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Mixed mode monolithic sorbent in pipette tip for extraction of ractopamine and clenbuterol prior to analysis by HPLC-UV and UHPLC-Q Exactive™ Plus Orbitrap MS

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Abstract

This work developed a fast and simple method for the quantification of two important β-agonists, ractopamine (RAC) and clenbuterol (CLEN) in animal urines. The method was based on micro-extraction by a mixed mode monolithic material and analysis by HPLC-UV and UHPLC-Q Exactive™ Plus Orbitrap mass spectrometer. A mixed functional monolith with nonpolar ester-based structure containing polar carboxylic groups was thermally synthesized in situ in the pipette tips. The material combines both hydrophobic and ionic interactions. Parameters including conditions and composition of reagents for the in-pipette tip monolith synthesis, as well as the extraction process, were investigated and optimized. The procedure for extraction is 800 μL washing solvent of 10:90 (*v/v*) ACN:water and 150 μL eluting solvent of 30:70 (*v/v*) ACN:200 mM acetate buffer pH 4.0. Extraction efficiencies of 92% and 100% for RAC and CLEN, respectively, were achieved within 5 min with total organic solvent consumption of 395 μL. The extracts of spiked cattle and swine urines were analyzed by HPLC-UV and UHPLC-Q Exactive™ Plus Orbitrap mass spectrometer. Good recovery with acceptable precision was observed. The mass spectrometry data confirmed efficient matrix removal by the synthesized extraction sorbent allowing routine analysis by the HPLC-UV method.

Keywords: Micro-solid phase extraction, Monolith, Ractopamine, Clenbuterol, HPLC analysis

Introduction

β-Agonists are sympathomimetic agents that interact with beta adrenoceptors in mammal muscles. Clenbuterol (CLEN) and ractopamine (RAC) are the common β-agonists used as animal feed additives by farmers to reduce fat and increase muscle mass in animal production (Herpin et al. 2018; Peng et al. 2018; Xiu-Juan et al. 2013). The residual β-agonists in food can cause adverse effects on human health, such as muscular tremors, cardiac palpitation, nervousness, and headache (Chu et al. 2017; Wang et al. 2016a, 2016b). Thus, they are forbidden in many countries, such as the European Union (EU) and China (Zhang et al. 2016). However, their illegal use is still found in areas around the world.

Several techniques including high-performance liquid chromatography-ultraviolet detection (HPLC-UV) (Qiu et al. 2016; Xu et al. 2010; Zhu et al. 2014), liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Li et al. 2015; Shao et al. 2009; Wang et al. 2017; Wang et al. 2016a, 2016b), capillary electrophoresis (Chen et al. 2008; Wang et al. 2015; Wang et al. 2010), electrochemical method (Wu et al. 2012), Raman

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spectroscopy (Xiao et al. 2015; Zhai et al. 2012), and colorimetric detection (He et al. 2011; Zhou et al. 2013) have been developed for the analysis of these compounds in various kinds of samples, such as pork (Qiu et al. 2016; Wang et al. 2016a, 2016b; Xu et al. 2010), pig liver (Shao et al. 2009; Zhu et al. 2014), swine urine (He et al. 2011; Li et al. 2015; Wang et al. 2017; Zhai et al. 2012), and animal feeds (Wang et al. 2015; Wang et al. 2010; Wu et al. 2012; Zhou et al. 2013).

In order to analyze trace RAC and CLEN in biological samples such as urine, serum, and meat, a sample preparation procedure is usually required for preconcentration and matrix removal before the analysis step. Various techniques have been reported including solid phase extraction (SPE) (Qiu et al. 2016; Shao et al. 2009; Wang et al. 2017; Xu et al. 2010; Zhu et al. 2014), liquid-liquid extraction (Li et al. 2015; Zhai et al. 2012), solvent extraction (Wu et al. 2012; Zhou et al. 2013), and immunoaffinity extraction (Cai and Henion 1997). Among these methods, SPE is commonly used due to its simplicity and high extraction efficiency with relatively low organic solvent consumption. However, simultaneous extraction of RAC and CLEN could not be performed using common C18 SPE, as the RAC is not readily retained on the hydrophobic phase. Special sorbents such as molecularly imprinted polymer (Qiu et al. 2016; Xiao et al. 2015; Xu et al. 2010), mixed mode cation exchanger (Zhu et al. 2014), monolithic material (Wang et al. 2016a, 2016b), hydrophilic phase (Shao et al. 2009), and graphene (Wang et al. 2017) have been reported for simultaneous extraction of RAC and CLEN. Although monolithic material has been previously reported, the method is complicated and time consuming (Wang et al. 2016a, 2016b). Briefly, the monolithic material is thermally polymerized for 24 h, then mechanically ground and sieved, followed by drying under vacuum for another 24 h.

