Ultrafast Analysis of Food Preservatives Using Automated Column Regeneration and Dual-Needle Injection

High-throughput analysis using the dual-needle function of the Agilent 1290 Infinity II Multisampler

Application Note

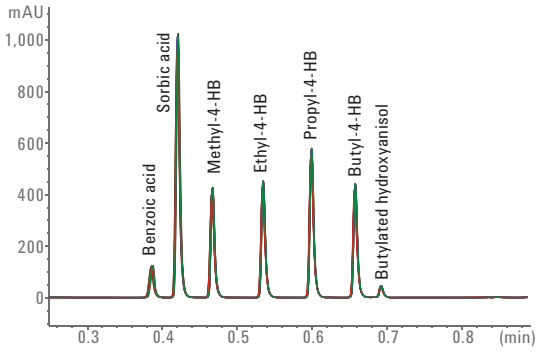
Food Testing and Agriculture

Authors

Sonja Schneider and Melanie Metzlaff
Agilent Technologies, Inc.
Waldbronn, Germany

Abstract

The combination of UHPLC systems with sub-2 µm (STM) particle columns enables ultrafast separations with minimal runtimes. The highest throughput is shown for ultrafast gradients with over 60 % reduction of total analysis time using alternating column regeneration. This was accomplished with parallel injection by the second needle installed in the Agilent 1290 Infinity II Multisampler, which has a dual-needle option for the analysis of food preservatives. Both inter- and intraneedle, as well as inter- and intracolumn precision of retention time and peak area were excellent. In addition, ultralow carryover and highly precise injection volume linearity was shown for the analysis of chlorhexidine and caffeine, respectively.



Agilent Technologies

Introduction

Methods for conventional high performance liquid chromatography (HPLC) are routinely used for food monitoring as one of the most reliable and rugged analysis techniques. There is an increased need for faster analyses with higher resolving power. The combination of UHPLC systems with sub-2 µm (STM) particle columns enables ultrafast separations, even below 1 minute runtime. Typically, a UHPLC cycle time consists of sample injection, gradient, column wash, and equilibration. The total cycle time can be reduced by using automated column regeneration (ACR) to save time for column wash and equilibration. ACR uses a setup of two pumps in the system to enable simultaneous UHPLC regeneration of the first column during the gradient run on the second column.

For additional time optimization, a second injection needle installed in the Agilent 1290 Infinity II Multisampler, with the dual-needle option, offers a significant reduction of analysis time for short runs in the high-throughput mode called Smart Overlap. This provides for simultaneous analysis on the first flow path, and overlapped sample draw using the second flow path preparing the next run. Figure 1 shows the hydraulic concept of the 1290 Infinity II Multisampler.

This Application Note shows the ultrafast analysis of food preservatives optimized for high throughput using alternating column regeneration in combination with parallel injection with a second needle installed in the 1290 Infinity II Multisampler.

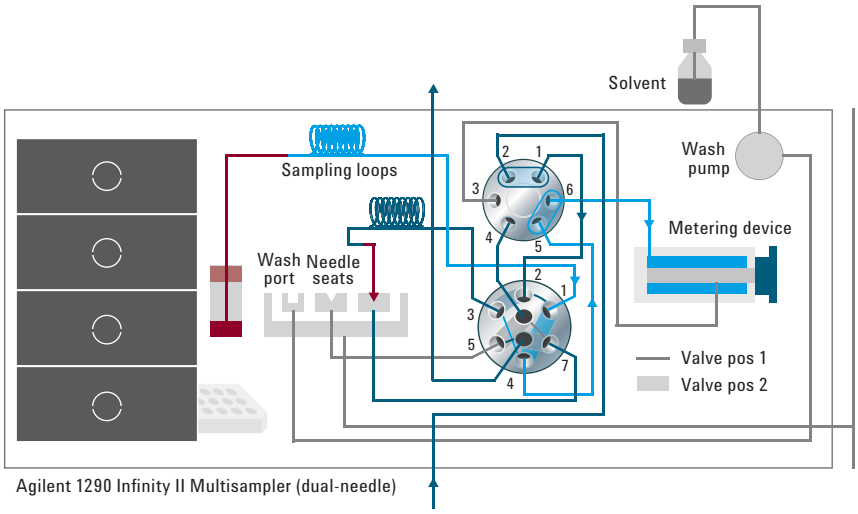


Figure 1. Hydraulic concept of the Agilent 1290 Infinity II Multisampler.

