JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Chemometric Optimization of Cation-Selective Exhaustive Injection Sweeping Micellar Electrokinetic Chromatography for Quantification of Ractopamine in Porcine Meat

Chun-Chi Wang,[†] Chia-Chia Lu,[†] Yen-Ling Chen,[‡] Hui-Ling Cheng,[§] and Shou-Mei Wu*,[†]

[†]School of Pharmacy, and [‡]Department of Fragrance and Cosmetic Science, College of Pharmacy, Kaohsiung Medical University, Kaohsiung 807, Taiwan

[§]Pharmaceutical Optimization Technology Division, Biomedical Technology and Device Research Laboratories, Industrial Technology Research Institute, Hsinchu 300, Taiwan

ABSTRACT: An online stacking capillary electrophoresis (CE) method, cation-selective exhaustive injection sweeping micellar electrokinetic chromatography (CSEI-sweep-MEKC), is developed and optimized for analysis of ractopamine (RP) and its homologue dehydroxyractopamine (DRP) in porcine meat. Chemometric experimental design was used to achieve the best possible optimization and reduce the number of trials and errors. The CSEI-sweep-MEKC method enables nanogram per gram level analysis, with limits of detection (LODs) in meat of 5 ng/g for RP and 3 ng/g for DRP (S/N = 3). A higher conductivity buffer (HCB) zone was injected into the capillary, allowing for the analytes to be electrokinetically injected at a voltage of 9 kV for 12 min. Using 125 mM sodium dodecyl sulfate and 15% methanol in the sweeping buffer, RP and DRP were well-separated. The method was validated with a linear calibration curve of 10−300 ng/g ($r > 0.994$). In comparison to the normal capillary zone electrophoresis method (1 psi for 10 s), this stacking strategy resulted in 900 times sensitivity enhancement. This technique was further applied for analyzing seven kinds of commercial meats, and the residual RP was detected in one (5.76 ng/g of RP). The data were corresponding to the data analyzed by the commercial testing kit and mass spectrometry spectra. This method was successfully used on real samples and is considered feasible for serving as a tool for routine examination in markets.

KEYWORDS: *Ractopamine, porcine meat, cation-selective exhaustive injection sweeping MEKC, chemometric experimental design*

INTRODUCTION

Ractopamine hydrochloride (RP), a β-adrenergic agonist, works as a repartitioning agent that accelerates protein synthesis, making the carcass leaner and heavier and enhancing the feeding efficiency in animals.[1] The use of RP as a feed additive has been approved by the U.S. Food and Drug Administration (FDA) in 1999 and 2003, to promote leanness of finishing swine and cattle.[2,3] However, the residual RP in meat becomes consumed by humans. Possible side effects of RP include nausea, tremor, dizziness, vomiting, and cardiac palpitations, and in severe cases, it may even cause cardiac arrest and death.[4] The Codex Alimentarius Commission held a session in 2012 regarding the maximum residual limits (MRLs), indicating that the maximum amounts of residuals allowed in matrices of RP were 10 μg/kg for muscles and fat, 10 μg/kg for liver, and 90 μg/kg for kidneys.[5]

To comply with strict regulations in certain countries, various analytical methods for screening, confirmation, and quantification of RP have been developed. Liquid chromatography−mass spectrometry (LC−MS) or liquid chromatography−tandem mass spectrometry (LC−MS/MS) are the most common methods that have been used to detect and quantify RP in meat, plasma, urine, or animal hair.[6−9] However, to remove interferences in the biological matrix, samples often need to go through cumbersome pretreatments with multiple liquid−liquid extractions or solid-phase extractions.[10−13] Other novel methods that employ biosensors[14] and molecularly imprinted polymers[15,16] also require tedious procedures and have been found to be relatively expensive.

Capillary electrophoresis (CE) is a powerful analytical tool that has several merits, including rapid separation, high efficiency, high resolution, and small sample and solvent requirements. Until now, only a few researchers have used CE for detection of RP, and they have not been able to reach enough sensitivity.[17−19] Chevolleau and Tulliez only used the RP (10 μg/mL) as an analyte to develop their CE method.[17] The limit of detection (LOD) of RP analyzed by the CE method with electrochemical detection was 90 ng/mL.[18] Although the CE method equipped with amperometric detection could be applied for detecting 0.7 ng/mL RP, the RP sample was not spiked in the biometrics.[19] To overcome the major disadvantage of poor sensitivity in CE, a sensitive online stacking CE method, cation-selective exhaustive injection sweeping micellar electrokinetic chromatography (CSEI-sweep-MEKC), is developed in this work. This technique was first developed by Terabe and Quirino in 2000[20,21] and greatly enhances a hundred- to million-fold of sensitivity when compared to capillary zone electrophoresis (CZE). Samples dissolved in water or low-conductivity matrix are exhaustively electrokinetically injected into a higher conductivity buffer

Received: March 9, 2013
Revised: May 27, 2013
Accepted: May 29, 2013
Published: May 29, 2013

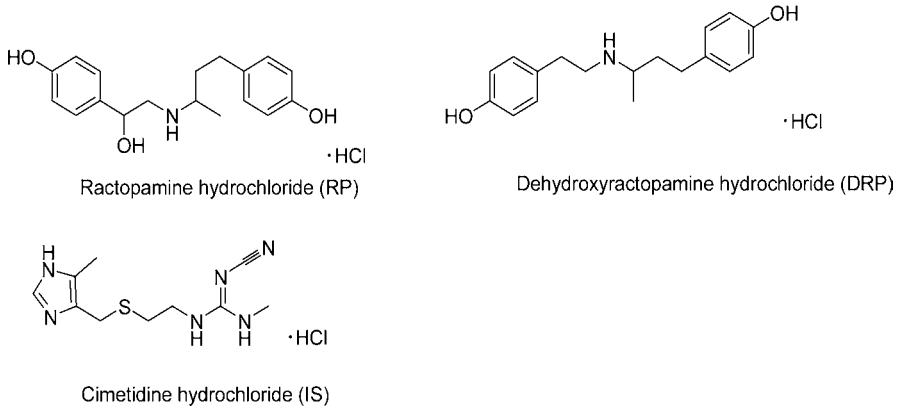

Figure 1. Structures of RP, DRP, and IS.