Detection of RAC and CLEN in urine enables simple control of their abuse in animal production. This work aims to synthesize a mixed mode monolithic material in a pipette tip as a simple micro-SPE for extraction of polar RAC and nonpolar CLEN in animal urine. The extracts are analyzed using HPLC with UV detection and high-resolution mass spectrometry. Important parameters in the in situ synthesis of monolithic micro-SPE (μ-SPE) and extraction process are investigated. The analysis by HPLC-UV is compared with data from the highly selective Orbitrap high-resolution mass spectrometer to confirm the extraction selectivity of the developed monolithic μ-SPE.

Experimental

Materials and reagents

The 1-mL polypropylene pipette tips were manufactured by Axygen (USA). Ractopamine hydrochloride (RAC), clenbuterol hydrochloride (CLEN), acetonitrile (ACN), methanol (MeOH), triethylamine (TEA), methacrylic acid (MAA), ethylene dimethacrylate (EDMA), azobisisobutylronitrile (AIBN), 1-decanol, glacial acetic acid, and toluene were acquired from Sigma-Aldrich (USA). Phosphoric acid, potassium dihydrogen phosphate, disodium hydrogen phosphate, sodium hydroxide, and ammonium acetate were obtained from Carlo Erba (Rodano, Ml, Italy). Phosphate, ammonium acetate, and acetate buffers were prepared in DI water and pH adjusted with 0.1 M NaOH before making up to the desired volume. Stock and working solutions of RAC and CLEN were prepared daily in MeOH and DI water, respectively.

Instruments

Monolithic polymerization was carried out in a hot air oven (Model ULE600, Memmert, Germany). The HPLC-UV analysis was performed using an Agilent 1100 HPLC instrument with 20-μL sample loop injector (Rheodyne® Model 7725i, USA) and ultraviolet-visible (UV-VIS) detector (Model HP 1100, USA). The UHPLC-Q Exactive™ Plus Orbitrap HRMS was performed using a Thermo Scientific LC system (Dionex Ultimate 3000 RSLC, USA) coupled with a high-resolution mass spectrometer (Q Exactive™ Plus Orbitrap, Thermo Scientific, USA). Commercial C18 SPE cartridges were purchased from VertiPakTM, Thailand. Morphology of the monolith was characterized by a scanning electron microscope (SEM) (Model Quanta450, Thermo Fisher Scientific, USA). An FT-IR spectrometer (TENSOR 27-Hyperion, Bruker, Germany) was used to characterize functional groups of the synthesized monolithic sorbent.

Preparation of IT monolithic μ-SPE

Bare pipette tips were washed with 300 μL/tip MeOH and dried before use. The ends of the cleaned tips were sealed with Parafilm® and Scotch® tape before filling with a series of polymerization solutions. The composition of the polymerization solutions comprising of MAA, EDMA, toluene, 1-decanol, and AIBN was varied by changing the ratio of the monomers to porogenic solvents (see Table 1). A 60 aliquot of the solution was transferred into the tips and the ends were sealed with Parafilm® and Scotch® tape before heating in an oven at 75°C for 70 min. The polymerized monolithic material was washed with 300 μL of MeOH before use.

Chromatographic conditions

HPLC-UV

The HPLC-UV method for analyses of RAC and CLEN uses Zorbax SB-C8 column (4.6 mm×75 mm, 3.5 μm, Agilent, USA). The optimum conditions were a mobile phase of 25:75 (*v*/*v*) ACN:20 mM acetate buffer pH 4.0

Table 1 Weight of monomers, porogens, and initiator of the polymerization solutions

Percent of monomer: porogen	Monomer (g)		Porogen (g)		Initiator (g)	Permeability	Preparation success rate[a]
	MAA	EDMA	Toluene	1-Decanol	AIBN		
15:85	0.0155	0.1360	0.1022	0.7480	0.0015	High	30%
18:82	0.0191	0.1635	0.0984	0.7230	0.0018	Medium	95%
21:79	0.0222	0.1898	0.0955	0.6965	0.0021	Less	95%
23:77	0.0230	0.2080	0.0924	0.6776	0.0023	Less	95%

[a]Percent monolith formed with good morphology and good adhesion to the tips (n = 20)

containing 2 mM TEA, a flow rate of 0.7 mL min^{-1}, an injection volume of 20 μL, and a UV detection at 210 nm.

UHPLC-Q Exactive™ Plus Orbitrap HRMS

For the UHPLC-Q Exactive™ Plus Orbitrap HRMS method, the separation was carried out using a Hypersil Gold C18 column (2.1 mm ×100 mm, 1.9 μm, Thermo Scientific, USA). The optimum conditions were a mobile phase of 20:80 (*v/v*) ACN:20 mM ammonium acetate pH 4.0, a flow rate of 0.3 mL min^{-1}, and an injection volume of 5 μL. The MS parameters were adopted from Li et al. (2016a, 2016b) with electrospray ionization in positive mode at 3.5 kV, capillary temperature of 350°C, sheath gas flow at 40 L min^{-1}, and auxiliary gas of 15 L min^{-1}. The mass analysis was MS/dd-MS^2 (data-dependent MS^2) mode, operated at the mass resolution of 70,000 FWHM with a scan range of m/z 150,000 to 400,000.

