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RESEARCH PAPER

Direct detection of chloramphenicol in honey by neutral desorption-extractive electrospray ionization mass spectrometry

X. Y. Huang · X. W. Fang · X. Zhang · X. M. Dai · X. L. Guo · H. W. Chen · L. P. Luo

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Abstract Herein, we constructed a platform of neutral desorption-extractive electrospray ionization mass spectrometry (ND-EESI-MS) for direct and rapid detection of chloramphenicol (CAP) in honey samples diluted with methanol. Under the optimized working conditions, the quantitative information of CAP residues was acquired effectively by EESI-Ion Trap MS^n. Using heated methanol-N_2 as spray reagent, we reduced the limit of determination (LOD) from 73.3 ng/mL to 0.3 ng/mL, and the CAP detection is linear in the range of 1–5000 ng/mL (R=0.9947). For the honey samples with CAP of 10, 100, and 1000 ng/mL, the recoveries were 133.0, 80.6, and 101.1 %, and the relative standard deviations were 5.96, 8.82, and 8.71 %, respectively. The reproducibility assays showed the stability of this method. Therefore, this ND-EESI-MS method is powerful for direct, rapid, and quantitative CAP analysis in honey samples with high sensitivity, precision, and specificity.

Keywords Neutral desorption-extractive electrospray ionization · Mass spectrometry · Chloramphenicol · Honey · Methanol spray · Direct analysis

X. Y. Huang · X. Zhang · X. M. Dai · X. L. Guo · L. P. Luo (✉)
State Key Laboratory of Food Science and Technology, School of Life Sciences and Food Engineering, Nanchang University, 999 Xuefu Road, Nanchang, Jiangxi 330031, China
e-mail: lluo2@126.com

X. W. Fang · H. W. Chen (✉)
Jiangxi Key Laboratory for Mass Spectrometry and Instrumentation, East China Institute of Technology, 418 Guanglan Ave, Nanchang Economic & Technological Development Zone, Jiangxi 330013, China
e-mail: chw8868@gmail.com

Introduction

With a long history of beekeeping, China has been the first apiculture country in today's world. In China, more than 8 million groups of bees generate over 0.3 million tons of honey annually, which is 20 % of total world output, ranking first in the world. The honey export, the value of which is about 100 million US$, remains the first in the world, and more than 90 % exports were to the USA, the European Union, and Japan [1]. In recent years, pesticide residues and chemical pollutants have been significant factors affecting the import and export of Chinese bee products. Chloramphenicol (CAP), an efficient antibiotic with broad-spectrum, was applied widely as a disease prevention drug in husbandry, fishery, and beekeeping. But CAP in animal-derived food has the potential to damage the hematopoietic function of human bone marrow, leading to neutropenia, aplastic anemia, or hemolytic anemia, severe cases of which could be fatal [2–4]. Therefore, the European Community banned its use in food-producing animals since 1994 [5]. CAP residues in honey were mainly caused by the using of drugs containing CAP in colony disinfection [6]. At the beginning of 2002, for the reason of CAP residues exceeding the maximum residue limit, the EU banned Chinese honey to enter its market. This led to the chain reaction from other countries and had a serious influence on Chinese honey exports. With the development of detection technology, the EU and Japan constantly reduced the minimum required performance limit (MRPL) in imported animal-derived food. They especially called for no CAP residue in imported honey [7]. Because of the existence of complex matrix, it is difficult for CAP to be detected in bee products, highlighting the need for CAP detection in honey.

Multiple sensitive and specific detection methods for CAP residues in foodstuff have been developed recently, such as ELISA [8–10], HPLC [11, 12], GC-MS [13, 14], and LC/MS [15–20]. Generally, the LODs of these methods are less than

the MRPL of EU commission, 0.3 ng/mL [7]. An optimized ELISA method has obtained a low LOD, 0.042 ng/mL [10]. After use of QuEChERS cleanup and LC/MS procedures, the determination of CAP in honey results in a limit of reporting level (LCL), 0.20 ng/g [20]. Based on laser diode thermal desorption (LDTD) and atmospheric pressure chemical ionization coupled to tandem MS (APCI-MS/MS), a modified method has reduced the LOD to 0.19 ng/g [21]. Another improved LC/MS method starts from an immune-affinity column (IAC) cleanup procedure. The LOD of this method is 0.16 ng/g for honey and 0.05 ng/g for prawns [19]. Recently, some methods based on nanoparticle biosensors have been developed for CAP detection in honey and other foodstuff, also leading to good detection limits and linear ranges [22, 23] For these current methods, complicated sample pretreatments, such as liquid-liquid extraction [21], affinity adsorption [19, 21], and nanoparticles preparation [22, 23] are necessary, as well as long analysis time and complicated operation. MS analysis methods, with lots of advantages, including the speed of analysis, the high specificity, the low limit of detection (LOD), and lack of any requirement for analyte-specific reagents, were considered as powerful methods for analyzing complex mixtures [24]. Hence, improving MS analysis methods for the simple and rapid CAP detection have a positive significance. For the liquid samples with complex matrix, the extractive electrospray ionization mass spectrometry (EESI-MS) is a method with direct ionization, rapid MS, and without sample pretreatment [25–31]. Our previous studies showed that using the neutral desorption-extractive electrospray ionization mass spectrometry (ND-EESI-MS) technology, those sticky samples, such as honey, olive oil, and toothpaste, could be analyzed rapidly and directly, without any sample pretreatment [32, 33]. In this paper, via a homemade neutral desorption (ND) apparatus combined with EESI-MS, we developed a modified ND-EESI-MS technology to detect CAP rapidly and efficiently in honey samples.

