



Automated Method Development with the 1200 Infinity Series LC

Infinitely better
for multiple
LC methods

Agilent 1200 Infinity Series
Multi-method Solutions



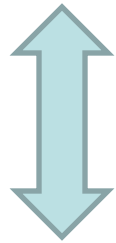
Sebastian Krahe
Produktspezialist LC & Dissolution
Agilent Technologies, Deutschland



Methodenentwicklung in Unternehmen

Aufwand vom Start bis zur validierten Methode

- Geräteauslastung
- Personalauslastung
 - Methoden einstellen
 - Datenanalyse



- Automation
 - der Testläufe
 - der Datenauswertung
- Quality-by-Design
- Datenauswertetools

Vertrauenswürdigkeit der endgültigen Methode

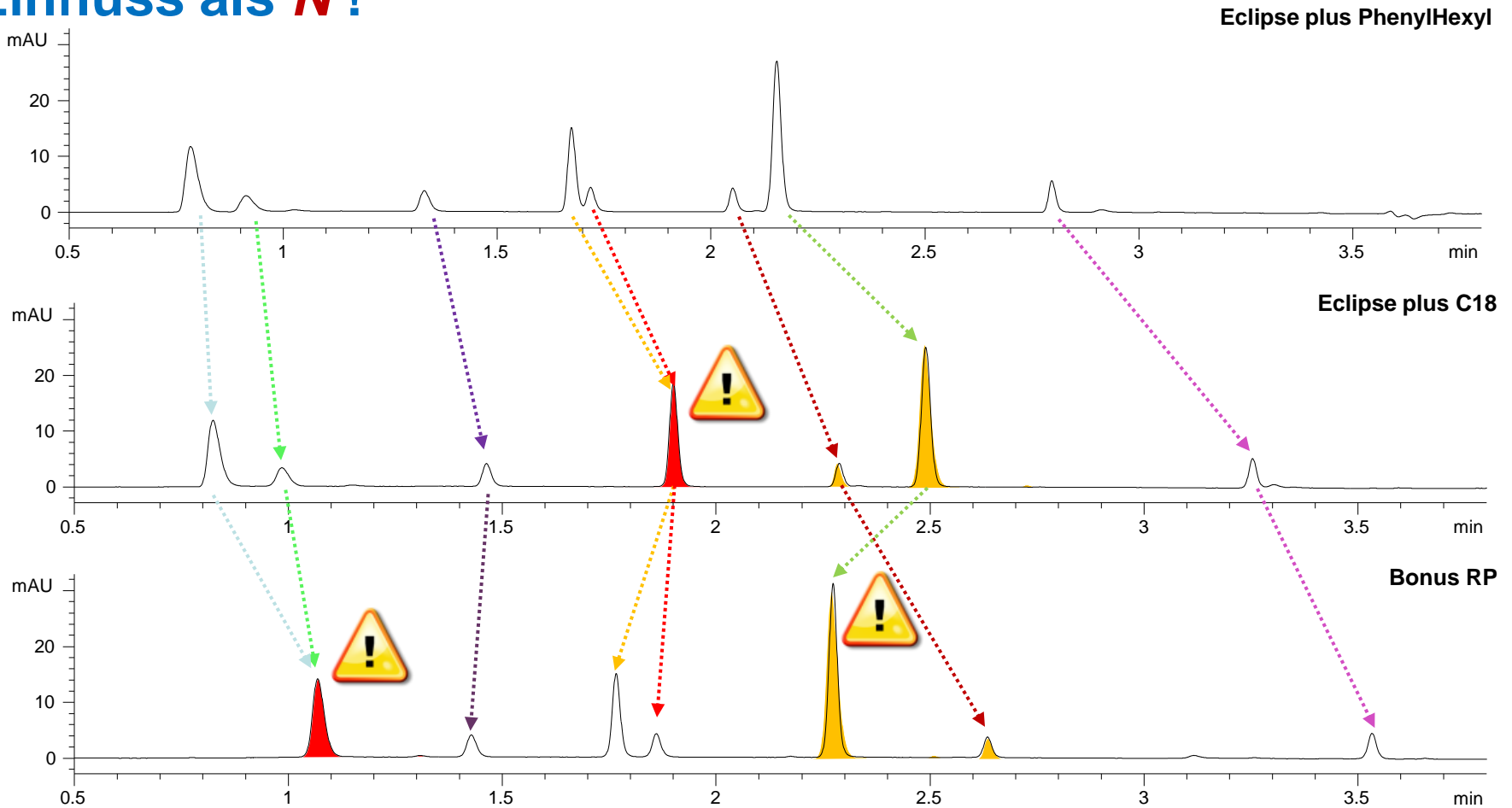
- Sind alle Analyten gefunden?
 - Auflösung
 - Selektivität
- Methodenrobustheit?



Fehler führen zu Zeit- und Geldverlusten!

Warum verschiedene Trennbedingungen?

Veränderungen an der Selektivität α haben einen größeren Einfluss als N !

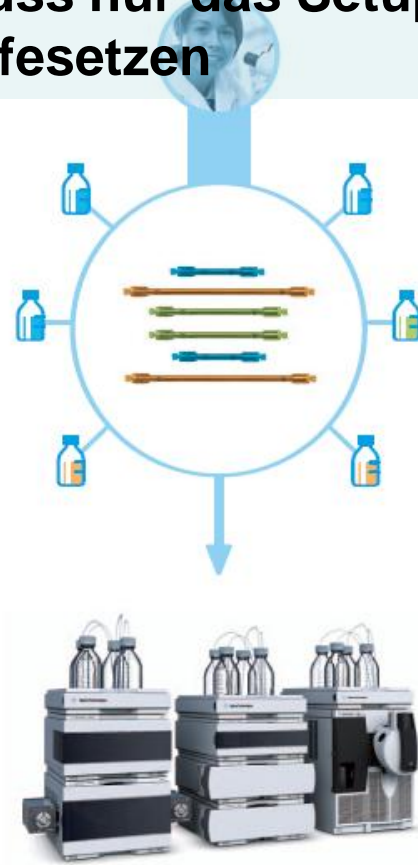


Method Development - a continuous change of separation conditions!

Ohne Automation: stetige Einflussnahme durch den Anwender



Mit Automation: der Anwender muss nur das Setup einmal aufsetzen



Agilent 1200 Infinity Series Multimethodenlösung

**1290 Infinity Thermostatted
Column Compartment Cluster**



Bis zu 8 Säulen frei wählbar

Bis zu 26 Eluenten frei wählbar

1200 bar

**>1000 unterschiedliche
Trennbedingungen!**

4-6 verschiedene Temperaturzonen

**für 2.1 – 4.6 mm ID Säulen bis zu
einer Länge von 300 mm**

**Verschiedenste Konfigurationen
möglich**

Verschiedene Detektoren verfügbar

Der **1290 Infinity TCC Cluster** ist das Herzstück des Agilent 1200 Infinity Multimethodenlösung für Methodenentwicklung und Multimethodenanwendungen.

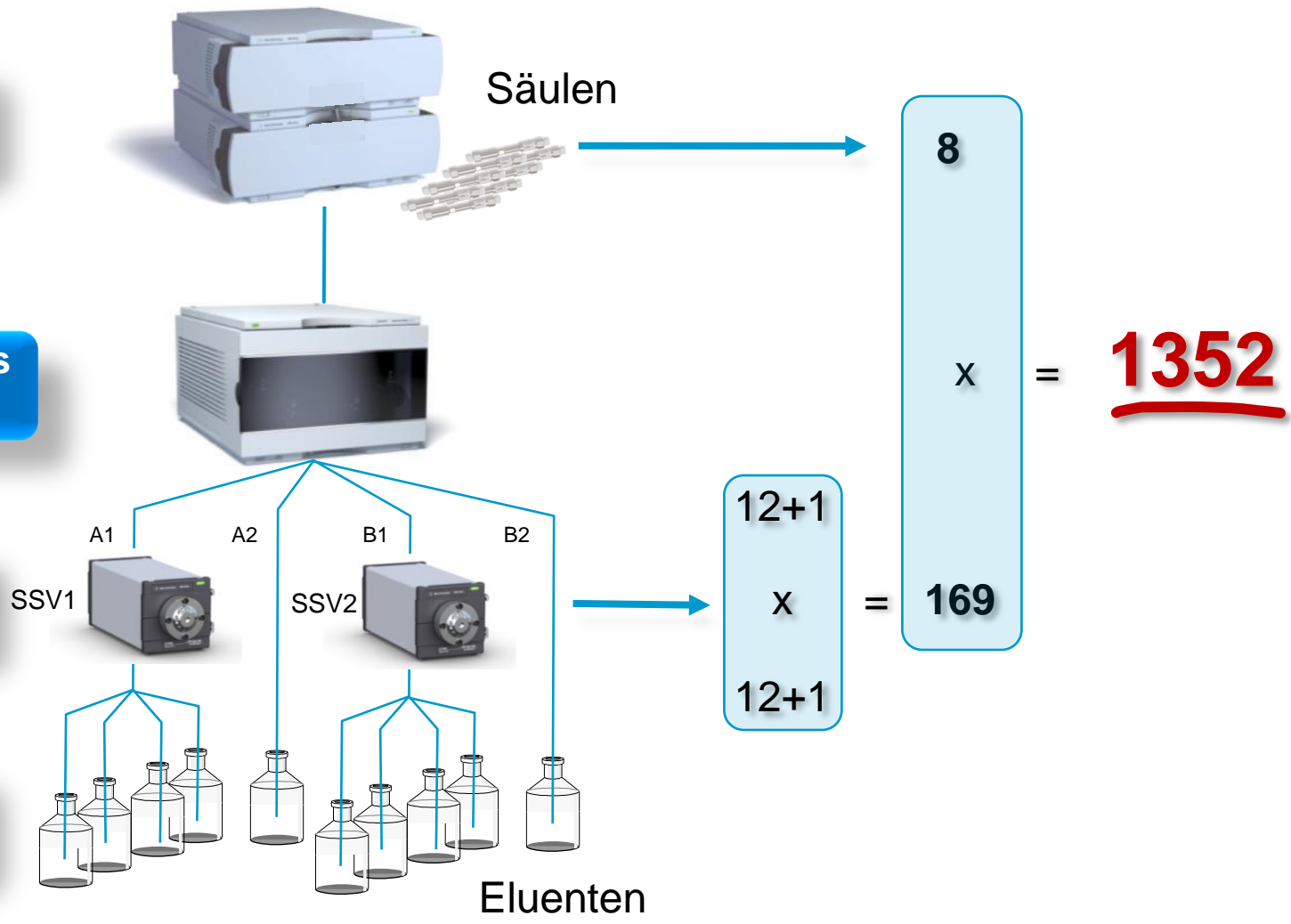
Mehr als 1000 einzigartige Kombinationsmöglichkeiten

1290 Infinity TCC-Cluster

1200 Infinity Series Pumpe

Externe Eluenten Ventile

Bis zu 12 Eluenten



Agilent Schaltventile



Ventil fährt auf Druck heraus

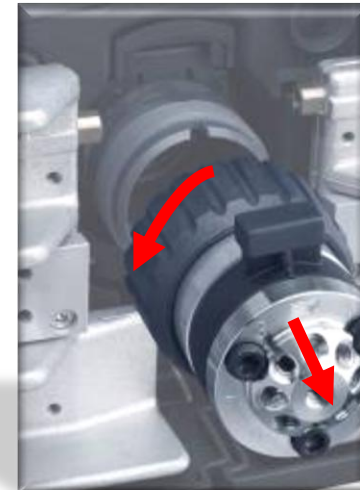
In 1200 bar oder 600 bar!

Ventil kann getauscht werden

RFID-tag speichert Ventiltyp, Druckbereich, Anzahl der Bewegungen



Einfacher Zugang zum Anschluss



Kompletter Austausch ohne Werkzeug

Agilent Schaltventile

Basiert auf der neuen Agilent Infinity LC

Als 1290 Infinity oder 1260 Infinity.

Auch 1100 and 1200 Series LC Systeme* können aufgerüstet werden!

Sie benötigen nur zwei 1290 Infinity TCCs und zwei Ventile.



1200 bar



600 bar

* 1100/1200 Series System können bis 600 bar betrieben werden.

Agilent Schaltventile

Neuer TCC Clustertreiber

Säulenauswahl mit einem Klick!



Method of **inCompartmentCluster (notAvailable)**

Columns

- SB-C18, 2.1x50mm, 1.8µm
- Eclipse Plus C18, 4.6x50mm, 1.8µm
- SB-C18, 3x50mm, 1.8µm
- SB-C18, 4.6x50mm, 1.8µm
- Eclipse XDB-C18, 4.6x150mm, 5µm
- Eclipse XDB-C18, 4.6x50mm, 1.8µm
- Waste
- Bypass

TCC1 G1316C(DE0000000) Inlet

TCC2 G1316C(PR00000054) Outlet

Temperature

- Not Controlled
- 20.0 °C
- As Detector Cell

Enable Analysis

- when front door open
- With any temperature
- When temperature is within ± 0.8 °C

Stoptime

- As Pump/Injector
- 1.00 min

Posttime

- Off
- 1.00 min

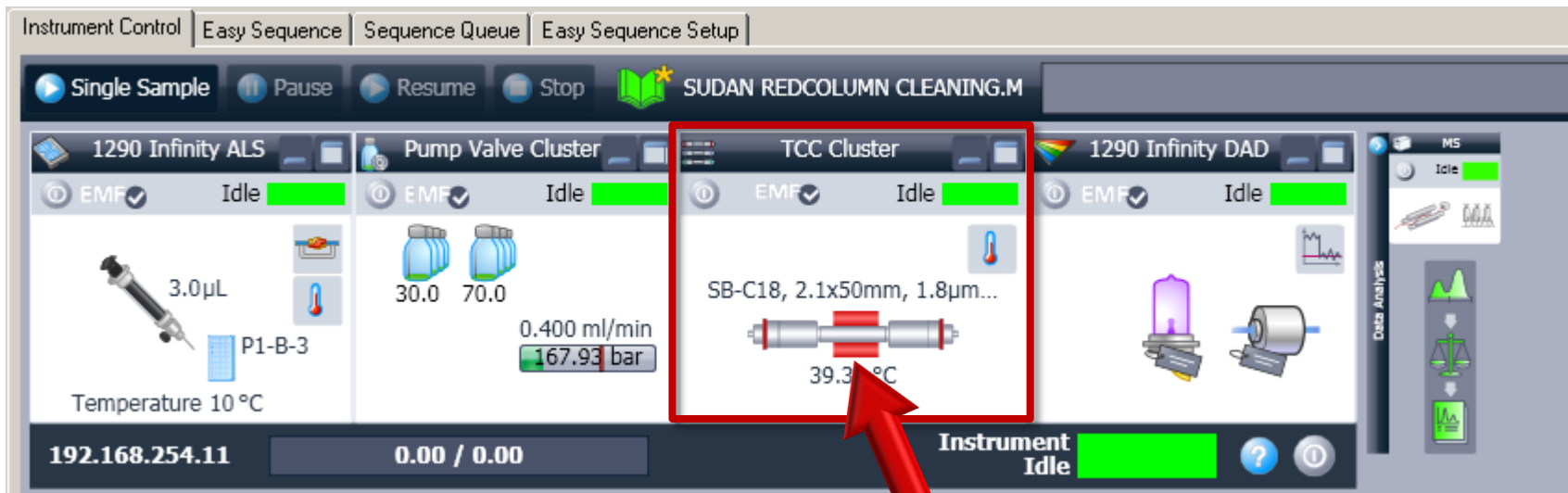
Timetable

Time	Function	Parameter

Buttons: Add, Remove, Clear all, Cut, Copy, Paste, Ok, Cancel

Agilent Schaltventile und Clustertreiber

Säulenauswahl nach Namen, nicht nach Ventilposition



Angewählte Säule ist in der Instrument Control Sektion direkt sichtbar.

