Agr. Nat. Resour. 54 (2020) 529–536

AGRICULTURE AND NATURAL RESOURCES

Journal homepage: http://anres.kasetsart.org

Research article

Confirmatory determination of chloramphenicol in honey using a molecularly imprinted polymer in a cleanup step with liquid chromatography-tandem mass spectrometry detection

Pongsak Lowmunkhong[a], Somyote Sutthivaiyakit[b], Pakawadee Sutthivaiyakit[a,*]

[a] *Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand*

[b] *Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Ramkhamhaeng University, Bangkok 10240, Thailand*

Article Info

Article history:
Received 2 September 2019
Revised 22 November 2019
Accepted 29 November 2019
Available online 30 October 2020

Keywords:
Chloramphenicol,
Honey,
Liquid chromatography-tandem mass spectrometry (LC-MS/MS),
Microwave heating,
Molecularly imprinted polymer (MIP)

Abstract

A sensitive solid-phase extraction method was developed using a new molecularly imprinted polymer (MIP) in a cleanup step with detection using liquid chromatography-tandem mass spectrometry (LC-MS/MS) for confirmatory determination of chloramphenicol (CAP) in honey. The MIP was prepared using a noncovalent and microwave-heating-induced polymerization method at 80°C. 4-Vinylpyridine, a mixture of trimethylolpropane trimethacrylate and 1,4-divinylbenzene, 1,1-azobis(cyclohexane carbonitrile) and Pluronic P123 were used as a monomer, crosslinkers, an initiator and a surfactant, respectively, in a mixture of acetonitrile and water. The polymer was used as a solid-phase extraction sorbent in the cleanup step of honey extracts prior to injection into an LC-MS/MS system. Honey samples were spiked at 0.30 µg/kg, 0.50 µg/kg and 1.0 µg/kg with d_5-CAP as the internal standard, and the mean recoveries of the spiked honey samples were in the range 91.9–104.6% with intra-day and inter-day precisions less than or equal to 7.9% and 9.3%, respectively.

Introduction

Chloramphenicol (CAP; Fig. 1) is an inexpensive broad-spectrum antibiotic with a nitrobenzene moiety, and it inhibits the synthesis of bacterial proteins without directly affecting a large number of other metabolic processes (Jardetzky, 1963). In human medicine, it has adverse side effects such as causing aplastic anemia, neuritis and encephalopathy with dementia (Botsoglou and Fletouris, 2001). Thus, its use is banned in food-producing animals in many countries, such as those in the European Union (EU) and in the USA and Canada (Taka et al., 2012). The EU has set the minimum required performance limit (MRPL) for CAP in foods of animal origin at 0.3 µg/kg (Commission Decision 181/2003/EC, 2003). Consequently, a selective and sensitive method is needed for monitoring CAP residues in food. Among the various techniques for the unambiguous confirmation of the zero-tolerance residue limit, liquid chromatography with tandem mass spectrometry (LC-MS/MS) is attractive, as it provides not only sensitivity but also selectivity that complies with the EU guidelines

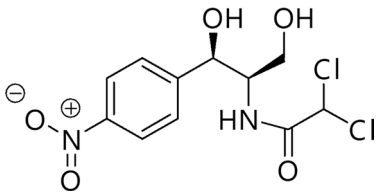

Fig. 1 Chemical structure of chloramphenicol

* Corresponding author.
E-mail address: fscipws@ku.ac.th (P. Sutthivaiyakit)

https://doi.org/10.34044/j.anres.2020.54.5.10

(Commission Decision 2002/657/EC, 2002). However, honey is a complex natural product that requires sample pretreatment to remove potential interferences prior to injection into an LC-MS/MS system. Moreover, several methods have been reported based on LC-MS/MS using commercial sorbents (Forti et al., 2005; Ortelli et al., 2004, Rønning et al., 2006, Liu et al., 2016).

Molecularly imprinted polymers (MIPs) are a popular sorbent used in sample cleanup, as they provide more selectivity than other types of solid phase extraction sorbents (Rejtharová and Rejthar, 2009; Shirmer and Meisel, 2009; Wang et al., 2014). MIPs are synthetic polymers generated by copolymerizing a prepolymerization solution of a monomer and template with an excess of a cross-linking monomer. Following copolymerization, the template is removed, thereby generating specific cavities that are complementary to the template in size, shape and functionality. For example, Wang et al. (2014) synthesized MIPs by first copolymerizing a prepolymerized solution of acrylamide and CAP in ethyl acetate with ethylene glycol dimethylacrylate and then grafting the resultant polymers onto chitosan (CHI). The CHI-MIP was then used as a sorbent in the pretreatment and cleanup of CAP from spiked honey and milk samples. However, in the preparation of MIPs in these reports, microwave heating was not used.

Microwave heating is often used in the synthesis of organic compounds and is gaining popularity in the synthesis of MIPs as it reduces the reaction time and provides monodispersity for the resultant polymer (Lidström et al., 2001; Zhang et al., 2012; Viveiros et al., 2018). The aim of the present study was to develop a confirmatory method for the determination of CAP in honey based on the use of a new MIP synthesized using a microwave heating method.

