アプリケーションノート

LC-MS/MS Determination of Nitrofuran Metabolite Residues in Honey

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Abstract

In this application note an LC-MS/MS method is described for the quantification of nitrofuran metabolite

residues in commercially available honey.

Introduction

Apiculturerelies on antibiotics to prevent disease propagating through the densely populated bee colonies. The overuse of antibiotics in honey bee colonies can cause high levels of residues in honey products, which becomes a public health issue. Additionally, bacteria that have developed resistance to the applied antibiotics can pose an increased threat to both human and animal health. Consequently, antibiotics become less effective against bacteria and there will be fewer alternatives available for the successful treatment of infection. Unscrupulous producers search for these alternative antibiotics such as nitrofurans to treat disease.

Nitrofuran antibiotics metabolize rapidly with an *in vivo* half-life in the order of hours, making parent drug detection ineffective. An LC-MS/MS method is described for the quantification of nitro furan metabolite residues [AOZ, AMOZ, 1-Aminohydantion(AH), Semicarbizide (SC)] in commercially available honey. The metabolite residues were extracted from the honey samples by first dissolving the honey in HCl. The samples were cleaned, derivatized, and then enriched using Oasis HLB solid-phase extraction (SPE) devices. The metabolite residues were resolved chromatographically using a XTerra MS C_{18} Column. Positive ion electrospray mass spectrometry (MS) was used to quantify and confirm the parent ion $[M+H]^+$ and fragments for each target analyte.

Experimental

Preparation of Honey Samples

The honey samples were prepared for analysis using a two step SPE process (Figure 1). The first step provides a simple pass through clean-up to fractionate the analytes from the bulk of the matrix. This dramatically improves the subsequent derivitazation procedure. The second SPE protocol provides additional clean-up as well as providing a sample enrichment factor of 10 to 1 (2 g Honey into 200 mL).

A 2 g sample of honey was diluted with 5 mL of 0.12 M HCl and prepared using the procedu reoutlined in Figure 1. The eluent was quantitatively collected and 300 mL of 50 mM 2-nitro benzaldehyde in DMSO is added for derivitization. The sample was hydrolyzed and derivatized for 18 h at 37 °C. The sample was cooled to room temperature and adjusted to pH 7 by addition of 6 mL of 0.1 M K_2HPO_4 prior to SPE step 2.

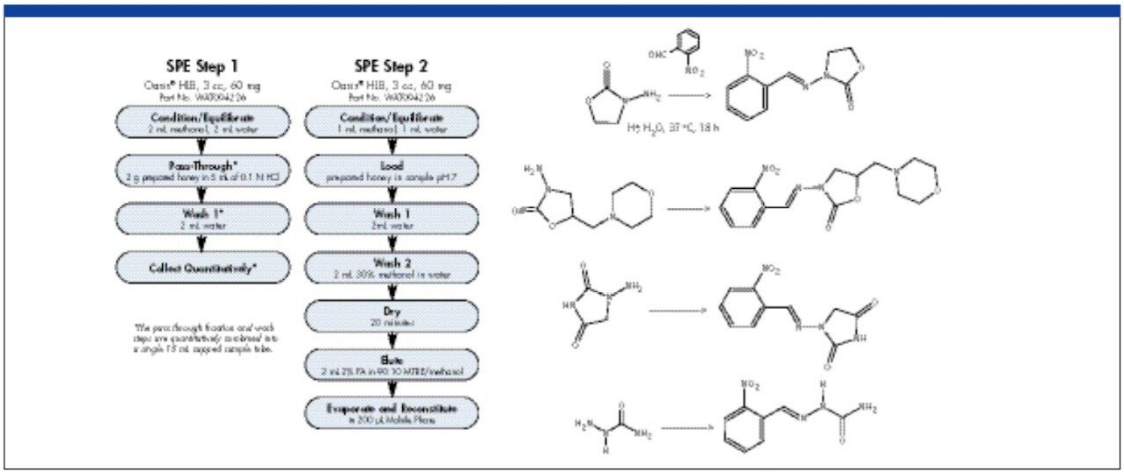

Figure 1. SPE Protocol Summary. SPE Step 1 removes polyphenolic constituents, waxes, and organic contaminants. The sugars and the underivatized nitrofuran metabolites pass through the cartridge. This cartridge is discarded. The sugars are removed post-derivatization in SPE Step 2 before the final SPE elution of the derivatized nitrofuran metabolites.