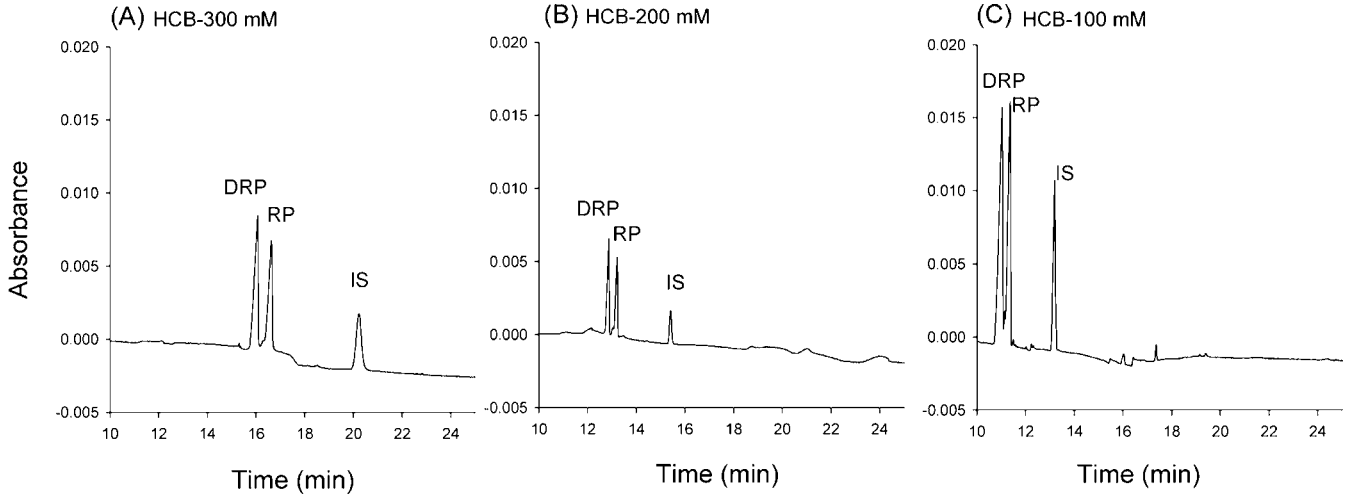

Figure 2. Electropherograms for analysis of RP and DRP spiked in porcine under (A) 300 mM, (B) 200 mM, and (C) 100 mM phosphate buffer (pH 2.75) as HCB. Other experimental conditions: separation buffer, 55 mM phosphate (pH 2.75) containing 25% MeOH; sweeping buffer, 55 mM phosphate (pH 2.75) containing 15% MeOH and 125 mM SDS; sample injection, 9 kV for 12 min; separation voltage, −25 kV; uncoated fused silica capillary, 40 cm effective length; and wavelength, 230 nm. Conditions: sample concentration, 500 ng/mL; IS concentration, 50 ng/mL.

(HCB) zone for a long time. Afterward, the analytes can be swept and separated for detection by micelles, from surfactant-containing buffers. However, a lot of CE parameters in CSEI-sweep-MEKC affect the separation efficiency. Thus, the aid of chemometric experimental design was used to find out the optimal CE conditions under which RP and its homologue dehydroxyractopamine hydrochloride (DRP) in porcine meat should be analyzed and quantified. Fractional factorial design (fFD) and response surface methodology (RSM) were used as effective tools to facilitate the process of optimization of parameters.[22,23] In comparison to the univariate approach, by changing one factor at a time for optimization, the chemometric designs provided better efficiency, productivity, and lower costs. This method is expected to be a feasible tool for the detection of residual RP in commercial meat products.

MATERIALS AND METHODS

Chemicals and Drugs. All chemicals used were of analytical-reagent grade. RP, DRP, and cimetidine hydrochloride (IS) (Figure 1) were purchased from Sigma-Aldrich (Sigma, St. Louis, MO). Sodium dihydrogen phosphate, sodium acetate trihydrate, ethyl acetate (EtOAC), methanol (MeOH), sodium dodecyl sulfate (SDS), HCl, and NaOH were purchased from Merck (Merck, Darmstadt, Germany). Milli-Q water (Millipore, Bedford, MA) was used for preparation of the buffer and other aqueous solutions.

Sample Preparation. Stock solutions (1 mg/mL) of RP, DRP, and IS were prepared by dissolving them in methanol and appropriately diluting with water for further use. Blank meat was purchased from a local company with SGS RP-free certification. Real samples were obtained from various sources, including traditional markets and supermarkets. All meat samples were kept frozen at −70 °C until analysis. Upon use, 1 g of homogenized meat was weighed and spiked with an identical amount of RP and DRP and 50 ng of IS (the amount of 50 ng of IS was chosen according to the appropriate peak signal), and then the meat sample would be pretreated by enzyme for digestion and extracted by ethyl acetate according the following procedures. The fixed amount of 50 ng of IS involving all procedures of pretreatment and extraction would be used to accurately reflect the recovery in extraction and quantify the RP and DRP in the meat.

