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Original Article

# Analysis of heterocyclic amines in meat products by liquid chromatography – Tandem mass spectrometry

*Shi-Han Jian* [a,1], *Pei-Jou Yeh* [a,1], *Chung-Huang Wang* [b], *Hsin-Chang Chen* [c], *Sung-Fang Chen* [a,*]

[a] *National Taiwan Normal University, Department of Chemistry, Taiwan*
[b] *Product and Process Research Center, Food Industry Research and Development, Taiwan*
[c] *Institute of Food Safety and Health, National Taiwan University, Taiwan*

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ABSTRACT

Heterocyclic amines (HCAs), a class made up of more than 25 compounds, are unintended hazardous substances that are generated by the heating or processing of proteinaceous foods at high temperatures. The International Agency for Research on Cancer (IARC) has classified four such HCAs (IQ, MeIQ, MeIQx, and PhIP) as being probable or possible human carcinogens. In this study, two sample preparation strategies, liquid–liquid extraction (LLE) with solid-phase extraction (SPE) and a rapid, easy, cheap, effective, rugged, and safe extraction (QuEChERS) method, were investigated for the determination of 11 types of HCAs in meat products by LC-MS/MS. The HCAs in the samples were first extracted with acetonitrile by LLE, and followed by SPE. In the case of QuEChERS extraction, acetonitrile is used as the LLME solvent, and PSA, C18EC and $MgSO_4$ were used as the dSPE sorbent. Both methods showed good performance with respect to precision (RSD < 15.15%), accuracy (79.80–117.64%), recovery (52.39–116.88%), limit of quantitation for a spiked meat extract (0.01–10 ppb) and correlation coefficients (>0.993). The QuEChERS extraction strategy provided a better linear dynamic range and superior sensitivity in comparison with the LLE-SPE approach. HCAs were successfully quantified in real samples using the two proposed approaches by LC-MS/MS.



* *Corresponding author*.
E-mail address: sfchen@ntnu.edu.tw (S.-F. Chen).
[1] These authors contributed equally to this study and share first authorship.
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## 1. Introduction

Heterocyclic amines (HCAs) are a series of chemical compounds that contain at least one heterocyclic ring in which one or more of the carbon atoms in the ring are replaced by other atoms. They are produced during the cooking of meat at high temperature [1,2]. During the cooking process, the amino acids and creatine in meat undergo pyrolysis and denaturation and participate in a series of Maillard reactions, leading to the formation of HCAs. There are a number of types of heterocyclic amines. For example, IQ, IQx, and pyridine analogs are classified as thermic HCAs and are mainly formed from the thermal pyrolysis of amino acids, creatine, and sugars in meat at 150–300 °C [3]. Pyridoindole, pyidoimidazole, phenylpyridine, teraazafluorantene and benzimidazole analogs are classified as pyrolytic HCAs and are formed during the pyrolysis of amino acids or proteins at temperatures higher than 300 °C. In this study, we determined 11 types of HCAs in meat products, including IQ, MeIQ, 8-MeIQx, PhIP, MeAαC, AαC, Harman, Norharman, Trp-P-1, Trp-P-2 and Glu-P-1. The types, polarity, optimized extraction solvents, and optimized compositions for the dSPE of 11 HCAs are listed in Table S1.

Recent epidemiological studies indicate that diet is an important factor in the development of human cancer [4]. It has been reported that the charred parts of broiled fish and meat have mutagenic activity and that this mutagenic activity is produced during the cooking process [5]. Mutagenic compounds can lead to mutations that cause various cancers, including colorectal cancer [6], prostate cancer [7], breast cancer [8], pancreatic cancer [9], and gastric cancer [10]. MeIQ, MeIQx, and PhIP are classified as Group 2B compounds by the International Agency for Research on Cancer (IARC) that are possibly carcinogenic to humans and IQ is classified as a Group 2A compound that is probably carcinogenic to humans. The National Toxicology Program also suggested that MeIQ, 8-MeIQx, IQ, and PhIP are materials that may cause cancers in humans.

