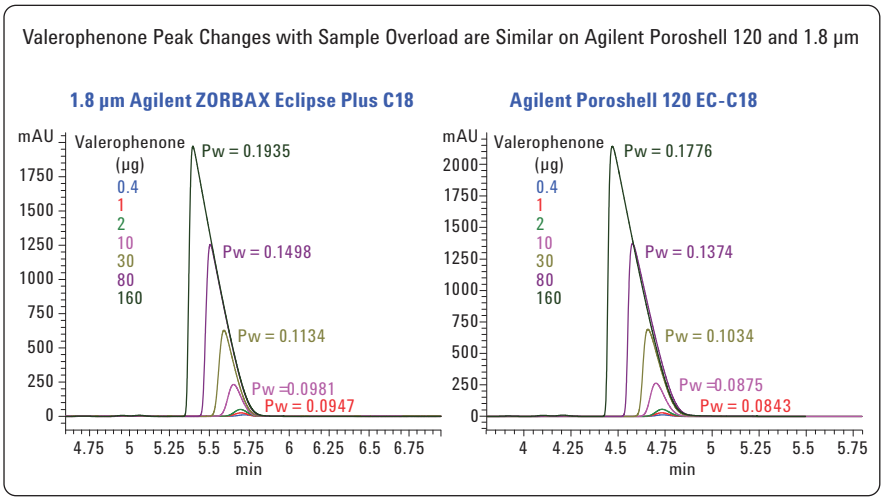


Figure 10. Overlay showing valerophenone peak shape change with sample overload on 3 mm × 100 mm columns.

Based on the loading curves for each of the compounds on each the columns tested, sample loads that produce peaks with twice the peak width at half-height were calculated. The results are shown in Table 1. The data shows that the superficially porous Poroshell 120 and totally porous 1.8-µm Eclipse Plus have similar loading abilities for all compounds tested, while the 3.5-µm Eclipse Plus can load about twice the sample mass in all cases. The superficially porous Ascentis Express was also tested, showing similar initial peak shape (Figure 11), but lower loading capacities with each compound, when compared to the Poroshell 120. Poroshell 120 was found to load about double the sample mass of a similar dimension Ascentis Express column before overloading occurred.

Table 1. Benzoic Acid, Nortriptyline and Valerophenone Loading Studies w/Calculated Mass on Column at Overload

	Benzoic Acid Loading			**Nortriptyline Loading**			**Valerophenone Loading**		
	Average Peak Width at 1/2 Height (s)	*Peak Width at Overload (2 x Ave PW) (s)*	*Mass Load on Column at Overload (µg)*	*Average Peak Width at 1/2 Height (s)*	*Peak Width at Overload (2 x Ave PW) (s)*	*Mass Load on Column at Overload (µg)*	*Average Peak Width at 1/2 Height (s)*	*Peak Width at Overload (2 x Ave PW) (s)*	*Mass Load on Column at Overload (µg)*
Agilent Poroshell 120 EC-C18, 3 mm × 100 mm, 2.7 µm	0.100	0.199	29.0	0.106	0.211	0.515	0.083	0.166	137
Supelco Ascentis Express C18, 3 mm × 100 mm, 2.7 µm	0.103	0.205	16.2	0.085	0.170	0.334	0.074	0.148	79.2
Agilent ZORBAX Eclipse Plus C18, 3 mm × 100, 1.8 µm	0.113	0.225	31.2	0.118	0.235	0.327	0.094	0.188	149
Agilent ZORBAX Eclipse Plus C18, 3 mm × 100, 3.5 µm	0.164	0.327	62.6	0.142	0.285	0.799	0.124	0.249	280

