Analysis of heterocyclic aromatic amines using selective extraction by magnetic molecularly imprinted polymers coupled with liquid chromatography – Mass spectrometry

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Analysis of heterocyclic aromatic amines using selective extraction by magnetic molecularly imprinted polymers coupled with liquid chromatography — Mass spectrometry

Shih-Ci Jhu, Jing-Ying Wanga, Hsueh-Ting Wanga, Sung-Fang Chen

Abstract

Heterocyclic aromatic amines (HCAs) are highly carcinogenic and mutagenic chemicals. This study reports on the development of magnetic molecularly imprinted polymers (MMIPs) for the purification and quantification of HCAs. A novel magnetic molecularly imprinted polymer was successfully prepared using a surface molecular imprinting method using functionalized Fe particles as the magnetic cores. 2-Amino-3-methylimidazo[4,5-f]quinoline (IQ) was used as a molecular template; methacrylic acid (MAA), ethylene glycol dimethyl acrylate (EGDMA), 2, 2′-Azobis (2-methylpropionitrile) were used as the functional monomer, crosslinker, and initiator, respectively. The use of the template/functional monomer/crosslinking agent at a ratio of 1:4:20 resulted in a product with better adsorption properties (3.24 mg/g). The HCAs were successfully detected and quantified in processed meat samples by MISPE and LC-MS/MS. Under the final optimized detection conditions, the proposed method offered good linearity (R > 0.995) for the five HCAs with an acceptable level of precision, and an LOQ of 0.05 ng/g was successfully achieved.

Keywords: Heterocyclic amines, LC-MS/MS, Magnetic molecularly imprinted polymers, Solid-phase extraction

1. Introduction

Heterocyclic aromatic amines are chemicals that contain at least one heterocyclic ring that also contains two or more different elements in the ring, in addition to the presence of one or more amine groups. During the high temperature processing of meat, the amino acids participate in the Maillard reaction and a series of other reactions, resulting in the eventual formation of HCAs. The formation of heterocyclic aromatic amines in processed meats can be classified into two main categories. Free amino acids, creatine and hexoses are hydrolyzed and cyclized to produce pyrrole and pyridine derivatives at 100 °C—300 °C, which results in the formation of thermal reaction materials (thermic HCAs or IQ type). In addition, pyrolytic HCAs or non-IQ types are mainly formed during thermal decomposition reactions between amino acids and proteins at temperatures above 300 °C.

IQ is classified as Group 2A compound by the International Agency for Research on Cancer (IARC) and is thought to be carcinogenic to humans. For the detection of HCAs in a complicated matrix, it is necessary to first perform a sample clean-up to eliminate compounds that might interfere with the analysis. Different sample pretreatment methods have been used for the extraction of HCAs. In recent years, the most frequently applied sample pretreatment for heterocyclic amines (HCAs) has been based on solid-phase extraction (SPE) [1] and QuEChERS [2]. Other extraction methods, such as liquid–liquid extraction (LLE) [3], dispersive liquid–liquid microextraction (DLLME) [4], microwave-assisted solvent extraction (MASE) [5], supercritical fluid extraction (SFE) [6] have also been used.

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reported. However, these methods all have some drawbacks that include low selectivity, tedious operation, or high cost.

Molecularly imprinted technology (MIPs) is an extending application based on the “antigen–antibody binding theory”. They are specialized polymeric materials, that are synthesized based on the correlation in a template, a functional monomer and a crosslinking agent. MIPs contain specific recognition sites with the memory of the shape, size and functional groups of the template molecule. MIPs are able to rebind a specific template molecule with a high degree of affinity and selectivity, but some limitations of MIPs have restricted their widespread application. These include difficulties in removing the template, a low efficiency of the imprinting site, inadequate adsorption and incomplete separation.

In the past, MIPs were mainly synthesized by bulk polymerization [9,10] or precipitation polymerization [11,12]. Synthesis involving bulk polymerization causes the formation of a product with an irregular shape and an insufficient number of binding sites. The above-mentioned shortcomings can be improved by the use of precipitation polymerization, but the synthesis process typically involves the use of large amounts of solvent. In recent years, several synthetic methods such as suspension polymerization [13,14], emulsion polymerization [15,16], and surface imprinting [17,18] have been developed in attempts to solve these shortcomings.

More recently, the surface imprinting strategy which involves the polymerization of layers of MIPs on the surface of various support materials has attracted increasing interest. Molecular imprinted technology was combined with magnetic separation techniques for preparing magnetic molecular imprinted polymers [17,18]. Compared to MIPs, MMIPs not only retain the advantages of the MIPs mentioned above, but also have the benefits of high surface areas and outstanding magnetism. MMIPs have high contact surface areas which enhances efficiency, and they can be easily separated using an external magnet, eliminating the need for tedious centrifugations. Silica gel, nanoparticles, quantum dots, etc are currently in use as core materials.

