



Application Note



**Determination of beta-agonists compounds in feed using
AFFINIMIP®SPE Beta-Agonists**



INTRODUCTION

This application note has been carried out by Laberca, the French national reference laboratory for different classes of substances such as (growth promoters, Dioxins, PCBs, PAHs, etc.).

Authors:

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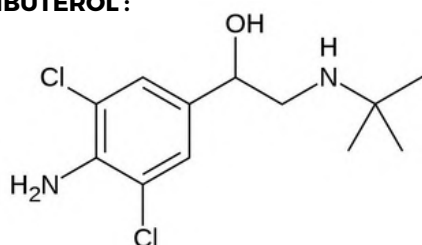
LABERCA Oniris, 101 Route de Gachet, 44307 Nantes, France

CONTEXT

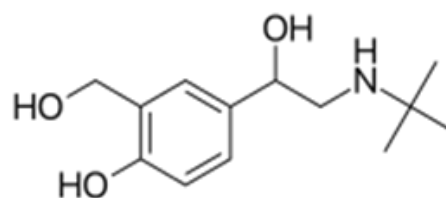
Regulation 2017/625 lays down common rules for official controls in the European Union (EU) to ensure compliance with and proper enforcement of legislation in the agri-food chain in order to protect human health, animal health and welfare and plant health. Beta-agonists belong to a family of chemical substances derived from catecholamines and which act on production animals by increasing their muscle mass while decreasing the amount of adipose tissue. As such, their use is strictly prohibited in the EU. Their control requires the implementation of effective and efficient analytical protocols.

Tested molecules : beta agonists (salbutamol, zilpaterol, ractopamine, clenbuterol)

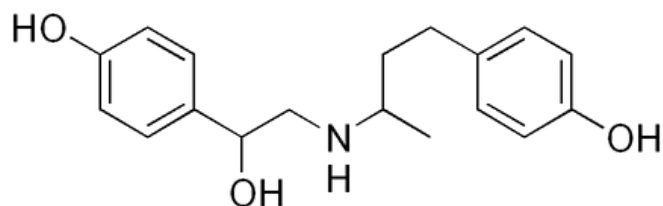
CLENBUTEROL :



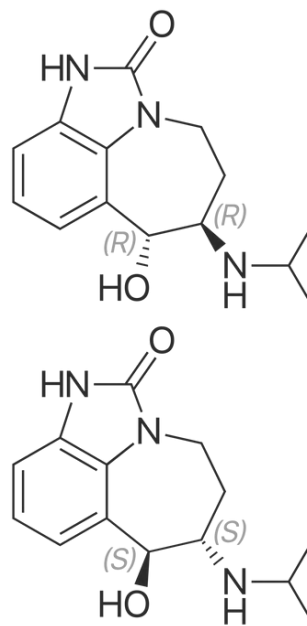
SALBUTAMOL :



RACTOPAMINE :



ZILPATEROL :



ANALYTICAL STRATEGY [1*]

Sample: 2g of animal feed

Solid/Liquid extraction
Methanol/acetate buffer
(0.2M), pH=5.2

Liquid/Liquid extraction
N-pentane

Filtration 0.45µm

**Extraction with
solid phase MIP**

**UPLC-MS/MS
(ESI+/Mode MRM)**

[1*] L. Herpin,*, E. Bichon, L. Rambaud, F. Monteau, B. Le Bizec, Comparison between liquid chromatography and supercritical fluid chromatography coupled to mass spectrometry for beta-agonists screening in feeding stuff; Journal of Chromatography B 1086 (2018) 130–137 - <https://doi.org/10.1016/j.jchromb.2018.04.023>

ANALYTICAL TECHNIQUE

UHPLC-MS/MS Waters Xevo TQ-S (ESI+)

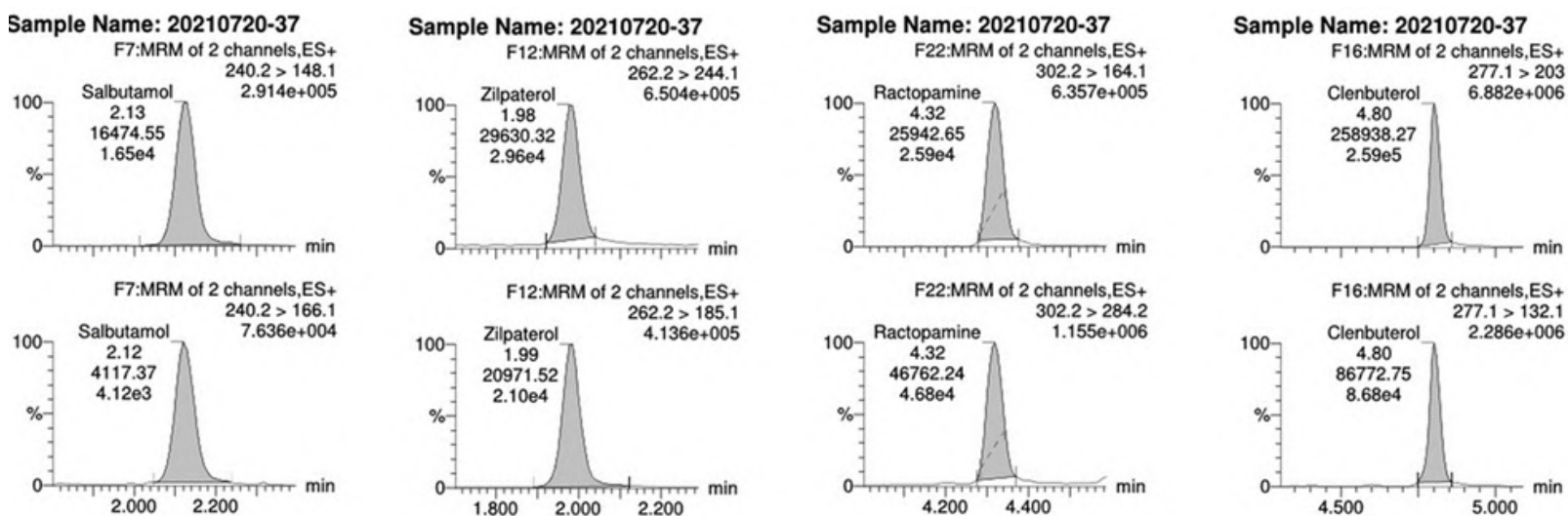
UHPLC column : Thermo Scientific Hypersil Gold C18, 100x2.1mmx1.9µm

