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Analysis of Parabens in Soy Sauce and Vinegar by HPLC Using Agilent Chem Elut S Supported Liquid Extraction (SLE) Cartridges

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Abstract

This Application Note demonstrates the use of Agilent Chem Elut S 12 cc supported liquid extraction (SLE) cartridges for the analysis of seven parabens from soy sauce and vinegar by LC/DAD detection. Both traditional liquid/liquid extraction (LLE) and four commercially available SLE products were tested for comparison. As a technique, SLE provides a simplified workflow compared to traditional LLE, with significant savings in time and effort. Chem Elut S uses a novel synthetic media demonstrating improved performance with the consideration of recovery, reproducibility, capacity, and cleanliness.

Introduction

LLE is a traditional sample preparation technique that can be applied to many aqueous sample extractions by altering the ion strength of the aqueous phase and the extraction solvent polarity. It has demonstrated the ability to extract analytes from various aqueous matrices with good recovery and reproducibility. However, LLE can be time-consuming and labor-intensive in sample mixing for analyte partition, clear phase separation for removing the organic phase, and emulsion formation. LLE can also be much more challenging for multiple extractions, which requires significantly more time and effort, and can be difficult for high-throughput laboratories seeking automated workflows.

SLE is an improved technique that can replace LLE with the advantages of time and effort savings, no emulsion formation, and easy automation. Conventional SLE products use natural diatomaceous earth (DE) material, which is difficult to control for sorbent quality and particle consistency, which can cause product performance variability. The novel synthetic Chem Elut S material is manufactured in a controlled process with strict monitoring of sorbent quality and consistency. Chem Elut S has greatly improved product performance and reproducibility.

Parabens are *para*-hydroxybenzoic acid esters (Figure 1), which are widely added as preservatives in foods such as baked goods, sauces, juices, and jellies. The use of parabens can prolong the shelf life of food products by preventing mold, yeast, and bacteria growth. In some countries such as the US and China, parabens are recognized as safe at certain permissible levels, while they are banned in some other countries such as Malaysia.

There are some suggestions for food sample preparation using LLE in Chinese regulation method GB5009.31-2016.[1] However, the method is very time-consuming, with multiple steps of extraction, and is environmentally unfriendly due to large amounts of diethyl ether. This Application Note demonstrates the use of Chem Elut S 12 cc cartridges for the analysis of seven parabens in soy sauce and vinegar using ethyl acetate as the extraction solvent. Compared with the regulation method of LLE, the Chem Elut S method used a low volume of a less toxic solvent. This method also provided a simplified workflow, saving time and effort while achieving excellent performance and cleanliness.

Experimental

Reagent and chemicals

All regents and solvents were HPLC or analytical grade. Methanol (MeOH) was from Honeywell (Muskegon, MI, USA), ethyl acetate (EtOAc) was from Dikma (Lake forest, CA, USA), and sodium chloride was from Sinopharm Chemical Reagent (Shanghai, China). The paraben standards methyl paraben (MP), ethylparaben (EP), propylparaben (PP), isopropylparaben (iPP), butylparaben (BP), heptylparaben (HP), and benzylparaben (BnP) were from AccuStandard (New Haven, CT, USA). Soy sauce and vinegar were purchased from a local supermarket.
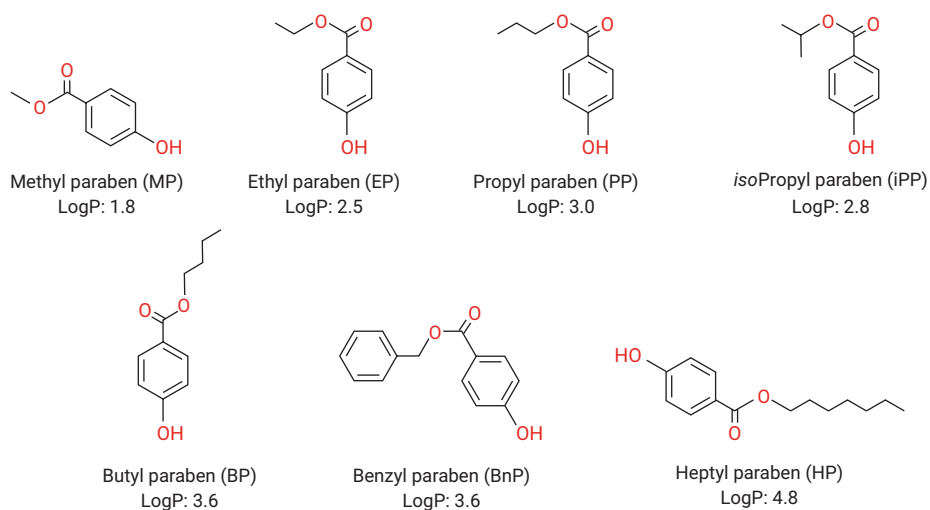