Materials and Methods

Materials

Trimethylolpropane trimethacrylate (TRIM), 4-vinylpyridine (4-VP) and 1,1-azobis (cyclohexane carbonitrile) (ABCN) were purchased from Sigma Aldrich (USA). 1,4-Divinylbenzene (DVB) was obtained from Merck (Germany). Chloramphenicol (CAP) and d_5-CAP were purchased from Sigma Aldrich (China) and Cambridge Isotope Laboratories, Inc., respectively. Pluronic P123 was a gift from BASF (USA). Ultra-pure water was obtained from a Millipore Simplicity™ water purification system (France). All other chemicals used were of analytical reagent grade. A 100 ppm CAP stock standard solution was prepared by dissolving 2.5 mg of CAP in 25 mL of methanol. The solution was diluted to the required concentrations with 10% methanol in water and stored at 4°C before use. A 10 parts per billion d_5-CAP solution was prepared by diluting 10 µL of 100 parts per million d_5-CAP stock solution with methanol to 100 mL.

Instrumentation

Polymerization was carried out on a microwave accelerated reaction system (MARS-5, CEM Corporation, USA). The size and surface morphology were investigated using scanning electron microscopy (SEM) with an FEI Quanta 450 (the Netherlands) operated at 15 keV with tungsten as a filament. Chromatographic analyses were performed on an LC-MS/MS consisting of an Applied Biosystems API 2000 triple quadrupole instrument (Thornhill, Canada) equipped with an electrospray ionization source, an Agilent 1100 LC binary pump (Agilent Technology, Germany), and an Agilent 1100 autosampler. Data acquisition was performed in the negative ionization mode and recorded using the Sciex Analyst software (1.5.1).

Liquid chromatography-electrospray ionization-tandem mass spectrometry analysis

LC separation was performed on a Lichrospher®100 RP 8 column (5.0 µm, 125 × 4 mm). Gradient elution was carried out with 3 mM ammonium acetate adjusted to pH 5 and methanol, and the linear program was as follows: 10% methanol to 30% methanol in 0.1 min; 30% to 40.0% in 0.9 min; and 40% to 70.0% in 2.0 min, which was maintained for 3 min at a flow rate of 1.0 mL/min. Then, the column was equilibrated at the initial LC conditions for 9 min prior to the next injection. The column temperature was held at 25°C, and the injection volume was 50 µL. Using a T-connector, the splitting ratio of eluate transferred from the column to the electrospray ionization (ESI) interface was 1:3. ESI-MS/MS detection was performed in the negative ionization mode for chloramphenicol and its deuterated internal standard. The mass analyzers Q1 and Q3 were operated at low resolution. The ESI source parameters were all optimized manually using the flow injection method until the highest signal was achieved.

Synthesis of chloramphenicol imprinted polymer

Nine vessels were used. In each vessel, the prepolymerization solution was first prepared by mixing 161.6 mg CAP with 210 mg 4-VP in 7 mL acetonitrile. The solution was degassed by sonication under a nitrogen atmosphere for 5 min and then stored in the dark for 12 hr, allowing for self-assembly between the template and monomer. Next, the prepolymerization solution was transferred to a microwave vessel and mixed with 1,364 mg TRIM, 520 mg DVB, 50 mg Pluronic P123 and 100 mg ABCN in a mixture of 7 mL acetonitrile and 50 mL water. The mixture was thoroughly homogenized, purged with a stream of nitrogen for 5 min and placed in a microwave. The microwave temperature programme was as follows: heating was performed from room temperature to 80°C within 3 min and then maintained at 80°C for an additional 120 min. The obtained polymers were extensively washed with 10% volume per volume acetic acid in methanol using microwave heating at 80°C for 45 min and then they were washed with methanol until no CAP residues were observed in the wash solution. The non-imprinted polymers (NIPs) were also prepared as described above but without the template.

Binding studies

The effect of the initial concentration on the adsorption capacity was investigated based on static equilibrium. Samples (each 20 mg) of

MIPs were mixed with varied concentrations of CAP (10-100 µg/L) in 2 mL dichloromethane. The mixture was shaken for 6 hr at room temperature followed by centrifugation at 3,000 rpm (1,000×*g*) for 5 min. The supernatant was separated and filtered through a 0.2 µm nylon membrane filter prior to LC-MS/MS injection.

Honey extraction and cleanup

A honey sample (1.00 ± 0.02 g) was weighed into a 15 mL polypropylene tube and spiked with 1.0 mL of 10 µg/kg d_5-CAP. This mixture was vortexed for 2 min and left at room temperature for 1 hr. Then, 2 mL of 4% NaCl were added to the mixture before vortexing for another 2 min. Then, 3 mL of ethyl acetate was added, followed by additional vortexing for 2 min before being centrifuged at 3,000 rpm for 5 min. The supernatant was transferred into a new tube. The extraction of the sample was repeated with 3 mL ethyl acetate. The supernatants were combined and evaporated to dryness under a stream of nitrogen at 45°C. The residue obtained was redissolved in 7 mL of 10% MeOH. This solution was then loaded onto an MIP cartridge containing 80 mg MIP that had already been preconditioned successively with 3 mL of methanol and 6 mL of water. The cartridge was washed with 3 mL of water. CAP was eluted with 4 mL of methanol. The eluate was evaporated under a stream of nitrogen at 45 °C until dryness. The residue was redissolved in 150 µL of 50% MeOH, and the solution was filtered through a 0.2 µm filter membrane into an autosampler vial.

Matrix-matched calibration curve and method validation

The matrix-matched calibration curve was based on the internal standard method. Calibration standards at 0 µg/kg, 0.2 µg/kg, 0.3 µg/kg, 0.5 µg/kg, 1.0 µg/kg and 2.0 µg/kg were prepared with a 1.00 ± 0.02 g sample containing 1.0 mL of 10 µg/L d_5-CAP. The matrix-matched standards were left at room temperature for 1 hr followed by processing as described in the honey sample extraction and cleanup. The area ratio of CAP to d_5-CAP was plotted against the concentration of CAP. Retention time and transition ion ratios were used to identify both CAP and d_5-CAP. Linearity, selectivity, precision (intra-day and inter-day) and recovery were determined. Since certified reference materials for CAP in honey are not available, the accuracy of the developed method was determined using blank honey fortified with CAP at 0.3 µg/kg, 0.5 µg/kg and 1.0 µg/kg. Additionally, the selectivity of the developed method was checked by analyzing 10 real samples in triplicate.

