

Improved SPE for LC-MS Determination of Ractopamine in Porcine and Bovine Liver: The Oasis MCX Method Using Otto SPEcialist

Nathan Stebbins, Jessica Lance, Joe Bruton, Jeremy Shia, Marina Obradovic, Kathryn Brennan, Claire Rhodes

Tyson Foods and WBA Analytical Laboratories, Waters Corporation

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 co-extractives from porcine and bovine liver extracts. This is executed via Oasis MCX 96-well SPE plate processed using Otto SPEcialist, a semi-automated positive pressure manifold prior to UPLC-MS/MS quantification of total ractopamine, a beta-agonist veterinary drug, with a limit of detection of 0.1 ng/g. This method quantifies ractopamine and ractopamine-glucuronide metabolites to accurately measure ractopamine in animal tissue. The use of Otto SPEcialist to process samples in 96-well plate format not only increases sample throughput and reproducibility, but also eliminates the risk of cross contamination when using manual vacuum manifold.

Benefits

- Processing samples using Otto SPEcialist with Oasis MCX in 96-well plate format provides improved cleanup
-

of porcine liver extracts with high recovery of target beta-agonist veterinary drugs

- The Otto SPEcialist positive pressure manifold improves workflow, data turnaround, and allows analysts more time for other responsibilities, while simultaneously improving repeatability between analysts and day-to-day improvement
- Samples prepared using the Otto SPEcialist in 96-well plate format had increased area count and signal-to-noise ratios compared to a modified method processed using a manual vacuum manifold in SPE cartridge format

Introduction

Ractopamine is a beta-adrenergic (beta-agonist) drug accepted as growth enhancing substances for cattle in the US and Canada. The US MRL (maximum residue limit) for ractopamine in porcine liver is 50 ng/g (US) and 40 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. Some countries exhibit zero-tolerance for these compounds and exports require certificates of analysis showing the absence of ractopamine below 0.1 ng/g (ppb). 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 in porcine and bovine liver and offal products.

Experimental

In this study, we describe the sample cleanup following AOAC method 2011.23¹ with the modification by substituting vacuum manifold with Otto SPEcialist, a positive pressure manifold, and Oasis MCX Cartridges with Oasis MCX 96-well plate.

Sample Preparation

Place a 5 g homogenized sample into a 50 mL centrifuge tube. Add 5 mL of methanol and vortex for 60 seconds. Centrifuge at 4000 rpm for 5 minutes. Transfer supernatant to a suitable polypropylene container (Extract 1). Re-suspend the pellet in a second 5 mL portion of methanol, then vortex and centrifuge as before. Collect the supernatant (Extract 2) and combine with Extract 1. Re-suspend the pellet in 5 mL portion of methanol, then vortex and centrifuge as before. Collect the supernatant (Extract 3) and combine with Extracts 1 and 2. Adjust the volume of the combined extracts to exactly 20 mL with methanol and centrifuge for 5 minutes. Transfer 8 mL combined extract to 15 mL tube and evaporate under nitrogen flush to remove methanol. Reconstitute in 0.8 mL 25 mM sodium acetate and add 20 μ L β -glucuronidase, then incubate for 2 hours at 65 °C. Add 0.8 mL methanol and centrifuge for 5 minutes at 4000 rpm before SPE cleanup.

