Determination of β2-Agonists in Pork with SPE Cleanup and LC-MS/MS Detection

Using Agilent BondElut PCX Solid-Phase Extraction Cartridges, Agilent Poroshell 120 column and Liquid Chromatography-Tandem Mass Spectrometry

Application Note

Food Safety

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Abstract

A method for simultaneous determination of 11 β2-agonist residues of clebuterol, salbutamol, ractopamine, terbutaline, salmeterol, propranolol, tulobuterol, cimaterol, mabuterol, mapenterol, and zilpaterol in pork has been developed and validated. The analytes are extracted by liquid-liquid extraction (LLE) and solid-phase extraction (SPE) and quantified by liquid chromatography coupled to electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) operating in positive ion multiple reaction monitoring (MRM) mode. The method provides a pg/g level of limit of detection (LOD) for all β2-agonists in pork. The dynamic calibration ranges for these compounds are obtained from 0.25 to 5 ng/g. The overall recoveries range from 82% to 105% with RSD values between 1.6% and 8.4%.

Introduction

The β2-agonists have been used worldwide as illegal growth promoters in meat production. They act as metabolic modifiers that convert energy normally used for fat production into energy for meat production. Recently, incidences of poisoning occurred due to high levels of the β-agonist (clenbuterol) in pork. This application note used Agilent's Bond Elut Plexa PCX SPE product to extract and enrich 11 β-agonists from pork and analysis by LC-MS/MS. Table 1 show the 11 β-agonist compounds name and structure.

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Table 1. The β2-Agonist Compounds Used in this Study

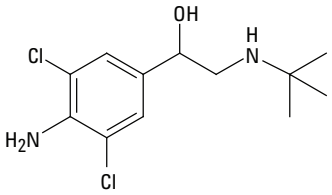
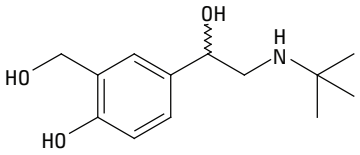
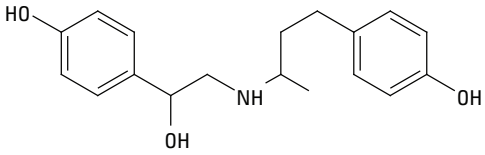
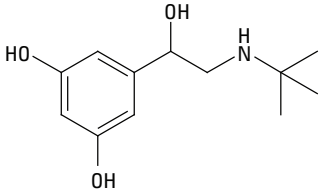
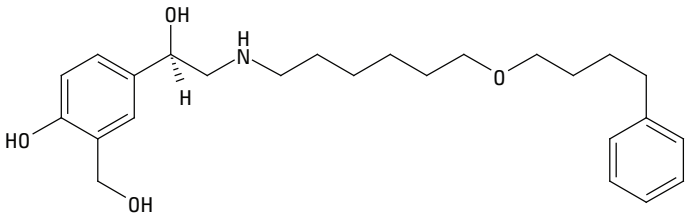
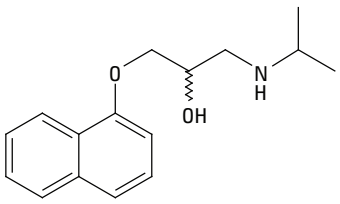
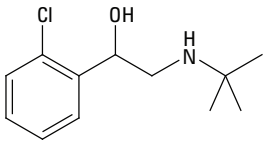
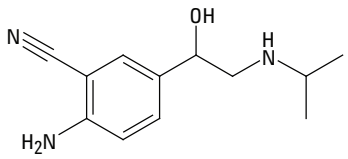
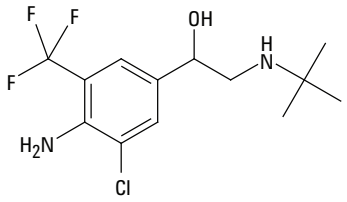
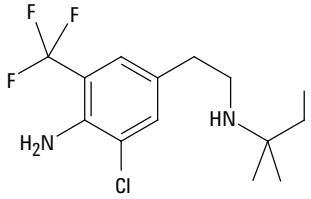
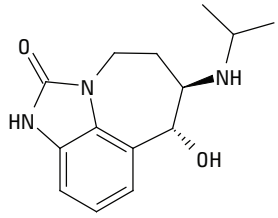
Compound	CAS No.	Structure
clenbuterol	21898-19-1	
salbutamol	18559-94-9	
ractopamine	90274-24-1	
terbutaline	23031-32-5	
salmeterol	94749-08-3	
propranolol	318-98-9	
tulobuterol	56776-01-3	