Materials and methods

Materials and apparatus

The ND apparatus and the EESI ion source were developed by Jiangxi Province Key Laboratory of Mass Science and Instrument [34]. The LTQ-XL type linear ion trap mass spectrometer (Finnigan, USA) was equipped with the Xcali-bur data-processing system.

Experiments were performed using a commercial LTQ-XL type linear ion trap mass spectrometer (Finnigan, USA) coaxially coupled with an EESI ion source were developed by Jiangxi Province Key Laboratory of Mass Science and Instrument and Xcali-bur software for instrument control and data processing, as described elsewhere [34]. Briefly, the EESI source (schematically shown in Fig. 1) and the LTQ mass spectrometer were set to work in negative ion detection mode. A gentle N_2 sheath gas flow (~1.0 MPa) were driven into heated liquid methanol (~70 °C) to form N2/methanol gas flow as spray reagent, which were used to bring CAP to the ionization region for the production of primary ions in the EESI source. The gas flow tube was heated to 450 °C, and the ionization region of the EESI source was maintained at 150~175 °C, ensuring that the extractant droplets brought by the N_2 gas flow were completely vaporized before ionization. This maximizes the ionization efficiency of CAP and results in a stable signal level. The deprotonated CAP ions (*m*/*z* 321) were created at ambient pressure and then introduced through the ion guide system into the LTQ mass analyzer for mass analysis.

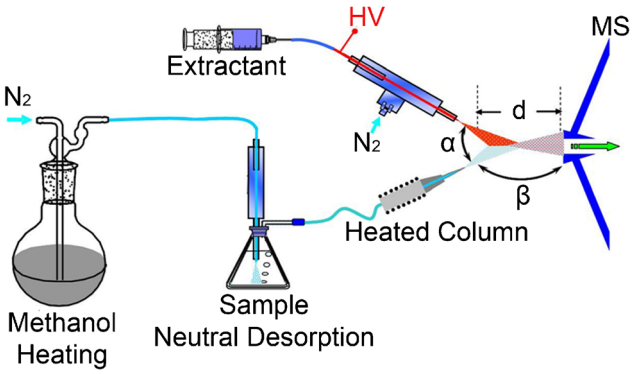

Fig. 1 Schematic diagram of ND-EESI-MS for CAP in honey sample

Methanol, ethanol, acetone, ethyl acetate, *n*-butanol, and *n*-hexane were chromatographic grade (SK Chemicals, Korea). Ultrapure water is home-made by a Simpak0r1 Millipore membrane filter system (Millipore, USA).

Five honey samples were provided by the Bee Institute of Jiangxi Agriculture University, Nanchang, China. One French honey sample was from Metro supermarket, Nanchang, China. The original area, collecting time, honey type, and its Baume degree of these six samples are shown in Table 1. All above honey samples were collected from *Apis mellifera* Linnaeus.

CAP standard solution

CAP standard (10 mg) with 99 % purity (Aladdin Reagents, China) was weighed into a 10-mL volumetric flask and dissolved in methanol to create a primary 1.0 mg/mL standard for storage. The primary standard solution was further diluted with methanol to furnish working solutions with concentrations in the range 1 to 5000 ng/mL.

Spiked honey sample preparation

The 1.0 mg/mL CAP standard solution was diluted to 10 μg/mL with methanol. A 5.0-mL honey sample (about 6.5 g) was

Table 1 Honey information in NDEEI-MS assays

Sample no.	Original area	Collected time	Honey type	Baume degree
1	Nanchang, Jiangxi Province, China	2013.9	Acacia honey	>41
2	Nanchang, Jiangxi Province, China	2013.6	Honey of various flowers	>41
3	Nanchang, Jiangxi Province, China	2013.5	Acacia honey	>41
4	Nanchang, Jiangxi Province, China	2013.5	Clover honey	>41
5	Jing'an, Jiangxi Province, China	2013.6	Chinese date honey	>42
6	France	2013.6	Honey of forest plant	>42

taken accurately. After adding 10 μg/mL CAP solutions, a series of spiked honey solution with CAP concentrations in the range 1 to 5000 ng/mL were obtained by diluting with methanol:water solution (1:1); 100 mL of each spiked honey solution was allocated into a 250-mL conical flask for experiments.