Softwareunterstützung

Agilent unterstützt einfache Softwarepaket bis hin zu hochkomplexen Lösungen für den höchsten Grad an Automatisierung

	Scouting (method and sequence set-up)	Automated optimization	Data-analysis	Peak-tracking	Robustness testing - QbD	Sophisticated reporting	Project handling	Data archiving	Price
Agilent Method Scouting Wizard	■								■
ChromSword - Auto	■	■	■		■				■
ChromSword - AutoRobust				■					■
ACD/Labs AutoChrom	■	■	■		■	■			■
Openlab CDS with IR		■			*				■
OpenLab ECM							■	**	■

*) ChemStation, Oct. 2010

**) Company wide data archiving!



ACD/Labs

Method Scouting Wizard – der einfache Weg

1. Projekt definieren

Bedingungen und Grundlagen der Methoden einstellen.

2. Säulenauswahl

Anzeige aller installierter Säulen.

3. Eluentenauswahl

Pumpe und Ventile werden automatisch erkannt.

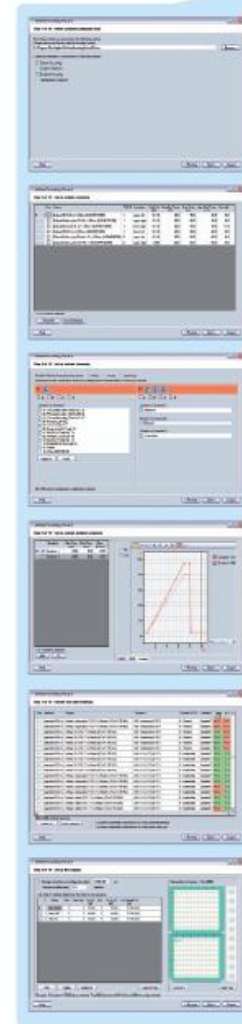
4. Einstellung der Gradienten

Eluentengradienten und Temperaturen.

5. Überprüfen des Testbedingungen

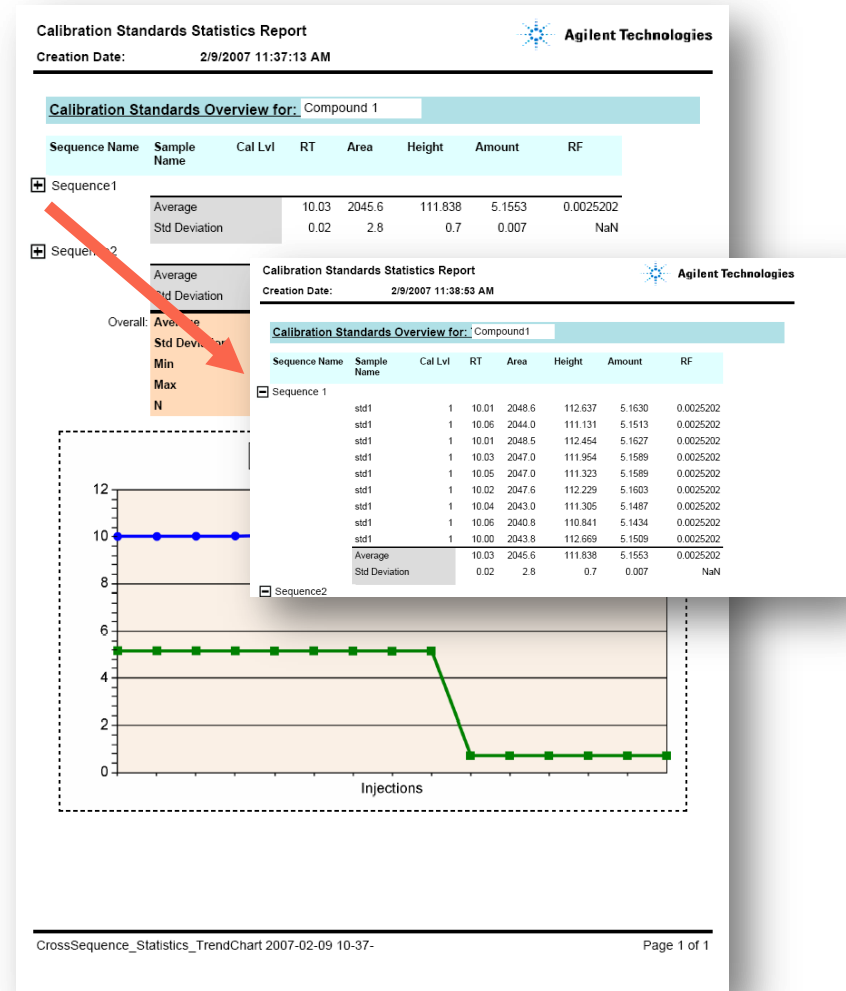
6. Proben einstellen

Injektionsvolumen und Wiederholungen angeben.



Intelligent Reporting in ChemStation – Oct. 2010

- Neues Standard Reporting Tool (Sequenzzusammenfassung und einzelne Injektionen)
- Klassische Reporte weiterhin verfügbar
- Verwendung von Modulen aus **OpenLAB ECM Intelligent Reporter**



Neuer Review in ChemStation

LCOffline (offline): Review, 1260methDev_Summary_MF6.rdl

File Report Log in/out View Abort Help

1260methDev_Summary_MF6.rdl

Review

ECM	Type	Line	Inj	Vial	Sample Name	Sample Type	Man...	Cal Level	Sample Info	Sample Am...	ISTD Amount	Multiplier
-		12	1	Vial 2	Tramadol	Sample	-	-	C=Poroshell 120 SBC18 S1=A1: water S2=B1: acetonitrile S3=D1: 2procent ...	0	0	1
-		13	1	Vial 1	sigma	Sample	-	-	C=EclipsePlus-C18 S1=A1: water S2=B1: acetonitrile S3=D1: 2procent TFA ...	0	0	1
-		14	1	Vial 2	Tramadol	Sample	-	-	C=EclipsePlus-C18 S1=A1: water S2=B1: acetonitrile S3=D1: 2procent TFA ...	0	0	1
-		15	1	Vial 1	sigma	Sample	-	-	C=EclipsePlus-C18 S1=A1: water S2=C1: Methanol S3=D1: 2procent TFA ...	0	0	1
-		16	1	Vial 2	Tramadol	Sample	-	-	C=EclipsePlus-C18 S1=A1: water S2=C1: Methanol S3=D1: 2procent TFA ...	0	0	1
-		17	1	Vial 1	sigma	Sample	-	-	C=Poroshell 120 SBC18 S1=A1: water S2=C1: Methanol S3=D1: 2procent T...	0	0	1
-		18	1	Vial 2	Tramadol	Sample	-	-	C=Poroshell 120 SBC18 S1=A1: water S2=C1: Methanol S3=D1: 2procent T...	0	0	1
-		19	1	Vial 1	sigma	Sample	-	-	C=SB C8 S1=A1: water S2=C1: Methanol S3=D1: 2procent TFA G=Gradie...	0	0	1
-		20	1	Vial 2	Tramadol	Sample	-	-	C=SB C8 S1=A1: water S2=C1: Methanol S3=D1: 2procent TFA G=Gradie...	0	0	1
-		21	1	Vial 1	sigma	Sample	-	-	C=SBAq S1=A1: water S2=C1: Methanol S3=D1: 2procent TFA G=Gradie...	0	0	1
-		22	1	Vial 2	Tramadol	Sample	-	-	C=SBAq S1=A1: water S2=C1: Methanol S3=D1: 2procent TFA G=Gradie...	0	0	1

1 of 2 <Enter search text> 100%

Method Development
Creation Date: 8/30/2010 2:45:32 PM

Agilent Technologies

Method Development Summary

Sequence: 1260METHTERNARY22 2010-08-17 10-34-57

Sample: sigma Signal: DAD1A

Peak #	Datefile	Column	Solvent1	Solvent2	Solvent3	Temp	Gradient
10	7-1 001-1101.D	SBAq	A1: water	B1: acetonitrile	D1: 2procent TFA	40.0 °C	Gradient 1 T
10	3-1 001-0501.D	Poroshell 120EC18	A1: water	B1: acetonitrile	D1: 2procent TFA	40.0 °C	Gradient 1 T
10	5-1 001-0801.D	SB CN	A1: water	B1: acetonitrile	D1: 2procent TFA	40.0 °C	Gradient 1 T
10	9-1 001-1401.D	SB C8	A1: water	B1: acetonitrile	D1: 2procent TFA	40.0 °C	Gradient 1 T
10	11-1 001-1701.D	Poroshell 120 SBC18	A1: water	B1: acetonitrile	D1: 2procent TFA	40.0 °C	Gradient 1 T
10	19-1 001-2901.D	SB C8	A1: water	C1: Methanol	D1: 2procent TFA	40.0 °C	Gradient 1 T
9	1-1 001-0201.D	Eclipse Plus C18	A1: water	B1: acetonitrile	D1: 2procent TFA	40.0 °C	Gradient 1 T
9	3-1 001-2001.D	EclipsePlus-C18	A1: water	B1: acetonitrile	D1: 2procent TFA	40.0 °C	Gradient 1 T
9	15-1 001-2301.D	EclipsePlus-C18	A1: water	C1: Methanol	D1: 2procent TFA	40.0 °C	Gradient 1 T
9	17-1 001-2601.D	Poroshell 120 SBC18	A1: water	C1: Methanol	D1: 2procent TFA	40.0 °C	Gradient 1 T
9	21-1 001-3201.D	SBAq	A1: water	C1: Methanol	D1: 2procent TFA	40.0 °C	Gradient 1 T
9	23-1 001-3501.D	SB CN	A1: water	C1: Methanol	D1: 2procent TFA	40.0 °C	Gradient 1 T

IR: LoadData <C:\Chem32\1\TEMP\reviewoffline.acaml>

Anonymous (localhost) LCOOffline 0 0 Ready

Stellen Sie Musterreporte und Dateien ein und Sie bekommen die Ergebnisse der kompletten Experimente.

Infinitely better reporting

Alle verfügbaren Informationen aus den Chromatogrammen können für das Reporting eingesetzt werden - across-samples, across-sequences, across-labs*! Tabellen und Diagramme sind verfügbar.

Method Development Summary Report
Creation Date: 10/24/2008 9:13:53 PM

Method Development Results Summary

Sequence: METOPROLOL1002008-07-30
Signal: DAD1 B, Sig=230,10 Ref=360,100
Min. Area%: 0.5

# Column / Solvent	# of peaks	Sum Resolution	Max RT
1-1 Chromatographic column Zorbax DB C18, 2.1x150mm, 1.5µm Solvents: 95% A1: H2O (water) 2%NaCl, 5% B1: ACN (5, 10% TFA)	13	3,469	7,769
1-2 Chromatographic column Zorbax DB C18, 2.1x150mm, 1.5µm Solvents: 95% A1: H2O (water) 2%NaCl, 5% B1: ACN (5, 10% TFA)	8	3,680	7,771
2-1 Chromatographic column Zorbax Eclipse Plus, 2.1x150mm, 1.5µm Solvents: 95% A1: H2O (water) 2%NaCl, 5% B1: ACN (5, 10% TFA)	11	6,270	3,989
2-2 Chromatographic column Zorbax Eclipse Plus, 2.1x150mm, 1.5µm Solvents: 95% A1: H2O (water) 2%NaCl, 5% B1: ACN (5, 10% TFA)	8	5,240	3,989
3-1 Chromatographic column Zorbax DB C18, 2.1x150mm, 1.5µm Solvents: 95% A1: H2O (water) 2%NaCl, 5% B1: ACN (5, 10% TFA)	10	6,012	4,463
3-2 Chromatographic column Zorbax DB C18, 2.1x150mm, 1.5µm Solvents: 95% A1: H2O (water) 2%NaCl, 5% B1: ACN (5, 10% TFA)	9	6,221	4,447
4-1 Chromatographic column Zorbax DB Pheno, 2.1x150mm, 1.5µm Solvents: 95% A1: H2O (water) 2%NaCl, 5% B1: ACN (5, 10% TFA)	9	10,976	2,750
4-2 Chromatographic column Zorbax DB Pheno, 2.1x150mm, 1.5µm Solvents: 95% A1: H2O (water) 2%NaCl, 5% B1: ACN (5, 10% TFA)	11	14,616	7,860
5-1 Chromatographic column Zorbax DB C18, 2.1x150mm, 1.5µm Solvents: 95% A1: H2O (water) 2%NaCl, 5% B1: ACN (5, 10% TFA)	7	4,355	6,951
6-1 Chromatographic column Zorbax DB C18, 2.1x150mm, 1.5µm Solvents: 95% A1: H2O (water) 2%NaCl, 5% B1: ACN (5, 10% TFA)	9	3,821	6,938
6-2 Chromatographic column Zorbax Eclipse Plus, 2.1x150mm, 1.5µm Solvents: 95% A1: H2O (water) 2%NaCl, 5% B1: ACN (5, 10% TFA)	23	5,951	8,223
6-3 Chromatographic column Zorbax Eclipse Plus, 2.1x150mm, 1.5µm Solvents: 95% A1: H2O (water) 2%NaCl, 5% B1: ACN (5, 10% TFA)	6	2,102	7,843
7-1 Chromatographic column Zorbax Eclipse Plus, 2.1x150mm, 1.5µm Solvents: 95% A1: H2O (water) 2%NaCl, 5% B1: ACN (5, 10% TFA)	21	7,971	6,919
7-2 Chromatographic column Zorbax Eclipse Plus, 2.1x150mm, 1.5µm Solvents: 95% A1: H2O (water) 2%NaCl, 5% B1: ACN (5, 10% TFA)	7	12,829	3,940
8-1 Chromatographic column Zorbax DB C18, 2.1x150mm, 1.5µm Solvents: 95% A1: H2O (water) 2%NaCl, 5% B1: ACN (5, 10% TFA)	6	5,648	6,714
8-2 Chromatographic column Zorbax DB C18, 2.1x150mm, 1.5µm Solvents: 95% A1: H2O (water) 2%NaCl, 5% B1: ACN (5, 10% TFA)	9	10,369	4,902
8-3 Chromatographic column Zorbax DB C18, 2.1x150mm, 1.5µm Solvents: 95% A1: H2O (water) 2%NaCl, 5% B1: ACN (5, 10% TFA)	16	6,971	7,973
8-4 Chromatographic column Zorbax DB Pheno, 2.1x150mm, 1.5µm Solvents: 95% A1: H2O (water) 2%NaCl, 5% B1: ACN (5, 10% TFA)	17	7,929	