Many methods have been introduced for the analysis of HCAs in processed food. An efficient sample pretreatment is essential because meat products are comprised of fat, proteins, cholesterol, carbohydrates, inorganic salts, and vitamins. Liquid–liquid extraction, solid phase extraction (SPE) [11], tandem solid phase extraction (two or more cartridges used serially) have been applied to purify HCAs. Moreover, several approaches for the separation and detection of heterocyclic amines have been reported, such as: liquid chromatography coupled with mass spectrometry (LC-MS), which permits mutagens to be detected at the low parts-per-billion-level because of its sensitivity [12]. These methods include, liquid chromatography-tandem mass spectrometry (LC-MS/MS) [13], which not only can detect low levels of HCAs, but can be used to confirm the identities of different compounds; liquid chromatography-ultraviolet (LC-UV), which may need more selective detection methods to increase the sensitivity of the assay [14]. Liquid chromatography-diode array detectors (LC-DAD) [15]; liquid chromatography-diode array detectors –tandem mass spectrometry (LC-DAD-MS/MS) [16]; gas chromatography-mass spectrometry (GC–MS), which was introduced for the analysis of nonpolar heterocyclic amines in cooked meat [17]. Among all of the above methods, liquid chromatography-tandem mass spectrometry (LC-MS/MS) appears to be the preferred method, due to its high selectivity and superior sensitivity.

The aim of this study was to develop an analytical method for the determination of HCAs in meat products that is superior to previous methods. Eleven HCAs including thermic HCAs and pyrolytic HCAs were used a model compounds, because of their potent carcinogenicity. Method validation, including linearity, sensitivity, accuracy, precision, recovery and matrix effects were also conducted for the intended use.

## 2. Methods and materials

### 2.1. Chemicals and materials

All chemicals and solvents were of HPLC or analytical grade. Acetonitrile, and Methanol were purchased from Merck (Germany). Formic acid, Hydrogen chloride (36.5–38%), Sodium chloride, and Magnesium sulfate was obtained from J.T.Baker (USA) was ACS grade. Ultrapure water (Milli-Q) was obtained from Millipore system (France). The heterocyclic aromatic amines (HCAs) were used as reference compounds: IQ, MeIQ, 8-MeIQx, PhIP, AαC, MeAαC, Harman, Norharman, Trp-P-1, Trp-P-2, and Glu-P-1 were purchased from Toronto Research Chemicals (Canada). Standard stock solutions of 10 μg/g in methanol were prepared and used for further dilution. 4,7,8-TriMeIQx was used as an internal standard (150 ng/g). The three types of sorbents used in QuEChERS are listed below: Primary and secondary amine (PSA) SPE bulk sorbent (Agilent Technologies, USA), SiliaBondR C18 (17%), 40–63 μm, 60 A (Silicycle® Inc, Canada), C18 Endcapped (C18EC), 57.9 μm, 59 A (Agilent Technologies, USA). Oasis® MCX Cartridge, 30 μm by Waters (USA). The analysis column was a Shim-pack GIST C18 (2.1 × 100 mm, 3 μm, 100 Å) from Shimadzu (Japan). Meat samples (pork floss, pork jerky, beef jerky, raw pork and raw beef) were purchased from local stores and stored in a freezer at −18 °C until used.

### 2.2. Parameters of LC-MS/MS analysis

LC separations were carried out on Agilent 1200 HPLC System. Separation was achieved on a Shim-pack GIST C18 (2.1 × 100 mm, 3 μm, 100 Å) at 37 °C. Mobile phase A consisted of 2% ACN and 0.1% FA and mobile phase B was 98% ACN and 0.1% FA. The flow rate was 0.2 mL/min. The injection volume was 5 μL. The applied chromatographic gradient was started at 0–0.5 min, 5% B; 0.5–14 min, linear gradient to 80% B; 14–15 min, a hold at 80% B; a 15–15.5 min drop to 5% B; 15.5–23 min for equilibrium with 5% B. The total analysis time was 23 min. Mass spectrometric analyses were performed on a AB Sciex API 4000™ triple quadrupole 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, 50 msec; pause time, 5.0070 msec; duration, 23.007 min. The mass spectrometric parameters were listed as below: gas 1, 60 psi; gas 2, 60 psi; curtain gas, 20 psi; temperature, 600 °C; collision gas: 4 psi; ionspray voltage: +5500 V; full width at half-maximum height, FWHM:

0.7 Da. Multiple reaction monitoring (MRM) transitions and optimal voltage parameters for 11 HCA compounds were given in Table S2.

