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Analysis of β-agonists in different biological matrices by liquid chromatography–tandem mass spectrometry

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Abstract

Introduction: Wide use is made of β-agonists in therapy due to their smooth muscle–relaxant properties. They also have a side effect of increasing muscle mass. Besides improving oxygen utilisation as bronchodilators, β-agonists increase protein synthesis and promote fat burning. The growth- and performance-enhancing effects are often exploited in illegal use. The guiding objective of this study was to develop a procedure for the determination of β-agonists by a single method in different types of matrices. **Material and Methods:** Five grams of homogenised samples were subjected to enzymatic hydrolysis with β-glucuronidase in ammonium acetate, pH 5.2. Purification was performed by solid phase extraction. Analytes were eluted with 10% acetic acid in methanol. The eluted β-agonists were analysed by high-performance liquid chromatography–tandem mass spectrometry. **Results:** Validation results met the requirement of the confirmation criteria according to European Commission Decision 2002/657/EC in terms of apparent recoveries (93.2–112.0%), repeatability (3.1–7.1%) and intra-laboratory reproducibility (4.1–8.2%). **Conclusion:** The method can be successfully applied in the detection and determination of clenbuterol, salbutamol, mabuterol, mapenterol, terbutaline, brombuterol, zilpaterol, isoxsuprine and ractopamine in feed, drinking water, urine, muscle, lung and liver matrices.

Keywords: β-agonists, residues, feed, food products, HPLC-MS/MS.

Introduction

Beta-agonists are a large group of chemical compounds with a common pharmacological effect of stimulating β-receptors, mainly in the bronchi (selective β-receptor agonists). Among these substances, three groups can be distinguished: aniline derivatives (clenbuterol, brombuterol, mabuterol and mapenterol), phenol derivatives (salbutamol, isoxsuprine and ractopamine) and resorcinol derivatives (terbutaline) (8). Some of these substances, are used in the treatment of bronchial asthma due to their spasmolytic properties. A side effect of prolonged use of β-agonists is muscle mass gain. These β-agonist actions can be illegally used in humans and animals to accelerate growth and increase muscle mass. In addition, regardless of the degree of selectivity, these drugs cause heart rate acceleration by stimulation of β2-receptors in the myocardium and blood vessels, which can cause cardiac arrhythmias, the appearance of sudden ventricular contractions causing loss of consciousness, and at higher doses even death (9, 19, 22, 24, 26, 28).

Due to adverse side effects, these compounds are not allowed to be used in slaughter animals (6). In Poland, monitoring of β-agonist residues in food products of animal origin is carried out based on the currently applicable European Community and internal regulations, and as part of a comprehensive program of chemical, biological and veterinary drug residue surveillance (6, 10, 11).

Residues of β-agonist substances are mainly detected and confirmed with gas and liquid chromatography methods, typically high-performance liquid chromatography, with tandem mass spectrometry (HPLC-MS/MS) (1, 3, 7, 9, 12, 13, 17, 18, 19, 22, 23, 25, 27–30). The aim of this study was to develop a method for the detection and determination of clenbuterol, salbutamol, mabuterol, mapenterol, terbutaline, brombuterol, zilpaterol, isoxsuprine and ractopamine in feed, drinking water and animal tissues from different species, *i.e.* pig, poultry and cattle based on extraction with molecular imprinted polymer (MIP) columns and analysis by liquid chromatography coupled with tandem mass spectrometry.

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Material and Methods

All reagents were of minimum analytical grade or higher. Materials were procured as follows: ammonium acetate and methanol (LC-MS grade) were from J.T. Baker (Deventer, the Netherlands). Formic acid, solid-phase extraction with molecular imprinted polymer (SPE-MIP) cartridges and β-glucoronidase were sourced from Sigma-Aldrich Chemie (Steinheim, Germany). Ultrapure water was filtered through a Milli-Q system manufactured by Millipore (Billerica, MA, USA). Nanosep MF 0.22 µm filters were supplied by Pall (Port Washington, NY, USA). Ammonia was obtained from POCH (Gliwice, Poland). Clenbuterol, salbutamol, mabuterol, mapenterol, terbutaline, bromobuterol, isoxsuprine, zilpaterol, ractopamine and the internal standards clenbuterol-D_9, isoxsuprine-D_5, mabuterol-D_9, mapenterol-D_{11}, ractopamine-D_6, and salbutamol-D_6 were purchased from Witega (Berlin, Germany).

