Improved Solid Phase Extraction for the LC/MS/MS Analysis of Beta-Agonist Residues from Animal Tissue

Dr. Antoni Rubies[1], Elisabeth Korte[2]
[1]Laboratori de l'Agencia de Salut Publica de Barcelona, [2]Varian Deutschland GmbH

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 newly developed 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 Cenbuterol, Clenproperol, Cimbuterol, Cyclohexylclenbuterol, Brombuterol, Tulobuterol, Fenoterol, Propanolol, Terbutaline 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.

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.

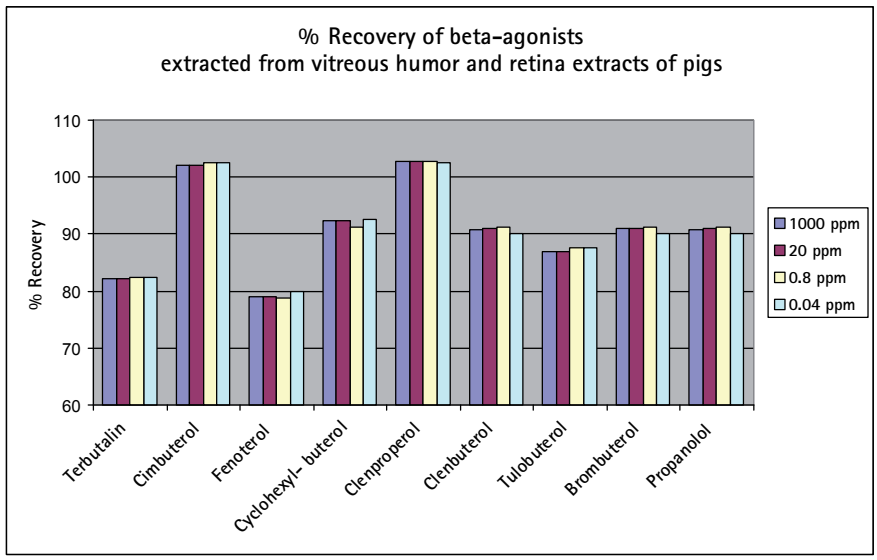

Table 1. Recoveries of spiked beta-agonists from vitreous humor and retina extracts of pigs. SPE clean up with Bond Elut Plexa PCX. Samples are analyzed with LC/MS/MS.

Sample Preparation

Apply 20 mL of 0.1 N HCl to 2 g of blanc 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 mins.
Centrifuge at 10 °C with 12000 rpm.
Apply supernatant to SPE cartridge.

SPE Method

Bond Elut Plexa™ PCX 200 mg in 6 mL tube
(Varian part # 12108206).
Condition cartridge: 3 mL CH_3OH, 3mL H_2O
Load sample.
Wash 3 mL H_2O
Wash 3 mL CH_3OH
Dry the cartridge for about 5 min.
Elute: 5 mL CH_3OH/ 5% NH_3 (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 the 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.

These data represent typical results.
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