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Rapid Detection of 7 Illegal Veterinary Additives in Animal Feed Using Oasis PRiME HLB Clean-up and UPLC-MS/MS

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APPLICATION BENEFITS

- Efficient, time-saving total solution for muti-residue analysis of veterinary drugs in animal feed formula
- Simple and rapid sample preparation with Oasis™ PRiME HLB
- Fast and sensitive UPLC™-MS/MS analysis

WATERS SOLUTIONS

ACQUITY™ UPLC I-Class System

Xevo™ TQ-S micro Mass Spectrometer

Oasis PRiME HLB Cartridge

KEYWORDS

Olaquindox, neomycin sulfate, sulfaquinoxaline, dihydropyridine, Oasis PRiME HLB Cartridge, formula feed, UPLC, MS, veterinary drug

INTRODUCTION

When discussing illegal additives in the feed, we immediately think of clenbuterol. Since the scandal of clenbuterol was exposed in 2011, the Chinese government has established a strict standard for the use of additives, and also tightened regulation for the illegal use of additives in feed. Nevertheless, some feed producers still have not stopped their illegal behavior. The "CCTV 3.15 party in 2017" exposed this situation and aroused great concern from the public. A reporter's survey found the abuse of veterinary drugs including olaquindox, neomycin sulfate, sulfaquinoxaline, and dihdropyridine in animal breeding.

Olaquindox is an alternative to clenbuterol, that can promote growth, reduce the feed and meat ratio, improve body size, and improve feed intake. It tends to be accumulated in animal tissue and leads to chromosomal abnormalities in cells if added to animal feed over a long period of time. However, the residues of these compounds also pose a health risk to the consumers.

Currently, the determination of olaquindox in the Chinese national standard (GB)[1] is mainly based on LC-UV and LC-MS/MS methods. Accurate quantification of Olaquindox is a challenge because of the complex matrices and potential to decompose during sample preparation and when exposed to light.

In this application note, a simple clean-up protocol using a novel SPE device was introduced for the analysis Olaquindox and six other illegal veterinary additives in animal feed. The extract was cleaned up by pass-through SPE using the Oasis PRiME HLB Cartridge prior to UPLC-MS/MS analysis. The spiked samples were quantified using an external standards method, and the recovery and reproducibility for each compound met the regulatory requirements of the quantitative method. This method is simple, rapid, accurate, suitable for the analysis of the highlighted veterinary drugs in animal feed.

EXPERIMENTAL

UPLC conditions

LC system:	ACQUITY UPLC I-Class
Column:	ACQUITY UPLC HSS T3, 1.8 µm, 2.1 x 100 mm
Temp.:	45 °C
Flow rate:	0.4 mL/min
Mobile phase A:	0.1% formic acid in water
Mobile phase B:	0.1% formic acid in methanol
Run time:	9 min
Injection vol.:	2 µL

Gradient:

Time	Flow rate (mL/min)	%A	%B
0.00	0.4	98	2
0.25	0.4	98	2
3.25	0.4	70	30
7.00	0.4	2	98
7.50	0.4	2	98
7.60	0.4	98	2
9.00	0.4	98	2

MS conditions

MS system:	Xevo TQ-S micro
Ionization mode:	ESI+
Capillary voltage:	3.0 kV
Desolvation temp.:	550 °C
Source temp.:	150 °C
Desolvation flow:	1000 L/h
Cone gas:	50 L/h

MRM conditions

Compound	Parent ion (*m/z*)	Product ion (*m/z*)	Cone voltage (V)	Collision energy (eV)
Olaquindox	264.1	143.0 212.1	32 32	30 23
Sulfaquinoxaline	301.0	92.0 155.9	32 32	30 13
Trimethoprim	291.1	123.0 230.1	40 40	27 28
Aminophylline	181.0	96.1 123.9	35 35	25 21
Diprophylline	255.1	123.9 181.0	35 35	35 22
Dexamethasone	393.2	355.2 373.2	20 20	10 10
Atropine	290.1	93.0 124.0	35 35	36 29

Sample preparation

Initial extraction

Step 1:	Weigh 1 g of feed sample into a 50 mL centrifuge tube;
Step 2:	Add 10 mL of extraction solvent (80% acetonitrile + 20% water) and shake well for 10 min;
Step 3:	Centrifuge at 6000 rpm for 5 min

Pass-through SPE clean-up

Step 1:	An Oasis PRiME HLB Cartridge (6 cc, 200 mg; p/n 186008057) was mounted on a pre-cleaned SPE vacuum manifold. Cartridge conditioning is not required and is not performed.
Step 2:	A 0.5 mL aliquot of the supernatant (sample extract) was passed through the Oasis PRiME HLB Cartridge and the eluant was discarded.
Step 3:	Install the collection tubes. Another 1 mL of supernatant was passed through the cartridge, and the eluant was collected. The eluant was diluted 1:3 with water and injected into Xevo TQ-S micro for analysis.