Pretreatment of Meat Samples. A total of 1 mL of sodium acetate (200 mM) adjusted to pH 5 by HCl and 10 μL (9.07 units) of β-glucuronidase/arylsulphatase (Sigma) were added to 1 g of the meat sample (the portion of ham). Enzymatic hydrolysis was carried out by incubation at 37 °C for 1 h. After centrifugation (800*g* for 5 min) to precipitate the hydrolyzed meat, the supernatant was transferred to another vial for liquid−liquid extraction. Before liquid−liquid extraction, the supernatant was alkalized to pH 9 with 10 M NaOH. A total of 600 μL of ethyl acetate was added for sample cleaning and extraction. After centrifugation at 15000*g* for 10 min, 450 μL of the supernatant was dried in a vacuum centrifuge machine (EYELA CVE-200D, Japan) and then the sample was reconstituted with water (60

dx.doi.org/10.1021/jf401064x | J. Agric. Food Chem. 2013, 61, 5914−5920

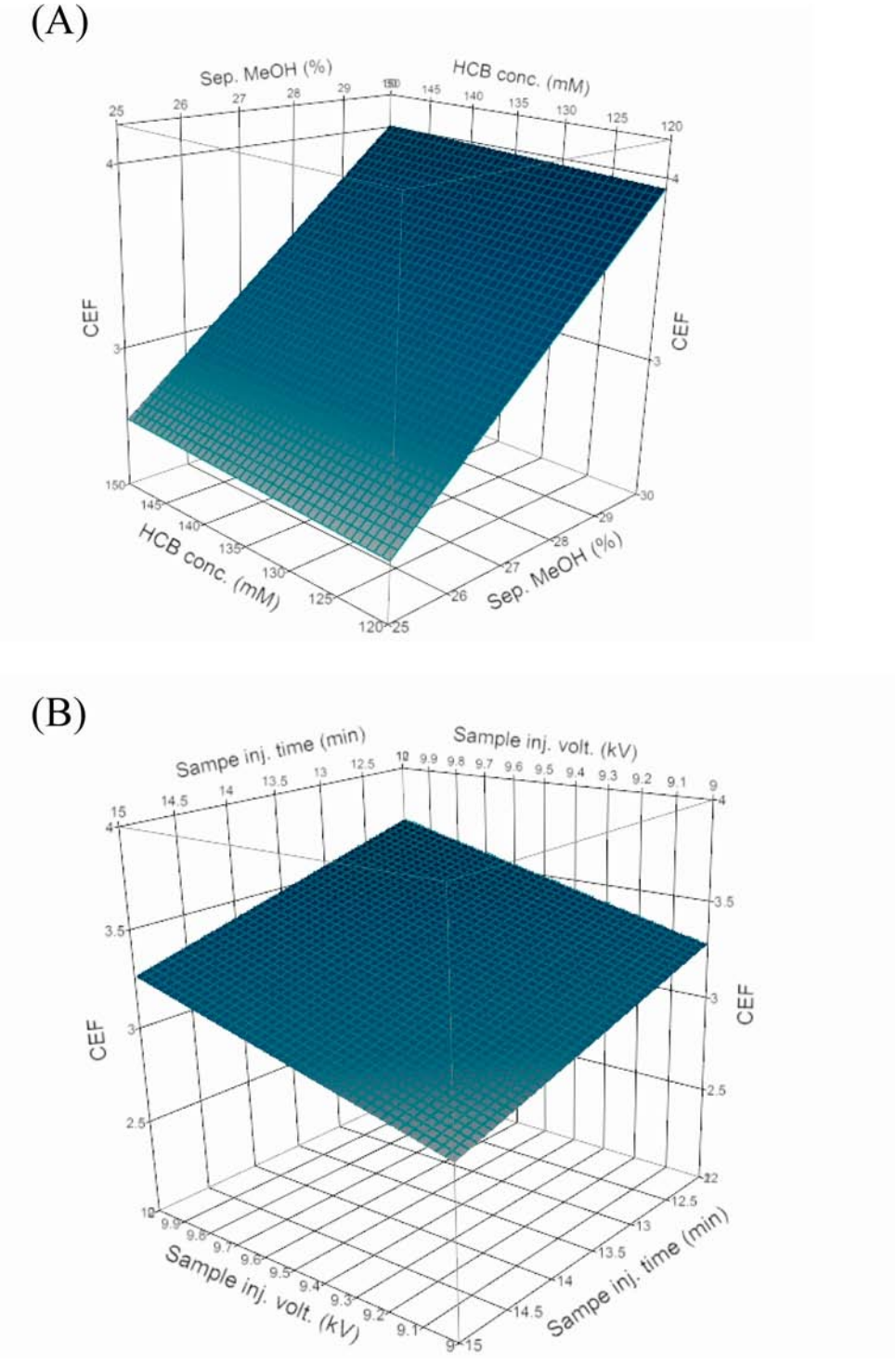

Figure 3. Response surface curve of the custom design: (A) HCB concentration and percentage of MeOH against CEF and (B) sample injection time and sample injection voltage against CEF.

μL) and introduced into the CE system. The water matrix in which there is no interference of ionic molecules is beneficial for electrokinetical injection of analytes.

A total of seven meat samples (the portion of ham) were collected from traditional meat stands and supermarkets. These specimens were stored at −70 °C until analysis using CSEI-sweep-MEKC. Before analysis, all meat samples were pretreated with enzymatic hydrolysis and extraction.

