Application Note: 465

Analysis of (Fluoro)quinolones in Honey with Online Sample Extraction and LC-MS/MS

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Key Words

- TurboFlow Technology
- Aria TLX-1
- TSQ Quantum Ultra
- Food Safety

Introduction

The global food market has become more competitive and equally cost responsive. The need for analytical procedures that permit high sample throughput as well as higher sensitivity allied to good reproducibility is growing by the day.^{1,2,3} A method using automated online extraction with tandem mass spectrometry is presented as an alternative to the commonly used, time-consuming solid-phase extraction (SPE) method.

Quinolones, including fluoroquinolones, are a group of synthetic antibacterial compounds used in the treatment of several diseases. There has been a significant and progressive increase in the use of quinolones in animal production, which has led to their residual presence in food. In the European Union, the maximum residue limits (MRLs) for several of these compounds are defined for different food matrices of animal origin, but not for honey.⁴ Furthermore, the presence of these compounds is an indication of unsafe practices of food production and deficient methods in the production of honey.

The complexity of the matrix plays a fundamental role on the adoption of the method of analysis. Thermo Scientific TurboFlow technology enables the reduction of sample preparation as well as the elimination of interferences from complex matrices such as honey.

Goal

To develop a sensitive and reproducible liquid chromatography tandem mass spectrometry (LC-MS/MS) method for the quantitation of 12 fluoroquinolones and 4 quinolones in honey using automated extraction by TurboFlow[™] technology.

Experimental

Sample Preparation

To a sample of 1 g of honey, 1 mL of water was added and the mixture was homogenized. The sample was then filtered directly to the HPLC vial using a 0.22 μ m polyethersulfone membrane syringe filter.

Different concentration levels were achieved by spiking the sample with different concentration levels of standard stock solution.

The total sample preparation time was 40 minutes for 12 samples.

TurboFlow Method Conditions:

System:	Thermo Scientific Aria TLX-1 controlled by Aria™ software (Figure 1)
Online Extraction:	TurboFlow Cyclone 50 x 0.5 mm
Mobile Phase A:	0.1 % formic acid in water
Mobile Phase B:	0.1 % formic acid in acetonitrile
Mobile Phase C:	10 mM ammonium formate in water
Mobile Phase D:	acetonitrile/isopropanol/acetone (4:3:3 v/v/v)
Injection Volume:	90 µL

HPLC conditions:

Analytical Column:	Thermo Scientific Hypersil GOLD 2.1 x 50 mm, 3 µm column at 40° C
Solvent A:	0.5 % formic acid in water
Solvent B:	0.5 % formic acid in methanol/acetonitrile (1:1 v/v)



Figure 1: Aria software with LC Method Editor



MS Conditions

MS analysis was carried out on a Thermo Scientific TSQ Quantum Ultra AM triple stage quadrupole mass spectrometer. The MS conditions were as follows:

lon Source Polarity:	Positive Ion Mode
Spray Voltage:	3000 V
Vaporizer Temperature:	350 °C
Sheath Gas Pressure (N ₂):	40 units
Auxiliary Gas Pressure (N ₂):	35 units
Capillary Temperature:	325 °C
Collision Gas (Ar):	1.5 mTorr
Q1/Q3 Peak Resolution:	0.7 u (unit mass resolution)
Scan Time:	0.025 s
Scan Width:	0.010 m/z
Data Acquisition Mode:	SRM

The optimization of Selective Reaction Monitoring (SRM) parameters was performed by direct infusion of standards in the positive electrospray ionization mode. Collision induced dissociation (CID) mass spectra were recorded for each analyte and the optimum collision energies were obtained for the selected ion transitions. Table 1 summarizes these parameters and Figure 2 displays the MS method controlled by Thermo Scientific Xcalibur software.

