

Matrix Blind Online Sample Extraction Coupled with Tandem Mass Spectrometry Method for Various Meat Matrices

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Overview

The goal of this study is to develop a matrix-blind TurboFlow LC-MS/MS assay with a wide analyte range in various meat and organ matrices.

Introduction

Different meat and organ matrices vary tremendously in phospholipid type and abundance resulting in widely variable recoveries. During the course of food safety monitoring it is very common to test the contaminant candidates in various matrices and this often requires multiple LC/MS methods to contend with the different matrices. Previous work utilizing two-dimensional TurboFlow chromatography has suggested that this technology is able to yield a method generic for a wide range of both analytes and biological fluids [1]. In this study, we demonstrate that this technology is capable of providing one method for antibiotics measurement in various meat and organ matrices as well.

Methods

Sample: Beef, chicken, pork, turkey meats and beef liver were purchased from a local grocery.

Method: The experiments were conducted using an Aria™ TLX-1 liquid chromatography system coupled to a TSQ Quantum™ Ultra mass spectrometer with a heated electrospray ionization II (HESI II) source. The meat samples were extracted using a TurboFlow MAX anion exchange extraction column (0.5 x 50 mm). Chromatography separation was performed using a Thermo Scientific Betasil Phenyl-Hexyl column. Mass spectrometry detection was performed under the selective reaction monitoring (SRM) mode with positive electrospray ionization.

Experimental Conditions:
Sample Preparation
The matrices were prepared using a very simple extraction procedure with acetonitrile (ACN). Four grams of each individual samples were homogenized with 30 mL of ACN and sat at room temperature for 10 min. The liquid phases were then centrifuged at 10,000 rpm for 15 min. The supernatants were stored at 4 °C for further analysis. Figure 1 shows the test compounds list. They represent three classes of common antibiotics: Sulfonamides, Tetracyclines, and Macrolides, which range from polar to highly nonpolar. A standard stock solution of sulfapyridine, sulfathiazole, doxycycline, oxytetracycline, demeclocycline and erythromycin was prepared in methanol at the concentration of 10 µg/mL. Calibrators in the concentration range 1 ng/mL to 500 ng/mL were prepared in individual obtained supernatant by serial dilution of the stock solution. Injection volumes were 25 µL.

Aria TLX-1 System Parameters
Columns: Thermo Scientific 0.5 x 50 mm TurboFlow MAX column
Thermo Scientific Betasil Phenyl-Hexyl column (3 x 100 mm, 3 µm particle size).

Mobile Phases
Loading Pump
Mobile Phase A: 1% Formic Acid (aq)
Mobile Phase B: 0.1 % Formic Acid in ACN
Mobile Phase C: 10 mM Ammonium Acetate, pH 9.0
Mobile Phase D: 50 mM Ammonium Acetate in MeOH with 0.1 % Formic Acid

Elution Pump
Mobile Phase A: 0.5 % Formic Acid and 0.04 % Trifluoroacetic acid (TFA) with 1 mM Nonfluoropentanoic acid
Mobile Phase B: 0.5 % Formic Acid and 0.04 % TFA in 1:1 ACN:MeOH

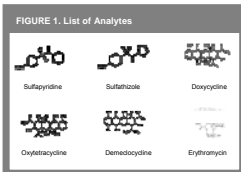


Table 1. Positive selective reaction mode (+SRM) transitions and other MS parameters for test compounds

Compound	Parent Ion	Fragment Ion Ret: Quantitation Ion	Collision Energy (eV)	S-Lens Offset
Sulfapyridine	250.045	91.902	29	98
		107.870	24	
		165.895	17	
		184.015	15	
Sulfathiazole	286.001	64.820	28	101
		91.960	28	
		107.853	22	
		155.930	15	
Doxycycline	448.121	153.710	30	117
		287.052	33	
		320.990	30	
		428.248	19	
Oxytetracycline	491.117	201.040	42	98
		283.035	42	
		428.259	17	
		444.335	16	
Demeclocycline	465.08	153.852	29	98
		288.982	31	
		430.154	00	
		448.192	16	
Erythromycin	734.176	82.983	35	109
		115.700	35	
		157.882	29	
		576.545	17	

Mass Spectrometer Parameters
MS analysis was carried out on a Thermo Scientific TSQ Quantum Ultra triple stage quadrupole mass spectrometer (Thermo Fisher Scientific, San Jose, CA). The MS conditions were as follows:
Ion Polarity: Positive ion mode
Spray Voltage (V): 4000
Vaporizer Temperature (°C): 400
Capillary Temperature (°C): 250
Sheath Gas Pressure (N₂): 60 units
Ion Sweep Gas Pressure (N₂): 0.2 units
Auxiliary Gas Pressure (N₂): 30 units
Scan Type: Selective Reaction Monitoring (SRM)
Chrom Filter Peak Width (s): 5.0
Collision Gas Pressure (mTorr): 1.2
Scan Width (m/z): 0.01
Scan Time (s): 0.05
Q1 (FWHM): 0.7 Da
Q3 (FWHM): 0.7 Da

Positive selective reaction mode (+SRM) transitions and other MS parameters for test compounds are shown in Table 1. The entire experiment was controlled by Aria OS 1.6.2 and the data was processed using Thermo Scientific LQuan 2.5.6 quantitative software after subtracting background using Thermo Scientific Xcalibur 2.0.7 SP1 data system software.