Methyl paraben (MP)
LogP: 1.8

Ethyl paraben (EP)
LogP: 2.5

Propyl paraben (PP)
LogP: 3.0

*iso*Propyl paraben (iPP)
LogP: 2.8

Butyl paraben (BP)
LogP: 3.6

Benzyl paraben (BnP)
LogP: 3.6

Heptyl paraben (HP)
LogP: 4.8

Figure 1. The structures and logP values of seven parabens investigated in soy sauce and vinegar.

Standards and solutions

A combined standard spiking solution was prepared at 1 mg/mL in MeOH, diluted with an appropriate volume of individual stock solutions, and stored in −20 °C until use. A 1% NaCl solution was prepared by dissolving 1 g of NaCl in 100 mL of Milli-Q water.

Instrument and equipment

The analysis was performed on an Agilent 1290 Infinity II LC with an Agilent 1290 Infinity II high speed pump, an Agilent1290 Infinity II multisampler, inline degasser, and an Agilent 1290 Infinity II diode array detector. Product cleanliness was evaluated using GC/MS full scan by an Agilent Intuvo 9000 GC combined with an Agilent 7000 triple quadrupole GC/MS. Agilent OpenLab and Agilent MassHunter software were used for data acquisition and analysis.

Other equipment and materials used for sample preparation included:

- Eppendorf pipettes and repeater
- Eppendorf centrifuge 5810R (Hamburg, Germany)
- Agilent Chem Elut S 12 cc, 3 mL (p/n 5610-2008)
- Four commercially available SLE 12 cc products
- SPEX SamplePrep 2010 Geno/Grinder (Metuchen, NJ, USA)
- Agilent Vac Elut 20 Manifold (part number 12234101)

Sample preparation

The combined standard spiking solution was used to spike the soy sauce and vinegar to determine the recoveries. The method used was revised from a published LLE and SLE method developed for measuring parabens in body wash.[2] Figure 2 describes the sample preparation. The soy sauce and vinegar samples were prepared accordingly before the extraction. Six replicates were performed for both SLE and LLE, respectively, at 20 ng/mL spiking level. Sodium chloride was added to increase the ion strength of the vinegar sample. Chem Elut S (12 cc) and four commercially available SLE cartridge products (12 cc) were investigated and compared. LLE was conducted in a centrifuge tube. To improve the extractive efficiency, a Geno Grinder was used for mixing the samples thoroughly. After centrifugation, the EtOAc layer was transferred to a clean glass tube with extra caution to avoid transferring part of the water layer, and dried down with nitrogen. Finally, the residue was reconstituted with 1 mL of MeOH for injection.
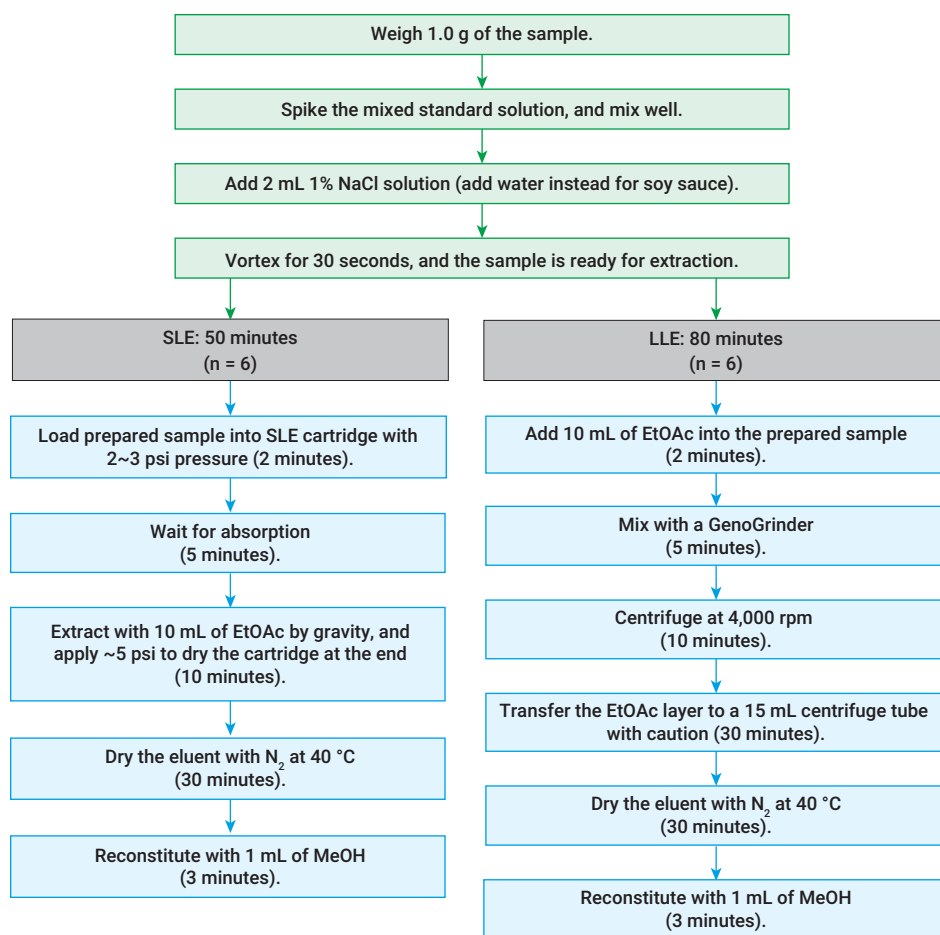