Table 1. The β2-Agonist Compounds Used in this Study (Continued)

Compound	CAS No.	Structure
cimaterol	54239-37-1	
mabuterol	54240-36-7	
mapenterol	54238-51-6	
zilpaterol	117827-79-9	

Experimental

Reagents and Chemicals

All reagents were MS, HPLC or analytical grade.

Acetonitrile and water were from Honeywell. The standards were purchased from National Institute for the Control of Pharmaceutical and Biological Products (NICPBP). Pork was purchased from a local supermarket.

Standard solutions (1.0 mg/mL) were made in methanol individually, and stored in freezer at –20 °C. A combined working solution (10 µg/mL) was made in acetonitrile-water (10:90) and also stored at 4 °C. The spiked solutions were then made weekly by appropriately diluting the combined working solution in water.

Equipment and Materials

Agilent 1200 HPLC system

Agilent 6460 Triple Quadrupole LC-MS/MS system

Agilent Bond Elut Plexa PCX cartridges, 60 mg, 3 mL (p/n 12108603)

Agilent Poroshell 120 EC-C18, narrow bore, 2.1 × 100 mm, 2.7 µm (p/n 695775-902)

Agilent Vac Elut 20 Manifold (p/n 12234101)

Sample Preparation

Liquid-Liquid Extraction

A 5 g amount of ground pork (± 0.01 g) was weighed into a 50 mL capped polypropylene tube. A 0.2 M sodium acetate (pH 5.2) solution, 20 mL was added and vortexed until mixed. This was followed by the addition of 250 µL β-glucuronidase (1000 U/mL), vortexed for 2 minutes to mix the sample thoroughly and hydrolyzed at 37 °C for 16 hours.

The hydrolysate was shaken for 15 minutes and centrifuged at 4000 rpm for 10 minutes, then 4 mL supernatant was transferred to another centrifuge tube. A 0.1 M perchloric acid solution, 5 mL was added and the pH was adjusted to 1 ± 0.3.

The tube was then centrifuged at 4000 rpm for 10 minutes prior to the supernatant being transferred to another tube. The pH was adjusted to 11 with 10 M sodium hydroxide.

A saturated sodium chloride solution 10 mL and 10 mL isopropanol-ethyl acetate (60:40) was added to the tube and shaken for 5 minutes. The tubes were centrifuged at 4000 rpm for 5 minutes prior to the organic layer being carefully transferred to another tube. Isopropanol-ethyl acetate addition, shaking, centrifuging and organic layer transfer was repeated twice with all supernants combined.

Samples were evaporated to dryness with nitrogen at 40 °C. The residue was dissolved in 5 mL 0.2 M sodium acetate (pH 5.2). The sample was then ready for SPE purification.

Solid-Phase Extraction

The procedure of SPE is shown in Figure 1. Agilent Bond Elut Plexa PCX cartridges were preconditioned with 3 mL of methanol and then equilibrated with 3 mL water. The 5 mL sample solution was then loaded onto a cartridge and passed through the cartridge by gravity (about 1 mL/min). The cartridges were washed with 2 mL water and 2 mL 2% formic acid in water. Full vacuum was applied to the cartridge for 3 minutes to completely dry the resin. The compounds were eluted with 5 mL 5% ammonia solution in methanol at a rate of 1 mL/min. The eluent was dried by nitrogen flow at 40 °C. The residue was reconstituted in 1 mL of 0.1% formic acid in water/acetonitrile (90:10). The sample was vortexed and ultrasonicated to completely dissolve the residue, filtrated with a 0.45 µm filter membrane. The sample was transferred to a 1.5 mL tube and centrifuged at 3000 rpm for 5 minutes. The sample was transfered to a 2 mL chromatography vial for analysis.

