General Description

Agilent Technologies has developed a number of key high performance liquid chromatography (HPLC) applications for the food market. This guide offers an overview of food sample analysis using HPLC, recommended starting conditions for application development, and information on Agilent literature concerning the various applications.

The first section in this document, the Application Overview, provides excerpts from Agilent publications that relate to HPLC applications for 13 major food groups. These highlights include detailed chromatographic conditions, chromatograms of standards, real-life samples, and information about the methods. To review a publication referenced in this guide, turn to the Application Reference Index for the publication’s number; entering this number at the Agilent Web site will give you access to the complete text.

The System Configurations section of this guide provides descriptions of the types of HPLC solvent delivery systems and detectors. The information in the Food Quick Reference Guide repeats the information provided in the previously mentioned sections; however, this section organizes the information by food group. Basic Principles of Liquid Chromatography offers an overview of separation modes, column dimensions and materials, sample preparation techniques, solid phase extraction, and method translation.

Please note that the described instruments are recommendations only.
Table of Contents

Application Overview 3
System Configurations
Carbohydrates, sugars, sugar alcohols 64
Dyes, colorants, pigments 64
Fats and oils 64
Flavors, sweeteners, organic acids 64
Herbal supplements, natural products, plant hormones 64
Preservatives 64
Proteins, peptides, amino acids 65
Regulated/hazardous drug substances 65
Regulated/hazardous miscellaneous substances 65
Regulated/hazardous natural toxin substances 65
Regulated/hazardous pesticides/herbicide substances 66
Fat-soluble vitamins 66
Water-soluble vitamins 66

Food Quick Reference Guide
Carbohydrates, sugars, sugar alcohols 67
Dyes, colorants, pigments 67
Fats and oils 67
Flavors, sweeteners, organic acids 67
Herbal supplements, natural products, plant hormones 67
Preservatives 68
Proteins, peptides, amino acids 68
Regulated/hazardous drug substances 68
Regulated/hazardous natural toxin substances 68
Regulated/hazardous pesticides/herbicide substances 69
Fat-soluble vitamins 69
Water-soluble vitamins 69

Basic Principles of Liquid Chromatography
Basic Principles of Liquid Chromatography 70
Concepts of the Rapid Resolution Systems and Methods 73
Method Translation 74
Sample Preparation Techniques 76

Application Reference Index
Carbohydrates, sugars, sugar alcohols 78
Dyes, colorants, pigments 78
Fats and oils 79
Flavors, sweeteners, organic acids 79
Herbal supplements, natural products, plant hormones 80
Preservatives 82
Proteins, peptides, amino acids 82
Regulated/hazardous drug substances 83
Regulated/hazardous miscellaneous substances 85
Regulated/hazardous natural toxin substances 85
Regulated/hazardous pesticides/herbicide substances 86
Fat-soluble vitamins 88
Mixed vitamins 89
Water-soluble vitamins 89
Mixed publications 90
Packaging 90
**Application Overview**

HPLC is increasingly applied to the analysis of food samples for additives and contaminants (see Table 1). The method enables complex mixtures to be separated into individual compounds and to be identified and quantified by suitable detectors and data handling systems. Separation and detection occurs at ambient temperature or slightly above and therefore the method is also well suited to compounds of low thermal stability. Although HPLC detectors in general are not as sensitive as gas chromatography (GC) detectors, HPLC is a sensitive method, mainly due to the possibility of injecting large sample amounts up to 1–2 mL per injection. This, and the nondestructive nature of many of the detection techniques, also enables the collection of fractions for further analysis, which is especially important for the analysis of proteins and synthesis environments. Modern sample preparation techniques, such as solid phase extraction (SPE) and supercritical fluid extraction (SFE), also enable high sensitivity HPLC analysis in the in the parts per trillion (ppt) range.

<table>
<thead>
<tr>
<th>Table 1 Food Compounds Typically Analyzed by HPLC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Applications</strong></td>
</tr>
<tr>
<td><strong>Group</strong></td>
</tr>
<tr>
<td>Carbohydrates, sugars, sugar alcohols</td>
</tr>
<tr>
<td>Dyes, colorants, pigments</td>
</tr>
<tr>
<td>Fats and oils</td>
</tr>
<tr>
<td>Flavors, sweeteners, organic acids</td>
</tr>
<tr>
<td>Herbal supplements, natural products, plant hormones</td>
</tr>
<tr>
<td>Preservatives</td>
</tr>
<tr>
<td>Proteins, peptides, amino acids</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Group</th>
<th>Major analytes</th>
<th>Matrix</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regulated/hazardous drug substances</td>
<td>Drugs</td>
<td>Water</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>Nitrofurans</td>
<td>Poultry, shrimp</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>Fluoroquinolones</td>
<td>Beef kidney</td>
<td>38</td>
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<tr>
<td></td>
<td>Chloramphenicol</td>
<td>Shrimp, honey</td>
<td>39</td>
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<td></td>
<td>Sulfa drugs</td>
<td>Meat</td>
<td>40</td>
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<td></td>
<td>Sulfonamides</td>
<td></td>
<td>41</td>
</tr>
<tr>
<td>Regulated/hazardous miscellaneous substances</td>
<td>HMF hydroxymethylfurfural</td>
<td>Bread, cereal, yogurt</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>Acrylamide</td>
<td>Drinking water</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>Chromium speciation</td>
<td></td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>Perchlorate</td>
<td>Water, vegetables</td>
<td>45</td>
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<tr>
<td></td>
<td>Arsenobetaine</td>
<td>Fish</td>
<td>46</td>
</tr>
<tr>
<td>Regulated/hazardous natural toxin substances</td>
<td>DSP algal toxins</td>
<td>Shellfish</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>Mycotoxin, fumonisin</td>
<td>Corn</td>
<td>49</td>
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<tr>
<td></td>
<td>Aflatoxins</td>
<td>Various</td>
<td>50</td>
</tr>
<tr>
<td>Regulated/hazardous pesticides/herbicide substances</td>
<td>44 pesticides</td>
<td>Vegetables, fruit</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>Acid herbicides</td>
<td>Water</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>Postharvest fungicides</td>
<td>Citrus</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>Chloronicotinyl insecticides</td>
<td>Vegetables, fruit</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>Phenylurea, triazine herbicides</td>
<td>Water</td>
<td>56</td>
</tr>
<tr>
<td>Fat-soluble vitamins</td>
<td>Retinol isomers</td>
<td></td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>Fat-soluble vitamins</td>
<td></td>
<td>59</td>
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<tr>
<td></td>
<td>Vitamin D3</td>
<td>Poultry feed</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Fat-soluble vitamins A,D, E</td>
<td></td>
<td>61</td>
</tr>
<tr>
<td>Water-soluble vitamins</td>
<td>Water-soluble vitamins</td>
<td></td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>Water-soluble vitamins</td>
<td>Cat food</td>
<td>63</td>
</tr>
</tbody>
</table>

The above information highlights only a fraction of the available Agilent application notes. Refer to the Application Reference Index (pages 78 to 90) at the back of this guide for a more comprehensive listing of available solutions. All of these application notes can be downloaded from the Agilent Web site www.agilent.com/chem.
Carbohydrates, Sugars, Sugar Alcohols

Major analytes
Carbohydrates

Matrix
Standard

Reference

System Summary
LC System
Isocratic
Detection
RID
Columns
ZORBAX NH₂, 250 mm × 4.6 mm
Column part number
880952-708 or 840300-908

Conditions
Column
ZORBAX NH₂
250 mm × 4.6 mm
(Agilent p/n 880952-708)
Mobile phase
ACN : H₂O, as indicated
Flow rate
1 mL/min
Detector
Refractive index (RID)
Carbohydrates, Sugars, Sugar Alcohols

Major Analytes
Sugar alcohols

Matrix
Beverage

Reference

System Summary

LC System
Isocratic

Detection
MSD negAPCI

Columns
150 mm × 2 mm Asahipak NH2-50 2D

Column part number
843300-908

Conditions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>150 × 2 mm Asahipak NH2-50 2D</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>Acetonitrile/Water (75/25)</td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.2 mL/min</td>
</tr>
<tr>
<td>Column temperature</td>
<td>40 °C</td>
</tr>
<tr>
<td>Injection volume</td>
<td>10 µL</td>
</tr>
<tr>
<td>Post column addition</td>
<td>Acetonitrile/CHCl3 (50/50) at 0.2 mL/min</td>
</tr>
<tr>
<td>Detector</td>
<td>MSD Quadrupole</td>
</tr>
<tr>
<td>Ionization</td>
<td>APCI (negative)</td>
</tr>
<tr>
<td>Scan range</td>
<td>m/z 100–500</td>
</tr>
<tr>
<td>Vaporizer</td>
<td>400 °C</td>
</tr>
<tr>
<td>Nebulizer pressure</td>
<td>40 psi</td>
</tr>
<tr>
<td>Fragmentor</td>
<td>20 V</td>
</tr>
<tr>
<td>Corona current</td>
<td>30 A</td>
</tr>
<tr>
<td>Drying gas</td>
<td>13 L/min, 350 °C</td>
</tr>
<tr>
<td>SIM (m/z)</td>
<td>negative</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>217, 219</td>
</tr>
<tr>
<td>Glucose, fructose</td>
<td>215, 217</td>
</tr>
<tr>
<td>Xylitol</td>
<td>187, 189</td>
</tr>
<tr>
<td>Sucrose</td>
<td>377, 379</td>
</tr>
<tr>
<td>Confirmation</td>
<td>MS spectral information and RT</td>
</tr>
</tbody>
</table>
## Carbohydrates, Sugars, Sugar Alcohols

### Major Analytes
- Dextran

### Matrix
- Standard

### Reference

### Conditions

<table>
<thead>
<tr>
<th>Sample preparation</th>
<th>Sample was dissolved in the mobile phase (concentration 0.1 %) and filtered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>PL aquagel-OH MXA, 7.5 mm × 300 mm, 8 µm (Agilent p/n 79911GF-MXA) in series with PL aquagel-OH 30A, 7.5 mm × 300 mm, 8 µm (Agilent p/n 79911GF-083)</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>Water</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1 mL/min</td>
</tr>
<tr>
<td>Column compartment</td>
<td>25 °C</td>
</tr>
<tr>
<td>Injection volume</td>
<td>100 µL</td>
</tr>
<tr>
<td>Detector</td>
<td>Refractive index (RID)</td>
</tr>
<tr>
<td>Polymer standards</td>
<td>Polyethylene oxide EasyCal standards in vials for calibration (Agilent p/n 5064-8280)</td>
</tr>
<tr>
<td>Software</td>
<td>ChemStation Plus with SEC data analysis software</td>
</tr>
</tbody>
</table>

### System Summary

**LC System**
- Isocratic

**Detection**
- RID

**Columns**
- PL aquagel-OH MXA, 7.5 mm × 30 mm, 8 µm
- PL aquagel-OH 30A, 7.5 mm × 30 mm, 8 µm

**Column part number**
- 79911GF-MXA and 79911GF-803

### Molecular weight data

<table>
<thead>
<tr>
<th>rid1A</th>
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</thead>
<tbody>
<tr>
<td>Mn: 9.0685e4 g/mol</td>
</tr>
<tr>
<td>Mw: 1.8436e5 g/mol</td>
</tr>
<tr>
<td>Mz: 3.8872e5 g/mol</td>
</tr>
<tr>
<td>Mv: 1.8436e5 mL/g</td>
</tr>
<tr>
<td>D: 2.9330e0</td>
</tr>
<tr>
<td>[n]: 0.000000</td>
</tr>
<tr>
<td>Vp: 1.4450e1 mL</td>
</tr>
<tr>
<td>Mp: 9.2727e4 g/mol</td>
</tr>
<tr>
<td>A: 2.9790e4 mL/V</td>
</tr>
</tbody>
</table>

**Report subsets - additional information on user-selected parts of chromatogram**

### Figures
- **Figure 1.** Analysis of dextran sample.
- **Figure 2.** Typical GPC report.
Carbohydrates, Sugars, Sugar Alcohols

Major Analytes
Starch

Matrix
Standard

Reference

System Summary

LC System
Isocratic
Detection
RID
Columns
Aq. GPC PSS Suprema 100 + 1000, 2 of 8 mm × 300 mm, 10 µm
Column part number
See application note

Conditions
Sample preparation Sample was dissolved in 1 mL eluent at 20 °C (concentration 0.1 % w/w). Dextran standards from Polymer Standards Services (PSS) were used for narrow standard calibration.
Column PSS Suprema 100 + 1000 in series, 8 mm × 300 mm, 10 µm
Mobile phase 0.1 M sodium nitrate
Flow rate 1 mL/min
Column compartment temperature 25 °C
Injection volume 100 µL
Detector Refractive index (RID)
Software ChemStation Plus with SEC data analysis software

<table>
<thead>
<tr>
<th>Starch</th>
<th>Mn</th>
<th>Mw</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Degradation I</td>
<td>1000</td>
<td>4700</td>
</tr>
<tr>
<td>Degradation II</td>
<td>910</td>
<td>6100</td>
</tr>
</tbody>
</table>
* Not calculated because of steric exclusion

Figure 1. Overlay of chromatograms of original starch after incorrect processing.
Carbohydrates, Sugars, Sugar Alcohols

Major Analytes
Carbohydrates

Matrix
Lemonade

Reference

System Summary
LC System
Isocratic
Detection
RID
Columns
BioRad HPXP
Column part number
Contact manufacturer

Conditions
Column 300 × 7.8 mm Bio-Rad HPXP, 9 µm
Mobile phase Water H₂SO₄
Column compartment temperature 80 ºC
Flow rate 0.7 mL/min
Detector Refractive index (RID)

Sample preparation Samples were directly injected.

HPLC method performance
Limit of detection < 80 ng with S/N = 2
Repeatability of RT RT over 10 runs < 0.05 %
areas over 10 runs 2 %
Dyes, Colorants, Pigments

Major Analytes
Sudan dyes

Matrix
Food

Reference

Reproducibility

<table>
<thead>
<tr>
<th>Standard (ppm)</th>
<th>RSD (%)</th>
<th>Accuracy (% avg)</th>
<th>RSD (%)</th>
<th>Accuracy (% avg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>6.04</td>
<td>97.31</td>
<td>5.76</td>
<td>97.61</td>
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<tr>
<td>0.4</td>
<td>6.98</td>
<td>101.95</td>
<td>5.64</td>
<td>100.27</td>
</tr>
<tr>
<td>0.8</td>
<td>4.61</td>
<td>104.75</td>
<td>6.12</td>
<td>103.78</td>
</tr>
<tr>
<td>1.6</td>
<td>5.17</td>
<td>102.53</td>
<td>5.99</td>
<td>105.92</td>
</tr>
<tr>
<td>2</td>
<td>6.12</td>
<td>96.77</td>
<td>4.74</td>
<td>94.72</td>
</tr>
</tbody>
</table>

Figure 2. TIC showing the four peaks of the Sudan Red dyes.

System Summary

LC System
Binary gradient

Detection
MS TOF posESI

Columns
ZORBAX XDB-C18, 2.1 × 50 mm, 1.8 µm

Column part number
922700-902

Experimental

Instrument
Agilent 1100 Series LC/MSD TOF with Agilent 1100 binary pump and well plate autosampler

LC Conditions
Column: ZORBAX XDB C18, 2.1 mm × 50 mm, 1.8 µm
Agilent p/n: 922700-902
Mobile phases:
A: H₂O with 5 mM NH₄OAc
B: Acetonitrile
Gradient:
0–3 min 95% B to 98% B
3–5 min 98% B
Post time:
3 min
Flow rate:
0.5 mL/min

MS Conditions
Ionization: ESI, Positive
Gas temp:
350 °C
Drying gas:
8 L/min
Nebulizer pressure:
45 psi
Capillary V (+ve):
4000 V
Dyes, Colorants, Pigments

Major Analytes
FDC food dyes, paraben

Matrix
Ricker

Reference

System Summary

LC System
Quaternary gradient

Detection
DAD

Columns
ZORBAX XDB-C18, 50 mm × 4.6 mm, 3.5 µm

Column part number
935967-902

1. Yellow #5  C_{16}H_{9}N_{4}Na_{3}O_{9}S_{2}  MW=534
2. Red #40  C_{18}H_{14}N_{2}Na_{2}O_{8}S_{2}  MW=496
3. Blue #1  C_{20}H_{34}N_{2}Na_{2}O_{9}S_{3}  MW=760
4. Propylparaben  C_{20}H_{41}O_{3}  MW=180
5. Red #3  C_{20}H_{41}Na_{2}O_{4}  MW=878

Conditions

Column: ZORBAX XDB-C18, 50 mm × 4.6 mm, 3.5 µm, (Agilent p/n 935967-902)

Mobile phase
A: 0.1% TFA with triethylamine (TEA)
B: Methanol

Gradient
17% B to 100 % B in 4 min

Flow rate
1 mL/min

Column temperature
Ambient

Detector
DAD, detection wavelength 254 nm
Dyes, Colorants, Pigments

Major Analytes
Cyanidins

Matrix
Cabbage

Reference

System Summary

LC System
Binary gradient

Detection
MSD posESI APCI

Columns
Inertsil ODS3, 2.1 mm × 250 mm, 5 µm
Column part number
Suggest SB-C18, 2.1 mm × 150 mm, 3.5 µm 935967-902

LC Conditions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Setting</th>
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</thead>
<tbody>
<tr>
<td>Column</td>
<td>250 × 2.1 mm Inertsil ODS3, 5 µm</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>A = 1% formic acid</td>
</tr>
<tr>
<td></td>
<td>B = Acetonitrile/water</td>
</tr>
<tr>
<td>Gradient</td>
<td>Start with 5% B</td>
</tr>
<tr>
<td></td>
<td>At 30 min 50% B</td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.2 mL/min</td>
</tr>
<tr>
<td>Column temp</td>
<td>40 °C</td>
</tr>
<tr>
<td>Injection vol</td>
<td>10 µL</td>
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</table>

MS Conditions

<table>
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<th>Parameter</th>
<th>Setting</th>
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<tbody>
<tr>
<td>Source</td>
<td>ESI</td>
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<tr>
<td>Ion mode</td>
<td>Positive</td>
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<tr>
<td>$V_{cap}$ Voltage</td>
<td>4000 V</td>
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<tr>
<td>Nebulizer</td>
<td>50 psig</td>
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<tr>
<td>Drying gas flow</td>
<td>10 L/min</td>
</tr>
<tr>
<td>Drying gas temp</td>
<td>350 °C</td>
</tr>
<tr>
<td>Corona</td>
<td>4 µA</td>
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<tr>
<td>Vaporizer temp</td>
<td>350 °C</td>
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<tr>
<td>Scan range</td>
<td>100–1200 amu</td>
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<tr>
<td>Step size</td>
<td>40.1</td>
</tr>
<tr>
<td>Peak width</td>
<td>0.15 min</td>
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<tr>
<td>Time filter</td>
<td>On</td>
</tr>
<tr>
<td>Fragmentor</td>
<td>200 V</td>
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Figure 2. Total and extracted ion chromatograms of red cabbage colorant.

Continued
Figure 2. The structure of major pigments in red cabbage colorant.

Figure 3. Mass spectra of major pigments in red cabbage colorant.

<table>
<thead>
<tr>
<th></th>
<th>R₁</th>
<th>R₂</th>
<th>MW</th>
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<tbody>
<tr>
<td>A</td>
<td>H</td>
<td>Sinapyl</td>
<td>979</td>
</tr>
<tr>
<td>B</td>
<td>Sinapyl</td>
<td>H</td>
<td>979</td>
</tr>
<tr>
<td>C</td>
<td>Ferulyl</td>
<td>H</td>
<td>949</td>
</tr>
<tr>
<td>D</td>
<td>p-Coumaryl</td>
<td>H</td>
<td>919</td>
</tr>
<tr>
<td>E</td>
<td>Sinapyl</td>
<td>Sinapyl</td>
<td>1185</td>
</tr>
<tr>
<td>F</td>
<td>Ferulyl</td>
<td>Sinapyl</td>
<td>1155</td>
</tr>
<tr>
<td>G</td>
<td>p-Coumaryl</td>
<td>Sinapyl</td>
<td>1125</td>
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</table>
Fats and Oils

**Major Analytes**
Phospholipids

**Matrix**
Soybean

**Reference**

**System Summary**

**LC System**
Prep/35900E

**Detection**
ELSD (ESA), some MSD

**Columns**
Prep SIL, 4.6 mm × 150 mm, 10 µm
21.2 mm × 150 mm, 10 µm

**Column part number**
See application note

**Phospholipid**

<table>
<thead>
<tr>
<th>Phospholipid</th>
<th>Abbreviation</th>
</tr>
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<tbody>
<tr>
<td>Phosphatidylethanolamine</td>
<td>PE</td>
</tr>
<tr>
<td>Phosphatidylserine</td>
<td>PS</td>
</tr>
<tr>
<td>Phosphatidylinositol</td>
<td>PI</td>
</tr>
<tr>
<td>Phosphatidylcholine</td>
<td>PC</td>
</tr>
</tbody>
</table>

**Figure 3.** Agilent prep 4.6 × 150 mm, 10-µm — Mixture of soy phospholipid standards, — Loadability.

**Figure 6.** Agilent prep 21.2 × 150 mm, 10-µm — Mixture of soy phospholipid standards, — Loadability.

**Conditions**
Mobile phase
A = 95:2.5:2.5% hexane-isopropanol-methanol
B = 40:60% isopropanol-methanol
Both solvents contain 10-mM ammonium acetate
Fats and Oils

Major Analytes

Triglycerides

Matrix

Standard

Reference


System Summary

LC System

Isocratic

Detection

MSD posAPCI

Columns

Develosil ODS OG-3, 4.6 mm × 75 mm

Column part number

Suggest SB-C18, 4.6 mm × 75 mm, 3.5 µm 866953-902

Conditions

Column Develosil ODS OG-3, 75 mm × 4.6 mm
Mobile phase Acetone/Water (98/2)
Flow rate 1 mL/min
Column temperature 40 °C
Injection volume 15 µL
Gradient At 0 min 20% A
at 30 min 100% A
Detector MSD Quadrupole, SL
Ionization APCI (positive)
Scan range m/z 100–1000
Vaporizer 400 °C
Nebulizer pressure 50 psi
Fragmentor 160 V
Drying gas 4 L/min, 350 °C
Confirmation MS spectral information and RT
Fats and Oils

Major Analytes
Triglycerides

Matrix
Edible oil

Reference

Chromatographic Conditions

- **Column**: 200 mm × 2.1 mm Hypersil MOS, 5 µm
- **Mobile phase**
  - A = 60:40 water:isopropanol + 25 mM ammonium formate
  - B = 10:10:80 water:isopropanol:n-butanol + 25 mM ammonium formate
- **Gradient**
  - Start with 30% B at 1.5 min
  - 30% B at 25 min 60% B at 28 min 100% B
- **Flow rate**: 0.25 mL/min
- **Column temp**: 50 °C
- **Injection vol**: 1.5 µL

**MS Conditions**

- **Source**: APCI
- **Ion mode**: Positive
- **Vcap**: 4000 V
- **Nebulizer**: 50 psig
- **Drying gas flow**: 4 L/min
- **Drying gas temp**: 325 °C
- **Corona**: 4 µA
- **Vaporizer**: 300 °C
- **Scan range**: 300–1100 m/z
- **Steps**: 0.1 m/z
- **Peakwidth**: 0.3 min
- **Time filter**: On
- **Fragmentor**: 80 V

System Summary

**LC System**
Binary gradient

**Detection**
MSD posAPCI

**Columns**
Hypersil MOS, 2.1 mm × 200 mm, 5 µm

**Column part number**
Suggest SB-C18, 2.1 mm × 150 mm, 3.5 µm 830990-902

**Figure 1.** TIC showing separation obtained for four C18 triglyceride standards. Note that cis and trans isomers are well resolved.