HPLC Conditions

Instrument:	Alliance 2695 Separations Module
Column:	XTerra MS C_{18}, 3.5 mm, 2.1 3 100 mm
Part number:	186000404
Flow rate:	0.20 mL/min
Mobile phase:	Isocratic 70% 20 mM ammonium formate pH 4.0, 30% ACN
Injection volume:	20.0 mL
Temperature:	30 °C

MS Conditions

Instrument:	Waters Micro mass Quattro Micro

Interface:	Positive Electrospray (ESI+)
Optics:	Capillary - 2.9 kV
Extractor:	4 V
RF Lens:	0.1 V
Source Block temperature:	150 °C
Desolvation temperature:	350 °C

MRM Parameters

Analyte	MRM	Cone (V)	Collision (V)
AOZ	236→134	28	12
AMOZ	335→291	28	12
SC	209→192	25	10
AH	249→178	28	15

Results and Discussion

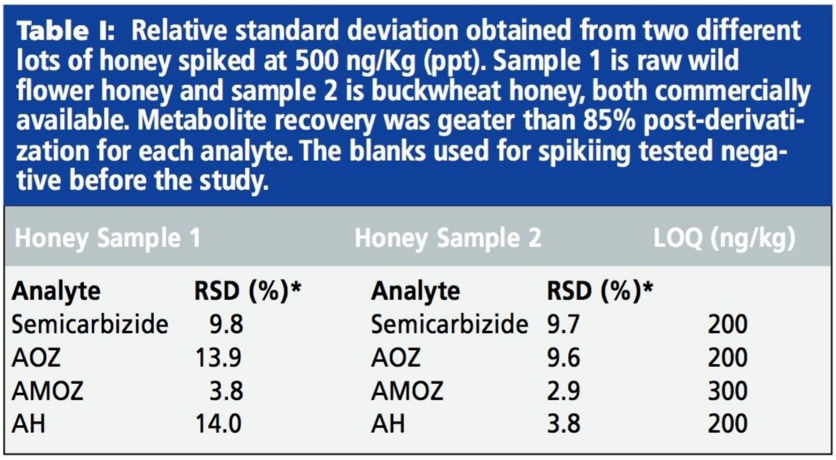

Table I: Relative standard deviation obtained from two different lots of honey spiked at 500 ng/Kg (ppt). Sample 1 is raw wild flower honey and sample 2 is buckwheat honey, both commercially available. Metabolite recovery was geater than 85% post-derivatization for each analyte. The blanks used for spikiing tested negative before the study.

Honey Sample 1		Honey Sample 2		LOQ (ng/kg)
Analyte	**RSD (%)***	**Analyte**	**RSD (%)***	
Semicarbizide	9.8	Semicarbizide	9.7	200
AOZ	13.9	AOZ	9.6	200
AMOZ	3.8	AMOZ	2.9	300
AH	14.0	AH	3.8	200

