Determination of Multiresidue Analysis of β-Agonists in Muscle and Viscera with QuEChERS

Yen-Ping Lin*, Ying-Lin Lee *, Chien-Ya Hung, Wen-Jeng Huang, Sheng-Che Lin
Department of Health, Tainan City Government

Abstract

Method of test for veterinary drug residues in foods-test of multiresidue analysis of β-agonists was according to official analytical method of food and drug administration, ministry of health and welfare. Liquid-liquid extraction (LLE) and solid-phase extraction (SPE) are used for sample pretreatment. Disadvantages of this method were time consuming and high solvent usage. On the basis of elevating analysis efficiency and economic need, our study established a method of multiresidue analysis of β-agonists in muscle with QuEChERS. The pretreatment time was thus shortened and solvent usage was minimized. In the present study, a method for analysis of seven β-agonists using the QuEChERS procedure followed by liquid chromatograph/tandem mass spectrometer (LC/MS/MS) and quantitated with multiple reaction monitoring. The results demonstrated that the coefficient of correlation of tissue calibration curve was higher than 0.99, the limit of quantification was 1 ppb. The average recoveries in spiked varied from 70 to 120 %, and the relative percent difference between duplicated analysis results was lower than 10 %. Finally, based on the results, it is concluded that the proposed method is an appropriated procedure for β-agonists with the advantage of high recovery in spiked, high precision, analysis time decreasing, solvent usage eliminating and cost saving.

Keywords: β-agonists, QuEChERS, multiresidue analysis, LC-MS/MS

1. Introduction

In recent years, the security of meat cause great public concern [2, 3] and methods to detect drug residues have become a domestic and international research focus. Effective determination methods are needed to guarantee the safety of meat [4, 5]. Veterinary drugs are widely used at therapeutic levels in the systems of livestock breeding for treating different diseases and one type of these, β-agonists, are originally used in the treatment of asthma and preterm labor in humans [6]. However, these compounds also promote lipolysis in muscle tissue to exhibit a significant nutrition redistribution function. resulting in higher feed efficiency and greater muscle to fat ratio in livestock [7-9]. β-agonists have been applied in the livestock industry such as pigs and ruminants to reduce carcass fat and increase muscle mass while improving growth rate and feed conversion [10-13]. Previous research indicated that the residues of six β-agonists have been accumulated in the retinal tissue of food-producing animals [14]. Moreover, it might cause acute poisoning for human to intake β-agonists deposited animal tissues, particularly in patients with such symptoms as muscular tremors, cardiac palpitation, nervousness, headache, muscular pain., etc [15]. Nonetheless, the β-agonists-contaminated tissues seem to be harmful and cause potential risk for human health [16]. While the therapeutic treatment of cattle with respiratory diseases is permitted, the use of β-agonists as growth promoters in cattle is forbidden in the EU and some countries[17]. There are still illicit usages of β-agonists in animal feeds in many countries [18]. Therefore, to monitor food safety, sensitive and specific analytical methods are needed to determine the level of β-agonists in meat. Analytical methods are essential to assess human exposure to β-agonists and support the enforcement of laws and regulations.

From a review of the literature the determination for monitoring β2-agonist residues include HPLC [19, 20], GC–MS [21, 22] and LC–MS or LC–MS/MS [23, 24]. Nowadays, liquid chromatography–tandem mass spectrometry (LC–MS/MS) is a most suitable technique for detection of multiclass veterinary drugs in foodstuffs, because it provides an unambiguous identification and a reliable confirmation [25]. For a method to be deemed confirmatory identification was carried out by relative retention times, identification points (IPs) of each analytes and relative ion ratios of selected multiple reaction monitoring (MRM) transitions. Despite the use of selective detection techniques such as MS, an appropriate sample preparation is a major challenge in analytical procedures in order to decrease interferences and avoid possible matrix effects. For extraction and purification, such techniques as immunoaffinity chromatography [26], liquid–liquid extraction (LLE) [27], solid phase extraction (SPE) [28] and matrix solid-phase extraction (MSPD) [29] are used. Most of the

current extraction methods are time consuming and tedious. However, the general SPE cleanup techniques lack specificity and selectivity and can retain analytes and interferents together, leading to interference and matrix effects and reducing the determination reliability. In addition, the large quantities of organic solvents may cause environmental pollution [30].

Consequently, analytical laboratories are increasingly interested in developing new analytical methods that are more rapid and enable higher sample throughput. In 2003, the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method for the multi-class and multiresidue analysis of pesticides in fruits and vegetables was first published by Anastassiades[*1]. The QuEChERS technique has quickly received widespread attention since its development. The technique has expanded beyond these traditional samples to include meat products, fish, blood, and even soil. The QuEChERS method is a well-known methodology for the extraction of several classes of drugs, including pesticides and veterinary drugs, from different matrices. This method minimises the time required for extraction and clean-up processes and is inexpensive; in addition, the QuEChERS procedure reduces the sample size and required quantities of laboratory glassware and requires low solvent consumption [31]. In contrast, the established QuEChERS pretreatment procedure without covering was very simple and economic and required only small amounts of organic solvents. The method can reduce the sample volume, solvent consumption and analytical time needed. It is necessary to develop QuEChERS method for β-agonists monitoring. The aim of this study was to develop a rapid and easy multi-residue analytical method with QuEChERS procedure for the determination of the levels of seven β-agonists in muscle and viscera samples.