### 2.3. Sample preparation

#### 2.3.1. Sample extraction

Two sample pretreatment strategies, including liquid–liquid extraction (LLE) combined with SPE and the QuEChERS method were investigated for the determination of 11 types of HCAs in meat products by LC-MS/MS. It is known that meat products typically contain large amounts of fat that may significantly hinder the analysis of HCAs. A simple and reliable liquid–liquid extraction process was applied for fat removal before carrying out the solid phase extraction. Three extraction solvents, including acetonitrile, ethyl acetate and chloroform were compared. The recoveries are summarized in Fig. 1. The findings indicate that acetonitrile was clearly the solvent of choice for this procedure and provided a better overall recovery efficiency. The fact that ethyl acetate and chloroform performed especially poorly for MeAαC and AαC can be attributed to the fact that they are less polar than the other HCAs which are relatively resistant to extraction. On the other hand, the recoveries of Trp-P-1 and Trp-P-2 using ethyl acetate were higher than expected. This may be because species with chemical structures that are similar cannot be distinguished by low resolution mass spectrometry. Thus, acetonitrile was selected as the extraction solvent for LLE.

#### 2.3.2. Extraction cleanup

*2.3.2.1. QuEChERS.* The QuEChERS extraction methodology was designed for the quantification of all HCAs in meat samples. A 2.0 g sample of meat, internal standard and 14 mL of $H_2O$ were placed in a 50 mL tube. The mixture was vortexed for 1 min and then ultrasonicated for 30 min. After adding 15 mL of acetonitrile, 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, and then centrifugation at 4000 rpm (4 °C) for 5 min. A 5 mL aliquot of the organic layer was then transferred to another 50 mL tube. In the dispersive solid phase extraction (dSPE) step, 250 mg of PSA, 250 mg of C18EC and 750 mg of anhydrous magnesium sulfate were added to the tube. The tube was vortexed for 1 min and centrifuged at 4000 rpm (4 °C) for 5 min. A 1 mL aliquot of the liquid extract was evaporated to dryness and the residue dissolved in 100 μL of mobile phase A before LC-MS/MS analysis.

*2.3.2.2. SPE.* The extraction step for the LLE-SPE method was as follows. A meat sample (2.0 g), internal standard and 14 mL of 0.1 M HCl were placed in a 50 mL tube. The mixture was vortexed for 1 min and ultrasonicated for 30 min. After adding 15 mL of acetonitrile, the tube was vigorously vortexed for 1 min to extract the sample. Next, 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, and centrifugation at 4000 rpm (4 °C) for 5 min. A 2 mL aliquot of the organic layer was transferred to an Oasis® MCX cartridge. In the first step, the cartridges were conditioned with 5 mL of MeOH and 5 mL of $H_2O$, washed with 2 mL of 0.1 M HCl and 2 mL of MeOH. The analytes were eluted with 2 mL of MeOH/$NH_4OH$ (95/5, v/v). The liquid extract was evaporated to dryness and the residue dissolved in 200 μL of mobile phase A before the LC-MS/MS analysis.

### 2.4. Method validation

#### 2.4.1. Calibration curves

Two types of calibration curves were prepared in this study. One was prepared using standard solutions of heterocyclic amines and the other was prepared by spiking the series of HCAs standards in the boiled beef. The latter curve is referred to as a quantitative calibration curve. The peak areas for the heterocyclic amines transitions were used for establishing calibration curves. The concentration of the stock was 10 ppm in methanol. A series of HCAs standards in methanol were prepared with concentrations of 0.005, 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 1, 2.5, 5, 10, 25, 50, 100, 250 ppb and 100 ppb of internal standard was added to each solution. The quantitative calibration curve was prepared by spiking the boiled beef with a series of HCAs standards, followed by applying the LLE-SPE

