Analysis of 10 β-Agonists in Pork Meat using Automated Dispersive Pipette Extraction and LC-MS/MS

HIGHLIGHTS: Reduced sample volumes, fast extraction times

SCX - XTR

INTRODUCTION

β-Adrenergic agonists are synthetic phenylethanolamine compounds used as bronchodilators and tocolytic agents. In many countries, including European Union countries (1) and China (2), these drugs have been banned due to their adverse effects on humans, such as food poisoning (3), cardiovascular and central nervous diseases (4). Regulations have either not set a maximum residue limit or have a zero-tolerance level for these compounds. Consequently, highly sensitive analyses are essential to support the enforcement of laws and regulations focusing on the detection and identification of these residues in animals via their biological matrices, animal products, feed and drinking water (1). Of all these samples, meat is a main source of consumer exposure to β-adrenergic agonists. Pork is the most widely eaten meat accounting for over 36% of the world meat intake (5). Therefore, sensitive methods for the analysis of β-adrenergic agonists in pork is a main focus in European and Chinese laboratories including industry, import regulation, and local quality control agencies.

Dispersive Pipette XTRaction is a patented technology that introduces the benefits of solid phase extraction in a simple to use pipette tip. Loose sorbent material is contained within a tip and the extraction occurs through aspirate and dispense steps. The purpose of this study was to develop a rapid multi-residue method that minimizes sample volume using automated dispersive pipette extraction to obtain sensitive quantitation of 10 β-agonists (cimaterol, terbutaline, salbutamol, isoxsuprine, ractopamine, cimbuterol, clenbuterol, brombuterol, mabuterol and mapenterol) in pork meat using LC-MS/MS analysis.

INSTRUMENTATION AND METHODS

Reagents and Standards

Ractopamine HCl, Isoxsuprine HCl, Clenbuterol HCl, and Cimbuterol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Terbutaline was purchased from Abcam (Cambridge, MA, USA). Cimaterol was purchased from Cayman Chemical Company (Ann Arbor, MI, USA), Salbutamol was purchased from VWR (Atlanta, GA, USA). Mapenterol, Mabuterol, and Brombuterol HCl were purchased from TRC-Canada (Toronto, Ontario, Canada). Internal standards Clenbuterol-d9 and Terbutaline-d3 were purchased from CDN Isotopes (Pointe-Claire, Quebec, Canada). XTR tips containing strong cation exchange sorbent (SCX) were purchased from DPX Technologies, LLC (Columbia, SC, USA).

All sample preparation was performed on a Hamilton Microlab Nimbus96

Instrumental Analysis

Analyses were performed using a Thermo TSQ Vantage™ triple quadrupole mass spectrometer (Milwaukee, WI) coupled to an Agilent 1260 Series HPLC (Agilent Technologies, Santa Clara, CA) equipped with an Agilent Poroshell EC-C18 column (3.0 × 50 mm, 2.7 µm) with column temperature held at 35°C and a 20 µL sample injection volume. The mobile phase was composed of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The gradient started at 4% B, ramped to 8% B at 2 minutes, and 40% B at 6.0 minutes, after which the composition was ramped to 95% B at 7.5 minutes, where it remained until 8.5 minutes and was re-equilibrated to 4% B. The total run time was 9.6 minutes. The column flow rate was 0.5 mL/minute. Mass spectrometer parameters were: electrospray voltage, 4000V; auxiliary gas pressure, 8 psi; sheath gas pressure, 30 psi; vaporizer temperature was 300 °C; capillary temperature was 300 °C

Sample Preparation

The selected 10 β-adrenergic agonists all contain amino groups with pKa's above 9. This structural property allows for high recoveries during cation exchange sample preparation. Multiple wash steps help to minimize matrix effects by removing interferents prior to the analyte elution. The dispersive mechanism provides highly efficient interaction promoting smaller amounts of sorbent and sample resulting in faster extraction times. Extraction was performed on a Hamilton NIMBUS96, allowing extraction of 96 samples in under 20 minutes. The pork matrix was spiked to prepare a calibration curve ranging from 0.2 ng/g to 50 ng/g.

