Application Note

748011X

High-Speed Analysis of Veterinary Drugs by Ultra High-performance Liquid Chromatography with Photodiode Array Detection

Introduction

Veterinary drugs including antibiotics, antibacterials, antiparasitics have widely been used in livestock and fishery industries and are contributing the productivity. From the food safety point of view, the Japanese Positive List System for Agricultural Chemical Residues in Foods came into force in May, 2006 and it set the criteria for monitoring of the residual chemicals and significantly extended the number of regulated chemicals from 31 to 240.

As one of the measurement methods for those animal drugs, there is a method of simultaneous detection of multi-components using the reversed phase chromatography and photodiode array detection (PDA detector).

In this report, 24 components were separated simultaneously by gradient method using the reversed phase ODS column of 2 mm particle and high sensitivity detection using PDA detector for UHPLC.

Keyword : UHPLC, Positive List System, Veterinary Drug, 2 µm particle, C18 column, PDA detector

Experimental

Equipment.		Conditions.	
Pump:	X-LC 3185PU x 2	Column:	X-PressPak V-C18 (2.0 mmID x 50 mmL, 2 µm)
Degasser:	X-LC 3080DG	Eluent A:	0.05% Phosphoric acid,
Mixer:	X-LC 3180MX		32 mM Sodium perchlorate in Water
Column oven:	X-LC 3067CO	Eluent B:	0.05% Phosphoric acid,
Autosampler:	X-LC 3159AS		32 mM Sodium perchlorate in Acetonitrile
Detector:	X-LC 3110MD	Gradient condition:	(A/B), 0 min→ (99/1)→ 1 min(59/41)→ 4.5 min(59/41)→ 7 min(40/60)→8.5 min(10/90)→ 9.5 min(10/90)→10 min(99/1) 1 cycle; 10 min
		Flow rate:	0.6 mL/min
		Column temp.:	40ºC
		Wavelength:	200-650 nm
		Injection volume:	1 µL
		Standard sample:	See below

The structures of 24 components for veterinary drug are shown in Fig. 1.

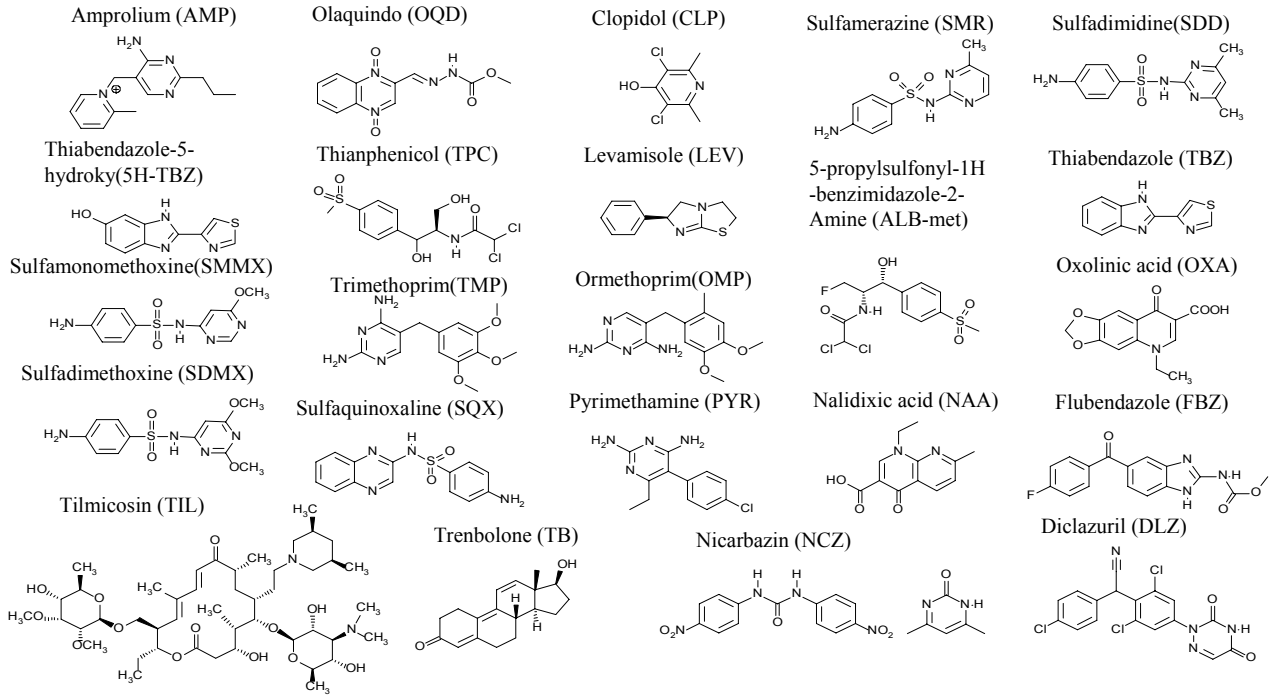

Fig. 1. Structures of formula of typical veterinary drug.

