Analysis of Nitrofuran Metabolites in Honey Using the SCIEX Triple Quad™ 3500 System

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Overview

A LC-MS/MS method for the simultaneous quantification of four Nitrofuran Metabolites (3-amino-2-oxazolidinone (AOZ) for furazolidone, 3-amino-5-methylmorpholino-2-oxazolidinone (AMOZ) for furaltadone, 1-aminohydantoin (AHD) for nitrofurantoin and semicarbazide (SEM) for nitrofurazone) on SCIEX Triple Quad™ 3500 was developed to detect Nitrofuran residues in honey samples, The method showed adequate linearity with correlation coefficients above r≥0.99 for all four analytes. The Minimum Required Performance Limit (MRPL) for Nitrofuran Metabolites in Honey was 1µg/kg.

Introduction:

Nitrofurans are broad spectrum antibacterial agents which were used in the treatment of bacterial infections in bee colony health. Nitrofurans have been prohibited in food-producing animals in the European Union and most other Countries for public health and safety concerns. The nitrofurans are unstable and easily metabolized within a few hours but Nitrofuran metabolites are highly stable in nature. Several methods have been described in the analysis of nitrofuran metabolite in honey samples by incubation period for derivatization with nitrobenzaldehyde in overnight or 16 hours at 37 ºC.

The LC-MS/MS method developed on SCIEX Triple Quad™ 3500 described here for the quantitation of nitrofuran metabolites in honey was found to meet the regulatory requirements of 1µg/kg.

Figure 1: SCIEX Triple Quad™ 3500

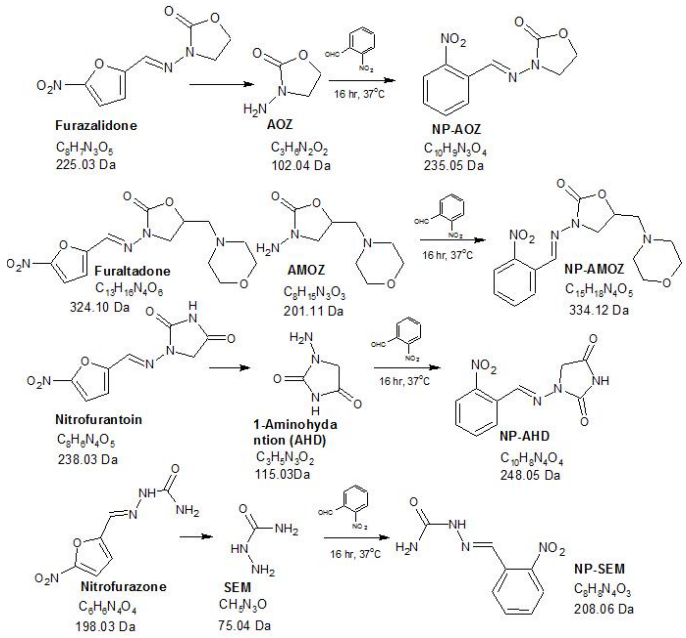

Figure 2: Structures of Nitrofuran, Nitrofuran metabolites and Nitrophenyl derivatives.

Materials and Methods

Chemicals

Nitrofuran metabolite standards were purchased from clearsynth and 2-Nitrobenzaldehyde was purchased from Sigma Aldrich ≥99% Purity. All other chemicals used were of LC-MS grade, commercially available.

Honey samples

Honey samples were procured from local market of Delhi and Gurgaon, India and were stored at room temperature until end of analysis.

Sample Preparation

Honey sample (1gm) was mixed with 3ml of HCl (0.1M) and 50mM of 2-Nitrobenzaldehyde (0.3ml), vortexed and incubated on ultrasonic bath for 16hr added 0.6ml of 1M K2HPO4 solution and added 10 ml of ethyl acetate, vortexed it, followed by centrifugation at 4000 rpm. The supernatant was evaporated to dryness, reconstituted with 1ml of Methanol: water (5:95) and 10µl is used for LC-MS/MS analysis.

