Highly Reproducible Extraction and Analysis of Sulfonamides from Honey using Strata™-X*-C Polymeric SPE Sorbent and Gemini® C18 HPLC Column

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Strata-X-C allows for the implementation of a very aggressive, 100 % organic wash, which is necessary in order to remove interfering matrix contaminants in difficult sample matrices like honey. A highly reproducible method has been developed using Strata-X-C SPE sorbent and a Gemini C18 HPLC column to retain and separate five different sulfa drugs.

Introduction

American and European Foulbrood (AFB and EFB) in honeybees is caused by two bacterial species, Paenibacillus larvae and Melissococcus pluton, respectively. For control of these bacteria, only a few antibiotics are recommended in Europe and the U.S.A. However, a variety of antibacterial agents are used, sulfa drugs being the most popular. Residues of these antibiotics are quite often found in honey samples and are of concern to consumers around the world due to toxic or allergic reactions. Earlier sample preparation methods involved using silica-based C18[1] or neutral polymers as sorbents[2] with an aqueous wash prior to elution of sulfonamides. In this communication, we describe a simple and effective method for cleanup and quantitation of antibacterial sulfonamides from honey using the polymeric strong cation exchange SPE sorbent Strata-X-C and a Gemini HPLC column.

Experimental Materials

Honey used was obtained from a local supermarket. All solvents and chemicals are from Sigma-Aldrich Biotechnology, Milwaukee, WI. The Strata-X-C cartridges (60 mg/3 mL) for solid phase extraction (SPE) and Gemini C18 columns (150 x 3.0 mm, 5 µm) for LC/MS are from Phenomenex, Torrance, CA. An Agilent 1100 HPLC system coupled to an API 3000™ mass spectrometer (ESI^+ source) was used for analysis of elution fractions from SPE.

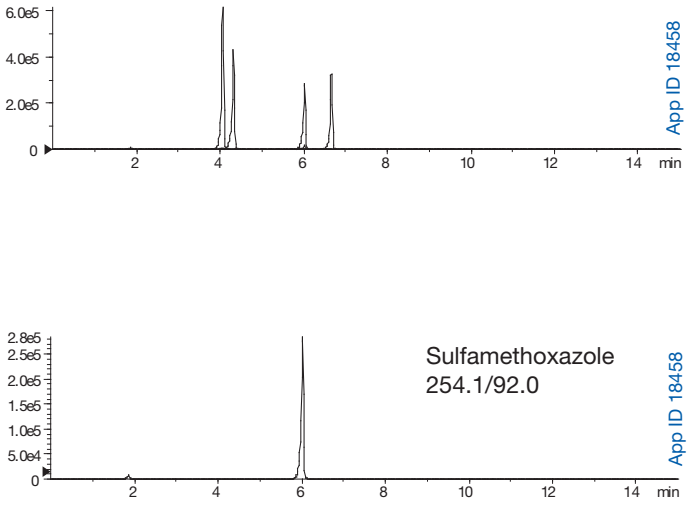
Solid Phase Extraction

The cartridge is conditioned with 2 mL methanol, followed by 2 mL of deionized water. The honey sample (1 gram) is acidified with 1 mL of 2 M hydrochloric acid, sonicated for 30 min and then treated with 0.3 M citric acid in water so as to make up the total volume to 5 mL. The sulfa drugs are spiked into this diluted honey solution and loaded onto the cartridge. Washing was done with 4 mL of water (in two aliquots), then with 4 mL of 50:50 methanol/acetonitrile (in two aliquots) and then the cartridge was dried for 2 min at 10" of Hg pressure. Elution was carried out with 2 mL of 2 % ammonium hydroxide in methanol. Internal standard was then added and the eluate evaporated under a slow stream of nitrogen at 40 °C. The residue was reconstituted into 100 µL of mobile phase.