Search for ND spray reagent

At the onset of the experiment, we used the direct traditional N_2 inletting method to desorb CAP, and found that the signal intensity of target ion was not satisfactory (NL 10^1). To enhance the signal intensity, we proposed a method that the samples were desorbed neutrally by spray reagent inletting into samples together with N_2 (Fig. 1). As CAP is soluble in methanol, ethanol, acetone, butanol, and ethyl acetate, we chose these solvents as candidates of the spray reagent. As CAP is insoluble in *n*-hexane, *n*-hexane was chosen as a control in the optimization assay.

The 100 ng/mL CAP standard solution was used in optimization assays, to compare the intensities of the total MS^2 signal for different spray reagents. The spray reagent search assay was carried out under initial conditions. Specifically, the EESI ion source was set at the negative ion mode; quality ranged from 50–500 Da; ionization voltage was 3.5 kV; the temperature of the ion-transport capillary was 200 °C; the pressure of the atomization agent (99.999 % N_2) was 1.0 MPa; the velocity of the extraction agent, methanol:water:ammonia (98:1:1), was 0.006 mL/min. During the CID assay, the width of the parent ion isolation was 1.2 Da, and the collision energy was 15 %. Other conditions were optimized by the system itself.

Apparatus setting and experimental conditions

For the spray solvent channel, we used the same materials and devices as the conventional EESI ion source. Because the negative ion mode was employed, methanol:water:ammonia (98:1:1) was used as the spray solvent or the extraction solvent. To make the sample molecules collide and react with the extraction reagent ions, we optimized the experimental conditions. The final conditions we selected were: the angle (α) between two spray channels, 60°; the angle between the sample inlet and the MS horizontal level (β), 150°; the distance between MS inlet and the two spray channels, 0.5 cm (Fig. 1).

During the experiment, the EESI ion source was set at the negative ion mode. The following source parameters were applied: quality, from 50–500 Da; ionization voltage, 2.5 kV, the temperature of the ion-transport capillary, 150 °C; the pressure of the atomization agent (99.999 % N_2), 1.0 MPa; the velocity of the extraction agent, methanol:water:ammonia (98:1:1), 0.008 mL/min. During the CID assay, the width of the parent ion isolation is 1.2 Da, and the collision energy is 18 %. The other conditions were optimized by the system itself.

Results and discussions

Search for spray reagent

As showed in Fig. 2, the signal intensity (198.0) was strongest when the ND spray reagent is methanol and the weakest (39.8) for *n*-hexane. This may be the result of CAP dissolving easily

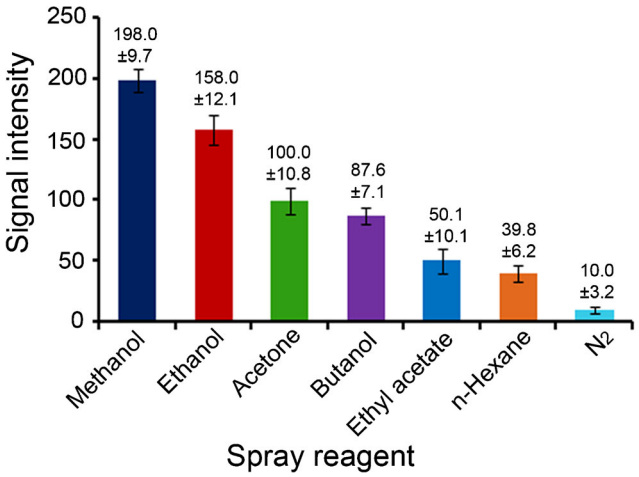

Fig. 2 The signal intensity of *m/z* 321 CID in different neutral desorption spray reagents

in methanol while being insoluble in *n*-hexane, and methanol has the strongest polarity in these organic solvents. But the MS signal was still stronger than the signal intensity obtained by traditional method in which only N_2 was used (10.0). These results suggested that the organic spray reagents could drive more CAP into gas phase or help to obtain better desorption efficiency later. Since the highest desorption efficiency was obtained when using methanol, we chose methanol as the ND spray reagent in this study.