Figure 2. Protocol used to extract parabens from soy sauce and vinegar using SLE and LLE.

HPLC conditions

HPLC columns were investigated for the separation of seven parabens, especially the isomers of PP and iPP. The Agilent InfinityLab Poroshell 120 PFP column demonstrated the best separation compared to C18 columns. Figure 3 shows the overlapped chromatograms of neat standard solution of seven parabens (20 ppb), and the matrix blanks of soy sauce and vinegar after LLE. The main interferences eluted before three minutes and did not interfere with the target parabens chromatographically.

Configuration	
Component	Agilent 1290 Infinity II high speed pump (G7120A) Agilent 1290 Infinity II multisampler (G7167B) Agilent 1290 Infinity II multicolumn thermostat (G7116B) Agilent 1290 Infinity II diode array detector (G7117B)
Column	Agilent InfinityLab Poroshell 120 PFP, 3.0 × 100 mm, 2.7 µm (p/n 695975-308)
Column Temperature	35 ℃
DAD Parameter	260 nm
Injection Volume	2 µL
Mobile Phase	A) water B) MeOH
Gradient	Time (min) %B Flow rate (mL/min) 0 30 0.5 1.0 45 0.5 4.0 65 0.5 6.0 95 0.5 8.0 95 0.5
Stop Time	10 minutes

Results and discussion

SLE versus LLE

Figure 2 details the extraction protocols and processing time for SLE and LLE. The total time required to prepare six replicates was 80 minutes for LLE and 50 minutes for SLE. LLE was more time-consuming and labor-intensive. The separation of the aqueous phase from the organic phase required extra attention, and longer drying time may be needed for LLE if a water layer was transferred accidently. As a result, it was challenging to prepare multiple samples simultaneously. However, SLE showed a much easier walk-away extractions workflow without special precautions, which improved the sample extraction productivity significantly.

