

Extraction and Analysis of Sulfonamides from Honey by LC/MS/MS using strata™ X-C Polymeric SPE Sorbent and Gemini™ C18 HPLC Column

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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, the 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 C18¹ or neutral polymers as sorbents² 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

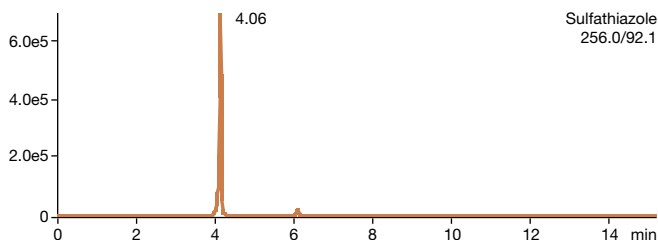
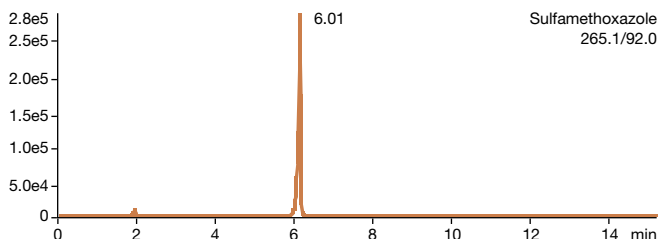
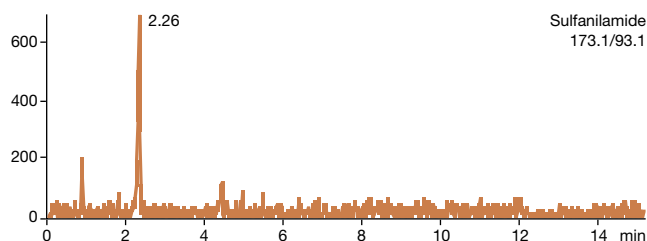
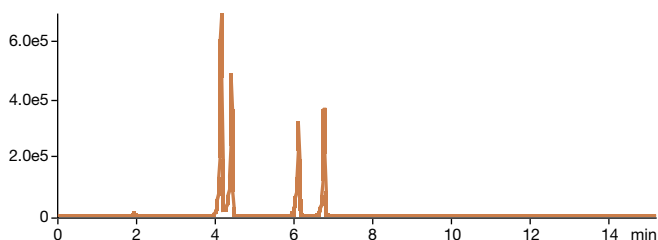
Materials: Honey used was obtained from a local supermarket. All solvents and chemicals are from Aldrich, Milwaukee, WI. The strata-X-C cartridges (60mg/3mL) for solid phase extraction (SPE) and Gemini C18 columns (150 x 3.0mm, 5µm) for LC/MS are from Phenomenex, Torrance, CA. An Agilent 1100 HPLC system 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 2mL methanol, followed by 2mL of deionized water. The honey sample (1 gram) is acidified with 1mL of 2M hydrochloric acid, sonicated for 30 min and then treated with 0.3M citric acid in water so as to make up the total volume to 5mL. The sulfa drugs are spiked into this diluted honey solution and loaded onto the cartridge. Washing was done with 4mL of water (in two aliquots), then with 4mL of 50:50 methanol/acetonitrile (in two aliquots) and then the cartridge was dried for 2min at 10" of Hg pressure. Elution was carried out with 2mL 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 100µL 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→93.1 | -0.62 | 2.4, 10.4 | 34% | 2-5% |
| Sulfathiazole | 100 | 256→92.1 | 0.09 | 2.08, 7.07 | 81% | 2-5% |
| Sulfamerazine | 100 | 265.1→108.2 | 0.54 | 1.16, 1.54, 2.0, 9.55 | 78% | 2-5% |
| Sulfamethoxazole | 100 | 254.1→92.0 | 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)



Liquid Chromatography:

Column: Gemini 5µm C18; Dimensions: 150 x 3.0mm; Part No.: 00F-4435-Y0-TN; 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: 600µL/min; Detection: API 3000 LC/MS/MS with ESI⁺ (TurboIon Spray), heater gas flow 7000cc/min, heater temp. 425°C



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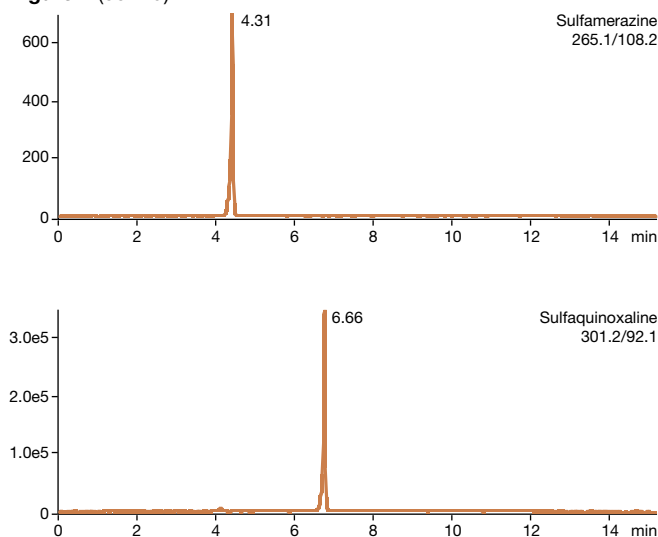
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Figure 1 (cont'd)



Result 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 yields³, 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 literature².

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

strata™X-C (selected dimensions)*

| Part No. | Dimension |
|----------------|--|
| 8B-S029-TAK-TN | strata-X-C 30mg/1mL Tubes (100/Box) |
| 8B-S029-UBJ-TN | strata-X-C 60mg/3mL Tubes (50/Box) |
| 8B-S029-FBJ-TN | strata-X-C 200mg/3mL Tubes (50/Box) |
| 8B-S029-HBJ-TN | strata-X-C 500mg/3mL Tubes (50/Box) |
| 8B-S029-ECH-TN | strata-X-C 100mg/6mL Tubes (30/Box) |
| 8B-S029-FCH-TN | strata-X-C 200mg/6mL Tubes (30/Box) |
| 8B-S029-HCH-TN | strata-X-C 500mg/6mL Tubes (30/Box) |
| 8B-S029-EDG-TN | strata-X-C 100mg/12mL GigaTubes (20/Box) |
| 8B-S029-HDG-TN | strata-X-C 500mg/12mL Giga Tubes (20/Box) |
| 8B-S029-JDG-TN | strata-X-C 1000mg/12mL GigaTubes (20/Box) |
| 8E-S029-AGB-TN | strata-X-C 96-Well Plate 10mg/well (2/Box) |
| 8E-S029-TGB-TN | strata-X-C 96-Well Plate 30mg/well (2/Box) |
| AH0-6023-TN | 12-Position SPE Vacuum Manifold |

Gemini™ 3µm C18 (selected dimensions)*

| Part No. | Dimension |
|----------------|-------------|
| 00B-4439-B0-TN | 50 x 2.0mm |
| 00B-4439-E0-TN | 50 x 4.6mm |
| 00F-4439-B0-TN | 150 x 2.0mm |
| 00F-4439-E0-TN | 150 x 4.6mm |

Gemini™ 5µm C18 (selected dimensions)*

| Part No. | Dimension |
|----------------|-------------|
| 00B-4435-B0-TN | 50 x 2.0mm |
| 00F-4435-Y0-TN | 150 x 3.0mm |
| 00F-4435-E0-TN | 150 x 4.6mm |
| 00G-4435-E0-TN | 250 x 4.6mm |

* Other dimensions and particle sizes available, please contact your Phenomenex representative for more information.