MethDev1 Page 1 of 2

Method Development Summary Report
Creation Date: 10/24/2008 5:17:12 PM

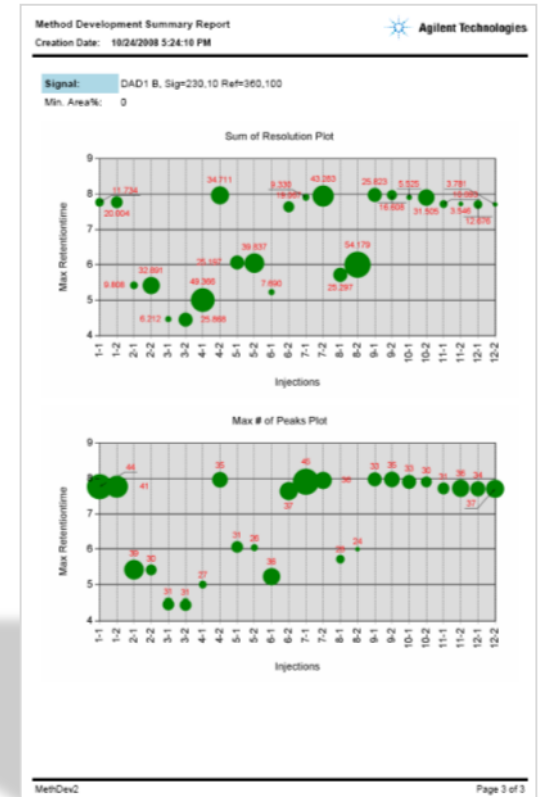
11 Sample 1 P1-A-01 Injection0811.M 1AA-1102.D 2
Chromatographic column: Zorbax Eclipse Plus, 2.1x150mm, 1.5µm
Solvents: 95% A1: H2O (water) 2%NaCl, 5% B2: MeOH (40, 10% TFA)

12 Sample 1 P1-A-01 Injection0812.M 1AA-1201.D 2
Chromatographic column: Zorbax DB C18, 2.1x150mm, 1.5µm
Solvents: 95% A1: H2O (water) 2%NaCl, 5% B2: MeOH (40, 10% TFA)

Max # of peaks found per signal: 55
Highest sum resolution per signal: 72,344

Data File	Signal	# of peaks	Sum Resolution
1AA-0101.D		44	11,7339
DAD1 B, Sig=230,10 Ref=360,100			
Peak RT	Tailing Factor	Resolution	Area%
DAD1 A, Sig=210,10 Ref=360,100		37	3,8950
Peak RT	Tailing Factor	Resolution	Area%
DAD1 D, Sig=260,10 Ref=360,100		22	47,6276
Peak RT	Tailing Factor	Resolution	Area%
0.248	-3.1416	1,0109	4022
0.294	-16.1762	1,0109	2031
0.381	0.7957	2,3312	4,9503
0.470	1.0233	2,3108	4,8956
0.844	0.9343	5,0491	1,311
2.195	-17.7206	10,0385	5504
2.308	1.8903	2,2352	1,2934
2.631	1.7904	4,0041	2,1559
2.639	-14.2448	1,0046	3071
2.720	-8.1042	1,2224	1,8477
2.854	4.4154	0,8508	78,3383
3.156	-23.6574	1,8856	561
3.252	-18.8536		3218
3.323	-25.0477		1,733
3.430	-7.0558		3,153
3.611	-8.2366		4,399
3.676	-18.5556		2,727

MethDev1 Page 2 of 11



*) across labs - only in combination with OpenLAB ECM

Infinitely better reporting

Viele Optionen zum Filtern und Sortieren der Ergebnisse...

z.B. Scouting Bedingungen sortiert nach maximalen Peakanzahl

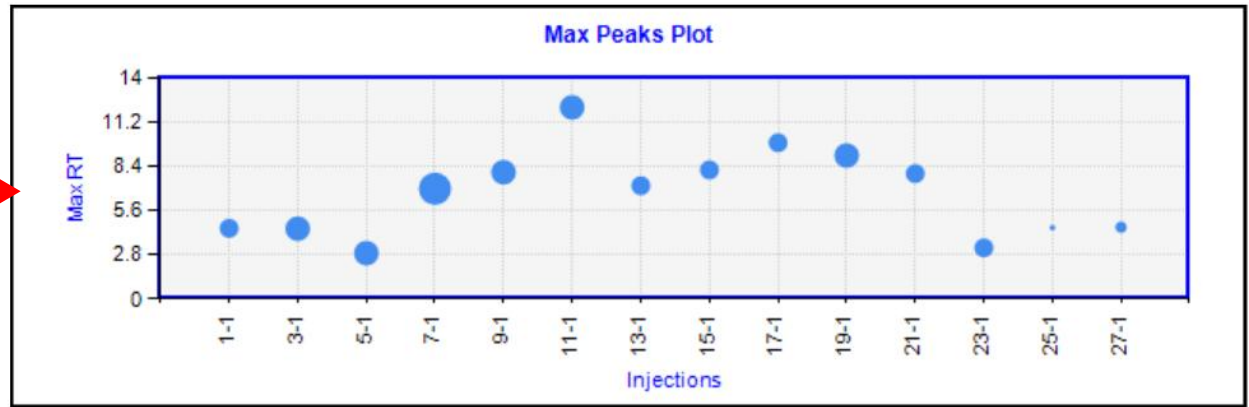


Method Development		Agilent Technologies						
Creation Date: 8/30/2010 3:57:39 PM								
Method Development Summary								
Sequence:		1260METHTERNARY22 2010-08-17 10-34-57						
Sample:		sigma			Signal:		DAD1A	
Peaks	#	Datafile	Column	Solvent1	Solvent2	Solvent3	Temp	Gradient
11	7-1	001-1101.D	SBAq	A1: water	B1: acetonitrile	D1: 2procent TFA	40.0 °C	Gradient 1 T
10	3-1	001-0501.D	Poroshell 120EC18	A1: water	B1: acetonitrile	D1: 2procent TFA	40.0 °C	Gradient 1 T
10	5-1	001-0801.D	SB CN	A1: water	B1: acetonitrile	D1: 2procent TFA	40.0 °C	Gradient 1 T
10	9-1	001-1401.D	SB C8	A1: water	B1: acetonitrile	D1: 2procent TFA	40.0 °C	Gradient 1 T
10	11-1	001-1701.D	Poroshell 120 SBC18	A1: water	B1: acetonitrile	D1: 2procent TFA	40.0 °C	Gradient 1 T
10	19-1	001-2901.D	SB C8	A1: water	C1: Methanol	D1: 2procent TFA	40.0 °C	Gradient 1 T
9	1-1	001-0201.D	Eclipse Plus C18	A1: water	B1: acetonitrile	D1: 2procent TFA	40.0 °C	Gradient 1 T
9	13-1	001-2001.D	EclipsePlus-C18	A1: water	B1: acetonitrile	D1: 2procent TFA	40.0 °C	Gradient 1 T
9	15-1	001-2301.D	EclipsePlus-C18	A1: water	C1: Methanol	D1: 2procent TFA	40.0 °C	Gradient 1 T
9	17-1	001-2601.D	Poroshell 120 SBC18	A1: water	C1: Methanol	D1: 2procent TFA	40.0 °C	Gradient 1 T
9	21-1	001-3201.D	SBAq	A1: water	C1: Methanol	D1: 2procent TFA	40.0 °C	Gradient 1 T

Infinitely better reporting

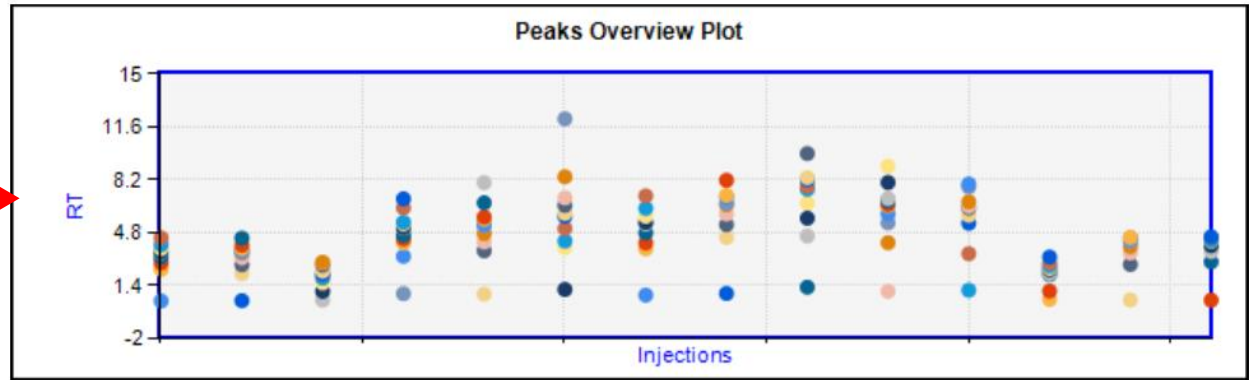
Viele Optionen zu grafischen Darstellung...

Experimente gegenüber maximale Retentionszeit und Peakanzahl (Bubblegröße)



Bubble size = # of found peaks

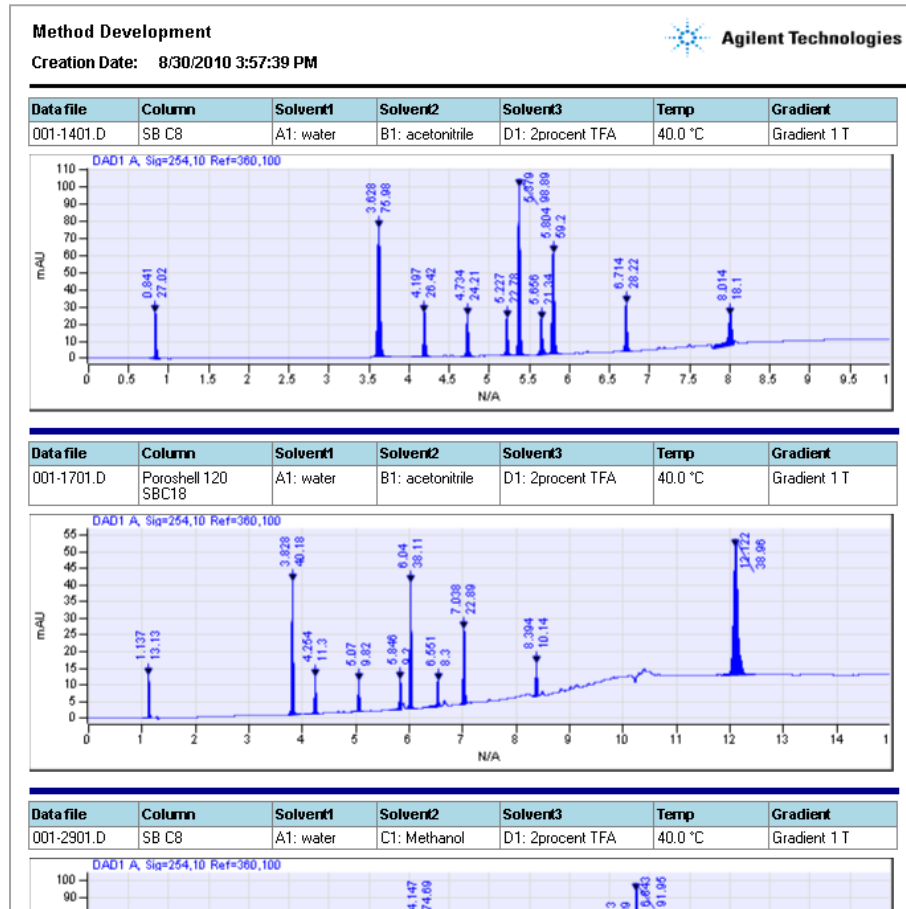
Experimente gegenüber Retentionszeit aller Peaks



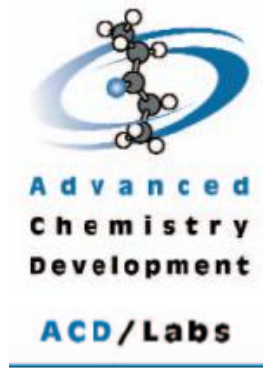
Infinitely better reporting

Chromatogramme mit Angabe der Testbedingungen

Datei, Säule, Eluenten, Temperatur, Gradientenname vom Method Scouting Wizard



Automated Method Optimization with Agilent Partners:



AutoChrom for ChemStation
Supports 1260 Infinity LC
1200 Series RRLC
1200 Series LC
1100 Series LC
1290 Infinity LC



ChromSword Auto and AutoRobust
for ChemStation
Supports 1290 Infinity LC
1260 Infinity LC
1200 Series RRLC
1200 Series LC
1100 Series LC

Applications – using the Method Scouting Wizard

Mit freundlicher Unterstützung von Dr. Matthias Farsch,
The Dow Chemical Company

Fallbeispiel 1: Analyse von Nitroaromaten

freigegeben!!!!