After a sample pretreatment procedure, liquid chromatography (LC) is typically used for separation with detection by various methods, such as LC-UV [19], LC-FL [20], HPLC-DAD [20], LC-MS [21–23]. Gas chromatography-mass spectrometry (GC–MS) was also used to determine HCAs [24]. Although it provides high separation efficiency and sensitivity, complicated and time-consuming derivatization steps are needed before the GC analysis. Compared with other detectors, MS provides accurate measurement results with a high specificity and superior sensitivity for the determination of HCAs.

In present work, MMIPs with core–shell structures were prepared using Fe3O4 particles as magnetic cores, mesoporous polymers as shells and IQ as the template. The prepared polymers were then characterized by XRD, FT-IR and SEM. Adsorption experiments related to isotherms and kinetics were also carried out to evaluate adsorption properties. Specificity was investigated using four structural analogs to verify the selectivity of the MMIPs for IQ. After optimizing the relevant conditions, a MISPE-LC/MS method based on this material was established and successfully used for the extraction and determination of IQ in processed meat products.

2. Materials and methods

2.1. Materials

2-Amino-3-methylimidazo[4,5-f]quinoline (IQ), 2-Amino-3,4-dimethylimidazo[4, 5-f]quinoline (MeIQ), 2-amino-3,8-dimethylimidazo[4,5-f]quinoline (8-MeIQx), 2-amino-3,4,7,8-tetramethylimidazo[4,5-f]quinoxaline (4,7,8-TriMeIQx), 1-methyl-9H-pyrido[3,4-b]indole (Harman), 9H-pyrido[3,4-B]indole (Norharman) were purchased from Toronto Research Chemicals (T.R.C.) (Toronto, Canada). Methacrylic acid (MAA), 2, 2’-Azobis (2-methylpropionitrile) (AIBN) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Ethylene glycol dimethacrylate (EGDMA), Tetraethyl Orthosilicate (TEOS) was purchased from ACROS (Pittsburgh, PA, USA), (3-Aminopropyl)trimethoxysilane (APTMS) were purchased from Alfa Aesar (Ward Hill, MA, USA). Acetonitrile (ACN), Methanol (MeOH) were purchased from Merck (Darmstadt, Germany). Ultra-pure water was obtained using a Milli-Q water system (Merck, Germany). Meat samples (pork floss, beef jerky, raw pork and raw beef) were purchased from local stores and stored in a freezer at 4 °C until used.

2.2. Equipment

A scanning electron microscope (SEM, JEOL, JSM-6510LV, Tokyo, Japan), a Fourier transform infrared spectrometer (FT-IR, Bruker, IS-2000S, Ettlingen, Germany), X-ray diffraction spectroscopy (XRD, Bruker, D8 advance, Karlsruhe, Germany), a Dynamic Light Scattering/Zeta Potential Analyzer (DLS, Otsuka, ELSZ-2000ZS, Osaka, Japan) were used in this work. Separations were carried out using a UPLC system (Sciex, ExionLC™ AD,
Framingham, MA, USA) equipped with Kinetex® C18 (2.1 × 100 mm, 2.6 μm, 100 Å, Phenomenex) column with the temperature controlled at 40 °C and mobile phase A consisting of 2% ACN and 0.1% FA and mobile phase B consisting of 98% ACN and 0.1% FA. The flow rate was set at 0.3 mL/min and the injection volume was 5 μL. The applied chromatographic gradient was started at 0–0.5 min, 0% B; 0.5–9 min, linear gradient to 80% B; 9–10 min, a hold at 80% B; a 10–10.5 min drop to 0% B; 10.5–15 min for equilibrium with 0% B. Mass spectrometric analyses were performed on a Sciex 5500+ QTRAP mass spectrometer equipped with an electrospray ionization source. The ionization was operated in the positive mode using multiple reaction monitoring (MRM) acquisitions. The MRM settings were as below: dwell time, 80 msec (IQ, MeIQ), 50 msec (8-MeIQx, Harman, Norharman, 4,7,8-TriMeIQx); pause time, 5.007 msec; duration, 14,997 min. The mass spectrometric parameters were as listed below: ionspray voltage, +5500 V; curtain gas (CUR), 20 psi; collision gas (CAD), 9 psi; gas 1 (GS1), 60 psi; gas 2 (GS2), 60 psi; temperature, 600 °C.