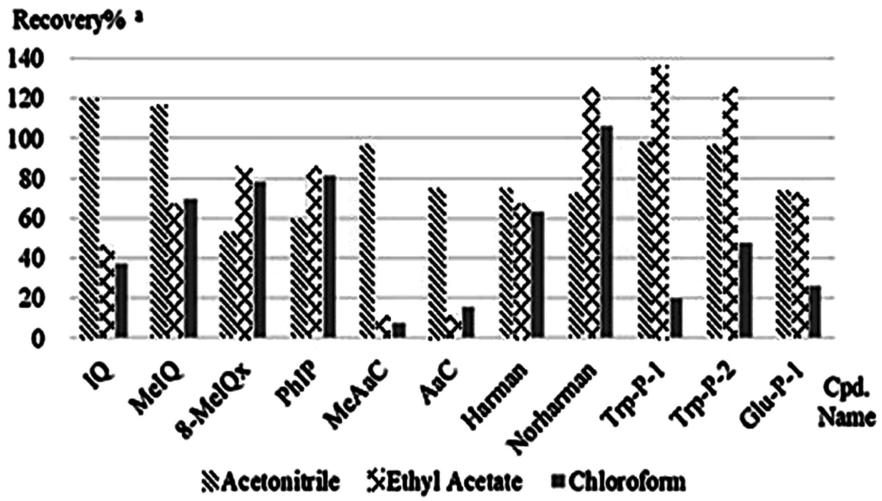


**Fig. 1 – Optimization of the solvents used for the liquid–liquid extraction.**

and QuEChERS methods, and the solution was injected into the LC-MS/MS system for analysis. The Analyst® 1.6.2 software was used for data acquisition.

#### 2.4.2. *Sample quantification*

In this experiment, three calibration curves were prepared by triplicate injections and weighted by 1/x. The LOD and LOQ values for the analytes were defined as S/N ratios of 3 and 10 respectively. The intraday precision was determined by analyzing concentrations of 10, 50, 100 ppb in triplicate in one day. The recovery was defined as the standards spiked in the blank compared with the theoretical concentration of the standards. The matrix effect was defined as the ratio between the slope of the quantitative calibration curve and the standard calibration curve.

## 3. Results and discussion

### 3.1. *Sample preparation*

Since the HCAs contain heterocyclic ring structures and amine groups, the Oasis® MCX Cartridge from Waters Inc. was used for solid phase extraction. The stationary phase is a mixed-mode polymeric sorbent containing both reversed phase (RP, benzyl) and weak cation exchange (WEX, $SO_3^-$) functionalities that would be expected to retain the HCAs well for the extraction. The experimental conditions basically followed the instructions specified by the manual with minor modifications. Cartridge equilibrating and washing volumes were examined for the use of 60 mg of the sorbent, the optimized conditioning was 10 mL of 1:1 MeOH/$H_2O$ without an acid modifier and 4 mL of 1:1 MeOH/$H_2O$ for washing.

There are only few reports of the use of the QuEChERS methodology for the analysis of HCAs in meat products. The objective was to establish an efficient QuEChERS-based method that would be comparable to the LLE-SPE method that was also examined in this study.

In the QuEChERS method, the samples are first homogenized, extracted with a liquid partition in the presence of excess salts which induce phase separation. An aliquot of the organic phase is then subjected to further clean up using dispersive SPE. Unlike conventional methods using SPE tubes, in dispersive SPE, sample cleanup is facilitated by mixing bulk amounts of SPE with the extract. Interfering substances tend to be retained when a dispersive SPE powder is used as the cleanup sorbent. A $C_{18}$, primary secondary amine (PSA) and graphitized carbon black (GCB) are widely used as sorbents for specific matrix cleanup and anhydrous $MgSO_4$ serves to remove excess water. To be specific, $C_{18}$ with reverse phase properties usually removes very non-polar/hydrophobic interfering substances, such as lipids and fats; PSA mainly removes polar/hydrophilic interfering substances, including saccharides/sugars and organic acids; GCB effectively removes pigments such as chlorophyll and carotenoids.

Several dSPE sorbents of QuEChERS for samples that are rich in fat have been investigated in previous studies. Among them, PSA, C18EC, and $MgSO_4$ were widely used. Various quantities and combinations of PSA, C18, C18EC, and $MgSO_4$ as the dSPE sorbent were compared in this study including: 250 mg PSA + 250 mg C18 + 750 mg $MgSO_4$, 250 mg C18 + 750 mg $MgSO_4$, 250 mg PSA + 750 mg $MgSO_4$, 250 mg PSA + 250 mg C18EC + 750 mg $MgSO_4$ and 250 mg C18EC + 750 mg $MgSO_4$. Since HCAs are relatively moderate polar compounds it is possible to remove extremely polar and non-polar substances using a PSA + C18EC mixture as the dSPE sorbent in QuEChERS. A sorbent mixture composed of PSA, C18EC and $MgSO_4$ provided satisfactory results. Two compounds, MeAáC and AáC, were especially difficult to recover when either C18EC and PSA were used alone.

An additional experiment was also conducted in which different ratios and quantities of PSA, C18EC and $MgSO_4$ were compared. The findings indicated that 250 mg PSA +250 mg C18EC + 750 mg $MgSO_4$ (1:1:3) gave a satisfactory result (Fig. 2). MeA$\alpha$C and A$\alpha$C tended to be adsorbed on a hydrophobic sorbent such as C18EC, which lead to their low recovery.