HPLC-MS/MS. The HPLC-MS/MS system consisted of an ExionLC HPLC system connected to an API 5500 Qtrap mass spectrometer (AB Sciex, Concord, Canada). Analyst 1.6.3 software controlled the HPLC-MS/MS system and Multiquant 3.2 was used to process the data (AB Sciex). The MS/MS was operated at the following parameters: desolvation temperature was set at 500°C, gas 1 (air) at 40 psi, gas 2 (air) at 40 psi, collision gas (N_2) at the high setting, nebuliser gas (N_2) at 40 psi, and curtain gas (N_2) at 40 psi. The voltages of the electron multiplier and the electrospray capillary were set at 2,100 and 4,500, respectively. Chromatography was performed in a Kinetex C8(2) column 3 µm × 2 mm, 100 mm connected to a SecurityGuard ULTRA Kinetex C8 precolumn 3 µm × 2 mm, 4 mm (both Phenomenex, Torrance, CA, USA). The mobile phase was composed of two solutions: A (0.1% formic acid) and B (methanol) in a gradient which started with 99% of A and 1% of B. From 0 to 2.0 min, the concentration of B was raised to 20% and left for 1.0 min. After that, the B concentration was raised to 50% and left for 2.0 min. Finally, after 0.5 min, it was decreased to 1% and left for 4 min. The flow rate was 0.4 mL/min. The column operating temperature was maintained at 15°C. The ions were monitored in scheduled multiple reaction monitoring (sMRM) mode with an MRM detection window of 40 s (Table 1).

Table 1. Mass spectrometry parameters for precursor ions and product ion transitions of β-agonists and internal standards

Analyte	Retention time (min)	Precursor ion (m/z)	Ion transition	Declustering potential	Collision energy (eV)
Brombuterol	4.2	366.8	213.9 292.8	56	43 47
Clenbuterol	4.1	276.8	203.0 132.1	76	23 47
Isoxsuprine	4.3	302.2	150.0 107.0	61	31 37
Mabuterol	4.3	310.9	237.0 217.0	20	25 35
Mapenterol	4.5	324.9	237.0 217.0	146	25 33
Salbutamol	3.2	239.9	148.0 166.1	131	25 19
Ractopamine	4.0	301.9	164.1 136.1	16	23 31
Terbutaline	3.2	225.9	152.1 125.1	121	23 35
Zilpaterol	3.1	261.9	185.0 202.0	26	35 27
Clebuterol-D_9	4.1	285.9	204.0	31	25
Isoxsuprine -D_5	4.3	307.2	150.0	61	23
Mabuterol-D_9	4.3	319.9	238.0	76	25
Mapenterol-D_{11}	4.5	335.9	238.0	76	25
Ractopamine-D_6	4.0	308.0	168.1	56	23
Salbutamol-D_6	3.2	248.9	148.0	136	27

Table 2. Concentration levels used for validation of the β-agonist detection method in different matrices

