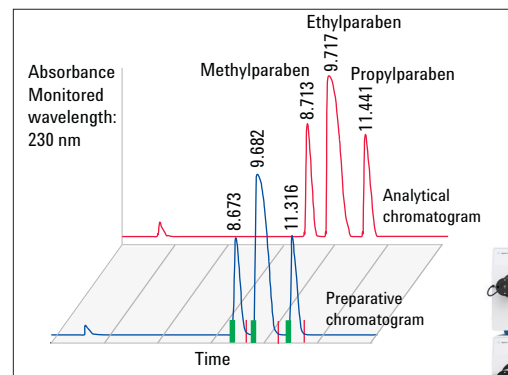


Scale-up with the Agilent SD-1 Purification System analytical and preparative runs on a single system

Technical Overview

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Abstract

This Technical Overview presents proof of performance of the Agilent SD-1 Purification System that offers a flexible and reliable system for a manual analytical to preparative scale-up process. Due to its exchangeable pump heads and capillary sets, the flow rate range rises up to 800 mL/min with excellent performance. Coupled with the dual path-length flow cell of the Agilent 325 Dual Wavelength UV-Visible Detector, which extends the dynamic absorbance range by a factor of 40, the Agilent SD-1 Purification System makes it possible to perform runs from analytical to preparative scale.

This Technical Overview demonstrates the flexibility and performance of this system, and explains how to develop a standard method for purification of several grams of a sample. A sample of three parabens in mixture (methyl, ethyl, and propyl ester derivatives) were resolved, as an example. The collected fractions were analyzed, and purities and recoveries were determined.



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Introduction

Purification by liquid chromatography was used for purification of precious samples, such as a reaction mixture from a tedious synthetic procedure, or a natural product extract from rare plant material. Optimum separation conditions were evaluated in analytical scale to save sample. After the optimization of the analytical separation method, a scale-up process to achieve high purity and recovery was performed. This Technical Overview describes the different steps.

The approach described provides an easy and reliable method which can be applied for any further purification work. For the generation of the preparative method, we applied the method transfer formulas, taking the dwell volumes of the system into consideration in order to retain the resolution of the separation. To validate the scale-up process, the analytical chromatogram was compared to the preparative chromatogram resulting from the purification of the three Paraben ester derivatives in the mixture.

An Agilent SD-1 Purification System equipped with a selection valve that allows choosing either the analytical or preparative flow path was used. This method demonstrates the high flexibility of this system from 1.5 mL/min to 177 mL/min without changing capillaries in the system flow paths or modification of the detection method.

Experimental

Instrumentation

- Two Agilent SD-1 Solvent Delivery Modules (G9302A and G9303A) equipped with 200 mL/min pump heads (G9303A#107) to setup a binary gradient system
- 1.2 mL dynamic mixer stainless steel (G9303A#144)
- Rheodyne manual 3-way valve (RE7030)
- Prep mast kit 218/SD-1, 200 mL preparative injector 1/16" (G9303A#165)
- Agilent 410 Autosampler (G9331A) for the analytical path way
- Agilent 325 Dual Wavelength UV-Visible Detector (G9309A)
- Flow cell 4 × 0.15 mm dual path-length (Super-prep) (G9309A#182)
- Agilent 440 Fraction Collector (G9340A) upgraded with the high flow kit (G9340A#190)

