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

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Introduction

Conventional high performance liquid chromatography (HPLC) methods are routinely used for food monitoring as one of the most reliable and rugged analysis techniques. Recently, an increased need for faster analyses with higher resolving power has been observed. The new possibilities of UHPLC systems with sub-2-micron (STM) columns allow ultrafast separations even below one minute run time. 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 to save the column wash and equilibration time. Further time saving is possible with a second injection needle installed in the autosampler.

Fig. 1. 1290 Infinity II Multisampler with dual-needle option

Experimental

The UHPLC analyses were performed on an Agilent 1290 Infinity II LC system:

- 1290 Infinity II High-Speed pump (2x)
- 1290 Infinity II Multicolumn thermostat
- Agilent 2-position/10-port ultrahigh pressure valve head, 1200 bar
- 1290 Infinity II Diode Array Detector
- 1290 Infinity II Multisampler with dual-needle option

To perform the column equilibration in parallel to the run, two identical columns and a second pump were used. The sample was a mix of seven typically used food preservatives, each 50 ng/µL.

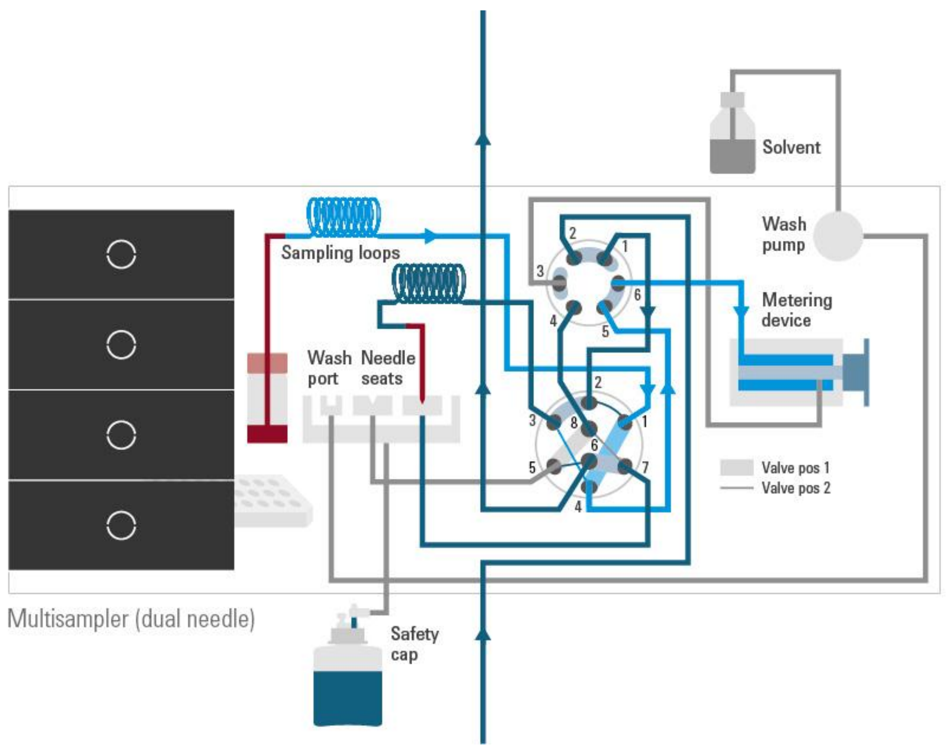

Fig. 2. Dual-needle option – hydraulic concept

The new 1290 Infinity II Multisampler with dual-needle option offers a significant reduction of total analysis time for short runs in the high throughput mode called "Smart Overlap". This allows simultaneous analysis on the first flow path and overlapped sample draw using the second flow path preparing the next run. Further time is saved with automated column regeneration using two alternating columns.

Experimental

Table 1. Chromatographic conditions

Chromatographic conditions for 2.1 x 50 mm, 1.8 µm column	
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
Stoptime both Pumps	1.1 minutes
Needle Wash Mode:	Standard Wash
Injection Volume:	1.00 µL
Needle Selection:	Alternating Needle
Enabled Smart Overlap:	Yes
Smart Overlap Wait Time:	0.70 min
Column Temperature	40 °C
Detection	Signal 260/40 nm, reference 380/100 nm, Data rate 160 Hz

Results and Discussion

To demonstrate the robustness and time saving of the setup described in the introduction, a preservative standard with six different compounds was used. The separation of these standards took only 0.7 minutes, see Figure 3.