**Figure 2.** The effect of raising the fragmentor voltage on a mixed triglyceride in coconut oil. The fragment masses and ratios are consistent with two C12 fatty acids and one C14 fatty acid.

**Figure 3.** Chromatographic Conditions

- **Column**: 200 mm × 2.1 mm Hypersil MOS, 5 µm
- **Mobile phase**
  - A = 60:40 water:isopropanol + 25 mM ammonium formate
  - B = 10:10:80 water:isopropanol:n-butanol + 25 mM ammonium formate
- **Gradient**
  - Start with 30% B at 1.5 min 30% B at 25 min 60% B at 28 min 100% B
- **Flow rate**: 0.25 mL/min
- **Column temp**: 50 °C
- **Injection vol**: 1.5 µL

**MS Conditions**

- **Source**: APCI
- **Ion mode**: Positive
- **Vcap**: 4000 V
- **Nebulizer**: 50 psig
- **Drying gas flow**: 4 L/min
- **Drying gas temp**: 325 °C
- **Corona**: 4 µA
- **Vaporizer**: 300 °C
- **Scan range**: 300–1100 m/z
- **Steps**: 0.1 m/z
- **Peakwidth**: 0.3 min
- **Time filter**: On
- **Fragmentor**: 80 V
Fats and Oils

Major Analytes
Triglycerides and their hydroperoxides

Matrix
Edible oil

Reference

System Summary
LC System
Quaternary gradient
Detection
DAD 3channel
Columns
Hypersil MOS, 2.1 mm × 200 mm, 5 µm
Column part number
Suggest SB-C18, 2.1 mm × 150 mm, 3.5 µm 830990-902

Conditions
Column 200 × 2.1 mm Hypersil MOS, 5 µm
Mobile phase A = water
B = ACN/methyl-tert.butylether (9:1)
Gradient At 0 min 87% B; at 25 min 100% B
Post time 4 min
Flow rate 0.8 mL/min
Column compartment 60 ºC
Injection vol 1 µL standard
UV absorbance 200 nm and 215 nm to detect triglycerides, 240 nm to detect hydroperoxides, and 280 nm to detect tocopherols and decomposed triglycerides (fatty acids with three conjugated double bonds)
Sample preparation Samples were dissolved in tetrahydrofuran (THF).

HPLC method performance
Limit of detection for >10 µg saturated triglycerides
Repeatability of RT over 10 runs <0.7 %
areas over 10 runs <6 %

Figure 1. Triglyceride pattern of aged sunflower oil. The increased response at 240 nm indicates hydroperoxides.

Figure 2. Analysis of olive oil. The response at 280 nm indicates a conjugated double bond and therefore poor oil quality.

Sunflower oil

Olive oil

Good quality

Poor quality

mAU

Time [min]

mAU

Time [min]
Flavors, Sweeteners, Organic Acids

Major Analytes
Flavor, sweetener, preservative

Matrix
Soft drinks

Reference

System
Agilent 1200 Series Rapid Resolution LC, consisting of:
- G1379B micro degasser
- G1312B binary pump SL
- G1367C HiP ALS autosampler SL, with thermostatic temperature control
- G1316B thermostatted column compartment SL
- G1315C UV/VIS diode array detector SL, flow cell as indicated in individual chromatograms

ChemStation 32-bit version B.02.01

Columns
Agilent ZORBAX SB-C18, 4.6 mm × 250 mm, 5 µm
Agilent ZORBAX SB-C18, 3.0 mm × 50 mm, 1.8 µm

Mobile phase conditions
Organic solvent: Acetonitrile (ACN) or ACN containing 0.1% formic acid
Aqueous solvent: 20 mm phosphoric acid in Milli-Q water, with ammonium hydroxide, or Milli-Q water containing 0.1% formic acid

Gradient conditions
Gradient slope: 2.8% per column volume
See individual chromatograms for flow rate and gradient time.

Samples
1. Standard mixture of sodium saccharin, caffeine, aspartame, vanillin, benzoic acid, sorbic acid, benzaldehyde, all 50 µg/mL in 1/1 methanol/water
2. Various soft drinks, decarbonated where applicable

Figure 1. Gradient separation of soft drink additives on 4.6 mm × 250 mm, 5-µm ZORBAX SB-C18.

Figure 2. Gradient separation of soft drink additives on 3.0 mm × 50 mm, 1.8-µm ZORBAX SB-C18.
Flavors, Sweeteners, Organic Acids

Major Analytes
Organic acids

Matrix
Foods

Reference

System Summary
LC System
Quaternary gradient
Detection
DAD
Columns
ZORBAX SB-Aq, 4.6 mm × 150 mm, 5 µm
Column part number
883975-914

Conditions
Column
ZORBAX SB-Aq, 4.6 mm × 150 mm × 5 µm
Mobile phase
20 mM aqueous phosphate buffer/acetonitrile = 99/1 (v/v)
Flow rate
1.0 mL/min
Injection volume
1 µL
Column temperature
25 °C
Detection
UV-DAD detection wavelength/window 210/8 nm, reference wavelength 360/80 nm
Sample preparation
Filtration over 0.45-µm filter
Flavors, Sweeteners, Organic Acids

Major Analytes
Flavoring agents

Matrix
Mouthwash

Reference

System Summary
LC System
Binary gradient
Detection
DAD
Columns
ZORBAX SB-Phenyl, 2.1 mm × 50 mm, 5 µm
Column part number
860975-912

Conditions
Column ZORBAX SB-Phenyl, 50 mm × 2.1 mm, 5 µm (Agilent p/n 860975-912)
Mobile phase 0.3% TFA:acetonitrile/water (65:35)
Flow rate 0.3 mL/min
Column temperature Ambient
Detector DAD, detection wavelength 254 nm

Cool Mint Listerine sample
1. Unknown
2. Benzoic acid
3. Methyl salicylate
4. Carvone
5. Unknown
6. Thymol
7. Anethole
Flavors, Sweeteners, Organic Acids

Major Analytes
Semivolatile flavors

Matrix
Standard

Reference

System Summary
LC System
Isocratic
Detection
DAD
Columns
ZORBAX XDB-Phenyl, 4.6 mm × 150 mm, 3.5 µm
Column part number
963967-912

Conditions
Column
ZORBAX XDB-Phenyl,
150 mm × 4.6 mm, 3.5 µm
(Agilent p/n 963967-912)
Mobile phase
Water : Methanol (40:60)
Flow rate
1 mL/min
Column temperature
35 °C
Detector
DAD, detection wavelength 254 nm

Aromatic sample
1. Acetophenone
2. Cinnamaldehyde
3. Eugenol
4. Cinnamaldehyde impurity
5. Ethyl cinnamate
6. p-Cymene
Flavors, Sweeteners, Organic Acids

Major Analytes
Aspartame, degradants

Matrix
Cola

Reference

System Summary
LC System
Isocratic
Detection
DAD
Columns
ZORBAX SB-C18, 4.6 mm × 75 mm, 3.5 µm
Column part number
866953-902

Conditions
Column ZORBAX SB-C18, 75 mm × 4.6 mm, 3.5 µm, (Agilent p/n 866953-902)
Mobile phase 0.1% TFA:acetonitrile/water (85:15)
Flow rate 1 mL/min
Column temperature 35 °C
Injection volume 1 µL
Detector DAD, detection wavelength 210 nm
Herbal Supplements, Natural Products, Plant Hormones

Major Analytes
Xanthine metabolites

Matrix
Standard

Reference

System Summary
LC System
Isocratic
Detection
DAD
Columns
Eclipse Plus C18, 4.6 mm × 150 mm, 5 µm
Column part number
959993-902

Analyte elution order:
1. 1-Methylxanthine
2. Theobromine
3. Theophylline
4. β-hydroxyethyltheophylline
5. 3-Propylxanthine
6. Caffeine

Figure 1. Separation of xanthines on various C18 columns.

Conditions
Column: Eclipse Plus C18, 4.6 mm × 150 mm, 5 µm, (Agilent p/n 959993-902)
Mobile phase:
A: 25 mM Phosphate
B: ACN (90:10)
Flow rate: 1 mL/min
Column temperature: 40 °C
Detector: DAD
Herbal Supplements, Natural Products, Plant Hormones

Major Analytes
Glycyrrhizin

Matrix
Licorice root

Reference

Figure 1. Structure of glycyrrhizinic acid.

System Summary
LC System
1200SL
Detection
DAD
Columns
ZORBAX SB-C18 RRHT, 4.6 mm × 150 mm, 1.8 µm
Column part number
829975-902

HPLC conditions
Instrument Agilent 1200SL Series Rapid Resolution System
Detector Multiple wavelength detector (MWD), 254 nm/100 BW, 450 nm reference
Mobile phase A = 1% Acetic acid in water
B = 1% Acetic acid in acetonitrile
Gradient conditions for ZORBAX SB-C18 columns
Conventional 4.6 mm × 250 mm, 5 µm
5% to 100% B in 50 minutes
RRHT 4.6 mm × 150 mm, 1.8 µm
5% to 100% B in 30 minutes
Flow 1.0 mL/min
Temperature Ambient
Herbal Supplements, Natural Products, Plant Hormones

**Major Analytes**
Xanthines

**Matrix**
Tea, chocolate

**Reference**

**System Summary**

**LC System**
1200SL

**Detection**
DAD 2-µL cell

**Columns**
RRHT, 1.8 µm various

**Column part number**
Various

**Compounds:**
1 1-methylxanthine
2 1,3-dimethyluric acid
3 3,7-dimethylxanthine
4 1,7-dimethylxanthine
* Preproduction batch

Figure 2. ZORBAX stationary phase selectivity comparisons for xanthines.

<table>
<thead>
<tr>
<th>Conditions</th>
</tr>
</thead>
</table>
| **Columns** | ZORBAX SB-C18, 4.6 × 50 mm, 1.8 µm  
SB-Phenyl, 4.6 × 50 mm, 1.8 µm  
ZORBAX Bonus-RP, 4.6 × 50 mm, 1.8 µm  
Bonus-RP, 4.6 × 100 mm, 3.5 µm |
| **Mobile phase** | A = 0.2% FA  
B = ACN w 0.2% FA |
| **Isocratic composition** | 98% A 2% B (v/v) |
| **Flow rate** | 1.5 mL/min |
| **Injection volume** | 2, 4, 6 µL, respectively |
| **Detector** | DAD, 254 nm |
| **Flowcell** | 3 µL, 2-mm flow path |
Herbal Supplements, Natural Products, Plant Hormones

Major Analytes
Ginsenosides part 1

Matrix
Root

Reference

System Summary

LC System
1200SL

Detection
MS TOF posESI

Columns
SB-C18, 2.1 mm × 150 mm, 1.8 µm

Column part number
820700-902

Table 1. Empirical Formulas and Achieved Mass Accuracies for the Structure Elucidation of Ginsenoside

<table>
<thead>
<tr>
<th>Measured mass</th>
<th>Calculated mass</th>
<th>Formula</th>
<th>Mass accuracy [mDa]</th>
<th>Mass accuracy [ppm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1109.6129</td>
<td>1109.6108</td>
<td>C_{49}H_{73}O_{22}</td>
<td>2.10</td>
<td>−1.90</td>
</tr>
<tr>
<td>1091.6012</td>
<td>1091.6002</td>
<td>C_{49}H_{71}O_{22}</td>
<td>1.00</td>
<td>−0.91</td>
</tr>
<tr>
<td>785.5047</td>
<td>785.5051</td>
<td>C_{49}H_{69}O_{19}</td>
<td>−0.40</td>
<td>0.53</td>
</tr>
<tr>
<td>767.5051</td>
<td>767.5046</td>
<td>C_{49}H_{67}O_{19}</td>
<td>0.40</td>
<td>−0.58</td>
</tr>
<tr>
<td>749.4854</td>
<td>749.4840</td>
<td>C_{49}H_{65}O_{18}</td>
<td>−1.40</td>
<td>1.88</td>
</tr>
<tr>
<td>425.3784</td>
<td>425.3783</td>
<td>C_{24}H_{47}O_{10}</td>
<td>0.10</td>
<td>−0.14</td>
</tr>
<tr>
<td>407.3679</td>
<td>407.3678</td>
<td>C_{24}H_{45}O_{10}</td>
<td>0.10</td>
<td>−0.30</td>
</tr>
<tr>
<td>343.1248</td>
<td>343.1240</td>
<td>C_{24}H_{37}O_{9}</td>
<td>0.80</td>
<td>2.23</td>
</tr>
<tr>
<td>325.1136</td>
<td>325.1135</td>
<td>C_{24}H_{35}O_{9}</td>
<td>0.10</td>
<td>−0.39</td>
</tr>
</tbody>
</table>

Methods:
The Agilent 1200 Series binary pump SL was operated under the following conditions:

Solvent A: Water + 0.1 % TFA
Solvent B: ACN + 0.1 % TFA
Flow: 0.5 mL/min
Gradient:
0 min 5 % B, 1 min 5 % B,
60 min 85 % B,
61 min 95 % B,
70 min 95 % B

Stop time: 70 min
Post time: 15 min
**Herbal Supplements, Natural Products, Plant Hormones**

**Major Analytes**
Anthocyanins

**Matrix**
Blueberry

**Reference**

---

**System Summary**

**LC System**
Prep

**Detection**
DAD

**Columns**
Prep-C18, 21.2 mm × 250 mm, 10 µm, and 4.6 mm × 250 mm, 5 µm

**Column part numbers**
410910-102 and 440905-902

---

**Herbal Supplements, Natural Products, Plant Hormones**

**Major Analytes**
Anthocyanins

**Matrix**
Blueberry

**Reference**

---

**Figure 1. Scalability of Agilent Prep columns.**

**Conditions**

**1A**
Column: Agilent Prep-C18, 21.2 × 250 mm, 10 µm
Temperature: Ambient
DAD wavelength: 525 nm
Injection: 2000 µL
Sample: Blueberry extract, 46.1 mg/mL total dissolved solids (~5 mg/mL anthocyanins)
Flow: 21.2 mL/min

**1B**
Column: Agilent Prep-C18, 4.6 × 250 mm, 5 µ
Temperature: Ambient
DAD wavelength: 525 nm
Injection: 100 µL
Sample: Blueberry extract, 46.1 mg/mL total dissolved solids (~5 mg/mL anthocyanins)
Flow: 1.0 mL/min

**Mobile phase**

A = 0.1% TFA in water
B = 0.1% TFA in methanol

**Gradient timetable**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% Solvent B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>23.0</td>
</tr>
<tr>
<td>35.00</td>
<td>26.0</td>
</tr>
<tr>
<td>85.00</td>
<td>53.5</td>
</tr>
</tbody>
</table>
Herbal Supplements, Natural Products, Plant Hormones

Major Analytes
Anthocyanins

Matrix
Standard

Reference

System Summary
LC System
Quaternary or binary gradient module
Detection
DAD
Columns
ZORBAX SB-C18, 4.6 mm, 5 µm and 3.5 µm
Column part number
See application note

<table>
<thead>
<tr>
<th>Conditions</th>
<th>ZORBAX SB-C18, 4.6 mm, 5 µm, (Agilent p/n 880975-902)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile phase</td>
<td>A: 3% phosphoric acid, B: methanol water</td>
</tr>
<tr>
<td>Gradient</td>
<td>23% B to 60% B in 97 min</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1 mL/min</td>
</tr>
<tr>
<td>Column temperature</td>
<td>30 °C</td>
</tr>
<tr>
<td>Injection volume</td>
<td>20 µL</td>
</tr>
<tr>
<td>Detector</td>
<td>DAD, detection wavelength 525 nm</td>
</tr>
</tbody>
</table>
Herbal Supplements, Natural Products, Plant Hormones

Major Analytes
Flavonoids, catechins

Matrix
Standard

Reference

System Summary

LC System
Quaternary or binary gradient module

Detection
DAD

Columns
ZORBAX SB-C8, 4.6 mm × 150 mm, 3.5 µm

Column part number
863953-906

Conditions

<table>
<thead>
<tr>
<th>Column</th>
<th>ZORBAX SB-C8, 4.6 mm, 150 mm × 3.5 µm (Agilent p/n 863953-906)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile phase A</td>
<td>0.1% trifluoroacetic acid in water 75%,</td>
</tr>
<tr>
<td>Mobile phase B</td>
<td>Methanol 25%</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1 mL/min</td>
</tr>
<tr>
<td>Oven temperature</td>
<td>40 °C</td>
</tr>
<tr>
<td>Injection volume</td>
<td>5 µL</td>
</tr>
<tr>
<td>Detector</td>
<td>DAD, detection wavelength 280 nm</td>
</tr>
</tbody>
</table>
Preservatives

Major Analytes
Flavors, sweeteners, preservatives

Matrix
Soft drinks

Reference

System Summary
LC System
1200SL
Detection
DAD
Columns
ZORBAX SB-C18, 4.6 mm × 250 mm, 5 µm; 3 mm × 50 mm, 1.8 µm (600 bar)
Column part numbers
827975-302 (1.8 µm), 880975-902 (5 µm)

Conditions
System
Agilent 1200 Series Rapid Resolution LC, consisting of:
G1379B micro degasser
G1312B binary pump SL
G1367C HiP ALS autosampler SL, with thermostatic temperature control
G1316B thermostatted column compartment SL
G1315C UV/VIS diode array detector SL, flow cell as indicated in individual chromatograms
ChemStation 32-bit version B.02.01
Columns
Agilent ZORBAX SB-C18, 4.6 mm × 250 mm, 5 µm
Agilent ZORBAX SB-C18, 3.0 mm × 50 mm, 1.8 µm

Mobile phase conditions
Organic solvent
Acetonitrile (ACN) or ACN containing 0.1% formic acid
Aqueous solvent
20 mm phosphoric acid in Milli-Q water, with ammonium hydroxide, or Milli-Q water containing 0.1% formic acid

Gradient conditions
Gradient slope
2.8% per column volume
See individual chromatograms for flow rate and gradient time.

Samples
1. Standard mixture of sodium saccharin, caffeine, aspartame, vanillin, benzoic acid, sorbic acid, and benzaldehyde, all 50 µg/mL in 1/1 methanol/water
2. Various soft drinks, decarbonated where applicable
Preservatives

**Major Analytes**
Paraben, phenoxyethanol

**Matrix**
Standard

**Reference**

![Figure 1. Separation of preservatives by reversed-phase HPLC.](image)

| Table 1. Structures and Concentrations of Preservative Compounds |
|------------------|------------------|
| **Compounds**    | **Concentrations** |
| 2PX: 2-Phenoxyethanol | (1.4 mg/mL) |
| MEP: Methylparaben  | (0.30 mg/mL) |
| ETP: Ethylparaben   | (0.07 mg/mL) |
| PRP: Propylparaben  | (0.04 mg/mL) |
| IBP: Isobutylparaben| (0.04 mg/mL) |
| BTP: Butylparaben   | (0.08 mg/mL) |

**Conditions**
- **Column**: ZORBAX Eclipse XDB-C18 Rapid Resolution, 4.6 mm × 150 mm, 3.5 µm
- **Mobile phase**: Solvent A: Water
  - Solvent B: Methanol
- **Gradient**
  - Time | % MeOH |
  - 0    | 38     |
  - 5    | 38     |
  - 6    | 60     |
  - 16   | 60     |
  - 17   | 62     |
  - 20   | 38     |
- **Flow rate**: 0.8 mL/min
- **Temperature**: 40 °C
- **Detector**: UV 254 nm
- **Injection volume**: 5 µL

**System Summary**

**LC System**
- Quaternary or binary gradient module

**Detection**
- DAD

**Columns**
- ZORBAX XDB-C18, 4.6 mm × 150 mm, 3.5 µm

**Column part number**
- 963967-902
Proteins, Peptides, Amino Acids

Major Analytes
Proteins

Matrix
Wheat

Reference

System Summary

LC System
Quaternary or binary gradient module

Detection
DAD

Columns
ZORBAX 300 SB-CN, 300 SB-C8

Column part numbers
883995-906, 883995-905

Conditions
Column ZORBAX 300 SB-CN, 300 SB-C8
4.6 mm × 150 mm
(Agilent p/n 883995-906, 883995-905)

Mobile phase As above with 0.1% TFA, water

Gradient 60 min

Flow rate 1.0 mL/min

Temperature 50 °C

Detector DAD
Proteins, Peptides, Amino Acids

Major Analytes
Proteins

Matrix
Wheat

Reference

System Summary

LC System
Quaternary or binary gradient module

Detection
DAD

Columns
ZORBAX 300 SB-C8, 4.6 mm × 150 mm, 5 µm

Column part number
883995-906

Conditions

<table>
<thead>
<tr>
<th>Component</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>ZORBAX 300 SB-C8, 150 mm × 4.6 mm, (Agilent p/n 883995-906)</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>A: 0.1% trifluoroacetic acid in water</td>
</tr>
<tr>
<td></td>
<td>B: 0.1% TFA in acetonitrile</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1 mL/min</td>
</tr>
<tr>
<td>Gradient</td>
<td>At 0 min 23% B at 120 min 48% B</td>
</tr>
<tr>
<td>Oven temperature</td>
<td>50 and 70 °C</td>
</tr>
<tr>
<td>Injection volume</td>
<td>15 µL</td>
</tr>
<tr>
<td>Detector</td>
<td>DAD, detection wavelength 210 nm</td>
</tr>
</tbody>
</table>
Proteins, Peptides, Amino Acids

Major Analytes
BSA (bovine serum albumin) digest, peptide

Matrix
Standard

Reference

System Summary

LC System
Binary gradient

Detection
DAD

Columns
Poroshell 300SB-C18, 2.1 mm × 75 mm, 5 µm
ZORBAX 300SB-C18, 2.1 mm × 150 mm, 5 µm

Column part number
660750-902 and 883750-902

Conditions
Column 300 SB-C18 (as indicated above)
Mobile phase A = 95% H₂O, 5% ACN, 0.1% TFA
B = 5% H₂O, 95% ACN, 0.07% TFA
Flow As above
Piston stroke 20 µL
Detection UV, 215 nm
Temperature 70 °C
Agilent 1100 well plate autosampler with delay volume reduction
Injection volume 20 µL (0.22 µg/1 µL)
Sample BSA Tryptic digest (15 hours, 70 pmol)
Proteins, Peptides, Amino Acids

Major Analytes
AAA amino acid

Matrix
Standard

Reference

System Summary

LC System
Quaternary or binary gradient module
Detection
FLD/OPA-FMOC
Columns
Eclipse-AAA, 4.6 mm × 75 mm and 4.6 mm × 150 mm, 3.5 µm; 3 mm × 150 mm, 3.5 µm; and 4.6 mm × 150 mm, 5 µm

Column part number
See application note

Mobile Phase
Mobile phase A 40 mM Na₂HPO₄ [5.5 g NaH₂PO₄, monohydrate + 1 liter water, adjust pH with NaOH solution (10 N)]
Mobile phase B ACN:MeOH:water (45:45:10, v/v/v)
It is convenient to make mobile phase A as a 10X stock solution with no pH adjustment. The solution can be kept for several weeks and can be diluted and titrated, as needed. All mobile-phase solvents should be HPLC grade

Pump Settings
Flow 2 mL/min
Stop time 14 min (75-mm column) or 26 min (150-mm column)
Post time Off
Gradients For 75-mm column length

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>9.8</td>
<td>57</td>
</tr>
<tr>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>12</td>
<td>100</td>
</tr>
<tr>
<td>12.5</td>
<td>0</td>
</tr>
<tr>
<td>14</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 1. Routine Analysis, High-Throughput Separation of 24 Amino Acids Using the Eclipse-AAA Protocol. The column dimensions are 4.6 × 75 mm, 3.5 µm. See Table 1 for peak identification. Detection: A. 338 nm (OPA amino acids). B. 262 nm (FMOC-amino acids).