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Results

The contour plot of the standard mixture of veterinary drugs is shown in Fig. 2. Twenty-four components are clearly separated within 10 minutes.

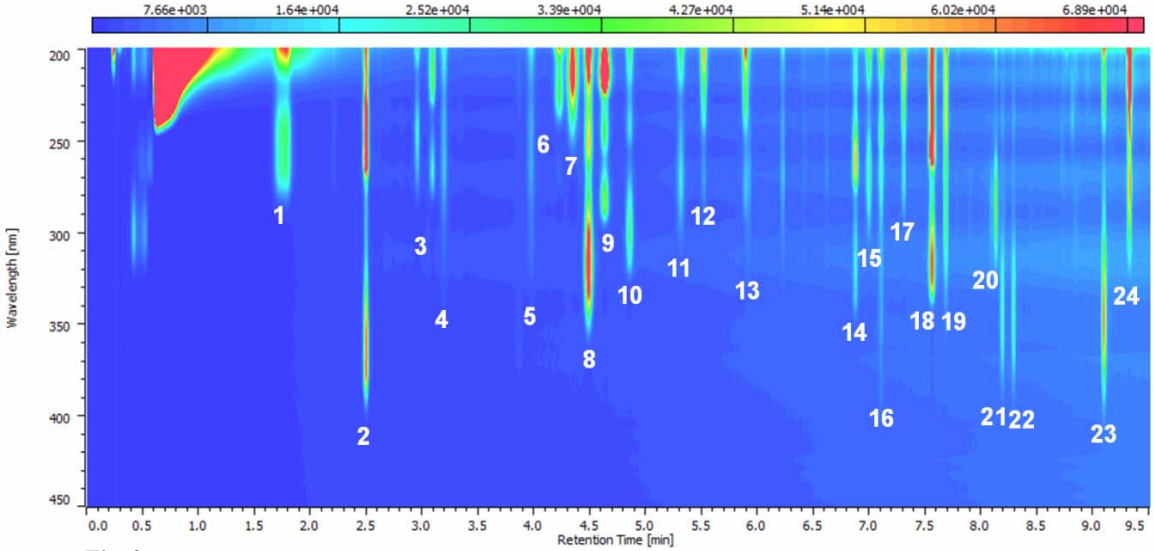

Fig. 2. Contour plot of standard samples for veterinary drugs. 1: AMP, 2: OQD, 3: CLP, 4: SMR, 5: SDD, 6: TPC, 7: LEV, 8: 5H-TBZ, 9: ALB-met, 10: TBZ, 11: SMMX, 12: TMP. 13: OMP, 14: OXA, 15: SDMX, 16: SQX, 17: PYR, 18: NAA, 19: FBZ, 20: TIL, 21: β-TB, 22: α-TB, 23: NCZ, 24: DLZ

The chromatogram at each wavelength is shown in Fig. 3.