ND-EESI-MS^n analysis of the CAP standard solutions

When the detected target molecules were in complicated matrix sample, false positive could be occurred, which need to be excluded by tandem mass spectrometry generally [30, 32]. Therefore, we used ND-EESI-MS assay to analyze CAP standard solution (100 ng/mL) and to study the CAP signals and the split mode under the experimental conditions. CAP is easily forms an $(M\text{-}H)^-$ negative ion under EESI negative ion mode, leading to a relatively strong *m*/*z* 321 signal peak (Fig. 3a). We chose *m*/*z* 321 for secondary MS (MS^2) analysis, and found that the main characteristic ions of parent ion *m*/*z* 321 were *m*/*z* 257, 194, 152, and 176 (Fig. 3b). This is in accordance with the characteristic fragments in previous studies [35–38]. We carried out MS^2 analysis of 100 ng/mL CAP standard solution by using EESI-MS^2 to ensure the reliability. The results showed that ND-EESI-MS^n spectrum of CAP was consistent with the EESI-MS^n spectrum. Hence, when a summit of *m*/*z* 321 is detected in an exact sample, and the main characteristic ion *m*/*z* 257 and *m*/*z* 194 are observed in the MS/MS spectrum, we then judge that this sample contains CAP.

Optimization of ND-EESI source

According to its MS behavior, we selected *m*/*z* 321 signal intensity under the negative ion mode to represent the detection efficiency of CAP.

Optimization of ionization voltage

The electrospray voltage and its polarity have significant impacts on the MS signal intensity. As shown in Fig. 4a, when the voltage was less than 1.5 kV, less positive charges were aggregated on the surface of droplets and mutual repulsion was relatively small between these droplets. Less repulsive force cannot overcome the surface tension of these liquid drops for them to form smaller droplets into the gas phase, resulting in low MS signal intensity. Along with the increase in voltage, more positive charges were accumulated on the droplet surfaces, and more ions were gained in the gas phase, resulting in rapidly increasing of target MS signal intensity. While the MS signal intensity was reduced as the voltage was larger than 3 kV. One possible reason was that too high voltage resulted in discharge at the spray mouth corona, and reduced ion formation efficiency and target MS signal intensity. Therefore, we selected 2.5 kV as the electrospray voltage.

Optimization of the temperature of the ion-transport pipe

The temperature of the ion-transport pipe of mass spectrometer determines the ion desolvation efficiency and further influences the target signal intensity. As shown in Fig. 4b, the target signal intensity rapidly increased along with the temperature ranged from 100 to 150 °C. While higher than 150 °C, the signal intensity gradually decreased. Therefore, we selected 150 °C as the temperature of the ion-transport pipe.

Optimization of sheath gas pressure for electrospray

Our experiments showed that target MS signal intensity was increased along with the increase of sheath gas pressure (Fig. 4c). The possible reason was that with the increase in N_2 pressure, the larger shear force on the charged droplet surface made it easier for the droplets to overcome the surface tension of the liquid drop, and for them to be atomized further and to form gas phase ions, leading to increased target MS signal intensity. When the pressure exceeds a certain value (such as 1 MPa), because of the formation of scattered ion-beam, most ions annihilated at the edge of MS inlet, and less ions got into MS, resulting in reduced target signal. So, we selected 1 MPa as the sheath gas pressure for electrospray.

Optimization of the flow rate of electrospray extractant

We found that the *m*/*z* 321 signal intensity was increased along with the flow rate of electrospray extractant when the latter was between 4 and 8 μL/min. While the flow rate was more than 8 μL/min, the *m*/*z* 321 signal intensity was decreased gradually (Fig. 4d). These results indicated that CAP could be extracted most efficiently when the flow rate was about 8 μL/min. Hence, we selected 8 μL/min as the flow rate of electrospray extractant.

Orthogonal experiment for further condition optimization

To further optimize ND-EESI-MS conditions to improve the sensitivity of this method, a dynamic orthogonal experiment was designed, based on the results of above single factor (static) optimization assays. This orthogonal experiment consisted of five factors, including the ionization voltage, the temperature of ion-transport pipe, the sheath gas pressure, the flow rate of extractant and the sample-side auxiliary gas pressure, and each factor has four levels (Table 2).

A L16 (4^5) orthogonal test was designed as Yin et al. [39], including 16 combinations. We chose the signal intensity of