LC Conditions

LC separation was achieved using the Shimadzu prominence system with an Eclipse plus C18 (4.6×150 mm) 5 µm column with a gradient of 1mM ammonium acetate as mobile phase A and Methanol as mobile phase B at flow rate of 0.4 mL/min. The injection volume was set to 10 µL. Gradient profile is given Table1.

Time (min)	Mobile phase A%	Mobile phase B%
0.01	95	5
0.50	45	55
3.50	45	55
4.00	95	5
12.00	Controller	Stop

Table 1: Mobile Phase Gradient

MS/MS Conditions

The SCIEX Triple Quad™ 3500 was operated in Multiple Reaction Monitoring (MRM) mode. The Turbo V™ source was used with an Electrospray Ionization (ESI) probe in positive polarity. Two selective MRM transitions were monitored for all nitrofuran metabolites using the Analyst® 1.6.2 Software and MultiQuant™ Software version 3.0.2. MRM transition is given in Table 2.

Compound	Precursor ion	Product ion Quantifier	Product ion Qualifier
AOZ	236.0	104.0	78.0
AMOZ	335.0	291.1	128.2
SEM	209.0	166.0	192.0
AHD	249.1	134.0	104.0

Table 2: MRM transitions

Results and Discussions

The results of repeatability data obtained for nitrofuran metabolites in the honey matrix is given in table 3 at different levels.

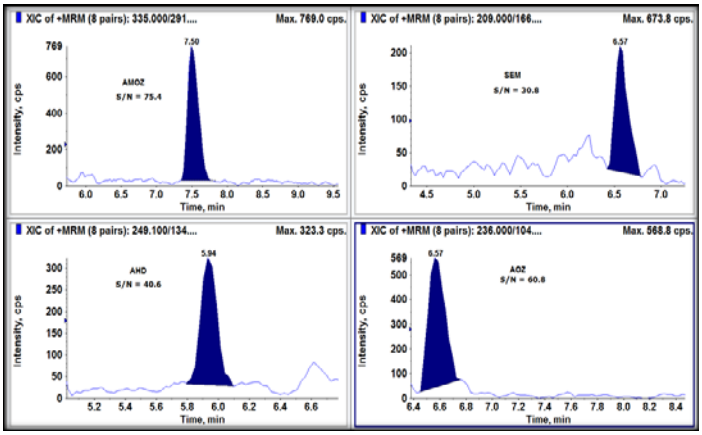

Figure 3: Signal to noise of AMZO, AHD, SEM and AOZ at MRPL level (1.0 ng/ml) in honey matrix sample

For all four Nitrofuran metabolites in honey the matrix based calibration curve shows excellent linearity (0.50 to 20.0 ppb), with a correlation coefficient r≥0.99 using linear regression and weighing factor 1/X. The SCIEX Triple Quad™ 3500 was found to be capable of analyzing concentrations well below the MRPL required by EU. The signal to noise ratio for all four nitrofuran metabolites at 1.0 ppb is ≥ 30. The signal to noise ratios and calibration curves are shown in Figure 3 and Figure 4.

Analyte	Repeatability			Recovery (n=6)		
	½ MRPL (0.5ppb)	MRPL (1.0ppb)	1.5MRPL (1.5ppb)	½ MRPL (0.5ppb)	MRPL (1.0ppb)	1.5MRPL (1.5ppb)
AOZ	6.01	7.00	4.28	113.47	95.05	89.89
AMOZ	12.16	4.46	4.33	83.80	103.88	96.11
SEM	4.49	7.31	9.44	109.67	98.13	91.33
AHD	4.96	7.58	8.22	114.90	105.40	105.00

Table 3: Repeatability (%CV) and recovery statistics in honey sample

Analyte	Calibration Range (ppb)	Linearity (r)	CCα	CCβ
AOZ	0.5 -20	0.9981	0.58	0.63
AMOZ	0.5 -20	0.9963	0.62	0.70
SEM	0.5 -20	0.9974	0.56	0.60
AHD	0.5 -20	0.9987	0.57	0.16

Table 4: Summary of CCα, CCβ and linearity in honey sample

Recovery experiments were performed in honey samples at ½ MRPL, MRPL and 1.5 MRPL level (n=6). The recovery of all nitrofuran metabolites was ≥ 80%. The recovery data for nitrofuran metabolites are shown in Table 3. The retention time (RT) of the AHD, AOZ, SEM, AMOZ, were 5.94, 6.57, 6.57 and 7.50 min, respectively.

