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 µm 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)

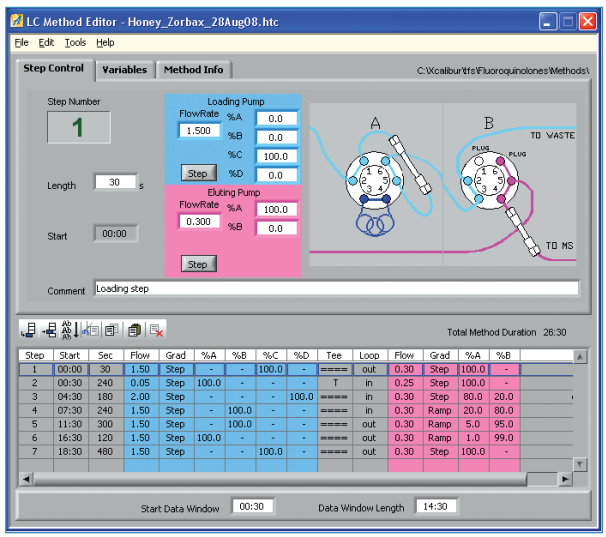

Figure 1: Aria software with LC Method Editor

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:

Ion 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.

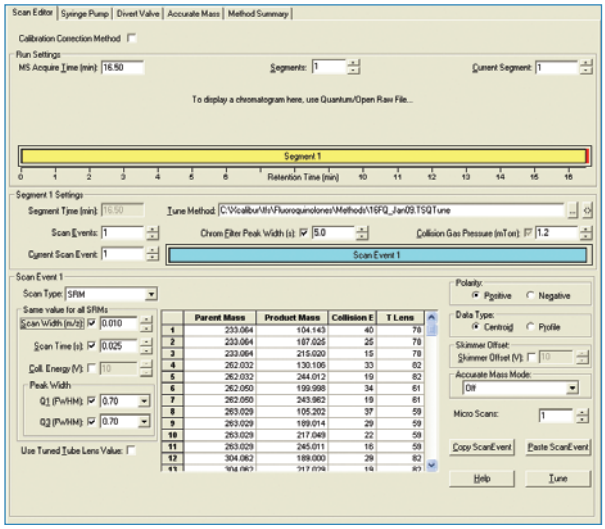

Figure 2: MS method showing the SRM transitions and other conditions

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

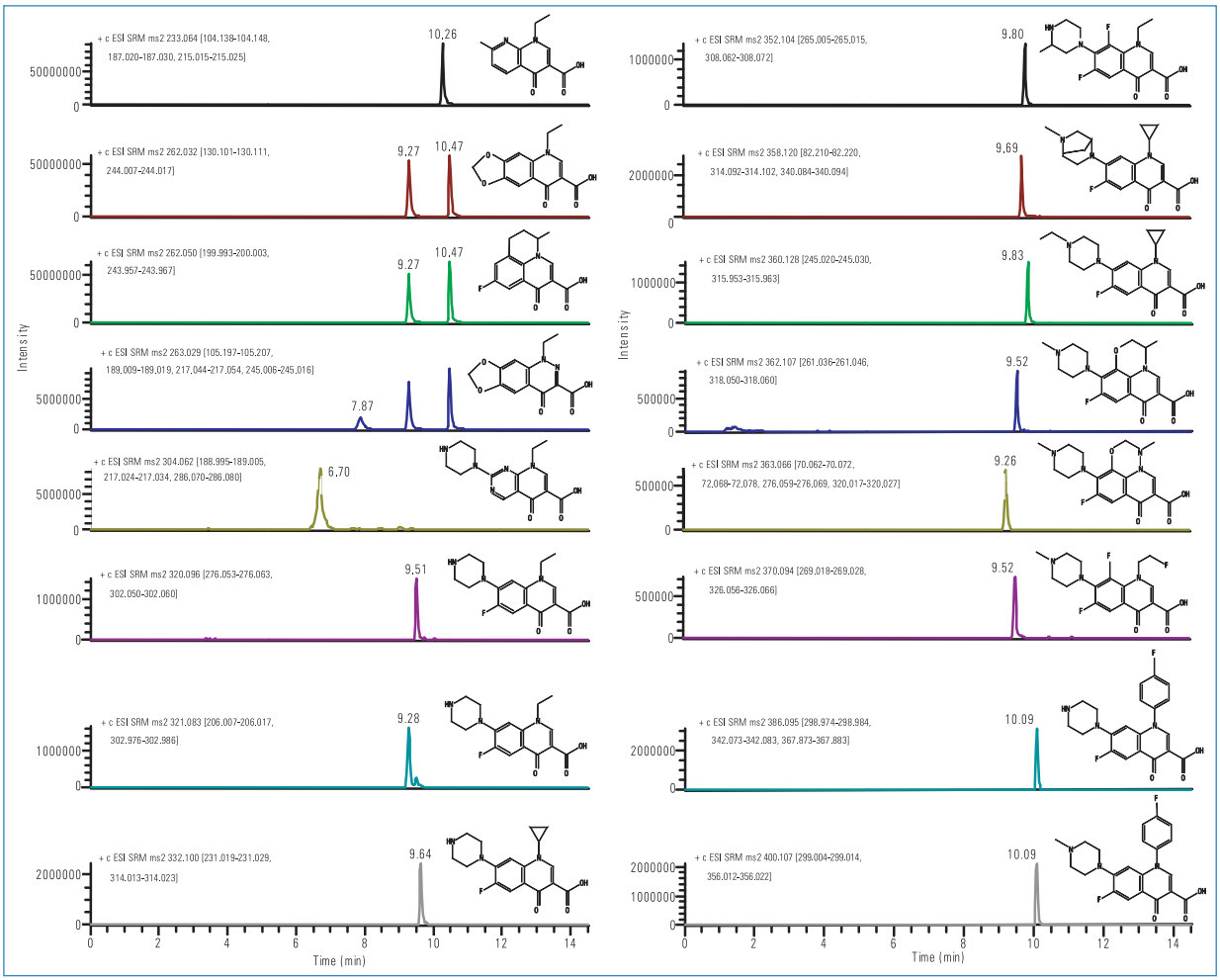

Figure 3: Representative SRM chromatogram (20 µg/kg) showing the selected ion transitions and retention times for the studied analyte

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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