Experimental

Instrumentation

The Agilent 1290 Infinity II LC consisted of:

- Agilent 1290 Infinity II High-Speed Pump (2x G7120A)
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116B)
- Agilent Internal Switching Valve, 2-position/10-port ultrahigh pressure, 1,200 bar, valve head (5067-4118)
- Agilent 1290 Infinity II Diode Array Detector (G7117B)
- Agilent 1290 Infinity II Multisampler (G7167B) with dual-needle option (#111)

To perform the column equilibration in parallel to the run, two identical columns and a second pump were used[1].

Columns

- Agilent ZORBAX SB C18, 4.6 × 150 mm, 5 µm, (p/n 7995218-595)
- Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 50 mm, 1.8 µm (p/n 959996-902)

Software

Agilent OpenLAB CDS ChemStation Edition for LC and LC/MS Systems, Rev. C.01.07 [27]

Solvents and samples

The sample was a mix of seven typically used food preservatives:

1. Benzoic acid, 250 ng/µL
2. Sorbic acid, 50 ng/µL
3. Methyl-4-hydroxybenzoate (methyl-4-HB), 50 ng/µL
4. Ethyl-4-hydroxybenzoate (ethyl-4-HB), 50 ng/µL
5. Propyl-4-hydroxybenzoate (propyl-4-HB), 50 ng/µL
6. Butyl-4-hydroxybenzoate (butyl-4-HB), 50 ng/µL
7. Butylated hydroxyanisol, 250 ng/µL

All solvents were LC grade. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22-µm membrane point-of-use cartridge (Millipak). The preservative standards were purchased from Sigma-Aldrich, St. Louis, Missouri, USA.

Table 1. Chromatographic conditions for injection volume linearity determination.

Parameter	Value
Column	Agilent ZORBAX SB C18, 4.6 × 150 mm, 5 µm
Sample	125 µg/mL Caffeine, 1:2 diluted five times
Mobile phase	Water + 0.1 % TFA/acetonitrile + 0.1 % TFA, 80/20
Flow rate	1 mL/min
Stop time	5 minutes
Needle wash mode	Standard wash
Injection volume	0.5, 1, 2, 4, 8, 16 µL
Needle selection	Alternating needle
Enable smart overlap	No
Column temperature	40 °C
Detection	273/4 nm, reference 360/100 nm, > 0.013 minutes (0.25 seconds response time) (20 Hz)

Table 2. Chromatographic conditions for carryover determination.

Parameter	Value
Column	Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 50 mm, 1.8 µm
Sample	Caffeine or chlorhexidine, each 1 mg/mL in water
Mobile phase	Water + 0.1 % TFA/acetonitrile + 0.1 % TFA, 80/20 for caffeine Water + 0.1 % TFA/acetonitrile + 0.1 % TFA, 67/33 for chlorhexidine
Flow rate	0.5 mL/min
Stop time	3 minutes
Needle wash mode	Standard wash
Injection volume	0.5 µL
Needle selection	Alternating needle
Enabled smart overlap	No
Column temperature	40 °C for caffeine, 50 °C for chlorhexidine
Detection	273/4 nm, reference 360/100 nm for caffeine, 257/4 nm, reference 360/100 nm for chlorhexidine, > 0.013 minutes (0.25 seconds response time) (20 Hz)

Results and Discussion

Proof of performance – dual-needle setup

The performance of the dual-needle setup in the 1290 Infinity II Multisampler was analyzed for carryover and injection volume linearity as two important parameters. Injection volume linearity was determined for the 100-µL metering device with 20-µL loops installed. A total of 62.5 ng of caffeine was injected in six different caffeine concentrations (125 µg/mL of caffeine was diluted five times), resulting in six different injection volumes from 0.5 to 16 µL. With the dual-needle concept, high injection volume linearity was achieved with both needles. Figure 2 shows the overlaid chromatograms of the different injection volumes. The precision of areas (RSD) was 1.2 % for injection volumes from 0.5 to 16 µL, and 0.7 % for injection volumes from 1 to 16 µL.

Table 3. Chromatographic conditions for high-throughput analysis of food preservatives.