Figure 4 shows the results of recovery and reproducibility at 20 ng/g in both soy sauce and vinegar with six replicates. Both LLE and synthetic SLE (Chem Elut S) methods generated good results in terms of analyte recoveries (88 to 110%) and reproducibility (RSD <10%).
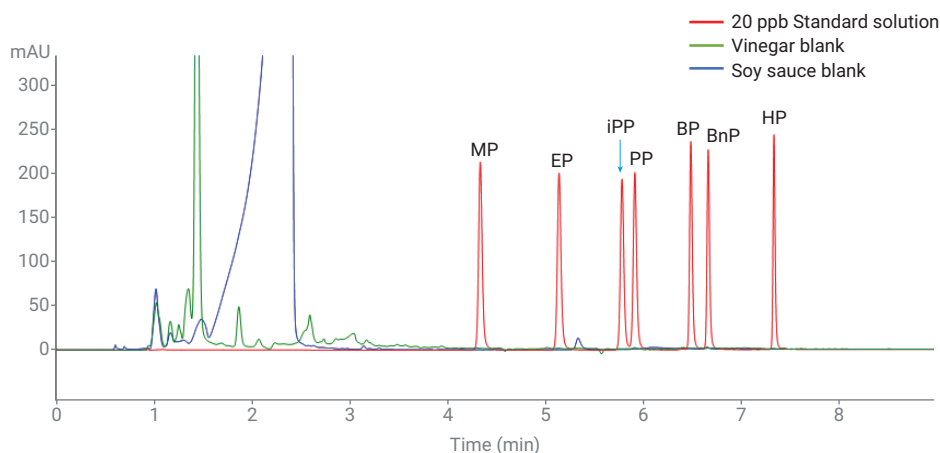

Figure 3. Typical chromatograms of parabens standard solution (red) for MP, EP, PP, iPP, BP, HP, and BnP, vinegar matrix blank after LLE (green), and soy sauce matrix blank after LLE (blue).

