

Improved SPE for the Analysis of Beta-Agonist Residues from Animal Tissue

Application Note

Food Testing and Agriculture

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Introduction

The capability to test for residues of veterinary drugs in animals is vital for the food industry. Food manufacturers are required to demonstrate the compliance of their products with stringent customer specifications. In addition, legislative requirements are becoming increasingly demanding, both in terms of the range of substances covered and the lower residue levels to which test systems must measure.

The objective of this application is to improve the sample preparation of growth promoting beta-agonists from vitreous humor and retina extracts of pigs. The preparation is based on solid phase extraction (SPE) using Bond Elut Plexa PCX, a polymeric sorbent with strong cation-exchange functionality.

The mixed-mode Plexa PCX sorbent concentrates the basic beta-agonists and eliminates neutral and acidic interferences. The improved cleanliness offers greater sensitivity and improves analytical recovery. The extracted analytes are clenbuterol, clenproperol, cimbuterol, cyclohexyl-butero, brombuterol, tulobuterol, fenoterol, propanolol, terbutalin and clenbuterol D9 (IS). The measured concentration is 1000 ppm, 20 ppm, 0.8 ppm, and 0.04 ppm. Samples are analyzed with LC/MS/MS.



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Sample Preparation

When extracting beta-agonists from vitreous humor and retina extracts of pigs, 0.1 N HCl has been proven to work most effectively. However, under these strong acidic conditions the beta-agonists are protonated and are not retained well on reversed-phase SPE sorbents.

To improve recovery on reversed-phase SPE, the pH of the acidic sample extract has to be adjusted to basic conditions. This pH adjustment is rather time consuming (~ 2 h for 20 samples) and the recoveries remain below 90%. High SPE recoveries using Bond Elut Plexa PCX, does not require pH adjustment and offers considerable time savings.

The polymeric sorbent combines reversed-phase with strong cation-exchange functionalities and binds the basic analytes under acid conditions. Recoveries for many compounds can be improved to > 90%. In addition, Bond Elut Plexa PCX reduces ion suppression because its highly polar, hydroxylated polymer surface is entirely amide-free and does not provide binding sites for macromolecules. As a result of this unique SPE particle surface, the binding of proteins and lipids can be minimized.

- Apply 20 mL of 0.1 N HCl to 2 g of blank vitreous humor and retina sample
- Spike with adequate standard solutions and internal standard (0.04 ppm, 0.8 ppm, 20 ppm, 1000 ppm)
- Ultrasonic mix for 10 min
- Centrifuge at 10 °C and 12000 rpm
- Apply supernatant to SPE cartridge

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SPE Method

- Bond Elut Plexa PCX 200 mg in 6 mL tube (p/n 12108206)
- Condition cartridge with 3 mL CH₃OH, 3 mL H₂O
- Load sample
- Wash with 3 mL H₂O
- Wash with 3 mL CH₃OH
- Dry the cartridge for about 5 min
- Elute with 5 mL CH₃OH/ 5% NH₃ (33%)

All samples are evaporated to dryness and reconstituted in 500 µL mobile phase. Inject 25 µL to LC/MS/MS.

Conclusion

The particular advantage of this method is the use of the combination of the chemical properties of mixed-mode Bond Elut Plexa PCX. A fast, simple extraction and clean-up procedure can be used, which saves time and improves clean-up effectiveness with respect to higher sensitivity and analyte recovery.

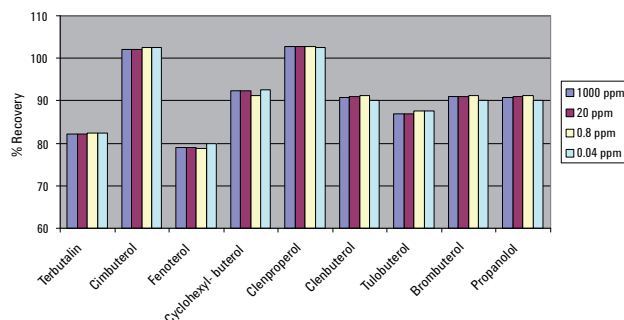


Figure 1. Recoveries of spiked beta-agonists from vitreous humor and retina extracts of pigs. SPE clean-up with Bond Elut Plexa PCX. Samples are analyzed with LC/MS/MS.

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