Sample pretreatment

Pooled swine and cattle urine samples were obtained from three local farms in Nakhon Ratchasima, Thailand. The samples were filtered through 0.2-μm nylon syringe filters and extracted by commercial C18 SPE cartridges or the developed IT monolithic μ-SPEs. The eluates were analyzed by the two HPLC methods.

Method validation

Calibration curves were constructed by analyzing standard solutions of both RAC and CLEN prepared in DI water at five different concentrations (see Table 2). Limits of detection (LOD) and quantification (LOQ) were determined from the concentration giving signal to noise ratio (peak height/baseline noise signal) of 3 and 10, respectively. Repeatability and reproducibility of the monolithic μ-SPE fabrication were estimated by extracting spiked pooled urine containing 100 ng mL^{-1} RAC and 100 ng mL^{-1} CLEN and analyzing by the HPLC-UV method. Five fabricated tips from the same batch and five tips from different batches were tested for within-batch and between-batch study, respectively. Precision in terms of %RSD was calculated from the concentration observed. A recovery study was carried out by spiking standard RAC and CLEN into pooled swine and cattle urine samples to obtain three different concentration levels (see Table 3). Reusability of a tip was investigated by performing 10 replicate extractions of a mixed standard solution of 100 ng mL^{-1} RAC and 100 ng mL^{-1} CLEN (n=3 tips). A carryover study was performed by loading 150 μL of the eluting solvent on the used monolithic tip (n=5 tips) and the eluent analyzed by the HPLC-UV method.

The enrichment factor (E.F.) and extraction efficiency (E.E.) were calculated by the following equations:

$$\text{E.F.} = \frac{C_2}{C_1}$$

$$\text{E.E.} = \frac{C_2 V_2}{C_1 V_1} \times 100$$

where $_1$ = analyte concentration before extraction (ng mL^{-1}), $_2$ = analyte concentration after extraction (ng mL^{-1}), $_1$ = sample loading volume (mL), and $_2$ = eluting solvent volume (mL).

Table 2 Analytical features of the IT monolithic μ-SPE coupled with HPLC-UV and UHPLC-Q Exactive™ Plus Orbitrap HRMS systems

	Concentration range (ng mL^{-1})	t_r (min)	Regression equation	r^2	LOD (ng mL^{-1})	LOQ (ng mL^{-1})
IT monolithic μ-SPE coupled with HPLC-UV						
RAC	25–250	5.34±0.68	$y = 0.6323x - 13.351$	0.9992	3.75	12.50
CLEN	10–200	7.02±0.67	$y = 0.9506x - 4.032$	0.9999	1.88	6.25
IT monolithic μ-SPE coupled with UHPLC-Q Exactive™ Plus Orbitrap HRMS						
RAC	2–250	2.23±0.01	$y = 1804862x + 4137950$	0.9969	0.34	1.13
CLEN	0.8–200	3.31±0.01	$y = 2765788x + 9745616$	0.9947	0.19	0.65

Table 3 Analytical results and percent recoveries of RAC and CLEN in pooled animal urine samples

Concentration of RAC (ng mL^{-1})				Concentration of CLEN (ng mL^{-1})		
Sample	**Added**	**Found**	**Recovery (%)**	**Added**	**Found**	**Recovery (%)**
IT monolithic μ-SPE coupled with HPLC-UV						
Swine urine	0	N.D.	–	0	N.D.	–
	31.8	32.8±0.02	103	16.8	16.9±0.03	101
	63.6	60.2±0.02	95	67.2	69.1±0.02	103
	159.0	159.7±0.09	100	168.0	167.5±0.01	100
Cattle urine	0	N.D.	–	0	N.D.	–
	31.8	30.6±0.03	96	16.8	15.7±0.03	93
	63.6	64.0±0.02	101	67.2	68.3±0.05	102
	159.0	158.3±0.08	100	168.0	165.0±0.16	98
IT monolithic μ-SPE coupled with UHPLC-Q Exactive™ Plus Orbitrap HRMS						
Swine urine	0	N.D.	–	0	N.D.	–
	5.3	5.2±0.01	98	3.1	3.1±0.05	100
	63.6	61.6±0.01	97	67.2	71.0±0.08	106
	159.0	159.0±0.09	100	168.0	169.8±0.29	101
Cattle urine	0	N.D.	–	0	N.D.	–
	5.3	5.2±0.02	98	3.1	3.0±0.03	97
	63.6	60.9±0.01	96	67.2	65.7±0.03	98
	159.0	157.9±0.02	99	168.0	169.7±0.17	101