Analyte	drinking water µg kg^{-1}	feed µg kg^{-1}	liver µg kg^{-1}	urine µg kg^{-1}	muscle µg kg^{-1}	lung µg kg^{-1}
Brombuterol	0.1; 0.2; 0.4; 1.6	2.5; 5.0; 10.0; 40.0	0.1; 0.2; 0.4; 1.6	0.1; 0.2; 0.4; 1.6	0.05; 0.1; 0.2; 0.8	0.1; 0.2; 0.4; 1.6
Clenbuterol	0.1; 0.2; 0.4; 1.6	2.5; 5.0; 10.0; 40.0	0.1; 0.2; 0.4; 1.6	0.1; 0.2; 0.4; 1.6	0.05; 0.1; 0.2; 0.8	0.1; 0.2; 0.4; 1.6
Isoxsuprine	0.25; 0.5; 1.0; 4.0	2.5; 5.0; 10.0; 40.0	0.25; 0.5; 1.0; 4.0	0.25; 0.5; 1.0; 4.0	0.25; 0.5; 1.0; 4.0	0.25; 0.5; 1.0; 4.0
Mabuterol	0.1; 0.2; 0.4; 1.6	2.5; 5.0; 10.0; 40.0	0.1; 0.2; 0.4; 1.6	0.1; 0.2; 0.4; 1.6	0.05; 0.1; 0.2; 0.8	0.1; 0.2; 0.4; 1.6
Mapenterol	0.1; 0.2; 0.4; 1.6	2.5; 5.0; 10.0; 40.0	0.1; 0.2; 0.4; 1.6	0.1; 0.2; 0.4; 1.6	0.05; 0.1; 0.2; 0.8	0.1; 0.2; 0.4; 1.6
Salbutamol	2.5; 5.0; 10.0; 40.0	2.5; 5.0; 10.0; 40.0	2.5; 5.0; 10.0; 40.0	0.5; 1.0; 2.0; 8.0	2.5; 5.0; 10.0; 40.0	0.5; 1.0; 2.0; 8.0
Ractopamine	0.5; 1.0; 2.0; 8.0	2.5; 5.0; 10.0; 40.0	0.5; 1.0; 2.0; 8.0	0.5; 1.0; 2.0; 8.0	0.5; 1.0; 2.0; 8.0	0.5; 1.0; 2.0; 8.0
Terbutaline	5.0; 10.0; 20.0; 80.0	2.5; 5.0; 10.0; 40.0	5.0; 10.0; 20.0; 80.0	1.5; 3.0; 6.0; 24.0	5.0; 10.0; 20.0; 80.0	1.5; 3.0; 6.0; 24.0
Zilpaterol	2.5; 5.0; 10.0; 40.0	2.5; 5.0; 10.0; 40.0	2.5; 5.0; 10.0; 40.0	0.5; 1.0; 2.0; 8.0	2.5; 5.0; 10.0; 40.0	0.5; 1.0; 2.0; 8.0

Sample Preparation. Urine, homogenised liver, muscle and lung samples in an amount of 5 ± 0.05 g each were mixed with 100 µL of the working solution of internal standards. A 5 mL volume of 0.05 M of acetate buffer (pH 5.2) and 50 µL of β-glucuronidase were added next, and the solution was mixed for approximately 60 s. The samples were hydrolysed by incubating overnight at 37 ± 3°C. Subsequently, 5 mL of 0.1 M acetate buffer (pH 6.7) was added and adjusted to pH ≈ 6.7 with ammonia or acetic acid. For other matrices (drinking water and feed), 5 ± 0.05 g of sample was mixed with 100 µL of the working solution of internal standards, then 5 mL of 0.1 M acetate buffer (pH 6.7) was added and the solution was mixed for 0.5 min on a vortex mixer at 349 × rcf and centrifuged at 2,930 × rcf for 15 min at 4°C before being transferred to the preconditioned SPE-MIP cartridges.

