Online sample preparation for the quantitative screening of multiple veterinary drug residues in chicken, beef and pork

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Overview

Purpose: To develop a rapid and sensitive screening method to detect and quantify multiple veterinary drug residues with automated online sample preparation.

Methods: Automated online sample preparation using Thermo Scientific TurboFlow technology coupled with the Thermo Scientific Quantum Ultra mass spectrometer.

Results: A TurboFlow™ online multi-residue screening method for veterinary drug residues in meat matrices was developed.

Introduction

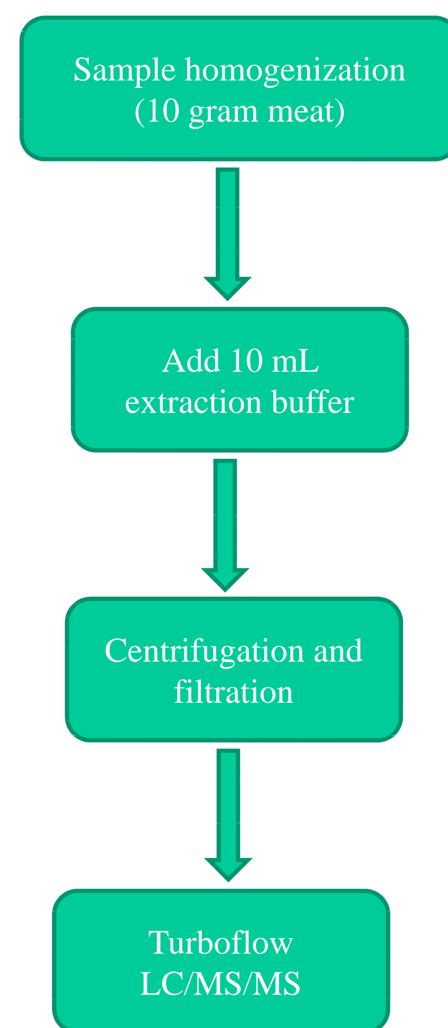
The presence of veterinary drug residues in meat and other edible tissues poses a potential health risk and safety for human. Many countries have implemented the regulations of acceptable drug residue levels in meat products. Therefore, a reliable and fast screening analysis is necessary to determine the levels of veterinary drug residues in meat and other edible tissue samples.

Generally, a liquid liquid extraction followed by solid phase extraction enrichment was used to extract the drug residues from meat matrices, but these methods are very time-consuming and labor-intensive. Moreover, these extraction methods usually work for individual compounds or a single compound class and they are not well suited for a multi-class, multi-residue screening analysis. TurboFlow chromatography has been successfully and widely used in the clinics for the online sample clean-up of plasma and urine[1]. Recently, the online sample preparation methods based on TurboFlow technology have been developed to quantitatively screen target compounds in milk[2], honey[3] and meat[4].

In the present work, a fast and simple online sample preparation coupled with LC/MS/MS was developed to screen quantitatively 22 drugs in chicken, pork, and beef. This TurboFlow technology based online sample preparation method demonstrated the great effectiveness of extracting drug residues from meat.

Methods

Sample Preparation



The matrix standard curve

Organic ground beef, chicken, and pork used in this study were obtained from a local grocery store. 10 gram homogenized meat were put into a 50 mL centrifuge tube, and 10 mL extraction buffer (0.2% Formic Acid in Acetonitrile and Water (80:20)). Vortex the whole mixture for 2 minutes and centrifuge at 5000g. The supernatant were collected and filtrated with a syringe filter (0.2 µm). Each mL of the supernatant was corresponding to 1 gram of meat.

A calibrant solution mixture was prepared at 20 µg/mL in extraction buffer. To prepare 200 ng/g sample, 10 µL of the calibrant mixture was added into 1000 µL of the extracted supernatant. A range of calibrators from 1 ng/g to 150 ng/g and three spike levels (20, 40 and 80 ng/g) were prepared by diluting the 200 ng/g sample with the prepared supernatants. Totally, 9 calibrators (1, 2, 5, 10, 25, 50, 75, 100, 150 ng/g) were prepared .

Instrumentation: Thermo Fisher Transend TLX-2 system coupled with Quantum Ultra Triple Quadrupole Mass Spectrometer.