2.3. Preparation of magnetic molecularly imprinted polymers (MMIPs) and NIP

2.3.1. Synthesis of Fe3O4 nanoparticles

Magnetic Fe3O4 nanoparticles were synthesized using a chemical coprecipitation method. FeCl3·6H2O (15 mmol) and FeCl2·4H2O (10 mmol) were initially dissolved in 80 mL H2O by sonication and the mixture was then stirred at 80 °C under a nitrogen atmosphere. A 50 mL aliquot of an ammonia solution (28%, v/v) was then added dropwise. After mechanical stirring for 30 min, the Fe3O4 nanoparticles were isolated magnetically and washed repeatedly with H2O until the washing solution reached neutral. The Fe3O4 nanoparticles were then dried at 60 °C for 6 h and collected for the next reaction.

2.3.2. Synthesis of Fe3O4@SiO2 nanoparticles

A 0.50 g portion of Fe3O4 nanoparticles was dispersed in an ethanol solution (80% v/v) and subsequently sonicated for 10 min. A 4 mL volume of TEOS was then added to the above mixture followed by adding 5 mL of an ammonia solution (28%, v/v) and the suspension was mechanically stirred and allowed to react at room temperature for 24 h. The resulting product was collected by magnetic decantation and washed thoroughly with H2O to remove unreacted reagents and finally dried in an oven at 60 °C.

2.3.3. Synthesis of Fe3O4@SiO2–NH2 nanoparticles

The 0.30 g Fe3O4@SiO2 nanoparticles were dispersed in 50 mL of methanol by sonication for 10 min and 3 mL of APTMS was then added to the mixture dropwise. The resulting suspension was then mechanically stirred and allowed to react at room temperature for 24 h. At the end of the reaction, the Fe3O4@SiO2–NH2 nanoparticles were separated by a magnetic field and washed thoroughly with methanol to remove unreacted reagents and finally dried in an oven at 60 °C.

2.3.4. Synthesis of magnetic molecularly imprinted polymers (MMIPs)

MMIPs was synthesized through surface imprinting polymerization, using IQ as the template, MAA as the monomer, EGDM as the cross-linker agent and AIBN as the initiator. A 0.05 mmol portion of IQ was dissolved in 10 mL of ACN/MeOH (9:1, v/v) and 17 μL MAA was then added to the solution. The resulting solution was degassed by sonication for 10 min and bubbled with nitrogen gas for 10min to remove oxygen. After allowing the pre-polymer to form in the solution for 24 h at 4 °C, 100 mg of Fe3O4@SiO2–NH2, 188.6 μL EGDM and 0.12 mmol of AIBN were added to the solution. The solution was degassed by sonication for 10 min in an ice bath and bubbled with nitrogen gas for 10min to remove oxygen. The resulting system was sealed and mechanically stirred at 60 °C for 24 h. The resulting MMIPs were collected magnetically, washed repeatedly with an acetic acid/methanol solution (10%, v/v) to remove the template until no template was detected by UV–vis. The resulting product was washed repeatedly with methanol until the washing solution reached a neutral pH and the product finally dried in an oven at 60 °C. The magnetic non-imprinted polymers (MNIPs) were synthesized using the same method without using IQ.

2.4. Adsorption test

2.4.1. Static adsorption

A 3 mg portion of the MNIPs/MMIPs were added to a 1.5 mL microtube, 1 mL of different concentrations of an IQ solution (1.0–50 mg/L) were added and the resulting solution shaken at RT for 80 min. The concentration of IQ in the supernatant was determined by LC-MS. The equilibrium adsorption capacity (Qe, mg/g) of the MMIPs/MNIPs was calculated according to the following equation (1):

\[
Q_e = \frac{(C_0 - C_e)V}{W}
\]
Qe (mg/g) is the adsorption capacity at the adsorption equilibrium, C0 (µg/mL) is the initial concentration of the IQ solution, Ce (µg/mL) is the concentration of IQ in the supernatant after adsorption, V (mL) is the volume of adsorption and W (g) is the weight of the MMIPs or MNIPs.

The binding curves for the polymer were described by a Langmuir and Freundlich model as shown in the equation below (2), (3):

\[
Q_e = \frac{Q_m K_L C_e}{1 + K_L C_e} \quad (2)
\]

\[
Q_e = K_F C_e^m \quad (3)
\]

where Qe (mg/g) represents the binding quantity for the polymer at equilibrium; Qm (mg/g) represents the theoretical maximum adsorption capacity for the polymer; Ce (µg/mL) represents the equilibrium solution concentration; KL and KF represents the Langmuir adsorption and Freundlich adsorption coefficient and m represents the Freundlich adsorption constant.

