Neue Agilent LC Produkte für unterschiedliche Einsatzgebiete

Sebastian Krahe
Produktspezialist LC
Agilent Technologies
The new Agilent 1290 Infinity Quaternary LC

How binary pump-like performance can enhance your lab efficiency
1290 Infinity Quaternary Pump
Various tools enabling the high performance
Multipurpose valve functions for highest comfort

Standard Application for standard Delay Volume
Agilent 1290 Infinity Quaternary Pump
Specifications & Benefits

Power Range

- For any kind of analysis

Composition Accuracy and Precision

< 0.15 % RSD or 0.02 min SD
0.4 % (1-99 % Composition B)

- High RT precision in gradient runs

Flow Accuracy and Precision

< 0.07 % RSD or 0.01 min SD
1.0 % or 10 µL

- High RT precision in isocratic runs

Composition Range

1-99 %

- Wide analytical range

Delay Volume

< 350 µL

- For fast quaternary gradients
Composition Accuracy and Precision

Step Gradient Analysis with Tracer

→ Different mixtures (H₂O, MeOH, ACN) at
→ different pressures and
→ different flow rates

Full application range!
You need different concentrations of modifiers in your analysis, would like to have just one stock-solution and do online dilution to profite from the quaternary mixing capability of your pump? Here is a simple tool – *BlendAssist*!

**Desired method conditions - example:**
1. 5 to 95% gradient of ACN with 0.1% TFA in Water and 0.08% TFA in ACN
2. 20 – 80% gradient of ACN with 0.5% TFA in Water and 0.4% TFA in ACN

Without BlendAssist you need to either pre-mix the required solvents or by using stock-solutions of TFA in Water and ACN to program complex gradients (%A, B, C, D).

**With BlendAssist:** just program your binary organic/aqueous gradient and define the dilution factor!
Ternary/Quaternary Gradients

Blend Assist, different Phosphate buffer concentrations

- **Procainamide**
- **Acetyl-Procainamide**
- **Disopyramide**
- **Quinidine**

Buffer Concentrations:
- 0.05 M buffer
- 0.025 M buffer
- 0.01 M buffer

mAU vs. Time (min)
Agilent 1290 Infinity 2D-LC Solution

A new flexible and user-friendly 2D-LC solution for the most complex samples
2D-LC - What is it?

2DLC: Injecting the effluent or a part of the effluent of one column to a second column, ideally with orthogonal separation behavior.

**Purpose:** increase total separation power.

Peak capacities **multiply** for orthogonal separation mechanisms!

Two different modes:

- **Comprehensive 2D-LC (“LCxLC”)**
  - Complex/unknown samples: bio-pharma, food, polymers....

- **Heart-cutting 2D-LC (“LC-LC”)**
  - Known samples/improving confidence: pharma, methdev....
2D-LC - What is it?

Standard 1-dimensional LC:

Pump → Autosampler → Column → Detector → 1D-Chrom.
2D-LC - What is it?

2-dimensional LC:

- Pump
- Autosampler
- Column 1
- Column 2
- Injector
- Pump 2
- Detector
2D-LC - comprehensive vs. heart-cutting 2D-LC

Comprehensive 2D-LC (LCxLC):

- LC$_1$
- LC$_2$

1st peak from 1st dimension
2nd peak from 1st dimension
3rd peak from 1st dimension

Agilent Technologies

The Measure of Confidence
2D-LC - comprehensive vs. heart-cutting 2D-LC

Heart-cutting 2D-LC (LC-LC):

- Only \textbf{parts} of the effluent of the first column will be injected to the second column.
- The gradients in the 2nd dimension can be much longer as in comprehensive 2D-LC.
- Loss of information.
  But better quality data for peak of interest.
2D-LC Acquisition Software
- supported 2D-gradients modes

- Isocratic
- Advancing Isocratic
- Std. Gradient
- Shifted Gradient

...and most combinations!
Application examples

- Advantage of shifted gradient features

RPLC x RPLC
Easy method set-up but only little orthogonality

Current state-of-the-art 2D-LC – narrow spread of peaks
Application examples
- Advantage of shifted gradient features

Resolution optimized!