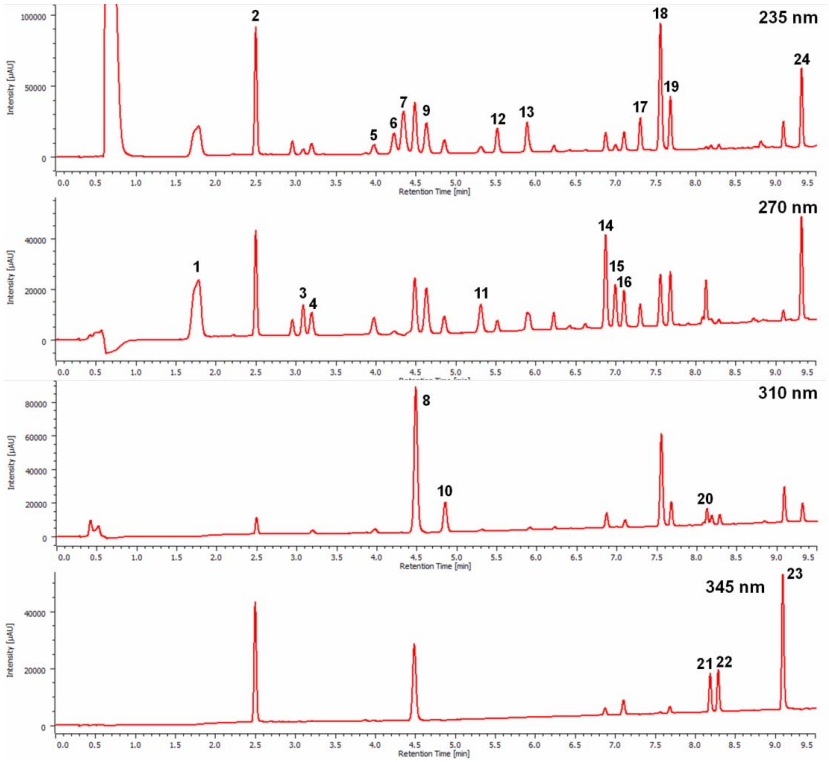

Fig. 3. Chromatograms of veterinary drug.
235 nm; 2: OQD, 5: SDD, 6: TPC, 7: LEV, 9: ALB-met, 12: TMP, 13: OMP, 17: PYR, 18: NAA, 19: FBZ, 24: DLZ.
270 nm; 1: AMP, 3: CLP, 4: SMR, 11: SMMX, 14: OXA, 15: SDMX, 16: SQX.
310 nm; 8: 5H-TBZ, 10: TBZ, 20: TIL.
345 nm; 21: β-TB, 22: α-TB, 23: NCZ

On-peak spectrum of each component is shown in Fig. 4.

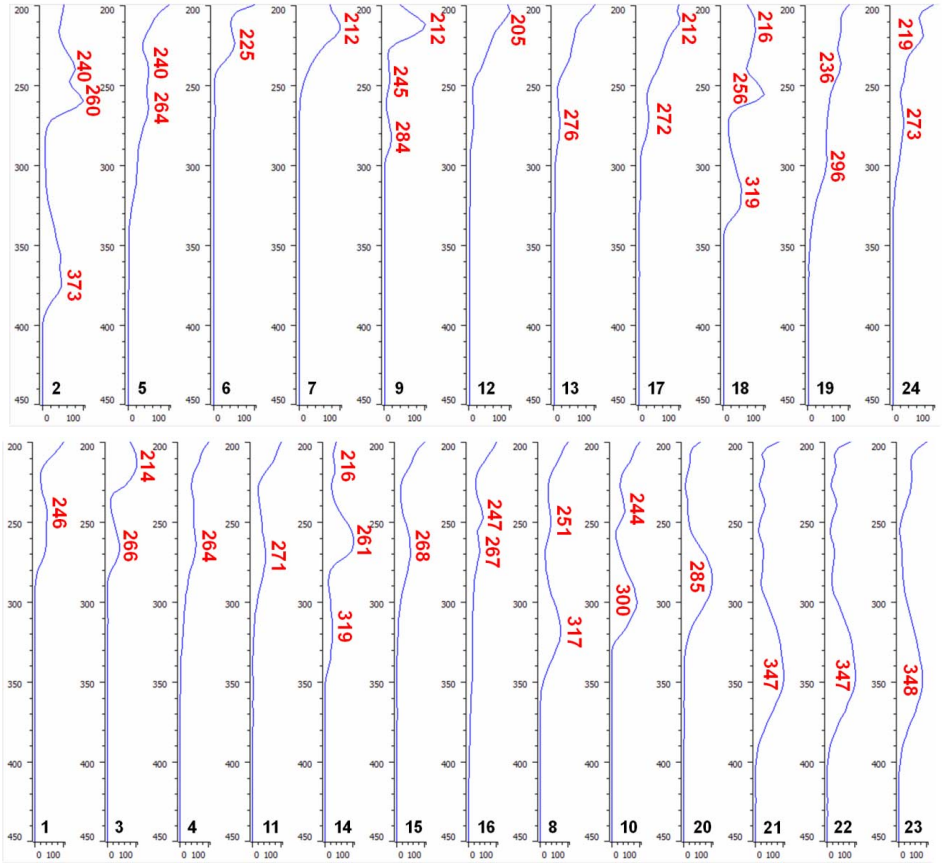

Fig. 4. On-peak spectra of veterinary drugs.
235 nm; 2: OQD, 5: SDD, 6: TPC, 7: LEV, 9: ALB-met, 12: TMP, 13: OMP, 17: PYR, 18: NAA, 19: FBZ, 24: DLZ.
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310 nm; 8: 5H-TBZ, 10: TBZ, 20: TIL.
345 nm; 21: β-TB, 22: α-TB, 23: NCZ

Table 1. Sample contents per 1 µL injection

Abbr.	ng	Abbr.	ng
AMP	79	OMP	8
OQD	40	OXA	8
CLP	8	SDMX	8
SMR	8	SQX	8
SDD	8	PYR	8
5H-TBZ	40	NAA	40
TPC	40	FBZ	12
LEV	58	TIL	40
ALB-met	58	β-TB	4
TBZ	8	α-TB	4
SMMX	8	NCZ	40
TMP	8	DLZ	40

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Fig. 5 shows the chromatograms of the extracts from the spiked pork meat with veterinary drugs. It is seen that the peaks of the main components are eluted and separated without interference by concomitants.