CESI-Sweep-MEKC. A Beckman P/ACE MDQ system (Fullerton, CA) equipped with an ultraviolet (UV) detector was used. The separations were performed in an uncoated fused-silica capillary (Polymicro Technologies, Phoenix, AZ) of 50 μm internal diameter and 40 cm effective length (total length of 50.2 cm). The detector wavelength was set at 230 nm. The temperature was maintained at 20 °C throughout the separations. The new capillary was preconditioned by rinsing methanol at 30.0 psi for 10 min, water at 30.0 psi for 5 min, 1 M HCl at 30.0 psi for 10 min, water at 30.0 psi for 5 min, 1 M NaOH at 30.0 psi for 10 min, and water at 30.0 psi for 5 min. The online stacking technique, CSEI-sweep-MEKC, was used to analyze RP and DRP. After the capillary was filled with separation buffer (55

dx.doi.org/10.1021/jf401064x | J. Agric. Food Chem. 2013, 61, 5914−5920

mM phosphate at pH 2.75 containing 25% MeOH), a long plug of HCB [125 mM phosphate at pH 2.75 containing 15% (v/v) MeOH] was injected at 5 psi for 40 s. Then, the sample was injected at 9 kV for 12 min, followed by switching of both ends of the vials to sweeping buffer [55 mM phosphate at pH 2.75 containing 25% (v/v) MeOH and 125 mM SDS], and then a separation voltage of −25 kV was applied.

Calibration and Method Validation. Calibration curves were established by spiking blank meat with RP and DRP over a range of 10−300 ng/g. Accuracy and precision were determined by spiking the meat with three different concentrations of 40, 75, and 250 ng/g. Intraday was defined as three repeated analyses in a single day (n = 3), and interday consisted of repeated analyses over five different days (n = 5). The LODs indicating the concentration of each analyte with the ratio of signal-to-noise equaling 3 (S/N = 3) in this research were determined by spiking the identical amount of reference standards into meats.

RESULTS AND DISCUSSION

CSEI-Sweep-MEKC. In this study, the stacking method of CSEI-sweep-MEKC combining two online CE enrichment techniques, including CSEI and sweeping, could enhance a hundred- to million-fold of sensitivity when compared to CZE.[20,21] First, the HCB would be introduced into the capillary, after the capillary was filled with separation buffer, and then the cationic analytes were exhaustively electrokinetically injected into the HCB zone. Finally, the analytes in the HCB would be separated and swept by SDS, which would interact with the analytes through electrostatic force or hydrophobicity and carry the analytes to the anode. According to the different combining strengths between SDS and analytes, RP and DRP could be concentrated and resolved within a short time.

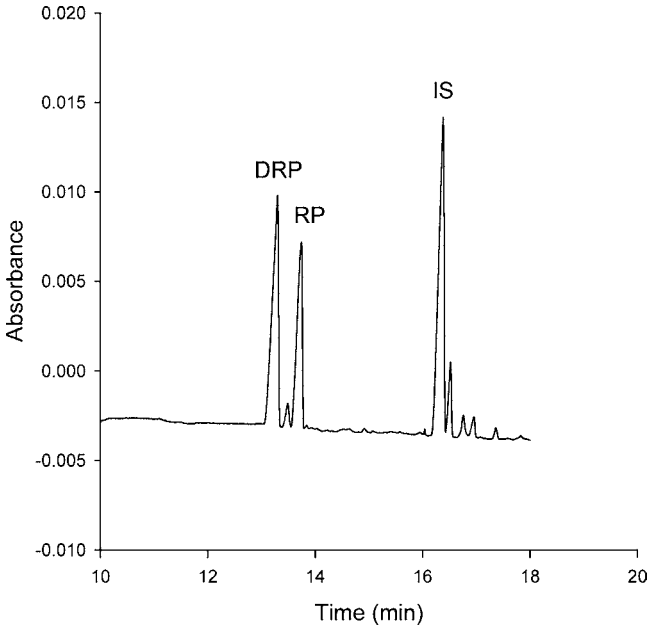

Figure 4. Typical electropherogram for analysis of RP and DRP spiked in porcine under the optimal conditions of CSEI-sweep-MEKC. Experimental conditions: separation buffer, 55 mM phosphate (pH 2.75) containing 25% MeOH; HCB (5 psi for 40 s), 125 mM phosphate (pH 2.75) containing 15% MeOH; sweeping buffer, 55 mM phosphate (pH 2.75) containing 15% MeOH and 125 mM SDS; sample injection, 9 kV for 12 min; separation voltage, −25 kV; uncoated fused silica capillary, 40 cm effective length; and wavelength, 230 nm. Conditions: sample concentration, 500 ng/mL; IS concentration, 50 ng/mL.

Table 1. Regression Analysis for the Determination of RP and DRP in Meat[a]

analysis	regression equation	coefficient of correlation (r)
	Intrabatch[b]	
RP	$y = (0.011 \pm 0.002)x - (0.103 \pm 0.031)$	0.997
DRP	$y = (0.008 \pm 0.002)x - (0.055 \pm 0.065)$	0.996
	Interbatch[c]	
RP	$y = (0.011 \pm 0.002)x - (0.023 \pm 0.095)$	0.995
DRP	$y = (0.008 \pm 0.002)x - (0.017 \pm 0.077)$	0.994

[a]The concentration for intra- and interday analyses for RP and DRP was 10−300 ng/g. [b]Regression equations of intrabatch analysis were calculated from assay values of prepared standards on a single day (n = 3). [c]The regression equations of interday analysis were calculated from the assay values of prepared standards on five different days (n = 5).

