Detection of β -agonists in pork tissue with novel electrospun nanofibers-based solid-phase extraction followed ultra-high performance liquid chromatography/tandem mass spectrometry

Lanling Chu ^{a,1}, Shenglan Zheng ^{b,1}, Bin Qu ^b, Shiwei Geng ^b, Xuejun Kang ^{a, c*}

^a Key Laboratory of Environmental Medicine and Engineering (Ministry of Education), School of Public Health, Southeast University, Nanjing 210096, China

^b Animal Products Quality Inspection and Test Center in Jiangsu Province, Nanjing 210036, China

^c Key Laboratory of Child Development and Learning Science (Ministry of Education), Research Centre for Learning Science, Southeast University, Nanjing 210096, China

ABSTRACT:

A selective analytical method based on packed-fiber solid-phase extraction and ultra-high performance liquid chromatography–tandem mass spectrometry (PFSPE-UPLC–MS/MS) has been developed for determination of six β -agonists (clorprenaline, bambuterol, clenbuterol, brombuterol, mabuterol, and penbuterol) in pork tissue. Polystyrene-polymeric crown ether (PS-PCE) composite nanofibers were fabricated by electrospinning and utilized to prepare the homemade extraction columns. With optimal conditions, all analytes were separated very well and the blank pork did not disturb the determination, and the linearity is good at a range of 5.0 ug/kg–25.0 ug/kg. The recoveries were in the range of 79.3%–110.1%. RSDs for intra-day were in the range of 1.5%–10.5% and RSDs for inter-day were in the range of 4.7%–11.8%. Above all, only 5 mg sorbent and 200 μ L elution solvent were favorable to directly extract all analytes in a complex matrix. The method is simple and cost-effective, and has the potential to be applied to quantitatively analyze the concentrations of polar species in food samples containing complex

¹ Joint first author. These authors contributed equally to the work.

* Corresponding author: E-mail: xjkang64@163.com; Tel: +86-025-83795664-1011; Fax: +86-025-83795929

matrix.

Keywords: β -Agonists; Nanofibers solid-phase extraction; Ultra-high performance liquid chromatography/tandem mass spectrometry; Pork tissue.

1. Introduction

Synthetic β -agonists have demonstrated efficiency in enhancing growth rates and feed efficiency (Johnson, Smith & Chung, 2014) in food producing animals, so they have been applied to the livestock industry such as pigs and ruminants to reduce carcass fat and increase muscle mass while improving growth rate and feed conversion (Cardoso & Taveira, 2002; Shook et al., 2009). Unfortunately, β -agonists deposited in animal tissues can cause acute poisoning when consumed by humans, particularly in people with symptoms of muscular tremors, cardiac palpitation, nervousness, headache, muscular pain, dizziness, nausea, vomiting, fever and chill (Brambilla et al., 2000). The presence of residues and associated harmful effects on humans makes the control of such veterinary drug residues an important measure in ensuring consumer protection (Beucher, Dervilly-Pinel, Prévost, Monteau & Le Bizec, 2015). Therefore, the determination method of β -agonists residues were urgently proposed.

Recently, many analytical methods for the determination of β -agonists residues in biological samples have been published, popularly using liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Li, Zhang, Wang, Wang & Su, 2015; Wang, Pu, Wang, Luo, Zhang & Zhang, 2015; Wang, Liu, Su & Zhu, 2015; Wang, Guo, Wang, Yuan & Zhao, 2015; Wu, Liu & Peng, 2014; Xiong, Gao, Li, Guo & Yang, 2015). As known to all, the sample pretreatment is a great of important procedure in the trace residues analysis especially for the residues of illegal drugs. Commonly, liquid-liquid extraction (Li et al., 2015), solid phase extraction (SPE) (Juan, Igualada, Moragues, León & Mañes, 2010; Li, Cao, Li, Wang & He, 2016; Nielen et al., 2008; Wang, et al , 2015; Xiong et al., 2015), immuno-affinity chromatography (Dos

Ramos, 2000; Junhua et al., 2015), molecularly imprinted polymer extraction (Wang et al., 2015), ion mobility-mass spectrometry (Beucher et al., 2015) or integrated procedure of above sample preparation method was applied into clean-up of the complex sample to decrease the sample matrix interference. Regrettably, above sample preparation methods, some are quite laborious, some need extremely expensive instrumentation, some were consuming the large volume of organic solvents, and some were suffering from the low selectivity or long analysis time. Fortunately, a novel separation technique, packed-fiber solid-phase extraction (PFSPE), has emerged recently which may help dealing with issues encountered in the complex foodstuff matrices.