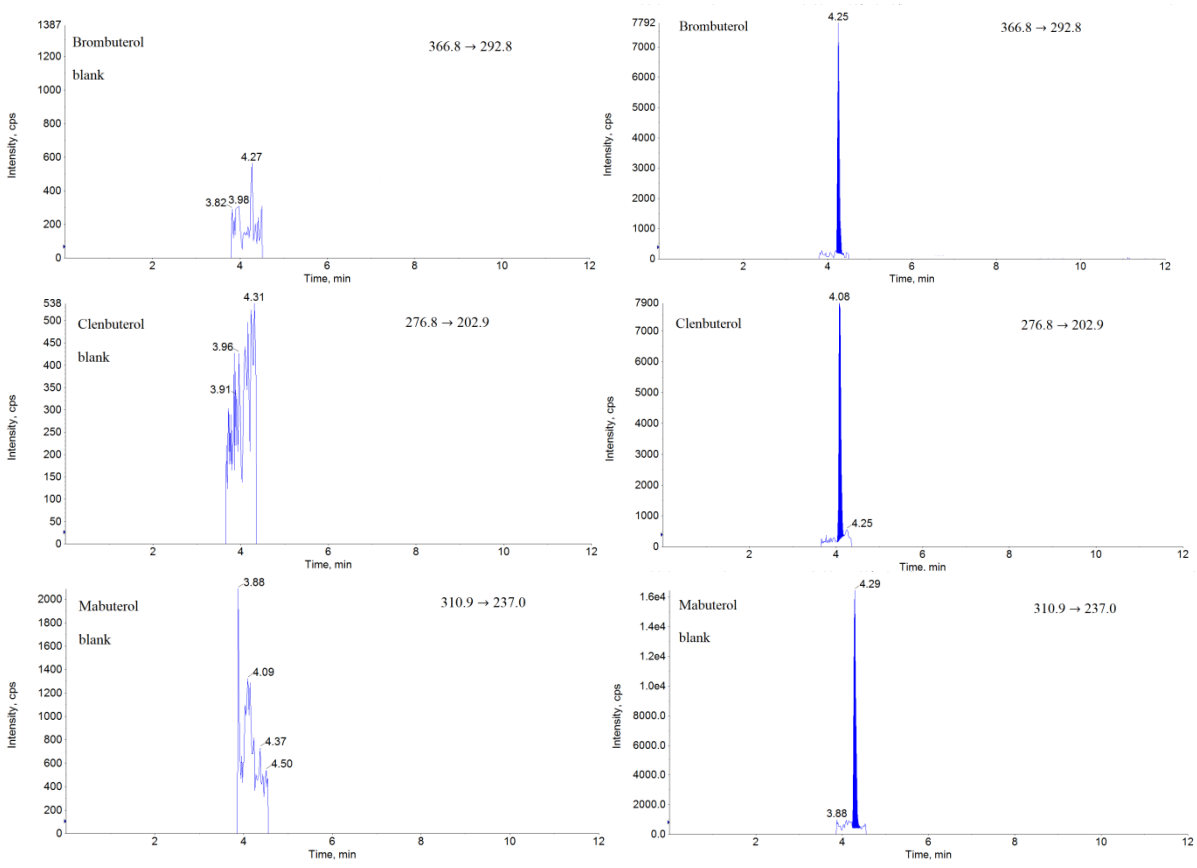
Cleanup. The SPE-MIP cartridges were placed on a vacuum manifold and the cartridges were sequentially conditioned with 1 mL of methanol, 1 mL of water, and 1 mL of acetate buffer. After loading the extract, the cartridges were washed with a mixture of 0.5% acetic acid in acetonitrile, 50 mM of ammonium acetate (pH 6.7), and acetonitrile/water (60/40, v/v). After drying, the substances were eluted with 10% acetic acid in methanol and then evaporated under a stream of nitrogen in a heating block at 45 ± 5°C. After evaporation, the dry residue was dissolved with 200 µL of methanol/water mixture (10/90, *v/v*) and filtered through Nanosep MF 0.22 µm filters before being placed into an autosampler vial.

Validation criteria. The method was validated according to the recommendations of Commission Decision 2002/657/EC (4), in like manner to previously described methods (20, 21). The following statistical parameters of the method were determined for each matrix: selectivity, linearity, repeatability, intra-laboratory reproducibility, limit of decision (CCα) and detection capability (CCβ).

The matrix effect was calculated for each of the compounds and each of the matrices at the second concentration level using the same method as previously described (21). The selectivity of the method was tested using reagent samples, blank - urine, liver, drinking water, lung, muscle and feed samples. The linearity of the standard curve was determined in a range depending on the matrix and compounds in question (Table 2). The same levels were used for repeatability, intra-laboratory reproducibility and apparent recovery.

Results

The procedure presented in the current article is selective and able to detect nine β-agonists in various matrices like feed, drinking water, urine, muscle and liver in one method (Fig. 1).



