Application Note: 481

Study of β-agonist Residues in Animal-derived Foods by LC-MS/MS

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Key Words

- Food residue analysis
- Veterinary drugs
- TSQ Quantum
- SRM (Selected Reaction Monitoring)

Introduction

Beta-adrenoceptor agonists (β-agonists) are bronchodilators that open air passages by relaxing the tightened muscles surrounding the bronchial tubes. These drugs are commonly prescribed to treat pulmonary diseases such as asthma, emphysema, and bronchitis. Additionally, at higher doses β-agonist drugs have anabolic effects and are very attractive as growth promoters and repartitioning agents in the livestock industry. Due to the potential health risks, β-agonists have been banned for use as growth promoters in livestock in the European Union.[1] Regulatory agencies require highly sensitive and specific analysis methods to ensure that β-agonist residues are not present in animal-derived foods.

In this application note, a sensitive LC-MS/MS method has been developed to detect β-agonist drugs in animal-derived foods including liver, pork, milk, and eggs. Ten β-agonists were identified: cimaterol, clenbuterol, clorprenaline hydrochloride, fenoterol, pentubutolol, propranolol, ractopamine, salbutamol, terbutaline, and tulobuterol.

Goal

To develop a sensitive and reproducible LC-MS/MS method to detect ten β-agonists drugs in animal-derived foods.

Experimental Conditions

Sample Preparation

A 2 g animal-derived tissue sample was weighed into a 50 mL centrifuge tube and 10 mL of 0.2 mol/L ammonium acetate (pH 5.2) was added. Then, 40 µL of β-glucosidase/arylsulfatase was added to digest the β-agonist drug residue. The mixture was vortexed for 3 minutes and incubated in a 37 °C water bath for 16 hours in the dark.

After incubation, the hydrolyzed sample was cooled to room temperature, vortexed for 3 minutes, and then centrifuged at 10,000 rpm for 10 minutes. The supernatant was transferred to another 50 mL centrifuge tube and 1 mL of 1 mol/L perchloric acid was added. The solution was centrifuged at 4000 rpm for 10 minutes. The supernatant was loaded to the solid phase extraction (SPE) column, which was previously conditioned with 5 mL of methanol. After drying, the analytes were eluted with 5 mL of 5% ammonium hydroxide methanol solution and evaporated to dryness under nitrogen at 40 °C. The residues were reconstituted in 1.0 mL of 20% acetonitrile aqueous solution. The resulting solutions were vortexed for 1 minute and then centrifuged at 10,000 rpm for 10 minutes. The upper clear solutions were transferred to another sample vial for LC-MS/MS analysis.

LC

HPLC analysis was performed using the Thermo Scientific Surveyor HPLC system. Each 10 µL of sample was injected onto a Thermo Scientific Hypersil GOLD column (150 mm x 2.1 mm, 5 µm). A gradient LC method used mobile phases A (5 mM ammonium acetate) and B (methanol) at a flow rate of 250 µL/min. Table 1 illustrates the gradient LC method.

Table 1. Gradient details

Retention Time (min)	A (%)	B (%)
0	90	10
0.5	90	10
5	10	90
10	10	90
10.1	90	10
12	90	10

MS

MS analysis was carried out on a Thermo Scientific TSQ Quantum triple stage quadrupole mass spectrometer with an electrospray ionization (ESI) probe. The MS conditions were as follows:

Ion source polarity:	Positive ion mode
Spray voltage:	4500 V
Sheath gas pressure (N_2):	30 units
Auxiliary gas pressure (N_2):	5 units
Capillary temperature:	350 °C
Collision gas pressure (Ar):	1.5 mTorr

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The SRM transitions that were monitored are summarized in Table 2.

Table 2. SRM transitions

Drug	Parent Ion *(m/z)*	Product Ions *(m/z)* [Collision Energy (V)]
Cimaterol	220	143 [22], 202 [10], 160 [18]
Clenbuterol	277	132 [27], 168 [26], 203 [16], 259 [8]
Clorprenaline hydrochloride	214.1	119.1 [27], 154 [17]
Fenoterol	304.1	107.1 [30], 135.1 [18]
Pentubutolol	292.2	201 [21], 236.2 [16]
Propranolol	260.2	183 [18], 116.2 [18]
Ractopamine	302	121 [20], 164 [14], 284 [8]
Salbutamol	240	148 [18], 166 [14], 222 [11]
Terbutaline	226	107 [31], 152 [35], 152 [15]
Tulobuterol	228.1	119 [28], 154 [16], 172 [14]

Results and Discussion

Figure 1 displays the SRM chromatograms for the ten β-agonists at 1 ng/mL. The limits of quantitation (LOQ) for clenbuterol, clorprenaline hydrochloride, fenoterol, pentubutolol, propranolol, ractopamine, salbutamol, and tulobuterol are 0.1 µg/kg in the liver, pork, milk, and egg samples. The LOQs for cimaterol and terbutaline are 0.5 µg/kg.

The extraction recovery rates of the β-agonist drugs are between 75% and 120%, which achieve the minimum detection requirements. Thus, the qualification method is accurate and reproducible.

Conclusion

This LC-MS/MS method is able to detect β-agonists drugs in animal-derived foods. The method yielded high recovery rates and enabled the accurate quantification of the residues. The sensitivity, extraction recovery, and reproducibility of this method meet international regulation and detection requirements.

References

1. European Community, Council Directive 96/22/EC concerning the prohibition on the use in stockfarming of certain substances having a hormonal or thyrostatic action and of ß-agonists, (Official Journal of the European Communities Official Journal L 125, 23 May **1996**, pp. 3-9).

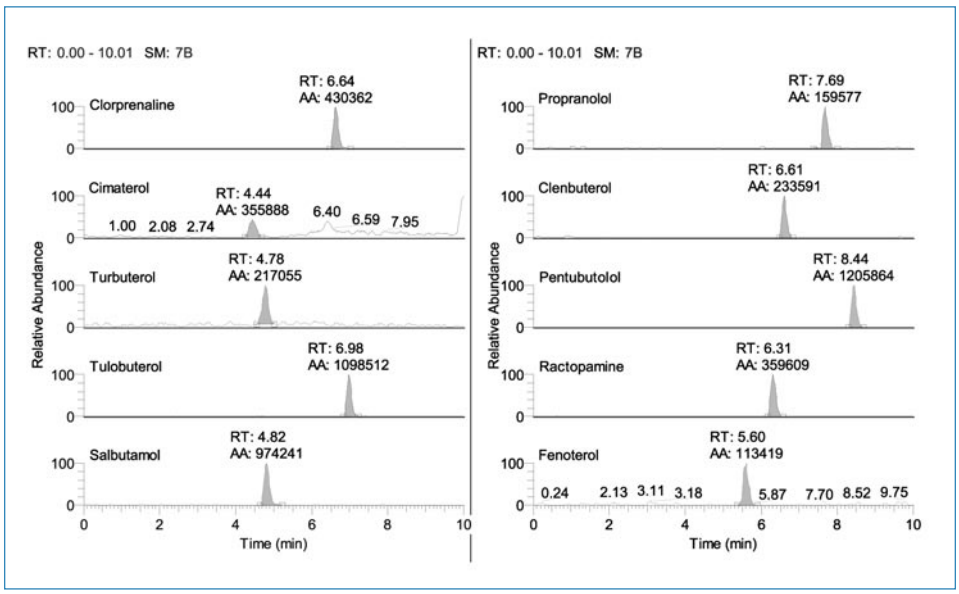

Figure 1. SRM chromatograms for the ten β-agonists at 1 ng/mL

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