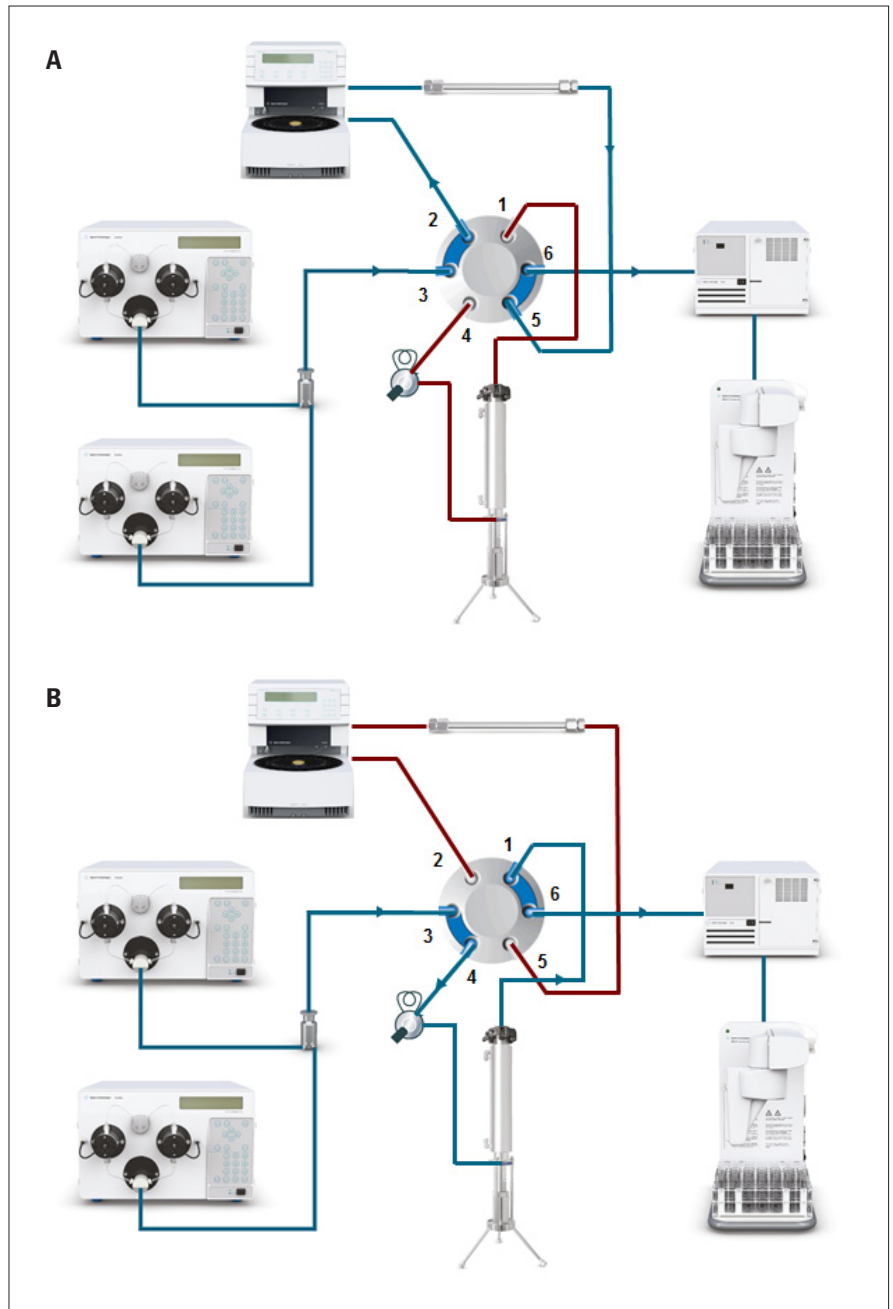


Figure 1
 Schematic of the Agilent SD-1 Purification System configuration: Position A for analytical flow path (top), and Position B for Preparative flow path(bottom).

Capillaries

- From SD-1 Solvent Delivery Modules to dynamic mixer and then to manual 3-way valve: stainless steel capillary, 1/16 in od, 0.04 in/1 mm id
- On the analytical flow path: PEEK tubing, 1/16 in od, 0.007 in/0.17 mm id, yellow
- On the preparative flow path: stainless steel capillary, 1/16 in od, 0.04 in/1 mm id
- From Rheodyne manual 3-way valve to Agilent 325 Dual Wavelength UV-Visible Detector: PEEK tubing, 1/16 in od, 0.02 in/0.5 mm id, orange
- From Agilent 325 Dual Wavelength UV-Visible Detector to Agilent 440 Fraction Collector: flexible tubing, 1/8 in od, 1/16 in id

Description	Part number
1/16" Stainless steel kit 0.040 in	5067-5196
PEEK tubing (yellow) 1/16 in od, 0.007 in/0.18 mm id, 5 m	5042-6462
PEEK tubing (orange), 1/16 in od, 0.5 mm id, 1.5 m	0890-1761
Sample tubing, PTFE, 2.0 mm id, 3.0 mm od, 5 m	5064-8020

Table 1
Capillary kits for plumbing the system.

Software

Agilent OpenLAB CDS ChemStation Edition Revision C.01.03

Sample

The purification mixture is a solution of methylparaben (74.2 mg/mL), ethylparaben (287.0 mg/mL) and propylparaben (69.8 mg/mL) in dimethyl sulfoxide. The total concentration is 431 mg/mL.

Chromatographic Conditions

Parameter	Conditions
Analytical column:	Agilent Prep-C18, 4.6 × 250 mm, 10 µm (p/n 440910-902)
Preparative self-packed column:	Load & Lock 4002 column (p/n PCG93LL 500 × 50) column dimension after packing: 50 × 243 mm
Preparative column media:	Agilent Prep-C18 (p/n 420910-902)
Mobile phase A:	Water + 0.05 % TFA
Mobile phase B:	Acetonitrile + 0.05 % TFA

Table 2
Chromatographic conditions.

The self-packed preparative column is packed with 300 g of Agilent Prep-C18, 10 µm bulk media packed at 1,000 psi bed compression¹.