2. Materials and methods

2.1 Samples

Samples (muscle and viscera) were purchased from local supermarkets and were confirmed to be free of the targeted drugs. The tissues were homogenized and stored at -20°C until analysis.

2.2 Chemicals, reagents and solution

All chemicals were of analytical grade. Acetonitrile, methanol (HPLC grade) and ammonium acetate was obtained from Merck (Darmstadt, Germany). Ultrapure water (Milli-Q, Millipore Corporation, Bedford, MA) was used to prepare all aqueous solutions.

All β-agonists (cimaterol, clenbuterol, ractopamine, salbutamol, terbutaline, tulobuterol and zilpaterol) and corresponding internal standards (cimaterol-d7, clenbuterol-d9,

ractopamine-d6, clenbuterol-d9, salbutamol-d6, terbutaline-d9 and zilpaterol-d7) were purchased from Sigma (St Louis, MO). The purity of all of these standards was not less than 98.0%. The chemical structures of all seven β-agonists are shown in Fig. 1.

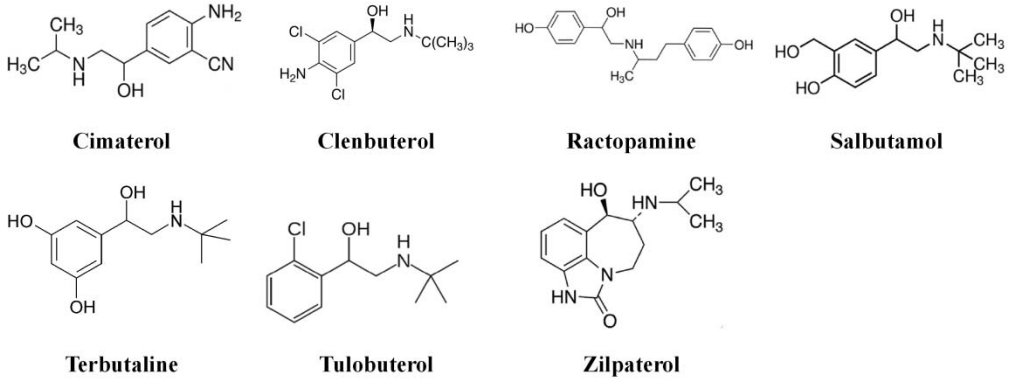


Fig. 1- Chemical structures of the β-agonists in this study.

2.3 Preparation of standards

Individual stock solutions (100 μg/mL) were prepared by dissolving 1 mg of each compound in 10 mL of methanol, and stored at −20°C in brown glass to prevent the photodegradation. Mixed standard solution at concentrations of 1 μg/mL of each standard was prepared by additive mixing 100 μL of each stock solution and diluting to 10 mL with methanol. The mixed standard solutions were stored in amber bottles at −20°C. Six mixed internal standard solutions were prepared and stored in the same way. All working solutions and calibration standards were obtained by gradient dilution of the intermediate solutions, in concentrations varying from 1 μg/mL to 1 ng/mL. Working standard solution of internal standards in a concentration of 1 μg/mL came by subsequent dilutions of their stock solutions in methanol. While not in use, the working solutions were kept at −20 °C and renewed weekly.

2.5 Sample preparation

Five gram finely chopped muscle and viscera homogenates were weighed into a 50mL Falcon tube and spiked as appropriate (target compounds and IS). Ten milliliter of 0.2 M sodium acetate solution was added, followed by shaking for 10 mins. After the addition of IS and 100 μL of β-glucuronidase-arylsulphatase, the sample was incubated at 37 °C water bath for 1 h before extraction. One milliliter of 1% acetate–acetonitrile was added and the mixture was stirred in a shaker for 30 s, subsequently. Then, ceramic homogenizer, 6 g of anhydrous magnesium sulphate and 1.5 g of anhydrous sodium acetate were added and the

tubes were shaken vigorously for 1 min. After centrifugation at 4032 x g, 15°C for 5 min, the supernatant was transferred to a new tube containing 900 mg of anhydrous magnesium sulphate, 150 mg of PSA and C18EC followed by high-speed homogenzied for 1 min and centrifuged again under the same conditions described above. The supernatant was evaporated to dryness by a nitrogen blowing concentrator in a water bath at 65°C. The muscle and viscera residues were re-dissolved with 1 mL of acetonitrile-H_2O solution (9:1, v/v) and acetonitrile, respectively. The sample extract was filtered through a 0.22μm PTFE filter into an auto-sampler vial for the LC-MS/MS analysis The scheme diagram of sample preparation was shown in Fig 2.