Results and Discussion

Synthesis of molecularly imprinted polymers for chloramphenicol using microwave-heating-induced polymerization

In the current work, a new MIP for chloramphenicol was synthesized and used as a cleanup sorbent to remove the existing matrix in the extract obtained from an ethyl acetate extraction of honey. 4-Vinylpyridine was chosen as a functional monomer that can form both hydrogen-bonding with CAP by the pyridine ring nitrogen atom and π-π stacking via aromatic rings. The mixed cross linkers were a 1:1 molar ratio of TRIM and DVB, which has been previously reported to provide polymers with suitable sizes for solid phase extraction applications (Yoshimatsu et al., 2007; Zhang et al., 2009). The synthetic route of MIP is shown in Fig. 2.

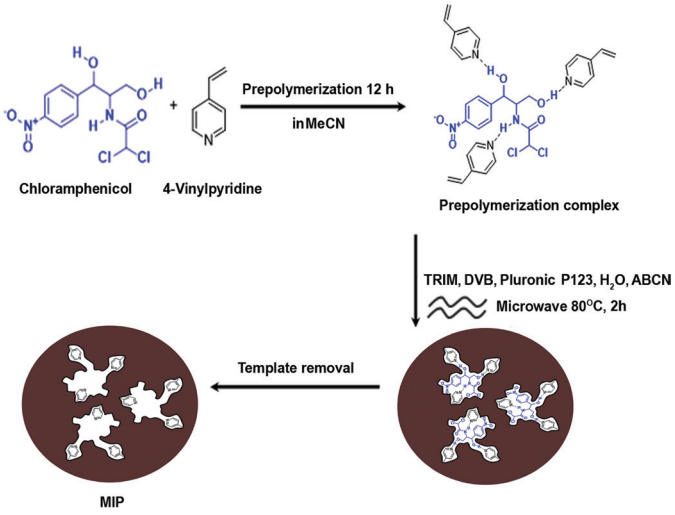

Fig. 2 A schematic drawing of a synthetic route for molecularly imprinted polymers

In the case of a noncovalent imprinting polymer, the solvent serves three purposes: to bring all components into one phase, to be a pore-forming agent; and to maximize the likelihood of template-monomer complex formation (Cormack and Elorza, 2004). Additionally, when microwave irradiation is used, its dielectric properties involve heating capability leading to a shorter reaction time and in some cases to the enhancement of initiator radicals (Kappe, 2004; Stange et al., 2007). In the current study, four solvents, namely, acetonitrile (MeCN), dimethyl sulfoxide (DMSO), tetrahydrofuran (THF) and toluene, were investigated as polymerization solvents. The dielectric constants and loss factors (tanδ) of toluene, THF, MeCN and DMSO are 2.38, 0.040; 7.58, 0.047; 37.5, 0.062 and 46.7, 0.825, respectively (Kappe, 2004). No polymerization occurred in toluene, and a very small yield of polymerization was obtained from THF; however, a high yield of polymerization was obtained from both DMSO and MeCN. The polymerization yields obtained in these solvents may have been due to both a heating effect and stabilization of template-monomer complex formation. The first factor favors a high dielectric loss and the latter favors a low polar aprotic solvent for H-bond interaction and a high polar solvent for hydrophobic interaction (Cormack and Elorza, 2004, O'Mahony et al., 2005). However, compared to DMSO, MeCN provided more uniformly spherical bead shapes with greater roughness, as shown in Fig. 3. The rough morphology can lead to a high surface area, leading to the enhancement of extraction capacity (Zhang et al., 2012). Thus, MeCN was chosen as the polymerization solvent in subsequent experiments.

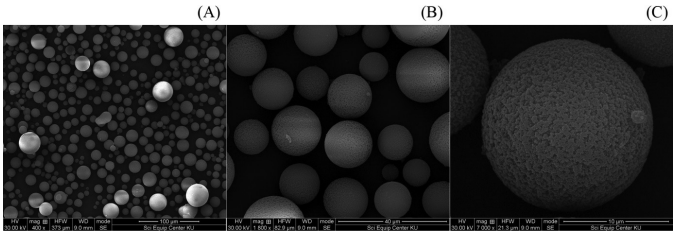

Fig. 3 Scanning electron microscopy images of molecularly imprinted polymers prepared using microwave heating at 80°C for 2 hr in a mixture of MeCN-water and taken at 15 keV with tungsten as a filament, Magnifications (A) 400×, (B) 1,800× and (C) 7,000×

Furthermore, different polymerization times (120 min or 180 min) at 80°C and different temperatures (70°C, 80°C, or 90°C) at a 120 min reaction time were also investigated. Compared to a polymerization time of 180 min, that of 120 min yielded smaller beads with a narrower size distribution. Thus, a polymerization time of 120 min was selected. Regarding the effect of temperature, at 70°C, polymerization was observed, but the yield was low, and at 90°C, a polymer lump formation was obtained. However, at 80°C, uniform spherical beads with a narrow diameter distribution (5–40 µm) were obtained, as shown in Fig. 3. Thus, a polymerization temperature of 80°C was selected for the synthesis of MIPs.