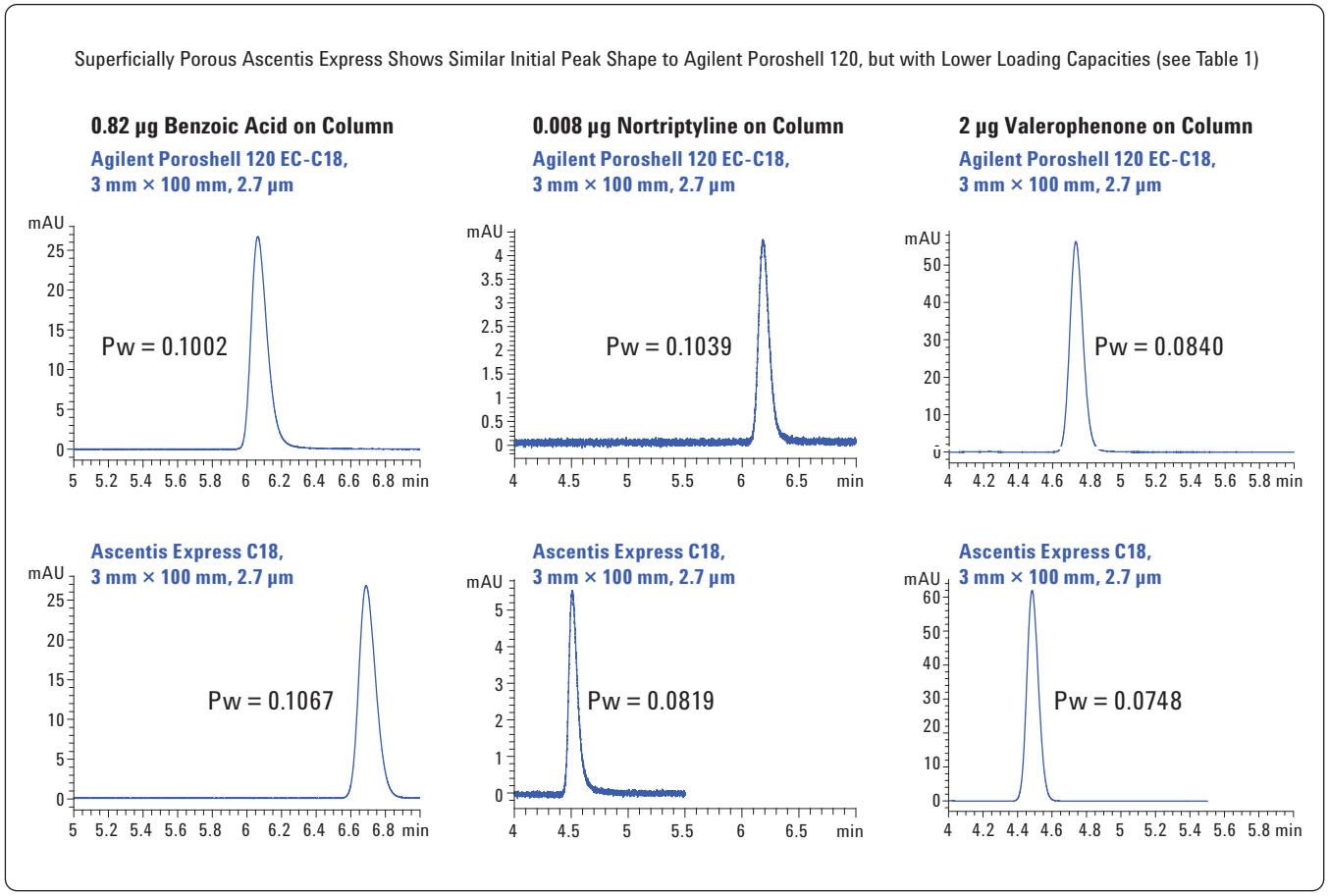


Figure 11. Agilent Poroshell 120 and Ascentis Express comparison under normal loading conditions with acidic, basic and neutral compounds.

An interesting observation from these loading studies is illustrated in Table 2. Overloading on a small particle column is not necessarily a reason to switch to a larger particle column to avoid overload. Table 2 shows the doubled peak width at which each column is determined to be overloaded, along with the calculated sample mass on-column needed to generate that peak width at overload. The third column for each of the respective loading studies gives the calculated peak width of the 3.5 µm totally porous column at the same mass load needed to double the peak width on the smaller particle columns. In nearly all cases, the 3.5 µm totally porous column produced wider peaks at the given sample load than both the overloaded superficially porous and sub-2-µm totally porous columns. This would suggest that even when high sample loading is required and sample overloading is unavoidable with small particle columns, superficially porous and 1.8-µm totally porous columns can still provide narrower peaks and potentially more resolution than a non-overloaded 3.5 µm totally porous column.

Table 2. Comparing Superficially Porous and sub-2-um Overloaded Peak Widths to Non-Overloaded 3.5-µm Peak Widths at Same Sample Mass Load

	Benzoic Acid Loading			**Nortriptyline Loading**			**Valerophenone Loading**		
	Peak Width at Overload (2 x Ave PW) (s)	*Mass Load on Column at Overload (µg)*	*3.5 µm Peak Width at Mass Load (s)*	*Peak Width at Overload (2 x Ave PW) (s)*	*Mass Load on Column at Overload (µg)*	*3.5 µm Peak Width at Mass Load (s)*	*Peak Width at Overload (2 x Ave PW) (s)*	*Mass Load on Column at Overload (µg)*	*3.5 µm Peak Width at Mass Load (s)*
Agilent Poroshell 120 EC-C18, 3 mm × 100 mm, 2.7 µm	0.199	29.0	0.244	0.211	0.515	0.245	0.166	137	0.190
Supelco Ascentis Express C18, 3 mm × 100 mm, 2.7 µm	0.205	16.2	0.203	0.170	0.334	0.210	0.148	79.2	0.160
Agilent ZORBAX Eclipse Plus C18, 3 mm × 100, 1.8 µm	0.225	31.2	0.250	0.235	0.327	0.208	0.188	149	0.197

The acidic, basic and neutral compounds used in this loading study show similar loading capacities for superficially porous and sub-2-µm totally porous columns. In all cases the symptoms of sample overload were similar:—retention time shifts inward, tailing increases, and peak width increases. However, despite the negative effects of sample overload on peak shape, superficially porous and sub-2-µm totally porous columns can still yield narrower peaks than larger 3.5 µm totally porous columns at the same sample mass load.

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Transfer of Methods between Poroshell 120 EC-C18 and ZORBAX Eclipse Plus C18 Columns