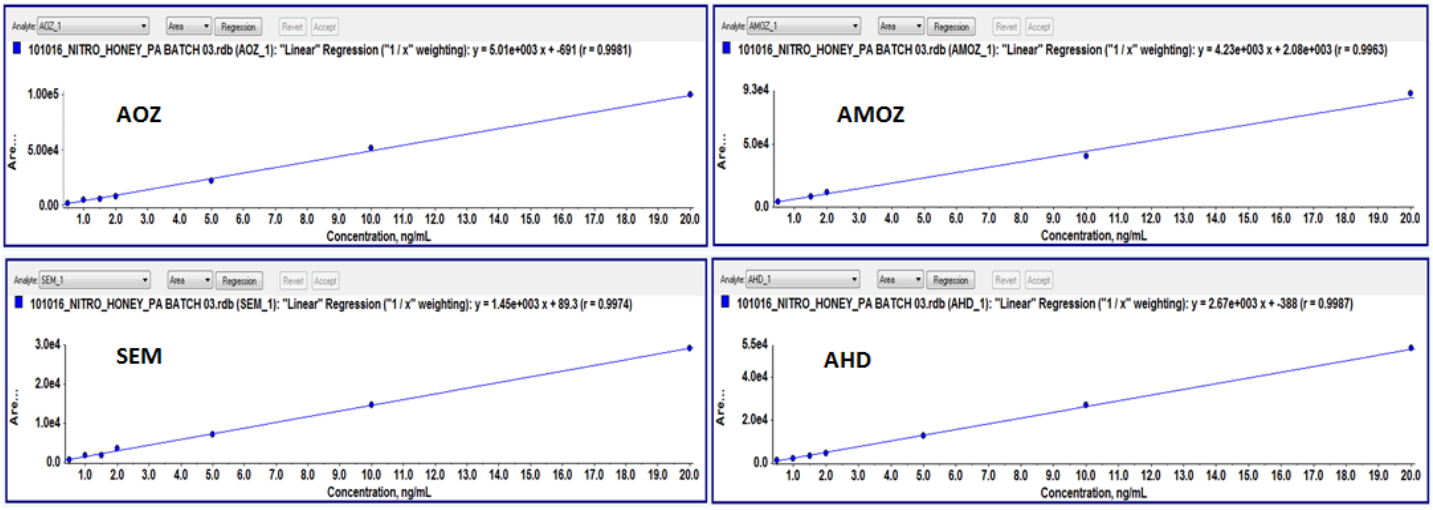

Figure 4: Matrix based calibration curve AOZ, AMOZ, SEM and AHD in Honey sample showing r = >0.99

Decision limit (CCα) and detection capability (CCβ) were calculated for all the four derivatives of Nitrofuran in Honey samples. The calculation was based on using linear regression model analyzing spiked honey samples at below MRPL level (Van Loco et al, 2007).

The calculated value of CCα and CCβ are given in table 4. The decision limit (CCα) and detection capability (CCβ) of all the metabolites were well below the MRPL.

Conclusions

- The developed quantitative method of Nitrofurans in honey on SCIEX Triple Quad™ 3500 was sensitive, linear, and reproducible.
- Trueness (Average recovery %) for this method found to be ≥ 80% at various MRPL levels.
- The method and data presented in the application note showcase the fast and accurate solution for the quantitation and identification of nitrofuran metabolites in honey samples for quality control check

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