Table 1.
Physicochemical Characteristics and Recovery Yields of Sulfa Drugs

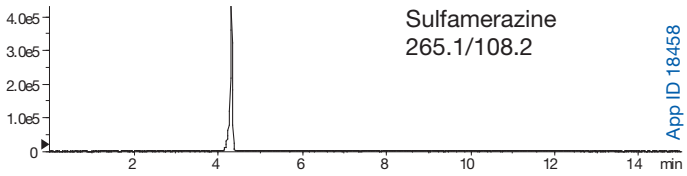
Analytes	Spiked Conc. (ng/mL)	MRM	log P of analyte	pK_a of analyte	% Recovered	% RSD
Sulfanilamide	100	173.1→93.1	-0.62	2.4, 10.4	34 %	2-5 %
Sulfathiazole	100	256.1→92.1	0.09	2.08,7.0	81 %	2-5 %
Sulfamerazine	100	265.1→108.2	0.54	1.16,1.54, 2.0, 9.55	78 %	2-5 %
Sulfamethoxazole	100	256.0→92.1	1.58	1.83,5.65	94 %	2-5 %
Sulfaquinoxaline	100	301.2→92.1	1.68	1.86,5.56	IS	2-5 %

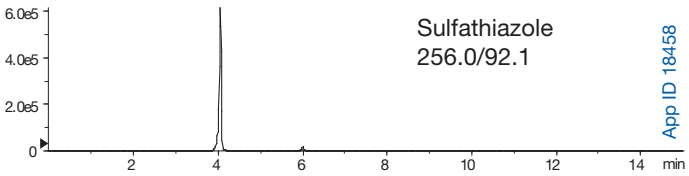
Figure 1.
LC/MS/MS of SPE extract of sulfa drugs
(see **Table 1** for MRM details)

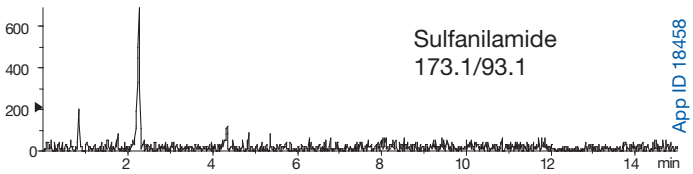


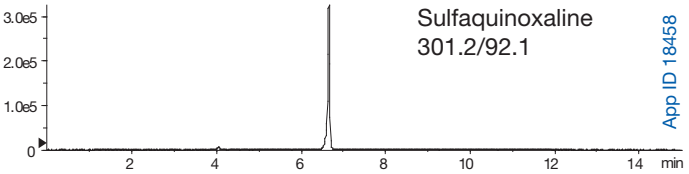
*Strata-X is patented by Phenomenex, Inc.

Figure 1. (cont'd)









Liquid Chromatography

Column: Gemini® 5 µm C18
Dimensions: 150 x 3.0 mm
Part No.: 00F-4435-Y0
Mobile Phase: A: 0.1 % Formic Acid in Water
B: 0.1 % Formic Acid in Acetonitrile
Gradient: A/B 90:10 to 30:70 in 8 min
Flow Rate: 0.6 mL/min
Detection: API 3000 LC/MS/MS with ESI^+ (TurbolonSpray®), heater gas flow 7000 cc/min, heater temp. 425 ˚C

Results and Discussion

Honey is a conglomeration of several classes of compounds that include carbohydrates, aliphatic carboxylic acids, amino acids, proteins and minerals. Such a matrix presents a challenge for isolating the antibiotic contaminants, especially sulfa drugs. Sulfa drugs carry aromatic amino groups, which can react with the sugars forming Schiff's bases. Hence acidic conditions are needed to break up such interactions. The cation exchange sorbent Strata™-X-C is ideal for eliminating the matrix components since sulfa drugs form strong ionic bonds with the sulfonic acid moieties on this sorbent. This facilitates stronger wash with organic solvents to get rid of the organic impurities while retaining the sulfa drugs. **Figure 1** and **Table 1** show the LC/MS/MS graphics and recovery data, respectively. Sulfanilamide is the only compound that shows lower recovery, due to its extremely polar characteristics (see Table 1 for log P values of the sulfa drugs studied). The strong organic wash (see experimental section) removes part of sulfanilamide, such a wash being necessitated by the complexity of the honey matrix. With Strata-X-C, sulfa drugs including sulfanilamide are all recovered from plasma matrices in excellent yields[3], demonstrating that the sorbent is capable of retaining sulfanilamide during pure methanol wash. Also, part of the lower recovery yield can be attributed to the aromatic primary amino moiety of sulfanilamide (the most basic amongst sulfa drugs) forming a Schiff's base with the sugars, an aspect already stressed in the literature[2].