Use of shifted gradient feature

Imagine to program this gradient manually!
With the Agilent 2D-LC Acquisition software a matter of a minute!
THE ART OF EMULATION
# Seamless Method Transfer 1260/1220 Infinity

*By unchanged critical specification*

<table>
<thead>
<tr>
<th></th>
<th>1100/1200 Series HPLC</th>
<th>1260 Infinity Quaternary LC</th>
<th>1200 Series RRLC – Std.</th>
<th>1260 Infinity Binary LC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max Flow Rate</td>
<td>5 mL/min</td>
<td>5 mL/min</td>
<td>5 mL/min</td>
<td>5 mL/min</td>
</tr>
<tr>
<td>Delay Volume</td>
<td>900-1200 µL</td>
<td>900-1200 µL</td>
<td>900-1200 µL*</td>
<td>900-1200 µL*</td>
</tr>
<tr>
<td>Capillary ID</td>
<td>0.17mm</td>
<td>0.17mm</td>
<td>0.17mm</td>
<td>0.17mm</td>
</tr>
<tr>
<td>Disp. Vol. w/o cell</td>
<td>15µl</td>
<td>15µl</td>
<td>15µl</td>
<td>15µl</td>
</tr>
<tr>
<td>Injection Principle</td>
<td>Variable Loop</td>
<td>Variable Loop</td>
<td>Variable Loop</td>
<td>Variable Loop</td>
</tr>
<tr>
<td>Inj.Volume – Std/Ext.</td>
<td>100 / 1500 µL</td>
<td>100 / 1500 µL</td>
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<td>100 / 1500 µL</td>
</tr>
<tr>
<td>Area RSD</td>
<td>&lt;0.25 %</td>
<td>&lt;0.25 %</td>
<td>&lt;0.25 %</td>
<td>&lt;0.25 %</td>
</tr>
<tr>
<td>Oven Design</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Column Length</td>
<td>300 mm</td>
<td>300 mm</td>
<td>300 mm</td>
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</tr>
</tbody>
</table>

* Smaller delay volumes possible

Optimized for 3 – 4.6mm ID Columns

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* Optimized for 3 – 4.6mm ID Columns

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* Smaller delay volumes possible
Seamless Method Transfer

*What if critical specification change ...?*

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<td>900-1200 µL</td>
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<td>900-1200 µL*</td>
<td>340 µL**</td>
<td>10 – 110 µL*</td>
<td></td>
</tr>
<tr>
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<td>0.17mm</td>
<td>0.17mm</td>
<td>0.12mm**</td>
<td>0.12mm*</td>
</tr>
<tr>
<td>Disp. Vol. w/o cell</td>
<td>15µl</td>
<td>15µl</td>
<td>15µl</td>
<td>15µl</td>
<td>7.5µL**</td>
<td>7.5µL*</td>
</tr>
<tr>
<td>Injection Principle</td>
<td>Variable Loop</td>
<td>Variable Loop</td>
<td>Variable Loop</td>
<td>Variable Loop</td>
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<td>Variable Loop</td>
</tr>
<tr>
<td>Inj.Vol. – Std/Ext.</td>
<td>100 / 1500 µL</td>
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<td>300 mm</td>
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</table>

* Optimized for 2.1 - 4.6mm ID
** Optimized for 2.1mm ID
Gradient Analyses with Different LC Systems
- Impact of delay volume and mixing behavior

The result:
- Difference in RT and Resolution
- One peak is missing!

Sample: 0.5% impurities in formulation (metoclopramide)
Xbridge C18, 150x3 mm, 3.5 µm dp,
0.45 ml/min, Eluent: A =0.25 % AmAc, B = ACN,
Gradient: 0-15 min; 5-57 % B
Method Transferability: 1290 Infinity LC
- System Emulation Technology

Concept

Select System to be emulated by a simple mouse click:

- Agilent 1100 Series
- Agilent 1200 Series LC
- Agilent 1260 Infinity LC
- Agilent 1220 Infinity LC
- Alliance (August 2012)
Intelligent System Emulation Technology ISET
Implementation

Select pump
Select sampler
Method transfer, pesticide example

2.1x100 mm Zorbax Eclipse Plus, 1.8 µm column, flow: 0.8 mL/min

(A) 1100 Quaternary 400 bar

(B) 1290 Infinity 1200 bar

(C) 1290 Infinity 1200 bar with ISET
30x wider linear UV range - Quantification of widely different concentration levels in one single run

Agilent 1200 Infinity High Dynamic Range (HDR-DAD) Solution

1260/1290 Infinity HDR-DAD
History of DAD Linearity Gain
The last 30 years

Detector Model (Intro Year)