- Trennung von 9 Komponenten
- 50x4.6 mm Säulen, 1.8 μm and 2.7 μm Partikel
- Austesten von Säulen und vier Eluenten in 6 Stunden

Applications – using the Method Scouting Wizard

Fallsbeispiel 2: Hochauflösende Analyse von Insektiziden

Vom Kunden nicht

zum Verteilen

freigegeben!!!!

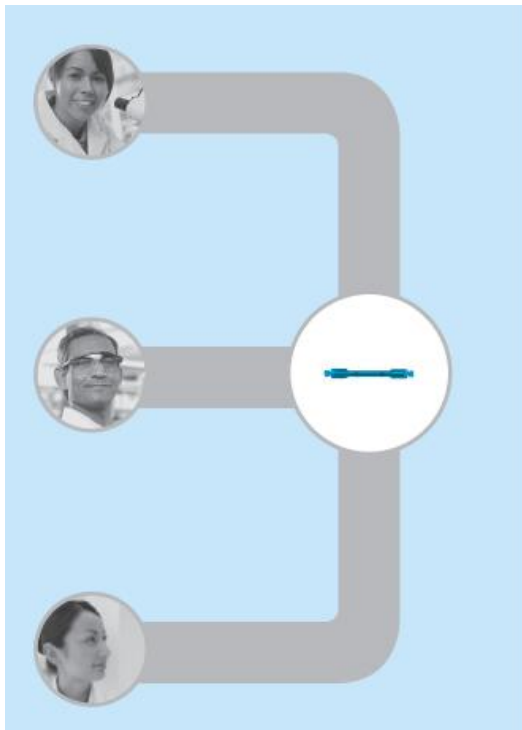
- Sehr komplexe Proben
- 150 x 2.1 mm Säulen, 1.8 μm und 2.7 μm Partikel
- **Screening von Säulen, Gradienten und Puffern über Nacht**

Mit freundlicher Unterstützung von Dr. Matthias Pursch, Dow Germany

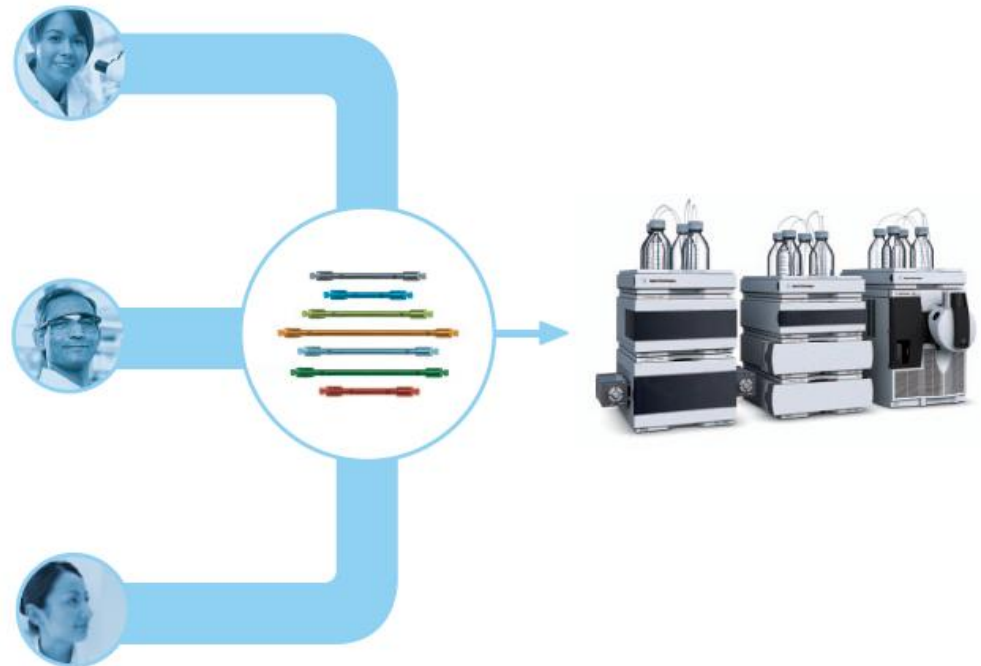


2. Anwendungsbereich - Multimethoden

Ohne Multimethodenmodul: nur ein Setup (Säule, Eluent) für verschieden Anwendungen nutzbar



Mit Multimethodenmodul: wählen Sie das beste Setup für Ihre Trennung per Mausklick



Typischer Laboralltag

Anwender 1:
Reaction
monitoring



Kurze Säule
mit normaler
Polarität

Anwender 2:
Analyse vor
Aufreinigung



Kurze Säule
mit mehreren
Polaritäten

Anwender 3:
Test auf
Zwischen-
produkten



Kurze Säule
mit hoher
Polarität

Anwender 4:
Reinheits-
bestimmung



Lange Säule
mit mittlerer
Polarität

Kurz: verschiedene Anwender benötigen unterschiedliche Trennbedingungen

Die Easy-Access SW ermöglicht das Setzen von Prioritäten, automatische Equilibrierung nach Säulenwechsel, Ergebnisse per email...

The image shows a chromatography system with four columns and a software interface. The software interface is titled "Agilent G2725AA Easy-Access Software" and includes the version "Version A.05.01 Build 280" and copyright information "Agilent Technologies, Inc. 2007". The interface displays a chromatogram. Below the system, four red arrows point to the columns, each with a label describing its characteristics.

Kurze Säule mit normaler Polarität

Kurze Säule mit mehreren Polaritäten

Kurze Säule mit hoher Polarität

Lange Säule mit mittlerer Polarität

Agilent Multi-method System und Easy-Access-Software

Anwender 1:
Reaction monitoring

Anwender 2:
Analyse vor
Aufreinigung

Anwender 3:
Test auf
Zwischen-
produkten

Anwender 4:
Reinheits-
bestimmung

Agilent Easy-Access - STATUS

1 run in queue (est. 12 mins to completion)

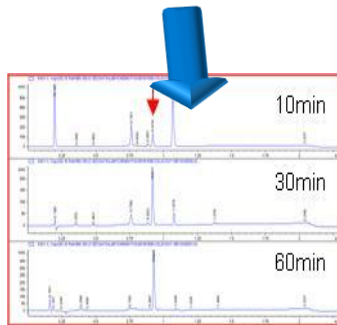
Sample Queue

Loc	Input	Notebook #	+/-	Method
1	P1-A-02	A-02		Purity check on 150mm length 161008-P

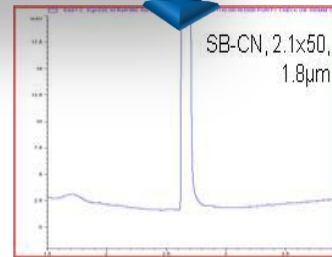
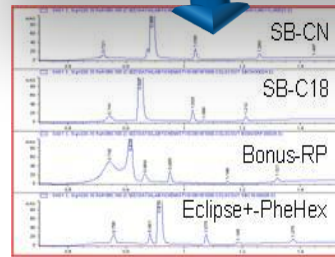
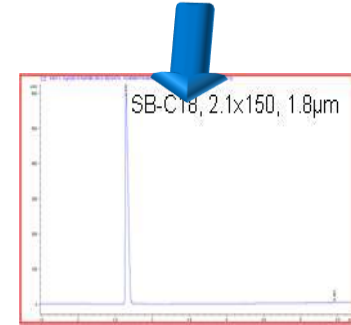
JobID	User	Group	LoadDate	
1	081016-134354	Arno	Chemist	10/16/08 13:43:54
2	081016-130122	susan	Chemist	10/16/08 13:01:22
3	081016-131458	Arno	Chemist	10/16/08 13:14:58
4	081016-103754	Arno	Chemist	10/16/08 10:37:54

Submit New Sample

Kurze Säule
mit normaler
Polarität



Lange Säule
mit mittlerer
Polarität



Optimum conditions for different users!

Das Agilent 1200 Infinity Multimethoden System

- **Reduziert die Zeit zum Umstellen des Systems**
- **Deutliche Vorteile bei der Nutzung der Nacht und des Wochenendes**
- **Methodenoptimierung in kürzerer Zeit**
- **Angebot an idealen Trennbedingungen**
- **für 600 oder bis zu 1200 bar!**



Haben Sie Fragen?



Appendix



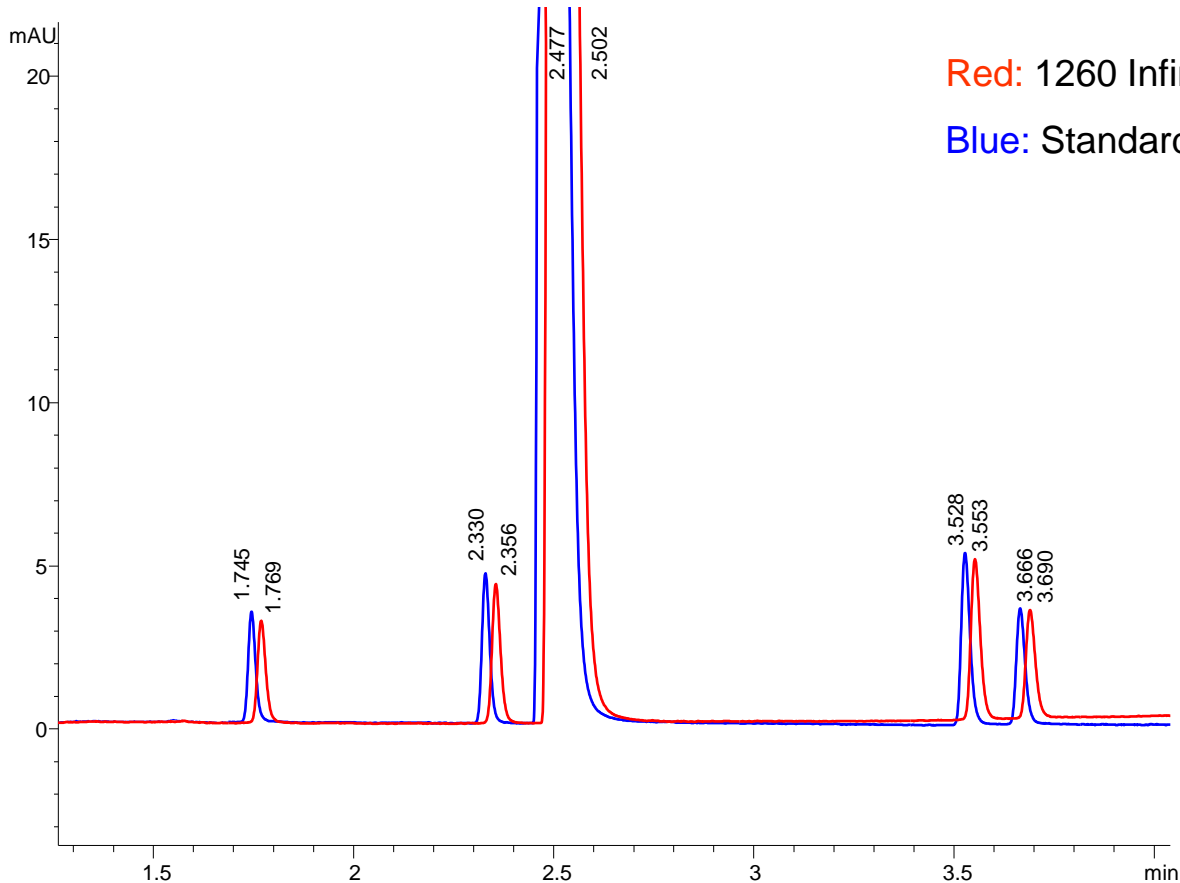
Preise

- **G2196AA Agilent ChemStation Method Scouting Wizard**
2082€
- **TCC mit Ventilmotor** **6392€**
- **Ventil 5900€ (600 bar), 7380€ (1200 bar)**
- **Easy Access** **4023€**

Performance

Additional extra column volume from additional capillaries and valves will cause some additional delay and peak broadening.

Performance for a **2.1x50mm, 1.8 μ m** column – most demanding in point of dispersion!



Red: 1260 Infinity Binary Method development system

Blue: Standard 1260 Infinity Binary system

Zorbax SB C18 **2.1x50 mm, 1.8 μ m**
Flow rate: 0.5 ml/min
Gradient: 10 to 45% in 4min,
Injection volume: 1 μ l

Performance

Performance for a **2.1x50mm, 1.8 μ m** column:

Peak number	Standard 1260 Binary system				Method development 1260 Binary system				Difference (MD vs. Std)			
	retention time (min)	Peak width (sec)	Symmetry	USB tailing	retention time (min)	Peak width (sec)	Symmetry	USB Tailing	retention time (sec)	Peak width	Symmetry (ratio)	USB tailing (ratio)
1	1.745	1.164	0.789	1.250	1.769	1.302	0.761	1.257	+1.44	+11.9%	96.5%	100.6%
2	2.330	1.236	0.771	1.224	2.356	1.350	0.767	1.224	+1.56	+9.2%	99.5%	100.0%
3	2.477	3.276	0.328	2.367	2.502	3.348	0.356	2.192	+1.50	+2.2%	108.5%	92.6%
4	3.528	1.398	0.715	1.317	3.553	1.482	0.734	1.292	+1.50	+6.0%	102.7%	98.1%
5	3.666	1.398	0.730	1.304	3.690	1.518	0.730	1.310	+1.44	+8.6%	100.0%	100.5%
average:									+1.50	+7.6%	101.4%	98.3%

With a 1290 Infinity system and/or larger columns (ID and/or length), larger particles and/or higher flow rates even less difference to a standard LC-system!