Parameter	Value
Column	Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 50 mm, 1.8 µm
Mobile phase	A) Water + 20 mM ammonium formate, pH 4.4 B) Acetonitrile
Flow rate	1.5 mL/min
Gradient pump	3 %B at 0 minutes 60 %B at 0.5 minutes 80 %B at 0.6 minutes 95 %B at 0.65 minutes 3 %B at 0.7 minutes
Equilibration pump	3 %B at 0 minutes 95 %B at 0.05 minutes 95 %B at 0.2 minutes 3 %B at 0.3 minutes
Stop time (both pumps)	1.1 minutes
Needle wash mode	Standard wash
Injection volume	1.00 µL
Needle selection	Alternating needle
Enable smart overlap	Yes
Smart overlap wait time	0.70 minutes
Column temperature	40 °C
Detection	260/40 nm, reference 380/100 nm, data rate 160 Hz

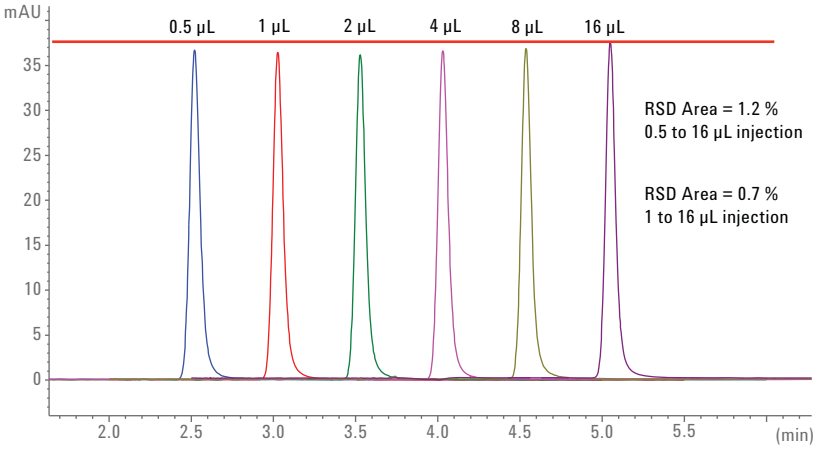


Figure 2. Injection volume linearity using alternating needles.

Carryover was measured after the injection of caffeine as well as chlorhexidine. First, carryover was measured by injecting 1,000 ng caffeine, followed by the injection of 1 µL water. Figure 3 shows the chromatograms of the caffeine analysis (A), and the water analysis before and after the injection of caffeine (B). No carryover was detected after the injection of 1,000 ng caffeine with both needles.

Second, carryover was measured by injecting 1,000 ng chlorhexidine, followed by the injection of 1 µL of water. Figure 4 shows the chromatograms of the chlorhexidine analysis (A), and the water analysis before and after the injection of chlorhexidine (B). Again, no carryover was detected after the injection of 1,000 ng chlorhexidine with both needles.