TurboFlow LC conditions

Turboflow column: Cyclone P 50 X 0.5 mm
Analytical Column: Accucore C18 (50 X3 mm, 2.6 µ)
Injection volume: 50 µL
Total run time: 8.58 minute

Mobile Phase: Loading solvent A: 0.1% formic acid in water, Loading solvent B: 0.1% formic acid in methanol, Loading solvent C: 1:1:1 Acetointrile : Aceton : Isopropanol

Eluting solvent A: 0.1% formic acid in water, eluting solvent B: 0.1% formic acid in methanol, Eluting solvent C: 1:1:1 Acetointrile : Aceton : Isopropanol

TurboFlow LC method

Step	Start	Sec	Flow	Grad	%A	%B	%C	%D	Tee	Loop	Flow	Grad	%A	%B	%C	%D
1	0.00	45	2.00	Step	100.0	-	-	-	====	out	0.70	Step	100.0	-	-	-
2	0.75	5	0.10	Step	100.0	-	-	-	====	out	0.70	Step	100.0	-	-	-
3	0.83	90	0.20	Step	100.0	-	-	-	T	in	1.30	Step	100.0	-	-	-
4	2.33	15	2.00	Step	-	-	100.0	-	====	out	0.80	Step	40.0	60.0	-	-
5	2.58	30	1.00	Step	-	-	100.0	-	====	in	0.80	Ramp	25.0	75.0	-	-
6	3.08	90	1.00	Step	-	-	100.0	-	====	in	0.80	Ramp	10.0	90.0	-	-
7	4.58	30	2.00	Step	-	100.0	-	-	====	out	0.80	Step	10.0	90.0	-	-
8	5.08	90	2.00	Step	50.0	50.0	-	-	====	in	1.00	Step	-	-	100.0	-
9	6.58	120	2.00	Step	100.0	-	-	-	====	out	0.70	Step	100.0	-	-	-

Table 1 shows all the MS conditions.

Detector	Thermo TSQ Quantum Ultra
Ionization	Heated ElectroSpray Ionization (HESI)
Vaporizer Temperature	450°C
Sheath Gas Pressure	30
Auxiliary Gas Pressure	0
Capillary Temperature	300°C
Collision Gas Pressure	1.5 mTorr
Spray Voltage	4000 V
Scan Type	SRM
Scan Width	0.1
Peak Width Q1 Da. (FWHM)	0.7
Peak Width Q3 Da. (FWHM)	0.7

Teble 2 shows all the SRM transitions of all the target drugs.

Analyte	SRM	Collision Energy (CE)	Tube Lens
Ractopamine	302.2-107.1 (C) 302.2-164.1 (Q)	29 23	83
Flumequine	262.1-126.1 (C) 262.1-202.1 (Q)	48 34	109
Oxolinic acid	262.1-160.1 (C) 262.1-216.1 (Q)	38 30	128
Clenbuterol	278.1-133.1 (C) 278.1-204.1 (Q)	31 17	135
Sulfamerazine	279.1-92.1 (C) 279.1-186.1 (Q)	33 18	103
Mabuterol	311.1-217.1 (C) 311.1-237.1 (Q)	26 17	112
Ciprofloxacin	332.1-245.1 (C) 332.1-288.2 (Q)	24 18	113
Penicillin G	335.1-160.1 (C) 335.1-176.1 (Q)	20 16	139
Ampicillin	350.1-106.3 (C) 350.1-192.1 (Q)	20 16	132
Penicillin V	351.1-114.1 (C) 351.1-160.1 (Q)	20 16	129
Enrofloxacin	360.2-245.1 (C) 360.2-316.2 (Q)	27 18	123
Brombuterol	366.9-214.0 (C) 366.9-292.9 (Q)	20 28	127
Sarafloxacin	386.1-299.1 (Q) 386.1-368.2 (C)	28 23	164
Dexamethasone	393.2-91.1 (C) 393.2-147.1 (Q)	58 32	178
Difloxacin	400.1-299.1 (Q) 400.1-382.2 (C)	29 24	145
Nafcillin	415.2-171.1 (C) 415.2-199.1 (Q)	35 15	142
Tetracycline	445.2-98.1 (C) 445.2-154.1 (Q)	38 28	162
Oxytetracycline	461.2-201 (C) 461.2-426.2 (Q)	38 18	123
Dicloxacillin	470-114 (C) 470-160.1 (Q)	40 17	141
Clortetracycline	479.2-444.2 (C) 479.2-462.2 (Q)	21 16	129
Phenylbutazone	309.1-120 (C) 309.1-92.1 (Q)	49 33	152
Oxacillin	402.2-114.1 (C) 402.2-160.1 (Q)	33 14	143