TCC and Pump-valve Cluster - Software control

- 1260 / 1290 Infinity only by **RC.net drivers!**
- ChemStation B04.02 DSP2 or higher
- EasyChrom Elite 3.3.2 SP2 or higher
- Method Scouting Wizard A02.02 (only with RC.net drivers)
- EasyAccess B02.01
- MassHunter planned for ca. January 2011

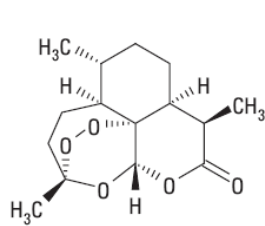
	CS B04.01 SP1*)	MSW A01.01*)	CS B04.02 DSP2		MSW A02.01	EZChrom Elite	MassHunter
			Classic	RC.net			
1290 Infinity	✗	✗	✗	✓	✓	✓ 3.3.2 SP2	Planned January 2011
1200 LC/RRLC	✓	✓	✓	✓	✓**))		
1100 LC	✓	✓	✓	✓	✓**))		



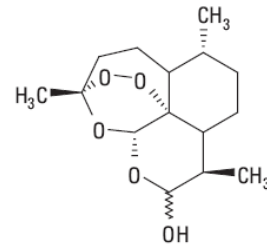
Applications – using ChromSword Auto

by courtesy of Rodger Stringham, William J. Clinton Foundation & William Long, Agilent

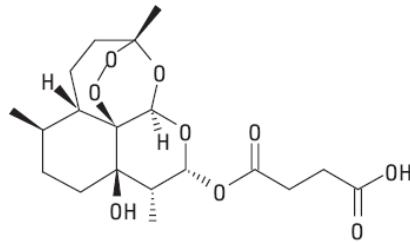
A mixture of four natural product-derivatives needed to be separated:



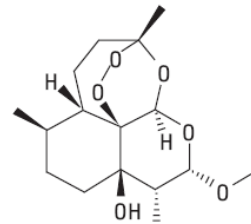
Artemisinin



Dihydroartemisinin



Artesunate



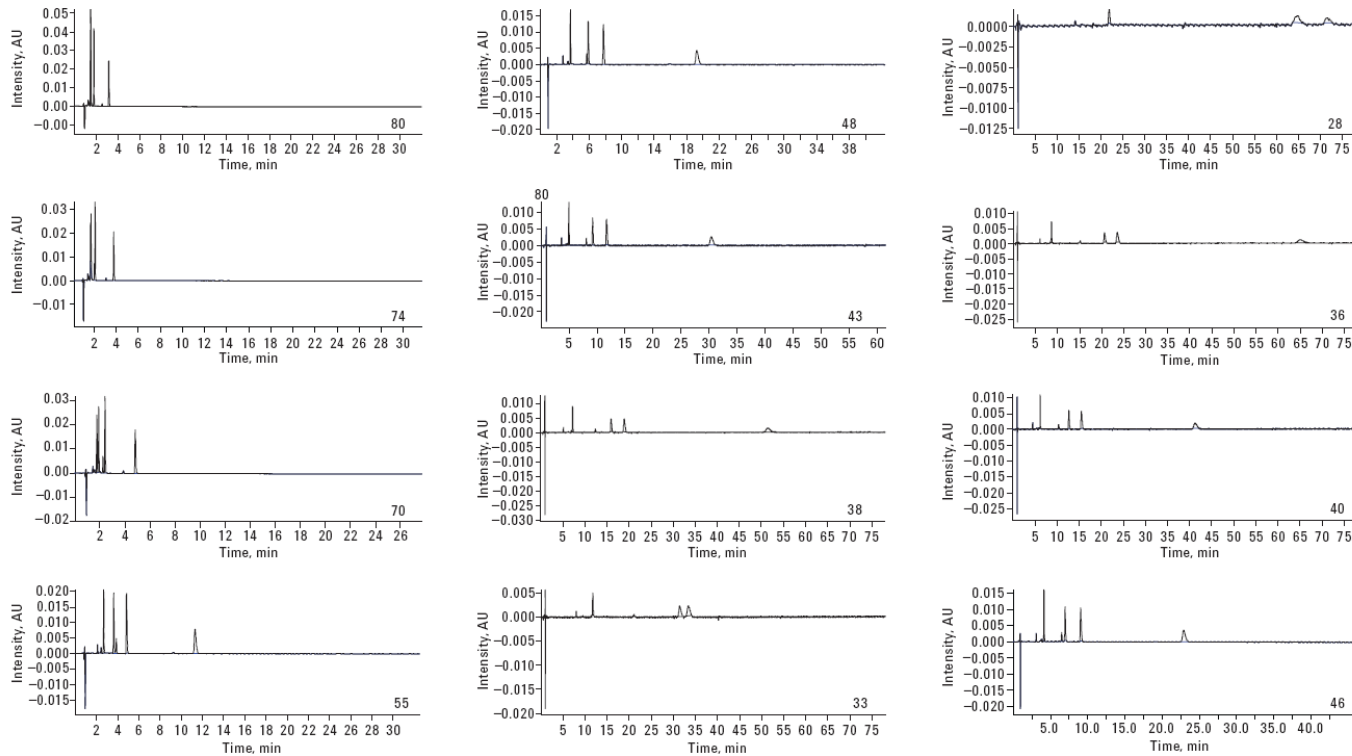
Artemether

Pub. No 5990-3617EN

Applications – example using ChromSword

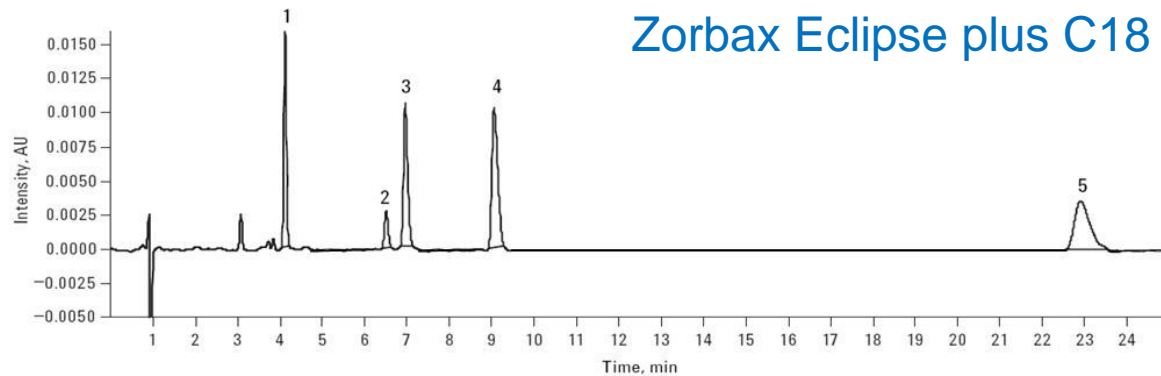
On a RRLC-based Method Development system three different columns have been screen automatically.

Goal was an **isocratic** method with **less than 30 min run time** and **baseline separation of all peaks**.



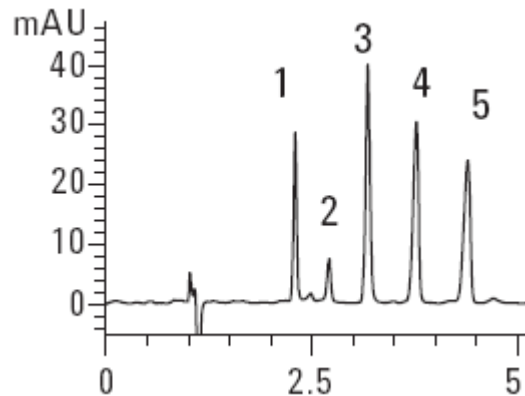
Applications – using ChromSword

After 12 hours **unattended** operation the method development goal was achieved:



- 1 Dihydroartemisinin
- 2 Dihydroartemisinin impurity
- 3 Artesunate
- 4 Artemisinin
- 5 Artemether

Zorbax StableBond CN



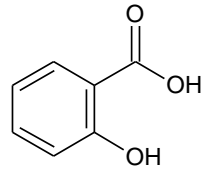
Minimum $R_s = 2.6$
Less than 5 min run-time with a SB-CN column

10 μ L 0.25 mg/mL each artemisinin compound
46 % acetonitrile/54 % water
 (each with 0.1 % formic acid) 1 mL/min 220 nm

Applications – using AutoChrom

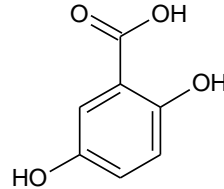
by courtesy of Margaret Antler, Andrey Vazhentsev, Advanced Chemistry Development, Inc. and William Long, Agilent

Analysis of Salicylic Acid In-Process Impurities (skin care product)



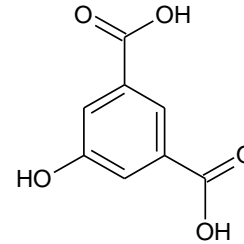
Salicylic Acid

pKa= 2.98



Gentisic Acid

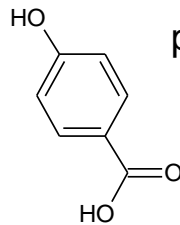
pKa= 2.97



5-Hydroxyisophthalic Acid

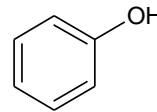
pKa= 3.41

pKa= 4.48

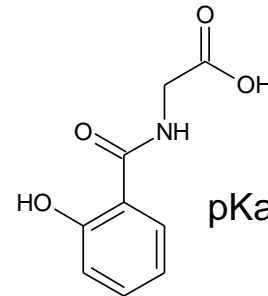


4-Hydroxybenzoic Acid

pKa= 10.01



Phenol



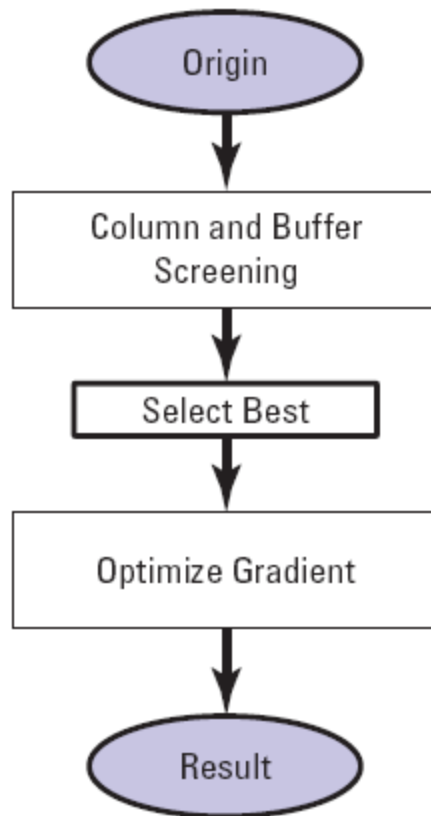
Salicylglycine

pKa= 3.54

Pub. No 5990-4809EN

Applications – using AutoChrom

Separation strategy defined in AutoChrom:



The screenshot shows the 'Configuration' window in AutoChrom, detailing the setup for 'Instrument 1'. The configuration includes:

- Column Compartments:**
 - 1200 Column Thermostat SM, 6 col.
 - SB-C18, 50 x 4.6 (1.8)
 - Eclipse Plus C18, 50 x 4.6 (1.8)
 - Bonus RP, 50 x 4.6 (1.8)
 - Eclipse Plus Phenyl Hexyl, 50 x 4.6 (1.8)
 - 1200 Column Thermostat SM, 6 col.
 - Eclipse Plus C8, 50 x 4.6 (1.8)
 - SB-Phenyl, 50 x 4.6 (1.8)
 - SB-Aq, 50 x 4.6 (1.8)
- 1200 Binary Pump SL, Pressure < 600 bars:**
 - A, idle = 50%, max = 100%**
 - 0.1 % TFA, *Weak*, 0.8803 liters
 - 0.1 % Formic Acid, *Weak*, 0.9486 liters
 - 0.1 % Acetic Acid, *Weak*, 0.9474 liters
 - pH 4.8 0.01M AcONH4, *Weak*, 0.9486 liters
 - pH 6.5 0.01M AcONH4, *Weak*, 0.9486 liters
 - Water, *Weak*, 0.8907 liters
 - B, idle = 50%, max = 100%**
 - MeCN, *Strong*, 0.5987 liters
 - MeOH, *Strong*, 1 liters
- Data Acquisition:**
 - 1100/1200 Diode Array Detector SL
 - SA 230
- 1200 High Performance Autosampler SL+, Inj. Volume < 40 ul:**
 - Standard
- Sample Containers:**
 - Plate *54VialPlate*, 54 items
 - A-1: Salicylic Acid Impurities, 1310 ul

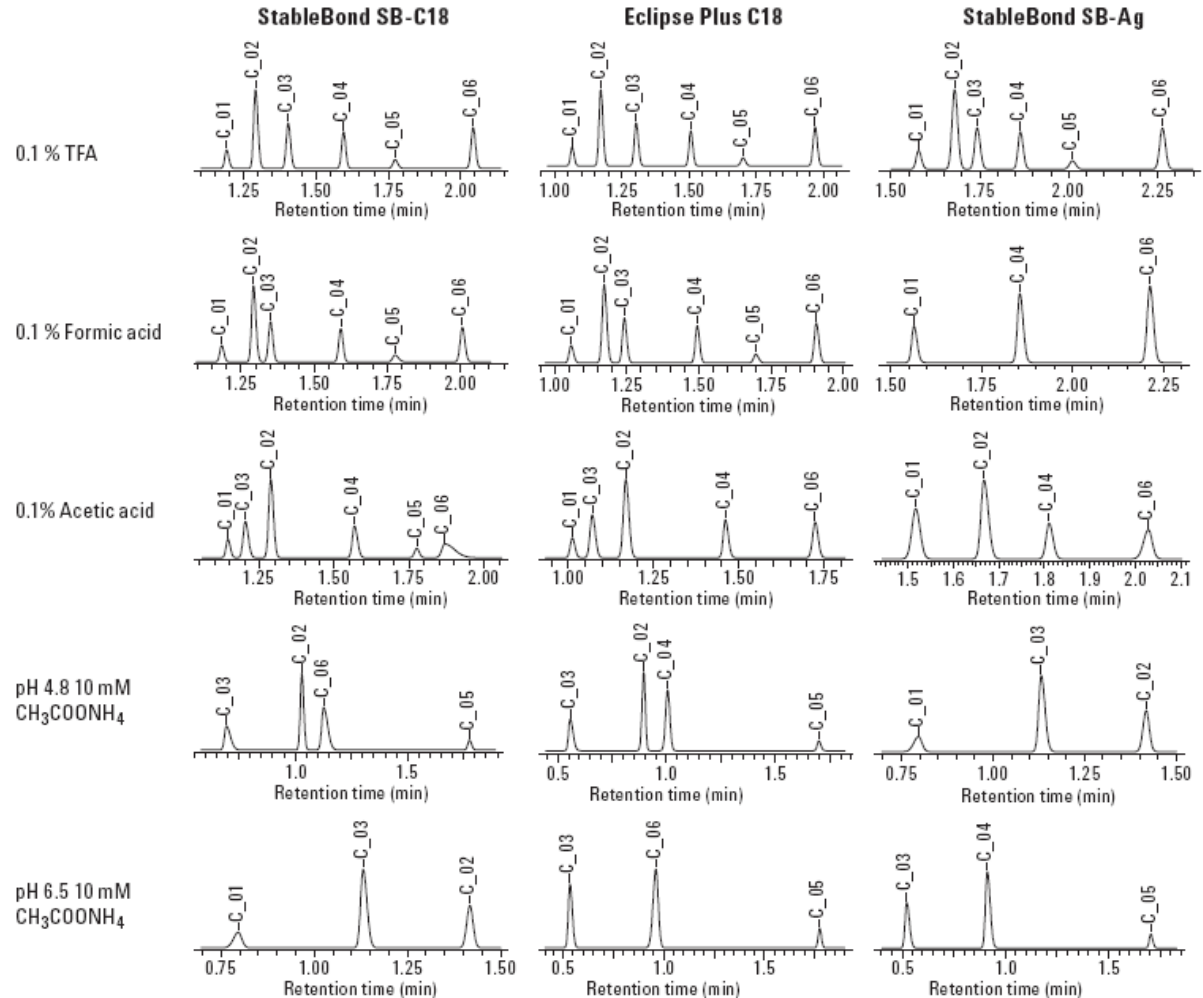


Applications – using AutoChrom

First wave of experiments:

3 columns

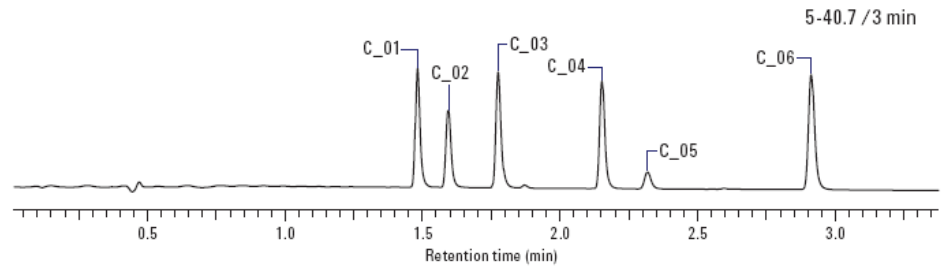
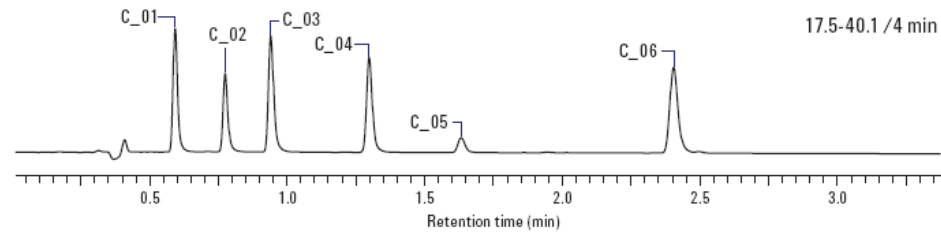
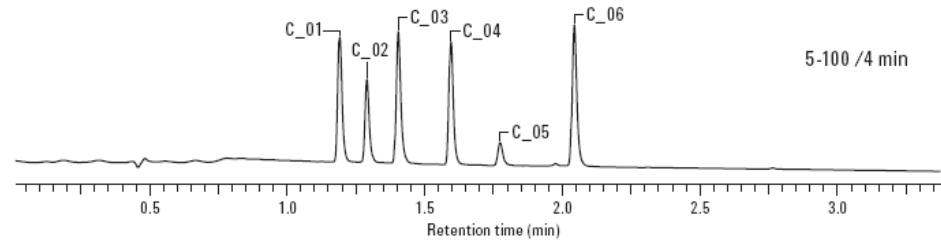
5 solvents



Applications – using AutoChrom

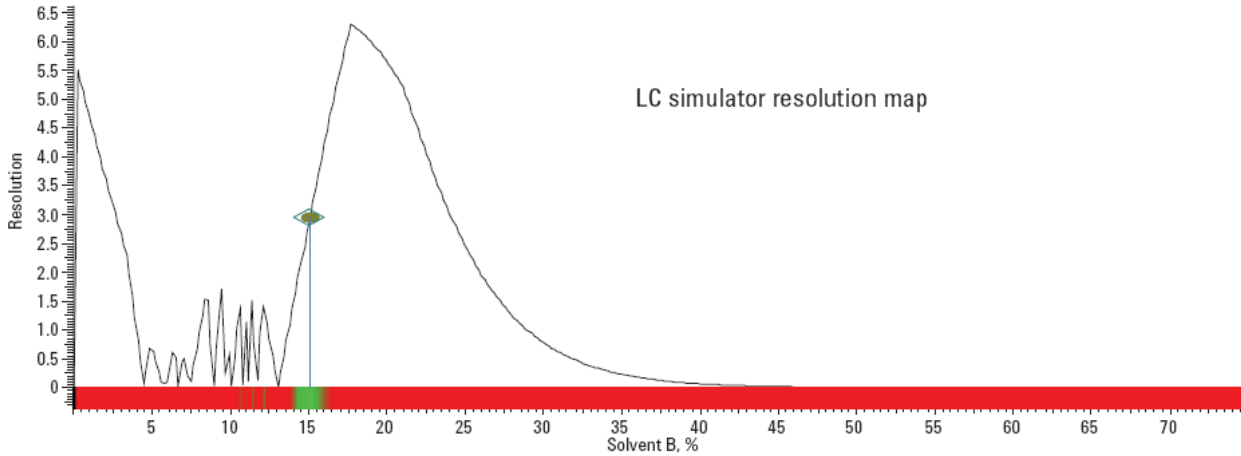
Automated Gradient optimization:

Experiments										
Experiment	Suit.	Suit.***	Total	Unlabeled	C_01	C_02	C_03	C_04	C_05	C_06
5-100% (4 min)	-	-	6/8		1.091	1.187	1.282	1.484	1.691	2.037
5-100% (4 min)	-	-	6/8		1.19	1.29	1.404	1.595	1.774	2.044
Salicylic Acid Impurities	-	-	6/8		1.19	1.29	1.404	1.595	1.774	2.044
Trial #1	-	-	0/8							
Trial #2	-	-	6/8		1.19	1.29	1.404	1.595	1.774	2.044
UV dad1.uv	-	-	6/8		1.19	1.29	1.404	1.595	1.774	2.044
UV TAC	-	-	0/8	6						
UV dad1A.ch	-	-	0/8	6						
17.5-40.1% (4 min)	-	-	6/8		0.597	0.786	0.964	1.361	1.705	2.449
17.5-40.1% (4 min)	-	-	6/8		0.595	0.777	0.942	1.3	1.635	2.405
Salicylic Acid Impurities	-	-	6/8		0.595	0.777	0.942	1.3	1.635	2.405
Trial #1	-	-	6/8		0.595	0.777	0.942	1.3	1.635	2.405
UV dad1.uv	-	-	6/8		0.595	0.777	0.942	1.3	1.635	2.405
UV TAC	-	-	0/8	6						
UV dad1A.ch	-	-	0/8	6						
5-40.7% (3 min)	-	-	6/8		1.538	1.631	1.773	2.057	2.245	2.832
5-40.7% (3 min)	-	-	6/8		1.482	1.595	1.772	2.152	2.319	2.91
Salicylic Acid Impurities	-	-	6/8		1.482	1.595	1.772	2.152	2.319	2.91
Trial #1	-	-	6/8		1.482	1.595	1.772	2.152	2.319	2.91
UV dad1.uv	-	-	6/8		1.482	1.595	1.772	2.152	2.319	2.91
UV TAC	-	-	0/8	6						
UV dad1A.ch	-	-	0/8	6						
15%	-	-	6/8		0.945	1.191	1.543	2.249	2.458	4.053
15%	-	-	6/8		0.715	0.944	1.194	2.014	2.299	5.349
Salicylic Acid Impurities	-	-	6/8		0.715	0.944	1.194	2.014	2.299	5.349
Trial #1	-	-	6/8		0.715	0.944	1.194	2.014	2.299	5.349
UV dad1.uv	-	-	6/8		0.715	0.944	1.194	2.014	2.299	5.349
UV TAC	-	-	0/8	6						
UV dad1A.ch	-	-	0/8	6						

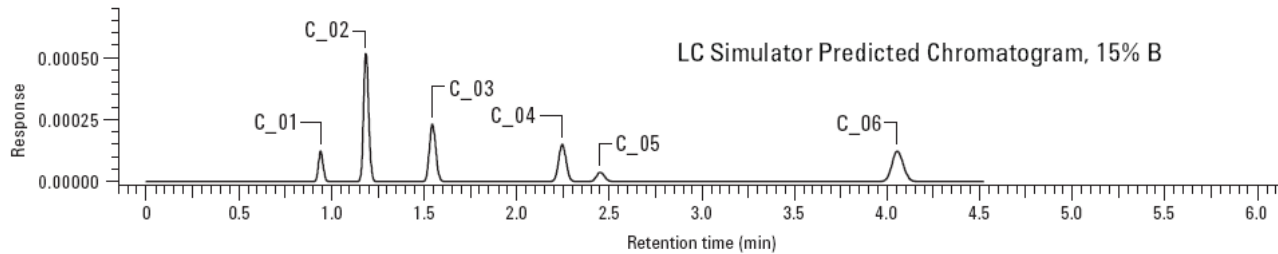


Applications – using AutoChrom

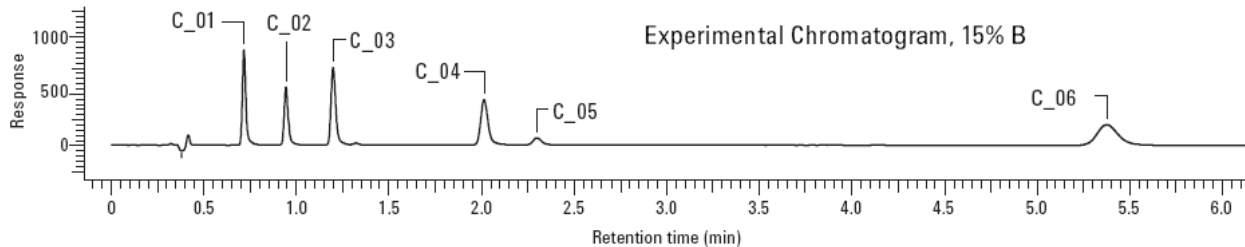
Retention model build in ACD/Labs LC-Simulator (part of AutoChrom)



LC Simulator



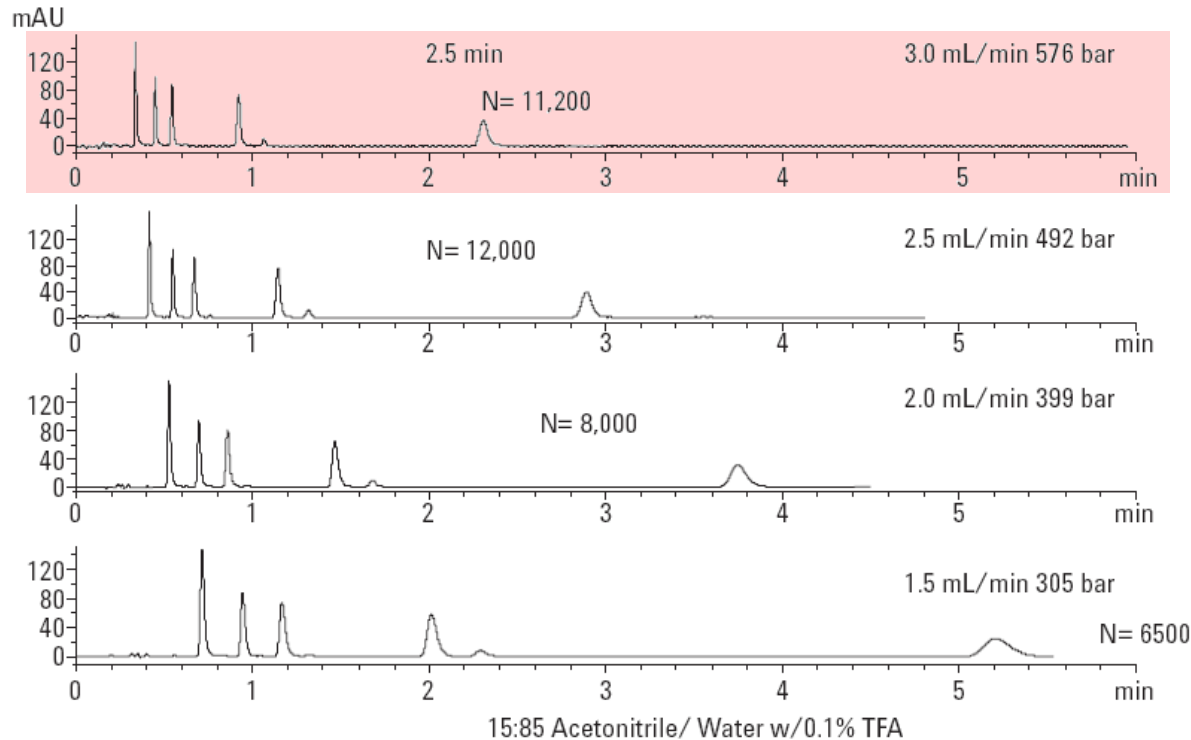
LC Simulator Prediction



Results from Chemstation

Applications – using AutoChrom

Final flow optimization – final run-time 2.5 min



Column and buffer screening performed **unattended overnight**. In total 20 h required until the final method was achieved. **AutoChrom managed and documented** all chromatographic conditions used during the development.