RESULTS AND DISCUSSION

METHOD RECOVERY AND STABILITY

The analyte recovery was determined by spiking standards into the blank matrix, a 1:1 mixture of rice and corn powders. The analytes were spiked at concentrations of low, medium (5 times low spike) and high levels (10 times low spike). The lowest spike for olaquindox was 10 ug/kg, sulfaquinoxaline was 0.5 ug/kg, trimethoprim and atropine was 2.5 ug/kg and the lowest spike for aminophylline, diprophylline and dexamethasone was 5.0 ug/kg). Each level of spiking was repeated in five replicates. All samples were processed according to the method described previously. The concentrations were calculated using a matrix-matched calibration curve. The recovery range of the high, medium, and low level samples ranged from 70.6% to 112%. The precision range of the high and medium level spike samples was 0.88% to 4.2% and the precision range was 4.3% to 8.8% for the low spike samples.

MATRIX EFFECTS AND MATRIX MATCHED CALIBRATION CURVE

The matrix effect was measured by comparing the peak area of solvent standards and post spiked samples in chicken feed and swine feed samples, where the spiked level was equal to 5 µg/kg for atropine and diprophylline, and 1 µg/kg for the other compounds.

Calibration curves ranged from 0.01 to 1.00 µg/L for sulfaquinoxaline, from 0.1 to 10 µg/L for olaquindox, aminophylline, diprophylline, and dexamethasone, and 0.05 to 5.0 µg/L for trimethoprim and atropine.

Table 1. Matrix effects of each compound and the correlation coefficients of their matrix matched calibration curves.

Veterinary drugs	Matrix effects (%)	Matrix matched calibration curve R^2
Olaquindox	(9.0)	0.9998
Sulfaquinoxaline	(14.9)	0.9997
Trimethoprim	7.2	0.9998
Atropine	16.1	0.9994
Aminophylline	(0.5)	0.9995
Diprophylline	9.6	0.9992
Dexamethasone	(14.7)	0.9991

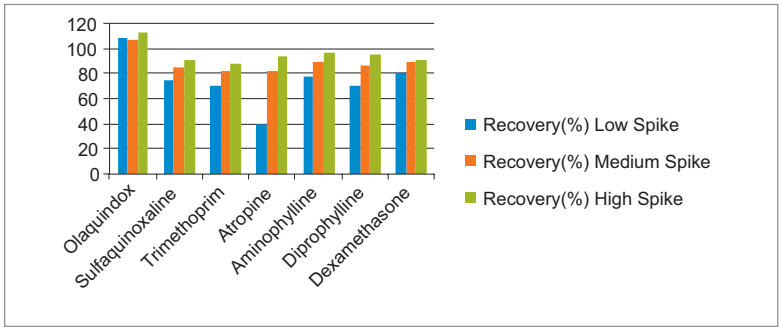

Figure 1. Summary of recoveries for spiked feed samples. The lowest spike for olaquindox was 10 ug/kg, sulfaquinoxaline was 0.5 ug/kg, trimethoprim and atropine was 2.5 ug/kg and the lowest spike for aminophylline, diprophylline and dexamethasone was 5.0 ug/kg).

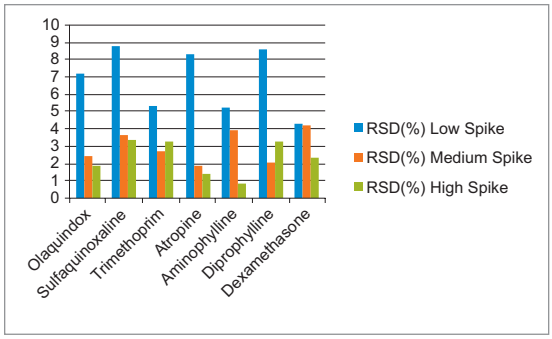

Figure 2. Precision of recoveries for spiked feed samples.