Instrument Conditions

HPLC Conditions

Column	Agilent Poroshell 120 EC-C18, 2.1 × 100 mm, 2.7 µm		
Flow rate	0.4 mL/min		
Column temperature	40 °C		
Injection volume	2 µL		
Mobile phase	Water (0.1% FA+2 mM NH_4Ac, A), acetonitrile (0.1% FA, B)		
Gradient	Time (min)	%A	%B
	0	98	2
	0.5	98	2
	4	65	35
	5	20	80
	6	10	90
	6.5	98	2
	7	98	2

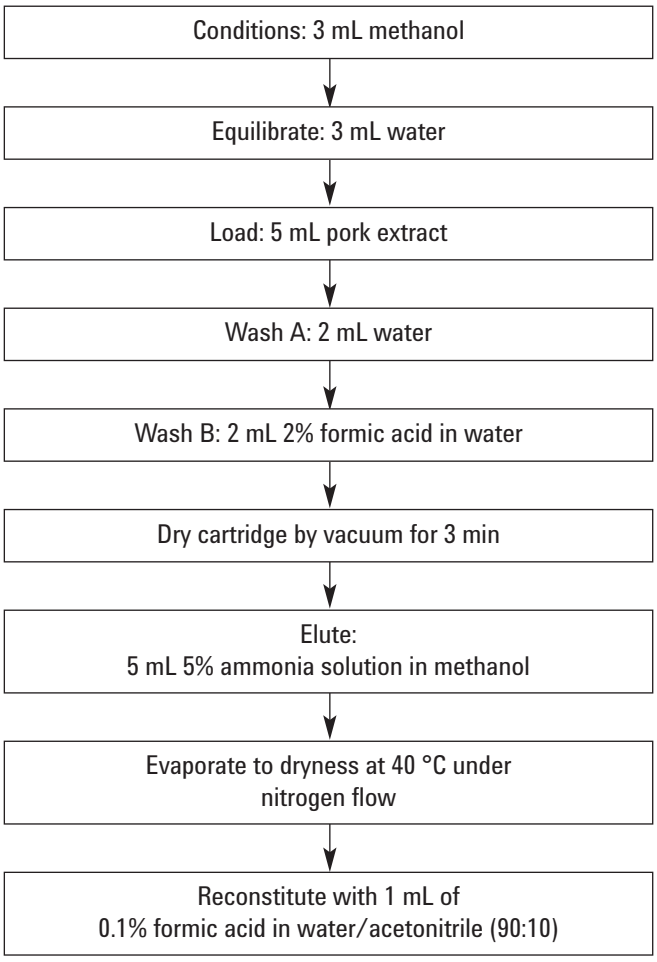


Figure 1. Pork extraction and enrichment –SPE procedure.

MS Conditions

These 11 compounds were monitored in the positive mode. The source conditions are shown in Table 2 and the MRM channels are shown in Table 3.

Table 2. MS Source Parameters for These 11 Compounds

Gas temp	350 °C
Gas flow	5 L/min
Nebulizer	45 psi
Sheath gas temp	400 °C
Sheath gas flow	12 L/min
Nozzle voltage	Positive: 0 V Negative: 1000 V
Capillary	Positive: 4000 V Negative: 3500 V

Table 3. Masses Monitored in the MRM for These 11 Compounds

Analyte	MRM channels (*m/z*)	Fragmentor (V)	CE (V)
clenbuterol	1) 277.2>203.1 2) 277.2>259.1	100	12 5
salbutamol	1) 240.2>148.2 2) 240.2>222.1	100	15 5
ractopamine	1) 302.2>164.2 2) 302.2>284.1	110	12 6
terbutaline	1) 226.1>152.2 2) 226.1>170.2	100	12 6
salmeterol	1) 416.3>380.3 2) 416.3>398.4	130	17 10
propranolol	1) 260.2>116.2 2) 260.2>183.2	120	15 15
tulobuterol	1) 228.1>154.1 2) 228.1>172.2	100	12 5
cimaterol	1) 220.1>160.2 2) 220.1>202.1	90	12 3
mabuterol	1) 311.2>237.1 2) 311.2>293.2	110	13 7
mapenterol	1) 325.3>237.1 2) 325.3>307.2	110	12 5
zilpaterol	1) 262.2>244.2 2) 262.2>202.2	100	7 17