Technical Overview

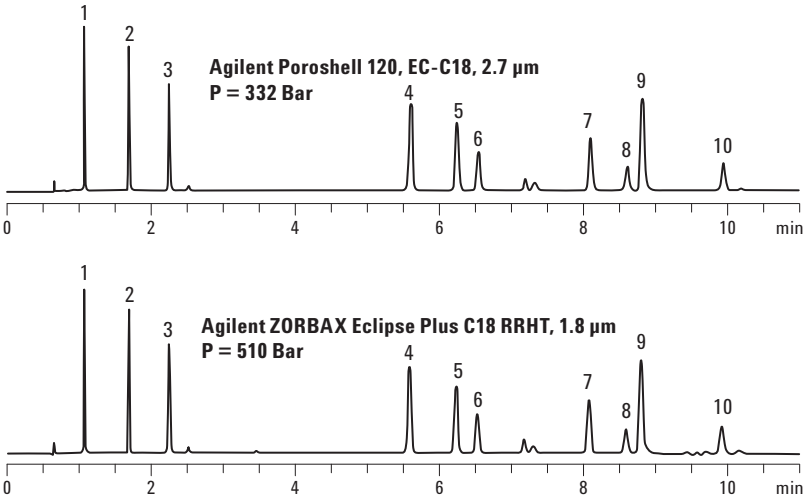
Introduction

The developement of superficially porous particles has led to the possibility of method transfer from larger 5-µm totally porous particles, as well as from sub-2-µm totally porous particles. One of the benefits of transferring from larger particle columns is significant time savings, as the superficially porous particles are optimally run at a faster flow rate achieving similar resolution with a much shorter column length [1–4]. The high efficiency of superficially porous particles is similar to sub-2-µm totally porous particles because of the short mass transfer distance and substantially narrower particle size distribution. Transferring methods from totally porous sub-2-µm columns may also be desirable. Many development laboratories have chosen to use sub-2-µm columns. However, in some cases the higher operating pressure required of sub-2-µm methods may not be transferable to all HPLC systems. In many cases methods using sub-2-µm columns can be directly transferred to superficially porous particle columns, without adjustment. This is particularly true when columns like the Agilent Poroshell 120 EC-C18 and Agilent ZORBAX Eclipse Plus C18 are manufactured to have similar bonding chemistries and use similar retention mechanisms. Additionally, superficially porous particle columns can perform the same analysis as sub-2-µm columns, while generating less backpressure. This allows analysts to increase flow rates for higher throughput, or to increase column length to enhance resolution without exceeding the system pressure limits.

One asset of the Agilent ZORBAX family of HPLC columns is the scalability of methods between particle sizes. This allows a quick and reliable transfer of methods from method development to preparative lab and high throughput analysis.

Several recent comparisons of Agilent Poroshell 120 EC-C18 and Agilent ZORBAX Eclipse Plus C18 have shown very similar chromatography. Poroshell 120 was designed to deliver 90 % of the efficiency of sub two micron columns such as Eclipse Plus C18 at approximately 60 % of the pressure. Superficially porous particles found in Poroshell 120 have the low pressure benefits of larger particles while achieving the performance of sub two micron particles.

Examples of this chromatographic similarity are shown using environmental phenols in Figure 1 with 0.1 % Formic acid and in Figure 2 in the analysis of soft drink additives using 10 mM ammonium acetate pH 4.8. In both cases, the retention order of the compounds are the same. The similarity of these two examples leads to the larger question, how similar are Poroshell 120 EC-C18 and Eclipse Plus C18, in terms of selectivity over a wider range of operating conditions and with a larger set of compounds including acids bases and neutral materials.



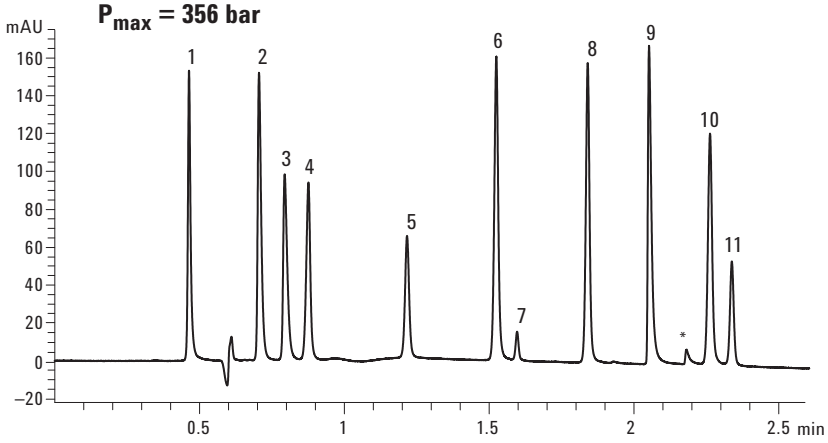
Conditions

Columns	Agilent Poroshell 120 EC-C18, 4.6 mm × 100 mm, 2.7 µm Agilent p/n 689975-902 Agilent ZORBAX Eclipse Plus RRHT C18, 4.6 mm × 100 mm, 1.8 µm Agilent p/n 959964-902
Mobile phase	A: 0.1% Formic acid B: MeCN + 0.1% Formic acid
Temperature	40 °C
Detection	275 nm
Injection volume	10 µL
Flow	2 mL/min
Initial	8% B, 10 min 30% B

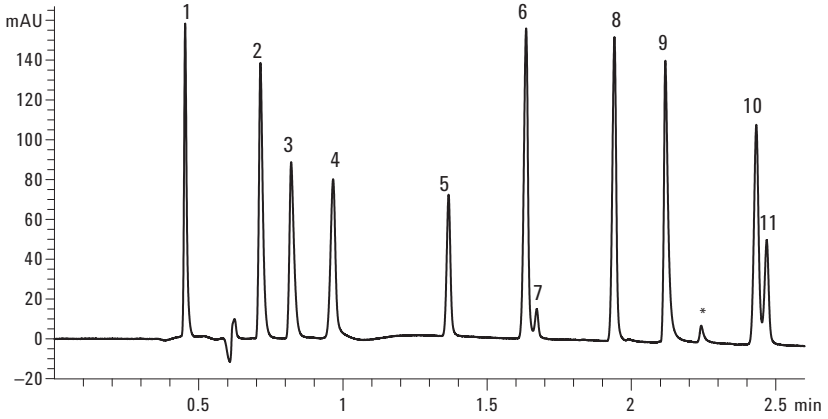
1. Hydroquinone	6. o-cresol
2. Resorcinol	7. 2-Nitrophenol
3. Catechol	8. 2,3 Dimethyl phenol
4. 4-Nitrophenol	9. 2,5 Dimethyl phenol
5. p-cresol	10. 1-Naphtol

Figure 1. Comparison of Agilent Poroshell 120 EC-C18 and Agilent ZORBAX Eclipse Plus C18 using acetonitrile and formic acid mobile phase for the analysis of environmental phenols.