2.4.2. Dynamic adsorption

A series of 3.0 mg portions of the MMIPs/MNIPs were added to 1.0 mL of a 20 mg/L solution of IQ. The mixtures were shaken for from 1 to 120 min respectively at room temperature and the following processes were used in the static adsorption experiment. The kinetics data were calculated from pseudo-first order and pseudo-second order equations based on equation (4) and (5):

\[
Q_t = Q_e (1 - e^{-k_1 t}) \quad (4)
\]

\[
Q_t = \frac{k_2 Q_e^2 t}{1 + k_2 Q_e t} \quad (5)
\]

where, t is the absorption time; Qe and Qt represent the amount of IQ bound to the polymers at equilibrium and t (min), respectively; k1 and k2 were analyzed using the pseudo first-order and pseudo second-order equations, respectively.

2.4.3. Selectivity of the MMIPs

Selectivity experiments were conducted using IQ, MelIQ, 8-MeIQx, Harman and Norharman as structural analogs. MMIPs or MNIPs samples (3.0 mg) were added to 1.0 mL of 20 mg/L solutions of the above compounds dissolved in ACN. The mixtures were then shaken for 60 min. The MMIPs or MNIPs were collected by an external magnet, a 1 mL aliquot of the supernatant was evaporated to dryness and the residue dissolved in 100 µL of mobile phase A prior to the LC-MS/MS analysis. The imprinting factor (IF) and selectivity coefficient (SC) were examined further according to the following equations (6) and (7):

\[
IF = \frac{Q_{MMIPs}}{Q_{MNIPs}} \quad (6)
\]

\[
SC = \frac{IF_M}{IF_F} \quad (7)
\]

2.5. Sample preparation

The extraction step for the LLE method was as follows. A 2.0 g sample of meat and 10 mL of H2O were placed in a 50 mL tube. The mixture was vortexed for 1 min and then ultrasonicated for 30 min. After adding 20 mL of ACN, the tube was vigorously vortexed 1 min to extract the samples. Subsequently, 3.0 g of anhydrous magnesium sulfate and 1.0 g of sodium chloride was added to the tube, followed by vortexing for 1 min, followed by centrifugation at 5000 rpm (4°C) for 10 min. A 10 mL aliquot of the organic layer was then transferred to another 50 mL tube and stored in a freezer at 4°C until used.

2.6. Application of MISPE to real samples

The MISPE purification procedure was used for the analysis of commercial pork floss and beef jerky samples purchased from Taiwan local market. The 10 mg MMIPs were conditioned with 10 mL ACN/MeOH (50/50, v/v), and the MMIPs were then isolated by a magnet and the supernatant removed and 10 mL of the actual sample solution was then added. The solution was shaken at room temperature for 80 min and the MMIPs were isolated by a magnet and the supernatant removed. The sample was washed with 10 mL can and the analytes were eluted with 10 mL of MeOH/AcOH (90/10, v/v). The liquid extract was evaporated to dryness and the residue dissolved in 100 µL of mobile phase A before the LC-MS analysis.

3. Results and discussion

In addition to being classified as 2A carcinogens by IARC, IQ also possesses the simplest structure of the thermic HCA, and the majority of the other thermic HCAs are derivatives of IQ. Therefore, IQ was chosen as the template molecule in this study, but the MMIPs that were synthesized could have more HCAs applications. The selectivity or
specificity of several HCAs toward the developed MMIP will be investigated and discussed.

In this study, surface imprinting was used to prepare MMIPs. As shown in Fig. 1. Fe$_3$O$_4$ nanoparticles were first prepared by a coprecipitation method. In order to avoid the oxidation of Fe$_3$O$_4$ to Fe$_2$O$_3$, a layer of SiO$_2$ was polymerized on the surface to prevent the oxidation of the nanoparticles. Finally, APTMS was used for surface modification. The amine group was covalently bonded to Fe$_3$O$_4$@SiO$_2$ to increase the hydrophobicity of the preparation to facilitate the polymerization reaction between the MIP layer and the magnetic core.

The adsorption capacity of MMIPs is affected by many factors, including the monomer that is used, synthesis ratio and the solvent being used. Based on a previous study [25,26], it was found that monomers such as methacrylic acid (MAA), 2-vinylpyridine (2-VP), 4-vinylpyridine (4-VP) with simpler configurations can reduce the steric effects that might be operative during the synthesis process. It can possibly form hydrogen bond and π-π interactions with the template molecule. The results are shown in Table S1 and Fig. S1a, for IQ molecules, the adsorption of MMIPs using three different monomers (MAA, 2-VP, 4-VP), 3.24 (mg/g), 2.69 (mg/g), 2.68 (mg/g), respectively. The results indicated that MAA showed an adequate level of hydrogen bonding and a low steric barrier against template molecules. Therefore, MAA was selected as the functional monomer for use in the further synthesis of MMIPs.