Although the separation conditions for LC-MS were not extensively optimized for these 11 HCA compounds in this work, a conventional C18 reversed phased column (2.1 mm i.d. $\times$100 mm L) using a ACN gradient was found to be efficient. Baseline separation was successfully achieved for all compounds and the overall analysis time was within 12 min (Fig. 3).

### 3.2. *Validation of the method*

Liquid chromatography/electrospray tandem mass spectrometry (LC/ESI-MS) with multiple reaction monitoring (MRM) acquisition was performed for method validation including linear regression, limit of detection (LOD), limit of quantitation (LOQ), precision, accuracy, recovery and matrix effect.

#### 3.2.1. *Calibration, LOD and LOQ*

Several calibration curves using different matrices and two pretreatments (LLE-SPE and QuEChERS) were constructed including a standard-spiked solvent blank, a standard-spiked blank matrix before the sample pretreatment and a standard-spiked blank matrix after sample pretreatment (matrix-matched) for 11 HCA compounds. The standard-spiked solvent blank calibration curve can be easily used as a reference, the matrix effect of HCAs from other sources can be compared and evaluated by calculating the recovery. The calibration curve of the standard-spiked blank matrix before the sample pretreatment can be carried out and similar to the standard addition method, it should provide the most accurate results, although precision and sensitivity might be compromised. The calibration curve of the standard-spiked blank matrix after sample pretreatment is sometimes called a matrix-matched calibration curve and is widely applied for high throughput sample screening for target compounds that are contained in a complicated matrix.

The linear regressions of the standard-spiked solvent are summarized in Table 1. The LOD and LOQ of the thermic IQ-type HCAs were determined to be within the 0.005–0.1 ppb and 0.025–0.25 ppb ranges respectively. The LOD and LOQ of the pyrolytic HCAs such as Harman were within 0.005–0.25 ppb and 0.01–1.00 ppb ranges. Two orders of linear dynamic ranges were obtained for the 11 HCA compounds with good correlation coefficients ($r > 0.9965$).

The linear regressions of standard-spiked blank matrix using LLE/SPE are summarized in Table 2. The LOD and LOQ of