1200 Infinity HDR-DAD

>30X Linearity
With HDR

(1986) 1.4
1040 to 1090

(1988) 1.7
1090 to 1050

(1995) 2.0
1050 to 1100

(2006) 1.4
1110 to 1200

(2010) 2.9
1200 to 1290/60

(2011) >30
1200 to HDR

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30x Wider Linear Range with HDR-DAD
3.7 mm and 60 mm and Max-Light flow cell

Computing the signals from
60 mm path-length for the low concentration
3.7 mm path-length for the high concentration
Comparison of conventional DAD vs. HDR DAD, 5µl injection

Red conventional DAD: 2 peaks out of linear range with 5µl injection

Green HDR-DAD: All peaks within linear range (up to 6AU)
Comparison of LOD

More reliable automated peak integration

<table>
<thead>
<tr>
<th>Compound</th>
<th>LOD with S/N=3 for Conventional DAD</th>
<th>LOD with S/N=3 for HDR- DAD solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorphenamine</td>
<td>~1ng</td>
<td>~0.1ng</td>
</tr>
</tbody>
</table>
What needs to be considered with narrow-bore columns:

Ultra Low Dispersion Kit Rev.2 for 1290 Infinity LC
Extra column volume, extra column dispersion

Smaller & more efficient columns (ID, L, particle size) => Smaller peak volume
Smaller peak volume requires smaller extra column volume

Example: 2.1 x 50 mm 1.8 µm particles

- **Standard LC**
  - 170 µm capillaries
  - 13 µL flow cell,
  - Poor fitting connections

- **1290 Infinity LC**
  - standard config.
  - 115 µm capillaries,
  - V(σ) 1 µL flow cell
Extra column volume, extra column dispersion

- **Facts**

- Effects are **huge** for isocratic runs with **early eluting peaks**

![Diagram of ALS and Detector with Extra column dispersion for isocratic separation]

**Example on 2.1 x 50 mm STM column**

**1290 Infinity LC** (universal, default setup)
- Extra-Column Volume = **9.7 µL**
- Standard Flow cell V(σ)= 1 µL

**1290 Infinity LC ULD** (lowest dispersion*)
- Extra-Column Volume = **3.9 µL**
- 75 µm capillaries, ULD flow cell V(σ)=0.6 µL

**Preliminary results:**

\[
\begin{align*}
R_s_{5,6} &= 2.38 \\
N_4 &= 5529 \\
N_8 &= 9697 \\
N_9 &= 9947 \\
R_s_{5,6} &= 2.77 \rightarrow 16\% \text{ increase} \\
N_4 &= 8864 \rightarrow 60\% \text{ increase} \\
N_8 &= 11251 \rightarrow 16\% \text{ increase} \\
N_9 &= 10898 \rightarrow 10 \% \text{ increase}
\end{align*}
\]
The Agilent 1260 Infinity SFC/UHPLC Hybrid System

Two orthogonal techniques with a single system

Simply switch the valve
Hybrid System in SFC Mode

LC Pump: G1310B or G1311B or G1312B

SFC-Binary Pump

Loop back Restrictor

SFC Waste

HPLC Waste

BPR

LC Pump out

Detector out

Autosampler in

SFC Pump outlet

The Measure of Confidence

Agilent Technologies
Hybrid System in UHPLC Mode

LC Pump: G1310B or G1311B or G1312B

Restrictor
Waste
SFC Pump inlet
SFC Pump outlet
BPR
SFC-Binary Pump
Autosampler in
Detector out
LC Pump out

The Measure of Confidence
Agilent Technologies
Overlay SFC versus UHPLC mode

1 - Caffeine
2 - Theophylline
3 - Cortisone
4 - Prednisone
5 - Hydrocortisone
6 - Prednisolone
7 - Sulfomerazine
8 - Sulfaquinoxaline
G6100 series MS connection to SFC

Advantages:
- Superior area reproducibility and robustness
- Acids can be added to enhance selectivity

Limitations:
- Only up to 3 ml/min

MS-setup with make-up flow pump G1310B
Are any other non-UV detectors suited for connection with SFC?

Evaporative Light-Scattering perfectly fits with SFC

For compounds which do not contain chromophores
Evaporative Light Scattering Detection (ELSD)
Introduction: Principle of Operation

The ELSD principle of operation employs three distinct stages:

- Nebulisation
- Evaporation
- Detection
Increased sensitivity, reduced baseline noise

The increased laser intensity coupled with a high gain photomultiplier and digital signal processing increases signal and reduces noise.