Note: (Q)= Quantitation Ion; (C) = confirmation Ion

Results and discussion:

In order to improve the recovery and minimize the matrix inference peaks, the transferring step of analytes from Turboflow column to analytical column was optimized. Figure 1 shows the extracted ion chromatogram of 22 drugs spiked in pork at 80 ppb level. No matrix interference peaks were observed for each of the analytes.

Table 3 shows the calibration ranges and the regression coefficients (r^2) for each target drug in pork. The LOQs for all the target drugs in meat matrices were set at those concentrations showing S/N (signal to noise ratio) greater than 10.

Table 4 shows the results of the method validation for Enrofloxacin at three different spike levels (20, 40 80 ng/g). These values are well within the acceptable ranges.

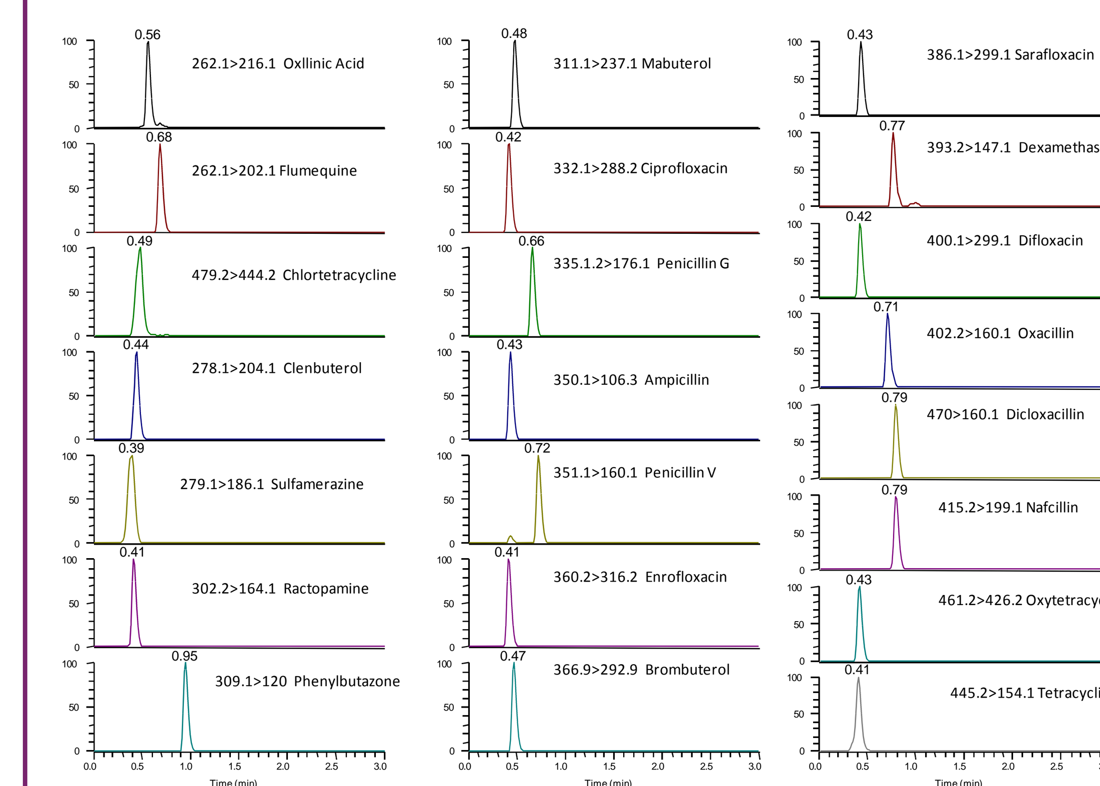


FIGURE 1. Extracted ion chromatogram of 22 drugs spiked in pork matrices at 80 ppb level.