Owing to their unique mechanical and chemical properties such as large surface area to volume ratio, both cheap and easy to obtain, electrospun nanofibers have the potential to serve as a good sorbent material for SPE-based techniques (Chigome & Torto, 2012). Surprisingly, since 2007 PFSPE has been successfully applied to the pharmaceutical analysis and pharmacokinetics studies of animals and humans (Kang, Chen, Zhang, Liu & Gu, 2008; Kang et al., 2007; Tian et al., 2012; Zhang, Kang, Chen, Pan, Yao & Gu, 2008), analysis of functional or contraband substances in foodstuff (Hu et al., 2014a; Si-Wei, Jian-Jun, Ting-Ting, Yu & Xue-Jun, 2014), and determination of environmental pollutants in various sample matrices (Deng, Kang, Chen, Wang, Gu & Lu, 2011; Qi, Kang, Chen, Zhang, Wei & Gu, 2008), *etc.* However, most published work has concentrated on the extraction of nonpolar compounds. Its application for the extraction of moderate polar or polar compounds is still limited because of the less polar property and neutral charge of fiber materials, which resulted in the poor analyte recoveries for polar compounds, especially in complex matrices.

During this study, a selective sorbent, polystyrene-polymeric crown ether composite electrospun nanofibers (PS-PEC nanofibers), were fabricated, characterized and applied to extract β -agonists. Crown

ether has been proved to be a versatile extractant (Khopkar & Gandhi, 1996). It can be immobilized on the solid phase by synthetic reaction to enhance extraction performance (Voloshina & Kamalov, 2014). In this work, the immobilization of PEC on the solid phase was finished by co-electrospinning of the mixture solution of PS polymer and PEC, and PFSPE coupled with high performance liquid chromatography–tandem mass spectrometry (PFSPE HPLC-MS/MS) has been used for the first time for simultaneous determination of six β -agonists in pork samples. Not only were compared on the extraction efficiency of PS nanofibers and PS-PEC nanofibers, the key PFSPE factors affecting the extraction efficiency of analytes, *i.e.*, the pH value and volume of eluent, were systematically optimized.

2. Experimental

2.1. Materials and reagents

Standards of β -agonists were obtained from Sigma-Aldrich (St. Louis, USA): clorprenaline, bambuterol, clenbuterol, brombuterol, mabuterol, penbuterol, clenbuterol-D₉ and mabuterol-D₉. Polystyrene (PS) and Polymeric Crown Ether (PCE) were provided by Department of Applied Chemistry, Nanjing University of Technology. HPLC grade methanol and acetic acid were obtained from Nanjing Chemical Agents Co. (Nanjing, China).

2.2. Instruments and apparatus

A pH meter (Shanghai, China) with the glass electrode was used for measuring the pH value. A high-voltage power supply (model DWP403-1AC, Tianjin, China), and a syringe pump were used for electrospinning. The nanofibers were characterized by the FT-IR Spectrophotometer NICOLET 5700 (Nicolet, US), Hitachi S-3000N scanning electron microscope (SEM, Tokyo, Japan) at an acceleration voltage of 6.0 kV, and Tecnai G20 transmission electron microscopy (TEM, FEI, America) at an acceleration voltage of 200 kV. Chromatographic separation was operated on the Waters Acquity UPLC

System (Waters Technologies, American) where a 100 mm × 2.1 mm × 1.7 μm C18 analytical column (BEH C18, Waters). [Detecting](#) β-Agonists was performed on a Thermo Finnigan TSQ quantum Discovery MAX connected to a quadrupole mass spectrometer with the positive electrospray ionization mode (ESI) source.

2.3. Fabrication of electrospun nanofibers

The electrospun PS nanofibers were prepared by loading PS solution into a glass syringe, which was fitted to a steel needle with a filed flat tip. A high-voltage generator was linked with the needle through a copper pin. A grounded iron drum mantled with a copper grid was served as the collection screen. A dense web of the fibers was collected on the copper grid. The detailed explanation [and](#) illustrated procedure was described in previously published literature ([Qi et al., 2008](#)).

PS-PEC nanofibers were fabricated as follows. First, [PS](#) (10%, w/v) solution was prepared by dissolving an appropriate amount of PS in the mixed solvent of dimethylformamide ([DMF](#)) and tetrahydrofuran (4:6, v/v), and [PEC](#) (5%, w/v) solution was obtained by dissolving an appropriate amount of PEC in the solvent of [DMF](#). Final electrospun solution was obtained by mixing above PS solution and PEC solution (5:2) with magnetic stirring. Next, the mixed solution was loaded into a glass syringe which was fitted with a steel needle with a tip diameter of 0.5 mm whose tip was filed flat. Then electrospinning performance was shown in [Supplemental Fig. 1](#) in this condition: an anodic voltage of 19.5 kV, 12.5 cm from the tip of the needle to the collecting equipment, the feed rate of 1.8 mL/h for precursor solution, and the experimental ambient conditions of temperature and humidity were 23 °C and 35%, respectively. [PS-PEC](#) nanofibers were collected on the collector which was covered by a piece of aluminum foil.