Binding characteristics of molecularly imprinted polymers and non-imprinted polymers

The binding parameters of both polymers were studied based on static equilibrium and calculated using Scatchard analysis (Wang et al., 2014) as provided in Equation 1:

$$\frac{Q}{[CAP]_f} = \frac{(Q_{max} - Q)}{K_d} \quad (1)$$

where Q is the amount of CAP bound to polymers, $[CAP]_f$ is the free concentration in solutions, Q_{max} is the apparent maximum number of binding sites, and K_d is the equilibrium dissociation constant. The plot of the ratio of the bound CAP concentration to the free CAP concentration against the bound CAP concentration produced a straight line, as shown in Fig. 4, where the slope is inversely related to K_d, and its intercept multiplied by K_d gives Q_{max}. For MIPs, the plot has two straight line segments, with the left segment showing a strong negative slope and the right segment displaying a gentle positive slope, suggesting heterogeneity of the binding sites (Wang et al., 2014). Furthermore, the plot shows both positive and negative slopes in the absence of a competing ligand, indicating that both cooperative and independent sites exist in the MIPs (Kalbitzer and Stehlik, 1979). In contrast, for NIPs, only a single positive slope was evident, indicating the presence of cooperative binding sites (Kalbitzer and Stehlik, 1979).

The upward slope of the Scatchard plot is largely indicative of the existence of negative cooperativity, which means that the interaction between binding sites leading to binding at one site decreases the affinity of others (Boardbar et al., 1996). These types of behavior were also reported by Ahmad et al. (2018) for atrazine-imprinted polymers. Table 1 depicts the values of K_d and Q_{max} for both polymers. Additionally, among the three K_d values, the positive K_d was the lowest, thus indicating that the strongest affinity sites were in the lower concentration range of MIPs, suggesting that the imprinting effect due to the template molecule increases the pre-existing binding capability of a polymer (Baggani et al., 2012). The molecular recognition of MIPs may have been due to both hydrogen bonding and the π-π stacking effect. The hydrogen bond between an amide hydrogen or the 1,3-dihydroxy moiety of CAP and the pyridine nitrogen atom of the 4-VP in MIPs could be a driving force for selectivity. Additionally, the π-π stacking interaction between the aromatic rings of CAP and 4-VP in the polar solvent could be a stabilization force (Molinelli et al., 2005; O'Mahony et al., 2005; Sun et al., 2008).

Optimization of the cleanup step

As honey is a complex matrix, its ethyl acetate extract needs to be subjected to cleanup pretreatment before injection into an LC-MS/MS system to increase the sensitivity and selectivity and to prolong the lifetime of the instrument. Important parameters were investigated: adsorption solvent and volume, amount of MIPs, washing solvent, eluent and eluent volume. Various solvents (MeOH, MeCN, toluene, dichloromethane and mixtures of dichloromethane and hexane, aqueous methanol and water) were investigated, and their percentage binding is shown in Fig. 5. MeOH and MeCN were poor adsorption solvents, while toluene, mixtures of dichloromethane and hexane, and aqueous methanol gave good adsorption. Since hexane is flammable, 10% MeOH was selected as an adsorption solvent.

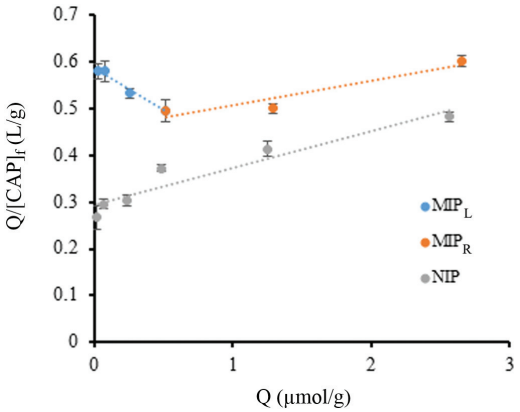

Fig. 4 Scatchard curves for chloramphenicol binding to molecularly imprinted polymers (MIPs, upper line) and non-imprinted polymers (NIPs, lower line)

Table 1 Mean (± SD) adsorption parameters of molecularly imprinted polymers (MIPs) and non-imprinted polymers (NIPs) (n = 3)

Polymer	K_d (µmol/L)	Q_{max} (µmol/g)
MIP_L	5.44 ± 0.44	3.20 ± 0.31
MIP_R	-19.19 ± 1.87	-8.74 ± 1.08
NIP	-12.58 ± 0.91	-3.71 ± 0.34

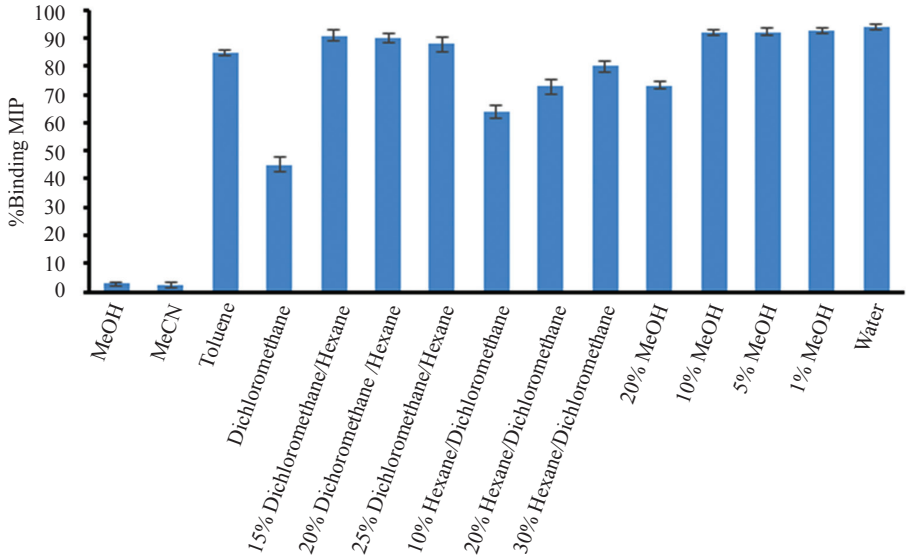

Fig. 5 Binding test for chloramphenicol in different solvents,where error bars are ± SD.