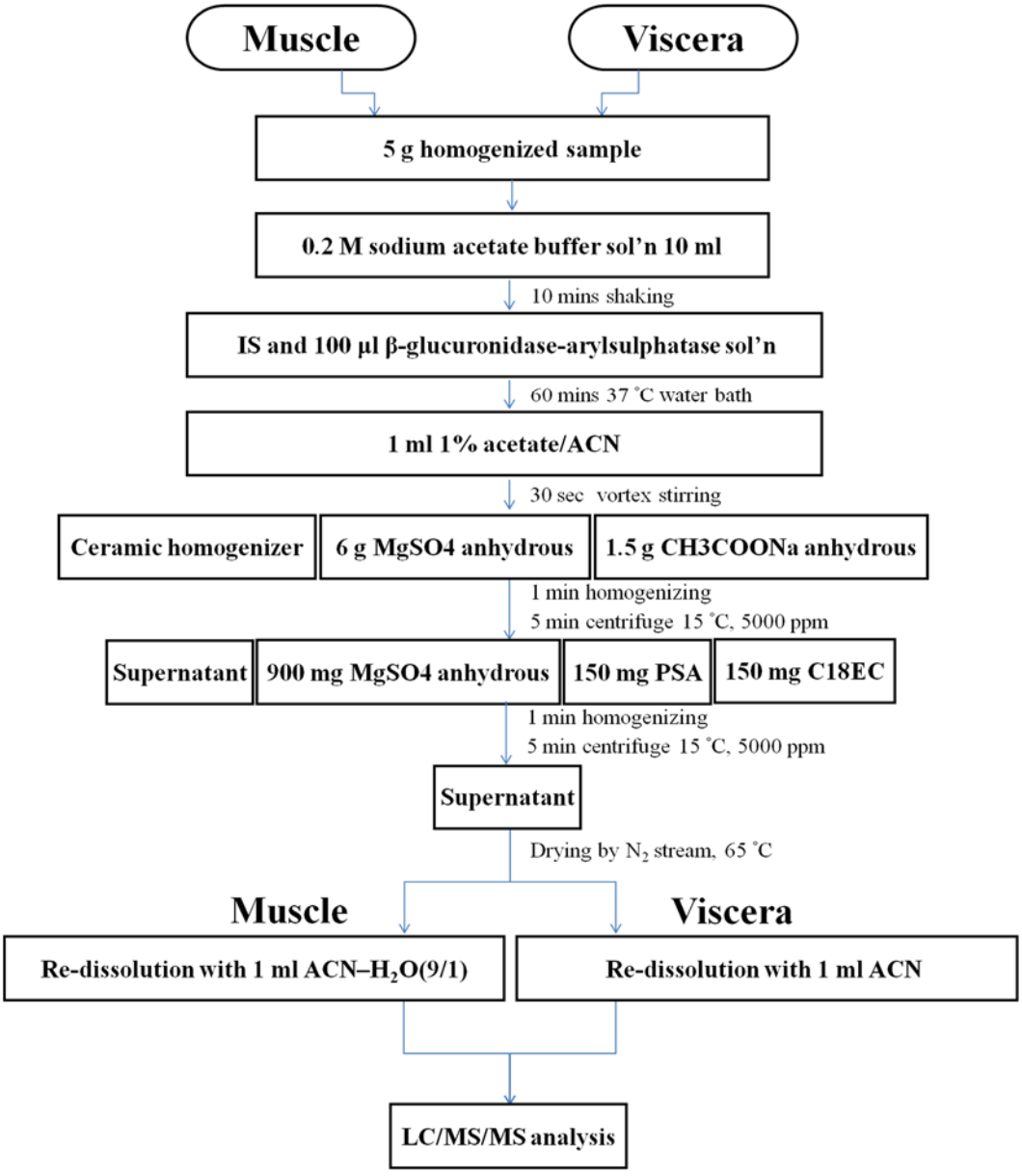


Fig. 2. The scheme diagram of sample preparation.

2.6 LC–MS/MS analysis

Liquid chromatography was performed on a Dionex Ultimate 3000 Rs system (Sunnyvale, CA, USA) coupled to an AB SCIEX Q TRAP 5500 mass spectrometer (Framingham, MA, USA). Chromatographic separation was performed using an Agilent Zorbox SB-C18 column (150 mm× 4.6 mm, 5 μm). The linear gradient elution was performed as Table 1. The injection volume was 10 μL. Mass analysis was carried out by an electrospray (ESI) source in positive mode. The operation conditions were as follows: ionspray voltage, 5.5 kV; source temperature at 650 ˚C; curtain gas, 20 psi; ion source gas 1, gas 2 both at 65 psi. The optimal MRM parameters are summarized in Table 2.

Table 1 Gradient program of the mobile phase for HPLC separation of the seven β-agonists in the present study(flow rate 1 ml/min).

Time (min)	5 mM ammonium acetate in methanol(%)
0	15
1	15
6	25
14	70
16	80
17	80
17.5	95
19.5	95
20	15
25	15

5 mM ammonium acetate in deionized water(%)+5 mM ammonium acetate in methanol (%)=100

Table 2 Parameters of MRM condition and retention times of the β-agonists.