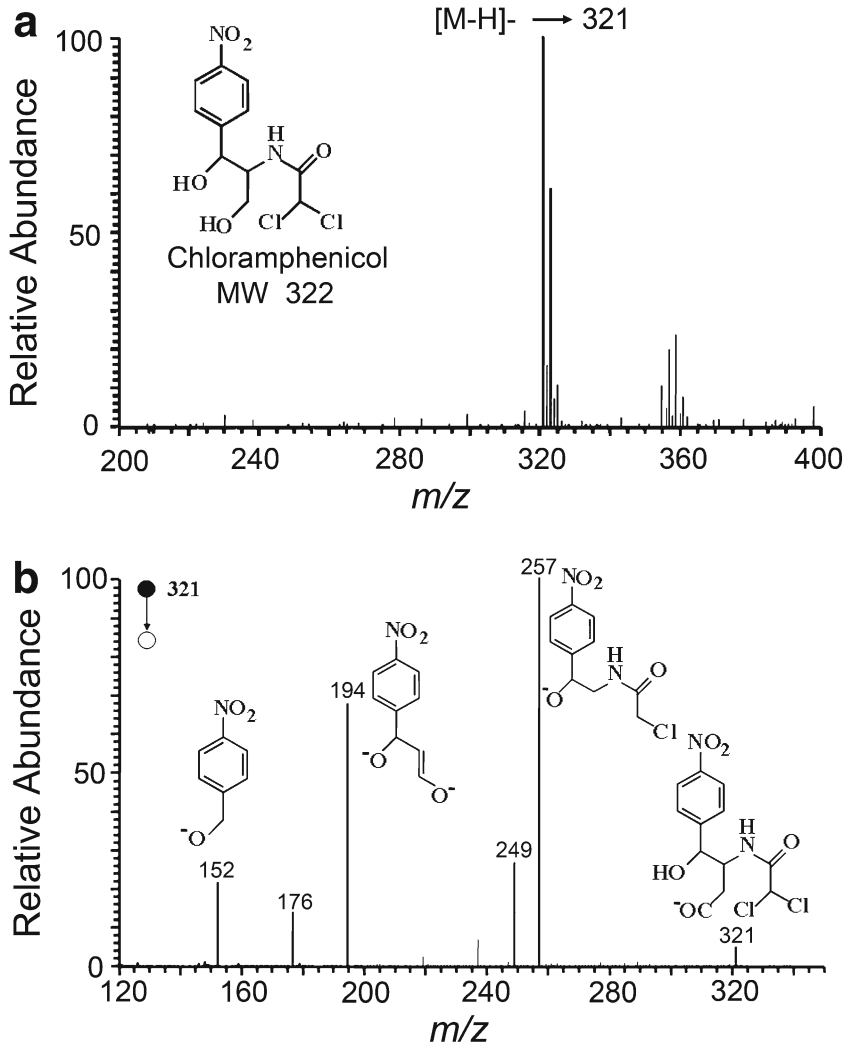

Fig. 3 ND-EESI-MS^n spectra of CAP standard solution. ND-EESI-MS spectrum (**a**) and MS^2 spectrum of *m/z* 321 (**b**)

the MS^2 characteristic ion *m/z* 257 of CAP (*m/z* 321) as the index to represent the detection efficiency of CAP in this test. Under the negative ion mode, signal intensities were obtained for all these combinations, as shown in Table 3, and each combination was detected for six times to confirm the reproducibility. As seen from Table 3, the influence of the above five factors decreases in the order: B>C>D>A>E, according to the *R* values, and the best combination is $A_1B_2C_2D_4E_4$. In other words, the maximum signal intensity would be obtained under the following conditions: the ionization voltage (2.5 kV), the temperature of ion-transport pipe (100 °C), the sheath gas pressure (1.2 MPa), the flow rate of extractant (10 μL/min) and the sample-side auxiliary gas pressure (1.0 MPa). To verify this, we detected CAP in 100 ng/mL under the above conditions, resulting in the signal strength of 488±17 (n=6), which showed significant differences with those of the 16 experimental combinations (P<0.01). We then performed CAP detection in 100 ng/mL spiked honey samples with methanol heating, leading to a 2.98 times detection efficiency. Therefore, the above conditions were adopted for ND-EESI-MS detection after this.

The ND-EESI mass spectra of real honey samples

Under the optimized experimental conditions, we analyzed the standard addition sample of CAP-honey (8 μg/mL) by ND-EESI-MS scanning. The parent ion peak of *m/z* 321 was observed in the EESI-MS spectrum, which represented the negative ion when CAP losing a proton (Fig. 5a). We observed the characteristic fragments *m/z* 257, 194, 152, and 176 (Fig. 5b) in the MS^2 spectrum of *m/z* 321, which was consistent with characteristic debris in previous literatures [35–38] and the MS^2 analysis of the standard CAP solution (Fig. 3b).

The linear range and the detection limit

For the series of concentration gradient of CAP-honey solutions (1~5000 ng/mL), to determine the linear range of the