Table 2. Precision, Accuracy, and Recovery of Detection of DRP and RP in Porcine Meat

	concentration known (ng/g)	concentration found (ng/g)	RSD (%)	RE (%)[a]	relative recovery (%)
		Intrabatch Analysis (n = 3)			
DRP	4.0×10^1	$(3.8 \pm 0.2) \times 10^1$	5.1	−4.3	95.3
	7.5×10^1	$(7.3 \pm 0.1) \times 10^1$	1.9	−3.1	97.3
	2.5×10^2	$(2.6 \pm 0.2) \times 10^2$	6.3	5.1	104.2
RP	4.0×10^1	$(4.3 \pm 0.1) \times 10^1$	2.4	7.4	107.5
	7.5×10^1	$(7.8 \pm 0.1) \times 10^1$	1.6	4.5	104.3
	2.5×10^2	$(2.6 \pm 0.2) \times 10^2$	8.4	6.2	103.7
		Interbatch Analysis (n = 5)			
DRP	4.0×10^1	$(3.8 \pm 0.5) \times 10^1$	12.3	5.6	94.8
	7.5×10^1	$(7.2 \pm 0.1) \times 10^1$	8.5	−4.1	96.7
	2.5×10^2	$(2.6 \pm 0.2) \times 10^2$	7.1	5.5	103.6
RP	4.0×10^1	$(4.3 \pm 0.3) \times 10^1$	7.5	7.2	106.8
	7.5×10^1	$(7.8 \pm 0.3) \times 10^1$	9.5	6.3	104.4
	2.5×10^2	$(2.6 \pm 0.3) \times 10^2$	9.6	7.4	103.6

[a]RE (% relative error) = (concentration found − concentration known) × 100/(concentration known).

Chemometric Experimental Design. Basic experimental conditions were established before the initiation of the screening design. CSEI-sweep-MEKC is an online stacking technique that involves many factors. To reduce time and cost involved in the procedure of method optimization, a chemometric experimental design was used to quickly find out the optimal conditions. Pretests were performed to select factors to be further optimized and to set reasonable limits for each factor. In this step, 10 factors were first investigated using fFD, and the results were as follows.

To minimize the impact of electroosmotic flow (EOF) in this system, the range of pH was set at 2.50−3.00. Dihydrogen phosphate was selected as the separation buffer because of its pH being within the working range, and the concentration range was set at 40−70 mM for better resolving power. MeOH was selected as the organic modifier, which could affect resolution and selectivity in CE, and, therefore, was investigated separately in each buffer and in HCB. Analytes could not be resolved without the addition of MeOH, but more than 40% (v/v) of MeOH leads to a longer migration time; therefore, it was kept between 10 and 40% (v/v). The long plug of HCB plays an important role in providing high conductivity, implying

dx.doi.org/10.1021/jf401064x | J. Agric. Food Chem. 2013, 61, 5914−5920

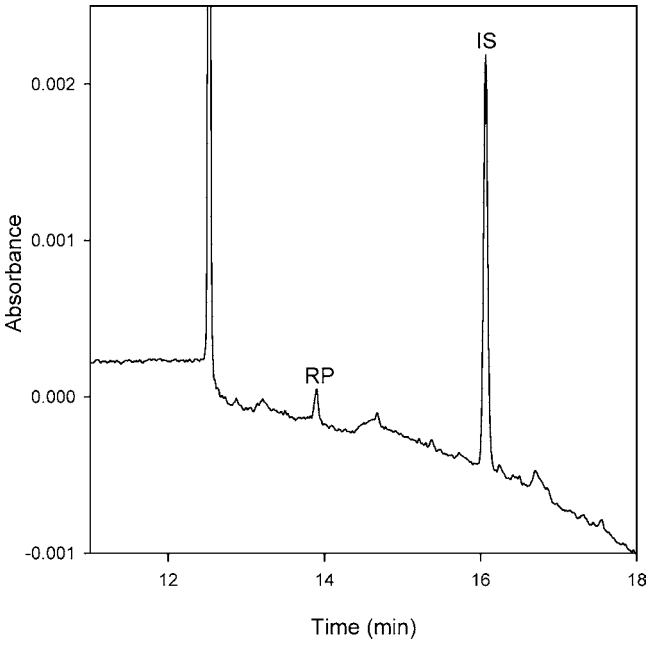

Figure 5. Electropherogram of the real sample in which RP was detected. For experimental conditions, please see Figure 4.

that a substantial amount of analytes can be injected and stacked at the HCB. Generally, better stacking results were obtained when the HCB concentration was three times as large as the separation buffer.[24] In the preliminary test, when the HCB concentration was 300 mM, the migration time became longer, whereas the peak height did not improve. Therefore, along with consideration of reducing joule heating, the HCB concentration range was set between 100 and 200 mM. Because HCB might be one of the most important factors that can affect the degree of preconcentration of the analytes, its length was also investigated. A longer plug can hold more analytes, with the cost of sacrificing separation length and reducing the resolution; a shorter plug limits the amount that can be injected, which affects sensitivity. Therefore, the injection pressure of HCB was set at 5 psi (345 mbar), and injection time was set between 30 and 50 s (13.4−22.3 cm capillary length, with the injection volume between 264 and 440 nL). CSEI-sweep-MEKC allows for the analytes to be electrokinetically injected into the capillary and stacked in HCB. Hence, the sample injection voltage and time are also important parameters that affect sensitivity. The injection voltage was set at 8−10 kV, and the injection time was between 10 and 20 min. In sweeping, surfactants have important roles of focusing and sweeping the stacked analytes toward the detector. SDS was selected because it is easily obtainable and inexpensive and also gives satisfactory results in terms of resolution and sensitivity. Therefore, concentrations of SDS were also investigated at 100−150 mM.