3.1. Characterization of MMIPs and MNIPs

The FT-IR spectra of the preparations are shown in Fig. 2a. The characteristic peak at 535 cm$^{-1}$ is assigned to Fe–O stretching and the peaks at 800 cm$^{-1}$ and 1072 cm$^{-1}$ as Si–O stretching vibration peaks. The peak at 3472 cm$^{-1}$ was the characteristic absorption peak for the N–H bond of APTMS. The peaks for C–O, C=O and the –CH stretching vibration were located at 1135 cm$^{-1}$, 1772 cm$^{-1}$ and 2996 cm$^{-1}$. The FT-IR spectra of the MMIPs and MNIPs were nearly identical because their chemical compositions were similar after the template molecules were removed from the MMIPs.

The XRD spectra are shown in Fig. 2b. In the 20 range of 10°–70°. Six relatively discernible diffraction peaks at 20 = 30.3°, 35.6°, 43.2°, 54.1°, 57.4°, and 63.1° were indexed as the diffractions of (220), (311),
(400), (422), (511) and (440), respectively. It is obvious that after modification with SiO₂, SiO₂@NH₂, MIPs and NIPs, Fe₃O₄@SiO₂, Fe₃O₄@SiO₂@NH₂, MMIPs and MNIPs still showed the same signals suggesting that the crystalline phase of Fe₃O₄ was not destroyed during the synthesis process.

The surface morphology and mean diameter of the MMIPs and MNIPs was examined by SEM and DLS (Fig. S2). The MMIPs and MNIPs have relatively spherical structures with a slight degree of agglomeration (which is probably related to the magnetic behavior of MMIP) with an average diameter of 52 μm and 49 μm thus providing a high contact surface that would lead to complete template removal.

3.2. Binding studies

3.2.1. Adsorption isotherms

The binding isotherm curves of the MMIPs and MNIPs are shown in Fig. 3a. The adsorptive capacity of the MMIPs or MNIPs increased along with the initial concentration of IQ until it reached a saturation level. The adsorptive maximum capacity of IQ on the MMIPs (Qₑ = 2.45 mg/g) was much higher than that for the MNIPs (Qₑ = 0.87 mg/g), which can be attributed to the presence of the specific imprinting phenomenon of the MMIPs. The MNIPs were prepared in an identical manner to that for the MMIPs except that the templates were not used during synthesis. Since the functional monomer (MAA) in the MNIPs could react with IQ through hydrogen bonding, the MNIPs may also hold some IQ molecules.

The adsorption isotherms were analyzed using the Langmuir and Freundlich isotherm models. The results indicated that the Langmuir model (R², MMIPs: 0.995, MNIPs: 0.984) gave a better fit than the Freundlich model (R², MMIPs: 0.873, MNIPs: 0.591) with regard to the adsorption of IQ to the MMIPs and MNIPs. We speculate that the recognition sites were uniformly distributed in the form of a monolayer on the surface of the MMIPs.

3.2.2. Dynamic adsorption

The kinetic curves for the MMIPs and MNIPs are shown in Fig. 3b. The level of adsorption of the MMIP and MNIPs increased rapidly during the first 30 min. The times to reach saturation for the MMIPs and MNIPs were roughly the same, both reached
adsorption equilibrium at about 45 min. However, compared with the MNIPs, the equilibrium adsorption of IQ to the MMIPs was significantly higher.

For both materials, the $R^2$ of pseudo-second order (0.995 and 0.998) were better than the $R^2$ of the pseudo-first order (0.968 and 0.844). In addition, the calculated $Q_e$ was basically consistent with the dynamic adsorption results, which indicates that pseudo-second-order kinetic model was better fitted for the adsorption of IQ on the surface of the MMIPs. According to the pseudo-second-order mechanism, chemical interactions could be the rate-limiting step in the adsorption process.

3.2.3. Selectivity of MMIPs

It is intended to explore specificity of synthesized MIP when IQ was used as a template. Compounds similar in structure to IQ (MeIQ, 8-MeIQx) were investigated. Harman and norharman were also tested because they are abundant and relatively different in structure also low in toxicity. They were used to investigate the selectivity of the MMIPs toward IQ, as shown in Fig. 4 and Table S3. Compared with MNIPs, the MMIPs show superior adsorption properties (IF) and selectivity. For the MMIPs, the results showed that when the structure of the competitive compounds have a greater difference from the IQ molecule, the adsorption effect of MMIPs become worse. This result further confirms that the strategy for synthesizing IQ as a template molecule was feasible. Although the MNIPs also exerted an adsorption effect on HCAs molecules, they do not contain precise recognition pores themselves, so the adsorption effect is less satisfactory.