Agilent Poroshell 120 EC-C18, 2.7 µm
P_{max} = 356 bar



Agilent ZORBAX Eclipse Plus C18 RRHT, 1.8 µm
P_{max} = 483 bar



Conditions

Columns	Agilent Poroshell 120 EC-C18, 3.0 mm × 100 mm, 2.7 µm Agilent p/n 695975-302 Agilent ZORBAX Eclipse Plus C18 RRHT, 3.0 mm × 100 mm, 1.8 µm Agilent p/n 959964-302
Mobile phase	A: 20 mM Ammonium acetate, pH 4.80 B: Acetonitrile
Gradient	14% B at t_o, ramp to 52% B in 2.1 min
Flow rate	0.851 mL/min
Temperature	30 °C

1. Ascorbic Acid
2. Acesulfame K
3. Saccharin
4. p-Hydroxybenzoic Acid
5. Caffeine
6. Benzoic Acid
7. Aspartame
8. Sorbic Acid
9. Quinine
10. Dehydroacetic Acid
11. Methylparaben

* Quinine Impurity

Figure 2. Comparison of Agilent Poroshell 120 EC-C18 and Agilent ZORBAX Eclipse Plus C18 using acetonitrile and ammonium acetate mobile phase for the analysis of soft drink addities.

Experimental

Method development is often based upon the use of a generic gradient. Using a short Agilent Poroshell 120 EC-C18, 4.6 x 50 mm column, several different mobile phases can be quickly evaluated. The generic gradient used in this work is run at 2.0 mL/min, starts at 5% organic and increases to 95% organic over 2 min and holds at this concentration for 1 min. Mass spectrometer compatible mobile phases consisting of volatile buffers such as ammonium formate buffer and ammonium acetate buffer are used. These buffers were prepared by dissolving sufficient ammonium formate or ammonium acetate in water to produce 10 mM solutions and titrating the solutions to the desired pH with the appropriate concentrated acid. The pH of these buffers covers a range between 3 and 6.5.

An Agilent 1200 Method Development Solution LC system was used for this work:

- G1312B Binary Pump SL
- G1367D Automatic Liquid Sampler (ALS) SL
- Two G1316C Thermostatted Column Compartments (TCC) SL
- G1315C Diode Array Detector (DAD) SL, using a G1315-60024 micro flow cell (3-mm path, 2-µL volume)
- ChemStation version B.04.01 was used to control the HPLC and to process the data.

Correlation data was calculated and plotted using Microsoft Excel 7.0.

Four Agilent Poroshell 120 EC-C18 columns were used in this work:

- Agilent Poroshell 120 EC-C18, 4.6 mm × 50 mm, 2.7 µm p/n 699975-902
- Agilent Poroshell 120 EC-C18, 3 mm × 100 mm, 2.7 µm p/n 695975-302
- Agilent ZORBAX Eclipse Plus C18, 4.6 mm × 50 mm, 1.8 µm p/n 959943-902
- Agilent ZORBAX Eclipse Plus C18, 3 mm × 100 mm, 1.8 µm p/n 959964-302

Table 1 summarizes the list of compounds studied for this work. These compounds were prepared in water or 50/50 water/acetonitrile and injected individually.

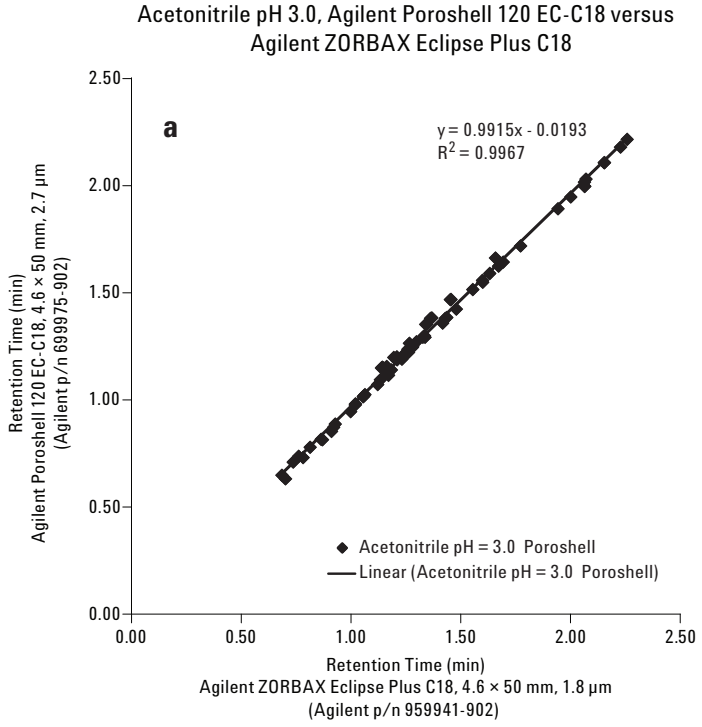
Table 1. Sixty-six Compounds Including Acids, Bases and Neutrals Prepared in 50/50 MeCN/Water and Injected onto 4.6 x 50 mm Columns Individually

List of tested compounds	
furazolidone	phenacetin
chloramphenicol	acetanilide
impramithue	phenol
norethindrail	resorcial
cortisone acetate	hydroquinone
chloramphenicol	4 nitro phenol
busirone hydrochloride	o cresol
benzocaine	1 napthol
pyrimethamine	imipramine hydrochloride
sulfaquinoxaline	3 4 dihydroxy l phenyl alanine
sulfamonomethoxine	dl phenyalanine
nimopidin	ephedrine hydrochloride
sulfadimethoxine	loperamide
sulfamethoxazole	dibenzofuran
sulfachloropyridazine	procaine hydrochloride
sulfamethoxypyridazine	exonazole nitrate
sulfamethizole	gembigrozil
sulfamerazine	beta estradiol
sulfathiazole	metoprolol
sulfadiazine	protriptyline
benzaldehyde	hydroxy sophthalic
phenanthrene	flufenamic acid
biphenyl	pramoxine hydrochloride
acenaphthene	naproxen
methoxy naphthalene	diphenhydramine
dimethoxy benzene	diflunisal
alpha hydroxyprogesterone	nisoldipin
progesterone	diclofenac
prednisolone	hydrocortisone
deoxycorticosterone	procainamide hydrochloride
chlorphenamine	lidocaine
berberine	terfenaine
chlortetracycline hydrochloride	chlorpheniramine maleate