After the preliminary experiment, relationships between factors and responses were determined with a minimal number of runs, calculated using the JMP Software (developed by SAS Institute, Cary, NC). The fFD equation used for investigating responses of factors was as follows:

$$\text{fFD} = \text{resolution}^2 \times (\text{peak height})/\text{migration time}$$

The resolution was set at 1−1.5; the peak height was maximized; and the migration time was minimized. The suggested values of each parameter generated by fFD were as followed: 30% (v/v) MeOH in separation buffer (55 mM phosphate at pH 2.75), 15% (v/v) MeOH in HCB (150 mM phosphate at pH 2.75), and 30% (v/v) MeOH in sweeping buffer (55 mM phosphate at pH 2.75) containing 125 mM SDS, and sample injection was performed at 9 kV for 15 min. On the basis of the results of this analysis, 4 of 10 factors were significant, with p values of less than 0.05, including the methanol percentage in sweeping buffer, concentration of HCB, injection voltage, and injection time. That indicated the large effects that these four factors exhibited in this CSEI-sweep-MEKC system; thus, the ranges of these four factors were narrowed, and eight extra runs obtained from the calculation of JMP were generated for further investigation.

Figure 6. Results for seven real samples using commercial test kits. Two lines represent no RP detected, and one line means that the result is positive.

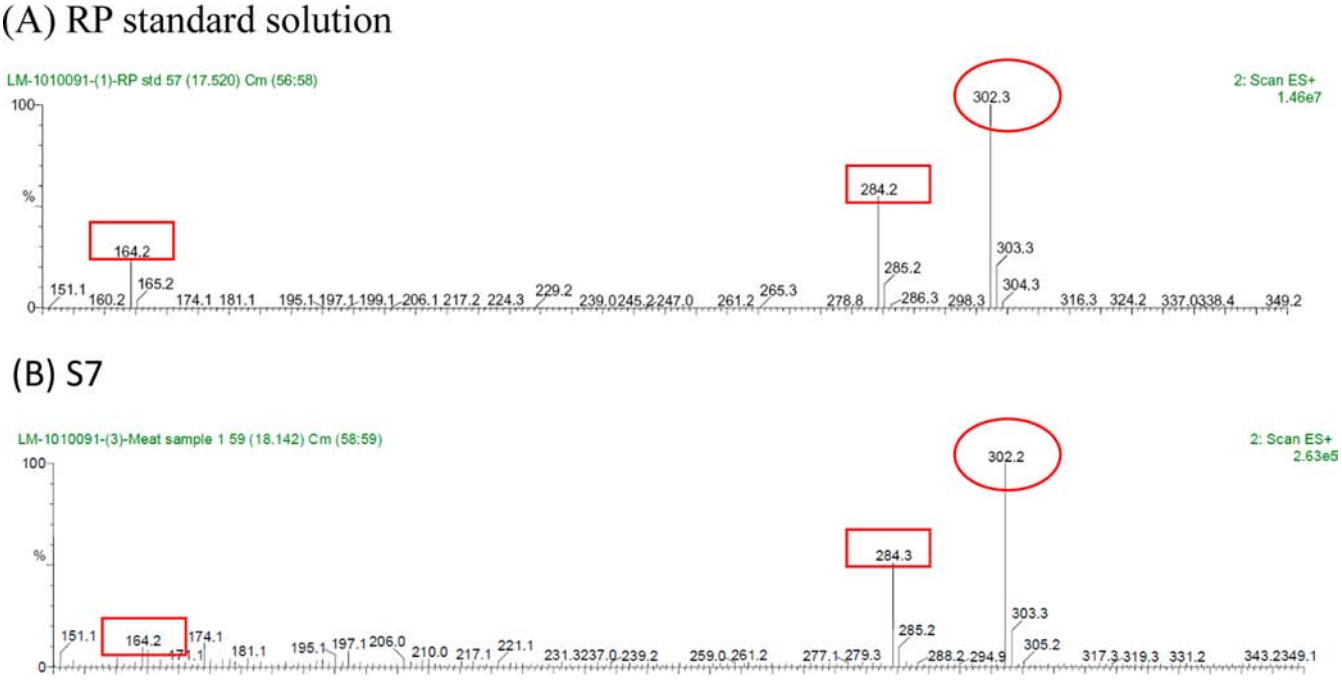

Figure 7. MS spectra of (A) RP standard solution and (B) S7. Column, Zorbax eclipse plus C18 Narrow Bore RR (2.1 × 100 mm, 3.5 μm); mobile phase, 0.1% formic acid in H_2O and 0.1% formic acid in methanol (50:50); flow rate, 0.2 mL/min; mode, positive electrospray mode; voltage, 3 kV [25 V (cone), 3 V (extractor), and 0.1 V (RF lens)]; source temperature, 98 °C; desolvation temperature, 248 °C; and gas flow rate, 600 L/h in desolvation and 100 L/h in the cone.

Figure 2 displayed the electropherograms using different concentrations of HCB from 100 to 300 mM, and the findings demonstrated that the HCB concentration had great effects on the migration and sensitivity. For further evaluation of the 4 factors, the equation of chromatographic exponential factor (CEF) was used to evaluate the outcome.

$$\mathrm{CEF} = \left[\left(\sum_{i=1}^{n-1}\left(1 - \mathrm{e}^{a(R_{\mathrm{opt}} - R_i)}\right)^2\right) + 1\right]\left[1 + \frac{t_{\mathrm{f}}}{t_{\mathrm{max}}}\right]$$