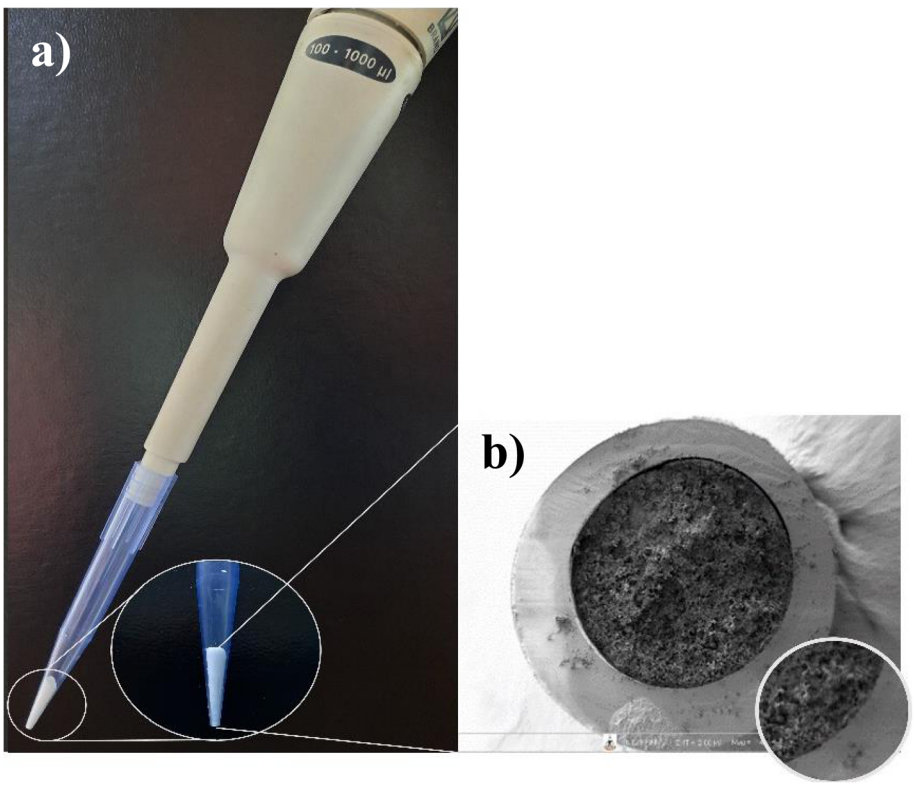

Fig. 1 **a** IT monolithic μ-SPE connected to autopipette and **b** SEM image of a representative IT monolith

Results and discussion

Synthesis and characterization of IT monolithic μ-SPE

RAC and CLEN are not easily extracted together by common C18 sorbent as RAC does not retain on the hydrophobic phase. Thus, we synthesized a monolithic material containing polar carboxylic groups with ester-based structure from co-polymerization of MAA and EDMA in micro-pipette tips for the extraction (see Fig. 1). Preliminary results showed that increasing polymerization temperature from 60 to 75°C reduced the polymerization time from 4 h to 70 min. To obtain sorbent with suitable permeability, the composition of the polymerization solution was optimized by varying the ratio of the monomers to the porogenic solvents from 15:85 to 23:77 (*w/w*) (see results in Table 1).

The composition of the polymerization solution affected not only the permeability but also the adhesion of the material to the wall of the pipette for polymerization time of 70 min; a high amount of the porogens (85%) resulted in detachment of the material from the end of the tip due to the gel-like monolith structure formed, as had been reported before (Li and Li 2015). The immobilization of the monolith was improved by increasing the monomer ratio from 18 to 23%. In addition, good morphology and adhesion of the monolith were observed with a success rate of 95%. The SEM image in Fig. 1b shows that the monolith formed inside the tip with good wall attachment. The optimal composition of monomers to porogens giving suitable permeability and good stability was found to be 18:82 (*w/w*). FT-IR study confirmed the polymerization of MAA with EDMA with absorption peaks at ~1146 cm^{-1} from C–O, ~1723 cm^{-1} from C=O, ~2800–2900 cm^{-1} from C–H, and ~3567 cm^{-1} from O–H bonds, respectively, as reported in previous work (Al-Hetlani et al. 2020).

Development of IT monolithic μ-SPE method for RAC and CLEN extraction

Extraction using commercial C18 SPE cartridge was first investigated. As expected, only the nonpolar CLEN was extracted. The polar RAC was weakly retained on the sorbent and was eluted off by the washing step with water.

The MAA-EDMA monolith was chosen and synthesized due to its mixed mode characteristics having hydrophilic carboxylic and hydrophobic ester-based groups in the structure. The polar RAC and nonpolar CLEN were both retained and extracted together. By comparing the chromatograms of solutions containing both RAC and CLEN with and without extraction, complete retention of RAC and CLEN on the monolithic sorbent was confirmed (Fig.S1). After loading with samples, the monolith was washed with a solvent to remove materials that may be retained on the sorbent. Finally, the adsorbed analytes were eluted off by a suitable solvent. To prevent loss of the trapped analytes in the washing step, the ACN concentration in the washing solution was optimized (Fig.S2). A solution that contained 10% ACN was chosen as it was sufficient to remove weakly adsorbed matrices without desorbing the trapped analytes.