Results and Discussion

Linearity and Limit of Detection

Solutions used to create external calibration curves were prepared by using a combined working solution to spike matrix blank (0.25, 0.5, 1.0, 2.0 and 5.0 ng/g). Matrix blanks were created by taking pork through the entire procedure, including hydrolysis, LLE and SPE procedures. The results for the calibration curves are shown in Table 4. The limits of detection (LOD) were chosen as the concentration of each compound that gave a signal to noise (S/N) ratio greater than 3:1. The LODs are also shown in Table 4.

Table 4. Linearity and LODs of β2-Agonists

Compound	Regression equation	R^2	LOD in pork (ng/g)
clenbuterol	Y = 0.5576x – 0.0045	0.998	0.03
salbutamol	Y = 0.5804 – 0.0266	0.997	0.01
ractopamine	Y = 0.6780x – 0.0278	0.999	0.01
terbutaline	Y = 0.6121x – 0.0143	0.999	0.02
salmeterol	Y = 0.1657x – 0.0056	0.996	0.05
propranolol	Y = 0.2017x + 0.0055	0.999	0.03
tulobuterol	Y = 0.6985x – 0.0080	0.998	0.02
cimaterol	Y = 1.0993x + 0.0169	0.999	0.03
mabuterol	Y = 0.9587x – 0.0088	0.995	0.03
mapenterol	Y = 0.8206x – 0.0102	0.995	0.02
zilpaterol	Y = 0.0620x + 0.0069	0.999	0.08

Recovery and Reproducibility

The recovery and reproducibility for the method were determined at three levels, pork spiked to a concentration of 0.5, 1.0, and 2.0 ng/g. The analysis was performed with six replicates at each level. The recovery and reproducibility data is shown in Table 5. The chromatograms of spiked pork extracts (1.0 ng/g) are shown in Figure 2.

Table 5. Recoveries and Reproducibility of β2-Agonists in Pork

Compund	Spiked level (ng/g pork)	Recovery (%)	RSD (n=6)
clenbuterol	0.5	88.4	2.3
	1.0	95.1	5.4
	2.0	93.7	1.9
salbutamol	0.5	98.6	7.3
	1.0	95.3	4.1
	2.0	90.2	6.7
ractopamine	0.5	95.9	6.8
	1.0	103.6	2.9
	2.0	100.9	7.3
turbutalilne	0.5	102.7	8.4
	1.0	96.9	2.7
	2.0	89.1	5.4
salmeterol	0.5	93.5	6.2
	1.0	92.7	3.5
	2.0	87.8	5.0
propranolol	0.5	104.9	6.8
	1.0	97.3	6.7
	2.0	104.2	2.7
tulobuterol	0.5	82.8	6.1
	1.0	89.3	4.4
	2.0	93.6	5.9
cimaterol	0.5	88.7	8.0
	1.0	90.6	3.3
	2.0	96.4	4.6
mabuterol	0.5	92.7	6.0
	1.0	103.8	7.6
	2.0	100.5	4.9
mapenterol	0.5	98.9	8.5
	1.0	92.1	2.5
	2.0	96.3	4.7
zilpaterol	0.5	103.7	5.2
	1.0	89.6	4.3
	2.0	92.4	3.9