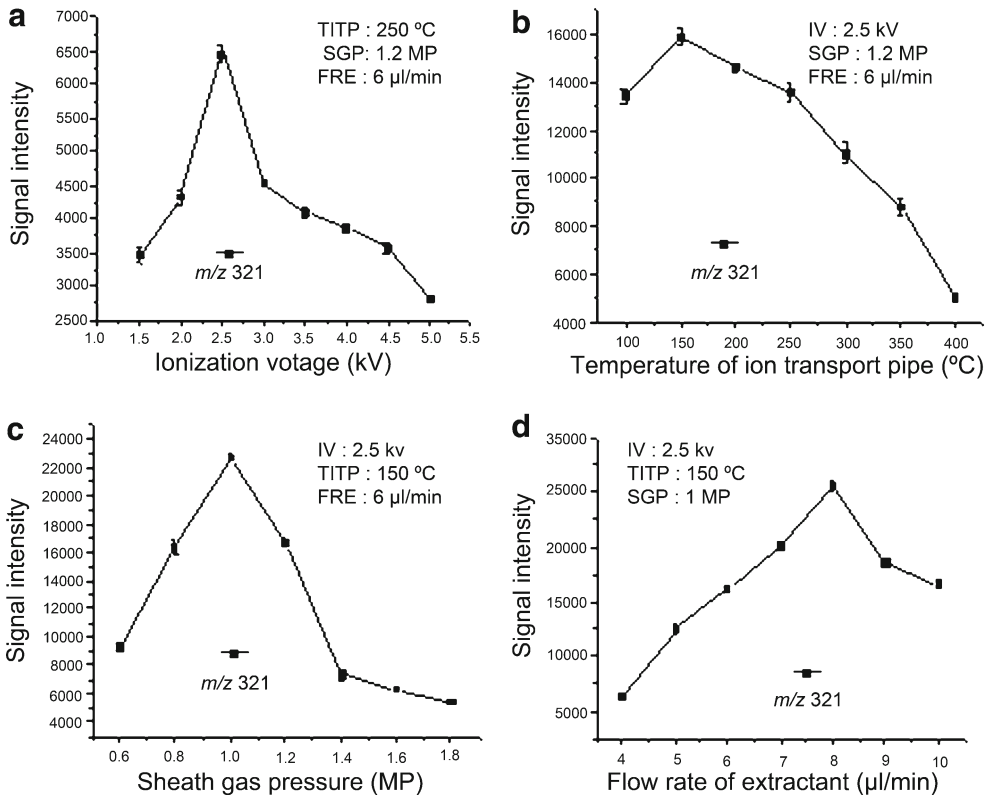

Fig. 4 The parameters that affect detection of CAP. The ionization voltage (IV, **a**), the temperature of ion-transport capillary (TITC, **b**), the sheath gas pressure (SGP, **c**), and the flow rate of extractant (FRE, **d**)

method and the detection limit, we carried out MS analysis in accordance with the above methods and conditions. In order to exclude those false positive signals and to deduct the corresponding blank back ground signals, we employed quantitative analysis for secondary characteristic fragment ions, *m/z* 321 and *m/z* 257. Every standard sample of each concentration was analyzed seven times, we then obtained a curve according to the mean value of the absolute signal intensity of *m/z* 257 and the corresponding CAP concentration. The results in our experiments showed that a good linear relationship was brought between the ionic intensity and the CAP concentration when the latter is 1~5000 ng/mL. The linear regression equation was $Y=0.1399X+1.1125$, $R=0.9947$.

For the MS analysis of 1 ng/mL spiked honey samples, net corresponding signal intensity was 6.41 for six parallel determinations. The precision (RSD) was 10.30 %, and six times standard deviation was 0.66 ($S/N=3$). According to $LOD=c3\sigma/S$ [31] (c is the standard concentration; σ is the standard deviation; S is the net corresponding signal average intensity), the calculated detection limit of spiked honey is 0.3 ng/mL using this method, achieving the EU MRPL [7]. This detection limit seems to be higher than those in some other previously published approaches, such as ELISA (0.042 ng/mL) [10], LC/MS method starts from IAC cleanup procedure (0.16 ng/g) [19], QuEChERS cleanup and LC/MS procedures (0.20 ng/g) [20], and LDTD-APCI-MS/MS (0.19 ng/g) [21]. In these published methods, different CAP cleanup procedures were carried out to reduce the disturbance from complicated matrix in honey [10, 19–21]. The results in this study showed that greatly stronger CAP MS signals were obtained when using (heated) methanol as the spray reagent (Fig. 2). In this study, for CAP-methanol solutions detected directly using

Table 2 Factors and their test levels in the orthogonal experiment

Factor Level	A Ionization voltage (kV)	B Temperature of ion transport pipe (°C)	C Sheath gas pressure (MPa)	D Flow rate of extractant (μL/min)	E Sample-side auxiliary gas pressure (MPa)
1	2.5	80	1.0	7	0.6
2	3.0	100	1.2	8	0.8
3	3.5	150	1.4	9	0.9
4	4.0	200	1.6	10	1.0