In order to obtain complete elution and study the retention mechanism, the composition of the eluting solvent including organic solvent (ACN) content, type, pH, and concentration of buffer was investigated. It was found that complete elution could only be achieved at acidic condition (Fig. 2a) which is expected since the carboxylate and secondary amine functional groups would be fully protonated at low pH and so releasing the analytes. The elution of RAC was also improved by changing from phosphate to acetate buffer and by increasing the concentration of the buffer from 20 to 200 mM (Fig. 2b). The results showed that RAC is retained on the monolithic sorbent mainly due to ionic interaction, while CLEN interacted with the sorbent via hydrophobic interaction. To obtain the highest enrichment factor, the volume of eluting solvent was kept minimal at 150 μL as required for the HPLC-UV analysis. Using this eluting volume, the minimum ACN concentration needed was found to be 30:70 (*v/v*).

The optimum conditions for extraction are 800 μL washing with 10:90 (*v/v*) ACN:water and 150 μL eluting with 30:70 *(v/v)* ACN:200 mM acetate buffer pH 4.0. The enrichment factors for 2.00-mL sample loading are 12 and 13 for RAC and CLEN and extraction efficiency of 92% and 100%, respectively. However, the enrichment factor can be readily enhanced by increasing sample loading volume as shown in Fig. 3. The extraction required only 395 μL of organic solvent consumption with an extraction time of 5 min. Repeatability of within-batch fabrication was 3.13% and 2.65% for RAC and CLEN, respectively. Batch-to-batch reproducibility was 2.83% and 1.27% for RAC and CLEN, respectively. The tips can be reused for standard solutions at least 10 times with no carryover or loss of extraction performance (%RSD were 2.36 and 4.64 for RAC and CLEN, respectively). Similar with the conventional particulate-packed-based SPE sorbents, it is not recommended to reuse the fabricated tip for unknown samples as adsorption of the previous sample matrix could have occurred.

IT monolithic μ-SPE with analysis by HPLC-UV and UHPLC-Q Exactive™ Plus Orbitrap HRMS

HPLC-UV is available in laboratories due to its wide applications and relatively low cost. We therefore developed a HPLC-UV method for the quantification of the two common β-agonists and compared its performances with a UHPLC-high-resolution mass spectrometer which