Chem Elut S versus competitors' SLE

We compared the performance of Chem Elut S and four commercial SLE products on the seven parabens in soy sauce and vinegar at 20 ng/g spiking level. Competitors 1, 3, and 4 were DE-based sorbent, and competitor 2 was a synthetic SLE sorbent. There was not a big difference between products for soy sauce, but Chem Elut S showed an obvious advantage for vinegar, especially for the most hydrophobic paraben: HP. Chem Elut S and competitor 2's product showed a slight advantage (RSD <5%) overall. Generally, Chem Elut S is equivalent to or slightly better than the other four SLE products when analyzing HP (Figure 5).
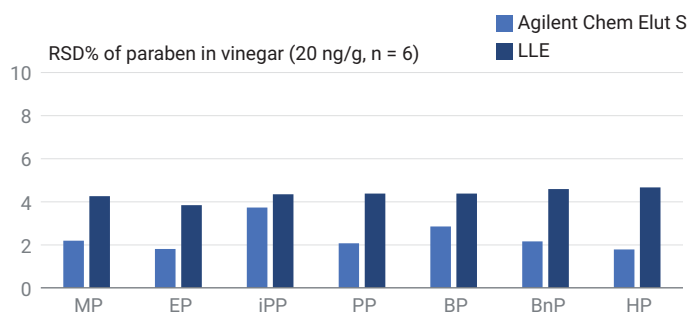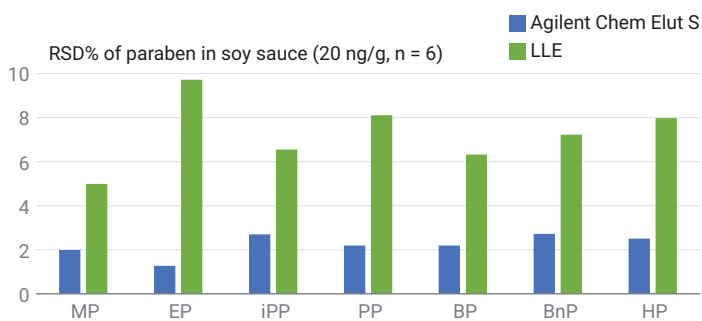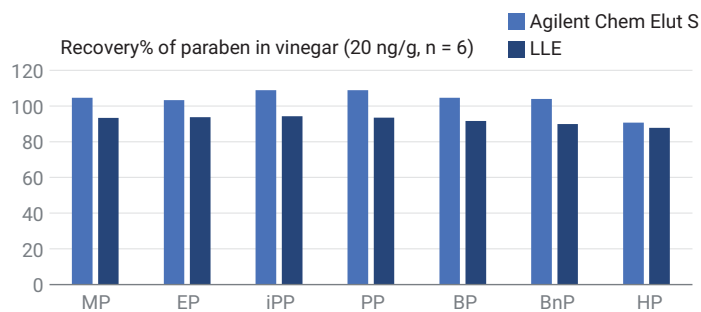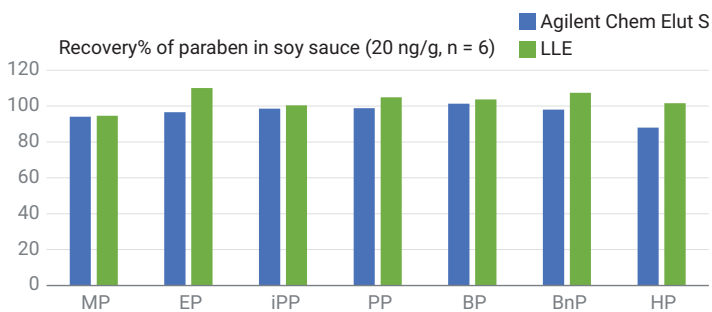
Figure 4. Recovery and RSD results from Agilent Chem Elut S and LLE methods at 20 ng/g spiking level with six replicates.
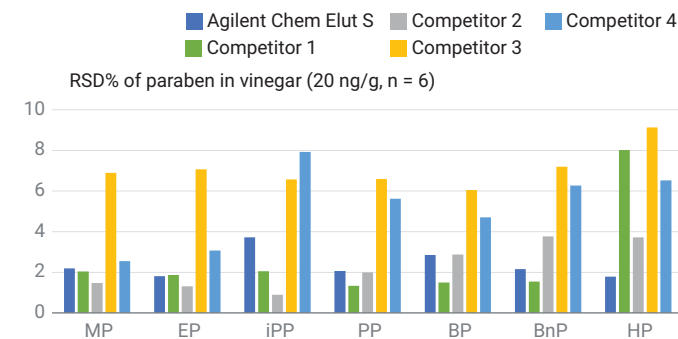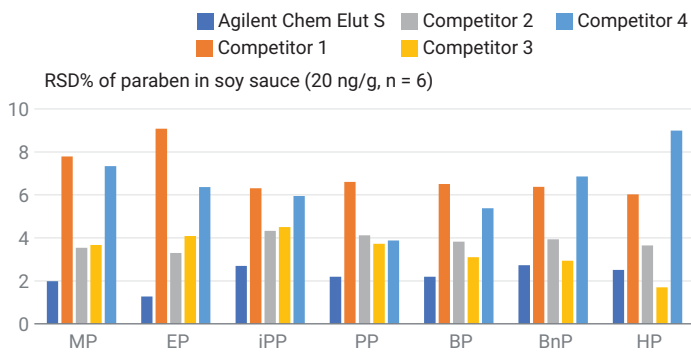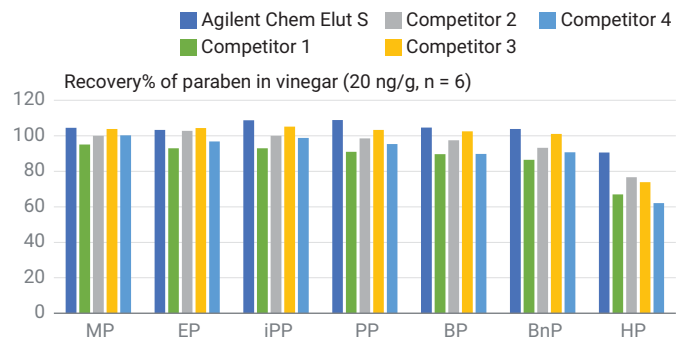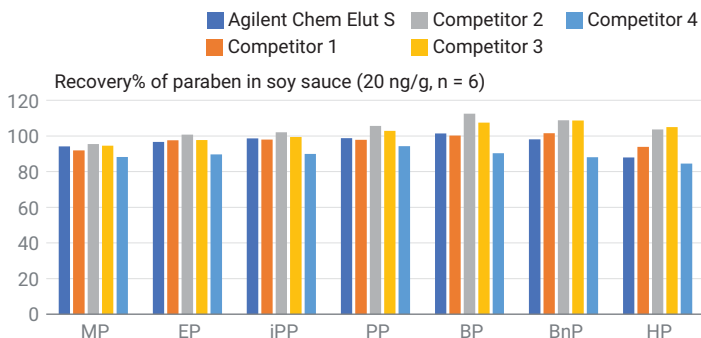

Figure 5. Recovery and reproducibility results from Agilent Chem Elut S and four competitors' SLE at 20 ng/g spiking level with six replicates.