Table 3 The results of five factors and four levels orthogonal experiment

No.	Factor					Signal intensity (n=6)
	A	B	C	D	E	
1	A_1	B_1	C_1	D_1	E_1	302±19
2	A_1	B_2	C_2	D_2	E_2	302±14
3	A_1	B_3	C_3	D_3	E_3	296±20
4	A_1	B_4	C_4	D_4	E_4	230±16
5	A_2	B_1	C_2	D_3	E_4	358±10
6	A_2	B_2	C_1	D_4	E_3	314±9
7	A_2	B_3	C_4	D_1	E_2	238±7
8	A_2	B_4	C_3	D_2	E_1	166±10
9	A_3	B_1	C_3	D_4	E_2	277±12
10	A_3	B_2	C_4	D_3	E_1	231±13
11	A_3	B_3	C_1	D_2	E_4	213±10
12	A_3	B_4	C_2	D_1	E_3	246±20
13	A_4	B_1	C_4	D_2	E_3	227±16
14	A_4	B_2	C_3	D_1	E_4	320±13
15	A_4	B_3	C_2	D_4	E_1	313±9
16	A_4	B_4	C_1	D_3	E_2	229±14
K_1	1130	1164	1058	1106	1012	
K_2	1076	1167	1219	908	1046	
K_3	967	1060	1059	1114	1083	
K_4	1089	871	926	1134	1121	
k_1	282.5	291	264.5	276.5	253	
k_2	269	291.8	304.8	227	261.5	
k_3	241.8	265	264.8	278.5	270.8	
k_4	272.3	217.8	231.5	283.5	280.3	
Range (R)	40.7	74	73.3	56.5	27	
Order	B>C>D>A>E					
Excellent level	A_1	B_2	C_2	D_4	E_4	

The best combination: $A_1B_2C_2D_4E_4$

this method (the spray reagent methanol was not heated), the LOD was 8.7 pg/mL, largely lower than that of direct detection in honey solutions. This result indicated that matrix effect exists for honey samples. This result also suggested that if this ND-EESI-MS method was coupled with some CAP extraction methods, lower detection limits would be expected for CAP detection in foodstuff.

Under the same experimental conditions, for the neutral desorption using the method of N_2 being accessed into samples directly, detection limit of CAP in honey was 73.3 ng/mL. Therefore, the method in this study reduced the detection limit compared with traditional detection methods.

To test the reliability of the above calibration curve, recovery rate experiment was performed. For spiked CAP-honey solution samples with concentration of 10, 100, and 1000 ng/mL (six times for each concentration), the recovery rates were 133.0 % (RSD 5.96 %), 80.6 % (RSD 8.62 %), and 101.0 % (RSD 8.71 %), respectively. To further test the method reliability, interday (three times for 1 day) and intraday (continuous 3 days) assays were carried out. For the spiked honey samples with 10, 100, and 1000 ng/mL CAP, detected CAP concentrations were 10.3 (RSD 3.8 %), 90.9 (RSD 4.7 %), and 926.2 (RSD 3.4 %), respectively, in interday assays. In intraday assays, detected CAP concentrations for above honey-CAP solutions were 11.9 (RSD 7.4 %), 98.8 (RSD 4.1 %) and 917.7 (RSD 6.7 %), respectively. These results suggested that this NDEEI-MS method is reliable for the CAP detection in honey sample.

To further test the anti-matrix effect of this NDEEI-MS method, we performed CAP detection in different honey samples (sample 1, 2, and 3) with the same CAP concentration (100 ng/mL), results in 103.3, 94.4, and 108.2 ng/mL. Furthermore, for different dilutions of same honey sample (sample 1, 10×, 20× and 40×), deduced CAP concentrations were 90.0, 103.3, and 110.8, according to the detection results and the above linear regression equation (Y=0.1399X+1.1125, R=0.9947). These results suggested that matrix effect seemed to show no difference for various honey samples, and