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

SPE Cleanup

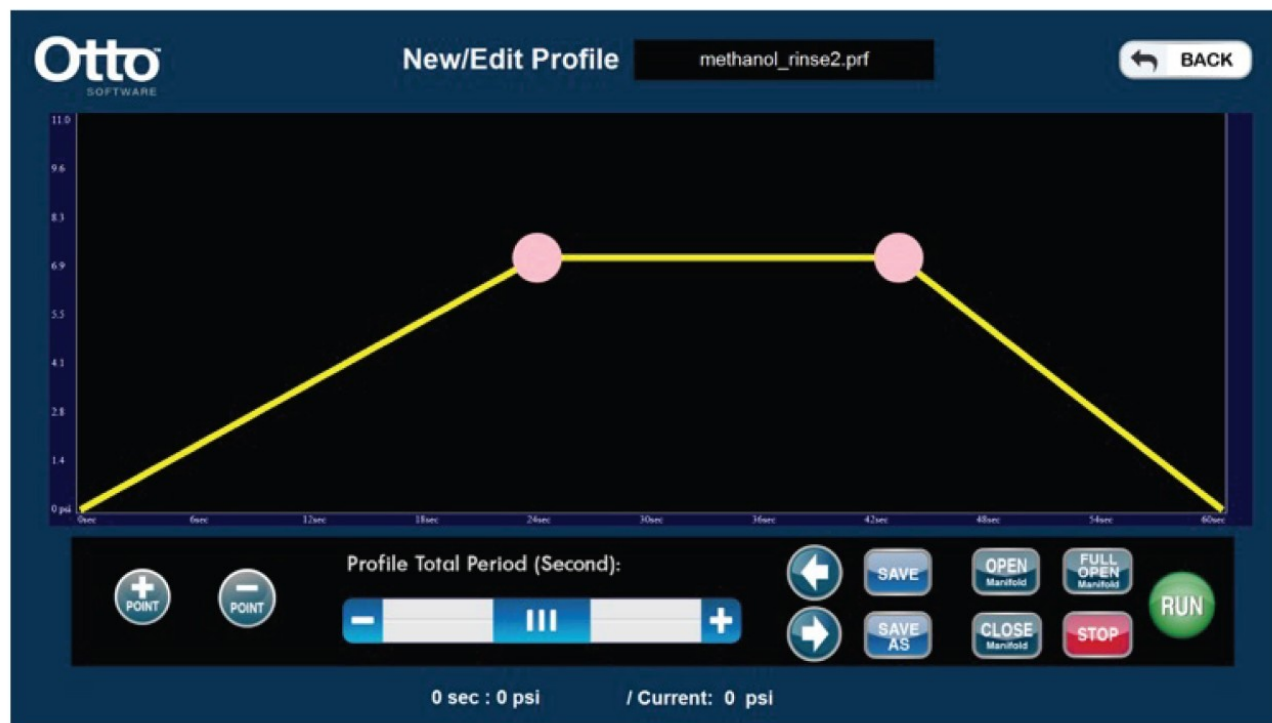
The Oasis MCX 96-well plate (60 mg, 60 μ m, p/n:186000678 < <https://www.waters.com/nextgen/us/en/shop/sample-preparation--filtration/186000678-oasis-mcx-96-well-plate-60-mg-sorbent-per-well-60--m-1-pk.html> >) was mounted on waste reservoir for condition, load, and wash steps. The waste reservoir was then replaced with the 2 mL collection plate (p/n:186002482 < <https://www.waters.com/nextgen/us/en/shop/vials-containers--collection-plates/186002482-96-well-sample-collection-plate-2-ml-square-well-50-pk.html> >) to collect eluate in elution step. SPE is performed according to the following protocol:

Condition:	1 mL Methanol
Load:	All of the combined supernatant (1.62 mL)
Wash:	1 mL Methanol
Elute:	0.8 mL of 5% Ammonium hydroxide in methanol