3.3. Optimization of conditions for MISPE

3.3.1. Type of extraction solvent

To select the appropriate solvent for eluting, three critical factors were considered: I) the polarity of the solvent, II) elution strength and solubility of the target analyte, while leaving the retained impurities behind, and III) its adaptability with the analytical instrument. Several types of elution solvents including pure H₂O, EtOH, MeOH and 10%–40% AcOH/MeOH solution were examined. Maximum extraction yield was achieved using 10% AcOH/MeOH (Fig. S3a). Moreover, the addition of acetic acid led to facilitating and accelerating the breakdown of hydrogen bonds between the trapped analyte and the sorbent.

3.3.2. Reusability performance of MMIPs

Reusability results are shown in Fig. S3b. The recovery was still higher than 98% after five adsorption–desorption cycles, indicating that the MMIPs possessed good stability and have the potential for being reused.

3.4. Establishment and validation of a MISPE-LC/MS method

3.4.1. Linearity and range

Under the optimized conditions, the feasibility and efficiency of the present method were estimated by investigating the linearity and dynamic range of the method. The linear range of the method was 0.05–50 ng/g and the coefficient of determination was 0.995, indicating that this method has a good correlation and linearity. The linear regressions of the matrix-matched calibration curve are summarized in Table 1a. In addition, the LOD and LOQ were determined using 3 and 10 times signal to noise ratios.

3.4.2. Intra-day and inter-day precision

IQ standards were spiked with a blank matrix and then subjected to the MISPE-LC/MS analysis. Three different concentrations with 250, 500 and 1000 mg/L of IQ standards were used to evaluate intra-day and inter-day precision. As shown in Table 1b. The

Fig. 4. Molecular structures of the 5 tested HCA compounds (a). Adsorption capacities of 5 HCAs on MMIPs and MNIPs (b).
recovery rate of the sample was between 84.7% and 108.5%, the RSD% was between 2.4% and 6.6%, indicating that this method had an acceptable level of recovery and precision.

### 3.5. Analysis of processed meat samples

The developed MISPE purification method was applied to the analysis of commercial pork floss and beef jerky. A 2.5 mg/kg IQ fortified meat sample was prepared by spiking known concentration of IQ into homogenous sample during extraction step for the LLE method, and it was extracted using the optimized MISPE purification procedure subjected to LC-MS analysis. Compared with the theoretical adsorption situation ($Q_e = 2.07 \text{ mg/g}$), the recovery for the real sample is roughly 60%. This may due to the fact that 1) the adsorption of the MMIPs on HCAs was hindered in a more complex environment and 2) The MMIPs still have a certain degree of non-specific adsorption, so the matrix molecules were still competing during the adsorption process, causing a reduced adsorption capacity for the target HCAs. Nevertheless, even with the suppressed adsorption capacity, the MMIPs still show a high degree of specificity and adsorption capacity compared with the MNIPs (Fig. S4).

### 3.6. Comparison with other methods

The analysis of HCAs in meat products in recent years is illustrated in Table 2. The popular choices of HCAs pretreatment have mainly relied on SPE or QuEChERS for the purification process, due to their good stability and reproducibility. More recently, various reusable adsorbents have been developed for the cost and waste reduction. A novel synthesized MMIP using IQ molecules as templates was developed in this study. Compared with other approaches, our method demonstrated good selectivity and superior detection limit with a wide linear dynamic range for a complicated matrix for IQ-analogue detection and monitoring. Furthermore, the MMIPs material can be easily incorporated as adsorbents for MISPE purification.

### Table 1a. LOD, LOQ and linear regression of the quantitative calibration curve.

<table>
<thead>
<tr>
<th>HCAs</th>
<th>LOD (ng/g)</th>
<th>LOQ (ng/g)</th>
<th>Linear range (ng/g)</th>
<th>Linear equation</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>IQ</td>
<td>0.025</td>
<td>0.05</td>
<td>0.05—50</td>
<td>$y = 0.01167x + 0.0010$</td>
<td>0.997</td>
</tr>
<tr>
<td>MeIQ</td>
<td>0.025</td>
<td>0.05</td>
<td>0.05—50</td>
<td>$y = 0.00583x + 0.0029$</td>
<td>0.997</td>
</tr>
<tr>
<td>8-MeIQx</td>
<td>0.025</td>
<td>0.05</td>
<td>0.05—50</td>
<td>$y = 0.00008x + 0.0019$</td>
<td>0.998</td>
</tr>
<tr>
<td>Harman</td>
<td>0.025</td>
<td>0.05</td>
<td>0.05—100</td>
<td>$y = 0.01839x + 0.0041$</td>
<td>0.998</td>
</tr>
<tr>
<td>Norharman</td>
<td>0.025</td>
<td>0.05</td>
<td>0.05—100</td>
<td>$y = 0.01444x + 0.0035$</td>
<td>0.998</td>
</tr>
</tbody>
</table>

*a* Limit of detection (LOD): $\text{S/N} \geq 3$.