2.4 Preparation of PFSPE column

[The](#) novel PFSPE column, as shown in [Fig. 1](#), was designed for pretreatment of the aqueous sample. 5

mg of nanofibers was packed into the column with an inside diameter of 1.5 mm. A conical liquid storage cartridge was attached to the column. The pressurizer was available by using a syringe with a modified tip which was fitted with the liquid storage cartridge.

2.5. Sample preparation

2 g of the homogenized pork sample was weighted into a 10 mL polypropylene centrifuge tube and mixed with 100 μ L internal standard solution (clenbuterol-D₉ and mabuterol-D₉, 100 ng/mL). After adding 5 mL (5%, V/V) of perchloric acid aqueous solution to the sample to precipitate the protein then homogenated, the tube was capped and oscillated for 20 min, then centrifuged for 5 min at 10000 rpm. The pH value of the supernatant was adjusted and then applied to PFSPE procedure

2.6. PFSPE procedure

The nanofibers should be activated before use. The extraction columns were conditioned by washing with 100 μ L of methanol, followed by 100 μ L of deionized water. After that, the nanofibers were ready for sample pretreatment. Sample supernatant prepared was loaded into the cartridge and pushed through the pre-conditioned adsorbent by air pressure forced by a gas-tight plastic syringe (10 mL), which was fitted to the top of the extraction cartridge. The analytes retained on the fibers were quantitatively eluted with 200 μ L of methanol. The flow rate was carefully controlled in a slow dropwise manner in the adsorption and desorption procedures. The desorption solution was added 300 μ L of 0.1% (V/V) formic acid aqueous solution and vortexed for 1min. Finally, 10 μ L of adjusted eluate was analyzed immediately by HPLC–MS/MS. All operations were performed at room temperature.

2.7 Analytical conditions

Chromatographic separation was carried out on a Waters Acquity UPLC system described as above. The column oven temperature was set at 25°C, flow rate was at 0.2 mL/min, and injection volume was 10 μ L.

The mobile phase consisted of 0.1% formic acid in water (A) and 0.2% formic acid in acetonitrile (B). The initial composition was 90 % A and 10 % B. A gradient elution was performed where phase B was increased linearly to 35 % in the first 3.0 min, next increased to 80% in another 2.0 min then kept for 1.5 min and finally returned to the initial composition in 0.2 min. The column was then equilibrated for 2.8 min before next injection.

Mass spectrometric acquisition was carried out on Thermo Finnigan TSQ quantum Discovery MAX described as above. The optimum ionization of β -agonists was achieved in a positive mode of ion-spray voltage of 3.5 kV. Nitrogen (99.999%) was used as nebulizing gas. Symmetrical heaters were operated at 350 °C with sheath gas flow rate at 15 L/min, assistant flow rate at 5 L/min and argon as collision gas (1.5 torr). The retention time, precursor ion, product ions and collision energy for each analyte are listed in [Supplemental Table 1](#).

2.8. Method validation

Quantification was performed using the internal standard method. The blank pork was regarded as that the pork was determined using the national standard method (GB, 2008) and the target β -agonists did not exit. Standard solutions were prepared containing six β -agonists in the concentrations of 1, 5, 10, 15, 20 and 25 μ g/kg, and then a series of standard solution was mixed with 100 μ L of internal standard solution (Clenbuterol-D₉, Mabuterol-D₉, 100 ng/mL). The extraction of mixed samples was then performed as described above. The calibration curves were constructed by plotting the peak-area ratio of β -agonists to internal standard against the concentrations of β -agonists.

The spiked blank solution was prepared containing β -agonists at concentrations of 3, 12.5, 17.5 μ g/kg, and then spiked solution was mixed with 100 μ L of internal standard solution (Clenbuterol-D₉, Mabuterol-D₉, 100 ng/mL). The extraction of mixed samples was then performed as described above.

Recoveries were determined as percentages of the measured concentrations (calculated from the standard curve equation) against the spiked concentrations.

The intra-day, inter-day precision and accuracy were evaluated for blank samples spiked with six β -agonists at the concentration of 12.50 $\mu\text{g/kg}$ according to the above pretreatment method. The inter-day precision was evaluated on five successive days. The precision of the method was reported as the relative standard deviation (RSD) of repeatability of the analyte concentration. Limit of detection (LOD) was estimated by the concentration that gave a signal equal to the blank plus 3 times the standard deviation, while limit of quantification (LOQ) is defined as the amount of the analytes that gave a signal equal to the blank plus 10 times the standard deviation.