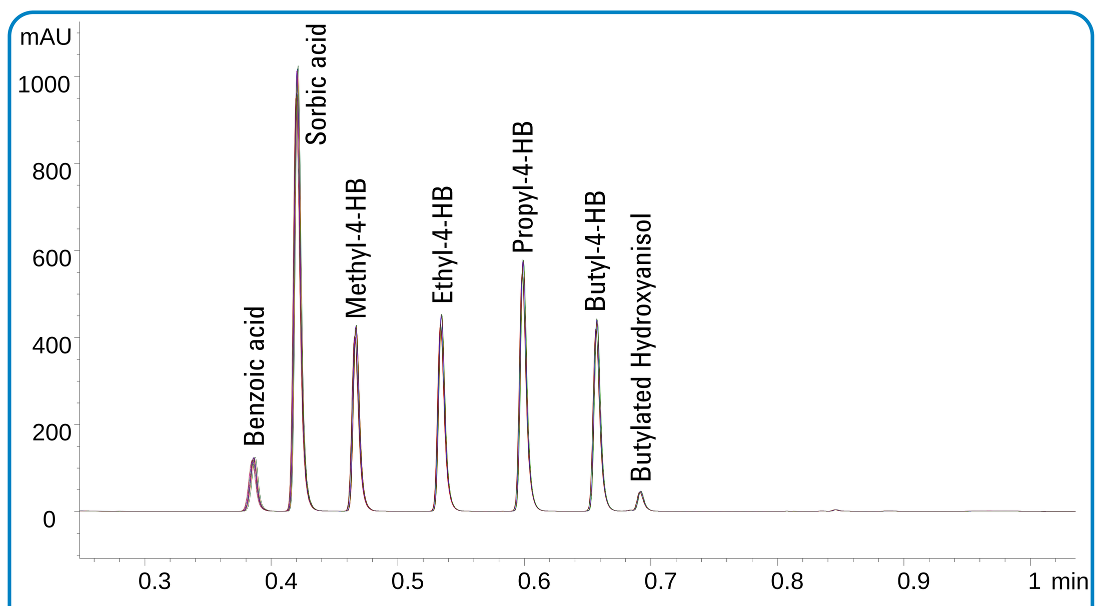

Fig. 3. Overlay of 14 subsequent runs of the preservatives separation with dual-needle setup and automated column regeneration

By parallel injection of the next sample as well as equilibration of the column, the overall cycle time could be decreased by over 60 %, see Figure 6. Furthermore, the intra- and inter-injection needle precision of retention time and peak area for seven consecutive runs were excellent. The same is true for intra- and inter-column precision, as well as combined in a dual-needle setup with automated column regeneration, see Table 2 and Figure 3.

Table 2. Chromatographic conditions

		Column 1, Needle 1		Column 1, Needle 2		Column 1, Dual Needle		Column 2, Dual Needle		Dual-needle automated column regeneration	
Substance	RT	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	0.347	0.11	0.347	0.159	0.406	0.18	0.44	0.132	0.234
Sorbic acid	0.428	0.08	0.377	0.08	0.377	0.245	0.35	0.125	0.427	0.093	0.312
Methyl-4-HB	0.476	0.052	0.36	0.052	0.361	0.15	0.377	0.077	0.427	0.074	0.273
Ethyl-4-HB	0.544	0.037	0.376	0.037	0.375	0.071	0.358	0.043	0.429	0.044	0.568
Propyl-4-HB	0.610	0.02	0.36	0.02	0.356	0.042	0.351	0.017	0.408	0.041	0.257
Butyl-4-HB	0.670	0.015	0.379	0.015	0.379	0.027	0.367	0.016	0.445	0.027	0.554
Butylated Hydroxyanisol	0.702	0.011	0.309	0.011	0.309	0.02	0.357	0.014	0.491	0.049	0.815

Results and Discussion

With the dual-needle concept, high injection volume linearity was achieved with both needles, see Figure 4.