R_{opt} is the optimum resolution, set at 1.5; n is the expected number of peaks; R_i represents the resolution between two adjacent peaks; t_{f} is the migration time of the last peak; t_{max} is the maximum acceptable time, set at 20 min; and the a value was used to adjust the relative weighting of resolution and migration time. A smaller CEF value means a shorter migration time and better resolution.[25] Figure 3 shows responses of the last four factors. Figure 3A showed that the percentage of MeOH in the separation buffer had a great influence. However, a much higher percentage of MeOH lowers the current, suppresses EOF, and hence, prolongs separation time. Finally, 25% (v/v) was chosen as the optimal amount to be added in the separation buffer for more efficient analysis, and 125 mM phosphate was selected as the HCB. Figure 3B shows that both sample injection time and voltage had smaller curvature effects on CEF. Although the higher injection voltage and longer injection time result in better CEF, it was felt that increasing both the injection voltage and time to a certain degree may exceed the holding capacity of the HCB. At last, the sample injection was carried out at 9 kV for 12 min. Figure 4 shows a typical electropherogram of RP and DRP using the optimized experimental conditions for separation of analytes spiked in porcine. The optimal conditions were as follows: separation buffer of 55 mM NaH_2PO_4 (pH 2.75) and containing 25% (v/v) MeOH; HCB (125 mM phosphate at pH 2.75 containing 15% MeOH) was injected at 5 psi for 40 s; the sample was electrokinetically loaded at 9 kV for 12 min; and the sweeping buffer was 55 mM phosphate (pH 2.75) consisting of 25% (v/v) MeOH and 125 mM SDS.

Method Validation. The established method was validated on extracts obtained from porcine meat spiked with RP, DRP, and IS. The linearity was determined from triple injections of the solutions from 10 to 300 ng/g in porcine meat (Table 1). The regression equations of RP were $y = (0.011 \pm 0.002)x - (0.103 \pm 0.031)$ and $y = (0.011 \pm 0.002)x - (0.023 \pm 0.095)$ in intrabatch ($n = 3$) and interbatch ($n = 5$), respectively, and the regression equations of DRP were $y = (0.008 \pm 0.002)x - (0.055 \pm 0.065)$ and $y = (0.008 \pm 0.002)x - (0.017 \pm 0.077)$ in intrabatch ($n = 3$) and interbatch ($n = 5$), respectively. In intra- and interbatch assays, the averages of the slopes were equal to 0.011 and 0.008 for RP and DRP, respectively. The results indicated the good repeatability of this method for the calibration assay. The correlation coefficients for the calibration curves were greater than 0.994 for intra- and interbatch analyses. With the wavelength set at 230 nm, LODs in meat were 5 ng/g for RP and 3 ng/g for DRP and LODs in standard solution were 2 ng/mL for RP and 1 ng/mL for DRP calculated on the basis of a S/N ratio of 3:1 (9 kV for 12 min). In comparison to the previous CE method for detection of RP (LOD of 90 ng/mL),[18] this method involved a lower LOD. Additionally, sample extraction was not dependent upon the complicated procedures of solid-phase extraction[12,13] and was accomplished by single liquid−liquid extraction. For assessment of precision and accuracy, three concentrations (40, 75, and 250 ng/g) of RP and DRP were chosen. As seen in Table 2, for intrabatch analysis ($n = 3$), values of RSD and RE were below 12.3% in intra- and interday assays. The relative recovery was located at the range of 107.5−94.8%. The sensitivity enhance-

dx.doi.org/10.1021/jf401064x | J. Agric. Food Chem. 2013, 61, 5914−5920

ment was about 900-fold in the case of LODs of the CSEI-sweep-MEKC method compared to the normal CZE (sample injection of 1 psi for 10 s) using standard solution.

Application. The method developed with the CSEI-sweep-MEKC technique was applied to analyze several real meat samples purchased from traditional meat stands and supermarkets. A total of seven meat samples were collected. All samples were subjected to enzymatic hydrolysis, extraction, and CE analysis. RP or DRP peaks were not observed in six of the seven real porcine samples (S1−S6); only one sample (S7) had a RP peak with a concentration of 5.76 ng/g (Figure 5). Commercial test kits that have a detection limit of 2 ng/g (Taiwan Advance Bio-Pharm, Inc., Taipei, Taiwan) were used for comparison purposes. Six samples in which analyte peaks were not detected by CSEI-sweep-MEKC were tested and yielded negative results (two lines, S1−S6, Figure 6). Although S3 looked like only having one line, two lines indeed existed in the kit of S3 (the other line was too slight). Only S7 was found to have positive data (one line) (Figure 6). The testing kits quickly provided information on whether the samples contained more than 2 ng/mL of RP. Figure 7 showed the mass spectrometry (MS) spectra of the RP standard, and S7 demonstrated the existence of RP. The data were equal to the data detected by this CSEI-sweep-MEKC method and demonstrated that the CSEI-sweep-MEKC method has potential for detection of RP in real meat samples. This simple and fast method was found suitable for serving as a routine tool for examination of RP in meat.

AUTHOR INFORMATION

Corresponding Author

*Telephone: 886-7-3121101, ext. 2164. Fax: 886-7-3210683. E-mail: shmewu@kmu.edu.tw.

Funding

We gratefully acknowledge the support of the National Science Council of Taiwan in funding this work.

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Walker, D. K.; Titgemeyer, E. C.; Drouillard, J. S.; Loe, E. R.; Depenbusch, B. E.; Webb, A. S. Effects of ractopamine and protein source on growth performance and carcass characteristics of feedlot heifers. *J. Anim. Sci.* **2006**, *84*, 2795−2800.

(2) U.S. Food and Drug Administration (FDA). *Fed. Regist.* **2000**, *65*, 4111.

(3) U.S. Food and Drug Administration (FDA). *Fed. Regist.* **2003**, *68*, 54658.

(4) Smith, D. J. The pharmacokinetics, metabolism, and tissue residues of β-adrenergic agonists in livestock. *J. Anim. Sci.* **1998**, *76*, 173−194.