Compound	ESI	Retention time (min)	Precusor ion (m/z)	Product ions (m/z)	Decluster potential (V)	Entrance potential (V)	Collision energy (eV)	Collision cell exit potential (V)
Cimaterol	+	4.40	220	202, 160	45	10	12, 10	13
Clenbuterol	+	7.62	277	203, 259	58	10	20,15	13
Ractopamine	+	6.34	320.01	284, 107	59	10	18, 33	13
Salbutamol	+	4.53	240.01	148, 222	45	10	24, 13	13
Terbutaline	+	4.45	226	152, 125	60	10	20, 32	13
Tulobuterol	+	8.48	228	154, 118	40	10	22, 33	13
Zilpaterol	+	4.70	262.2	244, 185	54	10	19, 33	13

2.7 Method validation

For the method validation, various parameters such as linearity, accuracy, precision and limits of quantification (LOQ) were evaluated. The peak area of the most intense transition versus the concentration was used to establish the linear regression equation. The linearity of the method was evaluated based on tissue calibration.

2.7.1 Linearity

The tissue calibration curves were used for quantification and testing the linearity of the method developed. The blank muscle and viscera sample were used to fortified at five concentrations from 1~50 μg/kg for the target analytes. Three replicates of each concentration were performed. Sample preparation procedure was as above. The tissue calibration curves for β-agonists were constructed by calculating the ratio of each peak area relative to the corresponding IS. The linearity of the LC-MS/MS method was evaluated assessing the regression coefficient measured for each analyte. The acceptance criterion was that the coefficient of correlation (R^2) must be more than 0.99.

2.7.2 Accuracy and precision

Accuracy and precision results were expressed as percentage recovery and coefficient of variation (CV%). Recovery and repeatability were assessed by spiking blank muscle and viscera samples at two concentration levels (1.5 and 3.0 μg/kg) for target analytes in five replicates at each level.

2.7.3 Limits of quantification(LOQ)

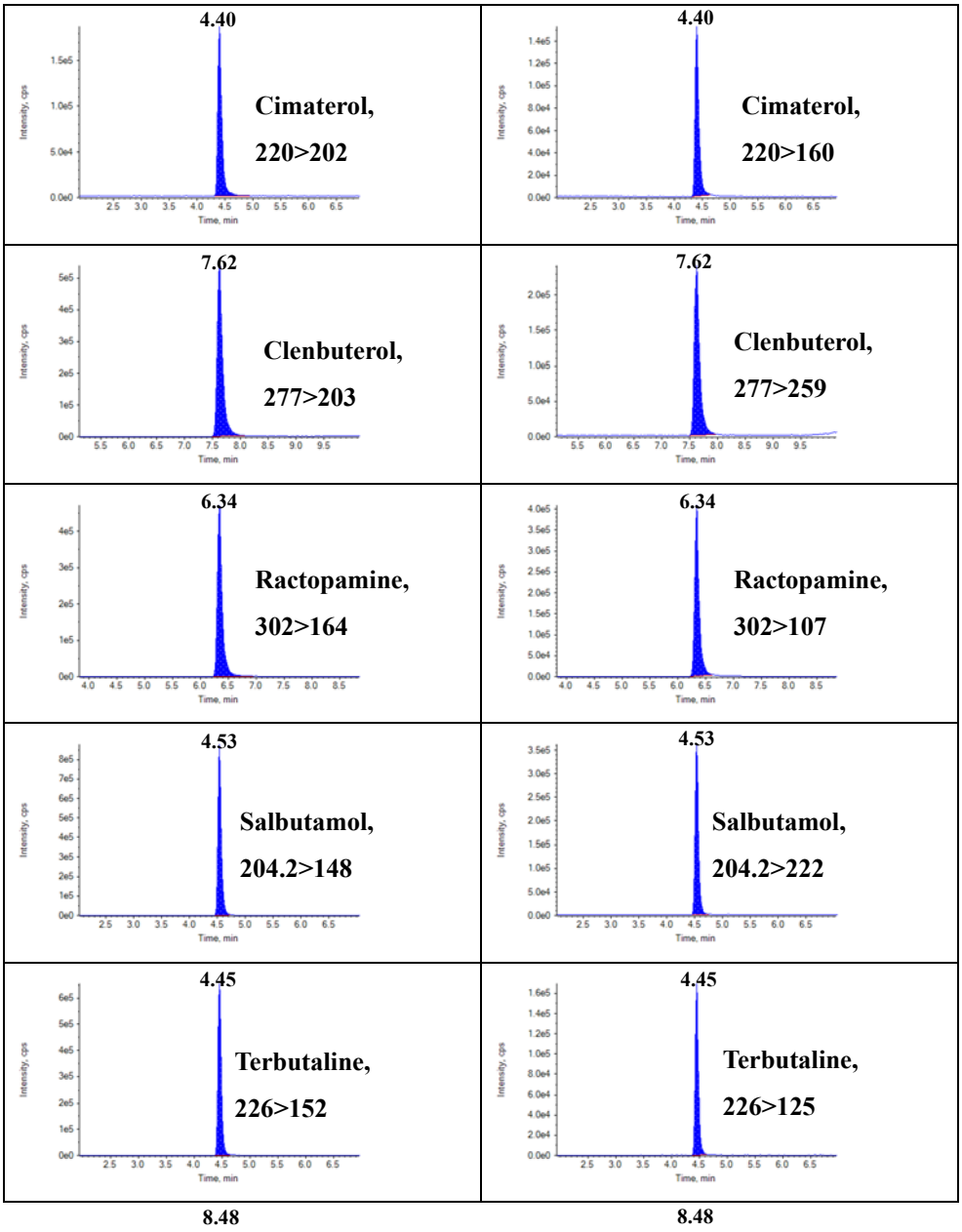
LOQ were calculated by analyzing blank samples fortified at 1 μg/kg and determined as the lowest concentration of an analyte for which the signal-to-noise ratio was over 10.