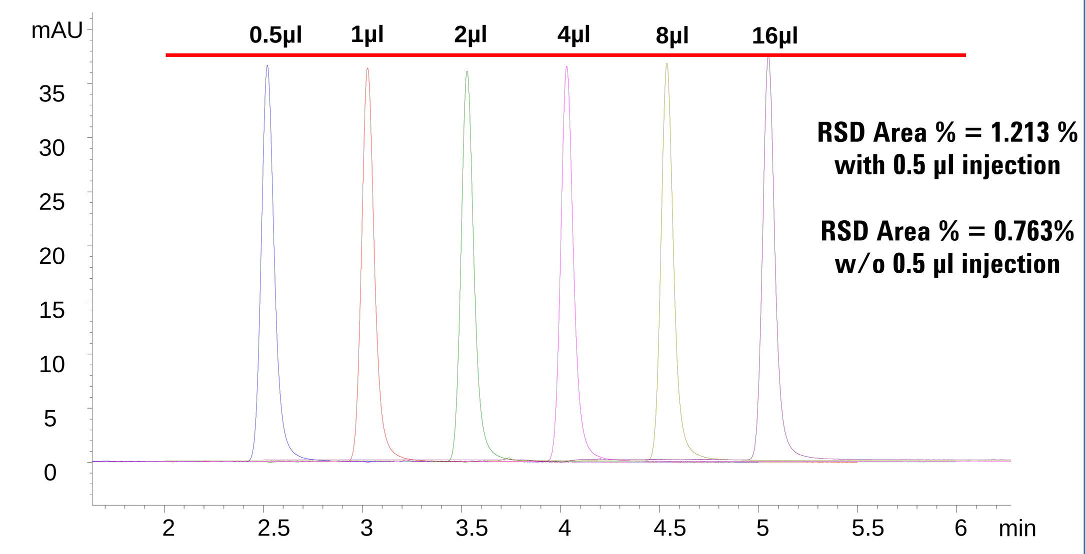

Fig. 4. Injection volume linearity – both needles alternating

In addition, the two independent flow paths enable ultralow carry-over, see Figure 5.