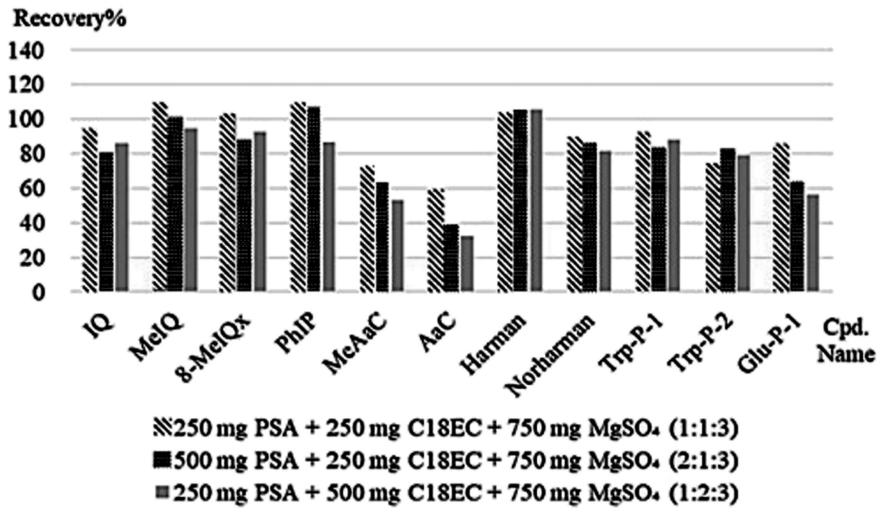


**Fig. 2 – Optimization of dSPE sorbent composition for the QuEChERS.**

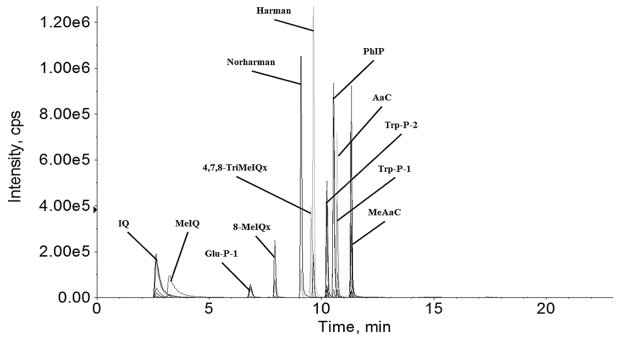


**Fig. 3 – Extract ion chromatogram (XIC) of 11 HCA compounds and internal standard.**

the thermic IQ-type HCAs were within 0.1–5.0 ppb and 0.25–10.0 ppb ranges, and the LOD and LOQ of pyrolytic HCAs were within 0.05–5.0 ppb and 0.1–10.0 ppb ranges. At least one order of linear dynamic ranges was obtained with correlation coefficients ($r > 0.9940$). These results were not as good as the ones achieved for the case of the standard-spiked solvent blank.

### 3.2.2. *Linear regressions of standard-spiked blank matrix using QuEChERS*

The linear regressions of standard-spiked blank matrix using QuEChERS and LC-MRM MS analysis are summarized in Table 3. The LOD and LOQ of thermic IQ-type HCAs were within the 0.005–0.50 ppb and 0.025–2.5 ppb ranges. The LOD and LOQ of the pyrolytic HCAs, such as Harman, were within 0.005–2.5 ppb and 0.01–5.0 ppb ranges. The analytical performances including LOD, LOQ, linear dynamic range and correlation coefficients were comparable, although not better, than the results achieved by solvent blank.

It should be noted that the performance of the QuEChERS method was comparable to the values obtained for the standard-spiked solvent blank. Comparing to SPE, QuEChERS excels in LOD, LOQ and dynamic range that provides superior sensitivity.

### 3.2.3. *Accuracy and precision*

HCA standards were spiked in a blank solvent or blank matrix, LLE/SPE or QuEChERS were conducted, followed by LC-MRM MS analysis. The accuracies and precisions of standard-spiked solvent blank are shown in Table S3. Acceptable accuracies

**Table 1 – LOD, LOQ and linear regression of the quantitative calibration curve for standards using LC-MRM MS analysis.**

| | Concentration (ppb) | | | Linear equation | r |
|---|---|---|---|---|---|
| | LOD[a] | LOQ[b] | Calibration range | | |
| IQ | 0.1 | 0.25 | 0.25–100 | $y = 0.0145\ x + 0.00242$ | 0.9965 |
| MeIQ | 0.1 | 0.25 | 0.25–100 | $y = 0.0133\ x + 0.00136$ | 0.9986 |
| 8-MeIQx | 0.1 | 0.25 | 0.25–100 | $y = 0.0109\ x + 0.00376$ | 0.9965 |
| PhIP | 0.005 | 0.025 | 0.025–100 | $y = 0.0151\ x + 0.00146$ | 0.9989 |
| MeAαC | 0.01 | 0.025 | 0.025–100 | $y = 0.0226\ x + 0.000926$ | 0.9985 |
| AαC | 0.01 | 0.025 | 0.025–100 | $y = 0.0278\ x + 0.00119$ | 0.9986 |
| Harman | 0.005 | 0.01 | 0.01–100 | $y = 0.0425\ x + 0.00197$ | 0.9987 |
| Norharman | 0.025 | 0.05 | 0.05–100 | $y = 0.0341\ x + 0.000259$ | 0.9973 |
| Trp-P-1 | 0.005 | 0.025 | 0.025–100 | $y = 0.0204\ x + 0.00059$ | 0.9980 |
| Trp-P-2 | 0.01 | 0.025 | 0.025–100 | $y = 0.0131\ x + 0.000805$ | 0.9981 |
| Glu-P-1 | 0.25 | 1 | 1–100 | $y = 0.0029\ x + 0.00126$ | 0.9976 |

[a] $S/N \geq 3$
[b] $S/N \geq 10$

**Table 2 – LOD, LOQ and linear regression of the quantitative calibration curve for the standard-spiked blank matrix using LLE/SPE and LC-MRM MS analysis.**