Red: Liquid extracted from the pork added with animal drugs
Blue: Liquid extracted from the natural pork

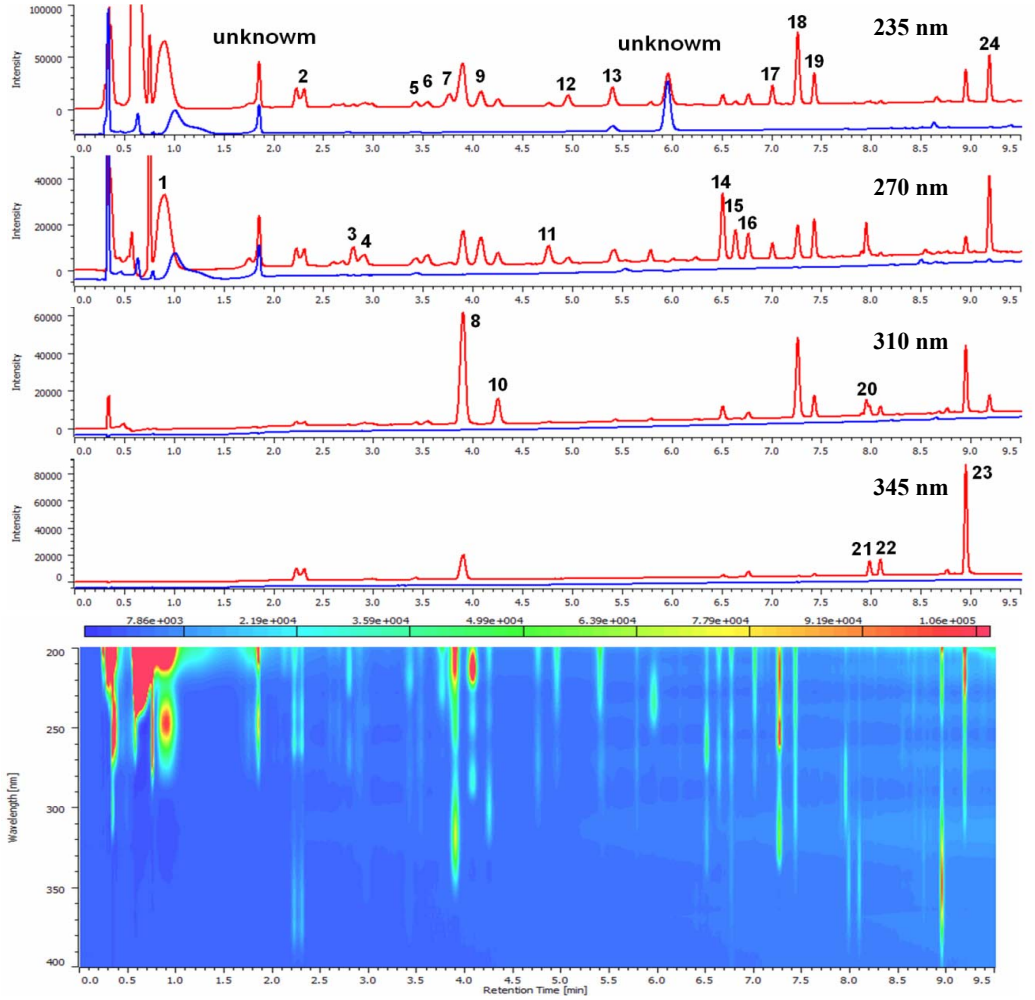

Fig. 5. Chromatograms of the liquid extracted from the pork to which animal drugs are added.

Preparation.

(1) Weigh 2.5 g of pork.
(2) Dissolve the following samples: Acetonitrile: 12 mL, Standard mixture of animal drugs : 3 mL, n-Hexane saturated with acetonitrile: 10 mL, Anhydrous sodium sulfate: 5 g
(3) Apply ultrasonic extraction for 30 minutes.
(4) Apply centrifugal separation at 3000 rpm for 5 minutes.
(5) Apply centrifugal separation for residual material by adding 10 mL of acetonitrile and for n-Hexane in the organic layer at 3000 rpm for 5 minutes.
(6) Mix the acetonitrile layer in (4) and acetonitrile layer in (5), and add 5 mL of n-propanol.
(7) Concentrate at 40°C by solvent elimination.
(8) Dissolve in Water/Acetonitrile (80/20).
(9) Add 0.25 mL of n-Hexane saturated with acetonitrile.
(10) Apply centrifugal separation at 3000 rpm for 5 minutes.
(11) Use Water/Acetonitrile layer as the sample to be jnjected.

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