Subsequently, the amount of MIPs required for efficient retention of CAP was investigated. Different amounts of MIPs ranging from 50 mg to 120 mg were packed into various cartridges. The results showed that 80 mg MIPs were adequate, producing 93% recovery. Further increases in the amount of MIPS provided no improvement in retaining CAP. The cleanup step was further optimized with a washing step by varying the percentage of MeOH in water in the range 0–20%. When washing with water, the recovery of CAP from MIP was the highest. An increase in the percentage of MeOH in the washing step reduced the retention of CAP. This suggested that the π-π stacking effect enhanced the selectivity of molecular recognition in an imprinted polymer (Molinelli et al., 2005; Xu et al., 2010). Thus, water was chosen as the washing solvent. As the elution process is the reverse process of adsorption, among the solvents tested and shown in Fig. 5, MeOH and MeCN were good eluents for CAP. Nevertheless, the former was chosen due to its lower price and lower toxicity. Next, the volume of eluent was varied in the range 3–10 mL. The recovery of CAP increased with an increase in the volume of MeOH from 3 mL to 5 mL and then remained almost constant. Thus, 5 mL of MeOH was used.

Liquid chromatography-tandem mass spectrometry

The precursor ions of both CAP and d_5-CAP were first determined by operating Q1 in the scan mode. Next, for the selected precursor, the following parameters were optimized: declustering potential focusing potential), entrance potential and collision cell entrance potential. For the selected precursor ion, a product ion scan was then performed to achieve two appropriate product ions, and the two most abundant and stable product ions were selected. For each multi-reaction monitoring (MRM) transition, the collision energy and the collision cell exit potential were then optimized. Finally, the ESI source parameters (curtain gas, collision activated dissociation gas, ion spray voltage, nebulizer gas, heater gas and temperature) were all optimized using flow injection analysis. The optimized parameters for MS/MS of CAP and d_5-CAP are presented in Table 2.

The Q1 scan spectrum of CAP (Fig. 6) shows $[M–H]^-$, $[M+2–H]^-$ and $[M+4–H]^-$ ions at *m/z* values of 320.9, 323.0, and 325.2, respectively, verifying the presence of two chlorine atoms in CAP. Fig. 7 shows a typical product ion scan of the *m/z* 320.9 precursor ion of CAP. The two most abundant fragment ions were observed at *m/z* values of 256.6 and 152.0 and were chosen as the qualitative and quantitative ions, respectively.

Table 2 Tandem mass spectrometry ion transitions and corresponding parameters*

Analyte	Q1 Mass (Da)	Q3 Mass (Da)	Dwell time (ms)	DP (V)	FP (V)	EP (V)	CEP (V)	CE (V)	CXP (V)
CAP	320.9	151.8	200.00	-16.00	-340	-10.50	-16.00	-20.00	-22.00
CAP	320.9	256.8	200.00	-16.00	-340	-10.50	-16.00	-12.00	-16.00
CAP-d5	325.7	156.7	200.00	-16.00	-340	-10.50	-16.67	-23.00	-22.00

CAP = chloramphenicol, DP = declustering potential, FP = focusing potential, EP= entrance potential, CEP = cell entrance potential, CE = collision energy, CXP = cell exit potential;

*Source parameters: curtain gas, 25.0 psi; collision activated dissociation gas, 7.0 psi; ion spray voltage, -4,500 V (NI mode), nebulizer gas, 40.0 pounds per square inch (psi); heater gas, 40.0 psi; temperature, 450°C

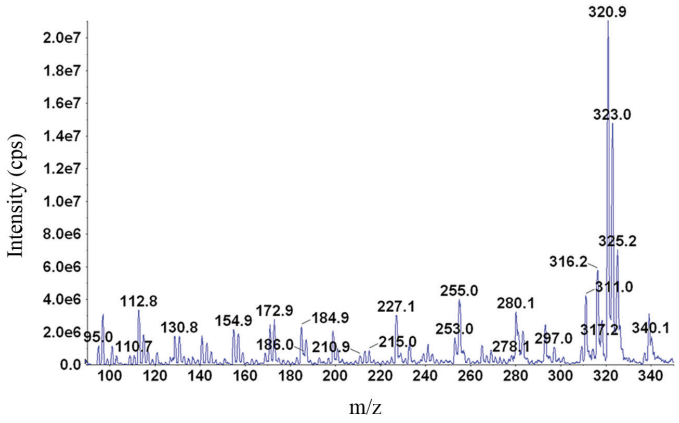

Fig. 6 Full scan mass spectrum of chloramphenicol

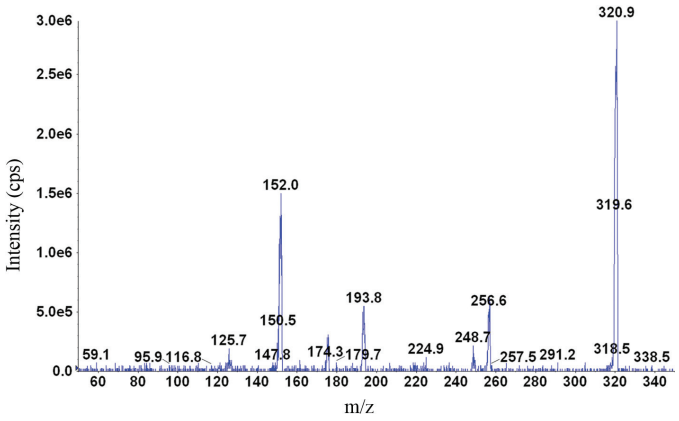

Fig. 7 Typical product ion scan mass spectrum of chloramphenicol using *m/z* 320.9 as the precursor ion