3.Results and discussion

3.1 Optimization of LC-MS/MS Parameters

The LC–MS/MS method was developed to provide confirmatory data for the analysis of seven β-agonists in animal muscle and viscera tissue. Separation was performed in a Agilent Zorbox SB-C18 column (150 mm× 4.6 mm, 5 μm). Chromatographic parameters such as choice of column, mobile phase composition, gradient conditions and flow rate were tested to obtain the best separation of β-agonists. With reference to previous reports, a mobile phase consisting of 5 mM ammonium acetate in water and methanol was chosen[32-34]. The addition of ammonium acetate greatly improved peak shape and helped to resolve closely eluted compounds. After optimization, the ammonium acetate concentration was set at 5 mM, and its addition into the aqueous phase further improved separation and provided

better overall peak shapes[9]. Because β-agonists belong to group A of Annex I, Council Directive 96/23/EC [35], a minimum of four identification points are required, which were obtained by monitoring one parent ion (1 point) and two transitions (each 1.5 points). The product ion with a stronger signal was selected as the ion for quantification, and the product ion with a weaker signal was selected as the ion for identification. The selected transitions for β-agonists and the optimal MS–MS conditions are described in Section 2.6. The gradient program in Table 1 was used for chromatographic separation. The flow rate was set at 1.0 mL/minute and the analysis could be completed in 25 minutes for the seven β-agonists. The MRM chromatograms of the seven β-agonists in the present study are shown in Fig. 3.



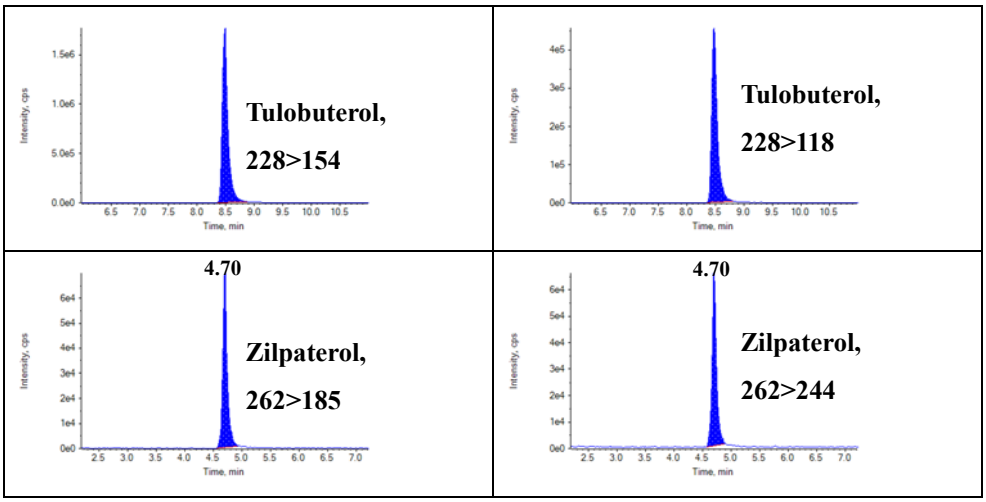


Fig. 3- Extract chromatograms of the seven β-agonists spiked at 3 μg/kg in muscle sample.