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Figure 2: MS method showing the SRM transitions and other conditions

Table 1: Selected ion transitions (m/z), collision energy (CE) and tube lens voltages (TL) for studied compounds

Analyte	Precursor Ion (m/z)	Product Ion (m/z)	CE (V)	TL (V)
1. Nalidixic Acid	233.064	104.143	40	78
		215.020 187.025	15 25	78 78
2. Oxolinic Acid	262.032	130.106 244.012	33 19	82 82
3. Flumequine	262.050	199.998 243.962	34 19	61 61
4. Cinoxacin	263.029	105.202 189.014 217.049 245.011	37 29 22 16	59 59 59 59
5. Pipemidic Acid	304.062	189.000 217.029 286.075	29 19 20	82 82 82
6. Norfloxacin	320.096	276.058 302.055	17 21	70 70
7. Enoxacin	321.083	206.012 302.981	29 21	65 65
8. Ciprofloxacin	323.100	231.024 314.018	36 22	74 74
9. Lomefloxacin	352.104	265.010 308.067	23 17	78 78
10. Danofloxacin	358.120	82.215 314.097 340.089	39 18 24	75 75 75
11. Enrofloxacin	360.128	245.025 315.958	26 19	72 72
12. Ofloxacin	362.107	261.041 318.055	27 19	109 109
13. Marbofloxacin	363.066	70.067 72.073 276.064 320.022	34 22 14 14	66 66 66 66
14. Fleroxacin	370.094	269.023 326.061	27 19	112 112
15. Sarafloxacin	386.095	298.979 342.078 367.878	28 18 22	105 105 105
16. Difloxacin	400.107	299.009 356.017	29 20	75 75

Results and Discussion

The analysis of food samples normally requires long preparation times due to the complexity of the matrices. The Thermo Scientific Aria TLX-1 system powered by TurboFlow technology enables reduction of the sample preparation time. It took only 40 minutes to prepare the batch of samples for LC-MS/MS analysis, instead of an average time of 6 hours when using Solid Phase Extraction (SPE). Even when dealing with complex matrices, such as honey, the use of the TLX-1 system enables the elimination of possible interferences and creates less noisy chromatograms (Figure 3). The results of a high-throughput, rapid, sensitive and linear method for the determination of 16 quinolones, including 12 fluoroquinolones, by LC-MS/MS using TurboFlow technology are presented (Table 2). The Limit of Detection (LOD) was calculated by using the statistical definition LOD = $Y_B + 3S_B$, where Y_B is the blank signal and S_B is the standard deviation of the blank.



Figure 3: Representative SRM chromatogram (20 µg/kg) showing the selected ion transitions and retention times for the studied analyte

Table 2: Linearity, sensitivity and precision of the method

Analyte	Range (µg/kg)	LOD (µg/kg)	RSD (%)	R2 (1/X)	
1	1-50	0.8	1.3 - 4.7	0.9943	
2	1-50	1.4	0.3 - 10.6	0.9909	
3	1-100	0.9	1.7 - 8.9	0.9902	
4	2-100	2.0	4.3 - 7.7	0.9918	
5	1-100	0.9	1.5 - 10.1	0.9964	
6	1-100	2.3	2.7 - 11.5	0.9925	
7	1-100	1.9	2.1 - 11.7	0.9928	
8	1-100	1.4	2.4 - 11.6	0.9967	
9	1-100	0.5	0.2 - 13.7	0.9954	
10	1-100	1.1	2.3 - 13.6	0.9961	
11	1-100	0.8	1.5 - 16.9	0.9907	
12	2-100	1.3	2.1 - 11.5	0.9945	
13	1-100	2.6	2.4 - 13.9	0.9939	
14	1-50	1.5	6.0 - 16.8	0.9903	
15	1-100	1.1	1.1 - 11.2	0.9966	
16	1-100	0.8	1.9 - 10.4	0.9947	

The method proved to be linear in the range studied. Three replicates were used for each point of the calibration levels, which, in addition to the relative standard deviation values, demonstrate the precision of the method.

Conclusion

A rapid, sensitive and reliable method for the quantitation of 16 quinolones, including 12 fluoroquinolones, was developed using a TurboFlow method in combination with a TSQ Quantum Ultra[™] mass spectrometer. The use of TurboFlow technology enables a significant reduction of the sample preparation time. For 12 samples the preparation time was reduced from 5 hours to 40 minutes. Preliminary trials indicate this online extraction coupled with a TSQ Quantum Ultra is an excellent total solution for the quantification of a large number of compounds in food samples.

References and Acknowledgements

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AN63070_E 06/09



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