The fragment ion at *m/z* 152.0 was likely formed through cleavage of the benzylic carbon bearing a hydroxyl group, giving rise to a $[NO_2C_6H_4CHOH]^-$ ion, while the ion at *m/z* 256.6 was proposed to occur via the loss of a Cl radical from the $[M–H–CH_2O]^-$ ion (Bowie, 1990), as shown in Fig. 8.

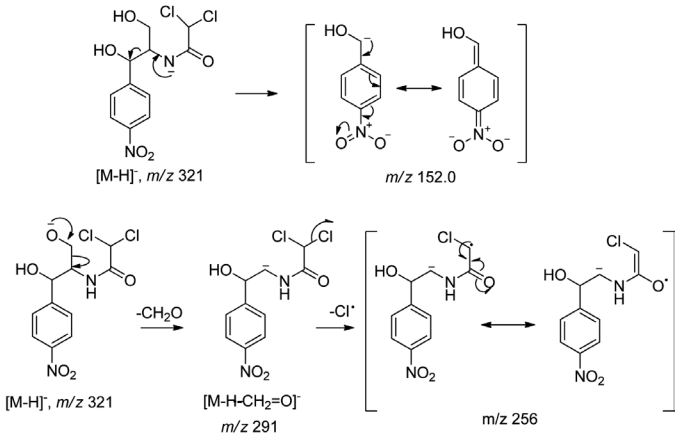

Fig. 8 A schematic drawing of the proposed mass fragmentation pathways for ions at *m/z* 256.6 and 152.0

Method validation

The selectivity of the developed method was investigated by analyzing different blank honey samples (Chen and Li, 2013). No interference peaks were observed at the retention time of the analyte. Fig. 9 shows a typical total ion chromatogram (MRM) for (A) a blank honey sample spiked with d_5-CAP as an internal standard and for (B) a blank honey sample spiked with 1 µg/kg CAP and d_5-CAP.

A matrix-matched calibration curve with d_5-CAP as an internal standard was constructed at five levels in the range 0–2.0 µg/kg. The ratio of the standard area to the internal standard area was plotted against the concentration of CAP spiked in blank honey using linear regression analysis. There was linearity in the concentration range 0–2.0 µg/kg with a regression coefficient greater than 0.99. The calibration equation was y = 0.3696x + 0.0167. The limit of detection and limit of quantitation based on three times and ten times the standard deviation obtained from 10 replicates of a blank honey spiked with 0.1 µg/kg CAP, respectively, were estimated to be 0.022 µg/kg and 0.074 µg/kg, respectively.

Precision and accuracy were investigated by measuring the relative standard deviation of both intra- and inter-day analysis for 3 d by analyzing blank honey spiked at 0.3 µg/kg, 0.5 µg/kg and 1.0 µg/kg. Each day, five replicates were performed. Relative standard deviations for intra- and inter-day analysis ranged from 4.4% to 7.9% and from 5.2% to 9.3%, respectively. Recoveries of CAP were in the range 91.9–104.6%. The results (Table 3) indicated the effectiveness of extraction, cleanup and detection for the current study.

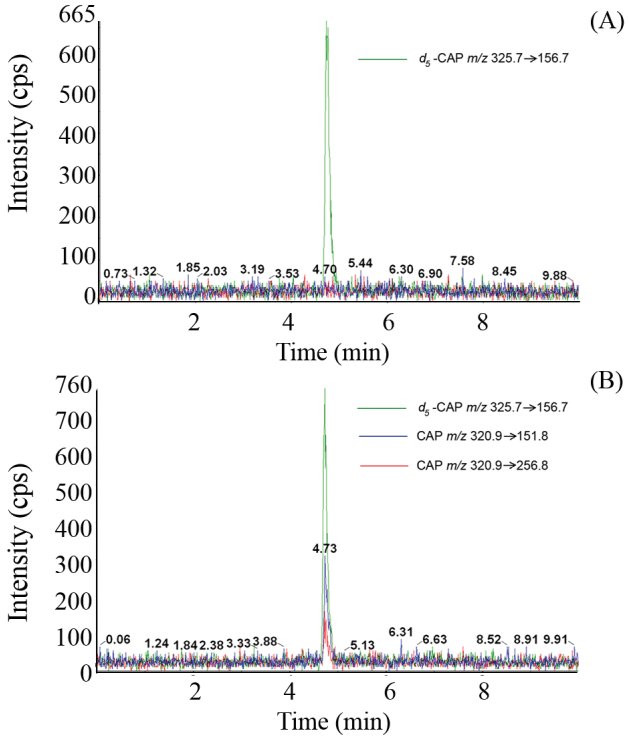

Fig. 9 Typical total ion chromatograms (multi-reaction monitoring): (A) blank honey sample spiked with d_5-CAP; (B) blank honey sample spiked with 1 µg/kg CAP and d_5-CAP

Table 3 Accuracy and precision of chloramphenicol in spiked blank honey

Spiked level	Intra-day precision (n = 5)		Inter-day precision (n = 3)	
(µg/kg)	Mean recovery (%)	RSD (%)	Mean recovery (%)	RSD (%)
0.30	104.6	7.9	100.2	9.3
0.50	91.9	7.8	96.9	9.0
1.00	102.6	4.4	102.7	5.2

RSD = relative standard deviation

Application to real samples

The method was applied to the analysis of 10 honey samples purchased from supermarkets in Bangkok, Thailand. Of the 10 samples, 4 had been imported from foreign countries. The following criteria were used to identify the presence of CAP contamination in honey: (a) retention time and (b) two MRM transitions and the proper ratio of these two transitions (Commission Decision 2002/657/EC, 2002). Table 4 shows that of the 10 samples, only one was contaminated with 0.50 µg/kg CAP.