Results

TurboFlow methods have been demonstrated to be able to remove endogenous compounds from complicated matrices effectively without time-consuming offline sample preparation, thus reducing ion suppression effects and increasing detection limits significantly. In food safety area, it is always an analytical challenge to develop a matrix blind method. This is the major purpose of this study. Figure 2 shows a representative chromatogram for the assay at 100 ng/mL. The results indicate that all compounds have very high recovery percentage (mostly within 100 to 20% range) in five matrices except erythromycin, whose signals are significantly suppressed in most matrices. The data shown in Table 2 further demonstrates that the tested compounds show very similar elution behaviors among different meat matrices. The phospholipids we monitored in the study (m/z 496, 504, 724) are very well separated from target compounds. We also noticed the loss of sensitivity for sulfapyridine and sulfathiazole with successive injections at high concentration calibrator (500 ng/mL), which is consistent with other researcher's observation [2].

FIGURE 2. The representative chromatogram for the assay at 100 ng/mL.

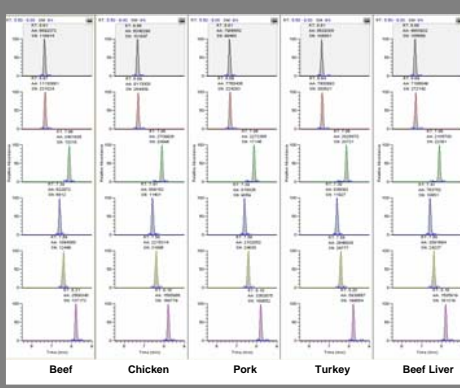


FIGURE 3. The representative recovery result for the assay at 100 ng/mL.

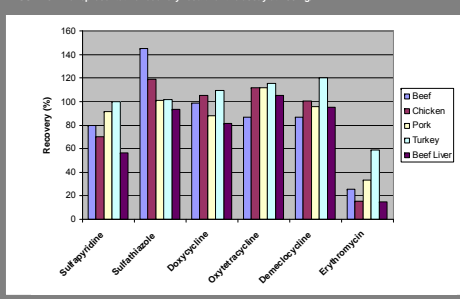


TABLE 2. Data summary showing the similar results over five matrices

Compounds	RT (min)	Beef		
		LOD (ng/mL)	ULOD (ng/mL)	R ²
Sulfapyridine	6.61-6.64	1	5	0.9767
Sulfathiazole	6.64-6.67	1	10	0.9782
Doxycycline	7.86	1	5	0.9922
Oxytetracycline	7.39	1	10	0.9948
Demeclocycline	7.56-7.58	5	10	0.9942
Erythromycin	8.20-8.21	1	5	0.9902
Chicken				
Sulfapyridine	6.64-6.66	1	5	0.9895
Sulfathiazole	6.66-6.69	1	5	0.9851
Doxycycline	7.86	1	1	0.9906
Oxytetracycline	7.39-7.41	1	5	0.9953
Demeclocycline	7.58-7.61	5	50	0.9941
Erythromycin	8.18-8.21	1	5	0.9888
Pork				
Sulfapyridine	6.61-6.66	1	5	0.9744
Sulfathiazole	6.66-6.69	1	10	0.9799
Doxycycline	7.86	1	5	0.9930
Oxytetracycline	7.39-7.41	1	5	0.9910
Demeclocycline	7.56-7.60	1	5	0.9891
Erythromycin	8.18	1	5	0.9928
Turkey				
Sulfapyridine	6.61-6.62	1	5	0.9906
Sulfathiazole	6.62-6.64	1	5	0.9870
Doxycycline	7.84-7.86	1	5	0.9922
Oxytetracycline	7.35-7.39	1	5	0.9896
Demeclocycline	7.56-7.58	1	5	0.9849
Erythromycin	8.18-8.21	1	5	0.9917
Beef Liver				
Sulfapyridine	6.64-6.66	1	5	0.9918
Sulfathiazole	6.66	1	5	0.9848
Doxycycline	7.86	1	5	0.9947
Oxytetracycline	7.39-7.41	1	10	0.9897
Demeclocycline	7.58-7.61	1	5	0.9951

Conclusions

In the current study, an online TurboFlow extraction method was developed which is mostly matrix blind for various meat matrices. The method is generic for a wide range of antibiotics compounds. The further work will be focused on the improvement of detection limit using a more sensitive mass spectrometer, such as Thermo Fisher TSQ Vantage triple quadrupole MS. To help compensate for variations in response, suitable internal standards will be included in the further study.

References

1. Espourteille, F.; Esposito, C. Matrix Blind Generic In-Line Method for Biological Fluids. ASMS 2008, Denver, CO.
2. van den Heever, J.; Thompson, T.; Noot, D. What You Can't See Can Hurt You: How MS/MS Specificity Can Bite Your Backside. Western Canada Trace Organic Workshop, 2007.

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