# IMPROVED SPE FOR LC-MS/MS DETERMINATION OF RACTOPAMINE IN PORCINE LIVER

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## INTRODUCTION

The β-agonist ractopamine is authorized for the production of some animals in a limited number of countries. For example, tolerances have been set in porcine liver in the USA and Canada at 50 and 40 µg/kg, respectively. The administration of such growth-promoting agents in food-producing animals is banned in many other countries due to concerns over human health. Some enforce zero-tolerance for these compounds and imports require certificates of analysis showing the absence of ractopamine. In the EU, specific prohibited or unauthorised pharmacologically active substances have had reference points for action (RPAs) set but none exist for β-agonists so minimum method performance requirements (MMPRs) for these substances have been provided by the EURLs. These are NOT enforcement limits but represent the minimum concentrations that official laboratories should be able to reliably determine. Laboratories should ensure that their CCβ for screening methods and CCα for confirmatory methods is lower than the MMPR for ractopamine in liver at 0.5 µg/kg.

This poster describes a method for the determination of ractopamine residues in liver. The use of OTTO SPEcialist to process extracts in a 96-well plate format not only increases sample throughput and precision, but also eliminates the risk of cross contamination when using manual vacuum manifold.

## METHOD

### Sample extraction

Samples were extracted using a modified version of AOAC method 2011.23. The vacuum manifold was substituted with Otto SPEcialist positive pressure manifold, and Oasis MCX SPE cartridges with an Oasis MCX 96-well plate.

Weigh 5 ±0.05 g sample into 50 mL plastic Falcon tube

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Add 5 mL MeOH, vortex (1 min), centrifuge (4000 rpm for 5 mins) and transfer supernatant to a suitable polypropylene container (Extract 1)

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Re-suspend the pellet in a 2nd 5 mL portion of MeOHl, then vortex, centrifuge as before and collect the supernatant and combine with Extract 1. Repeat step again and combine extracts.

↓

Adjust the vol the combined extracts to 20 mL with MeOH, centrifuge for 5 min, transfer 8 mL combined extract to 15 mL tube and evaporate under $N_2$

↓

Reconstitute in 0.8 mL 25 mM NaAc and add 20 uL β-glucuronidase, incubate for 2 hrs at 65°C

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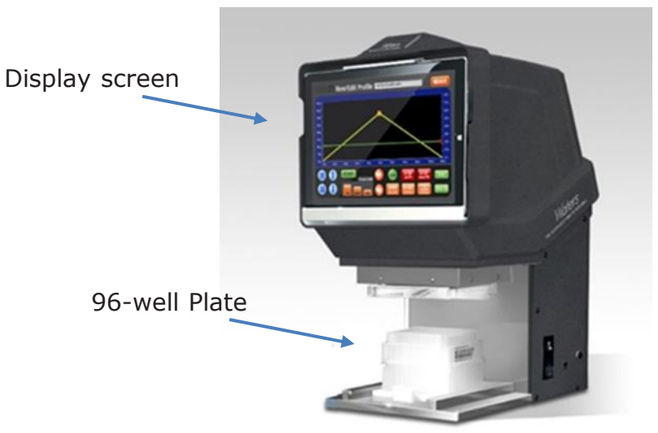
Add 0.8 mL MeOH and centrifuge (4000 rpm for 5 min) before SPE clean-up

### Clean-up using solid-phase extraction (SPE)

An Oasis MCX 96-well Plate (60 mg, 60 µm) was mounted on a drainless waste reservoir followed by a 2 mL square collection plate. Oasis MCX is a novel, mixed-mode polymeric sorbent that has been optimized to achieve higher selectivity and sensitivity for extracting basic compounds with cation-exchange groups. SPE was performed according to the following protocol:

| | |
|---|---|
| Condition: | 1 m MeOH |
| Load: | All the combined supernatant (1.62 mL) |
| Wash: | 1 ml MeOH |
| Elute: | 0.8 mL of 5% ammonium hydroxide in MeOH |