Results and Discussion

Analysis and Purification

To perform the separation of the three ester derivatives in mixture, a preliminary analysis was required as well as a loading study to identify the resolution of the separation and the loading which would be allowed for the purification sample.

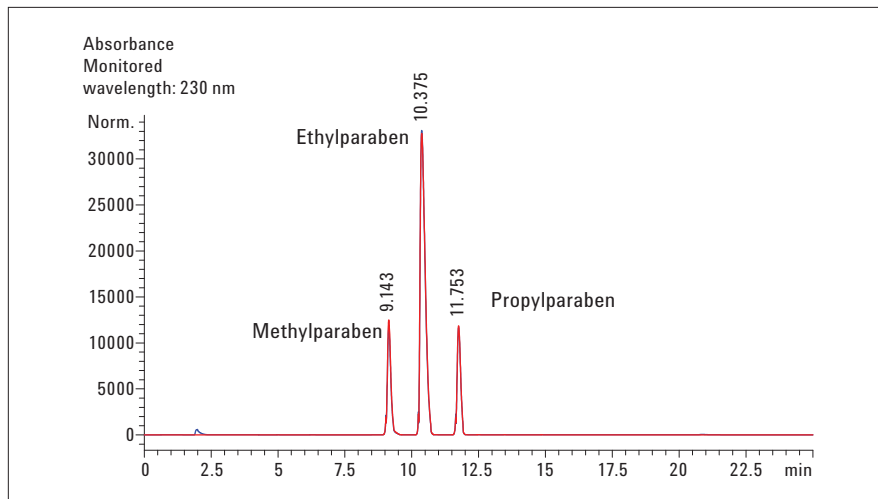


Figure 2
Analytical-scale separation of the sample mixture.

Parameter	Analytical column Agilent Prep-C18, 4.6 × 250 mm, 10 μm
Flow:	1.5 mL/min
Injection volume:	5 μL
Concentration:	431 mg/mL
Gradient:	0.00 min, 5 % B 0.01 min, 5 % B 16.01 min, 95 %B 19.00 min, 95 %B 19.50 min, 5 %B 25.00 min, 5 %B

Due to the dual path of the preparative flow cell of the Agilent 325 Dual Wavelength UV-Vis Detector, the dynamic detection range is enhanced by a factor of 40 (up to 80,000 mAU). Even with high concentrations (in this example 431 mg/mL), the preparative sample can be used for the analytical scouting work. This allows using the highly concentrated preparative sample to perform the analytical to preparative scale-up without any further dilution.

For a low concentrated analyte, the long path-length (4 mm for the super prep flow cell) will monitor the UV-Vis absorbance without saturation of the detector. In the case of higher sample concentration, this path length will be saturated and the short path-length (0.15 mm) will be used automatically to avoid saturation.

With this feature, the identification of the loading, which could be applied on the analytical column for this mixture, was easily determined by increasing the injection volume with 5- μ L steps. The maximum injection volume was determined at 45 μ L (19.3 mg of compounds mixture) on the 4.6 mm id column. The separation of the three compounds was obtained with a resolution of $R_s > 1.1$. This injection volume was used for the scale-up to the preparative column.

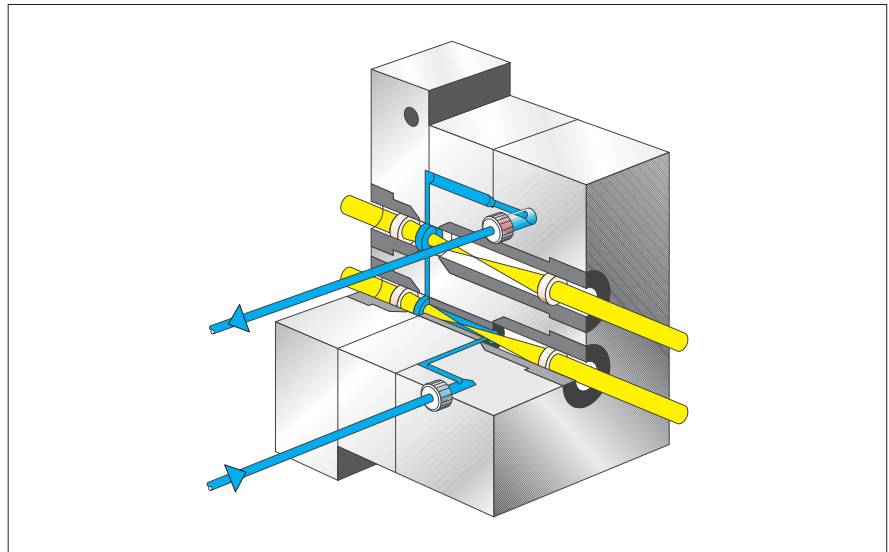


Figure 3
The dual path-length flow cell.