*b* Limit of quantification (LOQ): $\text{S/N} \geq 10$.

### Table 1b. Precision test of IQ in the blank matrix.

<table>
<thead>
<tr>
<th>IQ Conc. (mg/L)</th>
<th>Intra-day (n = 3)</th>
<th>Inter-day (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>250</td>
<td>108.5</td>
<td>102.5</td>
</tr>
<tr>
<td>500</td>
<td>98.7</td>
<td>98.8</td>
</tr>
<tr>
<td>1000</td>
<td>87.8</td>
<td>84.7</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Recovery %&lt;sup&gt;a&lt;/sup&gt;</th>
<th>108.5</th>
<th>98.7</th>
<th>87.8</th>
<th>102.5</th>
<th>98.8</th>
<th>84.7</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSD %&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.2</td>
<td>2.8</td>
<td>6.6</td>
<td>5.1</td>
<td>2.4</td>
<td>5.5</td>
</tr>
</tbody>
</table>

*a* Recovery% = (Amount of substance recovered/Amount of substance) × 100%.

*b* RSD% = (Standard deviation/Mean) × 100%.

### Table 2. Comparison of different methods used to determine HCAs.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sample pretreatment</th>
<th>Instrumental method</th>
<th>Linear range (ng/g)</th>
<th>LOQ (ng/g)</th>
<th>Recovery (%)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamburger patties</td>
<td>DLLME&lt;sup&gt;a&lt;/sup&gt;</td>
<td>HPLC</td>
<td>1–200</td>
<td>0.15</td>
<td>91</td>
<td>[4]</td>
</tr>
<tr>
<td>Bakery products</td>
<td>QuEChERS&lt;sup&gt;b&lt;/sup&gt;</td>
<td>UPLC-MS</td>
<td>1–80</td>
<td>0.3</td>
<td>62–75</td>
<td>[2]</td>
</tr>
<tr>
<td>Roasted duck</td>
<td>QuEChERS</td>
<td>UPLC-MS</td>
<td>0.05–10</td>
<td>0.03</td>
<td>76</td>
<td>[22]</td>
</tr>
<tr>
<td>Roasted pork</td>
<td>MCX&lt;sup&gt;c&lt;/sup&gt; cartrdges</td>
<td>UPLC-MS</td>
<td>0.13–68.5</td>
<td>0.148</td>
<td>71</td>
<td>[27]</td>
</tr>
<tr>
<td>Sausage</td>
<td>MCX cartrdges</td>
<td>UPLC-MS</td>
<td>0.03–63.6</td>
<td>0.075</td>
<td>88</td>
<td>[28]</td>
</tr>
<tr>
<td>Mutton</td>
<td>MPC&lt;sup&gt;d&lt;/sup&gt;</td>
<td>UPLC-MS</td>
<td>0.05–40</td>
<td>0.429</td>
<td>84</td>
<td>[29]</td>
</tr>
<tr>
<td>Fried chicken</td>
<td>CTC-COF@MCNT&lt;sup&gt;e&lt;/sup&gt;</td>
<td>UPLC-MS</td>
<td>0.05–50</td>
<td>0.083</td>
<td>74–108</td>
<td>[16]</td>
</tr>
<tr>
<td>Roasted pork</td>
<td>Fe&lt;sub&gt;3&lt;/sub&gt;O&lt;sub&gt;4&lt;/sub&gt;@PDA&lt;sup&gt;f&lt;/sup&gt;</td>
<td>UPLC-MS</td>
<td>1–500</td>
<td>0.211</td>
<td>70–108</td>
<td>[30]</td>
</tr>
<tr>
<td>Pork floss, Beef</td>
<td>MISPE</td>
<td>UPLC-MS</td>
<td>0.05–50</td>
<td>0.05</td>
<td>60</td>
<td>This study</td>
</tr>
</tbody>
</table>

*a* DLLME: Dispersive liquid–liquid microextraction.

*b* QuEChERS.

*c* MCX: Mixed-mode cation exchange.

*d* MPC: 3D magnetic porous carbon.

*e* CTC-COF@MCNT: Cyclotricatechylene covalent organic framework incorporated magnetic carbon nanotube.

*f* Fe<sub>3</sub>O<sub>4</sub>@PDA: Core-shell Fe<sub>3</sub>O<sub>4</sub>@polydopamine nanocomposites.
4. Conclusions

In this study, a magnetic molecularly imprinted polymer was synthesized using MAA, EDGMA, and AIBN for the selective extraction of IQ. It has successfully applied to processed meat products. This MMIPs possessed good selectivity and adsorption capacity toward the template molecule IQ. It also exhibited good stability and reusability as a material for the purification of IQ-analogue. The developed MISPE method has various advantages including sensitivity, simplicity, and low cost, and appears to be a promising method for the simultaneous determination of IQ-analogue in complicated matrices such as meat products.

