Journal of Chromatography A, 1235 (2012) 103–114

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Determination of N-methyl-1,3-propanediamine in bovine muscle by liquid chromatography with triple quadrupole and ion trap tandem mass spectrometry detection

Clare Ho, Wai-On Lee, Yiu-Tung Wong*

Analytical and Advisory Services Division, Government Laboratory, 7/F Ho Man Tin Government Offices, Ho Man Tin, Kowloon, Hong Kong Special Administrative Region

ARTICLE INFO

Article history:
Received 21 November 2011
Received in revised form 22 February 2012
Accepted 23 February 2012
Available online 28 February 2012

Keywords:
Morantel
N-methyl-1,3-propanediamine
Pyrantel
Pentafluoropropionic acid anhydride derivatization
Ion trap
Triple quadrupole

ABSTRACT

Morantel, pyrantel and their drug-related metabolites in food of animal-origin are regulated as sum of residues which may be hydrolysed to N-methyl-1,3-propanediamine (NMPA). In this study, an isotope dilution liquid chromatography tandem mass spectrometry (LC–MS/MS) method with pentafluoropropionic acid anhydride (PFPA) derivatization was developed for the determination of NMPA in bovine muscle. A stable isotope labeled internal standard N-methyl-d_3-3,3′-d_2-propane-1,3-diamine (NMPA-d_5) was synthesized as internal standard. NMPA was derivatized with PFPA to form an N,N′-bis (pentafluoroacyl) derivative (NMPA-PFPA) and analyzed by liquid chromatography triple quadrupole mass spectrometry (LC-QqQ-MS/MS) and liquid chromatography ion trap mass spectrometry (LC-IT-MS/MS) using negative ion electrospray ionization (ESI). Chromatographic behavior of several perfluorocarboxylic acid anhydride derivatives of NMPA and other structurally related diamines on C-18 and perfluorophenyl (PFP) columns was studied. Conversion of the parent drugs to NMPA under various hydrolysis conditions was evaluated. In addition, comparison of the matrix effect and linearity with isotopically labeled internal standard (I.S.) and analogous I.S. were performed and investigated. The method was validated using fortified bovine muscle samples. The apparent recovery (obtained after correction with an isotopically labeled I.S.) was between 89% and 97% and repeatability was less than 10%. The lowest LOD and LOQ (0.42 and 1.39 μg/kg, respectively) were obtained with LC-QqQ-MS/MS.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Morantel ((E)-1,4,5,6-tetrahydro-1-methyl-2-[2-(3-methyl-2-thienyl)ethenyl]pyrimidine MOTL) and pyrantel ((E)-1,4,5,6-tetrahydro-1-methyl-2-[2-(2-thienyl)ethenyl]pyrimidine, PYTL) (Fig. 1) are tetrahydro-pyrimidine anthelmintics intended to remove and control immature or mature gastro-intestinal endoparasites in ruminants. For the sake of consumer safety and to allow adequate withdrawal period, European Union (EU) [1] and USA [2,3] have established the maximum residue level (MRL) for MOTL in muscles of all ruminants and PYTL in porcine muscle, respectively (Table 1). Residue of MOTL in all ruminants is regulated as the sum of residues which may be hydrolyzed to the marker residue NMPA. It should be noted that no MRL was established by EU for PYTL whereas the MRL for PYTL in porcine tissue established by USA was based on the PYTL residue, instead of NMPA. The tolerance of PYTL was established before the USA initiated the current marker residue concept [4] and therefore PYTL was regulated as parent drug. In fact, previous metabolic studies showed that these two structurally related drugs metabolized rapidly into the same major marker residue NMPA after administration (Fig. 2) [5–7].

Up to now, most of the reported analytical methods for the determination of MOTL- and PYTL-related metabolites were based on derivatization with 4-fluoro-3-nitrobenzotrifluoride and analysis by gas chromatography (GC) coupled with mass spectrometry (MS) [4,8], flame ionization detector (FID) [4] and electron capture detector (ECD) [9,10]. The sample preparation procedures of these GC methods are lengthy and tedious. In addition, these methods are not selective enough. Direct analysis of NMPA by reversed phase LC–MS/MS is difficult. Even though NMPA should be effectively ionized by positive ion ESI, detection of the relatively low molecular weight analyte in hydrolyzed muscle extract encountered significant matrix interferences. Short-chain aliphatic diamine like NMPA is very polar which resulted in nearly non-retaining behavior on most reversed phase LC systems. The use of ion-pairing reagents such as pentafluoropropionic acid and heptafluorobutyric acid may be useful to achieve better retention and well resolved separation. However these reagents often suppress the ionization of the analytes in ESI [11,12]. Initial attempts have been made to analyze NMPA directly with hydrophilic interaction chromatography (HILIC) and positive ion ESI MS/MS but the peak shape was broad and sensitivity was low. Therefore, there is a need to develop a

* Corresponding author. Tel.: +852 25382013.
E-mail address: ytwong@govtlab.gov.hk (Y.T. Wong).

