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Applikationsbericht

Improved SPE for LC-MS Determination of Ractopamine and Zilpaterol in Bovine Liver: The Oasis PRiME MCX Method

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief describes a simple, rapid, and effective cleanup strategy to remove phospholipids from bovine liver extracts prior to UPLC-MS/MS determination of beta-agonist veterinary drugs.

Benefits

The Oasis PRiME MCX Cartridge provides improved cleanup of bovine liver extracts with high recovery of target beta-agonist veterinary drugs.

Introduction

Ractopamine and zilpaterol are betaandronergic (beta-agonist) drugs accepted as growth enhancing substances for cattle in the US and Canada. The MRL (or US tolerance) for ractopamine in bovine liver is 90 ng/g (US) and 40 ng/g (Canada). The MRL for zilpaterol in bovine liver is 12 ng/g (US) and 5 ng/g (Canada). These substances are not allowed for use in animal husbandry in the EU and in much of the rest of the world. To help ensure public health and safety, reliable analytical methods are necessary to determine residues of these compounds in tissue samples obtained from animals raised for human consumption. In this technology brief, a simple methanolic extraction, SPE cleanup, and UPLC-MS/MS analysis method is demonstrated for the determination of ractopamine and zilpaterol in bovine liver.

This matrix is challenging for residue analysis; bovine liver is a very good source of dietary lecithin (phospholipids); a gram of liver contains about 25 mg of phospholipids, about four times the amount typically found in muscle. The presence of this co-extracted substance in the methanolic extract can lead to interference in the UPLC-MS analysis, contamination of the analytical column and other components of the ACQUITY UPLC System, and contamination of the mass spectrometer itself.

Experimental

In this study, we compare the cleanup obtained using AOAC method 2011.23,[1] with cleanup obtained using a modification of that method (substitution of the Oasis PRiME MCX SPE Cartridge and protocol for the

original SPE protocol).

Sample preparation

Initial extraction/precipitation[1]:

Place a 5 g homogenized sample into a 50 mL centrifuge tube. Add 20 mL of methanol, vortex for 10 seconds and shake for 2 minutes. Centrifuge at 4000 rpm for 5 minutes. Transfer supernatant to a suitable polypropylene container (Extract 1). Re-suspend the pellet in a second 20 mL portion of methanol, then vortex shake and centrifuge as before. Collect the supernatant (Extract 2) and combine with Extract 1. Re-suspend the pellet in 10 mL portion of methanol, then vortex shake and centrifuge as before. Collect the supernatant (Extract 2) and combine with Extracts 1 and 2. Adjust the volume of the combined extracts to exactly 50 mL.

Note: This extraction protocol gives good recovery of the target compounds but also extracts significant amounts of phospholipids.

SPE cleanup

Mount an Oasis PRiME MCX Vac Cartridge (60 mg, 30 µm, p/n: 186008918) on a pre-cleaned vacuum manifold. (no conditioning/equilibration steps are required). The vacuum is set to 1–2 psi. SPE is performed according to the following protocol:

Load:	2 mL of the combined supernatant
Wash 1:	2 mL of aqueous buffer (100 mM ammonium formate, 0.1 % formic acid)
Wash 2:	2 mL of methanol
Elute:	2 mL of 5 % ammonia in methanol
Evaporate:	to dryness using gentle

	nitrogen
	flow (40 °C)
Reconstitute:	1 mL 20:80 methanol/water

UPLC conditions

LC system:	ACQUITY UPLC H-Class
Column:	ACQUITY UPLC BEH, 1.7 µm, 2.1 x 100 mm
Mobile phase A:	0.02% formic in water
Mobile phase B:	acetonitrile:MeOH (50:50)
Injection vol.:	4 µL
Column temp.:	40 °C
Weak wash:	10:90 acetonitrile:water (600 µL)
Strong wash:	50:30:40 water:acetonitrile:IPA (200 µL)
Seal wash:	10:90 acetonitrile:water

Gradient

Time (mL/min)	Flow	%A	%B
0.0	0.4	95	5
6.0	0.4	10	90
6.2	0.4	2	98

Time (mL/min)	Flow	%A	%B
9.1	0.4	2	98
9.3	0.4	95	5
12.0	0.4	95	5

MS conditions

Mass spectrometer:	Xevo TQ-S micro
Mode:	Positive Ion Electrospray, MRM
Source temp.:	120 °C
Desolvation temp.:	300 °C
Desolvation gas flow:	1000 L/hr
Cone gas flow:	30 L/hr
Collision gas flow:	0.15 mL/min
Data management:	MassLynx v4.2

Monitored transitions

Compound	MRM	Cone (v)	Collision (eV)
Zilpaterol	262.2 > 185.1	25	22
	262.2 > 201.1	25	18

Compound	MRM	Cone (v)	Collision (eV)
Ractopamine	302.2 > 164.1	35	15
	302.2 > 284.2	35	12

Table 1. Recovery data obtained from spiked bovine liver samples.

Results and Discussion

LC-MS/MS analysis

Ractopamine and zilpaterol recoveries were determined using LC-MS/MS. Conditions are presented in Figure 1.