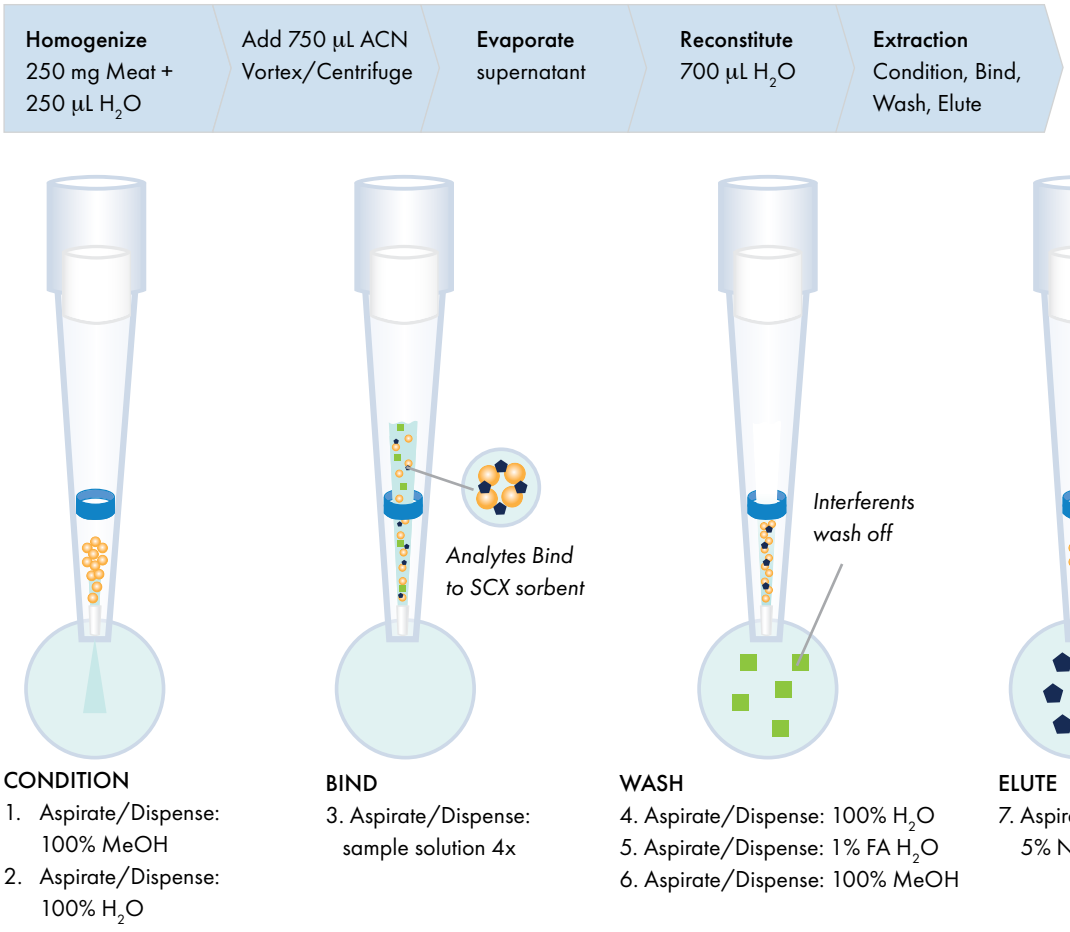

Figure 1. Sample pretreatment and Dispersive Pipette XTRaction protocol. After elution the solution is solvent evaporated and reconstituted in 100 µL of 5% methoanol, 0.1% formic acid in water

RESULTS AND DISCUSSION

A representative chromatogram for the separation of the 10 β-agonists is shown in Figure 2. The described method led to linear regression with a 1/x weighting factor and coefficients of determinations (R^2) over 0.99 for each β-agonist. The extraction process is rapid, minimizes matrix effects (<25%), and maximizes recoveries (>85%) as shown in Figure 3. The average within-run precision had a maximum of 11.8% at 5 ng/mL of isoxsuprine, and the between-run precision had a maximum at 0.5 ng/mL of bromobuterol at 21.0%. We believe the time between meat homogenization and extraction as well as the limited capacity of the solvent evaporator being used caused higher than expected values of within and between batch irreproducibility. These precision values adversely affected the LOD and LOQ calculations because the average slope and y-intercept standard deviation values were used to determine the LODs and LOQs for each compound as shown above (Table 1). Nevertheless, LODs and LOQs were calculated to be below 0.7 ng/g and 2.2 ng/g, respectively. Lower LODs and LOQs should be readily achievable for laboratories routinely performing these analyses.

Compound	Within Run Precision			Between Run Precision			LOD	LOQ
	0.5	5	10	0.5	5	10		
Terbutaline	4.2	4.5	2.4	5.1	6.6	3.1	0.3	0.8
Cimaterol	4.5	4.4	2.2	8.7	6.2	2.2	0.5	1.6
Salbutamol	4.3	8.5	4.0	14	11	12	0.2	0.6
Cimbuterol	6.6	6.4	3.5	14	12	5.6	0.4	1.1
Ractopamine	6.0	3.0	4.3	14	4.7	4.5	0.2	0.7
Clenbuterol	4.5	2.8	4.8	16	7.4	4.7	0.4	1.3
Bromobuterol	3.6	11	6.2	21	17	12	0.2	0.7
Isoxsuprine	5.3	12	3.3	16	21	2.9	0.7	2.2
Mabuterol	5.1	1.7	2.4	15	1.7	4.7	0.2	0.7
Mapenterol	5.6	4.6	2.2	12	5.4	9.9	0.3	0.8

Table 1. The within and between run precision (%) at three different concentrations (0.5, 5, and 10 ng/g) and the limit of detection (LOD) and quantitation (LOQ) in ng/mL for each β-agonist.