3.2 Optimization of sample preparation

Sample preparation plays an important role in an analytical method. Nowdays, various pretreatment methods have been proposed for monitoring the illegal use of β-agonists[26, 27, 36]. Due to the complexity of the biological matrices and the trace levels in real samples, salts and endogenous compounds cannot be fully removed leading to possible matrix effects. In addition, these techniques were time-consuming steps and the large quantities of organic solvents, including acetonitrile and methanol [3, 30], may cause environmental pollution. In contrast, the established QuEChERS pretreatment procedure without covering was very simple and economic and required only small amounts of organic solvents. The principle of the QuEChERS method relies on a sample cleanup using various dispersive solid-phase extraction (d-SPE) sorbents, including PSA, C18, and silica, as well as magnesium sulphate for the elimination of residual water prior to analysis [37]. In order to improve efficiency and reduce time-consuming sample preparation, QuEChERS method was developed for the cleanup of target analytes in the extracts of tissues. First, sodium acetate buffer solution extraction and enzymatic hydrolysis was performed. Previous study demonstrated that better recoveries were obtained for most of the compounds when acetonitrile acidified with 1% acetic acid was used as extract solvent[38]. In this work, 10 mL of acetonitrile acidified with 1% acetic acid was used as the extraction solvent. While the analytes are transferred into an organic phase, some more polar matrix impurities are left in the aqueous layer. 1.5 g sodium acetate and 6 g magnesium sulfate were used as a saltingout agent to partition analytes residues into the acetonitrile layer. Several sorbents such as PSA and C18 made analytes achieving satisfied recovery. The muscle and viscera extracts were evaporated under a stream of nitrogen at 65 °C and the final residue were dissolved in 1 mL of

acetonitrile-H_2O solution (9:1, v/v) and acetonitrile, respectively. The optimised sample preparation protocol enabled high percentage recoveries for seven β-agonists.

3.3 Method validation

3.3.1 Linearity

Linearity of analytical method was validated using the tissue calibration curves for each compound at different concentration levels to avoid matrix effects. Table 3 shows tissue calibration parameter of the correlation coefficients (R^2). In Table 3, the correlation coefficients for all seven β-agonists in muscle and viscera sample were higher than 0.99, which revealed a good linearity in the concentration range for each β-agonists.

Table 3 Linearity and limits of quantification of the β-agonists

β-agonists	Muscle		Viscera	
	R^2	LOQ (ng/g)	R^2	LOQ (ng/g)
Cimaterol	0.998	1	0.997	1
Clenbuterol	1.000	1	0.999	1
Ractopamine	0.998	1	0.997	1
Salbutamol	0.998	1	0.995	1
Terbutaline	0.996	1	0.995	1
Tulobuterol	0.999	1	0.998	1
Zilpaterol	0.993	1	0.993	1

3.3.2 Recovery

The rates of recovery of each compound from muscle and viscera samples were evaluated at two different concentration levels (1.5 and 3 μg/kg) by determining the ratios of the measured and added amounts of the target analyte. The results of the recovery test for the seven β-agonists in muscle and viscera samples are listed in Table 4 and Table 5. In muscle sample, the recovery rate of 1.5 and 3.0 μg/kg spiked level ranged from 90.5 to 101.2% and 87.6 to 102.5%, respectively. In viscera sample, the recovery rate of two spiked level varied from 90.7 to 110.3% and 91.7 to 111.5%, respectively. These results show the accuracy is satisfactory.

Table 4 Recovery rates and CVs of the β-agonists from muscle samples.

β-agonists	Spiked level (ng/g)	Recovery (%)	CV (%)
Cimaterol	1.5	90.5	5.4
	3.0	95.1	3.1
Clenbuterol	1.5	99.2	1.7
	3.0	87.6	5.8
Ractopamine	1.5	98.5	4.3
	3.0	100.3	4.6
Salbutamol	1.5	101.2	4.2
	3.0	90.8	5.8
Terbutaline	1.5	96.3	6.3
	3.0	100.2	3.5
Tulobuterol	1.5	97.9	7.7
	3.0	102.5	3.2
Zilpaterol	1.5	94.4	3.2
	3.0	97.4	6.4

Table 5 Recovery rates and CVs of the β-agonists from viscera samples.

β-agonists	Spiked level (ng/g)	Recovery (%)	CV (%)
Cimaterol	1.5	102.0	3.2
	3.0	95.0	4.2
Clenbuterol	1.5	101.1	2.5
	3.0	102.1	2.1
Ractopamine	1.5	99.6	5.7
	3.0	103.6	3.6
Salbutamol	1.5	110.3	4.2
	3.0	106.9	3.4
Terbutaline	1.5	100.8	4.2
	3.0	111.5	2.9
Tulobuterol	1.5	102.4	1.8
	3.0	105.5	6.2
Zilpaterol	1.5	90.7	6.6
	3.0	91.7	6.9

3.3.3 Precision

The precision of the assay were assessed at 1.5 and 3.0 μg/kg for each analyte in spiked sample and expressed as the coefficient of variation (CV). The results are presented in

Tables 4 and 5. The CV of 1.5 and 3.0 μg/kg spiked muscle sample ranged from 1.7% to 7.7% and 3.1% to 6.4%, respectively, and the corresponding values for viscera sample varied from 1.8% to 6.6% and 2.1% to 6.9%, respectively. Thus, all CV values were less than 10% at two different concentrations (1.5 μg/kg and 3 μg/kg).

3.3.4 LOQ

The limit of quantitation (LOQ), defined as the concentration at 10 times the signal intensity of noise. The LOQs of all β-agonists were 1 μg/kg for spiked muscle and viscera.

