SCF 521

Extraction of β-agonists from Bovine Liver Tissue

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Introduction

β-agonists are synthetic derivatives of naturally occurring compounds, such as adrenaline and noradrenalin. Clenbuterol is the only β-agonist licensed for the therapeutic treatment of respiratory conditions in animals. At multiples of the recommended therapeutic dose, β-agonists act as so-called repartitioning agents, the net results of which is the production of a leaner animal carcass. They have been widely used throughout Europe as illegal growth promoters in beef and veal production following the ban on anabolic agents in the mid-1980s.

Traditionally, β-agonists are extracted by methods such liquid to liquid extraction, matrix solid phase dispersion, and immunoaffinity chromatography, all of which are time consuming and require the use of toxic organic solvents. SFE is an alternative technique using supercritical carbon dioxide to extract β-agonists. SFE eliminates the use, exposure to, and disposal of hazardous solvents, while providing comparable extraction results to standard methods in less time.

This application describes a SFE method for the extraction of the principle β–agonist, clenbuterol, from beef liver. The optimized procedure involves a combination of SFE with enzyme immunoassay. The developed procedure is applicable to the determination of clenbuterol in liver samples from treated cattle.

Equipment

- ✓ Applied Separations' *Spe-ed*™SFE-2 or Helix Supercritical Extraction System

Materials

- ✓ *Spe-ed* Matrix (Cat. #7950)
- ✓ *Spe-ed* Wool (Cat. #7953)
- ✓ Carbon dioxide –SFE Grade

Method

Homogenize 1.5 g of fresh bovine liver in a blender. Add 2.0 g of *Spe-ed* Matrix to sample and mix for 30 sec. Prepare SFE vessel by first placing in a plug of *Spe-ed* Wool followed by the tissue-*Spe-ed* Matrix mixture. On top of the sample place another plug of *Spe-ed* Wool, then 3.5 g of *Spe-ed* Matrix, and top vessel with a plug of *Spe-ed* Wool. Use a stainless steel tamping rod to tightly pack the vessel, seal it, and then store extraction vessel at 4°C until ready to perform extraction. Before commencing with extraction, attach a 6 mL SPE column containing 2 g of neutral alumina to the micrometering valve.

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Extraction Conditions

Extraction vessel:	24 mL
Sample:	1.5 g
Pressure:	690 BAR
Temperature:	100ºC
Valve temperature:	120ºC
CO_2 Flow Rate:	2 L/min (gas)
Collection:	60 mL pre-weighed vial
Dynamic time:	40 minutes

Analyte Recovery

When the extraction sequence is complete, remove the SPE alumina column and elute with 4 mL of methanol/water (70+30).

Post-SFE Clean-up

Evaporate a fraction (1/5 or 1/10) of the elute under a gentle stream of nitrogen at 60 C and reconstitute in assay buffer. Analyze by enzyme immunoassay.

Conclusion

The supercritical carbon dioxide extraction of clenbuterol from liver samples of treated cattle offers a viable alternative to solvent-based procedures. The SFE method is quantitative and requires no post extraction clean up. The SFE method is also validated down to the MRL for clenbuternol of 0.5 ng per gram of fresh tissue.

References

M.J. O'Keeffe, M. O'Keeffe, J.D. Glennon, A.R. Lightfield, R. J. Maxwell, Supercritical fluid extraction of clenbuterol from bovine liver tissue, The Analyst, 123, (1998) 2711-2714.

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