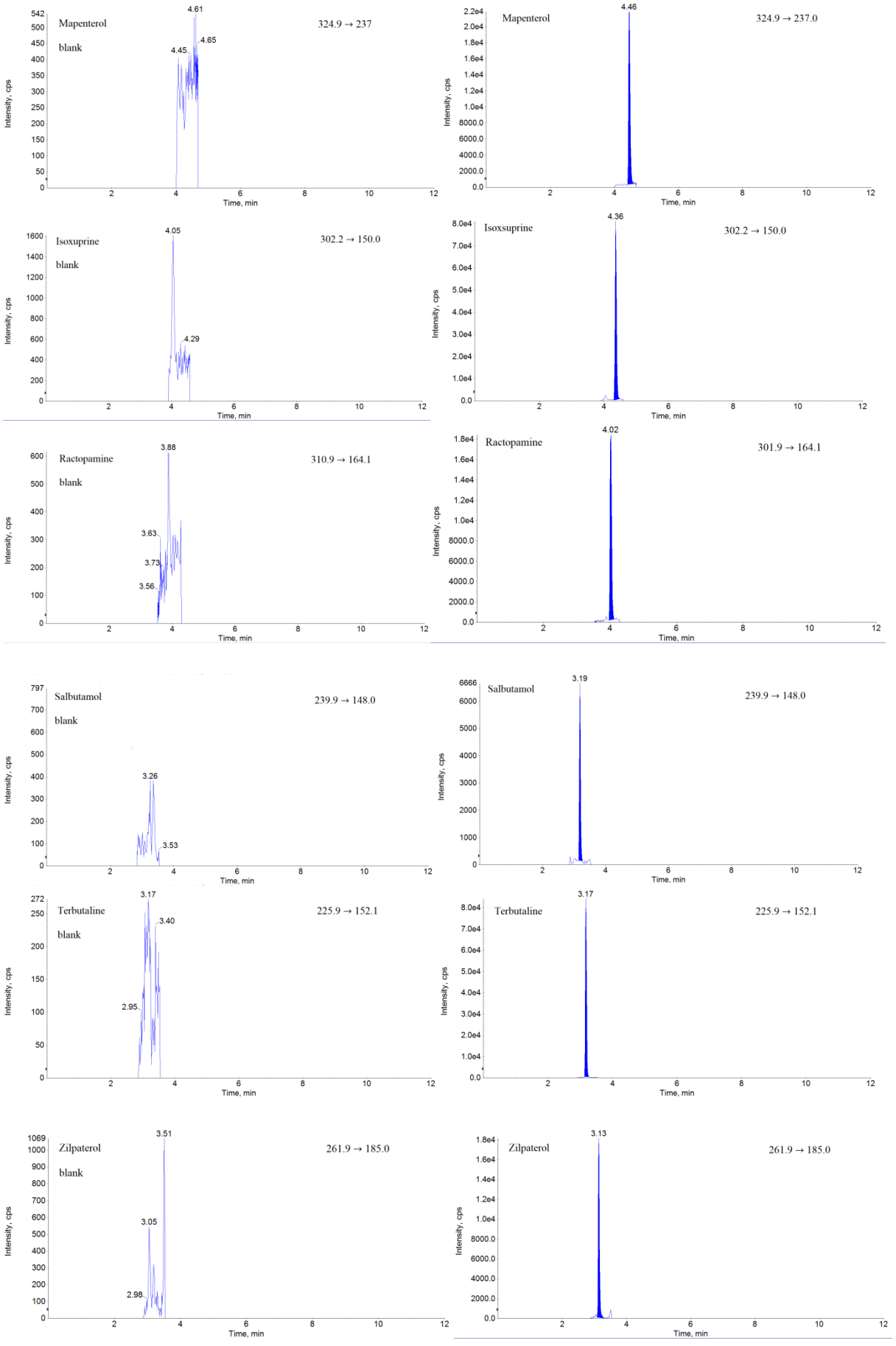


Fig. 1. Chromatograms of blank and spiked lung matrices with β-agonists at 0.2 μg kg^{-1} for clenbuterol, mabuterol, mapenterol, brombuterol, and at 1.0 μg kg^{-1} for salbutamol and zilpaterol, ractopamine, isoxsuprine, and terbutaline