4 Conclusion

In the present study, a simple, fast and sensitive multi-residue analytical method by LC–MS/MS was developed and validated for the simultaneous determination of seven β-agonists from two different tissues (muscle and viscera). Analytes were extracted using QuEChERS extraction procedure and analyzed with liquid chromatography electrospray ionization tandem mass spectrometric analysis. This method was validated with fortified blank samples and the extraction procedure was fully optimized. Good validation parameters such as linearity, recovery, precision and LOQs were obtained indicating the suitability of the proposed solvent extraction method for the analysis of β-agonists. The current method has the following advantages: simple, rapid, economic and low solvent consumption. QuEChERS is therefore a green technique for sample preparation. It is proved that the developed method is simple, rapid and reliable in routine β-agonists residues assessment.

7. References

[1] Taiwan FDA. Method of Test for Veterinary Drug Residues in FoodsTest of Multiresidue Analysis of β-Agonists. Available at: http://www.fda.gov.tw/TC/includes/GetFile.ashx?mID=133&id=2seven173&chk=3324b069-f792-4495-bfec-1a82419eb0f6.pdf [Accessed December24, 2015|] [in Chinese].

[2] Andrée S, Jira W, Schwind KH, Wagner H, Schwägele F. Chemical safety of meat and meat products. Meat Sci. 2010;86:38-48.

[3] Jenson I, Sumner J. Performance standards and meat safety — Developments and direction. Meat Sci. 2012;92:260-6.

[4] Baert K, Van Huffel X, Jacxsens L, Berkvens D, Diricks H, Huyghebaert A, Uyttendaele M. Measuring the perceived pressure and stakeholders' response that may impact the status of the safety of the food chain in Belgium. Food Res Int. 2012;48:257-64.

[5] Castro-Puyana M, Herrero M. Metabolomics approaches based on mass spectrometry for food safety, quality and traceability. Trends Analyt Chem. 2013;52:74-87.
[6] Hardman JG, Limbird LE, editors. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 9th ed. New York: McGraw-Hill; 1996.
[7] Stolker AAM, van Ginkel LA, Stephany RW, Maxwell RJ, Parks OW, Lightfield AR. Supercritical fluid extraction of methyltestosterone, nortestosterone and testosterone at low ppb levels from fortified bovine urine. J Chromatogr B Biomed Appl. 1999;726:121-31.
[8] Stolker AAM, Brinkman UAT. Analytical strategies for residue analysis of veterinary drugs and growth-promoting agents in food-producing animals—a review. J Chromatogr A. 2005;1067:15-53.
[9] Li T, Cao J, Li Z, Wang X, He P. Broad screening and identification of β-agonists in feed and animal body fluid and tissues using ultra-high performance liquid chromatography-quadrupole-orbitrap high resolution mass spectrometry combined with spectra library search. Food Chem. 2016;192:188-96.
[10] Bareille N, Faverdin P. Lipid Metabolism and Intake Behavior of Dairy Cows: Effects of Intravenous Lipid and β-Adrenergic Supplementation. J Dairy Sci. 1996;79:1209-20.
[11] Byrem TM, Beermann DH, Robinson TF. The beta-agonist cimaterol directly enhances chronic protein accretion in skeletal muscle. J Anim Sci. 1998;76:988-98.
[12] Cardoso LA, Taveira O. Effect of clenbuterol on growth, nitrogen and energy balances and endocrine status in food-restricted sheep. J S Afr Vet Assoc. 2002;73:127-30.
[13] Shook JN, VanOverbeke DL, Kinman LA, Krehbiel CR, Holland BP, Streeter MN, Yates DA, Hilton GG. Effects of zilpaterol hydrochloride and zilpaterol hydrochloride withdrawal time on beef carcass cutability, composition, and tenderness. J Anim Sci. 2009;87:3677-85.
[14] Gowik P, Julicher B, Ladwig M, Behrendt D. Measurement of beta-agonist residues in retinal tissue of food producing animals. Analyst. 2000;125:1103-7.
[15] Brambilla G, Cenci T, Franconi F, Galarini R, Macrì A, Rondoni F, Strozzi M, Loizzo A. Clinical and pharmacological profile in a clenbuterol epidemic poisoning of contaminated beef meat in Italy. Toxicology Letters. 2000;114:47-53.
[16] Zhang GJ, Fang BH, Liu YH, Wang XF, Xu LX, Zhang YP, He LM. Development of a multi-residue method for fast screening and confirmation of 20 prohibited veterinary drugs in feedstuffs by liquid chromatography tandem mass spectrometry. J Chromatogr B. 2013;936:10-7.
[17] Stachel CS, Radeck W, Gowik P. Zilpaterol—a new focus of concern in residue