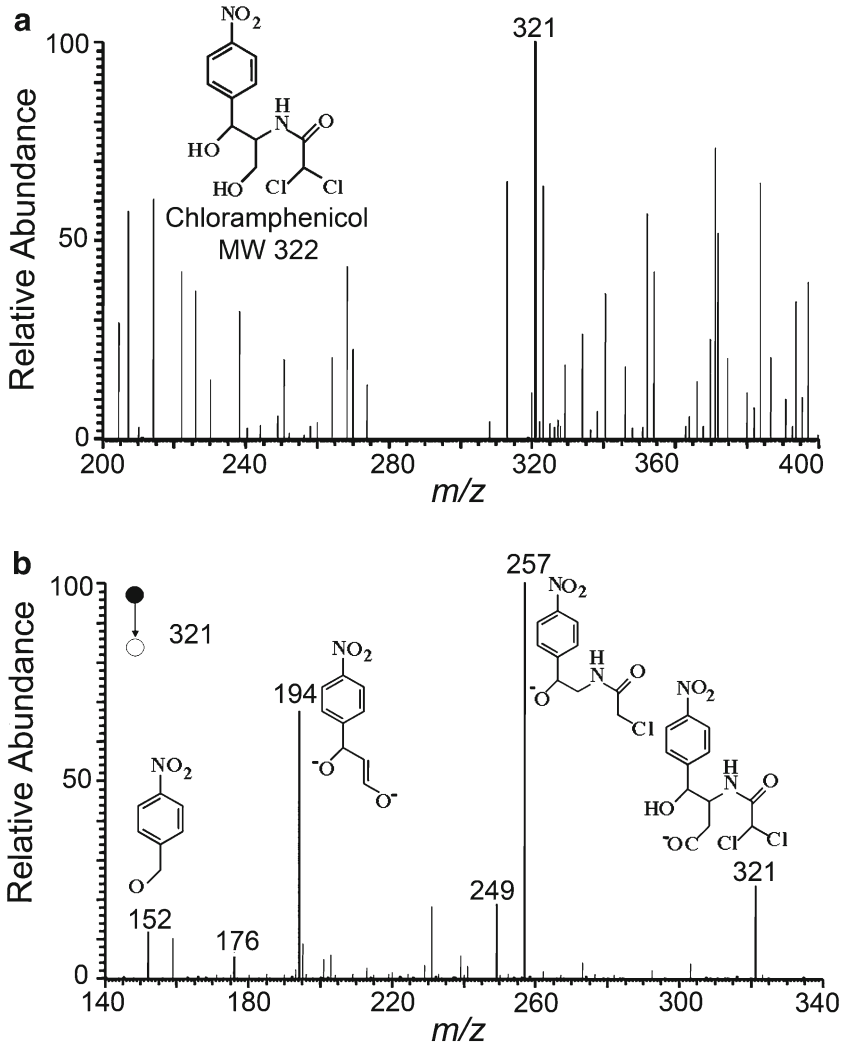

Fig. 5 The EESI-MS^n spectra of the standard addition sample of CAP-honey (8 μg/mL). The EESI-MS spectrum (**a**) and the MS^2 spectrum of *m/z* 321 (**b**)

this method could be used in CAP detection in honey with complicated matrix.

To test the capability of this ND-EESI-MS method for CAP detection in real honey samples, CAP was added to six samples for concentration of 100 or 1000 ng/mL, in consideration of no CAP was detected in these samples. The results were shown in Table 4, suggesting that this method is useful in real honey samples detection.

Analysis speed and stability

In our experiment, full spectrum scan time was set at 100 ms, and tandem mass spectrometry collision dissociation time was 30 ms, and the average time was 5 min for a single sample testing. While the detection time using GC-MS [13, 14], LC/MS [15–20], ELISA [8–10], HPLC [11, 12], and APCI-MS/MS [21] are generally longer than 3 h. An example is a newly

Table 4 CAP detection for spiked real honey samples

Sample No.	CAP concentration (ng/mL)	Detected CAP concentration (ng/mL)	RSD% (n=6)	Recovery (%)
1	100	103.3	8.94	103.3
2	100	94.4	8.46	94.4
3	100	108.2	9.00	108.2
4	1000	1092.4	7.92	109.2
5	1000	973.0	5.56	97.3
6	1000	917.7	7.00	91.8

developed method [21] that is based on laser diode thermal desorption (LDTD) and atmospheric pressure chemical ionization coupled to tandem MS (APCI-MS/MS). Although this is a rapid and direct method, sample preparation is necessary. In this method, honey sample was first diluted with NaCl saturated water and then the liquid-liquid extraction was done by ethyl acetate containing 500 μg/mL stearic acid. The sample was vortexed and centrifuged and a small aliquot of organic layer was then spotted into a 96-well plate to be dried in ambient air for LDTD analysis [21]. Another method is based on an IAC cleanup procedure followed by LC/MS/MS. Honey is dissolved in buffer solution and centrifuged, and an aliquot applied to an IAC, and then CAP is removed from the IAC with neat methanol for direct analysis by electrospray LC/MS/MS in the negative ionization mode [19]. For those upcoming nanosensor detection methods [22, 23], complicated nanosensor preparation is needed, as well as CAP extraction procedure. Therefore, compared with the existing detection methods, this is a method requiring less time, good repeatability, and with only simple sample pretreatment process.

All these results suggested the heated methanol spray reagent could help CAP be desorbed and enhance MS signal intensity for CAP determination, especially when methanol is heated. Using (heated) methanol as spray reagent or not greatly differs in signal intensity (Fig. 2), suggesting the availability of this method.

Conclusion

In summary, the results in our experiment indicate that the rapid detection of CAP in honey samples using an ND-EESI-MS method was realized. Under the optimized conditions, we obtained the CAP information in honey samples without complicated pretreatment. This method is of high sensitivity, fast analyzing speed, and strong specificity, providing a useful reference for the detection of CAP and other antibiotic residues in animal-derived food. This method also has a potential application value and positive significance in the regions of the safety of agricultural products, import, and export, etc. Furthermore, in consideration of stronger signals caused by using (heated) organic solution as the spray reagent, there is no doubting that this ND-EESI-MS method could be effectively coupled with those current CAP cleanup procedures for lower LODs. This method might be also potentially useful when it is coupled to other more accurate and sensitive mass analyzers for lower detection limits of CAP.

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