(5) Joint FAO/WHO Food Standards Programme Codex Alimentarius Commission. *Report of the Sixty-Seventh Session of the Executive Committee of the Codex Alimentarius Commission*; FAO Headquarters: Rome, Italy, July 2−7, 2012; 35th Session.

(6) Blanca, J.; Munoz, P.; Morgado, M.; Mendez, N.; Aranda, A.; Reuvers, T.; Hooghuis, H. Determination of clenbuterol, ractopamine and zilpaterol in liver and urine by liquid chromatography tandem mass spectrometry. *Anal. Chim. Acta* **2005**, *529*, 199−205.

(7) Shishani, E. I.; Chai, S. C.; Jamokha, S.; Aznar, G.; Hoffman, M. K. Determination of ractopamine in animal tissue by liquid chromatography−fluorescence and liquid chromatography/tandem mass spectrometry. *Anal. Chim. Acta* **2003**, *483*, 137−145.

(8) Garcia, P.; Paris, A. C.; Gil, J.; Popot, M. A.; Bonnaire, Y. Analysis of β-agonists by HPLC/ESI−MS^n in horse doping control. *Biomed. Chromatogr.* **2011**, *25*, 147−154.

(9) Liu, X.; He, X.; Moore, C.; Wang, G.; Coulter, C. Highly sensitive and specific liquid chromatography−tandem mass spectrometry method for testing ractopamine in cow and sheep urine. *J. Anal. Toxicol.* **2009**, *33*, 289−293.

(10) Ramos, F. J. D. β2-Agonist extraction procedures for chromatographic analysis. *J. Chromatogr., A* **2000**, *880*, 69−83.

(11) Liu, C.; Ling, W.; Xu, W.; Chai, Y. Simultaneous determination of 20 β-agonists in pig muscle and liver by high-performance liquid chromatography/tandem mass spectrometry. *J. AOAC Int.* **2011**, *94*, 420−427.

(12) Zhang, Q.; Su, Y.; He, Q.; Shen, X.; He, L.; Zhang, N.; Zeng, Z. Molecularly imprinted solid-phase extraction for the selective HPLC determination of ractopamine in pig urine. *J. Sep. Sci.* **2011**, *34*, 3399−3409.

(13) Hu, Y.; Liu, R.; Li, Y.; Li, G. Investigation of ractopamine-imprinted polymer for dispersive solid-phase extraction of trace β-agonists in pig tissues. *J. Sep. Sci.* **2010**, *33*, 2017−2025.

(14) Thompson, C. S.; Haughey, S. A.; Traynor, I. M.; Fodey, T. L.; Elliott, C. T.; Antignac, J. P.; Le Bizec, B.; Crooks, S. R. Effective monitoring for ractopamine residues in samples of animal origin by SPR biosensor and mass spectrometry. *Anal. Chim. Acta* **2008**, *608*, 217−225.

(15) Wang, S.; Liu, L.; Fang, G.; Zhang, C.; He, J. Molecularly imprinted polymer for the determination of trace ractopamine in pork using SPE followed by HPLC with fluorescence detection. *J. Sep. Sci.* **2009**, *32*, 1333−1339.

(16) Tang, Y. W.; Fang, G. Z.; Wang, S.; Li, J. L. Covalent imprinted polymer for selective and rapid enrichment of ractopamine by a noncovalent approach. *Anal. Bioanal. Chem.* **2011**, *401*, 2275−2282.

(17) Chevolleau, S.; Tulliez, J. Optimization of the separation of β-agonists by capillary electrophoresis on untreated and C18 bonded silica capillaries. *J. Chromatogr., A* **1995**, *715*, 345−354.

(18) Wang, W.; Zhang, Y.; Wang, J.; Shi, X.; Ye, J. Determination of β-agonists in pig feed, pig urine and pig liver using capillary electrophoresis with electrochemical detection. *Meat Sci.* **2010**, *85*, 302−305.

(19) Li, L.; Du, H.; Yu, H.; Xu, L.; You, T. Application of ionic liquid as additive in determination of three β-agonists by capillary electrophoresis with amperometric detection. *Electrophoresis* **2013**, *34*, 277−283.

(20) Quirino, J. P.; Terabe, S. Approaching a million-fold sensitivity increase in capillary electrophoresis with direct ultraviolet detection: Cation-selective exhaustive injection and sweeping. *Anal. Chem.* **2000**, *72*, 1023−1030.

(21) Quirino, J. P.; Terabe, S. Sample stacking of cationic and anionic analytes in capillary electrophoresis. *J. Chromatogr., A* **2000**, *902*, 119−135.

(22) Altria, K. D.; Clark, B. J.; Filbey, S. D.; Kelly, M. A.; Rudd, D. R. Application of chemometric experimental designs in capillary electrophoresis: A review. *Electrophoresis* **1995**, *16*, 2143−2148.

(23) Skartland, L. K.; Mjos, S. A.; Grung, B. Experimental designs for modeling retention patterns and separation efficiency in analysis of fatty acid methyl esters by gas chromatography−mass spectrometry. *J. Chromatogr., A* **2011**, *1218*, 6823−6831.

(24) Isoo, K.; Terabe, S. Analysis of metal ions by sweeping via dynamic complexation and cation-selective exhaustive injection in capillary electrophoresis. *Anal. Chem.* **2003**, *75*, 6789−6798.

(25) Frias-Garcia, S.; Jesus Sanchez, M.; Rodriguez-Delgado, M. A. Optimization of the separation of a group of triazine herbicides by micellar capillary electrophoresis using experimental design and artificial neural networks. *Electrophoresis* **2004**, *25*, 1042−1050.