analysis. Anal Chim Acta. 2003;493:63-7.
[18] Preželj A, Obreza, A. , Pečar, S. Abuse of clenbuterol and its detection. Curr Med Chem. 2003;10:281-90.
[19] Morales-Trejo F, León SV-y, Escobar-Medina A, Gutiérrez-Tolentino R. Application of high-performance liquid chromatography–UV detection to quantification of clenbuterol in bovine liver samples. J Food Drug Anal. 2013;21:414-20.
[20] Sirhan AY, Tan GH, Wong RCS. Method validation in the determination of aflatoxins in noodle samples using the QuEChERS method (Quick, Easy, Cheap, Effective, Rugged and Safe) and high performance liquid chromatography coupled to a fluorescence detector (HPLC–FLD). Food Control. 2011;22:1807-13.
[21] Di Corcia D, Morra V, Pazzi M, Vincenti M. Simultaneous determination of β2-agonists in human urine by fast-gas chromatography/mass spectrometry: method validation and clinical application. Biomed Chromatogr. 2010;24:358-66.
[22] Gallo P, Brambilla G, Neri B, Fiori M, Testa C, Serpe L. Purification of clenbuterol-like β2-agonist drugs of new generation from bovine urine and hair by α1-acid glycoprotein affinity chromatography and determination by gas chromatography–mass spectrometry. Anal Chim Acta. 2007;587:67-74.
[23] Du X-D, Wu Y-L, Yang H-J, Yang T. Simultaneous determination of 10 β2-agonists in swine urine using liquid chromatography–tandem mass spectrometry and multi-walled carbon nanotubes as a reversed dispersive solid phase extraction sorbent. J Chromatogr A. 2012;1260:25-32.
[24] González-Antuña A, Domínguez-Romero JC, García-Reyes JF, Rodríguez-González P, Centineo G, García Alonso JI, Molina-Díaz A. Overcoming matrix effects in electrospray: Quantitation of β-agonists in complex matrices by isotope dilution liquid chromatography–mass spectrometry using singly 13C-labeled analogues. J Chromatogr A. 2013;1288:40-7.
[25] Dasenaki ME, Thomaidis NS. Multi-residue determination of 115 veterinary drugs and pharmaceutical residues in milk powder, butter, fish tissue and eggs using liquid chromatography–tandem mass spectrometry. Anal Chim Acta. 2015;880:103-21.
[26] Shen J, Zhang Z, Yao Y, Shi W, Liu Y, Zhang S. Time-resolved fluoroimmunoassay for ractopamine in swine tissue. Anal Bioanal Chem. 2007;387:1561-4.
[27] Keskin S, Özer D, Temizer A. Gas chromatography-mass spectrometric analysis of clenbuterol from urine. J Pharm Biomed Anal. 1998;18:639-44.
[28] Wang L, Zeng Z, Wang X, Yang J, Chen Z, He L. Multiresidue analysis of nine

β-agonists in animal muscles by LC-MS/MS based on a new polymer cartridge for sample cleanup. J Sep Sci. 2013;36:1843-52.
[29] Qiao F, Du J. Rapid screening of clenbuterol hydrochloride in chicken samples by molecularly imprinted matrix solid-phase dispersion coupled with liquid chromatography. J Chromatogr B. 2013;923–924:136-40.
[30] Shishani E, Chai SC, Jamokha S, Aznar G, Hoffman MK. Determination of ractopamine in animal tissues by liquid chromatography-fluorescence and liquid chromatography/tandem mass spectrometry. Anal Chim Acta. 2003;483:137-45.
[31] Xu Z, Hu Y, Hu Y, Li G. Investigation of ractopamine molecularly imprinted stir bar sorptive extraction and its application for trace analysis of β2-agonists in complex samples. J Chromatogr A. 2010;1217:3612-8.
[32] Nakajima T, Nagano C, Sasamoto T, Hayashi H, Kanda M, Kanai S, Takeba K, Matsushima Y, Takano I. Development and Validation of Rapid Analysis Method for Multi-Class Veterinary Drugs in Livestock Products by LC-MS/MS. Shokuhin Eiseigaku Zasshi. 2012;53:243-53.
[33] Noguchi S, Terada H, Tamura Y. Simultaneous Determination of Veterinary Drugs in Livestock Foods and Seafoods Using Liquid Chromatography/Tandem Mass Spectrometry. Shokuhin Eiseigaku Zasshi. 2008;49:177-88.
[34] Kajita H, Hatakeyama E. Simultaneous Determination of Residual Veterinary Drugs in Livestock Products and Fish by Liquid Chromatography with Tandem Mass Spectrometry. Shokuhin Eiseigaku Zasshi. 2008;49:381-9.
[35] Directive 96/23/EC. Office for Official Publications of the European Communities; 1996 [4].
[36] Wang X, Wang S, Cai Z. The latest developments and applications of mass spectrometry in food-safety and quality analysis. Trends Analyt Chem. 2013;52:170-85.
[37] Boscher A, Guignard C, Pellet T, Hoffmann L, Bohn T. Development of a multi-class method for the quantification of veterinary drug residues in feedingstuffs by liquid chromatography-tandem mass spectrometry. J Chromatogr A. 2010;1217:6394-404.
[38] Zhang, Yaqian *et al*. Multi-class, multi-residue analysis of trace veterinary drugs in milk by rapid screening and quantification using ultra-performance liquid chromatography–quadrupole time-of-flight mass spectrometry Journal of Dairy Science , 2015;98:8433-44.