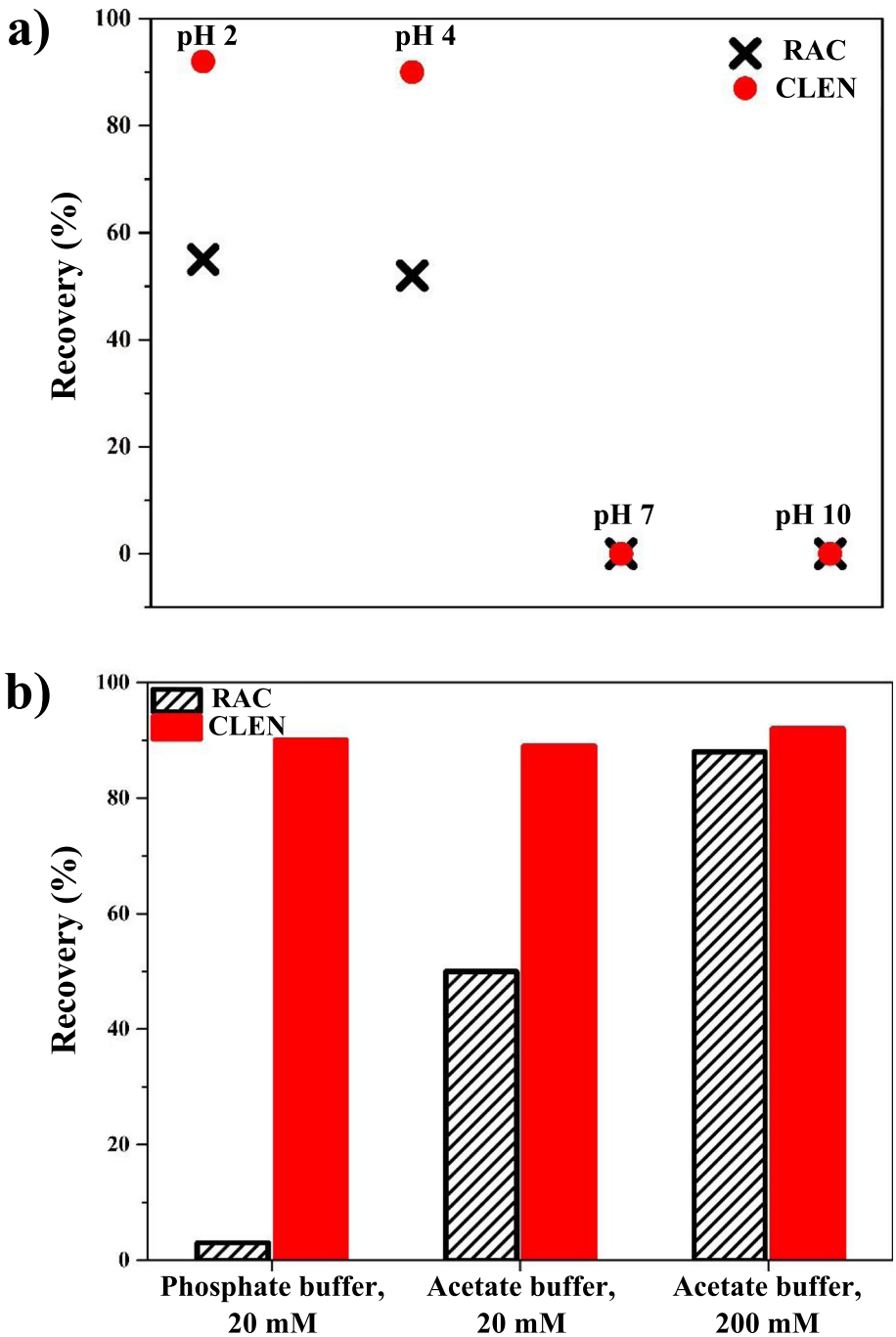

Fig. 2 Effect of **a** pH and **b** types of buffer and buffer concentrations of the eluting solvent on the extraction of spiked urine sample at 1000 ng mL^{-1} RAC and 1000 ng mL^{-1} CLEN. Conditioning solvent, 300 μL MeOH and 50 μL water; washing solvent, 800 μL 10:90 (*v/v*) ACN:water; sample loading, 2.00 mL; eluting solvent, 150 μL 30:70 (*v/v*) ACN:buffer solution. HPLC-UV condition: column C8, mobile phase 25:75 (*v/v*) ACN:20 mM acetate buffer pH 4.0 containing 2 mM triethylamine, injection volume of 20 μL, flow rate of 0.7 mL min^{-1}, and detection at 210 nm

is the most selective and sensitive analytical method. The two HPLC methods were employed with the developed IT monolithic μ-SPE for the analysis of RAC and CLEN in spiked animal urines.

In the HPLC-UV method, a mixture of ACN and 20 mM acetate buffer at the ratio of 25:75 (*v/v*) was employed as the mobile phase. The pH of the buffer was varied from pH 4.0 to 6.0 with TEA to prevent peak tailing. Using the Zorbax SB-C8 column, the two compounds were separated with a mobile phase of 25:75 (*v/v*) ACN:20 mM acetate buffer pH 4.0 containing 2 mM TEA. For UHPLC-Q Exactive™ Plus Orbitrap HRMS,

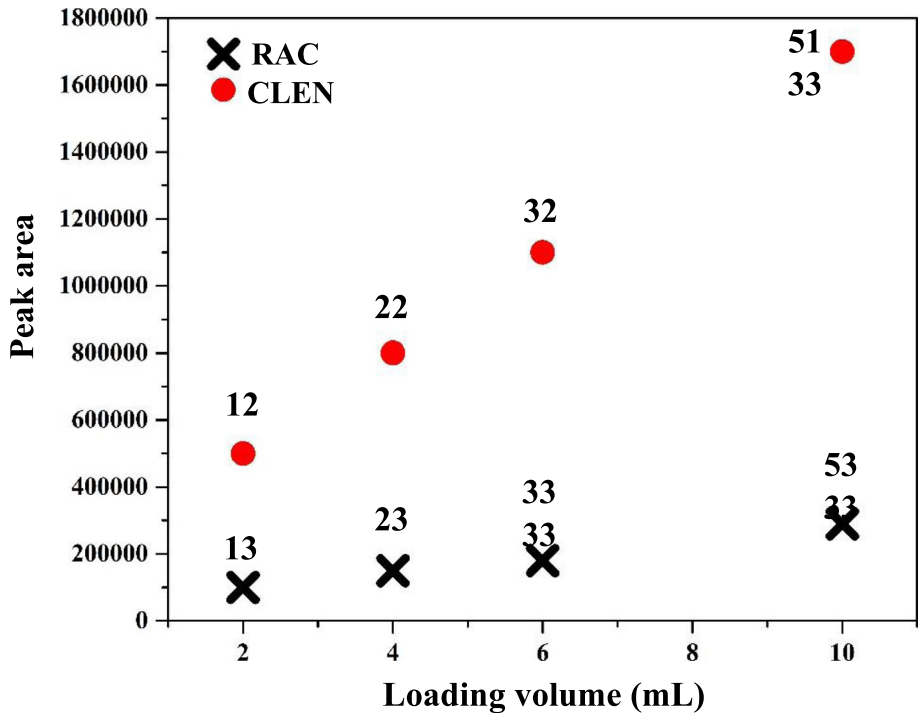

Fig. 3 Effect of sample loading volume on peak areas and enrichment factors of spiked urine sample containing 100 ng mL^{-1} RAC and 100 ng mL^{-1} CLEN, using optimum condition