Table 1 shows SPE recovery data obtained from six replicate analyses of bovine liver extracts. The extracts were spiked at 1, 6, and 20 ng/g ractopamine (equivalent to 5, 30, and 100 ng/g in a liver sample) and at 0.2, 1.2, and 4.0 ng/g zilpaterol (equivalent to 1, 6, and 20 ng/g in a liver sample). Recovery was measured by comparing the peak areas obtained from extracts spiked before SPE cleanup with peak areas obtained from extracts spiked after SPE cleanup (external standard calibration). The chromatograms shown in Figure 1 show typical response in a matrix sample after cleanup for zilpaterol (equivalent to 1 ng/g in bovine liver), and for ractopamine (equivalent to 5 ng/g in bovine liver). The chromatograms in Figure 2 show the effectiveness of the Oasis PRiME MCX Cartridge for removal of ≥90% more phopholipids from the liver extracts compared with the original SPE protocol.[1]

After the elution step (with ammonia/methanol), the sample was evaporated and reconstituted in 20:80 methanol/water; this step facilitates the LC-MS/MS determination of zilpaterol, a much more polar compound compared with ractopamine.

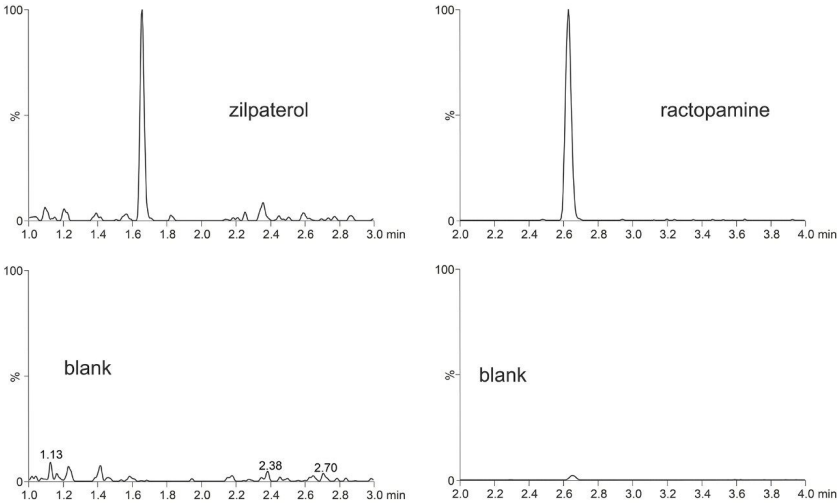

Figure 1. Typical chromatograms showing response in a matrix sample after cleanup for zilpaterol (left, equivalent to 1 ng/g in bovine liver) and for ractopamine (right, equivalent to 5 ng/g in bovine liver).

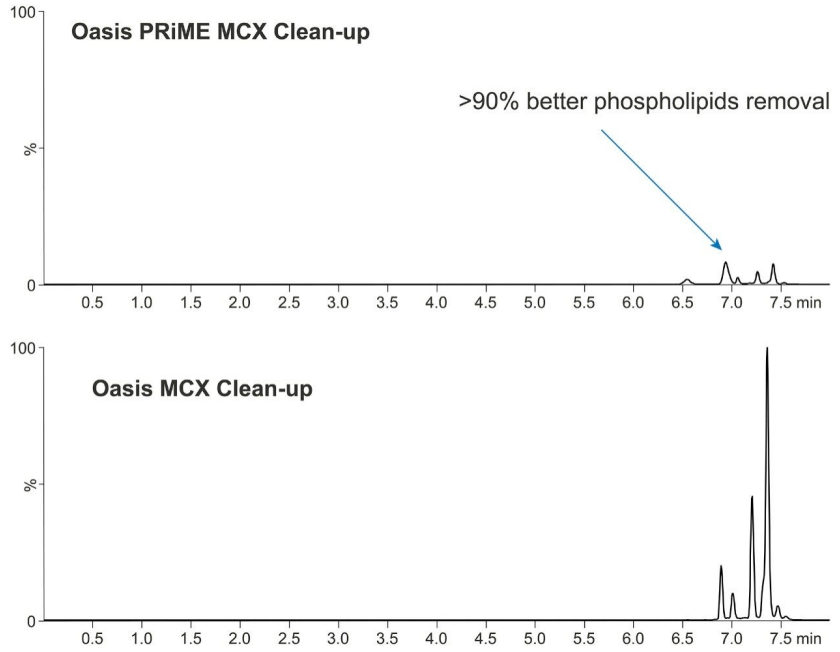

Figure 2. LC-MS/MS chromatograms showing improved removal of phospholipids from bovine liver extracts (transitions monitored: 496.4, 520.0, 522.0, and 524.0 m/z, all to 184.4 m/z).

Conclusion

- The Oasis PRiME MCX Cartridge is effective for cleanup and enrichment of methanolic extracts of bovine liver prior to LC-MS/MS determination of ractopamine and zilpaterol.
- High recoveries of both compounds were obtained.
- Compared with the prior SPE cleanup protocol (AOAC method), 90% more phospholipids were removed from the extracts.

References

1. AOAC Official Method 2011.23. *Determination and confirmation of parent and total ractopamine in bovine, swine, and turkey tissues*.

Featured Products

ACQUITY UPLC H-Class PLUS System <https://www.waters.com/10138533>

Xevo TQ-S micro Triple Quadrupole Mass Spectrometry <https://www.waters.com/134798856>

MassLynx MS Software <https://www.waters.com/513662>

Available for Purchase Online

Oasis PRiME MCX 3 cc Vac Cartridge, 60 mg Sorbent per Cartridge, 30 µm Particle Size < https://www.waters.com/waters/partDetail.htm?partNumber=186008918>

ACQUITY UPLC BEH C18 Column, 130Å, 1.7 µm, 2.1 mm X 100 mm < https://www.waters.com/waters/partDetail.htm?partNumber=186002352>

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