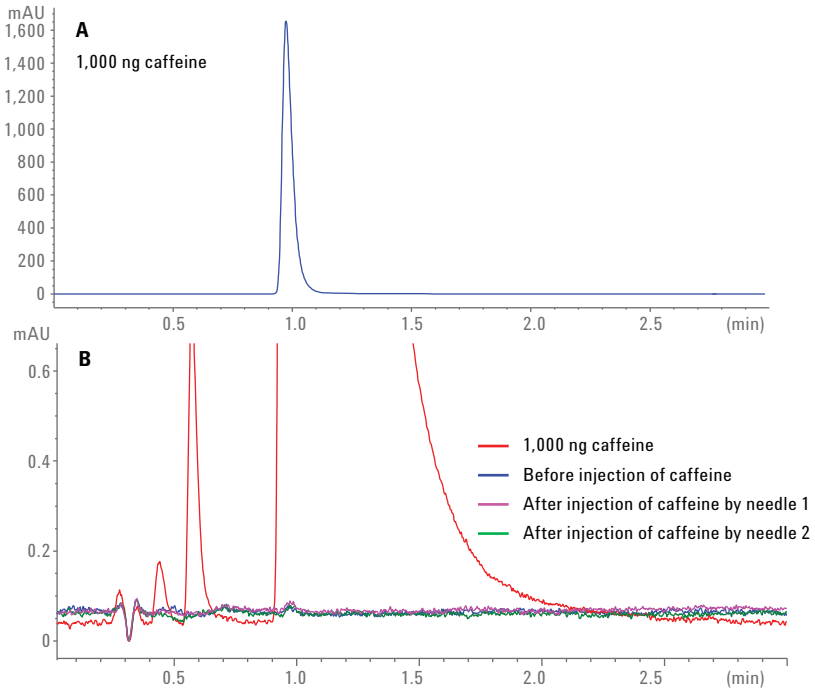


Figure 3. Carryover for the injection of 1,000 ng of caffeine (A). No carryover was detected (B).

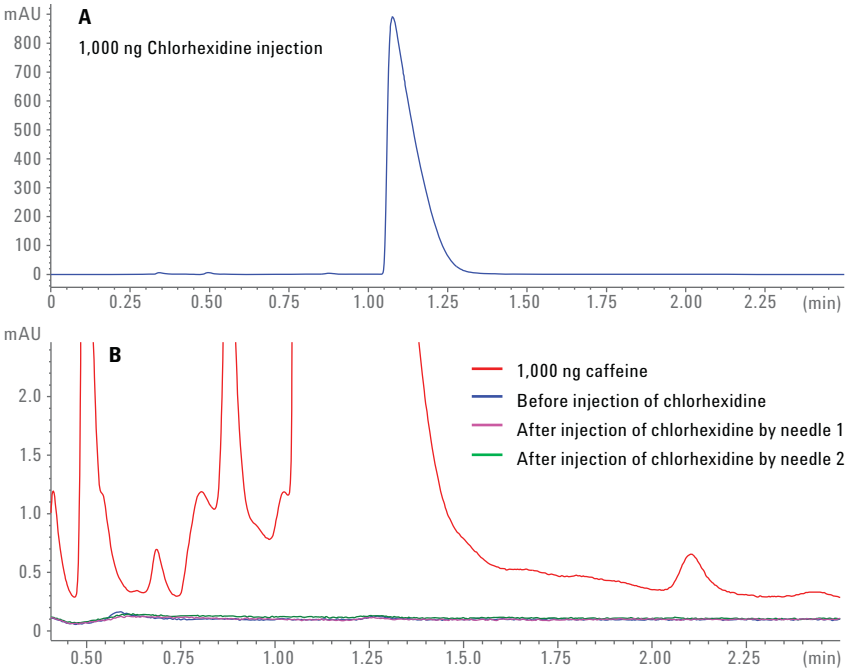


Figure 4. Carryover for the injection of 1,000 ng of chlorhexidine (A). No carryover was detected (B).

High-throughput analysis of food preservatives

To demonstrate the robustness and time savings of the setup, a preservative standard with seven different compounds was used. The separation of these standards took only 0.7 minutes (Figure 5).

The retention time (RT) and area precision was tested for every individual needle (intraneedle precision), for a combination of both needles (interneedle precision), and for the complete setup including ACR alternating two columns[1]. Figure 6 shows an overlay of 14 subsequent runs of the separation of the preservative standards, indicating excellent RT precision.

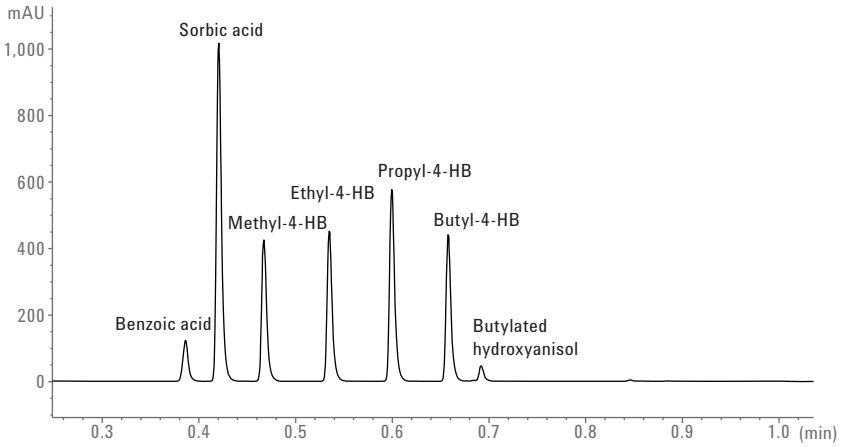


Figure 5. Ultrafast separation of seven typically used food preservatives.