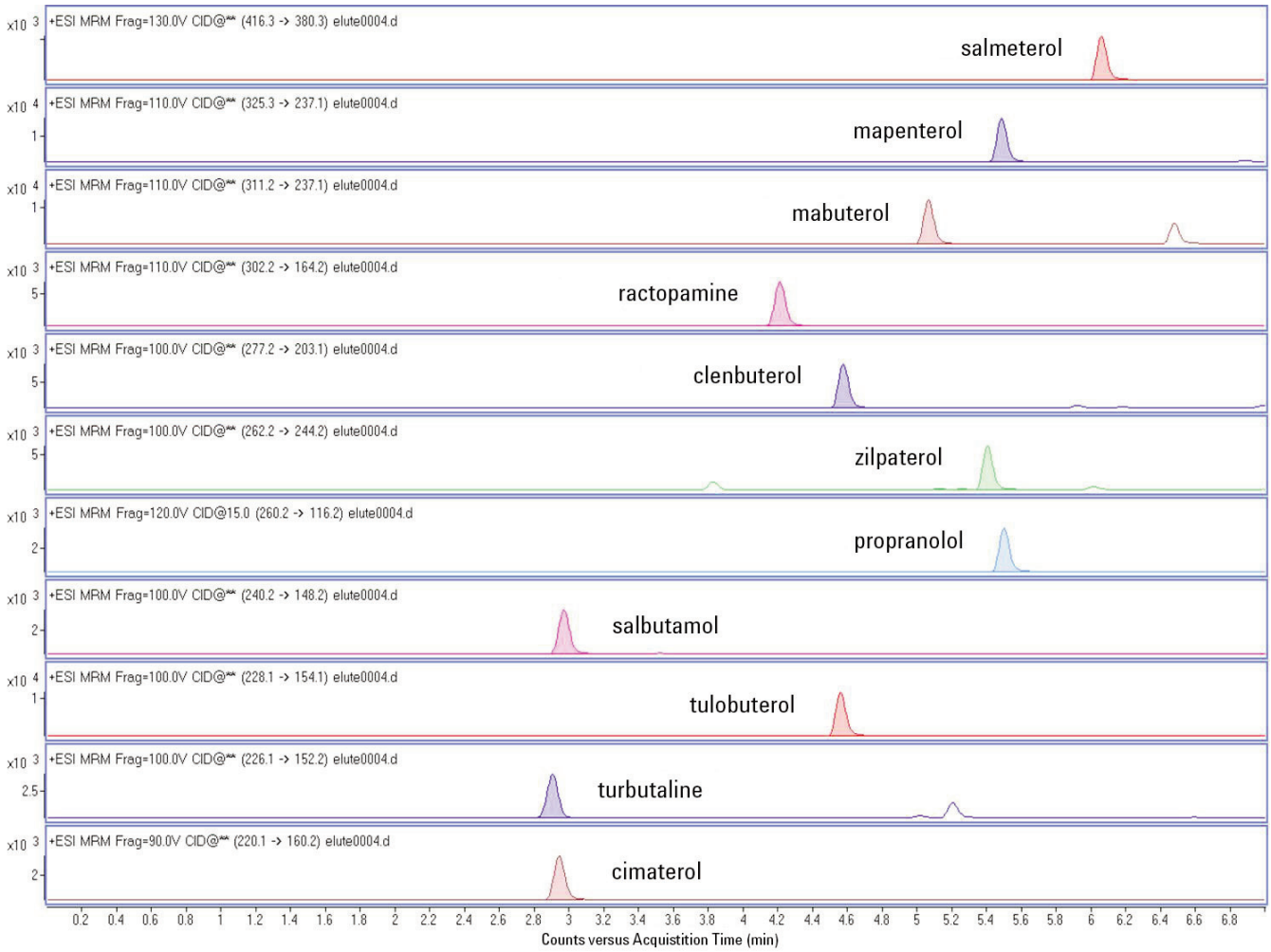


Figure 2. Chromatograms of 1.0 ng/g spiked pork sample extract.

Conclusion

The result of this study shows that Agilent Bond Elut Plexa PCX can be used as an effective method for purification and enrichment of multiple β2-agonists in complex matrix such as pork. The recovery and reproducibility results based on matrix spiked standards are acceptable for β2-agonists residue determination in pork under regulations. The impurities and matrix effect are minimal and do not interfere with the quantification of any target compound. The LOQ are significantly lower than the MRLs [1,2].

References

1. GB/T 21313-2007 "Analysis of β2-Agonists in Foods of Animal Origin by High Performance Liquid Chromatography Tandem Mass Spectrometry."
2. SN/T 1924-2007 "Determination of Clenbuterol, Ractopamine, Salbutamol and Terbutalin Residues in Foodstuffs of Animal Origin for Import and Export –HPLC-MS/MS Method."

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