

Parr M K¹, Blokland M H², Liebetrau F¹, Schmidt A H^{1,3}, Schänzer W⁴, Sterk S S²

Enantiomeric separation of clenbuterol as analytical strategy to distinguish abuse from meat contamination

Institute of Pharmacy, Freie Universität Berlin, Berlin, Germany¹; RIKILT Wageningen UR - Institute for Food Safety, Wageningen, Netherlands²; Steiner & Co., Deutsche Arzneimittel GmbH & Co.KG, Berlin, Germany³; Institute of Biochemistry, German Sport University, Cologne, Germany⁴

Abstract

Clenbuterol is a well known beta-agonist proven to be abused in animal husbandry and sports due to its anabolic properties. Smith et al. [1] reported that the racemic mixture of clenbuterol from preparations is enantiomerically enriched in meat due to differences in pharmacokinetics and pharmacodynamics in livestock. Thus, the ratio of enantiomers may be used in doping control to discriminate between the prohibited administration of preparations or (unintentional) ingestion of contaminated meat. The use of high-performance liquid chromatography (HPLC, Waters Aquity UPLC H-class) and supercritical fluid chromatography (SFC, Waters Aquity UPC²) is compared with respect to the analysis of the enantiomeric composition of clenbuterol residues in biological specimen at ultra-trace levels. Coupled to the same triple quadrupole mass analyzer (Waters TQD), both techniques, HPLC-MS/MS and SFC-MS/MS, allow for separation of the two enantiomers of clenbuterol on a vancomycin based column. Method comparison and validation of both methods demonstrated that either method is fit for purpose in urine and meat analyses. SFC appeared superior with respect to the speed of the analytical run and peak shape. Liquid-liquid extraction for urine samples or SPE for meat resulted in LOD <2.5 pg/mL in urine and <25 pg/g in meat for each enantiomer.

Meat from a calf feeding experiment (racemic clenbuterol administered before slaughter) was found enriched in R-clenbuterol (area ratio S/R = 0.70±0.02).

Further experiments will be needed to test the applicability to trace back the route of administration in athletes. Therefore a controlled administration of clenbuterol via incurred veal and a controlled administration of racemic clenbuterol from a preparation are planned.

Further details are available from the authors at request and included in a manuscript published soon.

References

[1] Smith, D. J. (2000) Stereochemical composition of clenbuterol residues in edible tissues of swine. *J Agric Food Chem* **48**, 6036-6043.

Acknowledgements

This study is funded by the World Anti-Doping Agency (11A18SS). Steiner & Co., Deutsche Arzneimittel GmbH & Co.KG, Berlin, Germany is acknowledged for the accessibility of the analytical equipment. It has been carried out with the support of Dr. Tobias Wage from Waters GmbH, Eschborn, Germany.