Figure 2. High-Resolution Analysis of 21 Amino Acids on the 5-µm and 3.5-µm ZORBAX Eclipse-AAA Column. Column dimensions are 4.6 × 150 mm. See Table 1 for peak identification. Detection: 338 nm (OPA amino acids).
Regulated/Hazardous Drug Substances

Major Analytes
Drugs

Matrix
Water

Reference

System Summary

LC System
1200SL

Detection
MSMS QQQ pos/neg ESI

Columns
Extend-C18, 2.1 mm × 100 mm, 1.8 µm

Column part number
728700-902

Conditions

<table>
<thead>
<tr>
<th></th>
<th>ZORBAX Extend-C-18, RRHT, 2.1 mm × 100 mm, 1.8 µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>40 °C</td>
</tr>
<tr>
<td>Mobile phases A</td>
<td>0.1% formic acid in water, add NH₄OH buffer</td>
</tr>
<tr>
<td>Mobile phases B</td>
<td>Acetonitrile (ACN)</td>
</tr>
<tr>
<td>Flow rate:</td>
<td>0.3 mL/min</td>
</tr>
<tr>
<td>Gradient:</td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>%B</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
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<tr>
<td>15</td>
<td>100</td>
</tr>
<tr>
<td>20</td>
<td>100</td>
</tr>
<tr>
<td>21.5</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 1. Negative ion mode TIC of 11 pharmaceuticals.
Regulated/Hazardous Drug Substances

Major Analytes
Nitrofurans

Matrix
Poultry, shrimp

Reference
Bernhard Wüst, Christian Sauber, and Hans (J.) A. van Rhijn
“Quantitation of Nitrofuran Metabolites in Shrimp and Poultry by LC/MS/MS Using the Agilent LC/MSD Trap XCT,”
Agilent Technologies, publication 5989-0738EN,
www.agilent.com/chem

System Summary

LC System
Binary gradient
Detection
MSn Trap XCT posESI
Columns
ZORBAX XDB-C8, 2.1 mm × 50 mm, 3.5 µm
Column part number
971700-906
HPLC Method performance
Overall amount RSD 4.3%
Overall recovery 100%

LC/MS/MS Method Details

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC</td>
<td>Agilent 1100</td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.4 mL/min</td>
</tr>
<tr>
<td>Column</td>
<td>ZORBAX XDB-C8, 2.1 mm × 50 mm, 3.5 µm</td>
</tr>
<tr>
<td>Mobile phases</td>
<td>A: Water + 0.1% acetic acid</td>
</tr>
<tr>
<td></td>
<td>B: Acetonitrile + 0.1% acetic acid</td>
</tr>
<tr>
<td>Gradient</td>
<td>0–14 min: 10% A - 45% A; 14–16 min: 45% A - 90% A</td>
</tr>
<tr>
<td>Injection</td>
<td>50 µL out of 500 µL</td>
</tr>
<tr>
<td>MS 1100 LC/MSD XCT Ion Trap</td>
<td></td>
</tr>
<tr>
<td>Ionization mode</td>
<td>Positive ESI</td>
</tr>
<tr>
<td>Nebulizer pressure</td>
<td>45 psi</td>
</tr>
<tr>
<td>Drying gas flow</td>
<td>12 L/min</td>
</tr>
<tr>
<td>Drying gas temperature</td>
<td>350 °C</td>
</tr>
<tr>
<td>Skimmer</td>
<td>20 V</td>
</tr>
<tr>
<td>Capillary exit</td>
<td>55 V</td>
</tr>
<tr>
<td>Trap drive</td>
<td>55</td>
</tr>
<tr>
<td>ICC</td>
<td>On</td>
</tr>
</tbody>
</table>
Regulated/Hazardous Drug Substances

Major Analytes
Fluoroquinolones

Matrix
Beef kidney

Reference

System Summary
LC System
Binary gradient

Detection
MSD posESI

Columns
ZORBAX XDB-C8, 4.6 mm × 150 mm, 5 µm

Column part number
993967-902

HPLC
Column
ZORBAX Eclipse XDB-C8, 150 mm × 4.6 mm, 5 µm (P/N 993967-906)

Solvent A
0.1% formic acid in water

Solvent B
0.1% formic acid in acetonitrile

Gradient
$t_0 = 20% B$
$t_1 = 20% B$
$t_8 = 90% B$
$t_15 = 90% B$

Post time = 2.0 min

Flow rate
0.4 mL/min

Injection volume
50 µL

Column temp
30 °C

MSD
Source
Electrospray ionization (ESI) (positive ion mode)

Ion dwell time
14 ions at 40 ms each

Fragmentation
Varies by ion

Drying gas flow
12 L/min

Nebulizer pressure
30 psi

Drying gas temperature
350 °C

Capillary voltage
4000 V
Regulated/Hazardous Drug Substances

Major Analytes
Chloramphenicol

Matrix
Shrimp, honey

Reference

System Summary
**LC System**
Binary gradient

**Detection**
MSD and MSn Ion Trap negESI

**Columns**
ZORBAX Eclipse XDB-C18, 4.6 mm × 150 mm, 5 µm

**Column part number**
993967-902

![Figure 2. Analysis of a standard solution containing 2.5 pg/mL CAP and 5 pg/mL CAP-d5 (IS) on the LC/MSD Trap together with the corresponding MS/MS spectra and the MS/MS spectrum resulting from an analysis of a standard solution containing 0.2 pg/mL CAP.](image)

**Conditions**
Mobile phase 10 mM ammonium acetate in water (solvent A)
Methanol/acetonitrile 1/3 (solvent B)

![Figure 4. Analysis of a blank honey sample containing 1 ppb CAP-d5.](image)
Regulated/Hazardous Drug Substances

Major Analytes
Sulfa drugs

Matrix
Meat

Reference

System Summary

LC System
Quaternary or binary gradient module
Detection
DAD
Columns
RP-18 Purospher, 250 mm × 4 mm, 5 µm
Column part number
79925PU-584

Conditions
Column
RP-18 Purospher, 250 mm × 4 mm, 5 µm
(Agilent p/n 79925PU-584)
Mobile phase
A: 0.7% phosphoric acid
B: Acetonitrile
Gradient
at 0 min 5% B
at 10 min 65% B
at 40 min 65% B
at 45 min 65% B
Post time
7 min
Flow rate
1 mL/min
Column temperature
40 °C
Injection volume
20 µL
Detector
DAD detection wavelengths at 338/10 nm, 264/8 nm and 360/8,
reference off

Figure 1. Chromatogram of standard solution, 2 ug/mL each analyte.

Figure 2. Chromatogram of extract of bovine muscle.
Regulated/Hazardous Drug Substances

Major Analytes
Sulfonamides

Matrix
Standard

Reference

System Summary

LC System
CapLC

Detection
DAD MSD posESI

Columns
ZORBAX SB-C18, 0.5 mm × 150 mm, 3.5 µm

Column part number
5064-8262

Conditions
Mobile phase
A = 0.1 % formic acid in water
B = 0.1 % formic acid in acetonitrile
Regulated/Hazardous Miscellaneous Substances

Major Analytes
HMF hydroxymethylfurfural

Matrix
Bread, cereal, yogurt

Reference

System Summary
LC System
Binary gradient G1312A or G1312B

Detection
MSD posAPCI

Columns
Bonus-RP, 2.1 mm × 100 mm, 3.5 µm

Column part number
861768-901

Conditions

<table>
<thead>
<tr>
<th>LC/MS</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow rate</td>
<td>0.2 mL/min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gradient</td>
<td>ZORBAX Bonus RP, 100 mm × 2.1 mm, 3.5 µm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mobile phase</td>
<td>0.01 mM acetic acid in 0.2% aqueous solution of formic acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Injection</td>
<td>20 µL out of 1000 µL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS conditions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ionization mode</td>
<td>Positive APCI</td>
<td></td>
<td></td>
</tr>
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<td>Nebulizer pressure</td>
<td>60 psi</td>
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<td></td>
</tr>
<tr>
<td>Drying gas flow</td>
<td>4 L/min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drying gas temperature</td>
<td>325 °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaporizer temperature</td>
<td>425 °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skimmer</td>
<td>20 V</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Capillary voltage</td>
<td>4 kV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fragmentor voltage</td>
<td>55 eV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dwell time</td>
<td>439 ms</td>
<td></td>
<td></td>
</tr>
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</table>
Regulated/Hazardous Miscellaneous Substances

Major Analytes
Acrylamide

Matrix
Drinking water

Reference

System Summary
LC System
Dual binary w/6-port valve for autoSPE
Detection
MS TOF posESI
Columns
ZORBAX SB-C18, 2.1 mm × 150 mm, 5 µm
Column part number
883700-922

Sample extraction

Conditions
Elution solvents
A: 97% HPLC water (0.01% formic acid)
B: 3% CH₃CN (0.01% formic acid)
Regulated/Hazardous Miscellaneous Substances

Major Analytes
Chromium speciation

Matrix
Metrohm

Reference

System Summary
LC System
Metrohm 818 pump, Agilent 7500 ISIS sampler
Detection
IC-ICPMS
Columns
Agilent Cr, 4.6 mm × 30 mm, 5 µm
Column part number
G3268A

Conditions
Cr column Agilent part number G3268A, 30 mm × 4.6-mm id
Mobile phase 5 mM EDTA (Na₂), adjust pH by NaOH
Flow rate 1.2 mL/min
Column temperature Ambient
Injection volume 50–500 µL
Sample preparation
Reaction 40 °C
temperature
Incubation time 3 h
EDTA concentration 5–15 mM, adjust pH by NaOH
System Summary

LC System
Metrohm IC
Detection
MSD negESI
Columns
See description of metrohm column on this page

HPLC Method Performance
Overall amount RSD 4.3%
Overall recovery 101.5%

Table 1. Operating Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection loop size</td>
<td>100 µL</td>
</tr>
<tr>
<td>Column</td>
<td>MetroSep ASUPP-5 (4 mm x 100 mm)</td>
</tr>
<tr>
<td>Eluent</td>
<td>3/7 v/v MeOH/30 mM NaOH, water</td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.8 mL/min</td>
</tr>
<tr>
<td>Tune mode</td>
<td>Negative mode “auto-tune”</td>
</tr>
<tr>
<td>V_cap</td>
<td>1400 V</td>
</tr>
<tr>
<td>Drying gas flow and</td>
<td>9 L/min @ 320 °C</td>
</tr>
<tr>
<td>temperature</td>
<td></td>
</tr>
<tr>
<td>Nebulizer pressure</td>
<td>20 psig</td>
</tr>
<tr>
<td>Fragmentor</td>
<td>140 V</td>
</tr>
<tr>
<td>Dwell time m/z 99</td>
<td>1 s</td>
</tr>
<tr>
<td>Dwell time m/z 101</td>
<td>1 s</td>
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Table 2. Metrohm-Peak Ion Chromatograph Parameters and Setup

<table>
<thead>
<tr>
<th>Hardware</th>
<th>Metrohm Advanced IC consists of Metrohm 788 Autosampler, 830 Interface with ICNet 2.3 software, 833 Suppressor Module, 819 Advanced IC Detector, 820 IC Separation Center, 818 IC serial dual-piston pump</th>
</tr>
</thead>
<tbody>
<tr>
<td>Setup</td>
<td>Metrohm ASUPP-5 (4 mm x 100 mm) p/n 6.1006.510</td>
</tr>
<tr>
<td>Eluent</td>
<td>3/7 v/v MeOH/30 mM NaOH, water</td>
</tr>
<tr>
<td>Regenerant solution</td>
<td>5/95 v/v MeOH/60 mM HNO₃</td>
</tr>
<tr>
<td>Rinse solution</td>
<td>5/95 v/v MeOH/H₂O</td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.8 mL/min</td>
</tr>
</tbody>
</table>
### System Summary

**LC System**
Isocratic G1310A

**Detection**
ICP-MS

**Columns**
See Hamilton described at right

### Conditions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
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<tbody>
<tr>
<td>Column</td>
<td>Hamilton PRP X-100</td>
</tr>
<tr>
<td>Mobile phase A</td>
<td>2.2-mM NH₄HCO₃</td>
</tr>
<tr>
<td>Mobile phase B</td>
<td>2.5-mM tartaric acid, 1% methanol</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1 mL/min</td>
</tr>
<tr>
<td>Column temperature</td>
<td>40 °C</td>
</tr>
<tr>
<td>Injection volume</td>
<td>50 µL</td>
</tr>
<tr>
<td>Gradient</td>
<td>At 0 min 20% A</td>
</tr>
<tr>
<td></td>
<td>at 30 min 100% A</td>
</tr>
<tr>
<td>Detector</td>
<td>HPLC-ICP-MS</td>
</tr>
<tr>
<td>RF power</td>
<td>1430–1550</td>
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<td>RF matching</td>
<td>1.89–1.92 V</td>
</tr>
<tr>
<td>Sampling depth</td>
<td>4.0–4.8 mm</td>
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<tr>
<td>Carrier gas flow</td>
<td>0.89–0.93 L/min</td>
</tr>
<tr>
<td>Make up gas flow</td>
<td>0.10–0.14 L/min</td>
</tr>
<tr>
<td>Optional gas oxygen</td>
<td>at 5%</td>
</tr>
<tr>
<td>Spray chamber temperature</td>
<td>0 °C</td>
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<tr>
<td>Cones</td>
<td>platinum</td>
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<tr>
<td>Isotopes monitored</td>
<td>75As, 103Rh, 77Se, 53Cr</td>
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<tr>
<td>Injector diameter</td>
<td>2.4 mm</td>
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<td>Nebulizer flow</td>
<td>100 L/min PFA</td>
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<td>Interface pumps</td>
<td>2</td>
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<tr>
<td>Confirmation</td>
<td>MS spectral information and RTs</td>
</tr>
</tbody>
</table>

---

**Reference**

---

![Figure 1. Chromatography A: 2.2 mM NH₄HCO₃, 2.5 mM tartaric acid, 1% MeOH, Hamilton PRP X-100 column. Concentration of standard ~ 5 ng/g as As.](image-url)
Regulated/Hazardous Natural Toxin Substances

Major Analytes
DSP algal toxins

Matrix
Shellfish

Reference

System Summary

**LC System**
Quaternary gradient

**Detection**
MSD pos/neg ESI

**Columns**
ZORBAX SB-C18, 3 mm × 150 mm, 5 µm;
9.4 mm × 50 mm, 5 µm semiprep

**Column part number**
883975-302 and 846975-202

---

**Figure 4.** LC/MS analysis of OA.

Continued
**LC/MS Method Details – Analytical**

**LC Conditions**
- **Instrument**: Agilent 1100 HPLC (Quaternary pump)
- **Column**: ZORBAX SB-C18, 3.0 mm × 150 mm, 5 µm
- **Mobile phase**:
  - A: Water (0.1% Formic acid)
  - B: Methanol
- **Gradient**:
  - 20% B at 0 min
  - 20% B at 5 min
  - 80% B at 20 min
- **Stop time**: 28 min
- **Post time**: 4 min
- **Flow rate**: 0.6 mL/min
- **Injection vol**: 10 µL

**MS Conditions**
- **Instrument**: Agilent LC/MSD
- **Source**: Positive/negative switching ESI
- **Drying gas flow rate**: 12 L/min
- **Nebulizer**: 60 psig
- **Drying gas temp**: 350 °C
- **V_cap**: 3000 V (positive and negative)

**LC/MS Method Details – Semipreparative**

**LC Conditions**
- **Instrument 1**: Agilent 1100 HPLC (quaternary pump)
- **Column**: ZORBAX SB-C18, 9.4 mm × 50 mm, 5 µm
- **Mobile phase**:
  - A: Water (0.1% formic acid)
  - B: Methanol
- **Gradient**:
  - 20% B at 0 min
  - 20% B at 5 min
  - 80% B at 20 min
- **Stop time**: 28 min
- **Post time**: 4 min
- **Flow rate**: 7.0 mL/min
- **Injection vol**: 100 µL (250 µL using multiple draw mode)
- **Instrument 2**: Agilent 1100 HPLC (isocratic pump) for makeup flow
- **Flow rate**: 0.8 mL/min (50% H2O + 50% MeOH + 0.1% formic acid)
- **Active splitter**: Split ratio 271:1

**MS Conditions**
- **Instrument**: Agilent LC/MSD
- **Source**: Negative ESI
- **Drying gas flow**: 12 L/min
- **Nebulizer**: 60 psig
- **Drying gas temp**: 350 °C
- **V_cap**: 3000 V (positive)

**MSD Fraction Collection Setup**
- **FC Mode**: Use method target mass; Adducts: (M–H)+

---

Figure 5. Comparative analysis of DSP toxins in shellfish.
**Regulated/Hazardous Natural Toxin Substances**

**Major Analytes**

Mycotoxin, fumonisin

**Matrix**

Corn

**Reference**


**System Summary**

**LC System**

Binary gradient

**Detection**

MSD posESI

**Columns**

ZORBAX XDB-C18, 2.1 mm × 150 mm, 5 µm

**Column part number**

993700-902

**Chromatographic Conditions**

<table>
<thead>
<tr>
<th>Column</th>
<th>ZORBAX Eclipse XDB, C18, 2.1 mm × 150, 5 µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile phase</td>
<td>A = 5 mM ammonium acetate in water</td>
</tr>
<tr>
<td></td>
<td>B = acetonitrile</td>
</tr>
<tr>
<td>Gradient</td>
<td>Start with 33% B</td>
</tr>
<tr>
<td></td>
<td>at 8 min 60% B</td>
</tr>
<tr>
<td></td>
<td>at 9 min 33% B</td>
</tr>
<tr>
<td>Flow rate</td>
<td>250 µL/min</td>
</tr>
<tr>
<td>Injection vol</td>
<td>5 µL</td>
</tr>
<tr>
<td>Column temp</td>
<td>40 °C</td>
</tr>
<tr>
<td>Diode-array detector</td>
<td>Signal 220, 4 nm; reference 550,100 nm</td>
</tr>
</tbody>
</table>

**MS Conditions**

<table>
<thead>
<tr>
<th>Source</th>
<th>ESI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ion mode</td>
<td>Positive</td>
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<tr>
<td>$V_{cap}$</td>
<td>4000 V</td>
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<tr>
<td>Nebulizer</td>
<td>30 psig</td>
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<tr>
<td>Drying gas flow</td>
<td>10 l/min</td>
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<tr>
<td>Drying gas temp</td>
<td>350 °C</td>
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<td>Scan range</td>
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<tr>
<td>Step size</td>
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</tr>
<tr>
<td>Peak width</td>
<td>0.15 min</td>
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<tr>
<td>Time filter</td>
<td>On</td>
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<td>Fragmentor</td>
<td>Variable 230 V (100-680)</td>
</tr>
<tr>
<td></td>
<td>100 V (680-800)</td>
</tr>
</tbody>
</table>

**Figure 2.** Mass spectra for fumonisin analogues.

**Figure 3.** Chromatographic separation of fumonisin analogues at 25 ng.
Regulated/Hazardous Natural Toxin Substances

Major Analytes
Aflatoxins

Matrix
Various

Reference

Method:
1. Sample Preparation and Derivatization
The clean-up and derivatization steps of the aflatoxins were performed online using a Gerstel MPS-3 autosampler with its new spe-capabilities. Two user defined injector programs were automatically executed one after the other.

<table>
<thead>
<tr>
<th>clean-up</th>
<th>derivatization</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOVE</td>
<td>MOVE</td>
</tr>
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<td>MOVE</td>
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<td>MOVE</td>
<td>MOVE</td>
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<tr>
<td>WASH</td>
<td>ADD</td>
</tr>
<tr>
<td>SHIFT</td>
<td>SHIFT</td>
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<td>ADD</td>
<td>ADD</td>
</tr>
<tr>
<td>ADD</td>
<td>ADD</td>
</tr>
<tr>
<td>MIX</td>
<td>MOVE</td>
</tr>
<tr>
<td>INJECT</td>
<td>INJECT</td>
</tr>
</tbody>
</table>

Figure 3. Program for automatic spe clean-up and derivatization.