3. Results and discussion

3.1. Morphology of nanofibers

PS-PCE composite nanofibers were proposed as the candidate sorbent in this study because of the efficient sample pretreatment of PS nanofibers in extraction (Chen, Kang, Sun, Deng, Gu & Lu, 2010; Chigome, Darko, Buttner & Torto, 2010; Hu et al., 2014b; Qi, et al., 2008; Zhang, et al., 2008), potential interaction between PCE polymer and polar compounds (Lee, Oh, Pathak, Paeng, Cho & Paeng, 2007), potential interaction between PCE polymer and agonists (Vaccher, Berthelot & Debaert, 1995), and selective interaction between PS-PCE nanofibers and catecholamine (Chen, Wang, Qu, Deng & Kang, 2015). Two types of fibers, PS single-component nanofibers and PS-PCE composite nanofibers, were used to explore the extraction mechanism.

PS-PCE nanofibers were identified by IR spectra, as shown in Fig. 2a. Compared to IR spectra of the PS nanofibers showed in Fig. 2a', many new peaks appeared in the IR spectra of PS-PCE nanofibers showed in Fig. 2a, which were similar to the IR spectra of PCE. According to the literature (Eaborn, 1979), the new

peaks at 1137 cm^{-1} , 1256 cm^{-1} , 1507 cm^{-1} and 540 cm^{-1} was respectively due to $\text{CH}_2\text{-O-CH}_2$ stretching vibration, C-N stretching vibration, C-O stretching vibration and N-H stretching vibration donated by PCE. It seems that PCE was successfully impregnated in the PS nanofibers.

As illustrated in Fig. 2b and Fig. 2c, the diameter of the PS-PCE nanofibers was 200-900 nm and the nanofibers were dense with network structure. Though PS nanofibers were modified with PCE, major morphological change was not observed in the view of the SEM images shown in Fig. 2b and Fig. 2b'. Interestingly, the surface of PS-PCE nanofibers is rough and their smoothness is decreased compared with PS nanofibers.

Fig. 2c and Fig. 2c' showed the TEM images of the fibers. It is clear that the surface morphology of the composite nanofibers is rough, as we discussed above. What's more, PS-PCE nanofibers (Fig. 2c) exhibited alternately dark and bright spots of nanofibers signifying numerous nanopores compared with PS nanofibers (Fig. 2c'). The composite nanofibers possess more porous structures, which should significantly increase the surface area of the fiber. The non-woven mats/fabrics composed of electrospun nanofibers offer unique abilities to supply an enormous number of pore sizes among the adjacent nanofibers, which should result in a significantly increased mass transfer during the adsorption as well as the analytes desorption process.

3.2 Chromatogram analysis of six β -agonists

Fig. 3a showed the chromatogram of blank pork spiked six β -agonists ($25.0\text{ }\mu\text{g/kg}$) and Fig. 3b displayed the chromatogram of blank pork. It can be seen from Fig. 3, six β -agonists were separated very well and no interfering substances were existed in blank pork.

3.3 Optimization of PFSPE parameters

3.3.1 Adsorption efficiency of PS and PS-PEC nanofibers

Two types of extraction columns of PS and PS-PCE nanofibers were prepared. Six β -agonists standard solution of every 20 ng/mL was loaded 1 mL into the extraction cartridge. After loading and eluting, targets were analyzed immediately by HPLC–MS/MS. The extraction efficiency of PS and PS-PEC nanofibers for the target compounds was displayed in Fig. 4a.

As shown in Fig. 4a, the extraction efficiency of six β -agonists onto the PS-PCE nanofibers was all superior to that onto the PS nanofibers. The reason was inferred that PCE molecule containing benzene rings, amide group and crown ether structure increases the adsorption sites of adsorbent for target molecules, and the interaction between adsorbent and targets generated polar force except non-polar force. It caused better adsorption efficiency of six β -agonists onto the PS-PCE nanofibers. Thus, in this study PS-PCE nanofibers were selected as optimal sorbents for the extraction of β -agonists in pork tissue.

3.3.2 Effect of pH

Six β -agonists standard solution of every 20 ng/mL was adjusted to desired pH value of 3, 5, 7, 10 and 13 before being loaded into the cartridge by 1mol/mL hydrochloric acid solution or saturated solution of NaOH. After loading and eluting, the targets were analyzed immediately by HPLC–MS/MS.