the separation was achieved on a Hypersil Gold C18 column with mobile phase modified from the HPLC-UV method and operating condition adopted from previous work (Li et al. 2016a, 2016b). The separation was achieved with the mobile phase of 20:80 (*v/v*) ACN:20 mM ammonium acetate buffer pH 4.0. In this method, the injection volume was less than in the HPLC-UV method as smaller packing particles were employed. The UHPLC-Q Exactive™ Plus Orbitrap HRMS had ten times lower LOD and LOQ with shorter analysis time. Analytical performances for the two methods are summarized in Table 2. As the UHPLC-Q Exactive™ Plus Orbitrap HRMS method has higher sensitivity, a wider linear calibration range can be achieved for both compounds. Typical values for residue urinary RAC in animal urines reported are between 13 and 4712 ng mL^{-1} during the administration period and between 2 and 853 ng mL^{-1} after cessation (Pleadin et al. 2011; Qiang et al. 2007; Tang et al. 2016). For CLEN, concentrations found are between 13 and 89 ng mL^{-1} and 0.5 and 43 ng mL^{-1} during administration and after cessation, respectively (Li et al. 2016a, 2016b; Pleadin et al. 2011; Pleadin et al. 2009). Therefore, the two techniques are capable of analysis of real samples. In addition, extraction allows enhancement of the sensitivity by increasing the sample loading volume.

Application to real samples

The IT monolithic μ-SPE together with the HPLC-UV and UHPLC-Q Exactive™ Plus Orbitrap HRMS analysis were applied to the analysis of RAC and CLEN in pooled cattle and swine urine samples. RAC and CLEN were not detected in samples from local farms by the two methods (Table 3 and Fig. 4). Analysis of spiked samples at three different concentrations was carried out for recovery investigation. Good percent recovery was obtained for the two HPLC methods. The results are not significantly different at 99% confidence using pair *t*-test (t_{stat} and t_{crit} were −0.131 and 2.977, respectively). Mass spectra from the UHPLC-Q Exactive™ Plus Orbitrap HRMS for RAC and CLEN extracted from urine samples are compared with those of pure standards (Fig. 5). The results confirm there were no interfering ions from the sample matrices.

Conclusions

The mixed functional group monolithic sorbent showed excellent extraction performance for simultaneous micro-extraction of the two important β-agonists, viz. RAC and CLEN, in swine and cattle urines prior to the analysis by HPLC with UV or high-resolution MS/MS. Good analytical performances in terms of accuracy, precision, and linearity were observed. The HRMS provided about ten times lower LODs and LOQs with wider linear range and shorter analysis time. The good correlation between HPLC with UV detection and the highly selective mass spectrometric detection confirms no matrix effect is present for the HPLC-UV system and thus sample

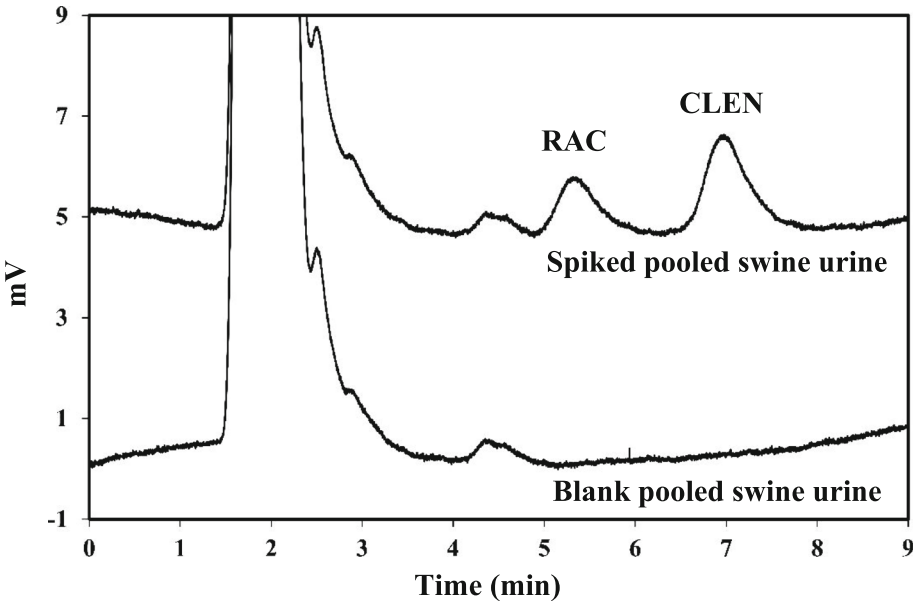

Fig. 4 Representative chromatograms of blank and spiked pooled swine urines containing 64 ng mL^{-1} RAC and 67 ng mL^{-1} CLEN extracted by the IT monolithic μ-SPE with HPLC-UV analysis. See caption of Fig. 2 for HPLC conditions

