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Highly sensitive simultaneous analysis of tetracyclines and β-lactams antibiotics in edible meat by LC/MS/MS

Ayaka MINAMIMOTO[1], Manami KOBAYASHI[1], Junichi MASUDA[1], Yoshihiro HAYAKAWA[2]

1 Shimadzu Corporation, Kanagawa, Japan., 2 Shimadzu Corporation, Kyoto, Japan

1. Introduction

Veterinary drugs are widely used for therapeutic and preventive purposes of murrain for cattle. The excessive use of antibiotics for livestock may generate drug-resistant bacteria. In addition, the residual antimicrobial agents may affect human health. Therefore, the limit of the maximum residue for veterinary drugs has been established by country or regions in order to provide assurance of safety for edible meat. A mass spectrometer coupled to a liquid chromatograph which has high throughput, sensitivity and selectivity is commonly utilized for veterinary drugs analysis. In this study, the analytical method with pretreatment procedure was newly developed to determine tetracyclines and β-lactams (e.g. penicillin and cephem) simultaneously in meat with high sensitivity and selectivity.

Fig. 1 LC-MS/MS system (Nexera™ X3 with LCMS-8060NX system)

2. Analytical procedure

Twenty of veterinary drugs were spiked in meat sample (each as 0.01 mg/kg i.e. each as 2 ng/mL of the final concentration in a vial after the pretreatment). Samples were pretreated by deproteinizing with acetonitrile and degreasing with hexane followed by extracting with EDTA-containing McIlvaine buffer. The extracted solution was purified through a solid phase column. The analysis was performed by triple quadrupole tandem mass spectrometer, LCMS-8060NX equipped with a Nexera X3 UHPLC system (Shimadzu Corporation, Kyoto, Japan) using a Shim-pack Scepter™ C18-120 [Metal free] (Shimadzu Corporation). LC and MS conditions are shown in Table1. The standard with sample matrix and water for dilution were co-injected using the automatic function integrated on the Nexera X3 system in order to quantify with a matrix-matched calibration.

Table 1 LC and MS conditions

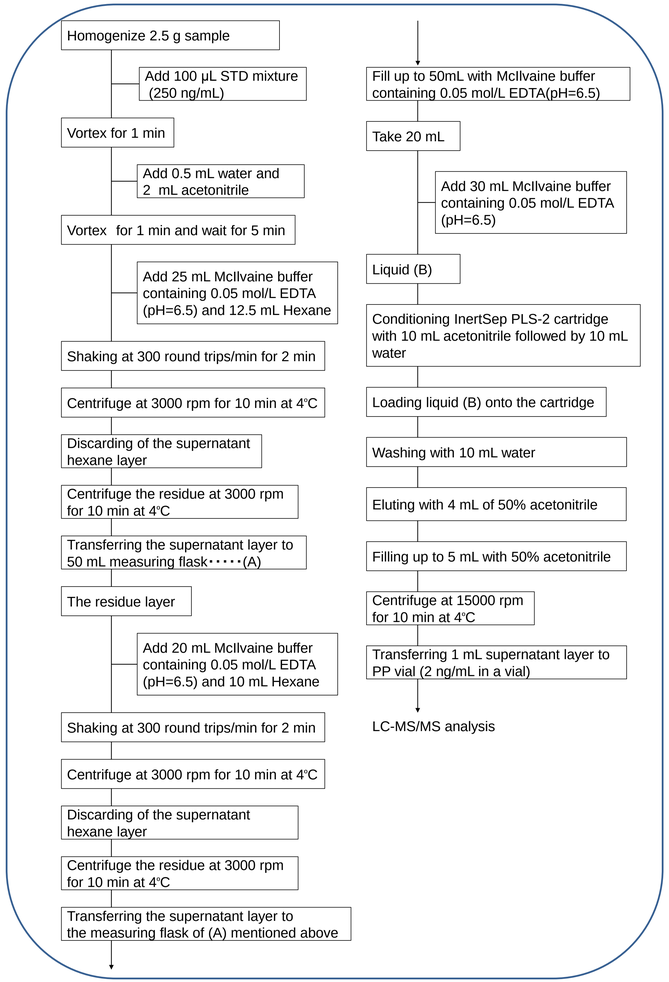
For HPLC

Column	Shim-pack Scepter C18-120 [Metal Free],1.9 µm (100 mm x 2.1 mm I.D., 1.9 µm)
Mobile Phase A	2 mmol/L Ammonium formate – Water containing 0.1% formic acid
Mobile Phase B	Methanol
Time Program	B Conc.5% (0.0 - 2.0 min)→70%(8.0 min) → 95%(10 min – 15 min)→5% (15.01 – 20 min)
Flow Rate	0.4 mL/min(0 - 10.6 min)→0.6 mL/min(10.61 – 15.5 min)→ 0.4 mL/min(15.51 -20 min)
Column Temp.	40℃
Injection vol.	Standard 1 µL+ Matrix 1 µL+ Water 45 µL or Pre-spiked sample 1 µL+ Blank 1 µL+ Water 45 µL

For LCMS

Ionization	: ESI (Positive) MRM mode	DL temp.	: 200℃
Nebulizer gas	: 2 L/min	HB temp.	: 450℃
Interface temp.	: 400℃	Heating gas	: 5 L/min
Drying gas	: 10 L/min	IF voltage	: 2 kV
Focus voltage	: 5 kV	Probe position	: +3 mm

Sample pretreatment



3. Results and Discussion

In Fig. 2 below, the simultaneous determination was achieved for all 20 veterinary drugs within 10 minutes. The matrix-matched calibration curve range for all 20 veterinary drugs were expected at least 1 to 40 ng/mL under the co-injection with matrix 1 uL, standard 1uL and water 45 uL. The matrix-matched calibration curves and MRM chromatograms of tetracycline, ampicillin, cefalexin and cefuroxime as a representative example are shown in Fig. 3 and Fig. 4 respectively. For all compounds, good linearity was obtained with a coefficient of determination R^2 as 0.996 or higher. The accuracy of calibration points was within 80.69 to 114.75%. The area repeatability (%RSD) of 2 ng/mL calibration points was confirmed in not more than 7.26% (n=6).