As Fig. 4b shown, alkaline condition is preferred to extraction of targets. It can be inferred that β -agonist molecule was non-polar and easy interaction with adsorption sites of sorbent in alkaline medium. While in acidic medium, β -agonist molecules were strongly polar and not conducive to adsorption onto the PS-PCE nanofibers. Furthermore, the sorbent including amino group might be protonated at low pH value, which could generate repulsion with the ammonium ions in the β -agonist molecules.

As far as alkaline medium, the extraction efficiency increased slightly with increasing pH value from 10 to 13 and the extraction efficiency of mabuterol decreased with the pH 13. The reason may be that the increasing pH value increased ionic strength of the medium. Generally speaking, if the adsorbent is

polymeric adsorbents, the increase of ionic strength in solution caused contraction of adsorbent and decrease of adsorbent porosity, which reduced the whole adsorption sites of sorbent (Lan, Bassi, Zhu & Margaritis, 2001; Xu, Zhou & Wang, 2008). So the higher pH value in sample solution caused lower extraction efficiency of β -agonists onto the nanofibers. Thus, in this study the optimum range of pH value for the β -agonists adsorption onto PS-PCE composite nanofibers was pH 10.

3.3.3 Effect of eluent volume

Methanol was selected as eluent because β -agonists could be well dissolved in methanol. Six β -agonists standard solution of every 20 ng/mL was adjusted to the desired pH value and conducted PFSPE procedure with 1mL standard solution. Then the nanofiber column adsorbed targets was eluted by methanol with different volume of 20 μ L, 50 μ L, 100 μ L, 200 μ L, 300 μ L. Every eluent was collected and then injected into UPLC-MS/MS and analyzed.

As shown in Fig. 4c, with increasing volume of eluent, the elution efficiency enhanced. As volume of 200 μ L eluent, the targets adsorbed onto the PS-PCE nanofibers could be effectively eluted. If continue to increase the volume of eluent, the elution effect hardly improved but the concentration ratio of eluent was dwindled. Thus, 200 μ L was selected as optimal volume of eluent for the PS-PCE nanofiber column.

3.4 Linearity and LOD

As shown in Table 1, a good linearity was found at the range 5.0-25.0 μ g/kg for six β -agonists. LODs of all analytes were in the range of 0.1-0.2 μ g/kg and LOQs were in the range of 0.3-0.6 μ g/kg, indicating the method is appropriate for the determination of β -agonists in pork samples. Even though only 10 μ L of extract is injected for analysis, these LODs and LOQs are lower than the national standard method and meet requirements (GB, 2008).

3.5 Recovery and precision

As shown in Table 2, the proposed sample handling exhibited relatively high extraction efficiency for all analytes. An acceptable range of recoveries was obtained, which were 83.3%-110.1% for the all target compounds in different spiked concentration samples. The intra-day and inter-day RSDs for the three spiked concentration samples was respectively 1.5%-10.5% and 4.7%-11.8%, all below 12%. The precision of the method is in accordance with the requirements of the β -agonists determination of pork samples.

3.6 Comparison of PFSPE with pretreatment method of national standard

PFSPE method was compared with the pretreatment method based on the national standard used for the determination of β -agonists in pork (GB, 2008). The comparison was in terms of sample amount, solvent use, time cost, LOD and recovery. As shown in Supplemental Table 2, PS-PCE nanofiber columns exhibited adequate LOD and recovery similar to the pretreatment method of national standard. In addition, PFSPE has a great economic advantage over the pretreatment method of national standard because less amount of reagents (only 0.5 mL) was used and the time cost greatly less (only 0.5 h). What's more, evaporation is not necessary after extraction. Therefore, PFSPE could be an ideal pretreatment method for traditional extraction methods especially for the complex matrix samples. The results also suggest that the developed method is simple, convenient, technically feasible, economically competitive, and environmentally friendly.

Additionally, the CRM (Certified Reference Material) pork sample could theoretically be adopted to further validate proposal method. It is more valuable and reliable to compare the measurement result on a CRM with the certified value. Unfortunately, the required CRM (even though containing one of the analytes) can't be found and purchased. It is unavailable so it is not feasible to use the CRM pork sample. Therefore, we solved this problem by spiking blank sample as conventional method because the spiked

method is simple and feasible with the same or similar function as the CRM.

4. Conclusion

In this study, a more convenient and selective protocol was presented to extract and determine six β -agonists in pork tissue by a novel PFSPE-HPLC–MS/MS procedure using the novel adsorbent of electrospun composite PS-PCE nanofibers packed into a sample pretreatment device. The composite nanofibers with increasing adsorption sites for polar target molecules enhanced adsorption effect. In addition, target compounds were extracted effectively from pork samples by optimizing the pH value of medium and the volume of eluent. Moreover, most of interfering substances in pork samples were removed, which reflected a good characteristic of selective extraction of β -agonists. This convenient method can be applied to the determination of actual pork samples because of the efficient sample pretreatment and clean-up effect. Results of this study showed that PFSPE-HPLC–MS/MS is a selective and cost-effective technique to determine β -agonists in pork products.