Quality by Design

Agilent 1200 Infinity Series LC Method Development System and ChromSword AutoRobust

The ChromSword Group
www.chromswordauto.com



Quality by Design – ChromSword AutoRobust

- AutoRobust controls the HPLC system and performs the **ROBUSTNESS** experiments fully automatically.
- Experiment design for the evaluation of HPLC method robustness is easily programmable with the **AutoRobust Wizard**.
- Results of experiments can be rapidly browsed and a **report is generated automatically** with Report Viewer.
- **AutoRobust** can be used as stand-alone program or add-on to the **ChromSword®Auto** software



Quality by Design – ChromSword AutoRobust

Enables fully **automatic** experiments for characterization of a method design space

Univariant (one-factor-at-a-time) tests

- 10 factors in 1-5 levels

Multivariant (several-factors-at-a-time)

- Full factorial design - up to 5 factors in 1-2 levels
- Full factorial design - up to 3 factors in 1-3 levels
- Plackett-Burman design – up to 7 factors in 1-5 levels

ChromSword® AutoRobust

Plackett-Burman design for 12 runs and up to 11 two-level factors[2]

Run	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	X ₈	X ₉	X ₁₀	X ₁₁
1	+	+	+	+	+	+	+	+	+	+	+
2	-	+	-	+	+	+	-	-	-	+	-
3	-	-	+	-	+	+	+	-	-	-	+
4	+	-	-	+	-	+	+	+	-	-	-
5	-	+	-	-	+	-	+	+	+	-	-
6	-	-	+	-	-	+	-	+	+	+	-
7	-	-	-	+	-	-	+	-	+	+	+
8	+	-	-	-	+	-	-	+	-	+	+
9	+	+	-	-	-	+	-	-	+	-	+
10	+	+	+	-	-	-	+	-	-	+	-
11	-	+	+	+	-	-	-	+	-	-	+
12	+	-	+	+	+	-	-	-	+	-	-



Quality by Design – ChromSword AutoRobust

Unique and maximal flexibility for characterization of a method design space

Any combinations and scenario are possible.

Example of a combined scenario:

1. one-factor-at-a-time tests to determine critical analytical parameters (CAP) – 10 factors



2. Full factorial design with CAPs – 2-3 factors



3. Plackett-Burman design – 7 factors for confidence

Quality by Design – ChromSword AutoRobust

AutoWizard set-up

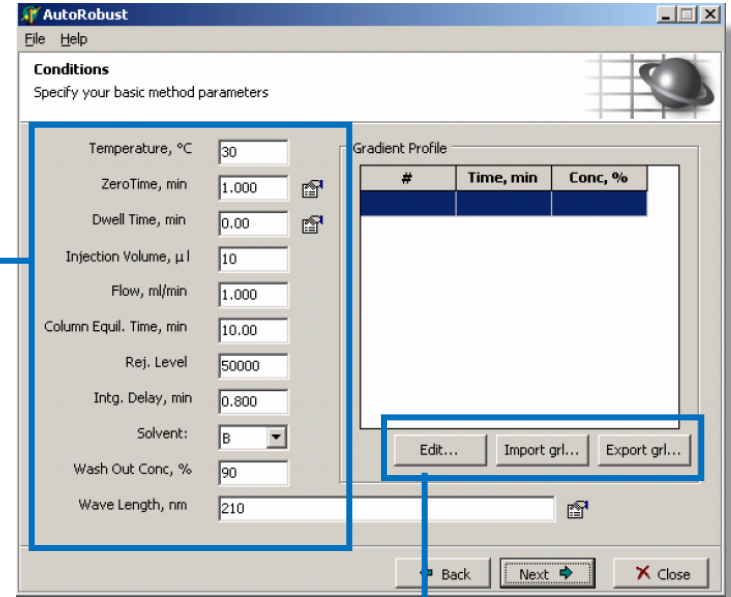
- a) **Configuration**
↓
specify columns (e.g. different batches or sorbents) and solvents (e.g. buffer compositions using a solvent switching valve)
- b) **Conditions**
↓
basic method parameters (input method manually or import from ChromSwordAuto®)
- c) **Tests**
↓
specify test plan – parameters to change during testing and a design – one or multi-parameter tests
- d) **Run**
↓
read and check your plan and run experiments
- e) **Report**

Quality by Design – ChromSword AutoRobust

b1: basic method parameters

parameter	used to	range
temperature	set oven tem. (°C)	0 – 100
zero time	set or calculate the column zero time (min)	0.01 – 10
dwel time	set or calculate the system dwell time (min)	0 – 5
injection volume	set injected amount (µL)	1 – 50
flow rate	set the flow rate (mL/min)	0.1 – 9
column equil. Time	set column equilibration time (min)	0 – 20
intg. delay	set time initial point to start integration of chromatogram (min). peaks with RT < int. delay value will not be integrated.	0 – 10
solvent	set solvent to be used (for quaternary pumps)	B, C, D
wash out conc.	set concentration of B, C, or D solvent to be used to wash column after finishing the method (%)	50 – 100
wavelength (or M/Z)	set wavelength of detector (nm) or wavelength range (DAD)	190 – 950

conditions



edit	specify gradient profile
import/export	import/export gradient profile as a *.grl (ChromSword format) file

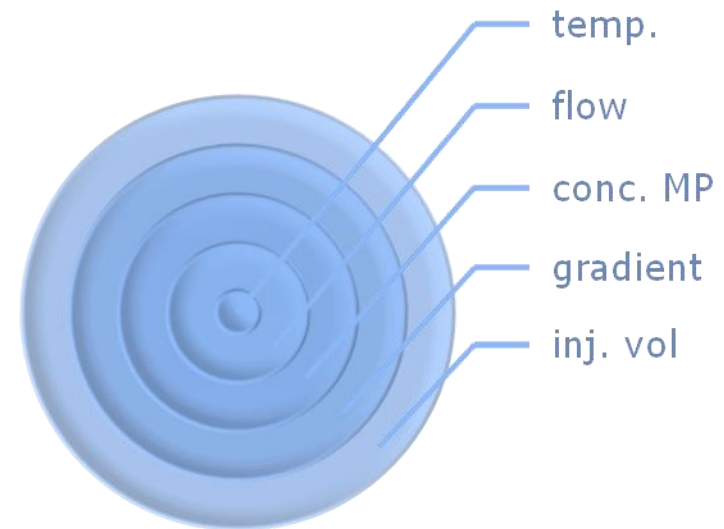
ChromSword® AutoRobust

Quality by Design – ChromSword AutoRobust

c1: AutoRobust test designs

n Method factors

1. temperature
2. flow rate
3. concentration
4. gradient break point positions
5. injection volume
6. equilibration time
7. Wavelength
8. column batch
9. buffer concentration/pH
10. solvent (different suppliers)
11. column stability

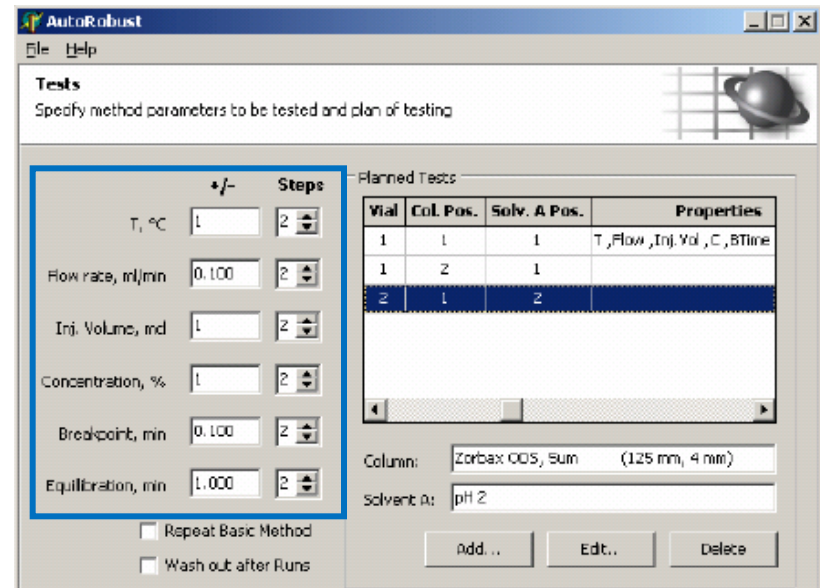


Quality by Design – ChromSword AutoRobust

c2: tests

parameter	+/- range	levels
temperature (°C)	1 - 5	0 - 5
flow rate (mL/min)	0.1 - 0.5	0 - 5
injection volume (µL)	1 - 5	0 - 5
concentration (%)*	1 - 5	0 - 5
break point (min)**	0.1 - 0.5	0 - 5
equilibration time (min)	0.1 - 2	0 - 5

tests

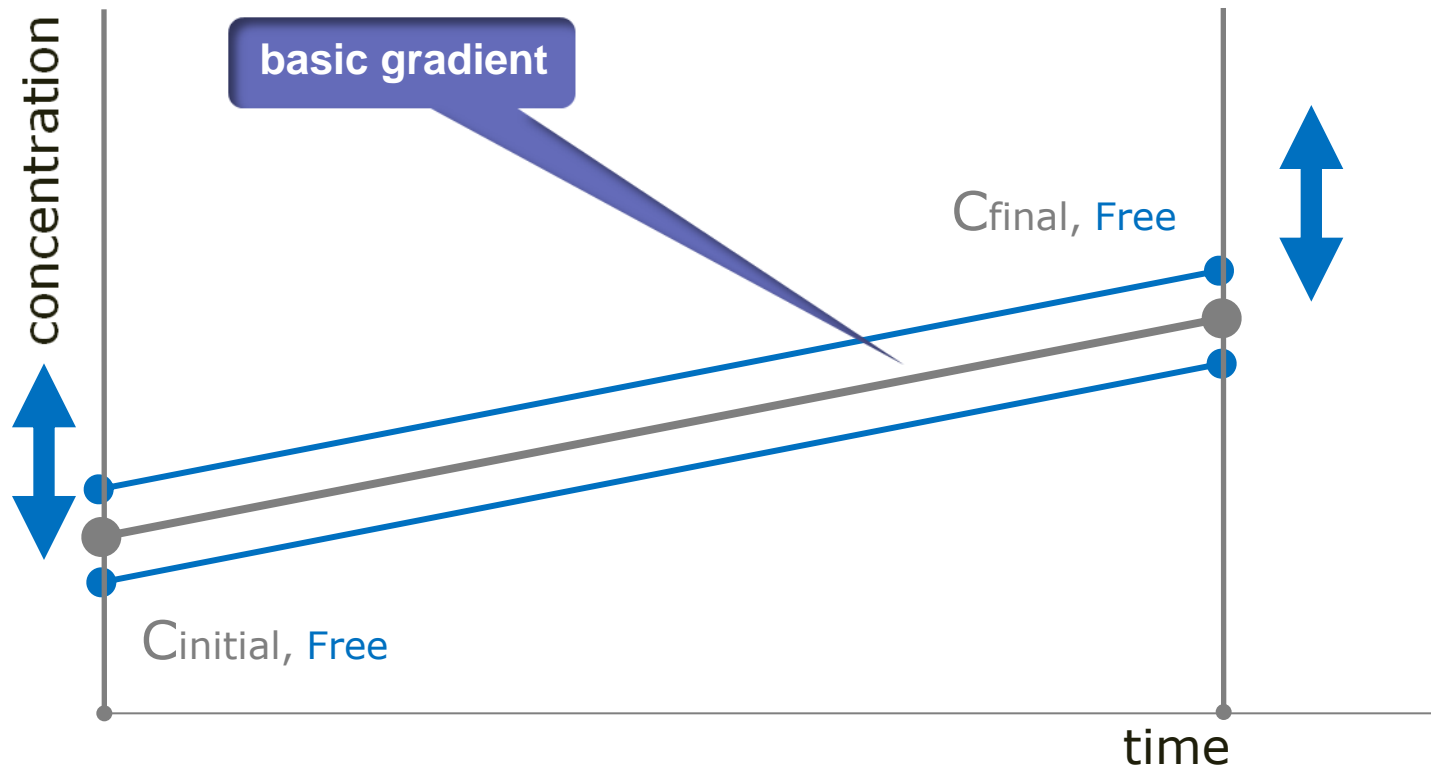


- * concentration of organic solvent
- * gradient method – changes concentration of all breakpoints by same value
- ** influence of gradient slope
- ** gradient method – change of gradient time for every breakpoint by same value