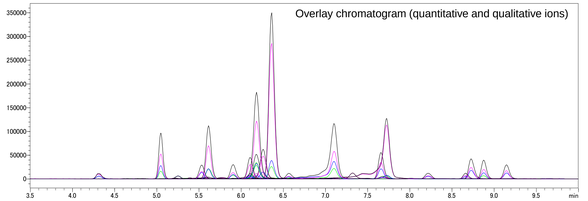


Fig. 2 MS Chromatogram of 20 veterinary drugs (2 ng/mL standard in chicken beast matrix)

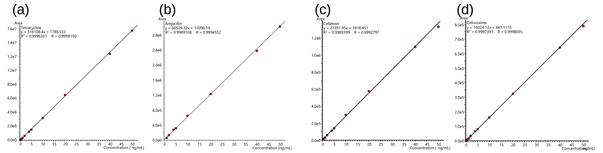


Fig. 3 The matrix-matched calibration curves of tetracycline(a), ampicillin(b), cefalexin(c) and cefuroxime(d) in chicken beast matrix

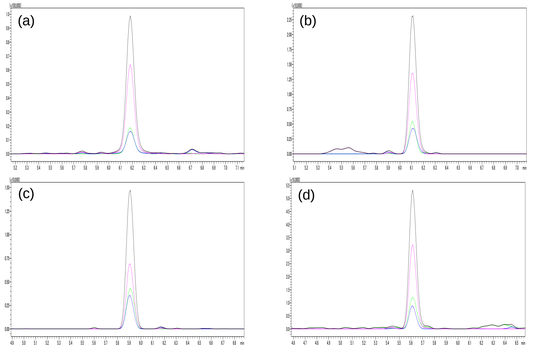


Fig. 4 MS Chromatogram of tetracycline(a), ampicillin(b), cefalexin(c) and cefuroxime(d) (1 ng/mL standard in chicken beast matrix)

The recovery of the pre-spiked sample was calculated by the matrix-matched calibration curve. Table 2 shows the result of recovery test for each samples. The recovery ratio for 80% of the compounds in chicken breast samples and that of 75% of the compounds in chicken thigh sample (each as n=3) were obtained around 70 to 120%. These results indicated that the analytical method developed by LCMS-8060NX equipped with Nexera X3 UHPLC could provide excellent sensitivity, linearity and recovery ration for tetracyclines, penicillins in addition to cephems in edible meat.

Table 2 The results of recovery tests (n=3)

Compound	Recovery (%) of Chicken breast				Recovery (%) of Chicken thigh			
	N=1	N=2	N=3	Ave.	N=1	N=2	N=3	Ave.
Chlortetracycline	76	86	80	81	77	84	76	79
Doxycycline	97	101	93	97	83	86	80	83
Oxytetracycline	91	102	110	101	85	106	83	92
Tetracycline	93	104	98	99	84	90	83	86
Amoxicillin	7	10	10	9	11	12	8	10
Ampicillin	109	98	100	102	70	75	70	72
Benzylpenicillin (Penicillin G)	102	106	102	104	67	73	65	68
Cloxacillin	90	97	85	91	85	88	85	86
Dicloxacillin	86	92	81	86	84	85	85	85
Mecillinam (Amdinocillin)	101	108	103	104	79	82	77	79
Oxacillin	89	100	89	93	72	75	72	73
Phenoxymethylpenicillin (Penicillin V)	79	89	88	85	68	77	70	72
Cefalexin	86	92	96	91	79	80	74	77
Cefazolin(Cephazolin)	95	96	111	101	84	87	86	86
Cefoperazone	117	98	133	116	75	83	82	80
Cefquinome	98	109	109	105	86	90	86	88
Ceftiofur	85	86	94	88	75	82	79	79
Cefuroxime	121	112	132	122	79	102	88	90
Cephalonium (Cefalonium)	126	105	118	116	81	93	91	88
Cephapirin(Cefapirin)	104	94	120	106	59	70	65	65

4. Conclusion

- We developed the pretreatment protocol in addition to simultaneous analysis method by LC/MS/MS for veterinary drugs in edible meat.
- Twenty veterinary drugs were separated with a typical ODS column within 10 min.
- 80% of the compounds in chicken breast as well as 75% of that in chicken thigh were recovered between 70 to 120%.
- It is supposed the recovery of amoxicillin is low since it is difficult for high polar amoxicillin to be trapped on a solid phase column.(blue colored in the table above.)

Reference

- BUNSEKI KAGAKU, Vol.66, No.5, pp.369-374 (2017)
- Food Hygiene and Safety Science, Vol.61, No.4, pp.109-118 (2020)