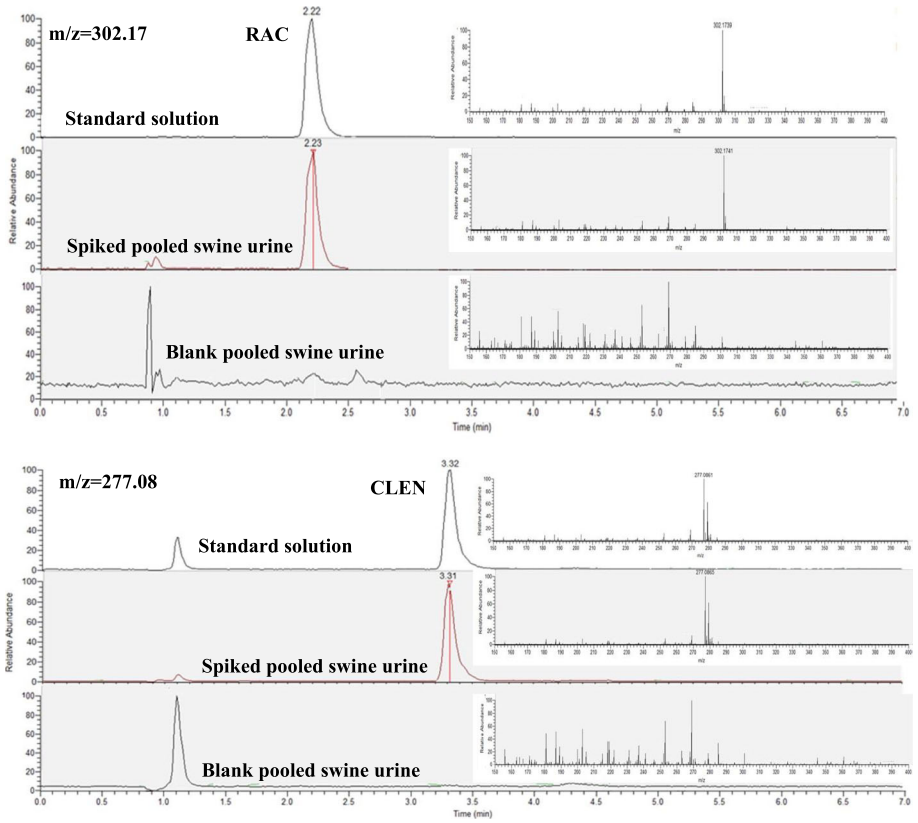

Fig. 5 Representative chromatograms and mass spectra of standard solution, blank, and spiked pooled swine urines containing 64 ng mL^{-1} RAC and 67 ng mL^{-1} CLEN extracted by the IT monolithic μ-SPE with UHPLC-Q Exactive™ Plus Orbitrap HRMS analysis

clean-up by the developed μ-SPE is efficient. As the analytical sensitivity can be readily enhanced by increasing the sample loading volume in the extraction step, coupling of the developed monolithic μ-SPE and HPLC-UV system can be applied for sensitive detection of RAC and CLEN in animal urines as a deterrent for illegal use of these compounds.

Abbreviations

ACN: Acetonitrile; AIBN: Azobisisobutylronitrile; CLEN: Clenbuterol; EDMA: Ethylene dimethacrylate; HPLC-UV: High-performance liquid chromatography-ultraviolet detection; IT monolithic μ-SPE: In pipette tip monolithic micro-solid phase extraction; LOD: Limit of detection; LOQ: Limit of quantification; MAA: Methacrylic acid; MeOH: Methanol; RAC: Ractopamine; SEM: Scanning electron microscope; SPE: Solid phase extraction; TEA: Triethylamine; UHPLC-Q Exactive™ Plus Orbitrap HRMS: Ultra-high-performance liquid chromatography-quadrupole Exactive™ Plus Orbitrap high-resolution mass spectrometer

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40543-021-00275-5.

Additional file 1: Fig. S1. Chromatograms of standard solution containing 1000 ng mL^{-1} RAC and 1000 ng mL^{-1} CLEN a) before and b) after loading on the IT monolithic μ-SPE. Separation condition: column C8, mobile phase of 25:75 (*v/v*) ACN:20 mM acetate buffer pH 4.0 containing 2 mM triethylamine, injection volume of 20 μL, flow rate of 0.7 mL min^{-1} and detection at 210 nm. **Fig. S2.** Effect of ACN concentration in washing solvent on extraction of spiked urine sample containing 1000 ng mL^{-1} RAC and 1000 ng mL^{-1} CLEN. Conditioning solvent: 300 μL MeOH and 50 μL water; sample loading: 2.00 mL; washing solvent: 800 μL of ACN and water mixture; eluting solvent: 150 μL 30:70 (*v/v*) ACN:200 mM acetate buffer pH 4.0.

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Authors' contributions

All authors have equal contribution to this research work. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Declarations

Competing interests

The authors declare that they have no competing interests.

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