Table 4 Analysis of chloramphenicol in honey samples using liquid chromatography-tandem mass spectrometry (n = 3)

Honey sample	Chloramphenicol found (µg/kg)	RSD (%)
1	<LOQ	–
2	nd	–
3	nd	–
4	<LOQ	–
5	nd	–
6	nd	–
7	<LOQ	–
8	nd	–
9	0.50	2.1
10	nd	–

*LOQ = limit of quantitation; nd = not detectable

The current developed method provides a new and alternative confirmation method for CAP detection in honey. A new chloramphenicol imprinted polymer (CAP-MIP) sorbent was synthesized based on microwave heating-induced polymerization of a preassembled mixture of CAP and 4-VP, TRIM and DVB in the presence of ABCN and Pluronic P123. Compared to previous methods, the present method had a shorter reaction time (approximately 2 hr instead of 14.5–24 hr) and the synthesized MIPs had a narrow diameter distribution, thus removing the need to grind and sieve prior to use (Guo et al., 2008; Wang et al., 2011; Shekarchi et al., 2013). The resulting MIPs could be successfully implemented in the cleanup step while providing adequate selectivity and sensitivity. Moreover, the use of CAP-MIP in the cleanup step made the method appropriate for routine analysis.

Acknowledgements

This research was funded by the Kasetsart University Research and Development Institute (KURDI), Bangkok, Thailand under grant number P-T(S) 137.56. The Center of Excellence for Innovation in Chemistry (PERCH-CIC) and the Ministry of Higher Education, Science and Research Innovation provided access to their LC-MS/MS system.

References

Ahmad, A.L., Che Lah, N.F., Low, S.C. 2018. Configuration of molecular imprinted polymer for electrochemical atrazine detection. J. Polym. Res. 25: 243. doi.org/10.1007/s10965-018-1595-2

Baggani, C., Giovannoli, C., Anfossi, L., Passini, C., Baravalle, P., Giraudi, G.A. 2012. A connection between the binding properties of imprinted and nonimprinted polymers: A change of perspective in molecular imprinting. J. Am. Chem. Soc. 34: 1513–1518. doi.org/10.1021/ja205632t

Boardbar, A.K., Sabouryy, A.A., Moosavi-Movahedi, A.A. 1996. The shapes of Scatchard plots for systems with two sets of binding sites. Biochem. Educ. 24: 172–175. doi.org/10.1016/0307-4412(95)00122-0

Botsoglou, N.A., Fletouris, D.J. 2001. Drug residues in foods: Pharmacology, food safety, and analysis. In: Fletouris, D.J. (Ed.). Marcel Dekker. New York, NY, USA, pp. 38–39.

Bowie, J.H. 1990. The fragmentations of even-electron organic negative ions. Mass Spectrom. Rev. 9: 394–379.

Chen, L., Li, B. 2013. Magnetic molecularly imprinted polymer extraction of chloramphenicol from honey. Food Chem. 141: 23–28. doi.org/10.1016/j.foodchem.2013.02.085

Commission Decision 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. 2002. Official Journal of the European Communities C(2002)3044 17.8. L221/8–L221/36.

Commission Decision 181/2003/EC of 13 March 2003 amending EU decision 2002/657/EC as regard the setting of minimum required performance limits (MRPLs) for certain residues in food of animal origin. 2003. Official Journal of the European Communities C(2003)764 15.3. L71/17–L71/18.

Cormack, P.A.G., Elorza, A.Z. 2004. Molecularly imprinted polymers: Synthesis and characterization. J. Chromatogr. B. 804: 173–182.

Forti, A.F., Campana, G., Simonella, A., Multari, M., Scortichini, G. 2005. Determination of chloramphenicol in honey by liquid chromatography-tandem mass spectrometry. Anal. Chim. Acta. 529: 257–263. doi.org/10.1016/j.aca.2004.10.059

Guo, L., Guan, M., Zhao, C., Zhang, H. 2008. Molecularly imprinted matrix solid-phase dispersion for extraction of chloramphenicol in fish tissues coupled with high-performance liquid chromatography determination. Anal. Bioanal. Chem. 392: 1431–1438. doi.org/10.1007/s00216-008-2454-5

Jardetzky, O. 1963. Studies on the mechanism of action of chloramphenicol. J. Biol. Chem. 238: 2498–2508.