2. LC/MS Method
The autosampler was integrated in an Agilent 1100 LC/MSD system, consisting of a binary pump, thermostatted column compartment, diode array detector and a LC/MSD (single quadrupole). The LC/MSD was used with electrospray ionization in positive ion mode. Chromatographic separations were performed on a Phenomenex Max RP (250*2.1 mm, 5 µm), using a flow rate of 0.3 mL/min in gradient mode (Eluent A: 0.1% formic acid, water; Eluent B: acetonitrile, water). Complete system control (including the autosampler) and data evaluation were carried out using the Agilent ChemStaion (Rev.A10.03)

System Summary
LC System
Agilent/Gerstel onlineSPE
Detection
MSD posESI
Columns
Phenomenex MAX RP, 2.1 mm × 250 mm, 5 µm
Column part number
Suggest ZORBAX SB-C18, 2.1 mm × 150 mm, 3.5 µm, 830990-902

Figure 3. Program for automatic spe clean-up and derivatization.

Figure 4. Monobrominated aflatoxins.

Figure 6. TICs of aflatoxins before (top) and after (bottom) derivatization.
Regulated/Hazardous Pesticides/Herbicide Substances

Major Analytes
44 pesticides

Matrix
Vegetables, fruit

Reference

System Summary
LC System
Binary gradient
Detection
MSMS QQQ posESI
Columns
Extend-C18, 2.1 mm × 100 mm, 1.8 µm
Column part number
728700-902

Figure 1. TIC of 33 pesticides standard in full scan mode (A) and product ion scan mode (B) at 1 µg/mL.

<table>
<thead>
<tr>
<th>LC Conditions</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Instrument</td>
<td>Agilent 1100 HPLC</td>
</tr>
<tr>
<td>Column</td>
<td>ZORBAX Extend C18, 100 mm × 2.1 mm, 1.8 µm (p/n 728700-902)</td>
</tr>
<tr>
<td>Column temp</td>
<td>40 °C</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>A = 0.1% formic acid + 5 mM ammonium formate in water</td>
</tr>
<tr>
<td>Gradient</td>
<td>B = Acetonitrile</td>
</tr>
<tr>
<td>Gradient</td>
<td>10% B at 0 min, 80% B at 30 min</td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.2 mL/min</td>
</tr>
<tr>
<td>Injection vol</td>
<td>5 µL</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MS Conditions</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Instrument</td>
<td>Agilent 6410 QQQ</td>
</tr>
<tr>
<td>Source</td>
<td>Positive ESI</td>
</tr>
<tr>
<td>Drying gas flow</td>
<td>10 L/min</td>
</tr>
<tr>
<td>Nebulizer</td>
<td>50 psig</td>
</tr>
<tr>
<td>Drying gas temp</td>
<td>350 °C</td>
</tr>
<tr>
<td>V&lt;sub&gt;e&lt;/sub&gt;</td>
<td>4000 V</td>
</tr>
<tr>
<td>Scan</td>
<td>m/z 100 to 550</td>
</tr>
<tr>
<td>Fragmentor</td>
<td>Variable 100 V</td>
</tr>
<tr>
<td>MRM ions</td>
<td>Shown in Tables 1 and 2</td>
</tr>
<tr>
<td>Collision energy</td>
<td>Shown in Tables 1 and 2</td>
</tr>
</tbody>
</table>
Regulated/Hazardous Pesticides/Herbicide Substances

Major Analytes
Acid herbicides

Matrix
Water

Reference

System Summary

LC System
AutoSPE/1200SL with dual binary pumps and 6-port valve

Detection
DAD

Columns
ZORBAX SB-C18, 5, 3.5, and 1.8 µm

Column part number
See application note

Conditions

Column EPA Method 555 with ZORBAX SB-C18 columns and fast DAD detector
ZORBAX SB-C18, 4.6 mm × 250 mm, 5 µm
Column temp 25 °C
Gradient 25 mM H₃PO₄, ACN, 10% to 90% ACN in 30 min
Gradient slope 7.8% ACN/column volume
Analysis flow rate 1 mL/min
Group A compounds 1 µL of 100 µg/mL
Total analysis time 60 min
Detection UV 230 nm, 10-mm 13-µL flow cell, filter 2 seconds (default)

Figure 2. Gradient separation of herbicides on 4.6 mm × 250 mm, 5-µm ZORBAX SB-C18.

Figure 5. High-speed gradient separation of herbicides on 2.1 × 80 mm, 1.8-µm ZORBAX SB-C18.

Conditions

EPA Method 555 with ZORBAX SB-C18 columns and fast DAD detector ZORBAX SB-C18, 2.1 mm × 80 mm, 3.5 µm
Column temp 50 °C
Gradient 25-mM H₃PO₄/ACN, 10% to 90% ACN in 2.7 min
Analysis flow rate 0.72 mL/min
Detection UV 230 nm, 3-mm 2-µL flow cell, filter 0.2 seconds
Sample Aged 1 µL 100 µg/mL
Total analysis time 6 min

Continued
Experimental Conditions

System
Agilent 1200SL Series Rapid Resolution LC consisting of:
- G1379B micro degasser
- G1312B binary pump SL
- G1312A binary pump with solvent selection valve option, or
- G1354A quaternary pump
- G1367C HiP ALS autosampler SL, and
- G2258A dual loop prep autosampler 5 mL
- G1316B thermostatted column compartment SL with 6- or 10-port
  2-position switching valve
- G1315C UV/VIS diode array detector (DAD) SL, flow cell as indicated in
  individual chromatograms
- ChemStation 32-bit version B.02.01

Columns
- Agilent ZORBAX SB-C18, 4.6 × 250 mm, 5 µm
- Agilent ZORBAX SB-C18, 3.0 × 150 mm, 3.5 µm
- Agilent ZORBAX SB-C18, 2.1 × 80 mm, 1.8 µm
- Agilent ZORBAX SB-Aq, 4.6 × 12.5 mm, 5 µm

Mobile phase conditions
- Organic solvent  Acetonitrile
- Aqueous solvent  25 mm phosphoric acid in Milli-Q water

Gradient conditions
- Gradient slope  7.8 or 2.3% per column volume, as indicated. See
  individual chromatograms for flow rate and time

Sample
- EPA 555 Group A chlorinated phenoxy acid herbicides (picloram,
  chloramben, dicamba, bentazon, 2,4-D, dichlorprop, and 2,4,5-TP,
  acifluorfen), 100 µg/mL in methanol or diluted to 20 ng/L (20 ppb)
  in reagent water acidified with 25 mm phosphoric acid.
Regulated/Hazardous Pesticides/Herbicide Substances

Major Analytes
Postharvest fungicides

Matrix
Citrus

Reference

System Summary

**LC System**
Binary gradient

**Detection**
MS TOF, MSn Ion Trap, both posESI

**Columns**
ZORBAX XDB-C8, 4.6 mm × 150 mm, 5 µm

**Column part number**
993967-906

**LC/TOF MS Method**

- **LC pumps**: Agilent 1100 binary pumps, injection volume 50 µL with standard Agilent 1100 ALS
- **Column**: ZORBAX Eclipse XDB-C8, 4.6 mm × 150 mm, 5 µm, product number 993967-906
- **Mobile phases**: A = acetonitrile and B = 0.1% formic acid in water
- **Gradient**: 5 minutes isocratic at 10% A, followed by a linear gradient to 100% A in 30 minutes at a flow rate of 0.6 mL/min
- **Instrument**: Agilent LC/MSD TOF time-of-flight mass spectrometer with dual electrospray source, positive ESI, capillary 4000 V
- **Nebulizer**: 40 psig
- **Drying gas**: 9 L/min, 300 °C
- **Voltagess**: Fragmentor 190 V, skimmer 60 V, Oct DC1 37.5 V, OCT RF V 250 V
- **Reference masses**: m/z 121.0509 and 922.0098
- **Resolution**: 9500±500 at m/z 922.0098
- **Mass range**: 50–1000 m/z
- **Flow rate**: Reference A Sprayer 2 is at a constant flow rate during the run
Regulated/Hazardous Pesticides/Herbicide Substances

Major Analytes
Chloronicotinyl insecticides

Matrix
Vegetables, fruit

Reference

Figure 3. LC/MSD TOF analysis of the three chloronicotinyl insecticides at 0.5 mg/kg in a fortified tomato matrix. The total and extracted ion chromatograms are shown in upper and lower panels, respectively.

Figure 5a. Structure and fragmentation pathways for acetamiprid using LC/MSD Trap mass spectrometer and LC/MSD TOF. Only the m/z 126 ion was seen with LC/MSD TOF as a fragment ion.

System Summary

LC System
Binary gradient

Detection
MS TOF, MSn Ion Trap, both posESI

Columns
ZORBAX Eclipse XDB-C8, 4.6 mm × 150 mm, 5 µm

Column part number
993967-906

Agilent LC/MSD TOF

LC pump Agilent 1100 binary
Autosampler Agilent 1100 standard ALS
Injection volume 50 µL
Column ZORBAX Eclipse XDB C-8
4.6 mm × 150 mm × 5 µm
p/n 993967-906
Mobile phase A: acetonitrile
B: 0.1% formic acid in water
Gradient 15% to 100% A over 30 minutes
at 0.6 mL/min
Regulated/Hazardous Pesticides/Herbicide Substances

Major Analytes
Phenylurea, triazine herbicides

Matrix
Water

Reference

System Summary
LC System
Dual binary w/6-port valve for autoSPE

Detection
MSD pos/negAPCI

Columns
ZORBAX Eclipse XDB-C8, 2.1 mm × 50 mm, 3.5 µm

Column part number
971700-906

The flow path through the column compartment 10-port valve and the wellplate 6-port autosampler valve is shown in Figure 1.

HPLC Conditions

<table>
<thead>
<tr>
<th>Pump 1 (Analytical column)</th>
<th>Time (min)</th>
<th>Mobile phase A</th>
<th>Mobile phase B</th>
<th>Flow rate (mL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>0</td>
<td>90</td>
<td>10</td>
<td>0.5</td>
</tr>
<tr>
<td>4.5</td>
<td>90</td>
<td>0</td>
<td>90</td>
<td>0.5</td>
</tr>
<tr>
<td>17.5</td>
<td>30</td>
<td>70</td>
<td>90</td>
<td>0.5</td>
</tr>
<tr>
<td>17.6</td>
<td>90</td>
<td>10</td>
<td>90</td>
<td>0.5</td>
</tr>
<tr>
<td>22.0</td>
<td>90</td>
<td>10</td>
<td>90</td>
<td>0.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pump 2 (Precolumn)</th>
<th>Time (min)</th>
<th>Mobile phase A</th>
<th>Mobile phase B</th>
<th>Flow rate (mL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>0</td>
<td>90</td>
<td>10</td>
<td>0.5</td>
</tr>
<tr>
<td>5.0</td>
<td>90</td>
<td>10</td>
<td>90</td>
<td>0.5</td>
</tr>
<tr>
<td>5.1</td>
<td>90</td>
<td>10</td>
<td>90</td>
<td>0.1</td>
</tr>
<tr>
<td>18.0</td>
<td>90</td>
<td>10</td>
<td>90</td>
<td>0.1</td>
</tr>
<tr>
<td>18.1</td>
<td>90</td>
<td>10</td>
<td>90</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Column switching valve

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Valve Position</th>
<th>Temp (L) °C</th>
<th>Temp (R) °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>1</td>
<td>20.0</td>
<td>40.0</td>
</tr>
<tr>
<td>2.0</td>
<td>2</td>
<td>20.0</td>
<td>40.0</td>
</tr>
<tr>
<td>20.0</td>
<td>1</td>
<td>20.0</td>
<td>40.0</td>
</tr>
</tbody>
</table>

Continued
Results

Figures 2, 3, and 4 show an extracted ion chromatogram from spiked tap water containing atrazine, isoproturon, and diuron, respectively, at a concentration of 0.1 µg/L. Figure 5 also shows an extracted ion chromatogram, again from a spiked tap water containing prometryn and terbutryn at a concentration of 0.01 µg/L (5 pg on-column).

Validation of the method was done on 11 sample batches. Standards were prepared at three levels: 0.01, 0.10, and 0.40 µg/L. The borehole raw water was spiked at two levels: 0.01 and 0.10 µg/L. The potable tap water (which was derived from a surface water source) was also spiked at two levels: 0.01 and 0.10 µg/L. All samples were analyzed in duplicate in each batch in a random order.

Table 3. Method Performance Data

<table>
<thead>
<tr>
<th>Compound</th>
<th>Borehole raw water</th>
<th>Potable treated water</th>
<th>LOD (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%Recovery</td>
<td>%RSD</td>
<td>%Recovery</td>
</tr>
<tr>
<td>Metamitron</td>
<td>101.0</td>
<td>4.9</td>
<td>101.5</td>
</tr>
<tr>
<td>Chloridazon</td>
<td>99.4</td>
<td>6.7</td>
<td>98.4</td>
</tr>
<tr>
<td>Monuron</td>
<td>102.1</td>
<td>4.7</td>
<td>98.4</td>
</tr>
<tr>
<td>Carbetamide</td>
<td>100.6</td>
<td>6.4</td>
<td>96.4</td>
</tr>
<tr>
<td>Simazine</td>
<td>101.7</td>
<td>5.1</td>
<td>98.2</td>
</tr>
<tr>
<td>Cyanazine</td>
<td>99.5</td>
<td>4.2</td>
<td>99.3</td>
</tr>
<tr>
<td>Chlortoluron</td>
<td>99.2</td>
<td>5.3</td>
<td>99.3</td>
</tr>
<tr>
<td>Atrazine</td>
<td>98.1</td>
<td>3.4</td>
<td>97.0</td>
</tr>
<tr>
<td>Diuron</td>
<td>100.8</td>
<td>4.6</td>
<td>97.7</td>
</tr>
<tr>
<td>Isoproturon</td>
<td>100.8</td>
<td>4.3</td>
<td>98.5</td>
</tr>
<tr>
<td>Linuron</td>
<td>102.5</td>
<td>5.1</td>
<td>99.9</td>
</tr>
<tr>
<td>Propazine</td>
<td>101.4</td>
<td>5.0</td>
<td>100.5</td>
</tr>
<tr>
<td>Terbutylazine</td>
<td>100.5</td>
<td>6.3</td>
<td>99.5</td>
</tr>
<tr>
<td>Triazine</td>
<td>102.5</td>
<td>5.4</td>
<td>100.5</td>
</tr>
<tr>
<td>Prometryn</td>
<td>102.0</td>
<td>4.8</td>
<td>101.1</td>
</tr>
<tr>
<td>Terbutryn</td>
<td>102.3</td>
<td>6.0</td>
<td>100.8</td>
</tr>
</tbody>
</table>
Fat-Soluble Vitamins

Major Analytes
Retinol isomers

Matrix
Standard

Reference

System Summary
LC System
Isocratic
Detection
DAD
Columns
ZORBAX Sil, 4.6 mm × 250 mm, 5 µm
Column part number
880952-701

Conditions
Column
ZORBAX Sil, 4.6 mm × 250 mm
Agilent p/n 880952-701
Mobile phase
Dioxane, (MTBE), hexane
Injection volume
20 µL, 25 °C

A = Retinol Isomers
11, 13-di-cis-
13-cis-
9, 11, 13-tri-cis-
9, 13-di-cis-
11-cis-
7, 11-di-cis-
9-cis-
7, 9-di-cis-
7, 13-di-cis-
AT all trans retinol
B-F = Retinol Isomers

Courtesy of Dr. G. Nöll Physiologisches Inst. – Justus Liebig Uni. Giessen
Fat-Soluble Vitamins

Major Analytes
Fat-soluble vitamins

Matrix
Standard

Reference

System Summary

LC System
Isocratic

Detection
DAD

Columns
ZORBAX XDB-C8, 4.6 mm × 150 mm, 5 µm

Column part number
993967-906

Conditions

Column ZORBAX XDB-C8, 150 mm × 4.6 mm, 5 µm (Agilent p/n 993967-906)
Mobile phase A: water 5%, B: methanol 95%
Flow rate 1 mL/min
Oven temperature 50 °C
Detector DAD, detection wavelength 280 nm
**Fat-Soluble Vitamins**

**Major Analytes**
Vitamin D3

**Matrix**
Poultry feed

**Reference**

**System Summary**

**LC System**
Binary gradient

**Detection**
MSn Ion Trap posAPCI

**Columns**
Flow injection – no column

**Column part number**
NA

### Conditions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile phase</td>
<td>0.05% acetic acid : methanol (50:50)</td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.6 mL/min</td>
</tr>
<tr>
<td>Injection volume</td>
<td>10 µL</td>
</tr>
<tr>
<td>Detector</td>
<td>MSD ion trap</td>
</tr>
<tr>
<td>Ionization</td>
<td>APCI (positive)</td>
</tr>
<tr>
<td>Nebulizer pressure</td>
<td>40 psi</td>
</tr>
<tr>
<td>Drying gas</td>
<td>10 L/min, 350 °C</td>
</tr>
<tr>
<td>Skim</td>
<td>1 15.0 V</td>
</tr>
<tr>
<td>Cap exit offset</td>
<td>60.0 V</td>
</tr>
<tr>
<td>Averages</td>
<td>2</td>
</tr>
<tr>
<td>ICC</td>
<td>on</td>
</tr>
<tr>
<td>Max acc.time</td>
<td>200 ms</td>
</tr>
<tr>
<td>Target</td>
<td>50000</td>
</tr>
<tr>
<td>Confirmation</td>
<td>MS spectral information and RTs</td>
</tr>
</tbody>
</table>

Comparison of production spectra from a pure standard of vitamin D3 and an enriched poultry feed extract.
Fat-Soluble Vitamins

Major Analytes
Fat-soluble vitamins A, D, E

Matrix
Standard

Reference

System Summary
LC System
Quaternary gradient

Detection
DAD

Columns
ZORBAX Eclipse XDB-C18, 4.6 mm × 75 mm, 3.5 µm

Column part number
7995118-344

HPLC Performance

<table>
<thead>
<tr>
<th>Compound</th>
<th>LOD for S/N = 2 (mg/L)*</th>
<th>Precision of RT (RSD of 10 runs) (1000 mg/L)*</th>
<th>Precision of Area (RSD of 10 runs) (1000 mg/L)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinol</td>
<td>4.0</td>
<td>0.10</td>
<td>0.14</td>
</tr>
<tr>
<td>Cholecalciferol</td>
<td>2.5</td>
<td>0.04</td>
<td>0.16</td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>2.0</td>
<td>0.04</td>
<td>0.20</td>
</tr>
</tbody>
</table>

* Injection volume: 5 µL

Figure 1. Analysis of three fat-soluble vitamins.
Water-Soluble Vitamins

Major Analytes
Water-soluble vitamins

Matrix
Standard

Reference

System Summary

LC System
Quaternary or binary gradient module

Detection
DAD

Columns
ZORBAX SB-C8, 150 mm × 4.6 mm, 5 µm

Column part number
883975-906

Conditions
<table>
<thead>
<tr>
<th>Column</th>
<th>ZORBAX SB-C8, 150 mm × 4.6 mm, (Agilent p/n 883975-906)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile phase</td>
<td>A: 50-mM sodium phosphate:methanol (90 : 10)</td>
</tr>
<tr>
<td></td>
<td>B: 50-mM sodium phosphate:methanol (10 : 90)</td>
</tr>
<tr>
<td>Gradient</td>
<td>At 0 min 0% B</td>
</tr>
<tr>
<td></td>
<td>At 18 min 70% B</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1 mL/min</td>
</tr>
<tr>
<td>Column temperature</td>
<td>Ambient</td>
</tr>
<tr>
<td>Injection volume</td>
<td>10 µL</td>
</tr>
<tr>
<td>Detector</td>
<td>DAD, detection wavelength 245 nm</td>
</tr>
</tbody>
</table>
Water-Soluble Vitamins

Major Analytes
Water-soluble vitamins

Matrix
Cat food

Reference

System Summary
LC System
Quaternary or binary gradient module
Detection
DAD
Columns
See description at right

Conditions
Column: ZORBAX SB-C18, 75 mm × 4.6 mm, 3.5 µm (Agilent p/n 866953-902)
Mobile phase: A: 1-L water, 2.5-g hexane sulfonate, 2.5-mL acetic acid, 4-g NaH2PO4, 50-L triethylamine
B: 600-mL water, 2.5-g hexane sulfonic acid, 2.5-mL acetic acid, 50-L triethyamine, 400-mL acetonitrile
Gradient: At 0 min 0% B
at 3.6 min 0% B
at 6 min 20% B
at 13.5 min 55% B
at 14.4 min 90% B
at 16.5 min 90% B
at 16.8 min 0% B
at 20 min 0% B
Flow rate: 1.9 mL/min
Column temperature: Ambient
Injection volume: 20 µL
Detector: DAD, detection wavelength 275 nm
## System Configurations

### Application Area – Carbohydrates, Sugars, Sugar Alcohols

<table>
<thead>
<tr>
<th>Major analytes</th>
<th>LC solvent delivery system*</th>
<th>Detection**</th>
<th>Notes</th>
<th>Publication number</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>Isocratic G1310A</td>
<td>RID</td>
<td></td>
<td>5988-6351EN</td>
<td>5</td>
</tr>
<tr>
<td>Sugar alcohols</td>
<td>Isocratic G1310A</td>
<td>MSD negAPCI</td>
<td></td>
<td>5988-4236EN</td>
<td>6</td>
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<tr>
<td>Dextran</td>
<td>Isocratic G1310A</td>
<td>RID</td>
<td></td>
<td>5988-0118EN</td>
<td>7</td>
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<tr>
<td>Starch</td>
<td>Isocratic G1310A</td>
<td>RID</td>
<td></td>
<td>5988-0117EN</td>
<td>8</td>
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<tr>
<td>Carbohydrates</td>
<td>Isocratic G1310A</td>
<td>RID</td>
<td></td>
<td>5966-0637EN</td>
<td>9</td>
</tr>
</tbody>
</table>