Purification of the Mixture

Using the following system and column characteristics for the analytical-to-preparative method transfer calculation², we obtained the transferred gradient and injection volume for the preparative run shown in Table 3.

Once the analytical method has been transferred to the preparative system, the method developed by this approach can be used for any sample for direct scale-up from the 4.6 to the 50 mm internal diameter columns without modification of the capillaries of the system, or change of detector flow cell.

Thus, the resolution acquired in the analytical sample acquisition, highly dependent upon the concentration of the sample, the gradient, and the system parameters (dwel volume and columns specifications), will be retained in the preparative runs.

Figure 4 shows the 3D overlays of the chromatograms resulting from the analytical and preparative runs. The tick marks in the preparative chromatogram show the fractions collected with a collection threshold of 1,500 mAU. Several fractions of each compound were collected because of the high flow rate and the volume of the fraction vessels.

Table 4 shows the recoveries and purities of each compound collected during the purification sample.

The recoveries and purities were determined by quantitative analysis on an Agilent 1220 Infinity LC system. The combined fractions for each compound were analyzed, concentrations were automatically calculated with the ChemStation, and recoveries and purities were determined.

Preparative flow path		Analytical flow path	
Dwell _{PREP} = 12.0 mL	System characteristics	Dwell _{ANA} = 2.0 mL	Column characteristics
D _{col,PREP} = 50 mm		D _{col,ANA} = 4.6 mm	
L _{col,PREP} = 243 mm		L _{col,ANA} = 250 mm	
D _{part,PREP} = 10 µm		D _{part,ANA} = 10 µm	
Flow rate			
	177 mL/min		
Injection volume			
	5100 µL (2.20 g)		
Gradient			
	0.00 min, 5 %B		
	1.33 min, 5 %B		
	16.86 min, 95 %B		
	19.77 min, 95 %B		
	20.26 min, 5 %B		
	25.00 min, 5 %B		

Table 3

Gradient and injection volume determined by the method transfer formulas.

Note: The injection volume is calculated from the injection volume determined in the loading study (45 µL).

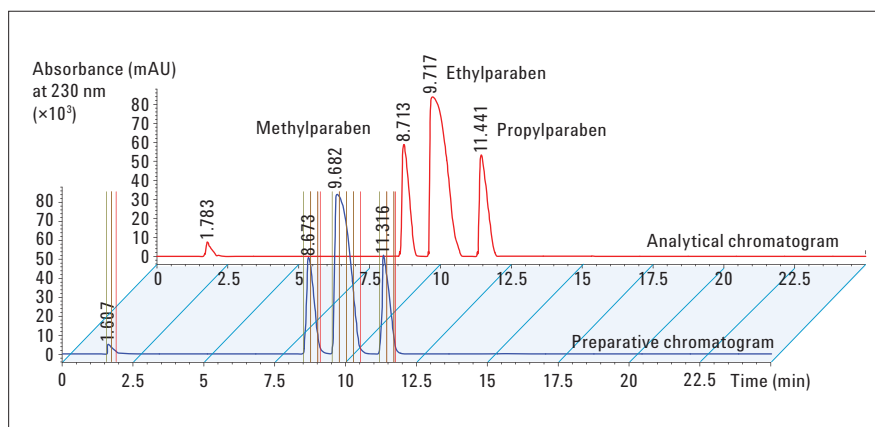


Figure 4

The preparative chromatogram is shown in blue, and the analytical chromatogram in red.

Substance	Theoretical mass (g)	Collected mass (g)	Recovery (%)	Purity (%)
Methylparaben	0.378	0.317	84	96
Ethylparaben	1.463	1.436	98	98
Propylparaben	0.356	0.258	72	97

Table 4

Results obtained after purification.

Conclusion

Due to the unique flow rate capabilities of the Agilent SD-1 Solvent Delivery Modules and the excellent dynamic range of the Agilent 325 Dual Wavelength UV-Visible Detector, we demonstrated the reliability and the excellence of this scale-up from analytical to preparative-scale purification. From an Agilent Prep-C18 Scalar, 4.6 × 250 mm, column to a self-packed 50 × 243 mm Agilent Load & Lock column, a sample mixture of three paraben ester derivatives was successfully resolved with good recoveries and high purities for gram-scale quantities. All this was achieved with no exchange of hardware components, such as capillaries or detector flow cells.

References

1. "A method to pack Agilent Prep C18 Media on Load & Lock Column", Agilent Technical Overview
2. "Analytical to Preparative HPLC Method Transfer", Agilent Technical Overview, publication number 5991-2013EN

www.agilent.com/chem/purification

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