Acknowledgements

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Appendix.

Supplementary figures and tables

![Graphs showing optimization results](image-url)

Fig. S1. Optimization of monomer (a), molar ratio (b) and porogenic solvent (c) for MMIP synthesis. (The equilibrium adsorption capacity \( Q_e \) (mg/g) was calculated as follows: \( Q_e = \frac{VW}{C_0 - C_e} \cdot \frac{C_0}{VW} \), where \( C_0 \) (µg/mL) is the initial concentration of IQ solution, \( C_e \) (µg/mL) is the concentration of IQ in the supernatant after adsorption, \( V \) (mL) is the volume of the adsorption and \( W \) (g) is the weight of MMIPs).
Fig. S2. The SEM images of MMIPs (a) and MNIPs (b). The DLS analysis of MMIPs (c) and MNIPs (d).

Fig. S3. Optimization of the extraction solvent (a), reusability of MMIPs (b).
References


Table S1. Selection of functional monomers for MMIP synthesis.

<table>
<thead>
<tr>
<th></th>
<th>MMIP_1</th>
<th>MMIP_2</th>
<th>MMIP_3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Template (mg)</td>
<td>9.9</td>
<td>17 (MAA)</td>
<td>21.5 (2-VP)</td>
</tr>
<tr>
<td>Monomer (µL)</td>
<td>17</td>
<td>21.3 (4-VP)</td>
<td></td>
</tr>
<tr>
<td>Linker (µL)</td>
<td>189</td>
<td>21.3 (4-VP)</td>
<td></td>
</tr>
<tr>
<td>Substrate</td>
<td>Fe₃O₄@SiO₂-NH₂</td>
<td>ACN : MeOH (9:1)</td>
<td></td>
</tr>
<tr>
<td>Solvent (mL)</td>
<td>ACN : MeOH (9:1)</td>
<td>24</td>
<td></td>
</tr>
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</table>

Table S2. Synthesis conditions of molar ratio and porogenic solvent for MMIPs.

<table>
<thead>
<tr>
<th>MMIPs</th>
<th>T:M:C</th>
<th>Template (mg)</th>
<th>Monomer (µL)</th>
<th>Linker (µL)</th>
<th>Solvent (mL)</th>
<th>Reaction time (hr)</th>
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</thead>
<tbody>
<tr>
<td>MMIP_1</td>
<td>1:4:20</td>
<td>9.9</td>
<td>17</td>
<td>189</td>
<td>ACN : MeOH (9:1)</td>
<td>24</td>
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<td>MMIP_4</td>
<td>1:8:40</td>
<td>9.9</td>
<td>34</td>
<td>378</td>
<td>ACN : MeOH (9:1)</td>
<td>24</td>
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<tr>
<td>MMIP_5</td>
<td>1:3:5</td>
<td>9.9</td>
<td>12.7</td>
<td>141.4</td>
<td>ACN : MeOH (9:1)</td>
<td>24</td>
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<tr>
<td>MMIP_6</td>
<td>1:20:99</td>
<td>189</td>
<td>21.6</td>
<td>189</td>
<td>ACN : MeOH (9:1)</td>
<td>24</td>
</tr>
<tr>
<td>MMIP_7</td>
<td>1:10:20</td>
<td>9.9</td>
<td>42.5</td>
<td>189</td>
<td>ACN : MeOH (9:1)</td>
<td>24</td>
</tr>
<tr>
<td>MMIP_8</td>
<td>1:4:20</td>
<td>9.9</td>
<td>17</td>
<td>189</td>
<td>ACN : MeOH (1:1)</td>
<td>24</td>
</tr>
<tr>
<td>MMIP_9</td>
<td>1:4:20</td>
<td>9.9</td>
<td>17</td>
<td>189</td>
<td>MeOH</td>
<td>24</td>
</tr>
</tbody>
</table>

Table S3. The selectivity coefficient of MMIPs and MNIPs for IQ interferences.

<table>
<thead>
<tr>
<th></th>
<th>IQ</th>
<th>MeIQ</th>
<th>8-MeIQx</th>
<th>Harman</th>
<th>Norharman</th>
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<tbody>
<tr>
<td>IF</td>
<td>2.95</td>
<td>1.85</td>
<td>2.5</td>
<td>1.87</td>
<td>2.00</td>
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<tr>
<td>SC</td>
<td>1.59</td>
<td>1.18</td>
<td>1.59</td>
<td>1.47</td>
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