Kalbitzer, H.R., Stehlik, D. 1979. On the analysis of competitive binding of various ligands to cooperative and independent binding sites of macromolecules. Z. Naturforsch. C. Biosci. 34: 757–769. doi.org/10.1515/znc-1979-9-1018

Kappe, C.O. 2004. Control microwave heating in modern organic synthesis. Angew. Chem. Int. Ed. Engl. 43: 6250–6284. doi.org/10.1002/anie.200400655

Lidström, P., Tierney, J., Wathy, B., Westman, J. 2001. Microwave assisted organic synthesis-a review. Tetrahedron 57: 9225–9283. doi.org/10.1016/S0040-4020(01)00906-1

Liu, H.Y., Lin, S.L., Fuh, M.R. 2016. Determination of chloramphenicol, thiamphenicol and florfenicol in milk and honey using modified QuEChERS extraction coupled with polymeric monolith-based capillary liquid chromatography tandem mass spectrometry. Talanta 150: 233–239. doi.org/10.1016/j.talanta.2015.12.045

Molinelli, A., O'Mahony, J., Nolan, K., Smyth, M.R., Jakusch, M., Mizaikoff, B. 2005. Analyzing the mechanisms of selectivity in biomimetic self-assemblies via IR and NMR spectroscopy of prepolymerization solutions and molecular dynamics simulation. Anal. Chem. 77: 5196–5204. doi.org/10.1021/ac050525f

O'Mahony, J., Molinelli, A., Nolan, K., Smyth, M.R., Mizaikoff, B. 2005. Towards the rational development of molecularly imprinted polymers: ^{1}H NMR studies on hydrophobicity and ion-pair interactions as driving forces for selectivity. Biosens. Bioelectron. 20: 1884–1893. doi.org/10.1016/j.bios.2004.07.036

Ortelli, D., Edder, P., Corvi, C. 2004. Analysis of chloramphenicol residues in honey by liquid chromatography-tandem mass spectrometry. Chromatographia 59: 61–64.

Rejtharová, M., Rejthar, L. 2009. Determination of chloramphenicol in urine, feed water, milk and honey samples using molecular imprinted polymer clean-up. J. Chromatogr. A. 1216: 8246–8253. doi.org/10.1016/j.chroma.2009.07.037

Rønning, H.T., Einarsen, K., Asp, T.N. 2006. Determination of chloramphenicol residues in meat, seafood, egg, honey, milk, plasma and urine with liquid chromatography-tandem mass spectrometry, and the validation of the method based on 2002/657/EC. J. Chromatogr. A. 1118: 226–233. doi.org/10.1016/j.chroma.2006.03.099

Shekarchi, M., Pourfarzib, M., Akbari-Adergani, B., Mehramizi, A., Javanbakht, M., Dinarvand, R. 2013. Selective extraction of lamivudine in human serum and urine using molecularly imprinted polymer technique. J. Chromatogr. B. 931: 50–55. doi.org/10.1016/j.jchromb.2013.04.041

Shirmer, C., Meisel, H. 2009. Chromatographic evaluation of polymers imprinted with analogs of chloramphenicol and application to selective solid-phase. Anal. Bioanal. Chem. 394: 2249–2255. doi.org/10.1007/s00216-009-2898-2

Stange, H., Greiner, A. 2007. Microwave-assisted free radical copolymerizations of styrene and methyl methacrylate. Macromol. Rapid Comm. 28: 504–508.

Sun, Z., Schüssler, W., Sengl, M., Niessner, R., Knopp, D. 2008. Selective trace analysis of diclofenac in surface and wastewater samples using solid-phase extraction with a new molecularly imprinted polymer. Anal. Chim. Acta. 620: 73–81. doi.org/10.1016/j.aca.2008.05.020

Taka, T., Baras, M.C., Bet, Z.F.C. 2012. Validation of a rapid and sensitive method for determination of chloramphenicol in honey by LC-MS/MS. Food Addit. Contam. Part A. Chem. Anal. Control Expo. Risk Assess. 29: 596–601. doi.org/10.1080/19440049.2011.625047

Viveiros, R., Rebocho, S., Casimiro, T. 2018. Green strategies for molecularly imprinted polymer development. Polymers 10: 306. doi:10.3390/polym10030306

Wang, T., Tong, J., Sun, M., Chen, L. 2011. Fast and selective extraction of chloramphenicol from soil by matrix solid-phase dispersion using molecularly imprinted polymer as dispersant. J. Sep. Sci. 34: 1886–1892. doi.org/10.1002/jssc.201100046

Wang, Y., Wang, E., Dong, H., Liu, F., Wu, Z., Li, H., Wang, Y. 2014. Synthesis of chitosan based molecularly imprinted polymers for pre-concentration and clean-up of chloramphenicol. Adsorpt. Sci. Technol. 32: 321–330. doi.org/10.1260/0263-6174.32.4.321

Xu, Z., Kuang, D., Feng, Y., Zhang, F. 2010. Combination of hydrophobic effect and electrostatic interaction in imprinting for achieving efficient recognition in aqueous media. Carbohyd. Polym. 79: 642–647. doi.org/10.1016/j.carbpol.2009.09.010

Yoshimatsu, K., Reimhult, K., Krozer, A., Mosbach, K., Sode, K., Ye, L. 2007. Uniform molecularly imprinted microspheres and nanoparticles prepared by precipitation polymerization: the control of particle size suitable for different analytical applications. Anal. Chim. Acta. 584: 112–121. doi.org/10.1016/j.aca.2006.11.004

Zhang, Y., Liu, R., Hu, Y., Li, G. 2009. Microwave heating in preparation of magnetic molecularly imprinted polymer beads for tract triazines analysis in complicated samples. Anal. Chem. 81: 967–976. doi.org/10.1021/ac8018262

Zhang, Z., Tan, W., Hu, Y., Li, G., Zan, S. 2012. Microwave synthesis of gibberellins acid 3 magnetic molecularly imprinted polymer beads for the trace analysis of gibberellins acids in plant samples by liquid chromatography-mass spectrometry detection. Analyst. 137: 968–977. doi.org/10.1039/C2AN16001C