Discussion

Differences in column performance have been studied by many including Wilson, Nelson, Gilroy, Dolan, Snyder and Carr [5,6]. The United States Pharmacopeia lists many columns [7] and a tool to determine how interchangeable columns may be. Characteristics such as silica chemistry and bonding can change selectivity. Silanol activity affects peak shape dramatically through secondary interactions. It also can affect selectivity through H-bonding or ion-exchange. These effects become more pronounced at higher pH than at lower pH [8]. Both Agilent ZORBAX Eclipse Plus C18 and Agilent Poroshell 120 EC-C18 Columns are made from silica produced by Agilent at the same facility that makes the final columns. Both are intended to be highly inert columns and have been designed to yield excellent peak shape with basic compounds. In addition to the effect of pH, silanol activity can also be affected by differences in solvent. Methanol is an H-bonding solvent that has weaker elution strength than aprotic acetonitrile [10]. By choosing a wide range of conditions, it is more likely that differences in selectivity will be revealed.

Figure 3 shows similar retention of 66 compounds on Agilent Poroshell 120 EC-C18 and Agilent ZORBAX Eclipse Plus C18 columns using a generic gradient analysis with a variety of compounds from different chemical classifications. The high correlation coefficient (R^2) indicates a high degree of similarity between the interactions involved in the separation on the two Agilent C18 columns, while a slope of approximately 1 implies similar interaction strengths [9,10].



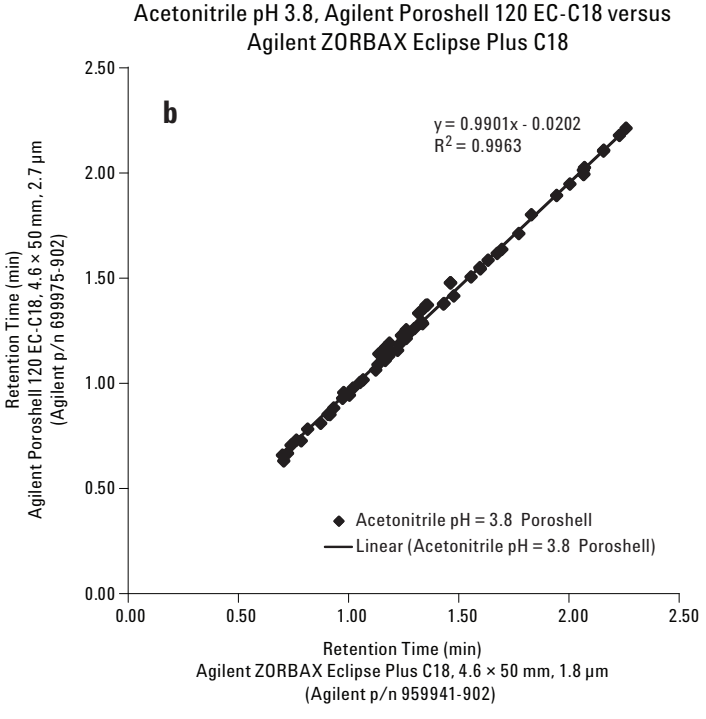
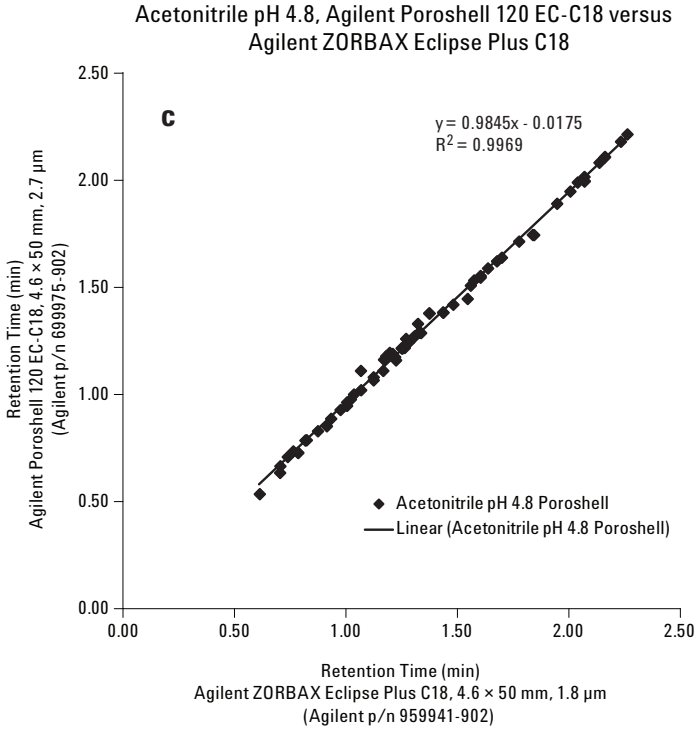
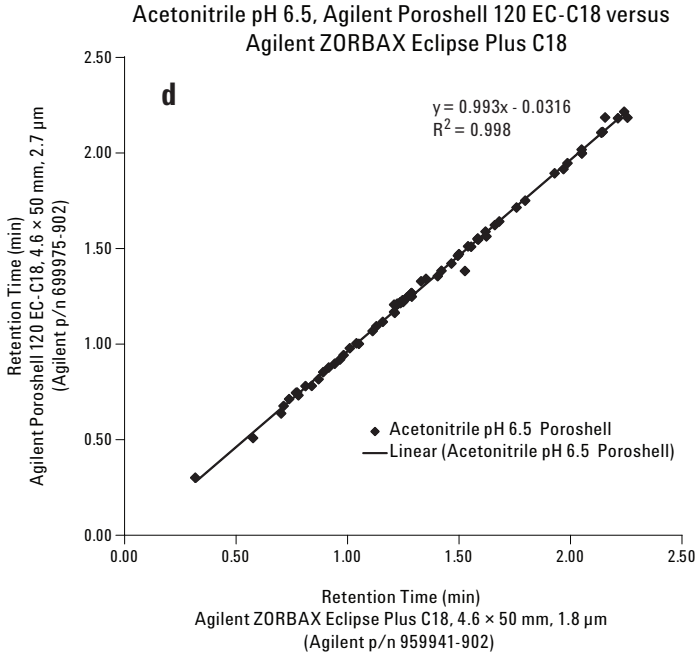