Table 3. Validation report for β-agonists

Matrix	Repeatability (RSD_r,%)	Within-lab reproducibility (RSD_{wR},%)	CCα (μg kg^{-1})	CCβ (μg kg^{-1})	Apparent recovery (%)	Concentration range (ng/mL)	Matrix effect (%)	Expanded uncertainty (μg kg^{-1})
					Clenbuterol			
drinking water	4.1 ± 3.8	4.5 ± 4.0	0.10	0.11	103.9 ± 4.3	0.1–1.6	4.3 ± 3.9%	0.20 ± 0.02
feed	6.2 ± 4.6	5.6 ± 6.1	2.50	2.70	104.5 ± 6.8	2.5–40.0	5.0 ± 3.9%	5.00 ± 1.14
liver	4.3 ± 4.1	5.1 ± 4.7	0.10	0.12	103.5 ± 6.2	0.1–1.6	6.1 ± 3.2%	0.20 ± 0.06
urine	4.6 ± 3.7	5.3 ± 4.4	0.10	0.11	102.6 ± 5.1	0.1–1.6	5.6 ± 2.7%	0.20 ± 0.04
muscle	5.9 ± 6.5	4.5 ± 7.6	0.05	0.06	109.1 ± 4.4	0.1–1.6	4.3 ± 6.5%	0.10 ± 0.02
lung	4.3 ± 3.8	4.8 ± 4.6	0.10	0.11	96.1 ± 4.9	0.1–1.6	5.3 ± 4.9%	0.20 ± 0.05
					Mabuterol			
drinking water	3.7 ± 3.6	4.7 ± 3.9	0.10	0.11	99.5 ± 4.8	0.1–1.6	4.0 ± 3.5%	0.20 ± 0.03
feed	5.6 ± 5.4	6.7 ± 5.6	2.50	2.62	106.2 ± 7.8	2.5–40.0	6.0 ± 3.3%	5.00 ± 1.25
liver	5.9 ± 5.8	7.3 ± 6.3	0.10	0.12	95.4 ± 7.3	0.1–1.6	6.3 ± 5.2%	0.20 ± 0.05
urine	5.2 ± 4.9	6.3 ± 5.1	0.10	0.11	103.0 ± 4.1	0.1–1.6	5.4 ± 4.9%	0.20 ± 0.04
muscle	6.3 ± 4.1	6.4 ± 5.0	0.05	0.06	95.9 ± 6.0	0.05–0.8	5.8 ± 5.0%	0.10 ± 0.02
lung	5.9 ± 3.9	6.6 ± 5.6	0.10	0.11	93.2 ± 4.6	0.1–1.6	6.3 ± 4.6%	0.20 ± 0.04
					Mapenterol			
drinking water	3.2 ± 3.4	4.1 ± 4.0	0.10	0.12	98.9 ± 4.8	0.1–1.6	4.0 ± 2.0%	0.10 ± 0.02
feed	5.4 ± 4.6	5.6 ± 7.1	2.50	2.61	105.1 ± 5.6	2.5–40.0	5.1 ± 2.9%	5.00 ± 1.14
liver	5.4 ± 5.0	6.4 ± 5.2	0.10	0.12	93.3 ± 5.6	0.1–1.6	5.4 ± 4.9%	0.20 ± 0.04
urine	5.3 ± 4.5	5.4 ± 4.9	0.20	0.12	102.0 ± 5.5	0.1–1.6	4.9 ± 5.1%	0.20 ± 0.04
muscle	4.1 ± 3.4	5.0 ± 4.2	0.05	0.06	97.0 ± 5.3	0.05–0.8	5.5 ± 4.1%	0.10 ± 0.02
lung	3.9 ± 3.7	5.4 ± 4.8	0.10	0.11	100.3 ± 6.1	0.1–1.6	4.8 ± 5.2%	0.20 ± 0.05
					Brombuterol			
drinking water	3.1 ± 3.3	4.2 ± 4.1	0.10	0.12	95.4 ± 4.3	0.1–1.6	4.0 ± 3.6%	0.20 ± 0.05
feed	5.6 ± 4.1	6.2 ± 4.0	2.50	2.67	103.2 ± 6.8	2.5–40.0	5.3 ± 3.9%	5.00 ± 1.24
liver	4.9 ± 4.7	5.3 ± 4.9	0.10	0.11	96.5 ± 6.8	0.1–1.6	6.1 ± 4.8%	0.20 ± 0.05
urine	4.5 ± 3.9	5.1 ± 4.1	0.10	0.11	99.6 ± 5.2	0.1–1.6	5.1 ± 4.6%	0.20 ± 0.04
muscle	4.6 ± 4.7	5.4 ± 4.8	0.05	0.06	105.4 ± 5.4	0.05–0.8	4.5 ± 5.3%	0.10 ± 0.03
lung	4.9 ± 3.5	5.4 ± 4.6	0.10	0.12	98.2 ± 4.9	0.1–1.6	5.3 ± 3.6%	0.20 ± 0.05
					Ractopamine			
drinking water	3.1 ± 3.7	4.6 ± 3.6	0.50	0.57	103.5 ± 3.9	0.5–8.0	3.9 ± 4.1%	1.00 ± 0.23