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References

Beucher, L., Dervilly-Pinel, G., Prévost, S., Monteau, F., & Le Bizec, B. (2015). Determination of a Large Set of β -Adrenergic Agonists in Animal Matrices Based on Ion Mobility and Mass Separations. *Analytical*

287   *Chemistry*, 87(18), 9234-9242.

288   Brambilla, G., Cenci, T., Franconi, F., Galarini, R., Macrì, A., Rondoni, F., Strozzi, M., & Loizzo, A. (2000).

289   Clinical and pharmacological profile in a clenbuterol epidemic poisoning of contaminated beef meat in

290   Italy. *Toxicology Letters*, 114(1), 47-53.

291   Cardoso, L. A., & Taveira, O. (2002). Effect of clenbuterol on growth, nitrogen and energy balances and

292   endocrine status in food-restricted sheep: research communication. *Journal of The South African*

293   *Veterinary Association*, 73(3), 127-130.

294   Chen, L. Q., Kang, X. J., Sun, J., Deng, J. J., Gu, Z. Z., & Lu, Z. H. (2010). Application of nanofiber-packed

295   SPE for determination of salivary - free cortisol using fluorescence precolumn derivatization and HPLC

296   detection. *Journal of Separation Science*, 33(15), 2369-2375.

297   Chen, L. Q., Wang, Y., Qu, J. S., Deng, J. J., & Kang, X. J. (2015). Selective extraction of catecholamines by

298   packed fiber solid - phase using composite nanofibers composing of polymeric crown ether with

299   polystyrene. *Biomedical Chromatography*, 29(1), 103-109.

300   Chigome, S., Darko, G., Buttner, U., & Torto, N. (2010). Semi-micro solid phase extraction with electrospun

301   polystyrene fiber disks. *Analytical Methods*, 2(6), 623-626.

302   Chigome, S., & Torto, N. (2012). Electrospun nanofiber-based solid-phase extraction. *TrAC Trends in*

303   *Analytical Chemistry*, 38, 21-31.

304   Deng, J., Kang, X., Chen, L., Wang, Y., Gu, Z., & Lu, Z. (2011). A nanofiber functionalized with dithizone by

305   co-electrospinning for lead (II) adsorption from aqueous media. *Journal of Hazardous Materials*, 196,

306   187-193.

307   Dos Ramos, F. J. (2000). β 2-Agonist extraction procedures for chromatographic analysis. *Journal of*

308   *Chromatography A*, 880(1), 69-83.

309 Eaborn, C. (1979). The chemistry of the carbon-carbon triple bond: edited by S. Patai, Wiley-Interscience,
 310 Chichester, New York, Brisbane and Toronto, 1978, 1065 pages (in two parts). *Journal of Organometallic*
 311 *Chemistry*, 171(2), C44.
 312 GB/T 22286-2008. Determination of β -agonists residues in foodstuff of animal origin—Liquid chromatography
 313 with tandem-mass spectrometric method [S]. 2008.
 314 Hu, W., Kang, X., Zhang, C., Yang, J., Ling, R., Liu, E., & Li, P. (2014). Packed-fiber solid-phase extraction
 315 coupled with high performance liquid chromatography - tandem mass spectrometry for determination of
 316 diethylstilbestrol, hexestrol, and dienestrol residues in milk products. *Journal of Chromatography B*, 957,
 317 7-13.
 318 Johnson, B. J., Smith, S. B., & Chung, K. Y. (2014). Historical Overview of the Effect of β -Adrenergic
 319 Agonists on Beef Cattle Production. *Asian-Australasian Journal of Animal Sciences*, 27(5), 757.
 320 Juan, C., Igualada, C., Moragues, F., León, N., & Mañes, J. (2010). Development and validation of a liquid
 321 chromatography tandem mass spectrometry method for the analysis of β -agonists in animal feed and
 322 drinking water. *Journal of Chromatography A*, 1217(39), 6061-6068.
 323 Junhua, L., Chunsheng, L., Meng, W., Yan, Z., Xiaofei, M., Hua, C., & Jinghui, Y. (2015). Development of an
 324 Ultrasensitive Immunochromatographic Assay (ICA) Strip for the Rapid Detection of Phenylethanolamine
 325 A in Urine and Pork Samples. *Journal of Food Science*, 80(4), T894-T899.
 326 Kang, X. J., Chen, L. Q., Zhang, Y. Y., Liu, Y. W., & Gu, Z. Z. (2008). Performance of electrospun nanofibers
 327 for SPE of drugs from aqueous solutions. *Journal of Separation Science*, 31(18), 3272-3278.
 328 Kang, X., Pan, C., Xu, Q., Yao, Y., Wang, Y., Qi, D., & Gu, Z. (2007). The investigation of electrospun
 329 polymer nanofibers as a solid-phase extraction sorbent for the determination of trazodone in human plasma.
 330 *Analytica Chimica Acta*, 587(1), 75-81.