0021-9673/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2012.02.060

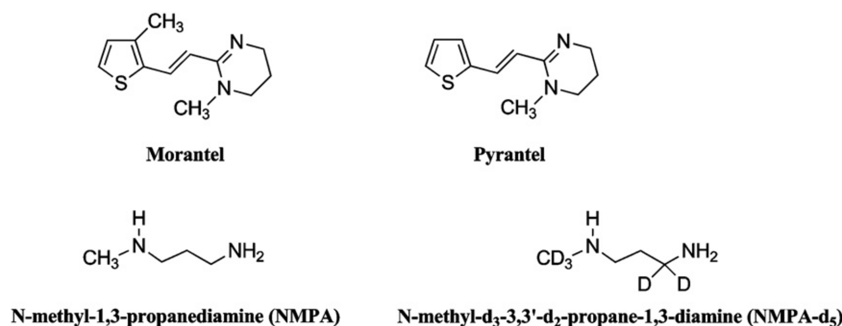

Fig. 1. Structures of MOTL, PYTL, N-methyl-1,3-propanediamine (NMPA) and N-methyl-d_3-3,3′-d_2-propane-1,3-diamine (NMPA-d_5, I.S.).

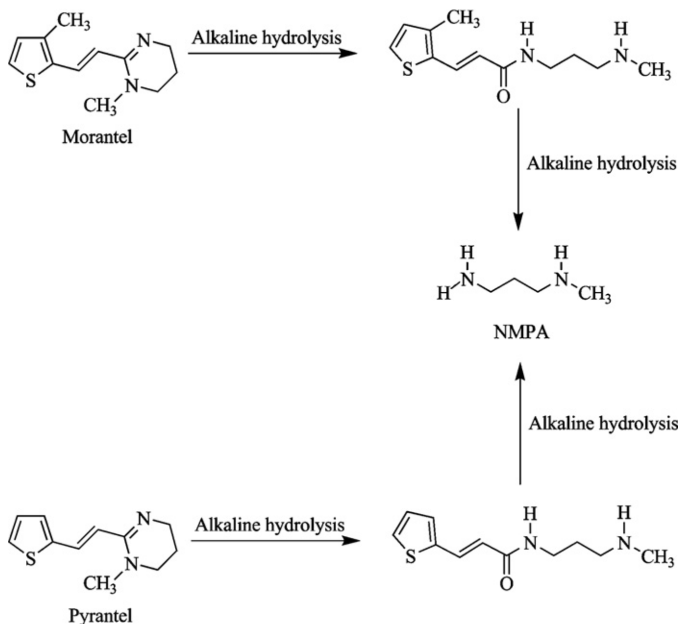

Fig. 2. Alkaline hydrolysis of MOTL and PYTL.

rapid, sensitive and selective analytical method for the determination of NMPA as marker residues of MOTL- and PYTL-related drug residues in food of animal-origin.

Perfluorocarboxylic acid anhydrides are derivatization reagents commonly employed in the determination of amines in biological samples by GC-ECD or GC-negative ion chemical ionization mass spectrometry (NCI-MS). Liquid chromatographic determinations of perfluorocarboxylic acid derivatives of amines have also been reported with UV [13,14] and MS [15,16] detection. However, these LC–MS/MS methods were performed with thermospray and plasmaspray ionization. A few LC–MS or LC–MS/MS methods were reported to determine diamines and aromatic amines as perfluorocarboxylic acid anhydride derivatives using negative ion ESI [17–19]. To our knowledge, there is no reported method using this state-of-the-art technique to determine NMPA as the marker residue of MOTL- and PYTL-related drug residues in food samples.

In this work, a sensitive and selective method for the determination of NMPA using PFPA derivatization and negative ion ESI LC–MS/MS detection was developed. MOTL- and PYTL-related drug residues were converted to NMPA after alkaline hydrolysis and derivatized with PFPA. The resulting derivative was analyzed by LC-QqQ-MS/MS and LC-IT-MS/MS in negative ion ESI mode. Interfering compounds found in blank bovine muscle were further confirmed by GC-electron ionization mass spectrometry (EI-MS). A deuterium labeled NMPA (NMPA-d_5) was synthesized as internal standard (Fig. 1) to improve method precision. Comparison of isotopically labeled compound and analogous compound as the I.S. was conducted and investigated. The analytical method was validated by evaluating the conversion of parent drugs to NMPA, hydrolysis conditions, matrix effect, linearity of calibration curve, limit of detection (LOD) and limit of quantification (LOQ), selectivity, recovery and repeatability.