Figure 3. Scatter plot of retention time of 66 compounds on Agilent Poroshell 120 EC-C18, 4.6 × 50 mm, 2.7 µm versus Agilent ZORBAX Eclipse Plus C18, 4.6 × 50 mm, 1.8 µm. (continued)





Conditions

Mobile phase	A: 10 mM Buffer
	B: Organic (ACN)
Gradient	5% B at t_0, ramp to 95% B in 2 min, hold 95% B for 1 min
Flow rate	2 mL/min
Sample	1 µL of 1 mg/mL standard in H_2O or H_2O/ACN

Figure 3. Scatter plot of retention time of 66 compounds on Agilent Poroshell 120 EC-C18, 4.6 × 50 mm, 2.7 µm versus Agilent ZORBAX Eclipse Plus C18, 4.6 × 50 mm, 1.8 µm.

Figure 4 shows scatter plots of the retention times of 66 compounds on Agilent Poroshell 120 EC-C18 versus Agilent ZORBAX Eclipse Plus C18 columns at different pH values between 3 and 6.5 in acetonitrile. Figure 2 shows scatter plots at different pH values between 3 and 6.5 in methanol. The slope and R^2 values for these combinations are summarized in Table 2. As illustrated, the correlation between the two plots is quite good. While retention times sometimes change with the ionic compounds, the changes are proportional on both columns. A slight difference in the slopes of the correlation curves may indicate some difference in H bonding interaction between Agilent ZORBAX Eclipse Plus C18 and Agilent Poroshell 120 EC-C18 when comparing the acetonitrile and methanol data (slope of 0.99 and slope of 1.01), but this is not likely to cause any problems in method transfer and is only measureable given the large number of experiments and compounds studied.

Generic Gradients using Methanol, Buffered with 10 mM Ammonium Formate or Ammonium Acetate between pH 3 and 6.5

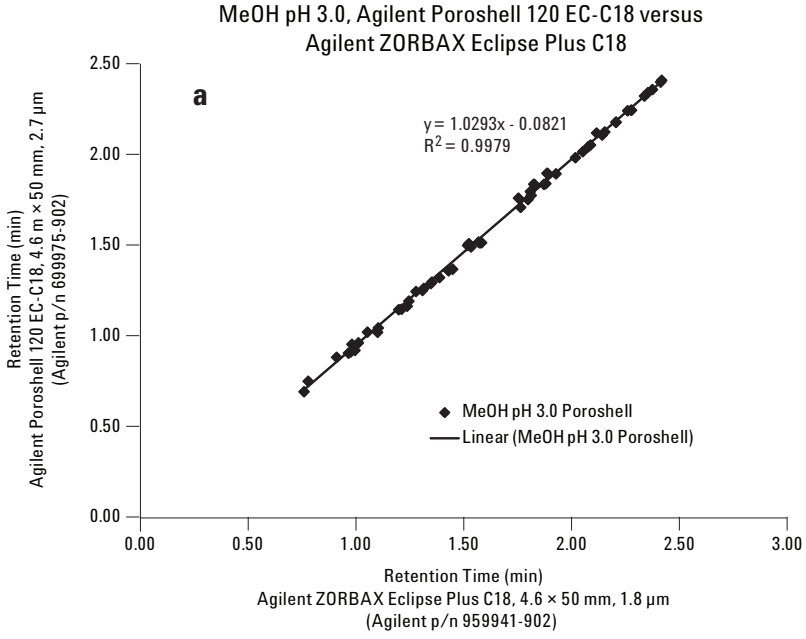
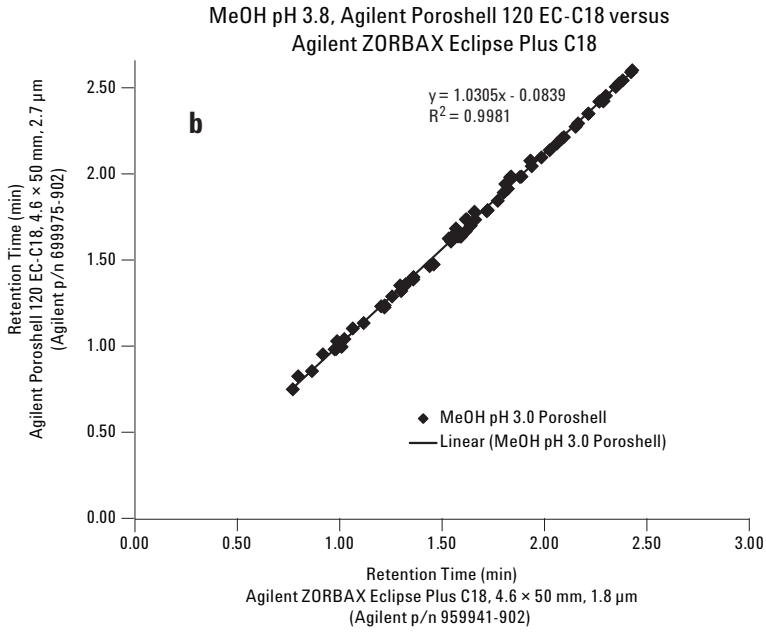