Injection: 10μL (Hydrocortisone 50ng-on column)
Column: Zorbax Eclipse XDB-C18 (5μm, 4.6x150mm)
Mobile Phase: Water/ACN (80/20)
Flow Rate: 1.0 m/min
ELSD: Neb 40°C, Evap 60°C, Gas 1.05SLM

Signal/noise calculated using OpenLab Chemstation v1.04

G4261B
S/N 158

G4260B
S/N = 5
<table>
<thead>
<tr>
<th></th>
<th>G4260B</th>
<th>G4261B</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1260 Infinity ELSD</strong></td>
<td>1290 Infinity ELSD (Cooling deleted)</td>
<td>1290 Infinity ELSD (Standard, cooled)</td>
</tr>
<tr>
<td><strong>Light Source</strong></td>
<td>Blue LED (480nm)</td>
<td>Blue Laser (405nm)</td>
</tr>
</tbody>
</table>
| **Features**         | • Hydrocortisone limit of detection 30ng  
                      | • RS232 Communication only            | • Hydrocortisone limit of detection 4ng  
                      |                                            | • 5-9 x improvement in sensitivity over current model  
                      |                                            | • Unique sub-ambient operation (greater response for volatile compounds)  
                      |                                            | • RS232 and LAN Communication            | • Voltage selection (110/240V) no longer required  
                      |                                            | • Variable gas flow for uniformity of response  
                      |                                            | • Fast data collection (up to 80Hz)  
                      |                                            | • Controlled via ChemStation / EZChrom  
                      |                                            | • Front Panel control of all parameters   (Temperature, gas flow, data rate..etc) |
The Agilent 1260 Infinity bio-inert quaternary LC

The New Standard in Bioanalysis

100% Bio-inert
✓ Precious sample does not touch metal surfaces
✓ pH range 1-13 (shortterm 14)
✓ 2 M salt, 8 M urea
✓ No stainless steel inmobile phase flow path
✓ New capillary technology

UHPLC capability
✓ 600 bar

Superior Ease of Use and Robustness
✓ Buffer Advisor Software
✓ Highly corrosion resistant
✓ Active seal wash
✓ Quaternary buffer mixing
✓ Agilent proven quality
✓ Agilent warranty and service quality (enhanced PM possible)
✓ Superior Bio-HPLC columns for biotherapeutic characterization

The choice for both, bioanalytical and biopurification up to 10 ml/min
Proof of concept: surface activity
ATP Analysis – 1260 Infinity standard LC system

Chromatographic conditions
Flow rate: 0.5 mL/min
Isocratic run with buffer A, B, C, D or E
Stop time: 5 minutes
Injection volume: 0.2 µL
Temperature TCC: 40 °C
Diode array detector: 254 nm
No column, PEEK restriction

Solvents
Buffer A: 10 mM ammonium acetate
Buffer B: 10 mM ammonium acetate +10% methanol
Buffer C: 10 mM ammonium acetate +50% methanol
Buffer D: 10 mM ammonium acetate +70% methanol
Buffer E: 10 mM ammonium acetate +90% methanol
Proof of concept: low surface activity
ATP Analysis – 1260 Infinity Bio-inert LC

Chromatographic conditions
Flow rate: 0.5 mL/min
Isocratic run with buffer A, B, C, D or E
Stop time: 5 minutes
Injection volume: 0.2 µL
Temperature TCC: 40 °C
Diode array detector: 254 nm
No column, PEEK restriction

Solvents
Buffer A: 10 mM ammonium acetate
Buffer B: 10 mM ammonium acetate +10% methanol
Buffer C: 10 mM ammonium acetate +50% methanol
Buffer D: 10 mM ammonium acetate +70% methanol
Buffer E: 10 mM ammonium acetate +90% methanol
...a Quat LC and Buffer Advisor enables Automation....

Mix 2 components directly to get desired pH

Dilute online or add salt for salt gradients
Using the 1260 Infinity LC for Protein SEC
Agilent 1260 Infinity Multi Detector Suite
Agilent 1260 Infinity Multi Detector Suite for Protein SEC

...helps to differentiate aggregates of monoclonal antibodies

**Figure 1.** Size distribution (by intensity) from two antibody samples, Abl-a (black) and its treated form Ab1-b (grey). Ab1-b is aggregated, while Ab1-a shows no evidence of larger components.
Danke für Ihre Aufmerksamkeit!