Table 3 summarizes the calibration ranges and Corr R^2 values for the linearity of the calibration curve for each compound from pork.

Compound Name	Calibration Range (ppb)	R^2
Sulfamethazine	1-150	0.9982
Tetracycline	5-150	0.9949
Ractopamine	1-150	0.9983
Ciprofloxacin	2-150	0.9995
Enrofloxacin	1-150	0.9992
Oxytetracycline	5-150	0.9973
Difloxacin	5-150	0.9944
Clenbuterol	5-150	0.9971
Ampicillin	2-150	0.9957
Sarafloxacin	2-150	0.9992
Brombuterol	1-150	0.9983
Mabuterol	1-150	0.9985
Chlortetracycline	5-150	0.9991
Oxolinic Acid	1-150	0.9973
Penicillin G	2-150	0.996
Flumequine	2-150	0.997
Oxacillin	5-150	0.9978
Penicillin V	2-150	0.9982
Dexamethasone	5-150	0.997
Nafcillin	1-150	0.9971
Dicloxacillin	5-150	0.9928
Phenylbutazone	5-150	0.9978

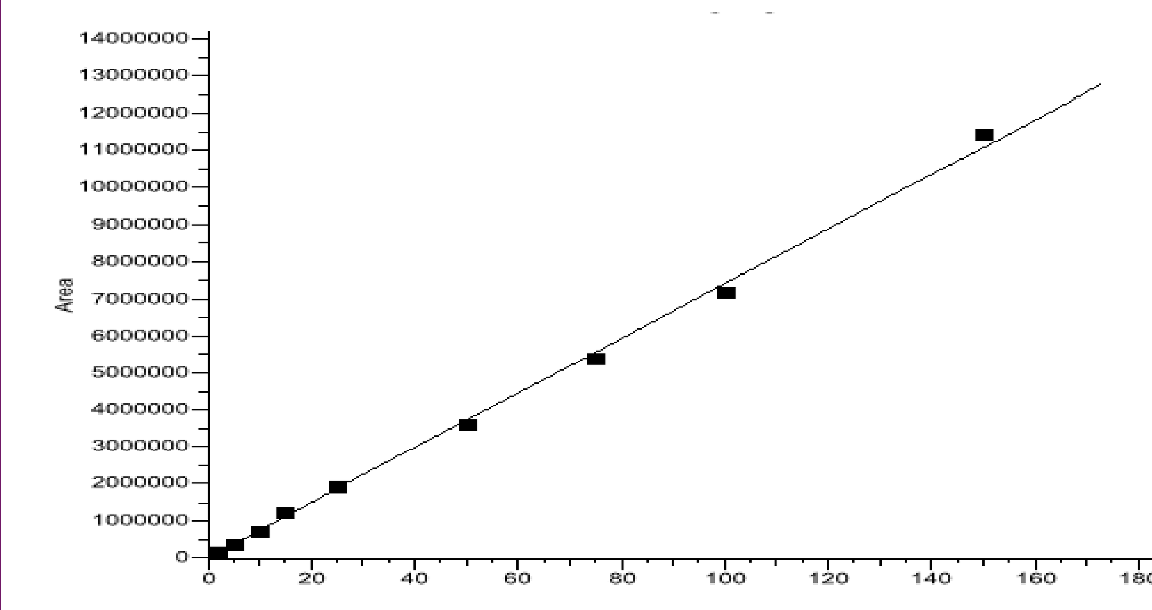


FIGURE 2. Representative calibration curve of ractopamine at Pork. Calibration range: 1 ppb – 150 ppb.

Table 4 summarizes the results of the method validation for enfloxacin in pork matriices at three different levels (20, 40 and 80 ng/g)

Enrofloxacin spike level (ng/g)	Within-run Accurancy (n=6, %)	Between-run Accurancy (n=6, %)	Within-run Precision (n=6, %)	Between-run Accurancy (n=6, %)
20	97.55	98.28	10.88	8.06
40	111.55	97.58	10.02	6.53
80	103.31	99.61	7.45	9.51

Conclusion

- A quantitative screening method with online sample clean-up was established.
- This method decreased the time required for the sample clean-up from meat matrices and the whole sample preparation time is less than half an hour.
- This method was partially validated at three different levels (20 ppb, 40 ppb and 80 ppb).

References

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