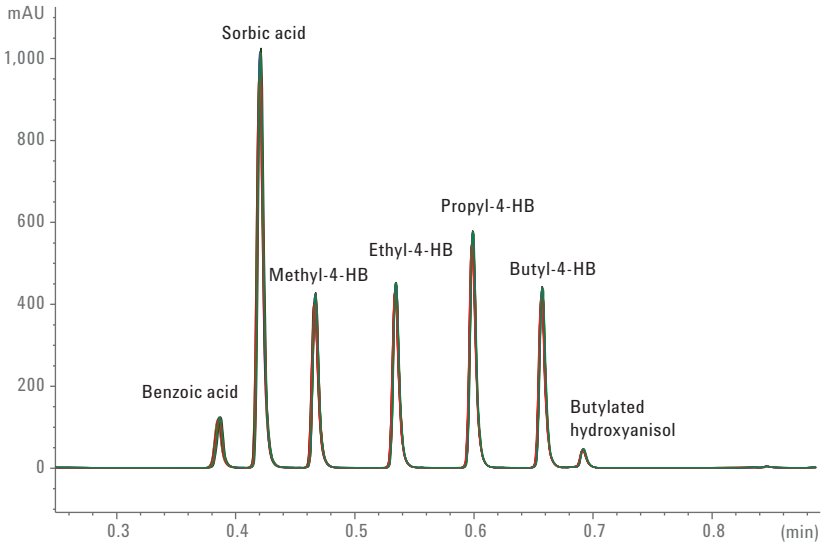


Figure 6. Overlay of 14 subsequent runs of the preservatives separation with dual-needle setup and automated column regeneration.

Table 4 shows all values for relative standard deviations (RSDs), calculated for intraneedle precision (column 1, needle 1, and column 1, needle 2), interneedle precision (columns 1 and 2, dual-needle), as well as for the precision in the complete setup (dual-needle automated column regeneration). The intraneedle area precision was below 0.4 % for both needles, tested with column 1. The interneedle precision was still excellent, with RSDs under 0.45 %. Even considering the variation of the complete setup with two different injections paths including two needles and two alternating columns, the area precision was excellent, with RSDs under 0.57 % except for the last peak, with 0.815 %. All RT RSDs were excellent, with values below 0.01 % for almost all peaks except for the fast eluting compounds.

The complete setup, including automated column regeneration and interlaced injection using two alternating injection needles, was evaluated regarding linearity, limit of detection (LOD), and limit of quantitation (LOQ). Six different concentration levels (from 50 to 0.1 µg/mL) were prepared from the stock solutions, and the linear relationship was determined between the peak area and the corresponding concentrations. LOD and LOQ were defined as the signal-to-noise ratio of 3:1 and 10:1, respectively. Both needles were used alternately, even for different concentrations in one sequence.

Table 4. Precision values for the different setups.

		Column 1, needle 1		Column 1, needle 2		Column 1, dual-needle		Column 2, dual-needle		Dual-needle automated column regeneration	
Substance	RT (min)	RSD RT (%)	RSD Area (%)	RSD RT (%)	RSD Area (%)	RSD RT (%)	RSD Area (%)	RSD RT (%)	RSD Area (%)	RSD RT (%)	RSD Area (%)
Benzoic acid	0.394	0.11	0.395	0.11	0.347	0.159	0.406	0.18	0.44	0.132	0.234
Sorbic acid	0.428	0.08	0.287	0.08	0.377	0.245	0.35	0.125	0.427	0.093	0.312
Methyl-4-HB	0.476	0.09	0.283	0.052	0.361	0.15	0.377	0.077	0.427	0.074	0.273
Ethyl-4-HB	0.544	0.046	0.304	0.037	0.375	0.071	0.358	0.043	0.429	0.044	0.568
Propyl-4-HB	0.610	0.015	0.288	0.02	0.356	0.042	0.351	0.017	0.408	0.041	0.257
Butyl-4-HB	0.670	0.013	0.304	0.015	0.379	0.027	0.367	0.016	0.445	0.027	0.554
Butylated hydroxyanisol	0.702	0.016	0.306	0.011	0.309	0.02	0.357	0.014	0.491	0.049	0.815

Table 5. Linearity and limits of detection and quantification of the seven food preservatives.