Quality by Design – ChromSword AutoRobust

ChromSword® AutoRobust

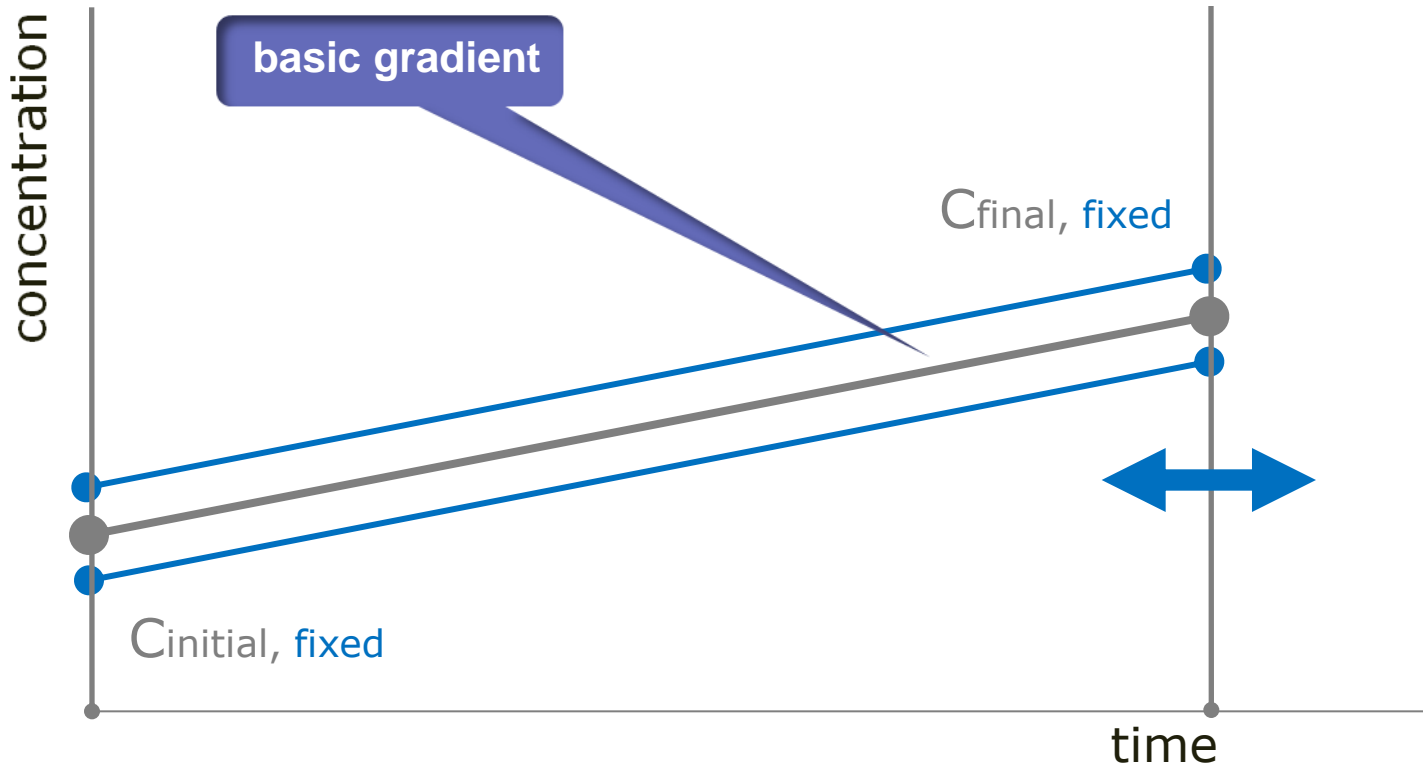
c: variation of concentration



Quality by Design – ChromSword AutoRobust

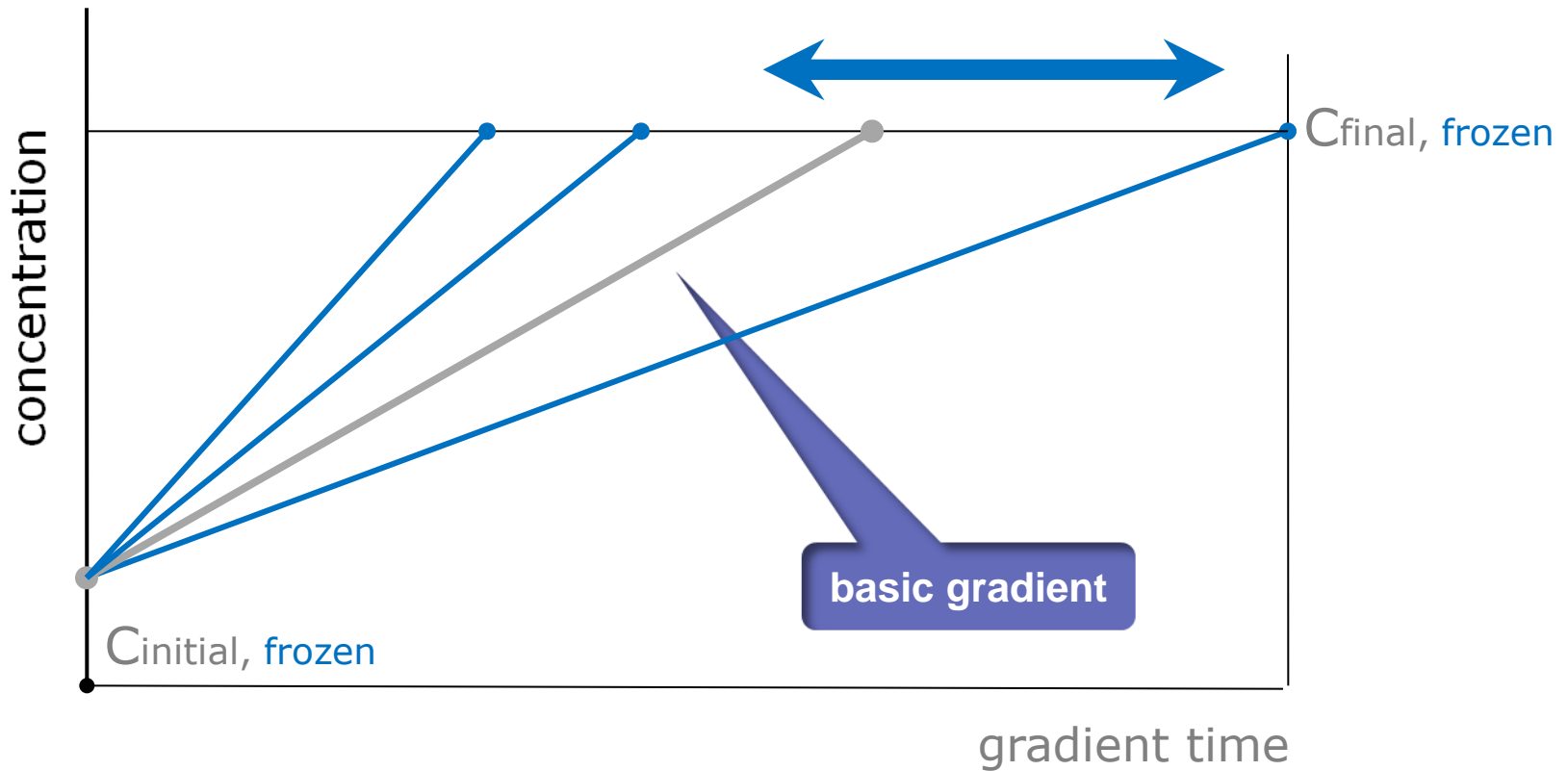
ChromSword® AutoRobust

C: variation of break point



Quality by Design – ChromSword AutoRobust

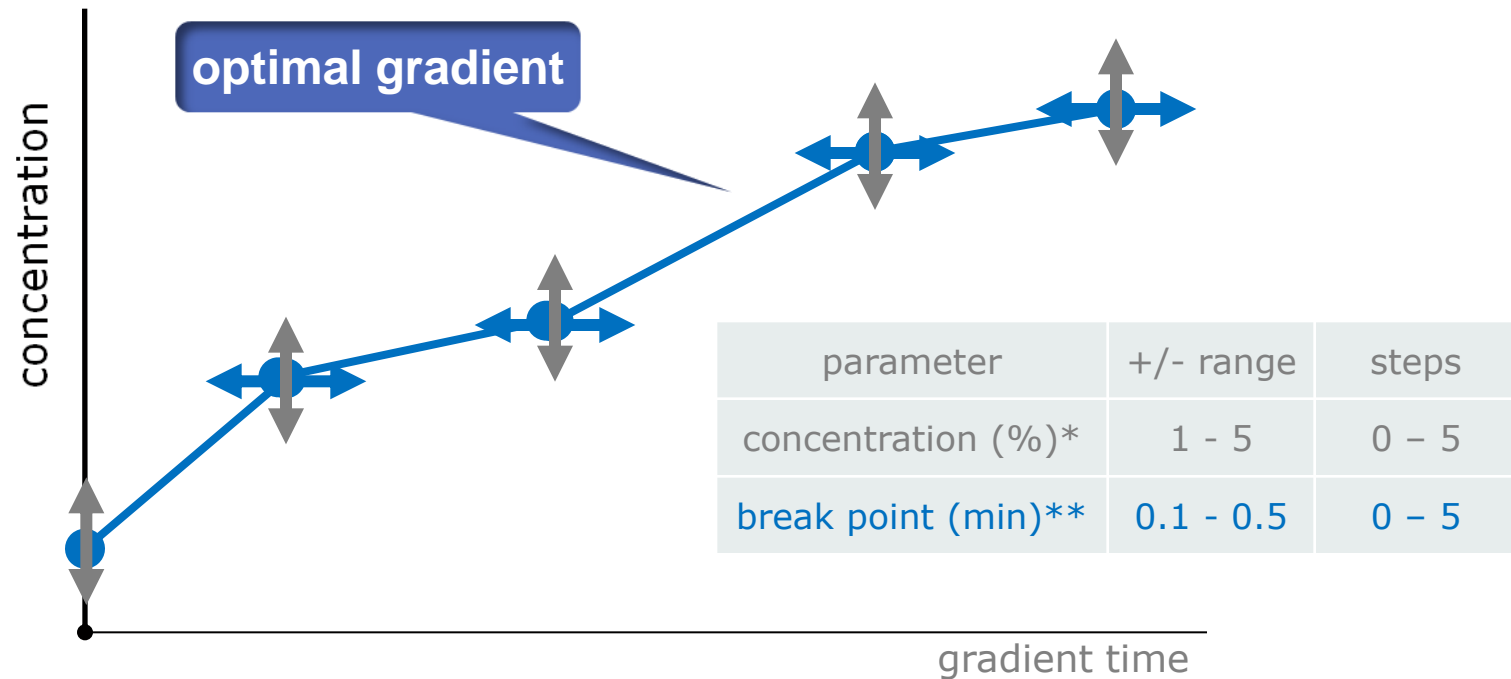
c: break point variation = gradient slope



ChromSword® AutoRobust

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c: variation of concentration & break points



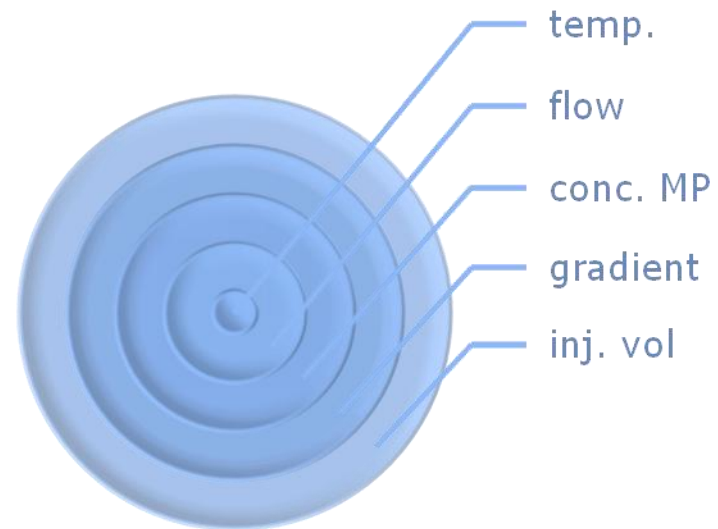
* concentration of organic solvent
gradient method – changes concentration of all breakpoints by same value

** influence of gradient slope
gradient method – change of gradient time for every breakpoint by same value

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c1: Automatic reporting for robustness studies

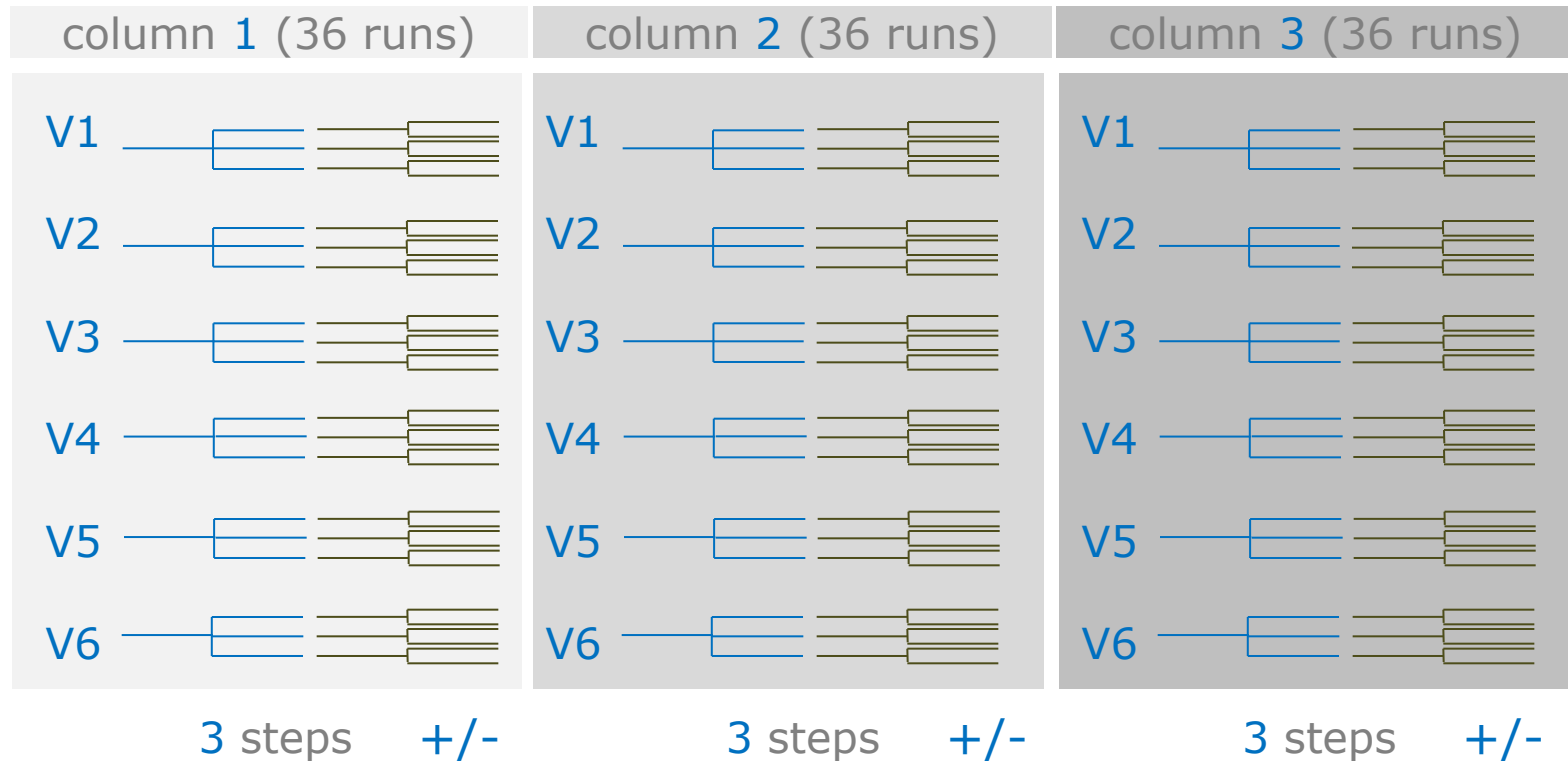
1. Chromatograms
2. Resolution for all peak pairs
3. Retention time
4. Area
5. Area Percent
6. Peak width
7. Peak height
8. Number of plates
9. Mean
10. Standard Deviation,
11. +/- %



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set-up time example

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3 columns x 6 variables V x 3 steps x 2 (+/-) + basic method (=3 columns x 6)

= 126 runs

set up in approx. 10 min

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Automation vs. routine work

- creation of sequences
- system and column wash out
- column equilibration
- testing of
 - gradient slope
 - concentration deviation effect
 - temperature effect
 - injection volume
 - different pHs
 - different columns
 - wavelength
- creation of reports



Agilent 1200 Infinity Series Method Development Systems and ChromSword AutoRobust can perform routine work **fully automatically**