### Application Area – Dyes, Colorants, Pigments

<table>
<thead>
<tr>
<th>Major analytes</th>
<th>LC solvent delivery system*</th>
<th>Detection**</th>
<th>Notes</th>
<th>Publication number</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sudan dyes</td>
<td>Binary gradient G1312A or G1312B</td>
<td>MS TOF posESI</td>
<td></td>
<td>5989-4736EN</td>
<td>10</td>
</tr>
<tr>
<td>FDC food dyes, paraben</td>
<td>Quaternary gradient G1354A</td>
<td>DAD</td>
<td></td>
<td>5988-6355EN</td>
<td>11</td>
</tr>
<tr>
<td>Cyanidins</td>
<td>Binary gradient G1312A or G1312B</td>
<td>MSD posAPCI</td>
<td></td>
<td>5968-2979E</td>
<td>12</td>
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</tbody>
</table>

### Application Area – Fats and Oils

<table>
<thead>
<tr>
<th>Major analytes</th>
<th>LC solvent delivery system*</th>
<th>Detection**</th>
<th>Notes</th>
<th>Publication number</th>
<th>Page</th>
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<td>Triglycerides and their hydroperoxides</td>
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### Application Area – Flavors, Sweeteners, Organic Acids

<table>
<thead>
<tr>
<th>Major analytes</th>
<th>LC solvent delivery system*</th>
<th>Detection**</th>
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<th>Publication number</th>
<th>Page</th>
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<td>Aspartame, degradants</td>
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### Application Area – Herbal Supplements, Natural Products, Plant Hormones

<table>
<thead>
<tr>
<th>Major analytes</th>
<th>LC solvent delivery system*</th>
<th>Detection**</th>
<th>Notes</th>
<th>Publication number</th>
<th>Page</th>
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### Application Area – Preservatives

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<th>Detection**</th>
<th>Notes</th>
<th>Publication number</th>
<th>Page</th>
</tr>
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</table>
**System Configurations (Continued)**

### Application Area – Proteins, Peptides, Amino Acids

<table>
<thead>
<tr>
<th>Major analytes</th>
<th>LC solvent delivery system*</th>
<th>Detection**</th>
<th>Notes</th>
<th>Publication number</th>
<th>Page</th>
</tr>
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### Application Area – Regulated/Hazardous Drug Substances

<table>
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<th>Major analytes</th>
<th>LC solvent delivery system*</th>
<th>Detection**</th>
<th>Notes</th>
<th>Publication number</th>
<th>Page</th>
</tr>
</thead>
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<td>Chloramphenicol</td>
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<td>MSD and MSn Ion Trap negESI</td>
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### Application Area – Regulated/Hazardous Miscellaneous Substances

<table>
<thead>
<tr>
<th>Major analytes</th>
<th>LC solvent delivery system*</th>
<th>Detection**</th>
<th>Notes</th>
<th>Publication number</th>
<th>Page</th>
</tr>
</thead>
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### Application Area – Regulated/Hazardous Natural Toxin Substances

<table>
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<tr>
<th>Major analytes</th>
<th>LC solvent delivery system*</th>
<th>Detection**</th>
<th>Notes</th>
<th>Publication number</th>
<th>Page</th>
</tr>
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<td>Mycotoxin, fumonisins</td>
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## System Configurations (Continued)

### Application Area – Regulated/Hazardous Pesticides/Herbicide Substances

<table>
<thead>
<tr>
<th>Major analytes</th>
<th>LC solvent delivery system*</th>
<th>Detection**</th>
<th>Notes</th>
<th>Publication number</th>
<th>Page</th>
</tr>
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<td>Chloronicotinyl insecticides</td>
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<td>Phenylurea, triazine herbicides</td>
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### Application Area – Vitamins, Fat-Soluble

<table>
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<th>Publication number</th>
<th>Page</th>
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### Application Area – Vitamins, Water-Soluble

<table>
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<th>LC solvent delivery system</th>
<th>Detection**</th>
<th>Notes</th>
<th>Publication number</th>
<th>Page</th>
</tr>
</thead>
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<td>DAD</td>
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</tbody>
</table>

* Selection of the injector, including accessory thermostat module, should be based on budget and additional user requirements. Consult your Agilent representative or agent.

Where isocratic is shown, choose an isocratic, binary, or quaternary pump module. Binary and quaternary gradient modules offer greater flexibility. Isocratic pumps are field-upgradeable to quaternary gradient pumps.

Where binary gradient is shown, quaternary gradients may often be substituted when using flow rates 1 mL/min or higher and with most 3- and 4.6-mm id columns. For very fast gradients with steep gradient slopes, as in a high-throughput analysis environment, the binary gradient module would be more suitable.

** Where DAD is shown, review the original application note carefully to determine if spectral content or multiwavelength monitoring is required. If not, you may substitute a multiwavelength or variable-wavelength detector. Choose standard flow cells for most 3- and 4.6-mm id column applications. For 2.1-mm applications, especially with 1.8-µm particle sizes, consider smaller volume flow cells to minimize extracolumn dispersion.

MS detectors require the user to specify the ionization source (that is, APCI, ESI, multimode ESI/APCI, AP-MALDI, APPI/APCI, dual-spray ESI [TOF], ChipCube ESI, and other sources) for nano or capillary LC operations.

Not all MS detector models permit concurrent positive/negative polarity switching. Review the original application note carefully and consider future needs to determine if this is required.
# Food Quick Reference Guide

## Carbohydrates, Sugars, Sugar Alcohols

<table>
<thead>
<tr>
<th>Page</th>
<th>Major Analytes</th>
<th>Matrix</th>
<th>LC system</th>
<th>Detection</th>
<th>Mobile phase</th>
<th>Literature reference</th>
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<td>ACN/water</td>
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<td>RID</td>
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## Dyes, Colorants, Pigments

<table>
<thead>
<tr>
<th>Page</th>
<th>Major Analytes</th>
<th>Matrix</th>
<th>LC system</th>
<th>Detection</th>
<th>Mobile phase</th>
<th>Literature reference</th>
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<tr>
<td>10</td>
<td>Sudan dyes</td>
<td>Food</td>
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<td>MS TOF posESI</td>
<td>ACN/water</td>
<td>5989-4736EN</td>
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<td>FDC food dyes, paraben</td>
<td>Ricker</td>
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<td>Cabbage</td>
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<td>ACN/water</td>
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## Fats and Oils

<table>
<thead>
<tr>
<th>Page</th>
<th>Major Analytes</th>
<th>Matrix</th>
<th>LC system</th>
<th>Detection</th>
<th>Mobile phase</th>
<th>Literature reference</th>
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<td>MSD posESI</td>
<td>Acetone/water</td>
<td>5988-4235EN</td>
</tr>
<tr>
<td>16</td>
<td>Triglycerides</td>
<td>Isocratic</td>
<td>Binary gradient</td>
<td>MSD posESI</td>
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<td>5968-0878E</td>
</tr>
<tr>
<td>17</td>
<td>Triglycerides and their hydroperoxides</td>
<td>Edible oil</td>
<td>Quaternary gradient</td>
<td>DAD 3channel</td>
<td>ACN/MTBE/ water</td>
<td>5966-0744EN</td>
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## Flavors, Sweeteners, Organic Acids

<table>
<thead>
<tr>
<th>Page</th>
<th>Major Analytes</th>
<th>Matrix</th>
<th>LC system</th>
<th>Detection</th>
<th>Mobile phase</th>
<th>Literature reference</th>
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<td>Soft drinks</td>
<td>1200SL</td>
<td>DAD</td>
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<td>DAD</td>
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<td>5989-1265EN</td>
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<td>20</td>
<td>Flavoring agents</td>
<td>Mouthwash</td>
<td>Binary gradient</td>
<td>DAD</td>
<td>ACN/water TFA</td>
<td>5988-6353EN</td>
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<td>Semivolatile flavors</td>
<td>Isocratic</td>
<td>DAD</td>
<td>MeOH/water</td>
<td>5988-6352EN</td>
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<td>22</td>
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<td>DAD</td>
<td>ACN/water TFA</td>
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## Herbal Supplements, Natural Products, Plant Hormones

<table>
<thead>
<tr>
<th>Page</th>
<th>Major Analytes</th>
<th>Matrix</th>
<th>LC system</th>
<th>Detection</th>
<th>Mobile phase</th>
<th>Literature reference</th>
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<td>DAD</td>
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<td>5989-4907EN</td>
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<td>DAD 2-µLcell</td>
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<td>DAD</td>
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<td>5989-0591EN</td>
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<td>DAD</td>
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<td>29</td>
<td>Flavonoids, catechins</td>
<td>Gradient</td>
<td>DAD</td>
<td>MeOH/water TFA</td>
<td>5988-6357EN</td>
<td></td>
</tr>
</tbody>
</table>
## Preservatives

<table>
<thead>
<tr>
<th>Page</th>
<th>Major Analytes</th>
<th>Matrix</th>
<th>LC system</th>
<th>Detection</th>
<th>Mobile phase</th>
<th>Literature reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>Flavors, sweeteners, preservatives</td>
<td>Soft drinks</td>
<td>1200SL</td>
<td>DAD</td>
<td>ACN/water</td>
<td>5989-5178EN</td>
</tr>
<tr>
<td>31</td>
<td>Paraben, phenoxyethanol</td>
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<td>DAD</td>
<td>MeOH/water</td>
<td>5989-3835EN</td>
</tr>
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</table>

## Proteins, Peptides, Amino Acids

<table>
<thead>
<tr>
<th>Page</th>
<th>Major Analytes</th>
<th>Matrix</th>
<th>LC system</th>
<th>Detection</th>
<th>Mobile phase</th>
<th>Literature reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>32</td>
<td>Proteins</td>
<td>Wheat</td>
<td>Gradient</td>
<td>DAD</td>
<td>ACN/water</td>
<td>5989-6358EN</td>
</tr>
<tr>
<td>33</td>
<td>Proteins</td>
<td>Wheat</td>
<td>Gradient</td>
<td>DAD</td>
<td>ACN/water</td>
<td>5989-6348EN</td>
</tr>
<tr>
<td>34</td>
<td>BSA (bovine serum albumin) digest, peptide</td>
<td></td>
<td>Binary gradient</td>
<td>DAD</td>
<td>ACN/water</td>
<td>5988-6081EN</td>
</tr>
<tr>
<td>35</td>
<td>AAA amino acid</td>
<td></td>
<td>Gradient</td>
<td>FLD/OPA-FMOC</td>
<td>ACN,MeOH, water, Na,HPO₄, NaOH</td>
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</tr>
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</table>

## Regulated/Hazardous Drug Substances

<table>
<thead>
<tr>
<th>Page</th>
<th>Major Analytes</th>
<th>Matrix</th>
<th>LC system</th>
<th>Detection</th>
<th>Mobile phase</th>
<th>Literature reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>36</td>
<td>Drugs</td>
<td>Water</td>
<td>1200SL</td>
<td>MSMS QQQ</td>
<td>ACN/water</td>
<td>5989-5319EN</td>
</tr>
<tr>
<td>37</td>
<td>Nitrofurans</td>
<td>Poultry, shrimp</td>
<td>Binary gradient</td>
<td>MSD Trap XCT</td>
<td>ACN/water</td>
<td>5989-0738EN</td>
</tr>
<tr>
<td>38</td>
<td>Fluoroquinolones</td>
<td>Beef kidney</td>
<td>Binary gradient</td>
<td>MSD posESI</td>
<td>ACN/water</td>
<td>5989-0596EN</td>
</tr>
<tr>
<td>39</td>
<td>Chloramphenicol</td>
<td>Shrimp, honey</td>
<td>Binary gradient</td>
<td>MSD and MSD Ion Trap negESI</td>
<td>MeOH/ACN, water AmmOAc</td>
<td>5988-9920EN</td>
</tr>
<tr>
<td>40</td>
<td>Sulfona drugs</td>
<td>Meat</td>
<td>Gradient</td>
<td>DAD</td>
<td>ACN/water</td>
<td>5988-7135EN</td>
</tr>
<tr>
<td>41</td>
<td>Sulfonamides</td>
<td>CapLC</td>
<td>DAD MSD posESI</td>
<td>ACN/water formic</td>
<td>Water acetic</td>
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</table>

## Regulated/Hazardous Miscellaneous Substances

<table>
<thead>
<tr>
<th>Page</th>
<th>Major Analytes</th>
<th>Matrix</th>
<th>LC system</th>
<th>Detection</th>
<th>Mobile phase</th>
<th>Literature reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>42</td>
<td>HMF hydroxymethylfurfural</td>
<td>Bread, cereal, yogurt</td>
<td>Binary gradient G1312A or G1312B</td>
<td>MSD posAPCI</td>
<td>Water acetic formic</td>
<td>5989-5403EN</td>
</tr>
<tr>
<td>43</td>
<td>Acrylamide</td>
<td>Drinking water</td>
<td>Dual binary w/6-port valve for autoSPE</td>
<td>MS TOF posESI</td>
<td>ACN/water formic</td>
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</tr>
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<td>44</td>
<td>Chromium speciation</td>
<td>Metrohm</td>
<td>Metrohm 818 pump, Agilent 7500 ISIS sampler</td>
<td>ICP-MS</td>
<td>water Na₂EDTA NaOH</td>
<td>5989-2481EN</td>
</tr>
<tr>
<td>45</td>
<td>Perchlorate</td>
<td>Water, vegetables</td>
<td>Metrohm IC</td>
<td>MSD negESI</td>
<td>MeOH/water 30 mm NaOH AmmHCO₃/ tartaric, methanol</td>
<td>5989-0816EN</td>
</tr>
<tr>
<td>46</td>
<td>Arsenobetaine</td>
<td>Fish</td>
<td>Isocratic G1310A</td>
<td>ICP-MS</td>
<td>ACN/water formic</td>
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</tr>
</tbody>
</table>

## Regulated/Hazardous Natural Toxin Substances

<table>
<thead>
<tr>
<th>Page</th>
<th>Major Analytes</th>
<th>Matrix</th>
<th>LC system</th>
<th>Detection</th>
<th>Mobile phase</th>
<th>Literature reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>47</td>
<td>DSP algal toxins</td>
<td>Shellfish</td>
<td>Quaternary gradient</td>
<td>MSD pos/negESI</td>
<td>MeOH/water formic</td>
<td>5989-2912EN</td>
</tr>
<tr>
<td>49</td>
<td>Mycotoxin, fumonisin</td>
<td>Corn</td>
<td>Binary gradient</td>
<td>MSD posESI</td>
<td>ACN/water</td>
<td>5968-2124E</td>
</tr>
<tr>
<td>50</td>
<td>Aflatoxins</td>
<td>Various</td>
<td>Agilent/Gerstel onlineSPE</td>
<td>MSD posESI</td>
<td>ACN/water</td>
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</tr>
</tbody>
</table>
## Food Quick Reference Guide (Continued)

### Regulated/Hazardous Pesticides/Herbicide Substances

<table>
<thead>
<tr>
<th>Page</th>
<th>Major Analytes</th>
<th>Matrix</th>
<th>LC system</th>
<th>Detection</th>
<th>Mobile phase</th>
<th>Literature reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>51</td>
<td>44 pesticides</td>
<td>Vegetables, fruit</td>
<td>Binary gradient</td>
<td>MSMS QQQ</td>
<td>ACN/water, amm. form.</td>
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</tr>
<tr>
<td>52</td>
<td>Acid herbicides</td>
<td>Water</td>
<td>AutoSPE/1200SL with dual binary pumps and 6-port valve</td>
<td>DAD</td>
<td>ACN/water H$_3$PO$_4$</td>
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</tr>
<tr>
<td>54</td>
<td>Postharvest fungicides</td>
<td>Citrus</td>
<td>Binary gradient</td>
<td>MS TOF, MSn Ion Trap both posESI</td>
<td>ACN/water formic</td>
<td>5989-2728EN</td>
</tr>
<tr>
<td>55</td>
<td>Chloronicotinyl insecticides</td>
<td>Vegetables, fruit</td>
<td>Binary gradient</td>
<td>MS TOF, MSn Ion Trap, both posESI</td>
<td>ACN/water formic</td>
<td>5989-1842EN</td>
</tr>
<tr>
<td>56</td>
<td>Phenylurea, triazine herbicides</td>
<td>Water</td>
<td>Dual binary w/ 6-port valve for autoSPE</td>
<td>MSD pos/negAPCI</td>
<td>MeOH/water formic</td>
<td>5989-0813EN</td>
</tr>
</tbody>
</table>

### Fat-Soluble Vitamins

<table>
<thead>
<tr>
<th>Page</th>
<th>Major Analytes</th>
<th>Matrix</th>
<th>LC system</th>
<th>Detection</th>
<th>Mobile phase</th>
<th>Literature reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>58</td>
<td>Retinol isomers</td>
<td>Isocratic</td>
<td>DAD</td>
<td>DAD</td>
<td>Dioxane, MeOH/water, MeOH/water, acetic, phosphate, TEA, hexane sulfonate</td>
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</tr>
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<td>59</td>
<td>Fat-soluble vitamins</td>
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<td>DAD</td>
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<td>MeOH/water</td>
<td>5988-6359EN</td>
</tr>
<tr>
<td>60</td>
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<td>Poultry feed</td>
<td>Binary gradient</td>
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<td>MeOH/water</td>
<td>5968-9408E</td>
</tr>
<tr>
<td>61</td>
<td>Fat-soluble vitamins A,D, E</td>
<td>Quaternary gradient</td>
<td>DAD</td>
<td>MeOH/water</td>
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</table>

### Water-Soluble Vitamins

<table>
<thead>
<tr>
<th>Page</th>
<th>Major Analytes</th>
<th>Matrix</th>
<th>LC system</th>
<th>Detection</th>
<th>Mobile phase</th>
<th>Literature reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>62</td>
<td>Water-soluble vitamins</td>
<td>Gradient</td>
<td>DAD</td>
<td></td>
<td>MeOH/water</td>
<td>5988-6365EN</td>
</tr>
<tr>
<td>63</td>
<td>Water-soluble vitamins</td>
<td>Cat food</td>
<td>Gradient</td>
<td>DAD</td>
<td>ACN/water, phosphate ACN/water, phosphate, TEA, hexane sulfonate</td>
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Basic Principles of Liquid Chromatography

Classical liquid chromatography (LC) was first used in 1903 by the Russian scientist Mikhail Tswett (1872-1919) to separate plant pigments. In his initial publications, Tswett called the new technique “chromatography” because the result of the analysis was “written in color” along the length of the adsorbent column.

Chromatography is a separation technique that places (or injects) a small amount of liquid sample into a tube, known as a column, that is packed with porous particles; this is called the stationary phase. The sample’s individual components are transported down the packed column by a liquid that is moved by gravity; this is called the mobile phase. The sample’s components are separated by the column packing through various chemical and/or physical interactions between their molecules and the packing particles and are moved through the column bed by the flowing solvent. The separated components are collected at the column exit and identified by an external measurement technique, such as spectrophotometry, which measures the intensity of the color, by gravimetric analysis, or by another technique that can measure the amount of each separated component. The modern form of column liquid chromatography is now referred to as “flash chromatography.”

HPLC is used for one of three reasons: qualitative analysis, quantitative analysis, and to prepare pure compounds. Qualitative analysis is used to identify the individual compounds in a sample. The most common parameter for identifying a compound is its retention time, the time it takes to elute from the column after injection. Depending on the detector used, identification may also be based on chemical structure, molecular weight, or some other molecular property.

The second reason for using HPLC is for quantitative analysis; that is, to determine what compounds are in a sample and to measure the amount (or concentration) of each one. There are two primary ways to interpret a chromatogram or perform quantitation. The first is to measure the height of a chromatographic peak from the baseline; the second is to determine the peak area. To quantitatively assess the compound, a sample or standard with a known amount of the compound of interest is injected under identical operating conditions, and its peak height or peak area is measured. This measurement is compared to the response of the same analyte in a subsequently analyzed sample mixture.

The final reason HPLC is used is to prepare a pure compound. A pure substance can be prepared for later use (for example, organic synthesis, identification, clinical studies, or toxicology studies) by collecting the chromatographic peaks at the detector’s exit and concentrating the compound (also called the analyte) by removing the solvent. This methodology is called preparative chromatography.

Instrumentation for HPLC

Although in principle LC and HPLC work the same way, the speed, efficiency, sensitivity, and ease of operation make HPLC vastly superior. The main components of an HPLC system are described below.
Pump
As part of the mobile phase, the HPLC’s pump forces a liquid through the liquid chromatograph at a specific flow rate, expressed in milliliters per minute (mL/min). Normal flow rates are between 0.2 and 2 mL/min; however, they can be lower for capillary and nanoseparations or higher for purification separations. Typical pumps can reach pressures between 6,000 and 9,000 psi (400 and 600 bar). During the chromatographic experiment, a pump can deliver a constant (isocratic) or dynamic (gradient) mobile phase composition.

Injector
The injector, which must withstand the system’s high pressures, introduces the liquid sample into the flow stream of the mobile phase without unduly interrupting or disturbing the system’s flow or pressure. Typical sample volumes are 1 to 20 microliters (µL). The injector must be able to withstand the system’s high pressures. An autosampler is an automatic injector for when there are many samples to analyze or when greater precision is required and manual injection is not practical.

Column
Considered the heart of the chromatograph, the column’s stationary phase separates the sample’s components of interest by using various physical or chemical parameters. The typically small particles (< 10-micron), densely packed inside the column, are what cause the separation of the sample’s components and the incidental high backpressure at normal flow rates. The pump must push hard to move the mobile phase through the column, and this resistance creates high pressure within the chromatograph.

Detector
The detector can see the individual molecules come out (elute) of the column, normally in dilute solution within the mobile phase. Modes of detection include UV/VIS, fluorescence; differential refractive index; evaporative light scattering; conductivity; electrochemistry in various forms, including oxidative or reductive measurements; and mass spectrometry. The detector measures the amount of the analyte and may provide orthogonal information (spectral data) relative to identification or confirmation so that the chemist can quantitatively analyze the sample’s components. The signal that the detector provides to a recorder or computer results in a liquid chromatogram.

Computer
Frequently called the data system, the computer usually controls all HPLC instrument’s system modules and acquires and stores the detector signal. In the final step, the computer processes the signal from the detector and uses it to determine the sample components’ elution time (qualitative analysis) and the sample’s amount (quantitative analysis).

HPLC Separation Modes
The four main types of columns offer separations based on a variety of analyte properties. The main examples or modes of separation are reversed phase; ion exchange; normal phase; and gel permeation, gel filtration, and size exclusion chromatography.

Reversed Phase (C18, ODS, C8, C4, CN, RP, and bonded phase, among others). Reversed phase separation is primarily used for compounds that are somewhat organic or aqueous soluble and that differ in solubility with respect to organic/aqueous mixtures.