| | Concentration (ppb) | | | Linear equation | r |
|---|---|---|---|---|---|
| | LOD | LOQ | Calibration range | | |
| IQ | 5 | 10 | 10–250 | $y = 0.0000618\ x + 0.00141$ | 0.9979 |
| MeIQ | 2.5 | 5 | 5–250 | $y = 0.0000482\ x + 0.000308$ | 0.9948 |
| 8-MeIQx | 0.25 | 1 | 1–250 | $y = 0.00104\ x - 0.00418$ | 0.9976 |
| PhIP | 0.1 | 0.25 | 0.25–100 | $y = 0.0224\ x + 0.00863$ | 0.9975 |
| MeAαC | 2.5 | 5 | 5–250 | $y = 0.00241\ x - 0.0129$ | 0.9976 |
| AαC | 1 | 5 | 5–250 | $y = 0.00158\ x - 0.0076$ | 0.9979 |
| Harman | 0.05 | 0.1 | 0.1–100 | $y = 0.0521\ x + 0.0186$ | 0.9940 |
| Norharman | 0.05 | 0.1 | 0.1–100 | $y = 0.0444\ x + 0.0327$ | 0.9963 |
| Trp-P-1 | 1 | 2.5 | 2.5–250 | $y = 0.00231\ x + 0.00817$ | 0.9978 |
| Trp-P-2 | 0.5 | 1 | 1–250 | $y = 0.00322\ x - 0.00189$ | 0.9974 |
| Glu-P-1 | 5 | 10 | 10–250 | $y = 0.000281\ x + 0.00147$ | 0.9980 |

and precisions were achieved (85.66–112.94%, 0.27–15.15% RSD %) with poorer precisions observed at lower concentrations.

The detailed results for the standard-spiked blank matrix before LLE/SPE and QuEChERS are also shown in Table S4 and Table S5. Similar outcomes were obtained with accuracies within 83.74–116.83%, precisions 1.04–14.84% (RSD%) for LLE/SPE and accuracies within 79.80–117.64%, precisions 0.92–14.25% (RSD%) for QuEChERS.

#### 3.2.4. *Intra-day and inter-day precision*

HCA standards were spiked in a blank matrix and LLE/SPE or QuEChERS were conducted followed by LC-MRM MS analysis. Three different concentrations with 10, 50 and 100 ppb of HCA standards were used to evaluate intra-day and inter-day precision. To evaluate intra-day precision, triplicate analyses were carried out on the same day and their RSD% were all found to be better than 10%, thus demonstrating that the method had a good repeatability. For inter-day precision, triplicate analyses per day were performed on three consecutive days and their RSD% were within the 5.32–14.35% range.

#### 3.2.5. *Recovery*

The recoveries of LLE/SPE and QuEChERS were tested on three concentration levels (low 10 ppb, medium 50 ppb, high 100 ppb) of HCA standards. The recoveries for 8-MeIQx and PhIP were low in the case of LLE/SPE, which can be attributed to an insufficient extraction using acetonitrile in the LLE. The recoveries of LLE/SPE from other HCA compounds were all within 75–116%. On the other hand, AαC and MeAαC was poorly recovered in the case of the QuEChERS method. For the other HCA compounds, their recoveries were within a satisfactory 83–112% range with RSD values less than 10%.

#### 3.2.6. *Matrix effect*

Several studies have reported on the quantitation of HCA compounds in different matrices, including meat products. In this study, the QuEChERS pretreatment combined with the LC-MS/MS analysis was found to provide superior analytical performance (LOD, LOQ, linear dynamic range) with good accuracy and precision.

Commercially processed meat products from local markets were purchased and used for the quantitation of HCAs. They included pork floss (sample 1, 6), pork jerky (sample 2, 7), and beef jerky (sample 3, 8), in which the pork was from a local source in Taiwan and the raw beef had been imported from Australia and New Zealand. Quantitative results using the LLE-SPE and QuEChERS procedures are listed in Table 4. Several of the HCAs in the processed meats could be detected and quantified by either method. Among them, IQ, harman, and norhaman can be detected and quantified using both LLE-SPE and QuEChERS methods with similar results (0.1–274.41 ppb). It should be noted that IQ and Trp-P-1 can be only quantified by the QuEChERS method in sample 6 and sample 3, since QuEChERS clearly provided superior sensitivity.