Chem Elut S sorbent provides higher water-holding capacity. As a result, the recommended sample loading volume is 3 mL, which is 1.5 times more than the recommended loading volume (2 mL) from other competitors' equivalent format cartridges. This feature certainly improved the method sensitivity, given similar analyte recoveries due to the larger sample size. Figure 6 shows the chromatograms for the same sample concentration (5 ng/g in vinegar) analysis using different SLE products. Chem Elut S (light green) demonstrates the highest peak response and sensitivity without obvious interferences, while co-extracts were observed from the results of competitors 2 (red) and 4 (blue).
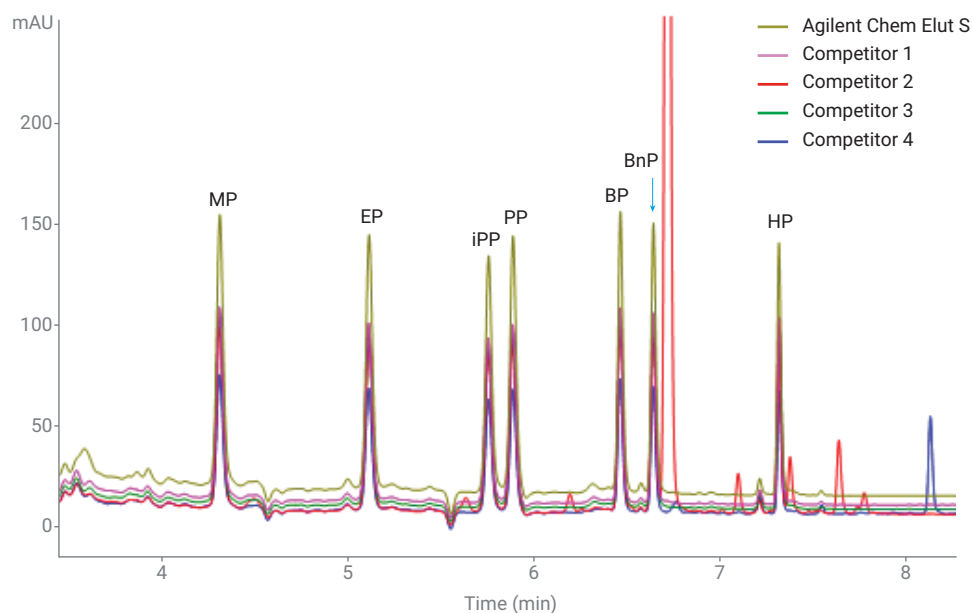

Figure 6. Chromatographic comparison of the same format (12 cc) cartridge with a sample of 5 ng/g parabens in vinegar.

There were unexpected contamination peaks found with some of the SLE products. To verify the source of the contamination, we tested the cleanliness of the SLE products with water instead of the matrix with a similar protocol. Figure 7 shows that the cleanliness of the products was investigated using both UV detection at 260 nm and GC/MS full scan detection. Competitor 2 (synthetic SLE) showed many more interferences from the cartridge with both UV detection and GC/MS full scan. Competitor 4 SLE cartridges also showed interference peaks. Chem Elut S and competitor 1 and competitor 3 products have fewer interferences. The results of UV detection are consistent with the interference peaks in the paraben analysis of soy sauce and vinegar in terms of retention time and peak height. This proves that the interferences were sourced from SLE products rather than food matrix.