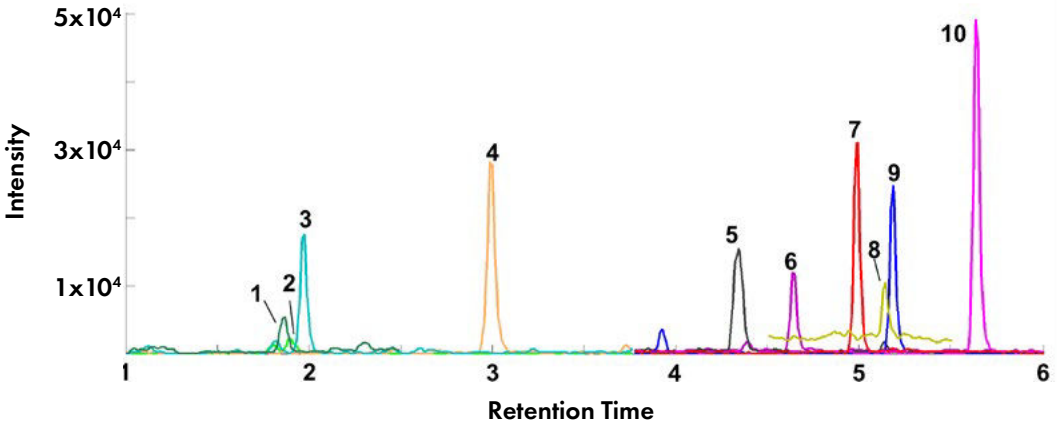

Figure 2. Extracted ion chromatogram of each β-agonist at 1 ng/g in pork meat. 1-Terbutaline, 2-Cimaterol, 3- Salbutamol, 4-Cimbuterol, 5- Ractopamine, 6- Clenbuterol, 7- Bromobuterol, 8- Isoxsuprine, 9-Mabuterol, 10-Mapenterol.

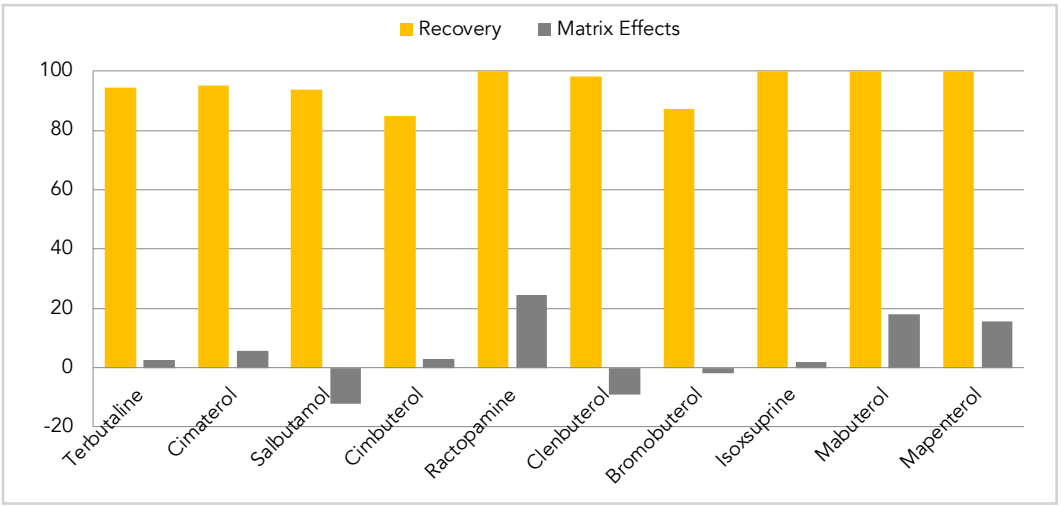

Figure 3. The percent recovery and matrix effects (ion suppression/ion enhancement) for the 10 β-agonists.

CONCLUSIONS

A rapid, environmentally friendly and reliable method has been developed using an automated dispersive pipette extraction followed by LC–MS/MS for sensitive quantitation of 10 β-agonists in pork meat. The benefits of this method include minimal sample volume as well as simplified sample pretreatment using DPX tips on an automated platform. The method is suitable for high-throughput monitoring of 10 β-agonists in pork meat and could be used routinely as a tool for regulatory compliance control in restricted markets like select European countries and China.

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