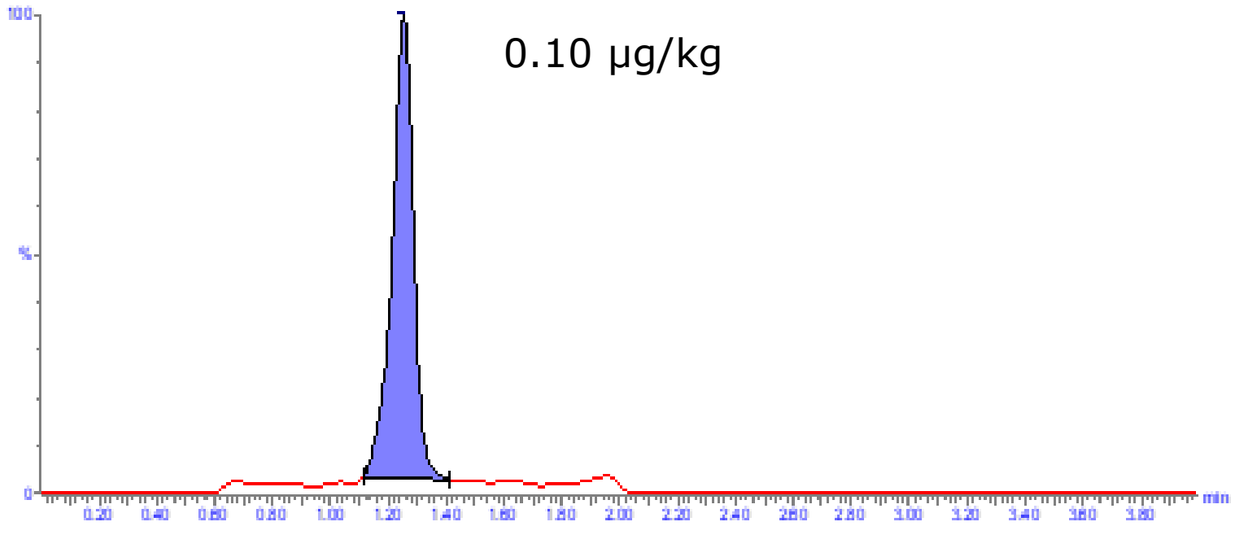

### Instrumental conditions

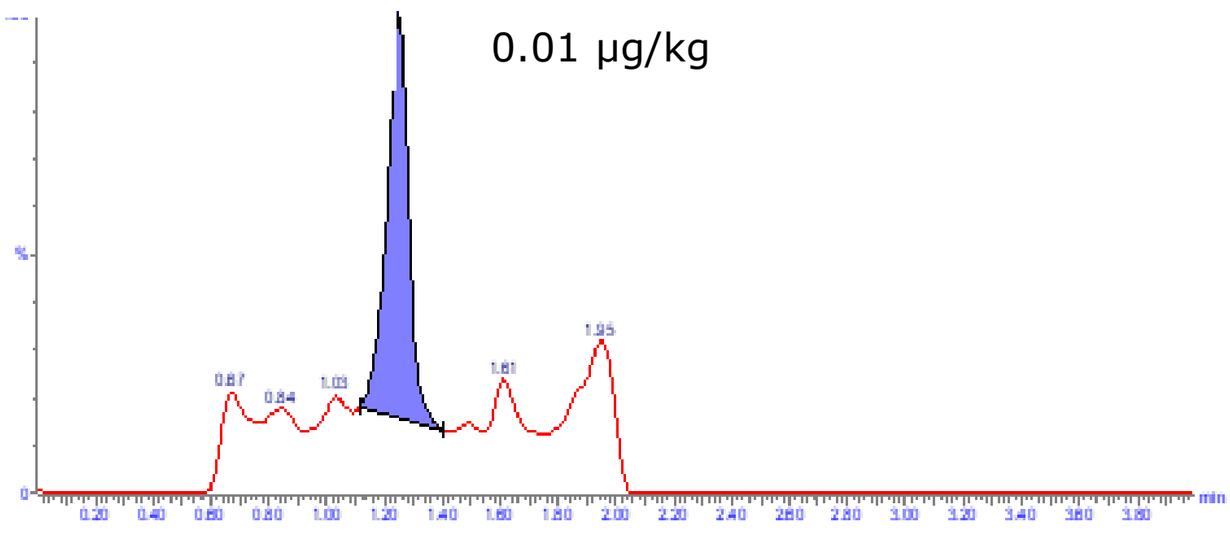
| | |
|---|---|
| LC System: | ACQUITY UPLC I-Class Plus (FL SM) |
| Column: | ACQUITY UPLC BEH C18 (2.1 x 20 100mm) |
| Mobile Phase A: | 0.1% Formic acid (*aq*) |
| Mobile Phase B: | Methanol |
| Injection volume: | 4 µL |
| Column temp: | 40°C |
| MS system: | Xevo TQ-XS |
| Ionization: | Electrospray |
| Polarity: | Positive ion mode |
| MRMs (CE): | *m/z* 302.2 > 164.1 (14 eV) |
| | *m/z* 302.2 > 121.0 (22 eV) |
| Cone voltage: | 35V |

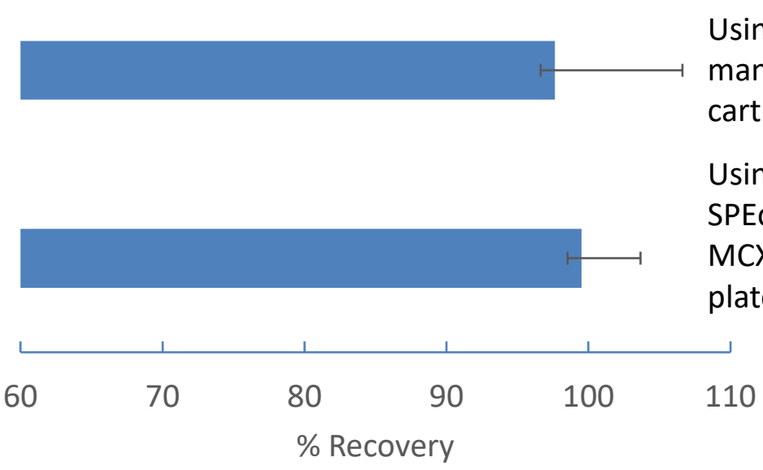
More details are presented in our app note (see QR code below)

## RESULTS AND DISCUSSION

Chromatograms for ractopamine spiked in porcine liver at 0.10 and 0.01 µg/kg




The recovery and repeatability for ractopamine from analysis of six replicates spiked porcine liver samples (0.1 µg/kg) using the standard SPE method was compared with that provided by the Otto SPEcialist positive semi-automated positive pressure manifold (PPM) with Oasis MCX 96 well plates. The mean values for recovery were similar but the repeatability was significantly improved using the PMM system.

## CONCLUSIONS

- The use of OTTO SPEcialist, a positive pressure manifold, with Oasis MCX 96-well plate is very effective for clean-up and enrichment of methanolic extracts of porcine liver samples prior to LCMS/MS determination of ractopamine at 0.1 µg/kg
- Compared to using Oasis MCX SPE cartridges on a vacuum manifold, the use of OTTO SPEcialist with plates provides faster, more repeatable results
- Significant cost savings were achieved through a reduction in volume of solvent and the elimination of consumables such as 0.2 µm filters and centrifuge tubes for sample enrichment

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