331 Khopkar, S. M., & Gandhi, M. N. (1996). Solvent extraction separations with crown ethers and cryptands.

332 Lan, Q., Bassi, A. S., Zhu, J. J., & Margaritis, A. (2001). A modified Langmuir model for the prediction of the

333 effects of ionic strength on the equilibrium characteristics of protein adsorption onto ion exchange/affinity

334 adsorbents. *Chemical Engineering Journal*, 81(1), 179-186.

335 Lee, M., Oh, S. Y., Pathak, T. S., Paeng, I. R., Cho, B., & Paeng, K. (2007). Selective solid-phase extraction of

336 catecholamines by the chemically modified polymeric adsorbents with crown ether. *Journal of*

337 *Chromatography A*, 1160(1), 340-344.

338 Li, T., Cao, J., Li, Z., Wang, X., & He, P. (2016). Broad screening and identification of β -agonists in feed and

339 animal body fluid and tissues using ultra-high performance liquid chromatography-quadrupole-orbitrap

340 high resolution mass spectrometry combined with spectra library search. *Food Chemistry*, 192, 188-196.

341 Li, Y., Zhang, W., Wang, R., Wang, P., & Su, X. (2015). Development of a Efficient and Sensitive Dispersive

342 Liquid – Liquid Microextraction Technique for Extraction and Preconcentration of 10 β 2-Agonists in

343 Animal Urine. *PLoS One*, 10(9), e137194.

344 Nielen, M., Lasaroms, J., Essers, M. L., Oosterink, J. E., Meijer, T., Sanders, M. B., Zuidema, T., & Stolker, A.

345 (2008). Multiresidue analysis of beta-agonists in bovine and porcine urine, feed and hair using liquid

346 chromatography electrospray ionisation tandem mass spectrometry. *Analytical and Bioanalytical*

347 *Chemistry*, 391(1), 199-210.

348 Qi, D., Kang, X., Chen, L., Zhang, Y., Wei, H., & Gu, Z. (2008). Electrospun polymer nanofibers as a

349 solid-phase extraction sorbent for the determination of trace pollutants in environmental water. *Analytical*

350 *and Bioanalytical Chemistry*, 390(3), 929-938.

351 Shook, J. N., VanOverbeke, D. L., Kinman, L. A., Krehbiel, C. R., Holland, B. P., Streeter, M. N., Yates, D. A.,

352 & Hilton, G. G. (2009). Effects of zilpaterol hydrochloride and zilpaterol hydrochloride withdrawal time on

353 beef carcass cutability, composition, and tenderness. *Journal of Animal Science*, 87(11), 3677-3685.

354 Si-Wei, D., Jian-Jun, D., Ting-Ting, W., Yu, W., & Xue-Jun, K. (2014). Determination of Quinolones in Spicy
 355 Soup Using Packed Fiber Solid Phase Extraction and High Performance Liquid
 356 Chromatography-Fluorimetric Detection. *Chinese Journal of Analytical Chemistry*, 42(8), 1171-1176.

357 Tian, T., Deng, J., Xie, Z., Zhao, Y., Feng, Z., Kang, X., & Gu, Z. (2012). Polypyrrole hollow fiber for solid
 358 phase extraction. *Analyst*, 137(8), 1846-1852.

359 Vaccher, C., Berthelot, P., & Debaert, M. (1995). GABA-B agonists: enantiomeric resolution of
 360 4-amino-3-(5-chlorothien-2-yl) butyric acid and analogues on chiral crown ether stationary phase. *Journal*
 361 *of Chromatography A*, 715(2), 361-365.

362 Voloshina, N. S., & Kamalov, G. L. (2014). Crown ethers immobilized on the surface of polymer materials and
 363 silicas. *Protection of Metals and Physical Chemistry of Surfaces*, 50(5), 587-598.

364 Wang, L., Pu, R. C., Wang, X. X., Luo, C. Y., Zhang, L. C., & Zhang, X. S. (2015). Multiresidue Determination
 365 of 2 β -Agonists Including Phenylethanolamine A in Animal-Derived Food by Ultra-High Performance
 366 Liquid Chromatography/Tandem Mass Spectrometry. *Journal of Chromatographic Science*, 53(6),
 367 925-931.