Conclusion

This Application Note conducted a comprehensive comparison of LLE and SLE using multiple SLE products in soy sauce and vinegar. SLE uses a simple extraction without sample emulsion formation, which reduces extraction time by 40% compared to LLE. Compared to LLE, Chem Elut S also demonstrated superior recovery and precision for parabens analysis in food, excellent water-holding ability, and qualified sorbent cleanliness. When compared to other commercially available SLE products, Chem Elut S demonstrated improved or equivalent precision and analyte recovery, especially with more hydrophobic analytes. Lastly, the Chem Elut S method delivered an easier workflow, higher sensitivity, better reproducibility, and eliminated sample contamination.
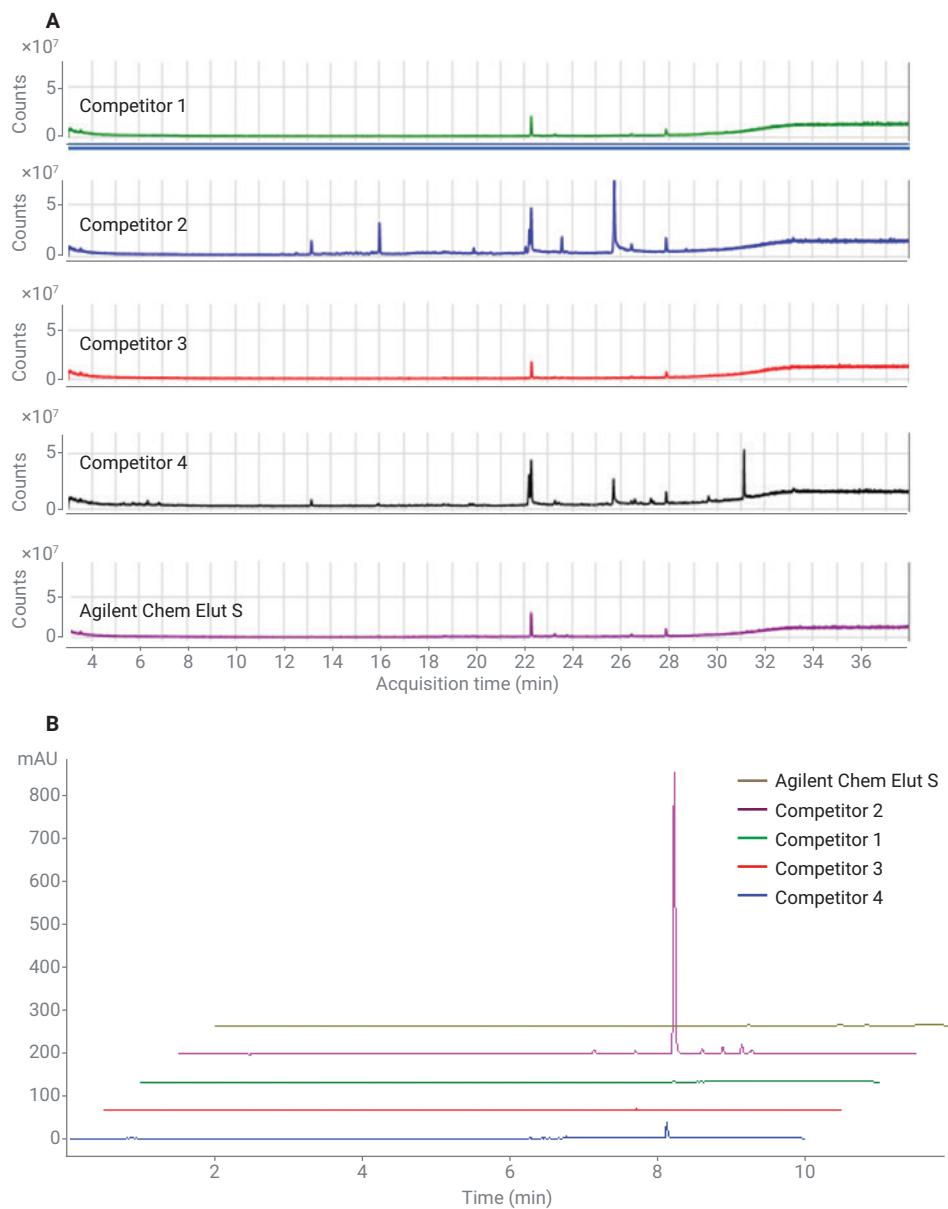
Figure 7. Cleanliness testing with neat solvent for 12 cc SLE cartridges by GC/MS full scan (A) and 260 nm UV detection (B).

References

1. Chinese National Food Safety Standard - Determination of p-Hydroxybenzoic Acid Esters in Foodstuffs: GB5009.31-2016.

2. Zimmerman, D. C.; *et al.* Determination of Parabens in Body Wash using Solid-Supported Liquid-Liquid Extraction, *Agilent Technologies Application Note*, publication number 5991-2735EN, **2013**.

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