Substance	Correlation factor	LOD (pg)	LOQ (pg)
Benzoic acid	0.99997	81.1	270.3
Sorbic acid	0.99997	10.9	36.4
Methyl-4 HB	0.99997	25.9	86.2
Ethyl-4 HB	0.99998	25.0	83.3
Propyl-4 HB	0.99997	20.0	66.7
Butyl-4 HB	0.99998	25.6	85.5
Butylated hydroxyanisol	0.99994	250.0	833.3

Table 5 shows the results of the evaluation. The setup showed high linearity, with correlation coefficients over 0.9999 for all standards. The LODs and LOQs were found in the double-digit picogram range for most compounds. Figure 7 displays the correlation for sorbic acid, as an example.

The parallel injection of the next sample as well as the parallel column regeneration achieved a decrease in the overall cycle time of over 60 %. For 100 injections, the time could be decreased from 260 minutes (sequential runs) down to 110 minutes (overhead time not included) (Figure 8). Additional overhead time has to be added to the analysis time for detector balancing, saving data, and other software-related processes. However, the ratio of the total time savings will not change if the overhead time is included, still resulting in a time savings of over 60 %. Even more time can be saved if the detector balancing is skipped, although this can be critical in terms of reproducibility.

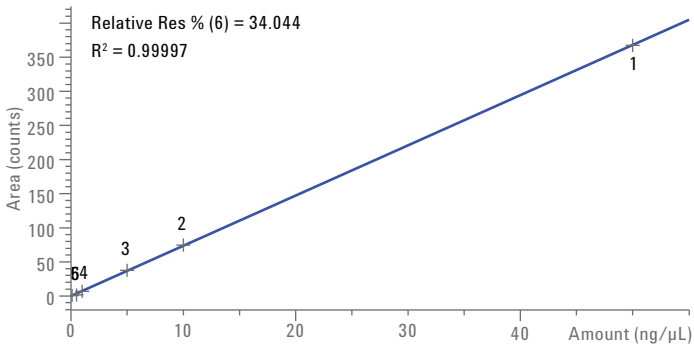


Figure 7. Linearity for sorbic acid using two alternating injection needles.

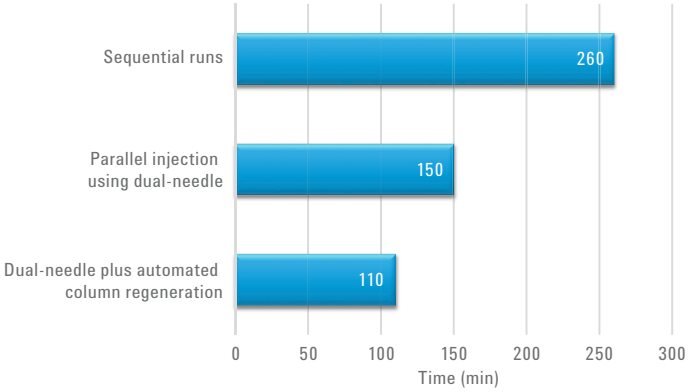


Figure 8. Time savings for ultrahigh throughput over 100 injections.

Conclusion

The Agilent 1290 Infinity II Multisampler can be equipped with a dual-needle option, enabling parallel injection by the second needle while the analytical method is still running through the first needle. This Application Note shows ultrafast gradients with an over 60 % reduction of analysis time using alternating column regeneration in combination with parallel injection by the second needle for the analysis of food preservatives. Both, inter- and intraneedle, as well as inter- and intracolumn precision of retention time and peak area were excellent. In addition, two important performance characteristics, carryover and injection volume linearity, showed superb results. No carryover was detected after the injection of caffeine and chlorhexidine. Highly linear injection volume linearity was achieved with both needles in the dual-needle concept.

Reference

1. Huesgen, A. G., Naegele, E., Automated alternating column regeneration on the Agilent 1290 Infinity LC, *Agilent Technologies Application Note*, publication number 5990-5069EN, **2009**.

www.agilent.com/chem

Published in the USA, September 1, 2015
5991-6150EN

Agilent Technologies