This mode of separation is by far the most widely used technique in LC today, due to column durability, the compatibility of mobile phases with typical sample matrices, and the availability of versatile separation mechanisms. The columns used for reversed phase separation are usually packed with a porous silica-based material that is covalently bonded with linear alkyl chains like octyldimethylsilane (a C8 column) or octydecyldimethylsilane (a C18 column, or ODS).

Mobile phases are generally mixtures of water-miscible reagents and solvents. Methanol (MeOH), acetonitrile (ACN or MeCN), and tetrahydrofuran (THF) are the common organic solvents. Water, phosphate buffers, acetonitrile buffers, and ion pairing reagents (such as alkylsulfonic acids or quarternary alkyl ammonium compounds) are commonly used.

Halide salts or corrosive acids (for example, hydrochloric acids, perchloric acid, and even sulfuric acid) are often avoided because of possible corrosive effects on the instrument’s stainless steel parts. Proper care and use of the instrument allows almost all types of reagents to be used; however, the use of these corrosive reagents can create maintenance problems.

Ion Exchange (IEX, cation exchange [CX], anion exchange [AX]). Ion exchange separations are most commonly used for organic carboxylic or sulfonic acids, sugars, proteins, classical amino acid techniques using post-column derivatization, DNA/RNA-related and oligonucleotides, inorganic anions and cations, and some small organic amines.
These columns are made of a silica or cross-linked styrene-divinyl benzene or methacrylate polymer base material with a charged site (anionic or cationic) covalently grafted onto the surface. The solvents used with this mode are predominantly buffers with small amounts of organic solvent sometimes added for improved solubility or selectivity (that is, to improve separation). Ion exchange is often used in the gradient separation mode (complex mixtures especially DNA-related, proteins, amino acids).

Normal Phase (adsorption and silica). The normal phase is the least commonly used technique today, representing less than 5% of total applications worldwide. The base material for these columns is silica; it is usually not derivatized, except sometimes cyanopropyl or aminopropyl groups are added. Normal phase is used almost exclusively with non-polar solvents that are typically not water miscible. The preferred separations for this mode include preparative chromatography (because of easy solvent removal from fractions), some fat-soluble vitamins like vitamins A and E, some synthesis products, and some polymer additives.

Gel Permeation, Gel Filtration, and Size Exclusion Chromatography (GPC, GFC, and SEC, respectively). All of these modes imply separations based on the size of a molecule in a particular solution, in this case the mobile phase. Columns may be based on silica or polymeric materials, with or without the addition of covalently bound functional groups, and are generally larger and more expensive than other columns for analytical separations.

GPC is commonly used for synthetic or natural polymers and GFC is used for biomolecules like proteins or large peptides. SEC describes the mechanism of separation most accurately. SEC separations are generally easy, require little mobile phase development, and are inherently isocratic, for good detectability. These columns are from three to 10 times larger than for other separation modes, yet the separation takes place relatively quickly because the columns do not (or generally should not) interact chemically with the sample components; thus, the sample components travel at the same or faster speed than the mobile phase. Separation times are from 10 to 60 minutes, depending on the range of molecular sizes in the sample. (Molecular weight is an analogous term that can be used in most situations.)

Column Dimensions and Materials

A modern column for HPLC is normally constructed of a stainless steel tube with a highly polished interior and end caps with integral or removable porous frits. The frit porosity is designed to allow solvent to flow through while preventing particles from entering and packing material particles from escaping. PEEK, TEFLON™, other inert polymers, glass, and other inert materials have also been used. With proper handling and reasonable attention to chemical compatibility and sample preparation, columns often deliver thousands of usable injections before their performance begins to fail.

The column dimensions and particle size of the packing material are as important as the separation mode. Typical analytical columns vary from about 1.0 to 6.0 mm internal diameter (id) and from as short as 10 to 300 mm long.

Small-diameter columns consume small volumes of solvent, may allow enhanced detection sensitivity, and operate at flow rates that are compatible with mass spectrometers (MS) and evaporative light-scattering detectors (ELSD). The small bed volume, however, makes the column susceptible to resolution losses due to extracolumn dispersion or band spreading. Special tubing, fittings, and flow cells may need to be used to minimize the extracolumn volume, maximizing the usable efficiency, which is always lower than the column’s theoretical efficiency.

In contrast, larger diameter columns operate at relatively high flow rates (1.5 to 3 mL/min, typically), have a relatively large bed volume, and consume significantly more mobile phase. Flow splitting may be required when these columns are used with MS- or ELSD-type detectors. Large-diameter columns enable larger injection volumes and sample capacity. It also may be possible to use these analytical size columns for low milligram scale purification. The larger bed volume yields comparatively small extracolumn dispersion effects.

In addition, very short columns make extremely fast separations possible, although lower efficiency (that is, resolving power) will be observed. Larger diameter long columns deliver the highest resolution but require more time and solvent to perform the same separations. For further discussion about converting methods from one column dimension to another, with appropriate adjustments in flow rate, gradient, or run times, see “Method Translation” below.

Particle size is another important physical variable that should be considered carefully. We have long known that smaller particle sizes and narrower particle size distributions allow higher efficiency, which, in turn, contribute to greater resolution in the separation. The increase in efficiency is inversely proportional to the particle size change, so halving the particle size doubles the efficiency, which increases actual resolution by about 1.4 (the square root of 2, per the standard resolution equation). Operating pressure, though, shows inverse but exponential increases; thus, halving the particle size is theoretically expected to increase pressure by 4 times. For these reasons, we try to
balance the resolution requirements against the negative impact that increased pressure may have on system reliability, column lifetime, and possibly increased analysis time in cases where maximum pressures are reached and desired operating flow rates must be reduced.

Concepts of the Rapid Resolution Systems and Methods

The Agilent 1200 Series Rapid Resolution LC concept has been developed to allow increased speed and resolution of chromatographic analyses while keeping system pressure at a minimum. The system provides faster analyses and higher resolution than conventional LC, which beneficially allows higher sample throughput and higher data quality. It optimizes performance while minimizing the risks that ultra-high pressure might impose on instrument reliability and longevity.

In general, shorter analyses and increased resolution and sensitivity may be reached by optimizing the column, column thermostating, and gradient delay volume and by reducing the extracolumn dispersion volume. These steps ensure the best possible system performance for high-speed and high-resolution separations while offering the extra benefits of solvent reduction and increased sensitivity. The user may enjoy a substantial improvement in the overall chromatographic process, especially if higher operating pressures are available and compatible with the column packing materials.

A new high-performance pump with flow rates from 0.05 to 5 mL/min and up to 600 bar pressure was developed for the new Agilent system. The system also features a high-performance degasser, a 600 bar low-dispersion autosampler, and new UV and MS detectors. It can be optimized for highest speed and resolution in both LC/UV and LC/MS applications and can run any traditional LC method, making the new Agilent system very flexible. Flow rates from 0.05 to 5 mL/min ensure flexibility from semi-micro to semi-preparative operation on the single platform. It accommodates all HPLC and STM-LC operational modes on one system and facilitates the use of existing and newly developed HPLC methods without the need for revalidation or extensive reconfiguration.

Optimizing the Instrument Setup for Different Column IDs

When performing high-throughput sample analyses, the major focus is on having short run times. The usual way to do this is to use very short columns to achieve high-column-volume-per-minute flow rates. In the flexible RRLC system concept, one can choose 2.1-mm, 3.0-mm, or 4.6-mm inside diameter (id) columns, with slightly different instrument configurations recommended for each. It is generally important to have the lowest possible extracolumn volume when using 2.1 mm id STM columns.

When compared to conventional system configurations using 4.6 mm, 5-um columns, the major difference is from the autosampler onward—the point where the sample enters the flow path and is subject to peak dispersion. The first step is to change from 0.17-mm id capillaries to smaller 0.12-mm id capillaries, a 50% reduction in extracolumn volume. Further means of reducing the extracolumn volume include a specially designed low-dispersion heat-exchanger in the thermostatted column compartment and small-volume flow cells with a specially designed inlet and outlet flow path for improved flush-out behavior. In general, all capillaries are kept as short as possible, and the use of connecting unions is minimized.

It has often been said that the column is “the heart of the system.” Indeed, proper column selection, based on a range of user requirements, is a critical step in the method-development or method-improvement process. The variable parameters are length, id, and particle size. Increased length or decreased particle size will increase the resolving power of
the column. Increasing the column length will result in a proportional increase in the operating pressure, solvent consumption, and analysis time. Reducing the particle size will result in an exponential increase in the pressure with minimal effect on solvent consumption or analysis time, providing that the pressure requirement does not exceed the system maximum or user preference. Decreasing the column id is a common approach to reducing solvent consumption with minimal effect on resolution and analysis time. However, efficiency may diminish slightly due to extracolumn effects, and care must be taken to minimize this detrimental parameter.

The 2.1-mm id column is very popular in LC/MS methods because the typical flow rate is ideal for the most popular ionization sources. The 3-mm id column offers a balance between the more demanding system requirements of a 2.1 mm id column and the high solvent consumption of a 4.6 mm id column. The nearly twofold increase in bed volume, over 2.1 mm columns, allows larger volume UV flow cells with longer paths to be used without loss of resolution, which can improve sensitivity. Depending on the ionization source and flow rate, a flow split before an MS detector might be required.

The most commonly used columns are the 4.6-mm id column. They typically have the most available stationary phases, tolerate extracolumn dispersion reasonably well, and allow flow cells with long paths to be used, often giving the best sensitivity on a purely signal-to-noise basis. However, 4.6 mm id columns have the highest solvent consumption per analysis. In reality, the smaller volume columns will invariably give higher sensitivity when the same sample mass is injected under comparable analysis conditions.

### Gradient Delay Volume

The Agilent binary pump SL can be optimized to favor lowest delay volume or maximum solvent mixing performance. Two flow paths are available, and only two fittings need to be moved. If the pump’s internal volume is quite large, the time until the gradient reaches the column and makes the compounds move along the stationary phase will be long. When operating with very small column volumes, it will require longer run times to compensate for this delay. For larger columns or long, shallow gradients (typical of peptide and other macromolecule separations), this is not a critical parameter. In general, one should consider how many column volumes of delay, not absolute volume, will be present and compare it proportionately to the total number of column volumes in the gradient analysis.

Like all Agilent samplers, the Agilent 1200 high-performance ALS sampler SL is also designed to allow a bypass mode (advanced delay volume reduction) to be assumed after the sample aliquot is completely beyond the injector plumbing. This gives the user more flexibility in controlling the small but sometimes significant delay volume associated with sending the gradient through the sample loop.

### Summary

System flexibility is typically ranked as highly as system performance and reliability. The RRLC system was developed with all of these parameters in mind, to offer dynamic performance without compromising precision, resolution, or speed. Using the available tools for method design and conversion (see other sections in this solutions guide and at www.agilent.com/chem), the user can preemptively design a method around a particular column and then preselect the optimum system configuration to ensure the best possible overall performance. Users with a wide variety of liquid chromatographic tasks can select one or more RRLC systems to operate a wide range of methods without worrying about the system limitations inherent in traditional general purpose instruments or narrowly focused specialty systems.

![Flow diagram](image)

**Figure 2.** How to change between standard and low delay volume configuration of the binary pump SL.

### Method Translation

It is sometimes advantageous or necessary to change a separation’s overall scale when adapting an existing method for a new purpose. This might include increasing the mass capacity (scaling an analytical separation for purification), increasing the sensitivity (reducing the column size to improve detectability by increasing the average peak concentration eluting to the detector), or increasing throughput. In every case, following simple mathematical guidelines will ensure that the method is scaled appropriately and will deliver the required capacity, sensitivity, resolution, and throughput according to your requirements.

Analysis methods developed on older columns packed with large 5- or 10-µm particles are often good candidates for modernization by simply replacing these large columns with smaller ones packed with smaller particle sizes. This can reduce analysis time and solvent consumption, improve sensitivity, and enable greater compatibility with mass spectrometer ionization sources.
Simplistically, a 250-mm long column that contains 5-µm particles can be replaced by a 150-mm long column packed with 3-µm particles. If the ratio of length (L) to particle size (dp) is equal, the two columns are considered to have equal resolving power. Solvent consumption is reduced by L1/L2 so a 250 mm column length separation converted to a 150 mm length results in about a 1.6-fold reduction in solvent usage per analysis. If an equal mass of analyte can then be successfully injected, the sensitivity should also increase by 1.6-fold due to reduced dilution of the peak as it travels through a smaller column of equal efficiency.

Liquid chromatography/mass spectrometry ionization sources, especially the electrospray ionization mode, have demonstrated greater sensitivity at lower flow rates than typically used in normal liquid chromatography/ultraviolet (LC/UV) optical detection methods, so it may also be advantageous to reduce the internal diameter of a column to allow timely analysis at lower flow rates. The relationship of flow rate between different column diameters is shown in Equation 1.

\[
\text{Flow}_{\text{col. } 1} \times \left( \frac{\text{Diam}_{\text{column } 2}}{\text{Diam}_{\text{column } 1}} \right)^2 = \text{Flow}_{\text{col. } 2} \quad (\text{eq. 1})
\]

The combined effect of reduced column length and diameter contributes to a reduction in solvent consumption and, again assuming the same analyte mass can be injected into the smaller column, a proportional increase in peak response. The injection mass is normally scaled to the size of the column, though, and a proportional injection volume would be calculated from the ratio of the void volumes of the two columns multiplied by the injection volume on the original column (see Equation 2).

\[
\text{Inj. vol}_{\text{col. } 1} \times \left( \frac{\text{Volume}_{\text{column } 2}}{\text{Volume}_{\text{column } 1}} \right) = \text{Inj. vol}_{\text{col. } 2} \quad (\text{eq. 2})
\]

For isocratic separations, the above conditions will normally result in a successful conversion of the method with little or no change in overall resolution. Several other parameters can be considered to improve the method conversion’s outcome. The first parameter is the column efficiency relative to flow rate, or, more correctly, efficiency relative to linear velocity, as commonly defined by van Deemter [1] and others. The second parameter is the often-overlooked effect of extracolumn dispersion on the column’s observed or empirical efficiency.

Although Van Deemter observed and mathematically expressed the relationship of column efficiency to a variety of parameters, we are most interested in his observation that in a well-packed HPLC column there is an optimum linear velocity for any given particle size and that the optimum linear velocity increases as the particle size decreases. The practical application is that a reduction in particle size, as discussed earlier, can often be further optimized by increasing the linear velocity, resulting in a further reduction in analysis time. This increased elution speed will decrease absolute peak width and may require an increase in data acquisition rates and reduction in signal filtering parameters to ensure that the chromatographic separation is accurately recorded in the acquisition data file.

The second important consideration is the often-overlooked effect of extracolumn dispersion on the observed or empirical efficiency of the column. As column volume is reduced, peak elution volumes are proportionately reduced. If smaller particle sizes are also used, there is a further reduction in the expected peak volume. The liquid chromatograph, and particularly the areas where the analytes will traverse, is a collection of various connecting capillaries and fittings that will cause a measurable amount of band spreading. From the injector to the detector flow cell, the cumulative dispersion that occurs degrades the column performance and results in observed efficiencies that can be far below the values that would be estimated by purely theoretical means. It is fairly typical to see a measured dispersion of 20 to 100 µL in an HPLC system. This has a disproportionate effect on the smallest columns and smallest particle sizes, both of which are expected to yield the smallest possible peak volumes. Care must be taken to minimize the extracolumn volume and, where practical, reduce the number of connecting fittings and the volume of injection valves and detector flow cells.

For gradient elution separations, where the mobile phase composition increases through the initial part of the analysis until the analytes of interest have been eluted from the column, successful method conversion to smaller columns requires that the gradient slope be preserved. While many publications have referred to gradient slope in terms of percent change per minute, it is more useful to express it as percent change per column volume. In this way, the change in column volume during method conversion can be used to accurately render the new gradient condition. If we think of each line of a gradient table as a segment, we can express the gradient by the following equation:

\[
\% \text{ Gradient slope} = \frac{(\text{End} \% - \text{Start} \%)}{\# \text{Column volumes}} \quad (\text{eq. 3})
\]

Note that using percent change per column volume rather than percent change per minute enables users to control gradient slope by altering gradient time and/or gradient flow rate. A large value for gradient slope yields very fast
gradients with minimal resolution, while lower gradient slopes produce higher resolution at the expense of increased solvent consumption and somewhat reduced sensitivity. Longer analysis time may also result unless the gradient slope is reduced by increasing the flow rate (within acceptable operating pressure ranges) rather than by increasing the gradient time. Resolution increases with shallow gradients because the effective capacity factor, $k^*$, is increased. Much like in isocratic separations, where the capacity term is called $k'$, a higher value directly increases resolution. The effect is quite dramatic up to a $k$ value of about 5 to 10, after which little improvement is observed. In the subsequent examples, we will see the results associated with the calculations discussed above.

Careful analysis of the existing gradient conditions, coupled with an awareness of the need to accurately calculate new flow and gradient conditions can lead to an easy and reliable conversion of existing methods to new faster or higher resolution conditions. In addition, awareness of extracolumn dispersion, especially with small- and high-resolution columns, will ensure good column efficiency, which is critical to a successful translation of the method.

Further reading can be found in application notes 5989-2908EN, 5989-4721EN, 5989-5176EN, 5989-5177EN, 5989-5178EN, RRLC system brochure 5989-4330EN, and 5989-5200EN.

Proper sample preparation is a key component of successful HPLC analysis. From techniques as simple as dissolution or dilution to complex, multistep matrix interference-removing procedures, the choices are abundant and diverse. The key goal of good sample preparation is to prepare the sample in an injectable form that is compatible with the operating conditions of the intended analysis. Miscibility and pH compatibility are primary elements at this step. Further goals include removing unwanted matrix components that may complicate or lengthen the analysis or reduce the useful life of the separation column or other system components.

### Filtration

Sample filtration is the most fundamental procedure, protecting the instrument and column from insoluble particulate materials. Selective precipitation of some matrix components, followed by filtration or centrifugation, may also be advised if some matrix components might precipitate on contact with the mobile phase or adsorb strongly or irreversibly to the column packing material. (add pub note describing available products here)

### Extraction

Liquid/liquid extraction techniques, with or without pH modification of the aqueous phase, have long been used to selectively extract general classes of compounds based on
solubility. While this is effective for a limited number of samples, it may be cumbersome and too labor-intensive for processing large numbers of samples unless appropriate automation methods (such as automatic pipetting and shaking) are applied in the scale-up phase. Depending on the matrix complexity, these extraction techniques may be ineffective at removing critical interferences and may be subject to troublesome emulsion formation that limits speed and reduces recovery and cleanup efficiency.

SPE, solid/liquid extraction, may be a convenient way to more extensively fractionate a sample mixture. It is a simple and generally small form of open-column liquid chromatography. By using one or more of the chromatographic processes-reversed phase, adsorption/normal phase, or ion exchange—it may be possible to process individual samples manually or use automation for offline processing of larger numbers of samples. (add pub note(s)/links for applications and products here)

http://www.chem.agilent.com/ecommerce/product/p2_cas_main.aspx?
or

Typical SPE formats include syringe barrels of various sizes, low profile SPE devices resembling membrane filtration cartridges, simple polymeric cartridges and mini-cartridges that may be attached to disposable syringes, and 96 well microtiter plate compatible devices.

In some cases, it is possible to move the SPE procedure directly to the liquid chromatograph as an integral part of the analysis. When the sample matrix is not highly loaded with particulates or dissolved matrix components, online SPE techniques usually use reusable media packed in guard column hardware. The fractionation is controlled through a switching valve and additional pump to deliver sample preparation and SPE column regeneration reagents independently of the main analysis hardware and column. This has been widely demonstrated for trace enrichment associated with drinking water analysis and has also been applied to more complex matrices where total mass loading is appropriate for online sample processing. For more information, access publications 5989-0813EN and 5988-9917EN.

**Gel Permeation Chromatography (GPC)**

GPC has also been used as an effective high-performance sample cleanup technique. Because the separation mechanism is one of size rather than chemistry, it is a useful way to remove lipids, proteins, and other larger molecules from samples containing broad classes of small molecule analytes. Nowhere has this been more frequently used than in the analysis of various molecule classes from environmental samples, especially sludge, soil, and other solid matrices, which are first thoroughly extracted to obtain the soluble analytes and a variety of larger molecules inherent in the matrix. Additional discussion is found in application notes 5989-5401EN and 5989-0181EN.

**Ideal Sample Preparation**

The ideal sample preparation removes unwanted or interfering matrix components, shortens and simplifies the analysis, reduces overall sample analysis time, lowers the per-sample cost, extends column life, and improves the overall performance of the analysis to which the purified sample is finally subjected. A wide selection of published techniques from peer-reviewed journals and suppliers of sample preparation products is available to help you adapt existing techniques and materials to your specific sample preparation requirements.

An appropriate combination of HPLC components, a suitable column chemistry and mobile phase chemistry, and effective sample preparation are the keys to developing and using a robust and reliable analysis or purification method that can deliver good results time after time and with a variety of operators at the controls. To view the various hardware and chemical products and references available from Agilent Technologies, refer to our general product catalog or visit us online at www.agilent.com/chem. Once registered, you can shop for chromatographic components, consumables, and supplies; download technical references; review FAQs; or contact us for further technical information.

Credits for material used here: Ron Majors and Mike Woodman, Agilent Technologies
The table below lists Agilent Technologies applications for the food industry. In this guide, the page number where you can review the most current application overview is listed after the publication number. The complete publication for all application overviews listed below can be viewed on the Agilent Web site, www.agilent.com/chem.