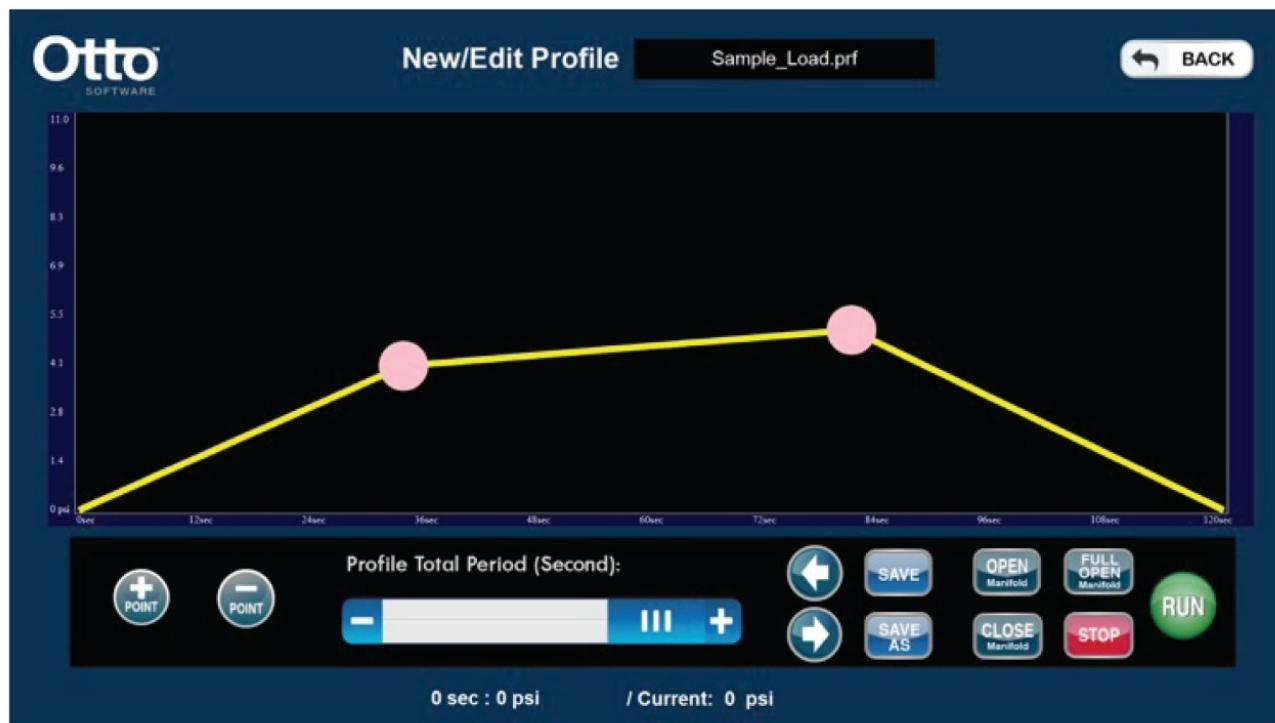


Figure 1. Otto SPEcialist

Pressure Profiles are shown here:



Condition Step



Load Step



Wash Step



Elute Step

UPLC Conditions

LC system: ACQUITY
 UPLC I-
 Class PLUS

Column: ACQUITY
 UPLC BEH,
 1.7 μm , 2.1 x
 50 mm

Mobile
 phase A: 0.1% Formic
 acid in water

Mobile phase B: LCMS grade methanol

Injection volume: 4 μ L

Column temperature: 40 $^{\circ}$ C

Weak wash: 10:90
Methanol:water
(600 μ L)

Strong wash: 50:50
Methanol:water
(800 μ L)

Seal wash: 10:90
Methanol:water

Gradient Table

Time (mL/min)	Flow	%A	%B
0.0	0.4	90	10
3.0	0.4	10	90
3.4	0.4	10	90
3.5	0.4	90	10
4.5	0.4	90	10

MS Conditions

Mass spectrometer:	Xevo TQ-XS
Mode:	Positive Ion Electrospray, MRM
Source temperature:	150 °C
Desolvation temperature:	550 °C
Desolvation gas flow:	1000 L/hr
Cone gas flow:	150 L/hr
Collision gas flow:	0.15 mL/min
Data management:	MassLynx v4.2

Compound	MRM	Cone (v)	Collision (eV)
Ractopamine	302.2>164.1	35	14
	302.2>121.0	35	22

Monitored Transitions

Results and Discussion

Ractopamine recoveries were determined using LC-MS/MS. Conditions are presented in Figure 2.

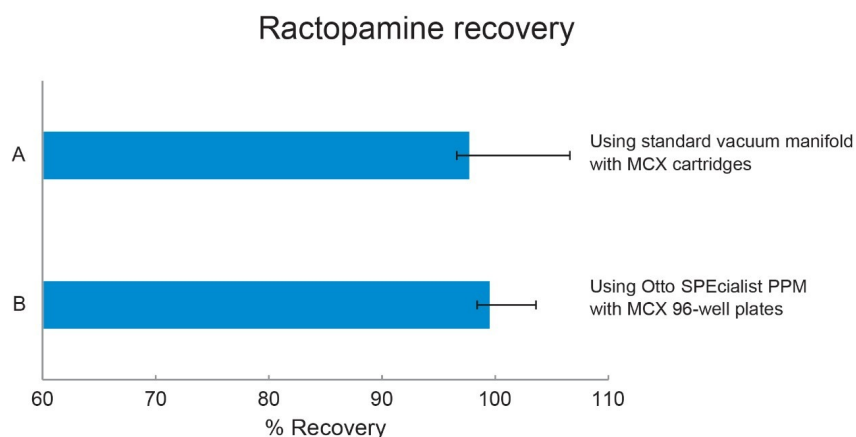


Figure 2. Comparison of recovery data from spiked porcine liver samples between (A) using standard vacuum manifold with Oasis MCX Cartridges and (B) using Otto SPEcialist semi-automated positive pressure manifold (PPM) with Oasis MCX 96-well plates

Figure 2 shows SPE recovery data obtained from six replicate analyses of porcine liver extracts spiked at 0.1 ng/g ractopamine. Two methods of solid phase extraction (SPE) are utilized for sample cleanup: (A) using standard vacuum manifold with Oasis MCX Cartridges, and (B) using Otto SPEcialist semi-automated positive pressure

manifold (PPM) with Oasis MCX 96-well plates. Both methods have excellent ractopamine recoveries. It should be noted that the standard deviation of the Otto SPEcialist is less than half of the Oasis MCX Cartridges indicating better data precision. This is critical for accurate quantification at trace quantities. Standards were spiked into porcine liver matrix blank and samples prior to the extraction.