Conclusion

The strong retention mechanism of Strata-X-C allows for the aggressive wash conditions necessary for the cleanup of difficult matrices and the reproducibly high recoveries accomplished. Coupled with the LC/MS/MS analysis using Gemini C18, the end result is a very sensitive method for analyzing Sulfonamides in honey.

References

1. A. Posyniak, J. Zmudzki, J. Niedzielska, T. Sniegocki and A. Grzebalska, *APIACTA*, **2003**, 38, 249-256.
2. A. Kaufmann, S. Roth, B. Tyser, M. Widmer and D. Guggisberg, J. *AOAC International*, **2002**, 85, 853-860.
3. S.Huq, J. Teuscher and K. Kallury, *LC-GC (Europe) Applications Book*, Sept. **2003**, pp.16-17.

Ordering Information

Sample Preparation

Part No.	Description	Unit
8B-S029-TAK	Strata™-X-C Tubes (30 mg/1 mL)	100/box
8B-S029-UBJ	Strata-X-C Tubes (60 mg/3 mL)	50/box
8B-S029-FBJ	Strata-X-C Tubes (200 mg/3 mL)	50/box
8B-S029-HBJ	Strata-X-C Tubes (500 mg/3 mL)	50/box
8B-S029-ECH	Strata-X-C Tubes (100 mg/6 mL)	30/box
8B-S029-FCH	Strata-X-C Tubes (200 mg/6 mL)	30/box
8B-S029-HCH	Strata-X-C Tubes (500 mg/6 mL)	30/box
8B-S029-HDG	Strata-X-C Giga™ Tubes (500 mg/12 mL)	20/box
8B-S029-JDG	Strata-X-C Giga™ Tubes (1 g/12 mL)	20/box
8E-S029-AGB	Strata-X-C 96-Well Plate (10 mg)	2 Plates/Box
8E-S029-TGB	Strata-X-C 96-Well Plate (30 mg)	2 Plates/Box
8E-S029-UGB	Strata-X-C 96-Well Plate (60 mg)	2 Plates/Box
AH0-6023	12-Position SPE Vacuum Manifold	ea

HPLC				SecurityGuard™ Cartridges (mm)	
Part No.	Description	Dimensions	Unit	4 x 2.0†	4 x 3.0†
00B-4435-B0	Gemini® 5 µm C18	50 x 2.0 mm	ea	AJ0-7596	–
00F-4435-Y0	Gemini 5 µm C18	150 x 3.0 mm	ea	AJ0-7596	–
00F-4435-E0	Gemini 5 µm C18	150 x 4.6 mm	ea	–	AJ0-7597
00G-4435-E0	Gemini 5 µm C18	250 x 4.6 mm	ea	–	AJ0-7597
00B-4439-B0	Gemimi 3 µm C18	50 x 2.0 mm	ea	AJ0-7596	–
00F-4439-B0	Gemimi 3 µm C18	150 x 2.0 mm	ea	AJ0-7596	–
00F-4439-Y0	Gemimi 3 µm C18	150 x 3.0 mm	ea	AJ0-7596	–
00B-4439-E0	Gemimi 3 µm C18	50 x 4.6 mm	ea	–	AJ0-7597
00F-4439-E0	Gemimi 3 µm C18	150 x 4.6 mm	ea	–	AJ0-7597

†SecurityGuard™ Analytical Cartridges require holder, Part No.: KJ0-4282

guarantee

If Strata-X-C SPE products and Gemini analytical HPLC columns do not provide at least equivalent results and separations as compared to products of similiar dimensions, phase, and particle size, return the product with comparative data within 45 days for a FULL REFUND.

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