feed	4.4 ± 3.1	5.6 ± 4.6	2.50	2.56	102.3 ± 5.2	2.5–40.0	6.0 ± 3.2%	5.00 ± 1.35
liver	4.0 ± 4.3	4.6 ± 4.9	0.50	0.52	103.6 ± 6.8	0.5–8.0	5.3 ± 6.0%	1.00 ± 0.26
urine	4.2 ± 3.8	5.3 ± 4.2	0.50	0.52	102.8 ± 5.1	0.5–8.0	5.8 ± 5.3%	1.00 ± 0.23
muscle	4.6 ± 4.9	5.4 ± 5.3	0.50	0.52	105.0 ± 4.7	0.5–8.0	6.5 ± 5.7%	1.00 ± 0.16
lung	4.9 ± 4.5	5.4 ± 4.9	0.50	0.56	103.8 ± 4.3	0.5–8.0	5.5 ± 3.9%	1.00 ± 0.26
					Salbutamol			
drinking water	4.2 ± 2.4	5.4 ± 4.0	2.50	2.54	104.9 ± 4.8	2.5–40.0	5.3 ± 3.1%	5.00 ± 1.25
feed	5.9 ± 4.6	6.5 ± 6.6	2.50	2.72	105.1 ± 6.9	2.5–40.0	6.0 ± 4.1%	5.00 ± 1.31
liver	5.3 ± 4.7	7.4 ± 5.2	2.50	2.61	93.3 ± 5.6	2.5–40.0	6.5 ± 5.4%	5.00 ± 1.45
urine	4.9 ± 4.8	6.4 ± 4.9	0.50	0.52	97.0 ± 6.3	0.5–8.0	5.4 ± 5.5%	1.00 ± 0.25
muscle	5.2 ± 3.3	5.7 ± 4.3	2.50	2.61	105.3 ± 4.6	2.5–40.0	6.5 ± 6.5%	5.00 ± 1.16
lung	4.9 ± 4.5	5.0 ± 4.6	0.50	0.52	101.5 ± 5.1	0.5–8.0	4.8 ± 5.6%	1.00 ± 0.33
					Zilpaterol			
drinking water	3.2 ± 2.4	4.1 ± 3.0	2.50	2.58	105.4 ± 4.8	2.5–40.0	3.0 ± 2.1%	5.00 ± 1.19
feed	4.1 ± 4.5	5.9 ± 4.6	2.50	2.73	104.5 ± 6.3	2.5–40.0	5.3 ± 3.9%	5.00 ± 1.40
liver	6.4 ± 5.7	7.7 ± 6.2	2.50	2.79	107.3 ± 5.6	2.5–40.0	6.3 ± 6.3%	5.00 ± 1.52
urine	7.1 ± 4.3	8.0 ± 4.9	0.50	0.56	100.3 ± 6.8	0.5–8.0	5.3 ± 4.6%	1.00 ± 0.23
muscle	5.1 ± 4.3	5.9 ± 4.8	2.50	2.69	112.0 ± 6.1	2.5–40.0	7.3 ± 5.5%	5.00 ± 1.64
lung	4.0 ± 3.5	8.2 ± 7.6	0.50	0.56	106.4 ± 5.3	0.5–8.0	7.0 ± 4.3%	1.00 ± 0.45
					Terbutaline			
drinking water	5.2 ± 4.4	6.0 ± 4.5	5.00	5.34	106.9 ± 3.8	5.0–80.0	5.1 ± 4.6%	10.00 ± 3.60
feed	6.2 ± 3.6	7.6 ± 3.9	2.50	2.70	107.5 ± 7.1	2.5–40.0	6.6 ± 4.3%	5.00 ± 1.72
liver	4.2 ± 4.7	6.1 ± 5.7	5.00	5.25	93.3 ± 5.1	5.0–80.0	5.7 ± 6.1%	10.00 ± 3.12
urine	6.1 ± 4.2	7.3 ± 5.0	1.50	1.54	102.0 ± 6.5	1.5–24.0	8.8 ± 4.7%	3.00 ± 0.86
muscle	3.4 ± 3.2	4.2 ± 3.6	5.00	5.63	105.3 ± 3.8	5.0–80.0	3.5 ± 3.5%	10.00 ± 4.1
lung	5.2 ± 3.5	6.0 ± 4.0	1.50	1.64	96.3 ± 5.1	1.5–24.0	6.3 ± 6.2%	3.00 ± 0.82
					Isoxsuprine			
drinking water	3.6 ± 4.3	4.4 ± 5.1	0.25	0.27	102.9 ± 4.9	0.25–4.0	3.2 ± 2.3%	0.50 ± 0.09
feed	3.6 ± 3.7	4.6 ± 4.0	2.50	2.59	104.5 ± 2.8	2.5–40.0	5.0 ± 4.9%	5.00 ± 1.19
liver	3.8 ± 4.0	4.9 ± 5.2	0.25	0.26	102.3 ± 4.3	0.25–4.0	4.6 ± 4.5%	0.50 ± 0.11
urine	4.9 ± 3.8	5.4 ± 4.5	0.25	0.26	106.2 ± 4.6	0.25–4.0	5.3 ± 4.0%	0.50 ± 0.12
muscle	3.6 ± 3.8	4.3 ± 4.1	0.25	0.26	98.0 ± 4.3	0.25–4.0	4.5 ± 3.5%	0.50 ± 0.08
lung	3.6 ± 4.5	5.8 ± 5.6	0.25	0.27	108.0 ± 6.2	0.25–4.0	4.3 ± 5.0%	0.50 ± 0.12