### Carbohydrates, Sugars, Sugar Alcohols

<table>
<thead>
<tr>
<th>Major Analytes</th>
<th>Matrix</th>
<th>LC System</th>
<th>Detection</th>
<th>Column(s)</th>
<th>Col. P/N</th>
<th>Mobile Phase</th>
<th>Notes</th>
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<tbody>
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<td>Carbohydrates</td>
<td>Isocratic</td>
<td>RID</td>
<td>ZORBAX NH₂</td>
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<td>ACN/water</td>
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<td>Beverage</td>
<td>Isocratic</td>
<td>MSD negAPCI</td>
<td>Asahipak NH₂-50 2D 2 mm × 150 mm</td>
<td>Substitute 843300-908</td>
<td>ACN/water</td>
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</tr>
<tr>
<td>Dextran</td>
<td>Isocratic</td>
<td>RID</td>
<td>PL aquagel-OH MXA</td>
<td>7.5 mm × 30 mm, 8 µm PL aquagel-OH 30A, 7.5 mm × 30 mm, 8 µm</td>
<td>79911GF-MXA and 79911GF-083</td>
<td>Water</td>
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<tr>
<td>Starch</td>
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<td>RID</td>
<td>Aq. GPC PSS Suprema</td>
<td>100 + 1000, 2 of 8 mm × 300 mm, 10 µm</td>
<td>See app. note 0.1M NaNO₃</td>
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### Dyes, Colorants, Pigments

<table>
<thead>
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</tr>
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<tbody>
<tr>
<td>5968-5144EN</td>
</tr>
<tr>
<td>5969-9797</td>
</tr>
</tbody>
</table>

### Application Reference Index

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## Dyes, Colorants, Pigments (Continued)

<table>
<thead>
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<th>Major Analytes</th>
<th>Matrix</th>
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<th>Col. P/N</th>
<th>Mobile Phase</th>
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</thead>
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<td>Dyes</td>
<td>DAD</td>
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## Fats and Oils

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<th>Matrix</th>
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<th>Column(s)</th>
<th>Col. P/N</th>
<th>Mobile Phase</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>5989-2848EN</strong> page 14</td>
<td>Phospholipids</td>
<td>Soybean</td>
<td>Prep/35900E</td>
<td>Elsd (ESA), some MSD</td>
<td>Prep Sil</td>
<td>MeOH, IPA, hexane, salt, water, CHCl₃</td>
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</tr>
<tr>
<td><strong>5988-4235EN</strong> page 15</td>
<td>Triglycerides</td>
<td>Isocratic</td>
<td>MSD posAPCI</td>
<td>Develosil ODS DG-3</td>
<td>4.6 mm × 75 mm</td>
<td>Acetone/water</td>
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<td><strong>5968-0878E</strong> page 16</td>
<td>Triglycerides</td>
<td>Edible oil</td>
<td>Bin. grad</td>
<td>MSD posAPCI</td>
<td>Hypersil MOS</td>
<td>IPA, n-ButOH, water, amm. formate</td>
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</tr>
<tr>
<td><strong>5966-0744E</strong> page 17</td>
<td>Triglycerides</td>
<td>Edible oil</td>
<td>Quat. grad</td>
<td>DAD 3 channel</td>
<td>Hypersil MOS</td>
<td>ACN/MTBE/water</td>
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</tr>
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<td><strong>5966-0635E</strong></td>
<td>Saponified to fatty acids</td>
<td>Food</td>
<td>DAD, derivatized</td>
<td>Hypersil MOS</td>
<td>2.1 mm × 200 mm, 5 µm</td>
<td>ACN, THF, water</td>
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<td><strong>5966-0634E</strong></td>
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<td>Rid</td>
<td>Hypersil MOS</td>
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<td><strong>5954-6269E obsolete</strong></td>
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<td>Old HP</td>
<td>DAD</td>
<td>Hypersil MOS</td>
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## Flavors, Sweeteners, Organic Acids

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<th>Matrix</th>
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<th>Detection</th>
<th>Column(s)</th>
<th>Col. P/N</th>
<th>Mobile Phase</th>
<th>Notes</th>
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</thead>
<tbody>
<tr>
<td><strong>5989-5178EN</strong> page 18</td>
<td>Flavor, sweetener, preservative</td>
<td>Soft drinks</td>
<td>1200SL</td>
<td>DAD</td>
<td>ZORBAX SB-C18</td>
<td>ACN/water</td>
<td>Amm, HPO₄</td>
</tr>
<tr>
<td><strong>5989-1265EN</strong> page 19</td>
<td>Organic acids</td>
<td>Foods</td>
<td>Quat. grad</td>
<td>DAD</td>
<td>ZORBAX SB-Aq</td>
<td>ACN/water</td>
<td>20 mM phosphate</td>
</tr>
<tr>
<td><strong>5988-6353EN</strong> page 20</td>
<td>Flavoring agents</td>
<td>Mouthwash</td>
<td>Bin. grad</td>
<td>DAD</td>
<td>ZORBAX SB-Phenyl</td>
<td>ACN/water</td>
<td>TFA</td>
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<tr>
<td><strong>5988-6352EN</strong> page 21</td>
<td>Semivolatilie flavors</td>
<td>Isocratic</td>
<td>DAD</td>
<td>ZORBAX XDB-Phenyl</td>
<td>963967-912</td>
<td>MeOH/water</td>
<td></td>
</tr>
<tr>
<td><strong>5988-6349EN</strong> page 22</td>
<td>Aspartame, degradants</td>
<td>Cola</td>
<td>Isocratic</td>
<td>DAD</td>
<td>ZORBAX SB-C18</td>
<td>ACN/water</td>
<td>TFA</td>
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### Flavors, Sweeteners, Organic acids (Continued)

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<th>Major Analytes</th>
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<th>Detection</th>
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<td>NaOAc</td>
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<td>Bitter naringenin, hesperidin</td>
<td>Citrus</td>
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<td>Hypersil BDS 4 mm × 125 mm, 5 µm</td>
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<td>H₂SO₄</td>
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<td>H₂SO₄</td>
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<td>DAD</td>
<td>BioRad HPX-87</td>
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### Herbal Supplements, Natural Products, Plant Hormones

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<th>Major Analytes</th>
<th>Matrix</th>
<th>LC System</th>
<th>Detection</th>
<th>Column(s)</th>
<th>Col. P/N</th>
<th>Mobile Phase</th>
<th>Notes</th>
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<td>Xanthine metabolites</td>
<td>Isocratic</td>
<td>DAD</td>
<td>Eclipse Plus C18 4.6 mm × 150 mm, 5 µm</td>
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<td>25 mm phosphate</td>
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<td>Glycyrrhizin</td>
<td>Licorice root</td>
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<td>DAD</td>
<td>ZORBAX SB-C18 RRHT, 4.6 mm × 150 mm, 1.8 µm</td>
<td>ACN/water</td>
<td>acetic</td>
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<tr>
<td>Xanthines</td>
<td>Tea, chocolate</td>
<td>1200SL</td>
<td>DAD 2 µL cell</td>
<td>RRHT 1.8 µm various</td>
<td>Various</td>
<td>ACN/water</td>
<td>formic</td>
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<tr>
<td>Ginsenosides Root</td>
<td>1200SL</td>
<td>MS TOF posESI</td>
<td>SB-C18 RRHT, 2.1 mm × 150 mm, 1.8 µm</td>
<td>ACN/water</td>
<td>TFA</td>
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<td>Anthocyanins</td>
<td>Blueberry</td>
<td>Prep</td>
<td>DAD</td>
<td>Prep-C18, 21.2 mm × 250 mm, 10 µm 4.6 mm × 250 mm, 5 µm</td>
<td>410910-102</td>
<td>MeOH/water</td>
<td>TFA</td>
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<td>Anthocyanins</td>
<td>Gradient</td>
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<td>ZORBAX SB-C18 4.6 mm, 5 µm, and 3.5 µm</td>
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<td>MeOH/water</td>
<td>phosphoric acid</td>
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<td>Flavonoids, catechins</td>
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<td>ZORBAX SB-C8 4.6 mm × 150 mm, 3.5 µm</td>
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<td>TFA</td>
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<td>Ginsenosides</td>
<td>Ginseng</td>
<td>1200SL</td>
<td>MS Ion Trap, TOF both posESI, and DAD</td>
<td>SB-C18 2.1 mm × 150 mm, 1.8 µm</td>
<td>820700-902</td>
<td>ACN/water</td>
<td>TFA</td>
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<td>MS Ion Trap posESI</td>
<td>SB-C18 2.1 mm × 150 mm, 1.8 µm</td>
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<td>ACN/water</td>
<td>TFA</td>
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<td>MSn posESI</td>
<td>SB-Aq 2.1 mm × 50 mm, 3.5 µm</td>
<td>871700-914</td>
<td>Water formic</td>
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<td>Ergot alkaloids</td>
<td>Fungal extract</td>
<td>Nano LC, semiprep LC</td>
<td>MSn Ion Trap posESI</td>
<td>Nano, unspecified</td>
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**80**
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<th>Detection</th>
<th>Column(s)</th>
<th>Col. P/N</th>
<th>Mobile Phase</th>
<th>Notes</th>
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<tr>
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<td>Goldenseal</td>
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<td>ACN/water TFA</td>
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<td>Formononetin</td>
<td>Red clover</td>
<td>Quat. grad</td>
<td>DAD/FLD</td>
<td>SB-C18</td>
<td>3 mm × 150 mm, 5 µm</td>
<td>883975-302</td>
<td>ACN/water HOAc</td>
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<td>Quat. grad</td>
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<td>Rhubarb</td>
<td>Prep</td>
<td>DAD</td>
<td>SB-C18</td>
<td>3 mm × 150 mm, 5 µm, 21.2 mm × 150 mm, 5 µm</td>
<td>883975-302</td>
<td>ACN/water HOAc</td>
</tr>
<tr>
<td>Ephedrines, alkaloids berberine, tetrandrine, fangchinoline, chlorogenic acid, etc.</td>
<td>Mahuang, mahonia, others</td>
<td>CE</td>
<td>CEMS</td>
<td>75 µm fused silica capillary</td>
<td>Various</td>
<td>Borate</td>
<td></td>
</tr>
<tr>
<td>Ginsenosides, saponins</td>
<td>Ginseng</td>
<td>MSn Ion Trap posESI</td>
<td>SB-C18</td>
<td>2.1 mm × 50 mm, 3.5 µm</td>
<td>863954-302</td>
<td>ACN/water acetic</td>
<td>5</td>
</tr>
<tr>
<td>Rhein, emodin laxative</td>
<td>Rhubarb</td>
<td>DAD</td>
<td>SB-C18</td>
<td>3 mm × 150 mm, 5 µm, 9.4 mm × 150 mm, 5 µm</td>
<td>883975-302</td>
<td>846975-202</td>
<td>ACN/water AmmOAc, H₂SO₄</td>
</tr>
<tr>
<td>Atropine hyoscyamine</td>
<td>Atropa Belladonna</td>
<td>DAD</td>
<td>SB-C8</td>
<td>4.6 mm × 75 mm, 3.5 µm</td>
<td>866953-906</td>
<td>ACN/water KH₂PO₄</td>
<td></td>
</tr>
<tr>
<td>Quinine quinidine</td>
<td>Cinchona (Cortex Cinchonae) bark</td>
<td>DAD</td>
<td>Purospher RP18</td>
<td>4 mm × 125 mm, 5 µm</td>
<td>ACN/water KH₂PO₄</td>
<td></td>
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</tr>
<tr>
<td>Quercetin, kaempferol, isorhamnetin</td>
<td>Gingko (Gingko biloba)</td>
<td>DAD</td>
<td>Hypersil ODS</td>
<td>4 mm × 125 mm, 5 µm</td>
<td>MeOH/water H₃PO₄</td>
<td></td>
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</tr>
<tr>
<td>Rhein, emodin</td>
<td>Rhubarb (Rheum palmatum)</td>
<td>DAD</td>
<td>Hypersil ODS</td>
<td>4 mm × 125 mm, 5 µm</td>
<td>ACN/water AmmOAc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ephedrine norephedrine stimulant</td>
<td>Ma Huang, Ephedra sinica stapf</td>
<td>DAD</td>
<td>SB-C8</td>
<td>4.6 mm × 75 mm, 3.5 µm</td>
<td>866953-906</td>
<td>ACN/water KH₂PO₄</td>
<td></td>
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### Herbal Supplements, Natural Products, Plant Hormones (Continued)

<table>
<thead>
<tr>
<th>Major Analytes</th>
<th>Matrix</th>
<th>LC System</th>
<th>Detection</th>
<th>Column(s)</th>
<th>Col. P/N</th>
<th>Mobile Phase</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>5966-2882E</strong></td>
<td>Protocatechuic acid, aldehyde, various tanshinones</td>
<td>Dan Shen extr.</td>
<td>DAD</td>
<td>SB-C8 4.6 mm × 75 mm, 3.5 µm</td>
<td>866953-906</td>
<td>ACN/water KH₂PO₄</td>
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<tr>
<td><strong>5966-2591EN</strong></td>
<td>Plant hormones</td>
<td>DAD</td>
<td>SB-C8 4.6 mm × 75 mm, 3.5 µm</td>
<td>866953-906</td>
<td>ACN/water TFA</td>
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<td></td>
</tr>
<tr>
<td><strong>5965-9802E</strong></td>
<td>Catechins</td>
<td>ECD</td>
<td>Hypersil BDS 4 mm × 250 mm, 5 µm cartridge column</td>
<td>Recommend XDB-C18 or SB-C18 chemistry</td>
<td>MeOH/water, NaNO₃, H₂SO₄</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Preservatives

| 5989-5178EN page 30     | Flavors, sweeteners, preservatives | Soft drinks 1200SL | DAD | ZORBAX SB-C18 4.6 mm × 250 mm, 5 µm, 3 mm × 50 mm, 1.8 µm (600 bar) | 827975-302 (1.8 µm) and 880975-902 (5 µm) | ACN/water AmmHPO₄ |                                            |
| 5989-3635EN page 31     | Paraben, phenoxyethanol             | Gradient DAD | ZORBAX XDB-C18 4.6 mm × 150 mm, 3.5 µm | 963967-902 | MeOH/water        |                                            |
| 5988-6366EN             | Paraben Food                         | DAD | SB-C18 4.6 mm × 30 mm, 3.5 µm cartridge | 833975-902 | ACN/water H₃PO₄  |                                            |
| 5986-0629E              | Preservative Wine dressing           | DAD | Hypersil BDS 4 mm × 125 mm, 5 µm | ACN/water H₂SO₄ |                                            |
| 5966-0628E              | Antioxidants Gum                     | DAD | Hypersil BDS 4 mm × 100 mm, 3 µm | ACN/water H₂SO₄ |                                            |

### Proteins, Peptides, Amino Acids

| 5988-6358EN page 32     | Proteins Wheat Gradient DAD | ZORBAX 300 SB-CN, 300 SB-C8 | 883995-906, 883995-905 | ACN/water TFA | 6                          |
| 5988-6348EN page 33     | Proteins Wheat Gradient DAD | ZORBAX 300 SB-C8 4.6 mm × 150 mm, 5 µm | 883995-906 | ACN/water TFA | 7                          |
| 5988-6081EN page 34     | BSA (bovine serum albumin) digest, peptide Bin. grad DAD | Poroshell 300 SB-C18 2.1 mm × 75 mm, 5 µm ZORBAX 300 SB-C18 2.1 mm × 150 mm, 5 µm | 660750-902, 883750-902 | ACN/water TFA | Fast                      |
| 5980-1193EN page 35     | AAA amino acid Gradient FLD / OPA-FMOC | Eclipse-AAA 4.6 mm × 75 mm, 3.5 µm 4.6 mm × 150 mm, 3.5 µm 3 mm × 150 mm, 3.5 µm 4.6 mm × 150 mm, 5 µm | See app. note | ACN, MeOH, water Na₂HPO₄, NaOH | 8  |
### Proteins, Peptides, Amino Acids (Continued)

<table>
<thead>
<tr>
<th>Major Analytes</th>
<th>Matrix</th>
<th>LC System</th>
<th>Detection</th>
<th>Column(s)</th>
<th>Col. P/N</th>
<th>Mobile Phase</th>
<th>Notes</th>
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<tr>
<td>5989-0015EN</td>
<td></td>
<td></td>
<td>DAD</td>
<td>300 SB-x, Poroshell SB-x</td>
<td>Various</td>
<td>ACN/water TFA</td>
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<tr>
<td>5988-7930EN</td>
<td></td>
<td></td>
<td>MSn Ion Trap AP/MALDI</td>
<td>MALDI</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>5988-0897EN</td>
<td></td>
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<td>MSn Ion Trap with library, DAD</td>
<td>300 SB-C18, 0.3 mm × 150 mm, 5 µm</td>
<td>5064-8291</td>
<td>ACN/water formic</td>
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<tr>
<td>5980-2155</td>
<td></td>
<td></td>
<td>CapLC</td>
<td>MSn Ion Trap posESI</td>
<td>180 µm 300A C18</td>
<td>Unknown</td>
<td>ACN/water formic</td>
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<tr>
<td>5986-0746</td>
<td></td>
<td></td>
<td>Beer</td>
<td>FLD OPA/ FMOC</td>
<td>Hypersil DDS, 2.1 mm × 200 mm, 5 µm</td>
<td>ACN, THF, water, NaOAc</td>
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### Regulated/Hazardous Drug Substances

<table>
<thead>
<tr>
<th>5989-5319EN page 36</th>
<th>Drugs</th>
<th>Water</th>
<th>1200SL</th>
<th>MSMS DQO</th>
<th>Extend-C18, 2.1 mm × 100 mm, 1.8 µm</th>
<th>728700-902</th>
<th>ACN/water AmmForm</th>
<th>Offline</th>
<th>SPE</th>
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</thead>
<tbody>
<tr>
<td>5989-0738EN page 37</td>
<td>Nitrofurans</td>
<td>Poultry, shrimp</td>
<td>Bin. grad</td>
<td>MSn Trap XCT posESI</td>
<td>ZORBAX XDB-C8, 2.1 mm × 50 mm, 3.5 µm</td>
<td>971700-906</td>
<td>ACN/water acetic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5989-0596EN page 38</td>
<td>Fluoroquinolones</td>
<td>Beef kidney</td>
<td>Bin. grad</td>
<td>MSD posESI</td>
<td>ZORBAX XDB-C8, 4.6 mm × 150 mm, 5 µm</td>
<td>993967-906</td>
<td>ACN/water formic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5989-9920EN page 39</td>
<td>Chloramphenicol</td>
<td>Shrimp, honey</td>
<td>Bin. grad</td>
<td>MSD and MSn Ion Trap negESI</td>
<td>ZORBAX XDB-C18, 4.6 mm × 150 mm, 5 µm</td>
<td>993967-902</td>
<td>MeOH/ACN/water AmmOA</td>
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<tr>
<td>5989-7135EN page 40</td>
<td>Sulfur drugs</td>
<td>Meat</td>
<td>Gradient</td>
<td>DAD</td>
<td>RP-18 Purospher, 4 mm × 250 mm, 5 µm</td>
<td>79925PU-584</td>
<td>ACN/water H3PO4</td>
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<tr>
<td>5989-2499EN page 41</td>
<td>Sulfonamides</td>
<td>CapLC</td>
<td>DAD MSD posESI</td>
<td>ZORBAX SB-C18, 0.5 mm × 150 mm, 3.5 µm</td>
<td>5064-8262</td>
<td>ACN/water formic</td>
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<tr>
<td>5989-4858EN</td>
<td>Estrogens</td>
<td>River sewage, treated sewage effluent</td>
<td>MS TOF negAPPI</td>
<td>Luna Phenyl Hexyl, 2.0 mm × 150 mm, 3 µm w/guard</td>
<td>Various</td>
<td>See app. note</td>
<td>Propanol/cyclohexane</td>
<td>10</td>
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<tr>
<td>5989-1302EN Poster</td>
<td>Nitrofuran</td>
<td>Poultry, shrimp</td>
<td>MS TOF posESI</td>
<td>ZORBAX XCB-C18, 2.1 mm × 50 mm, 3.5 µm</td>
<td>971700-902</td>
<td>ACN/water acetic</td>
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<td>11</td>
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<tr>
<td>5989-0182EN</td>
<td>Sulfonamides</td>
<td>Pork</td>
<td>MSD posAPCI</td>
<td>ZORBAX XDB-C8, 4.6 mm × 150 mm, 5 µm</td>
<td>993967-906</td>
<td>ACN/water formic</td>
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<td>12</td>
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<td>Major Analytes</td>
<td>Matrix</td>
<td>LC System</td>
<td>Detection</td>
<td>Column(s)</td>
<td>Col. P/N</td>
<td>Mobile Phase</td>
<td>Notes</td>
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<tr>
<td>Chloramphenicol</td>
<td>Fish</td>
<td>MSD negAPPI, DAD</td>
<td>ZORBAX XDB-C18 3 mm × 150 mm, 5 µm</td>
<td>993967-302</td>
<td>MeOH/water AmmOAc</td>
<td>13</td>
<td></td>
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<td></td>
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<tr>
<td>Nitrofurans</td>
<td>Poultry</td>
<td>MSD posESI, DAD</td>
<td>Inertsil ODS3 2.1 mm × 150 mm, 5 µm</td>
<td></td>
<td>ACN/water formic</td>
<td>14</td>
<td></td>
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<tr>
<td>Steroids</td>
<td>Water</td>
<td>MSn Ion Trap pos/negAPCI, posAPPI, DAD</td>
<td>ZORBAX XDB-C18 2.1 mm × 50 mm, 3.5 µm</td>
<td>971700-902</td>
<td>ACN/water AmmOAc</td>
<td>15</td>
<td></td>
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<tr>
<td>Tetracyclines</td>
<td>Meat, food</td>
<td>DAD</td>
<td>Hypersil BDS 4 mm × 100 mm, 3 µm</td>
<td>Recommend SB-C18 chemistry</td>
<td>ACN/water H₂SO₄</td>
<td>16</td>
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<tr>
<td>Antibiotics, sulfas</td>
<td>Meat</td>
<td>DAD</td>
<td>Purospher RP18 4 mm × 250 mm, 5 µm</td>
<td>Recommend SB-C18 chemistry</td>
<td>ACN/water H₃PO₄</td>
<td></td>
<td></td>
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<tr>
<td>Arsenic species</td>
<td>Urine, water</td>
<td>LC-ICPMS</td>
<td>ICPMS</td>
<td>G3288-80000 Arsenic column, 4.6 mm × 250 mm</td>
<td>G3288-80000 plus G3154-65002 (Guard Column)</td>
<td>EtOH, water, phosphate EDTA, NaOAc, NaN₃</td>
<td>17</td>
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<td>Methylmercury, mercury, ethylmercury</td>
<td>Water, seawater</td>
<td>LC/ICP-MS</td>
<td>ICPMS</td>
<td>ZORBAX XDB-C18 2.1 mm × 50 mm, 5 µm</td>
<td>960967-902</td>
<td>MeOH/water AmmOAc</td>
<td>2-mercaptoethanol</td>
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<tr>
<td>Acid herbicides, steroids</td>
<td>Water</td>
<td>MSD negESI</td>
<td>Extend-C18 2.1 mm × 150 mm, 3.5 µm</td>
<td>763750-902</td>
<td>ACN/water formic NH₃</td>
<td>18</td>
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<tr>
<td>Herbicide antibiotic, peptide bialaphos, bilanaphos</td>
<td>Bialaphos</td>
<td>MSn Ion Trap, MS TOF, both posAPCI</td>
<td>Infusion</td>
<td>NA</td>
<td>NA</td>
<td>18</td>
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<tr>
<td>Various including pesticides, drugs</td>
<td>Food</td>
<td>Various</td>
<td>Various</td>
<td>Various</td>
<td>Various</td>
<td>19</td>
<td></td>
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<tr>
<td>Amitrol</td>
<td>Groundwater</td>
<td>MSD posAPCI</td>
<td>SB-C18 3 mm × 150 mm, 3.5 µm</td>
<td>863954-302</td>
<td>MeOH/water AmmOAc</td>
<td>20</td>
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<tr>
<td>EPA 3640A Standard mix</td>
<td>Vegetable oil, broccoli, animal fat</td>
<td>GPC</td>
<td>DAD</td>
<td>Various Organic GPC</td>
<td>See app. note</td>
<td>Various</td>
<td>21</td>
<td></td>
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<tr>
<td>HMF hydroxy-methylfurfural</td>
<td>Bread, cereal, yogurt</td>
<td>Bin. grad</td>
<td>MSD posAPCI</td>
<td>Bonus-RP 2.1 mm × 100 mm, 3.5 µm</td>
<td>861768-901</td>
<td>Water acetic formic</td>
<td>Offline SPE</td>
<td></td>
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<tr>
<td>Acrylamide</td>
<td>Drinking water</td>
<td>Dual binary w/ 6-port valve for autoSPE</td>
<td>MS TOF posESI</td>
<td>ZORBAX SB-C18 2.1 mm × 150 mm, 5 µm</td>
<td>883700-922</td>
<td>ACN/water formic</td>
<td>43</td>
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Regulated/Hazardous Drug Substances (Continued)
<table>
<thead>
<tr>
<th>Major Analytes</th>
<th>Matrix</th>
<th>LC System</th>
<th>Detection</th>
<th>Column(s)</th>
<th>Col. P/N</th>
<th>Mobile Phase</th>
<th>Notes</th>
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<tbody>
<tr>
<td>5989-2481EN page 44</td>
<td>Chromium</td>
<td>Metrohm</td>
<td>Metrohm 818</td>
<td>IC-ICPMS</td>
<td>G3268A</td>
<td>Water Na₂EDTA</td>
<td>22</td>
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<tr>
<td>speciation</td>
<td>icht</td>
<td>pump, Agilent</td>
<td>7500 ISIS sampler</td>
<td>Agilent Cr</td>
<td></td>
<td>NaOH</td>
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<tr>
<td>5989-8816EN page 45</td>
<td>Perchlorate</td>
<td>Water, vegetables</td>
<td>Metrohm IC</td>
<td>MSD negESI</td>
<td>See app. note</td>
<td>MeOH/water 30 mm NaOH</td>
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<tr>
<td>5988-9893EN page 46</td>
<td>Arsenobetaine</td>
<td>Fish</td>
<td>Isocratic</td>
<td>ICP-MS</td>
<td>Contact manufacturer</td>
<td>AmmHCO₃/ tartaric</td>
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<tr>
<td>5988-3161EN</td>
<td>Bromate</td>
<td>Drinking water</td>
<td>ICP-MS</td>
<td>IC Dionex PA-100</td>
<td>Water, AmmNO₃</td>
<td>HNO₃</td>
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<tr>
<td>5968-3049</td>
<td>Bromate, iodate</td>
<td>Ozone-treated water</td>
<td>Yokagawa IC</td>
<td>ICP-MS or DAD post col. deriv.</td>
<td>IC See app. note</td>
<td>Water, carbonate/ bicarbonate</td>
<td>23</td>
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<tr>
<td>5966-0633</td>
<td>Anions</td>
<td>Water</td>
<td>DAD, indirect UV</td>
<td>Contact author for reversed phase column and mobile phase details</td>
<td>ACN/water NaOH plus UV mod</td>
<td>24</td>
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Regulated/Hazardous Natural Toxin Substances