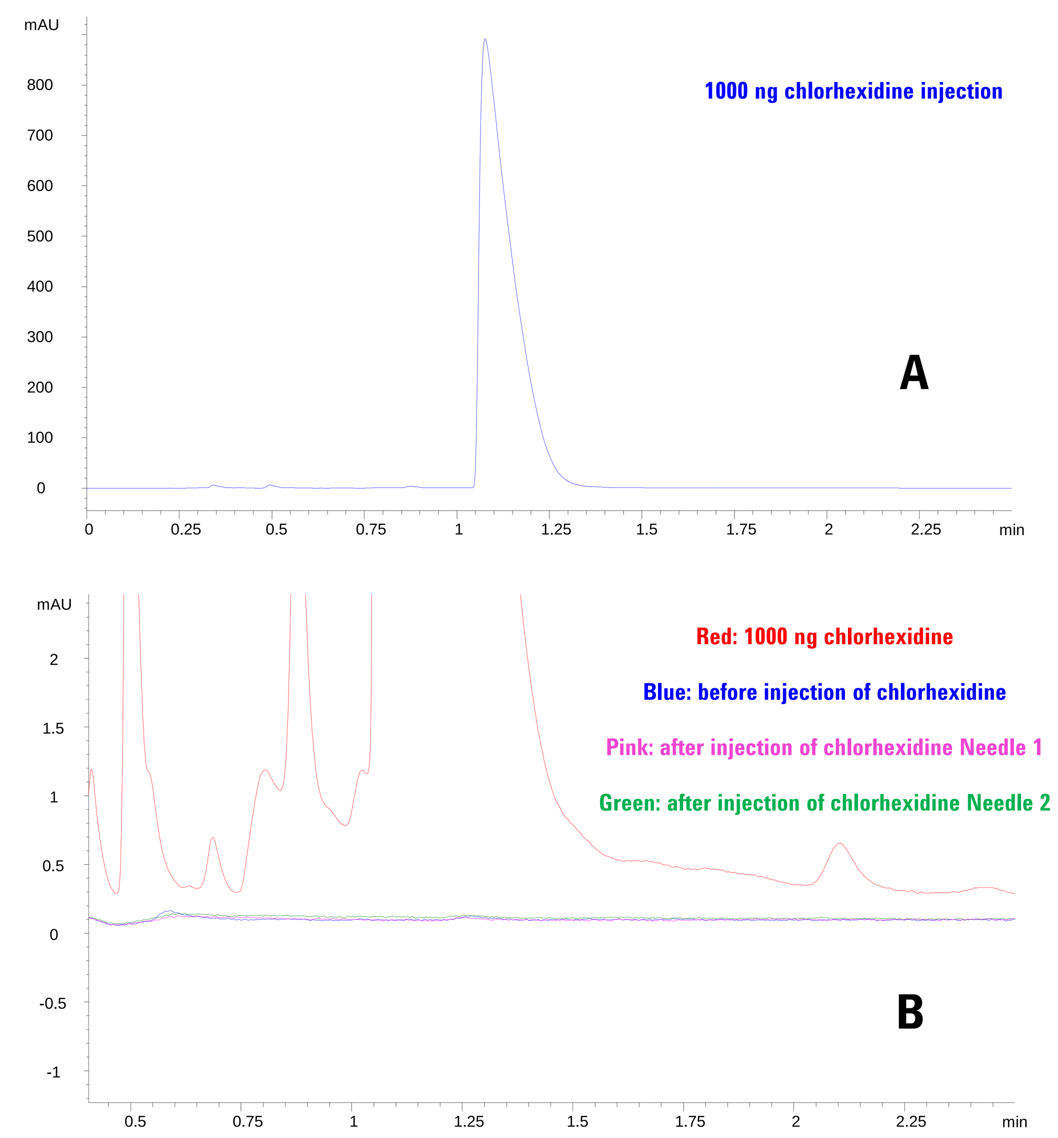

Fig. 5. Carryover for the injection of 1000 ng of chlorhexidine (A) – no carryover was detected (B)

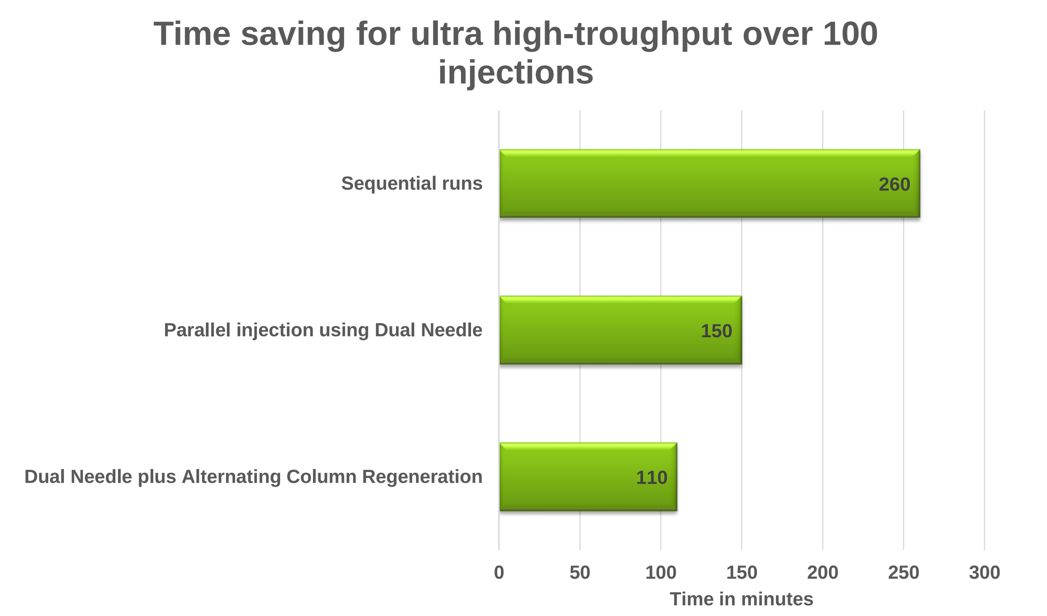

Fig. 6. Overall time saving: sequential runs compared to dual-needle and automated column regeneration

Conclusions

Highest throughput is shown for ultrafast 1.1 minute gradients by over 60 % reduction of total analysis time using alternating column regeneration in combination with parallel injection by the second needle installed in the new Agilent Multisampler with dual-needle option for the analysis of food preservatives. Both, inter and intra-needle as well as inter and intra-column precision of retention time and peak area were excellent.

In addition, ultra-low carry over and highly precise injection volume linearity was shown for the analysis of chlorhexidine and caffeine, respectively.