368 Wang, P., Liu, X., Su, X., & Zhu, R. (2015). Sensitive detection of β -agonists in pork tissue with novel
 369 molecularly imprinted polymer extraction followed liquid chromatography coupled tandem mass
 370 spectrometry detection. *Food Chemistry*, 184, 72-79.

371 Wang, X., Guo, T., Wang, S., Yuan, J., & Zhao, R. (2015). Simultaneous Determination of 11 β -Agonists in
 372 Human Urine Using High-Performance Liquid Chromatography/Tandem Mass Spectrometry with Isotope
 373 Dilution. *Journal of Analytical Toxicology*, 39(3), 213-218.

374 Wu, J., Liu, X., & Peng, Y. (2014). Determination of ractopamine in pig hair using liquid chromatography with

tandem mass spectrometric detection. *Journal of Pharmacological and Toxicological Methods*, 69(3), 211-216.

Xiong, L., Gao, Y., Li, W., Guo, T., & Yang, X. (2015). A method for multiple identification of four β -2-Agonists in goat muscle and beef muscle meats using LC-MS/MS based on deproteinization by adjusting pH and SPE for sample cleanup. *Food Science and Biotechnology*, 24(5), 1629-1635.

Xu, D., Zhou, X., & Wang, X. (2008). Adsorption and desorption of Ni^{2+} on Na-montmorillonite: effect of pH, ionic strength, fulvic acid, humic acid and addition sequences. *Applied Clay Science*, 39(3), 133-141.

Zhang, Y., Kang, X., Chen, L., Pan, C., Yao, Y., & Gu, Z. (2008). Fiber-packed SPE tips based on electrospun fibers. *Analytical and Bioanalytical Chemistry*, 391(6), 2189-2197.

Figure Captions

Fig. 1. Schematic of packed-nanofiber solid-phase extraction (PFSPE) device.

Fig. 2. IR spectra of (a) PS-PCE nanofibers and (a') PS nanofibers; SEM images of (b) PS-PCE nanofibers magnified 20k times and (b') PS nanofibers magnified 20k times; TEM images of (c) PS-PCE nanofibers and (c') PS nanofibers.

Fig. 3. Chromatograms: (a) blank pork spiked six β -agonists (25.0 $\mu\text{g/kg}$), and (b) blank pork.

Fig. 4. (a) Adsorption efficiency of six β -agonists onto PS and PS-PCE nanofibers extraction column; (b) Effect of pH value on extraction efficiency at a concentration of 20 ng/mL for each β -agonist; (c) Effect of the eluent volume on elution efficiency at the concentration of 20 ng/mL for β -agonist.

Table 1 Calibration curves of six β -agonists in pork.

Table 2 Recovery and interday, intraday precision in %RSD.

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Table 1 Calibration curves of six β -agonists in pork.

β -agonists	Regression equation	R^2	LOD ($\mu\text{g/kg}$)	LOQ ($\mu\text{g/kg}$)
Clorprenaline	$y=0.4213x+0.4952$	0.9991	0.2	0.6
Bambuterol	$y=5.4417x-3.9622$	0.9958	0.1	0.3
Clenbuterol	$y=1.0012x+4.9278$	0.9975	0.2	0.6
Brombuterol	$y=0.2976x+0.5371$	0.9929	0.2	0.6
Mabuterol	$y=0.5995x+1.7558$	0.9936	0.1	0.3
Penbuterol	$y=0.0534x-0.0811$	0.9987	0.1	0.3

Table 2 Recovery and interday, intraday precision in %RSD.

β -agonists	Spiked concentration ($\mu\text{g/kg}$)	Recovery (%)	Intraday ($n=5$)	Interday ($n=5$)
Clorprenaline	3.00	83.3	8.9	
	12.5	105.4	9.2	11.8
	17.5	87.2	10.5	
Bambuterol	3.00	103.2	6.3	
	12.5	96.8	5.8	9.4
	17.5	101.5	9.2	
Clenbuterol	3.00	105.4	7.3	
	12.5	99.6	4.5	7.5
	17.5	93.5	6.1	
Brombuterol	3.00	102.4	5.0	
	12.5	97.7	6.3	8.4
	17.5	101.1	7.3	
Mabuterol	3.00	103.4	5.9	
	12.5	110.1	6.2	10.6
	17.5	79.7	9.6	
Penbuterol	3.00	96.7	2.1	
	12.5	102.4	3.4	4.7
	17.5	100.7	1.5	