<table>
<thead>
<tr>
<th>Major Analytes</th>
<th>Matrix</th>
<th>LC System</th>
<th>Detection</th>
<th>Column(s)</th>
<th>Col. P/N</th>
<th>Mobile Phase</th>
<th>Notes</th>
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<tbody>
<tr>
<td>5989-2912EN page 47</td>
<td>DSP algal toxins</td>
<td>Shellfish</td>
<td>Quat. grad</td>
<td>MSD pos/ negESI</td>
<td>883975-302 and 846975-202</td>
<td>MeOH/water formic</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ZORBAX SB-C18 3 mm × 150 mm, 5 µm, 9.4 mm × 50 mm, 5 µm semiprep</td>
<td></td>
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<tr>
<td>5968-2124E page 49</td>
<td>Mycotoxin, fumonisin</td>
<td>Corn</td>
<td>Bin. grad</td>
<td>MSD posESI</td>
<td>993700-902</td>
<td>ACN/water AmmOAc</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ZORBAX XDB-C18 2.1 mm × 150 mm, 5 µm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>00060329.pdf page 50</td>
<td>Aflatoxins</td>
<td>Various</td>
<td>Agilent/ Gerstel onlineSPE</td>
<td>MSD posESI</td>
<td>Phenomenex MAX RP 2.1 mm × 250 mm, 5 µm</td>
<td>Suggest SB-C18 2.1 mm × 150 mm, 3.5 µm 830990-902</td>
<td>ACN/water formic</td>
</tr>
<tr>
<td>5989-3634EN</td>
<td>Aflatoxins</td>
<td>DAD</td>
<td></td>
<td>ZORBAX XDB-C18 4.6 mm × 150 mm, 3.5 µm</td>
<td>963967-902</td>
<td>MeOH, ACN, water</td>
<td></td>
</tr>
<tr>
<td>5968-3796E</td>
<td>Anatoxin A, alkaloid neurotoxin</td>
<td>(Algae) drinking water</td>
<td>MSD posESI</td>
<td>Inertsil ODS3 2.1 mm × 150 mm, 5 µm</td>
<td>Recommend SB-C18 or XDB-C18 chemistry</td>
<td>ACN/water AmmOAc</td>
<td>26</td>
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<tr>
<td>5986-2123E</td>
<td>Microcystis</td>
<td>Fresh (surface) water</td>
<td>MSD posESI</td>
<td>Mytisil ODS 2.1 mm × 100 mm, 5 µm</td>
<td>Recommend SB-C18 chemistry</td>
<td>ACN/water formic</td>
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### Regulated/Hazardous Natural Toxin Substances (Continued)

<table>
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<th>LC System</th>
<th>Detection</th>
<th>Column(s)</th>
<th>Col. P/N</th>
<th>Mobile Phase</th>
<th>Notes</th>
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<tr>
<td>5966-0632 Afattoxins, mycotoxins, patulina, etc</td>
<td>Various</td>
<td>DAD/FLD</td>
<td>Various</td>
<td>Various</td>
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<td>5952-5852 obsolete</td>
<td>Mycotoxins</td>
<td>Old HP</td>
<td>MS Thermospray (obsolete)</td>
<td>Vydac 201HSB 4.6 mm × 150 mm, 5 µm</td>
<td>Recommend SB-C8 or XDB-C8 chemistry</td>
<td>ACN/water AmmOHAc</td>
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<tr>
<td>5952-5852</td>
<td>Various fungal toxins</td>
<td>Various</td>
<td>MS Thermospray (obsolete)</td>
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<td>Old HP note</td>
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<td>5991-0692</td>
<td>Mycotoxins</td>
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<td>DAD w/ Library, FLD</td>
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### Regulated/Hazardous Pesticide/Herbicide Substances

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<th>Detection</th>
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<th>Mobile Phase</th>
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<tbody>
<tr>
<td>5989-5459EN page 51</td>
<td>44 pesticides</td>
<td>Vegetables, fruit</td>
<td>Bin. grad</td>
<td>MSMS QQQ posESI</td>
<td>Extend-C18 2.1 mm × 100 mm, 1.8 µm</td>
<td>728700-902</td>
<td>ACN/water AmmForm</td>
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<tr>
<td>5989-5176EN page 52</td>
<td>Acid herbicides</td>
<td>Water</td>
<td>AutoSPE/1200SL DAD with dual binary pumps and 6-port valve</td>
<td>ZORBAX SB-C18 5-, 1.8-µm columns</td>
<td>See app. note</td>
<td>ACN/water H2PO4</td>
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<td>5989-2728EN page 54</td>
<td>Postharvest fungicides</td>
<td>Citrus</td>
<td>Bin. grad</td>
<td>MS TOF, MSn Ion Trap, both posESI</td>
<td>ZORBAX XDB-C8 4.6 mm × 150 mm, 5 µm</td>
<td>993967-906</td>
<td>ACN/water formic</td>
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<tr>
<td>5989-1842EN page 55</td>
<td>Chloronicotinyl insecticides</td>
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<td>5989-0813EN page 56</td>
<td>Phenylurea, triazine herbicides</td>
<td>Water</td>
<td>Dual binary w/ 6-port valve for autoSPE</td>
<td>MSD pos/ negAPCI</td>
<td>ZORBAX XDB-C8 2.1 mm × 50 mm, 3.5 µm</td>
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<td>ACN/water formic</td>
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<td>Drinking water</td>
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<td>ACN, MeOH, water AmmOAc</td>
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<td>MSD pos/ negESI, DAD</td>
<td>ZORBAX XDB-C8 2.1 mm × 150 mm, 3.5 µm</td>
<td>930990-902</td>
<td>ACN/water formic</td>
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<td>5989-0184EN Amitrol herbicide</td>
<td>Water</td>
<td>MSD posAPCI</td>
<td>ZORBAX SB-C18 3 mm × 150 mm, 3.5 µm</td>
<td>863954-302</td>
<td>MeOH/water AmmOAc</td>
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<td>5988-8692EN Pesticides, antibacterials</td>
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<td>3-mm RP columns</td>
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<td>Various</td>
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<td>ZORBAX XDB-C8 2.1 mm × 50 mm, 3.5 µm</td>
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<td>MeOH/water formic</td>
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<td>ACN/water NaOAc</td>
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<td>MSD posESI</td>
<td>Extend-C18 2.1 mm × 150 mm, 3.5 µm</td>
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<td>ACN/water TDFHA (tetradecafluoro-heptanoic acid)</td>
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<td>ACN/water TDFHA (tetradecafluoro-heptanoic acid)</td>
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<td>ZORBAX XDB-C8 4.6 mm × 150 mm, 5 µm</td>
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<td>ACN/water formic</td>
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<td>5988-6635EN Pesticides phenoxyureas, carbanates</td>
<td>MSD ESi, APCI, APPi</td>
<td>ZORBAX XDB-C8 4.6 mm × 50 mm, 3.5 µm</td>
<td>935967-906</td>
<td>ACN, MeOH, acetic</td>
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<td>Water</td>
<td>MSD negESI</td>
<td>ZORBAX XDB-C18 2.1 mm × 150 mm, 3.5 µm</td>
<td>930990-902</td>
<td>ACN/water formic</td>
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<td>5988-4981EN Glyphosate, AMPA</td>
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<td>MSD posESI</td>
<td>ZORBAX XDB-C8 4.6 mm × 50 mm, 5 µm</td>
<td>946975-906</td>
<td>ACN/water AmmOAc</td>
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<td>Major Analytes</td>
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<td>Detection</td>
<td>Column(s)</td>
<td>Col. P/N</td>
<td>Mobile Phase</td>
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<td>Carbamates</td>
<td>Vegetable broccoli</td>
<td>MSD posESI</td>
<td>ZORBAX XDB-C18</td>
<td>2.1 mm × 150 mm, 5 µm, 4.6 mm × 150 mm, 5 µm</td>
<td>960967-902, 993967-902</td>
<td>ACN/water</td>
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<td>Simazine, thiobencarb, thiuram</td>
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<td>Inertsil ODS3</td>
<td>2.1 mm × 250 mm, 5 µm</td>
<td>MeOH/water</td>
<td>AmmOAc</td>
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<td>Glycosylated flavonoids</td>
<td>Plant, hostas</td>
<td>MSD negESI</td>
<td>300SB-C18</td>
<td>2.1 mm × 150 mm, 5 µm</td>
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<td>ACN/water</td>
<td>AmmOAc</td>
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<td>Organophosphate pesticides</td>
<td>MSD posESI, DAD</td>
<td>ZORBAX SB-C18</td>
<td>2.1 mm × 50 mm, 3.5 µm</td>
<td>MeOH/water</td>
<td>AmmOAc</td>
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<td>Sulfonylureas,</td>
<td>Surface water</td>
<td>MSn Ion Trap posESI</td>
<td>Metasil Basic</td>
<td>2.1 mm × 100 mm, 5 µm</td>
<td>ACN/water</td>
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<td>Rodenticides</td>
<td>Sausage, dog stomach contents</td>
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<td>ZORBAX XDB-C18</td>
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<td>MeOH/water</td>
<td>AmmOAc</td>
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<td>Carbaryl</td>
<td>Complex food homogenate</td>
<td>MSn Ion Trap posESI</td>
<td>ZORBAX XDB-C8</td>
<td>4.6 mm × 250 mm, 5 µm</td>
<td>990967-906</td>
<td>ACN/water</td>
<td>acetic AmmOAc</td>
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<td>Glyphosate</td>
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<td>IEX</td>
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<td>Triazines, phenylureas, methabenzthiazuron, diquat, paraquat, mercaptobenzothiazole</td>
<td>DAD</td>
<td>Hypersil BDS</td>
<td>3 mm × 100 mm, 3 µm</td>
<td>Recommend XDB-C18 chemistry</td>
<td>ACN/water</td>
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<td>Carbamates</td>
<td>Old HP</td>
<td>FLD w/post-col. deriv.</td>
<td>C18</td>
<td>4.6 mm × 250 mm, 5 µm</td>
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<td>Glyphosate</td>
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<td>FLD w/postcol. or postcol. deriv.</td>
<td>SAX-300</td>
<td>4.6 mm × 100 mm</td>
<td>79919QA-754</td>
<td>Water, phosphate</td>
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**Fat-Soluble Vitamins**

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<th>Matrix</th>
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<th>Detection</th>
<th>Column(s)</th>
<th>Col. P/N</th>
<th>Mobile Phase</th>
<th>Notes</th>
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<td>Retinol isomers</td>
<td>Isocratic</td>
<td>DAD</td>
<td>ZORBAX Sil</td>
<td>4.6 mm × 250 mm, 5 µm</td>
<td>880952-701</td>
<td>Dioxane, tBME, hexane</td>
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<tr>
<td>Fat-soluble vitamins</td>
<td>Isocratic</td>
<td>DAD</td>
<td>ZORBAX XDB-C8</td>
<td>4.6 mm × 150 mm, 5 µm</td>
<td>993967-906</td>
<td>MeOH/water</td>
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<tr>
<td>Vitamin D3</td>
<td>Poultry feed</td>
<td>Bin. grad</td>
<td>MSn Ion Trap posAPCI</td>
<td>Flow inject – no column</td>
<td>NA</td>
<td>MeOH/water</td>
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<td>Major Analytes</td>
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<td>Detection</td>
<td>Column(s)</td>
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<td>5968-2970 page 61</td>
<td>Fat-soluble vitamins A, D, E</td>
<td>Quat. grad</td>
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<td>ZORBAX XDB-C18 4.6 mm x 75 mm, 3.5 µm</td>
<td>7995118-344</td>
<td>MeOH/water</td>
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<td>5988-6354EN</td>
<td>Fat-soluble vitamins</td>
<td>VWD with wavelength switching</td>
<td>SB-C18</td>
<td>4.6 mm x 150 mm, 5 µm, XDB-C18 4.6 mm x 150 mm, 5 µm</td>
<td>883975-902</td>
<td>MeOH/ACN</td>
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<td>5988-6350EN</td>
<td>Fat-soluble vitamins A, D and E isomers, K1</td>
<td>VWD with wavelength switching</td>
<td>ODS</td>
<td>4.6 mm x 250 mm, 5 µm, ODS classic 4.6 mm x 150 mm, 5 µm</td>
<td>884950-543, 883952-702</td>
<td>MeOH/ACN</td>
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<td>5980-1390</td>
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<td>Rx-C18</td>
<td>4.6 mm x 75 mm, 3.5 µm</td>
<td>866967-902</td>
<td>MeOH/water</td>
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<td>5986-0745</td>
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<td>Multiwave-length</td>
<td>Hypersil MOS</td>
<td>2.1 mm x 100 mm, 5 µm</td>
<td>Suggest 2.1 mm x 150 mm, 3.5 µm Zorbax Sil</td>
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<td>Tocopherol isomers, Vitamin E Margarine</td>
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<td>Hypersil SI</td>
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**Mixed Vitamins**

| 5091-3194 | Vitamins A,B,C,E Various | ECD | Lichrospher, Hypersil | Various | |

**Water-Soluble Vitamins**

| 5988-6365EN page 62 | Water-soluble vitamins | Gradient | DAD | ZORBAX SB-C8 4.6 mm x 150 mm, 5 µm | 883975-906 | MeOH/water phosphate | |
| 5988-5761EN page 63 | Water-soluble vitamins | Cat food | Gradient | ZORBAX SB-C18 4.6 mm x 75 mm, 3.5 µm | 866953-902 | ACN/water acetic acetic phosphoric TEA hexane sulfonate | 47 |
| 5988-6364EN | Water-soluble vitamins | Tablets | DAD | SB-C18 4.6 mm x 250 mm, 5 µm, USP23, L1 | 880975-902 | MeOH/water acetic hexane sulfonate | |
| 5988-6363EN | Water-soluble vitamins | DAD | SB-C8 | 4.6 mm x 75 mm, 3.5 µm | 866953-906 | MeOH/water phosphoric hexane sulfonate | |
| 5968-2971 | Water-soluble vitamins | Chilled ALS | DAD | SB-C18 4.6 mm x 75 mm, 3.5 µm | 866953-902 | ACN/water phosphate | |
### Water-Soluble Vitamins (Continued)

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<td>Tablets</td>
<td>DAD</td>
<td>Hypersil BDS</td>
<td>4 mm × 100 mm, 3 µm</td>
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<td>ACN/water H₂SO₄</td>
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### Mixed Publications

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<td>Method transfer - example acid herbicides</td>
<td>1200SL</td>
<td>DAD</td>
<td>SB-C18 5-, 3.5- and 1.8-µm columns</td>
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<td>5999-4086EN</td>
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<td>Various Eclipse RP</td>
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<td>5999-1947EN</td>
<td>Food solution guide</td>
<td>Various</td>
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<td>ZORBAX general</td>
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<td>5998-9346E</td>
<td>Application overview of FLD</td>
<td>FLD</td>
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<td>5998-8565pdf</td>
<td>Peptides, aspartame, NSAIDs</td>
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<td>ZORBAX misc.</td>
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### Packaging

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<th>Detection</th>
<th>Column(s)</th>
<th>Col. P/N</th>
<th>Mobile Phase</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>5998-8610EN</td>
<td>Antioxidants</td>
<td>Polymer</td>
<td>MSD pos/ negAPCI DAD</td>
<td>ZORBAX XDB-C8, 4.6 mm × 50 mm, 3.5 µm</td>
<td>935967-906</td>
<td>ACN,MeOH, THF, water</td>
<td></td>
</tr>
<tr>
<td>5967-6102E</td>
<td>Bisphenol Esters</td>
<td>Food</td>
<td>MSD posESI</td>
<td>Hypersil ODS 2.1 mm × 200 mm, 5 µm</td>
<td></td>
<td>ACN/water AmmOAc</td>
<td></td>
</tr>
</tbody>
</table>
Notes for the Application Reference Index

1. 12 pp intro
2. Post-column addition of NaOH solution
3. Bromophenacyl bromide derivatizing agent
4. Precolumn deriv. w/ OPA/mercaptoethanol
5. MS and MSMS, some infusion, some chromatography
6. Compare various bonded phases
7. Column temperature vs. separation quality and selectivity
8. Precolumn derivatization
10. Offline automated SPE procedure, normal phase cleanup (SB-CN 4.6 mm × 50 mm 5 µm and guard with propanol/cyclohexane), GPC cleanup (PLgel 50A in MeCl2), postcolumn addition of reference mass solution
11. Precolumn offline derivatization
12. Detailed sample prep including offline SPE
13. Postcolumn dopant acetone
14. Offline precolumn derivatization, detailed sample prep
15. Large volume direct injection with good comparison of strong and weak diluents on peak shape
16. Detailed sample prep provided
17. Ion suppression problems studied, postcolumn addition used
18. Elaborate structural elucidation via Trap MSMS and TOF accurate mass analysis
19. Food safety primer_LC, GC, ICP
20. Derivatization hexylchloroformate, SPE trace enrichment
21. Loading guidelines, various solvents
22. Column normally bundled with ICP-MS. Contact your agent/representative for details
24. UV modifier, probably aromatic acid like 4-OH-benzoic or trimesic; some applns have used cetylpyridinium chloride for indirect or displacement UV of anions.
25. Bromination of B1 and G1 prior to separation
26. Online derivatization with FMOC; good details
27. Detailed sample preps and references in this review article
28. QuECHERS sample prep
29. Offline SPE trace enrichment
30. Liquid/liquid extraction and SPE
31. Ion suppression problems studied, postcolumn addition used, “fully automated offline SPE”
32. Derivatization, SPE trace enrichment
33. Novel ion pairing reagent
34. Novel ion pairing reagent
35. Post-column addition of APPI dopant
36. “Fully automated offline SPE”
37. Offline precolumn derivatization FMOC, post-column addition dilute formic acid
38. QuECHERS sample prep
39. Post-column addition of TEF in IPA
40. Compares DAD to MSN-SIM results
41. Tubulent flow trace enrichment
42. Detailed sample prep, compares DAD to MSD, and neg vs. pos and ESI vs. APCI
43. Complex and detail sample prep.
44. Pickering post-column system with Agilent LC/FLD
45. Pickering system postcolumn base hydrolysis followed by OPA
46. Pickering post column system with Agilent LC/FLD
47. Titriplex-V hot extraction
48. RRHT standalone brochure
49. Former rev (2004) of Food Solution Guide
50. Zorbax SB LC columns
51. Zorbax applications guide various markets
52. Applications book various markets
53. Zorbax Rapid Resolution Columns, various applications