Figure 4. Scatter plot of retention time of 66 compounds on Agilent Poroshell 120 EC-C18, 4.6 × 50 mm, 2.7 µm versus Agilent ZORBAX Eclipse Plus C18, 4.6 × 50 mm, 1.8 µm. (continued)



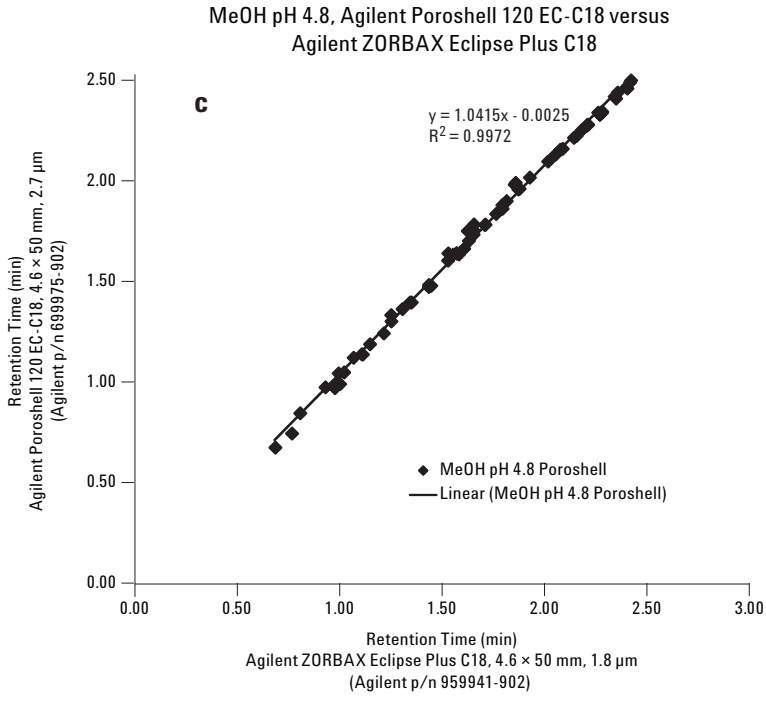
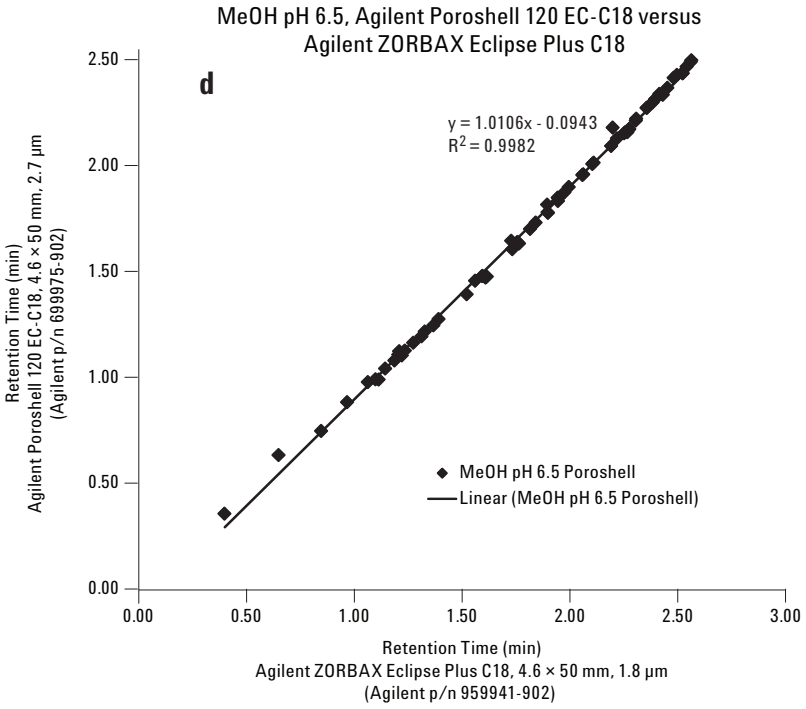


Figure 4. Scatter plot of retention time of 66 compounds on Agilent Poroshell 120 EC-C18, 4.6 × 50 mm, 2.7 µm versus Agilent ZORBAX Eclipse Plus C18, 4.6 × 50 mm, 1.8 µm. (continued)



Conditions

Mobile phase:	A: 10 mM Buffer
	B: Organic (MeOH)
Gradient:	5% B at t_0, ramp to 95% B in 2 min, hold 95% B for 1 min
Flow rate:	2 mL/min
Sample:	1 µL of 1 mg/mL standard in H_2O or H_2O/ACN

Figure 4. Scatter plot of retention time of 66 compounds on Agilent Poroshell 120 EC-C18, 4.6 × 50 mm, 2.7 µm versus Agilent ZORBAX Eclipse Plus C18, 4.6 × 50 mm, 1.8 µm.