The correlation coefficient of the standard curves prepared with the fortified samples for each β-agonist was ≥ 0.95. Depending on the β-agonist and matrix assayed, the coefficients of variation of repeatability for fortified samples were in a 6.8–11.3% range, while the range for reproducibility was 8.5–14.2%. The average apparent recovery was 93.2–112.0%. Determination of CCα and CCβ was according to Commission Decision 2002/657/EC; the former was in the range of 0.05–5.00 μg kg^{-1} and the latter 0.06–5.63 μg kg^{-1} depending on the compound and the matrix. The expanded uncertainty was calculated for each compound and each of the matrices at the second concentration level applying a coverage factor of 2, which provided a level of confidence of approximately 95% (Table 3). The calculated ion suppression of the matrix effects for β-agonists in all matrices did not exceed 15%.

Discussion

In this article we describe a method that allows parallel detection and determination of β-agonist residues in urine, liver, muscle, and lung tissue, feed, and drinking water in a single analytical run. The first step in the development of the method was the selection of chromatographic conditions, which was the optimisation of the conditions for analysis by liquid chromatography coupled with mass spectrometry. Electrospray (ESI) and atmospheric pressure chemical ionisation (APCI) were tested as alternatives, and the electrospray mode was selected for further use due to its higher reproducibility, selectivity, and sensitivity, which was were also confirmed by other authors (12, 13, 15, 18, 25, 29, 30).

With gradient elution, adequate separation of analytes from matrix interfering components was achieved. The selection of chromatographic conditions was based on the publications of other researchers (3, 15). The main step of the analytical method is the development of the isolation of analytes from the sample of biological material. The most important stages of analyte isolation in the whole analytical procedure are extraction and purification. After preliminary studies, the extraction method proposed by Boyd *et al*. (2), Leyssens *et al*. (16) and Hooijerink *et al.* (14) was abandoned and purification by liquid–solid extraction was investigated. From the obtained results it was found that all the cartridges selected for the study gave comparable recoveries, but the use of MIP columns yielded higher extract purity, required shorter analysis time (below 12 min), and ensured good result.

Simultaneously, studies were conducted on the effect of chemical and enzymatic hydrolysis on the recoveries of the analysed compounds. It was observed that the use of the hydrolysis step allows the release of salbutamol and salbutamol-like compounds from bonds with endogenous matrix components, which was observed in urine, muscle, lung and liver samples. In our procedure, enzymatic hydrolysis was chosen due to its simplicity. This is in agreement with reports in the literature (2, 3, 9, 14, 16).

A study comparing different methods for the isolation of analytes from biological material showed that the developed method provided good recovery for the β-agonists under investigation and, at the same time, sample processing was not very labour- and time-consuming, which prompted its validation. The validation parameters show that the method meets the appropriate acceptance criteria set by the European Union (4).

The obtained validation results presented above confirm the suitability of the method for conducting routine residue monitoring of these compounds in all presented matrices in the National Monitoring Plan in Poland.

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