Solvents : water + 0.1 % Formic Acid, Acetonitrile + 0.1 % Formic Acid

Target molecule	CAS Number	Transition 1	Transition 2	Internal Standard and diagnostic transition
Salbutamol	18559-94-9	240.2>148.1	240.2>166.1	Salbutamol-d6 (246.2>148.1)
Zilpaterol	119520-05-7	262.2>244.2	262.2>185.1	Salbutamol-d6 (246.2>148.1)
Ractopamine	97825-25-7	302.3>164.1	302.3>284.2	Ractopamine-d6 (308.2>168.1)
Clenbuterol	37148-27-9	277.2>203.2	277.2>132.1	Clenbuterol-d6 (283.2>204.2)

Example of extracted ion chromatograms:

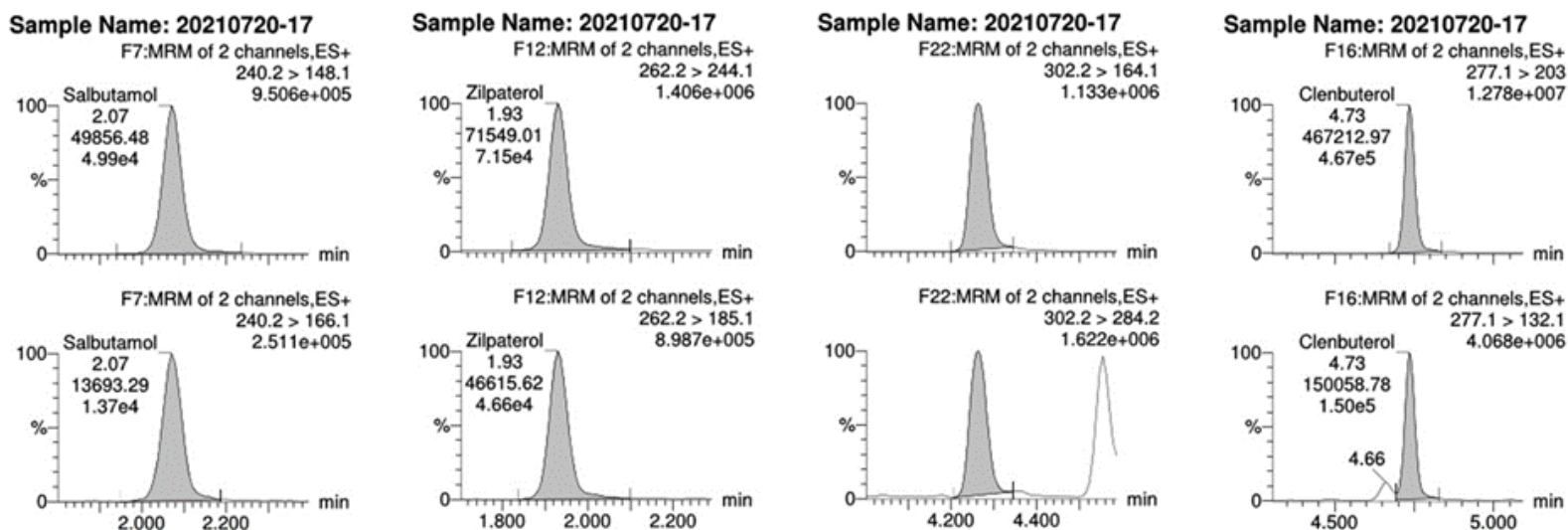
animal feed spiked with 10 µg/kg (test intake: 2 g.). MIP Affinisep.



ANALYTICAL TECHNIQUE

Example of extracted ion chromatograms:

animal feed spiked with 10 µg/kg (test intake: 5 g.). MIP Biotage.



EXPECTED PERFORMANCE

"EURL Guidance on minimum method performance requirements (MMPRs) for specific pharmacologically active substances in specific animal matrices", i.e. MMPR: 50 µg/kg.

The level of supplementation shown on the chromatograms presented is therefore 5 times lower than the proposed MMPR. The results obtained are therefore in line with the performances to be achieved for these 4 molecules in feed for production animals, both for the use of the MIP "Biotage" and "Affinisep".

In terms of applicability, the Affinisep MIPs show a more regular and easier flow, thus allowing the duration of the purification stage to be reduced.

Our solution AFFINIMIP® Beta-Agonists



Product references :

AFFINIMIP®SPE BETA-AGONISTS

- **DG104-03 for AFFINIMIP®SPE Beta-Agonists - 3mL - 50/pk**
- **DG104-03LRC1 for AFFINIMIP®SPE Beta-Agonists - LRC 10mL - 50/pk**