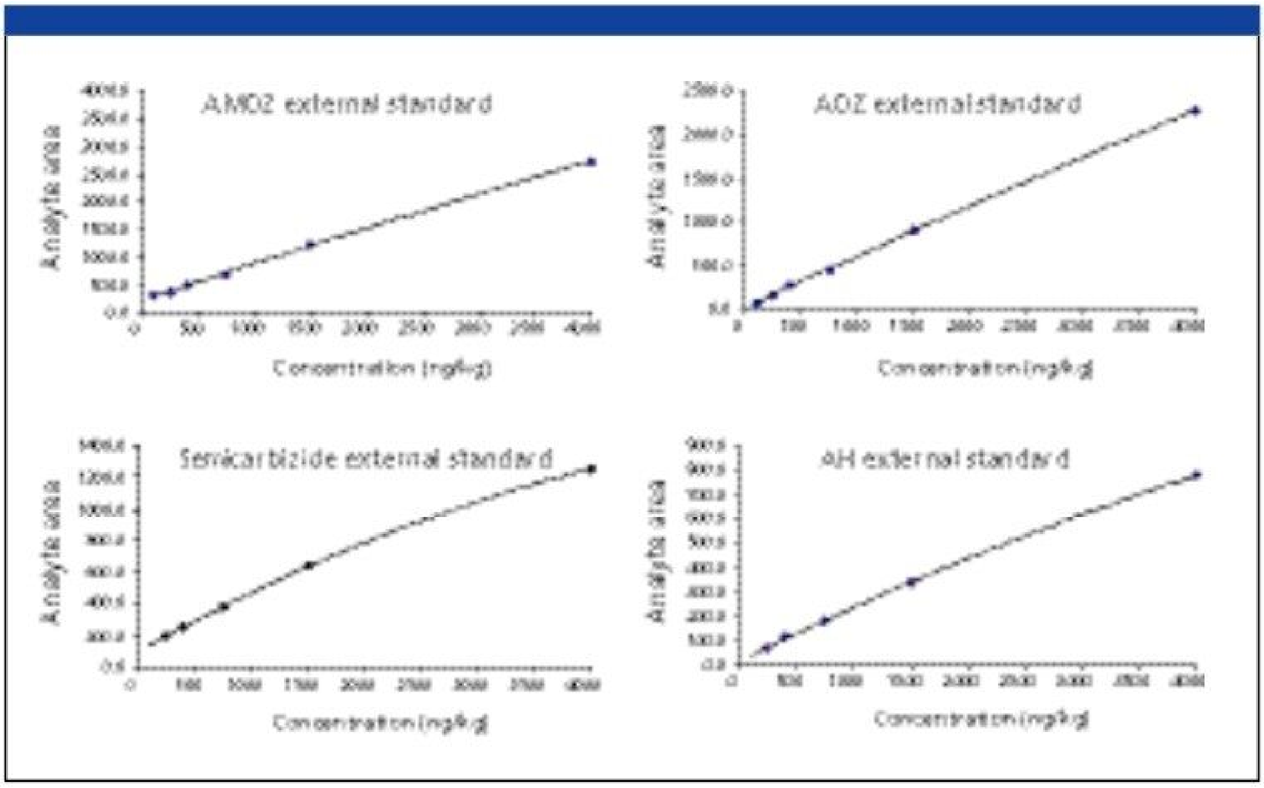

Figure 2. Representative calibration curves spiked into blank honey. Note: Suitable deuterated internal standards were not available at the time of this study. The resulting nonlinear calibration curves for SC and AH were fit to a quadratic function.


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Figure 3. Left-side column (SPE Step 1): The column on the left shows the retained matrix resulting from the initial sample pass-through. This column is discarded and the passed-through sample is then derivatized. Right-side column (SPE Step 2): The column on the right shows the sample clean-up after the final analyte elution.

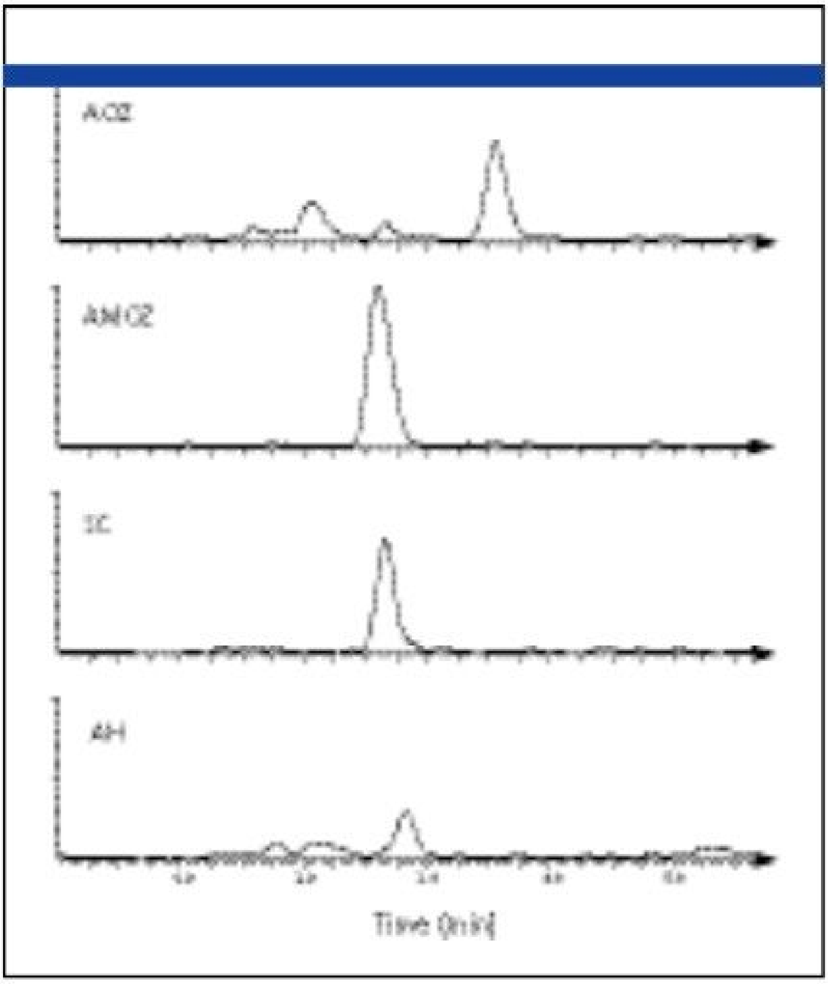

Figure 4. Representative chromatograms of a 400 ng/Kg spiked honey sample.

Conclusion

The SPE protocol described in this paper provides sample enrichment and cleanup acceptable for the routine determination of nitrofuran metabolites in honey. Results obtained from fortified honey samples indicate that the limit of quantification (LOQ) was below 300 ng/Kg.

Sample derivatization is necessary for chromatographic retention for the small, polar nitrofuran metabolites. Unfortunately, derivatization in the presence of unwanted matrix reduces the reaction efficiency and may increase matrix side-reactions that can interfere with the LC analysis. The two-step SPE procedure described in this method was optimized to minimize the matrix effects prior to derivatization. This procedure reduced the undesirable matrix interferences while minimizing the amount of derivatization reagent required to achieve a successful reaction.

References

1. Waters Application Note 720000847EN <https://www.waters.com/webassets/cms/library/docs/720000847en.pdf> , Waters Corporation, Milford, Massachusetts.
2. Waters Application Note 720000705EN <https://www.waters.com/webassets/cms/library/docs/720000705en.pdf> , Waters Corporation, Milford, Massachusetts.

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