**Table 3 – LOD, LOQ and linear regression of the quantitative calibration curve for the standard-spiked blank matrix using QuEChERS and LC-MRM MS analysis.**

| | Concentration (ppb) | | | Linear equation | r |
|---|---|---|---|---|---|
| | LOD | LOQ | Calibration range | | |
| IQ | 0.5 | 1 | 1–250 | $y = 0.00482\ x - 0.00208$ | 0.9988 |
| MeIQ | 0.5 | 2.5 | 2.5–250 | $y = 0.00582\ x - 0.00834$ | 0.9965 |
| 8-MeIQx | 0.25 | 1 | 1–250 | $y = 0.0134\ x - 0.00727$ | 0.9991 |
| PhIP | 0.005 | 0.025 | 0.025–100 | $y = 0.0225\ x + 0.0000865$ | 0.9989 |
| MeAαC | 0.005 | 0.25 | 0.25–100 | $y = 0.0139 - 0.000337$ | 0.9955 |
| AαC | 0.25 | 1 | 1–250 | $y = 0.0223\ x - 0.0167$ | 0.9973 |
| Harman | 0.005 | 0.01 | 0.01–100 | $y = 0.0802\ x + 0.0121$ | 0.9982 |
| Norharman | 0.005 | 0.01 | 0.01–100 | $y = 0.0316\ x + 0.0225$ | 0.9940 |
| Trp-P-1 | 0.005 | 0.025 | 0.025–100 | $y = 0.0309\ x - 0.000196$ | 0.9980 |
| Trp-P-2 | 0.1 | 0.25 | 0.25–100 | $y = 0.0145\ x + 0.000731$ | 0.9937 |
| Glu-P-1 | 2.5 | 5 | 5–250 | $y = 0.00271\ x - 0.0161$ | 0.9981 |

**Table 4 – Quantitative analysis of commercial meat products.**

| LLE-SPE | Concentration (ppb) (n = 3) | | | | | |
|---|---|---|---|---|---|---|
| | sample 1 | sample 2 | sample 3 | sample 4 | sample 5 | sample 6 |
| IQ | 271.50 | 73.78 | 88.66 | N.Q. | 32.40 | 63.64 |
| MeIQ | N.Q. | N.D. | N.D. | N.D. | N.D. | N.D. |
| 8-MeIQx | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| PhIP | N.Q. | N.Q. | N.D. | N.D. | N.D. | N.D. |
| MeAαC | N.D. | N.D. | N.D. | N.D. | N.D. | N.Q. |
| AαC | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| Harman | 261.59 | 86.68 | 81.79 | 176.74 | 51.29 | 142.91 |
| Norharman | 32.49 | 5.67 | 7.33 | 15.67 | 4.52 | 25.95 |
| Trp-P-1 | N.D. | N.D. | N.Q. | N.D. | N.Q. | N.Q. |
| Trp-P-2 | N.D. | N.D. | N.Q. | N.D. | N.Q. | N.D. |
| Glu-P-1 | 342.19 | 120.52 | 67.67 | 465.27 | 193.76 | 718.46 |
| QuEChERS | | | | | | |
| IQ | 274.41 | 71.56 | 82.69 | 3.38 | 31.07 | 62.04 |
| MeIQ | N.Q. | N.D. | N.D. | N.Q. | N.D. | N.Q. |
| 8-MeIQx | N.D. | N.D. | N.D. | N.D. | N.Q. | N.D. |
| PhIP | N.Q. | N.D. | N.Q. | N.D. | N.D. | 0.10 |
| MeAαC | N.D. | N.D. | N.Q. | N.D. | N.D. | N.D. |
| AαC | N.D. | N.D. | N.D. | N.Q. | N.D. | N.Q. |
| Harman | 254.81 | 42.50 | 75.06 | 161.77 | 35.46 | 149.73 |
| Norharman | 68.76 | 8.00 | 16.11 | 33.92 | 10.29 | 58.19 |
| Trp-P-1 | N.Q.[a] | N.Q. | 0.06 | N.Q. | N.D. | N.D. |
| Trp-P-2 | 0.53 | N.D. | N.Q. | N.Q. | N.D. | N.Q. |
| Glu-P-1 | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |

N.D.
[a] Which is under the LOD for each standard.

## 4. Conclusions

Two pretreatment processes, namely, LLE-SPE and QuEChERS, were investigated for the determination of 11 types of HCAs in meat products by LC-MS/MS analysis. The overall analytical performances were found to be satisfactory for most of the HCA compounds. It should be noted, however, that the QuEChERS method provided a better linear dynamic range with superior sensitivity. These two methods were also applied to the quantification of HCAs in commercially processed meat products. IQ, as well as others, were successfully detected and quantified within the concentration range of 0.06–718.46 ppb. A method using the QuEChERS preparation method coupled with LC tandem MS analysis was developed for the quantification of HCA in meat products. This not only provides a suitable method for the quantitation of HCA compounds, but should be also beneficial for monitoring potential carcinogens in such products and could improve public health consequences and the management of such species.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jfda.2018.10.002.

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