Table 2. Summary of Correlation Data

Acetonitrile	Methanol
a. pH =3.0 y = 0.9915x -0.0193 R² = 0.9967	a. pH =3.0 y = 1.0293x -0.0821 R² = 0.9979
b. pH =3.8 y = 0.9901x -0.0202 R² = 0.9963	b. pH =3.8 y = 1.0305x -0.0839 R² = 0.9981
c. pH =4.8 y = 0.9845x -0.0175 R² = 0.9969	c. pH =4.8 y = 1.0415x -0.002 R² = 0.9972
d. pH =6.5 y = 0.993x -0.0316 R² = 0.998	d. pH =6.5 y = 1.0106x -0.0943 R² = 0.9982

Another benefit of the Agilent Poroshell 120 columns over sub-2-µm columns is lower operating pressure. The pressure is related to the particle size of the column; larger particles naturally yield lower pressure than smaller particles. In addition to the particle size, the pressure generated inside a column is dependent upon several other factors including solvent linear velocity, and solvent viscosity at a given composition and temperature. While this is a gradient study, the most viscous solvent composition in this study occurs between 40/60 and 50/50 methanol/water. At 25 °C the viscosity of this solvent is 1.62 cP. The most viscous acetonitrile composition is 10/90 acetonitrile/water. At 25 °C the viscosity of this solvent is 1.01 cP [11]. As indicated in the references the viscosity of the solutions is inversely dependent on the temperature. The pressure verses linear velocity graphs for Agilent Poroshell 120 EC-C18 columns and Agilent ZORBAX Eclipse Plus C18 1.8 µm columns are shown for both solvent pairs as Figures 5 and 6. In this case 100 mm columns are used. As stated earlier, this benefit can allow the use of longer columns achieving the same pressure (and larger injection volumes), or higher flow rates.

Differences in selectivity are more likely to occur in cases where the pore size difference becomes more important, typically for compounds between 1500 and 2500 mw. Compounds such as PAHs that involve shape selectivity may also be problematic.

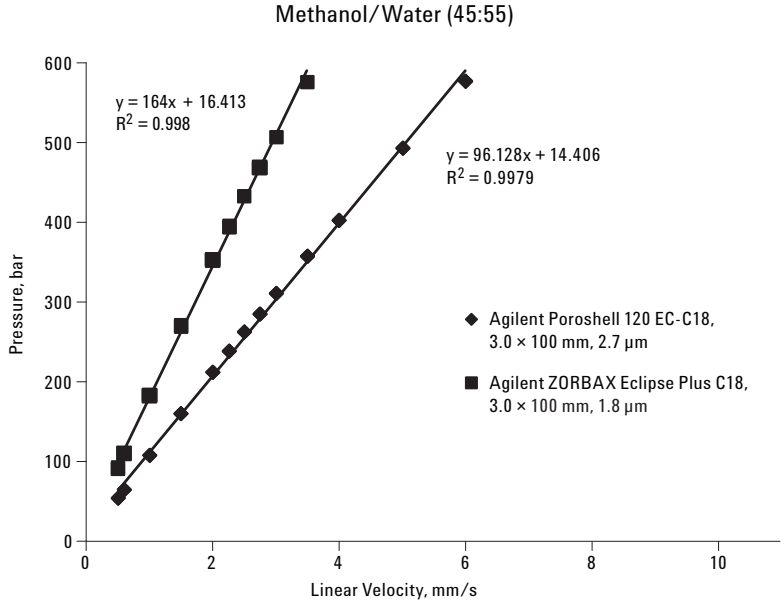


Figure 5. Pressure measured at varied linear velocities indicates lower operating pressure for Agilent Poroshell 120 than an a 1.8 µm column of similar length.

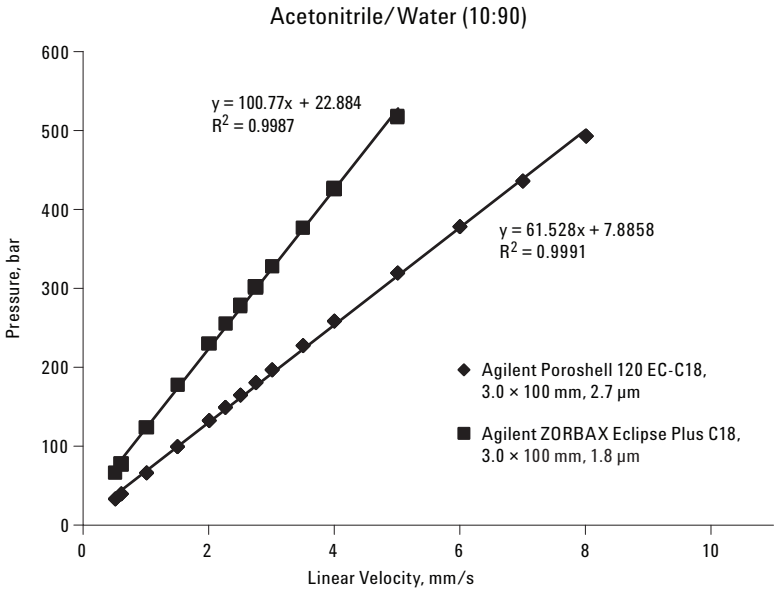


Figure 6. Pressure measured at varied linear velocities indicates lower operating pressure for Agilent Poroshell 120 than an a 1.8 µm column of similar length.

Conclusions

This work has demonstrated the equivalence of selectivity between Agilent ZORBAX Eclipse Plus C18 and Agilent Poroshell 120 EC-C18 columns across a wide range of pH and mobile phase conditions. Both column chemistries are manufactured using similar materials with similar proprietary bonding chemistries. Both columns were designed to achieve excellent peak shapes for bases without sacrificing low pH peak shape and performance for other compounds. The benefit of using Agilent Poroshell 120 EC-C18 columns is high efficiency at a lower backpressure. Based on this work, it is expected that if the need arises methods developed on Agilent ZORBAX Eclipse Plus C18 columns can be reliably transferred to Agilent Poroshell 120 EC-C18 columns and conversely with low risk.

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