The chromatograms in Figure 3 show typical response in a matrix sample after cleanup for ractopamine where the spiked concentrations are equivalent to 0.1 ng/g for A and B, and 0.01 ng/g for C and D in porcine liver. This is a comparison of samples (A and C) prepared using an MCX 96-well plate on the Otto SPEcialist PPM versus samples (B and D) prepared using MCX cartridges on a standard vacuum manifold. Samples prepared using OttoSPEcialist had 87% increase in area count with improved signal:noise, thus allowing TargetLynx to more accurately integrate peaks and reduce analyst time reading data. The lower area counts using Oasis MCX Cartridges are possibly due to loss from the extra steps of evaporation and reconstitution for sample enrichment. After the elution step using Otto SPEcialist, the collection plate was capped and inserted directly in LC-MS/MS system without filtering by 0.2 µm filters. This eliminates the evaporation/concentration step, reduces consumables, and improves turnaround time. Another significant benefit of the consumable 96-well plates is that it eliminates the risk of cross contamination compared to using manual vacuum manifold. For high throughput laboratories, the Otto SPEcialist has a return of investment of months, followed by significant long-term savings.

The LC gradient is diverted to waste from 0–0.6 and 2–4 minutes to reduce contamination from co-extractives on source and detector. This causes a sudden rise in the baseline, which is particularly evident in Figure 3D.

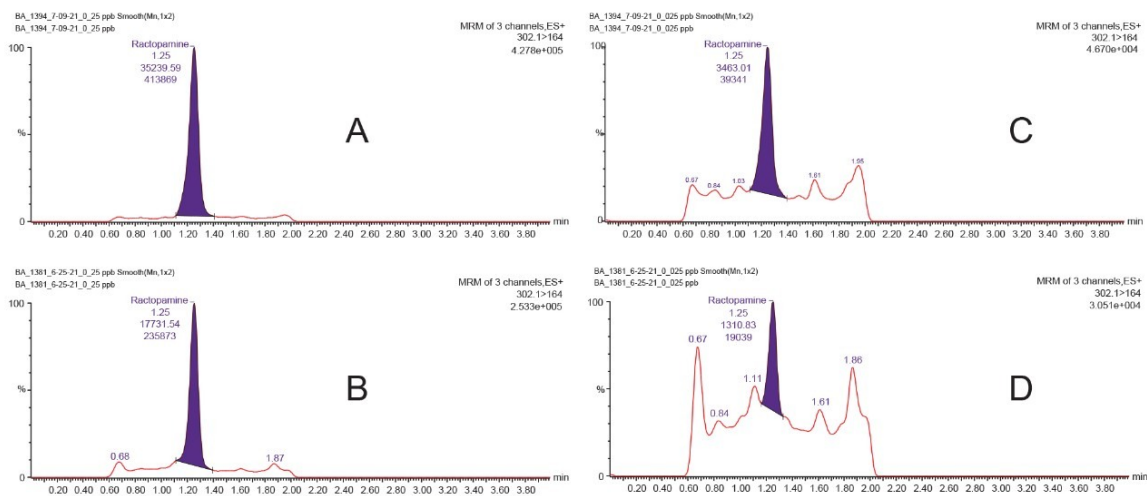


Figure 3. Chromatograms showing response in a matrix samples after cleanup for ractopamine where (A) and (C) using Otto SPEcialist with MCX 96-well plate, and (B) and (D) using vacuum manifold with MCX Cartridges. (A) and (B) spiked samples equivalent to 0.1 ng/g ractopamine in porcine liver, and (C) and (D) spiked samples equivalent to 0.01 ng/g ractopamine in porcine liver.

Conclusion

- The use of Otto SPEcialist, a positive pressure manifold, with Oasis MCX 96-well plate is very effective for cleanup and enrichment of methanolic extracts of porcine and bovine liver and offal samples prior to LC-MS/MS determination of ractopamine at 0.1 ng/g.
- Compared to using Oasis MCX Cartridges on a vacuum manifold, the use of Otto SPEcialist with plates provides faster, more repeatable results with increased peak response and signal to noise.
- Significant cost savings through the elimination of consumables such as 0.2 μ m filters and centrifuge tubes for sample enrichment.

References

1. AOAC Official Method 2011.23. Determination and Confirmation of Parent and Total Ractopamine in Bovine, Swine, and Turkey Tissues.

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[ACQUITY UPLC I-Class PLUS System <https://www.waters.com/134613317>](https://www.waters.com/134613317)

[Xevo TQ-XS Triple Quadrupole Mass Spectrometry <https://www.waters.com/134889751>](https://www.waters.com/134889751)

[Otto SPEcialist <https://www.waters.com/waters/nav.htm?cid=135073532>](https://www.waters.com/waters/nav.htm?cid=135073532)

[MassLynx MS Software <https://www.waters.com/513662>](https://www.waters.com/513662)

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