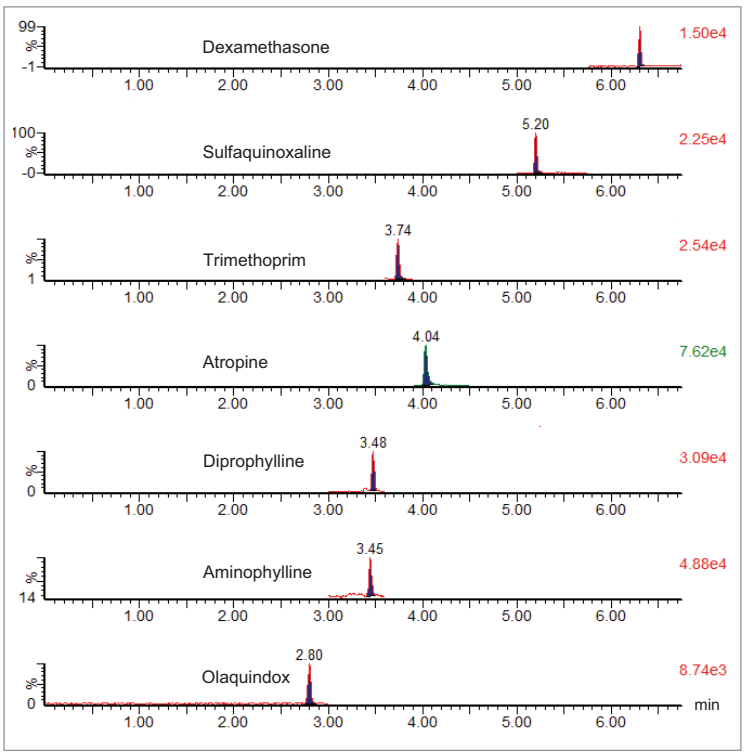

Figure 3. Typical chromatograms of spiked sample (sulfaquinoxaline spiked at 0.1 ppb; olaquindox, aminophylline, diprophylline, and dexamethasone spiked at 1.0 ppb; trimethoprim and atropine spiked at 0.5 ppb).

The established method was used for real sample analysis. Finally, an olaquindox content up to 1.9 to 18 mg/kg was detected in chicken feed and swine feed samples.

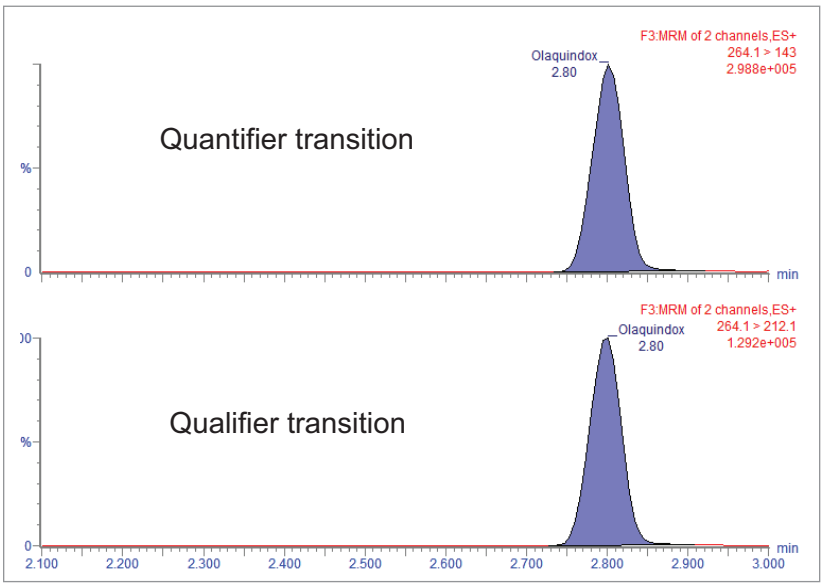

Figure 4. Chromatogram of olaquindox (1.9 mg/kg) in chicken feed.

CONCLUSIONS

- A simple and rapid analytical method was developed for the determination of seven illegal veterinary drug additives in animal feed. This method has been proven to achieve levels of detection that meet regulatory requirements.
- The Oasis PRiME HLB Cartridge provided effective clean-up and good recoveries for the target veterinary drugs in animal feeds.
- The ACQUITY UPLC I-Class System coupled with Xevo TQ-S micro offered good sensitivity and robust methodology.

Reference

1. Announcement No. 2086-5-2014 of the Ministry of Agriculture of the People's Republic of China: Determination of carbadox, mequindox, quinocetone and olaquindox in feeds – liquid chromatography – tandem mass spectrometry.

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