2. Experimental

2.1. Reagents and chemicals

Dichloromethane (DCM), diethyl ether, ethyl acetate (EtOAc) and toluene were of analytical grade from Labscan (Bangkok, Thailand). Acetonitrile (ACN) was of HPLC grade from Labscan (Bangkok, Thailand). Water was purified to 18.2 MΩ cm using Millipore Milli-Q Gradient system (Billerica, MA, USA). Potassium hydroxide pellet was purchased from VWR (Leuven, Belgium). N-methyl-1,3-propanediamine (NMPA) (≥97%) and N-methyl-d_3-3-aminopropionitrile were purchased from Fluka (Steinheim, Germany) and TRC (Toronto, Canada), respectively. 1,3-propanediamine dihydrochloride (1,3-PDA·2HCl) (98%), 1,5-pentanediamine (1,5-PDA) (95%), N-isopropyl-1,3-propanediamine (NIPA) (95%), MOTL (+)-tartrate (99.5%) and PYTL (+)-tartrate (99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA), 1,4-butanediamine (1,4-BDA) (99%) was obtained from Fluka (St. Louis, MO, USA). NEPA (97%) was purchased from Tianjin FineChem Co. Ltd (Tianjin, China). Trifluoroacetic acetic acid anhydride (TFAA) and pentafluoropropionic acid anhydride (PFPA) were purchased from Supelco (Bellefonte, PA, USA). Heptafluorobutyric acid anhydride (HFBA) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Table 1
MRLs for MOTL and PYTL residues in animal muscle.

Analytes	EU			USA		
	Marker residue	Animal species	MRLs (μg/kg)	Marker residue	Animal species	MRLs (μg/kg)
MOTL	Sum of residues which may be hydrolyzed to NMPA and expressed as MOTL equivalents	All ruminants	100	–	–	–
PYTL	–	–	–	PYTL	Porcine	1000

1 mg/mL of NMPA and NMPA-d_5 stock standard solutions were prepared in MeOH and diluted to appropriate volume with ACN before use. Stock solution (400 μg/mL) of 1,3-propanediamine (1,3-PDA) was prepared by dissolving appropriate amount of 1,3-PDA·2HCl in MeOH and diluted to appropriate volume with ACN before use. Stock solutions (400 μg/mL) of 1,5-PDA, 1,4-BDA, NEPA, NIPA, MOTL (+)-tartrate and PYTL (+)-tartrate were prepared in ACN and diluted to appropriate volume with ACN before use.

2.2. Apparatus

LC-QqQ-MS/MS analysis was performed on a Thermo Scientific Surveyor HPLC system composed of a Surveyor MS pump Plus, an on-line degasser and a Surveyor autosampler Plus coupled to a Thermo TSQ Quantum Access tandem mass spectrometer equipped with an ESI source (San Jose, CA, USA) interfaced with Xcalibur 2.1. LC-IT-MS/MS analysis was performed on an Thermo Scientific Surveyor HPLC system composed of a Surveyor LC pump Plus, an on-line degasser and a Surveyor autosampler Plus coupled to Thermo LCQ Deca XP MAX ion-trap mass spectrometer equipped with an ESI source (San Jose, CA, USA) interfaced with Xcalibur 1.4. The analytical LC columns used were from Phenomenex (Torrance, CA, USA). Kinetex PFP column (2.6 μm, 50 mm × 2.1 mm) and Kinetex C18 column (2.6 μm, 50 mm × 2.1 mm) were used for LC-QqQ-MS/MS. Kinetex PFP column (2.6 μm, 150 mm × 2.1 mm) was used for LC-IT-MS/MS. Alkaline hydrolysis was performed on a Zodiac CX-1110 personal organic synthesizer from Eyela (Tokyo, Japan). Distillation chiller B-741 from Bunchi (Flawil, Switzerland) was used with the personal organic synthesizer. Nitrogen evaporator N-EVAP 112 from Organomation Associates, Inc. (Berlin, MA, USA) was used for sample pre-concentration. Refrigerated centrifuge 5417R from Eppendorf (Hamburg, Germany), hotplate stirrer HC1202 from Bibby Sterilin (Staffordshire, UK), multi-pulse vortex mixer from Glascol (Terre Haute, IN, USA), and 0.45 μm PVDF syringe filter from Alltech (Deerfield, IL, USA) were used in sample preparation.

GC-EI-MS analysis was performed on a Hewlett Packard 6890 series GCMS system composed of a HP 5973 MSD, HP G2614A autosampler and Agilent 7683 series injector module from Agilent Technologies (Atlanta, GA, USA). GC-EI-MS confirmation of 1,4-BDA and 1,5-PDA was performed on a DB-5MS column (30 m × 0.25 mm, 0.25 μm film thickness) from Agilent J&W (Atlanta, GA, USA).

2.3. Preparation and characterization of NMPA-d_5

NMPA-d_5 was synthesized by using the synthetic route proposed by Häkkinen et al. [20]. To an ice-cooled suspension of $LiAlD_4$ (12.5 mL of 1 M solution in diethyl ether, 12.5 mmol), a solution of N-methyl-d_3-3-aminopropionitrile (0.270 g, 3.10 mmol in dried diethyl ether) was added dropwise with stirring over a period of 45 min. The reaction mixture was allowed to warm to room temperature and then heated to reflux for 3 h followed by stirring at room temperature for additional 20 h. The reaction mixture was quenched by dropwise addition of water (1 mL) and 5 M NaOH (5 mL) with stirring at 0–4 °C. The organic phase was collected and the residues were extracted with 2 × 20 mL diethyl ether and 2 × 20 mL EtOAc. The combined organic extracts were evaporated to ca. 1 mL. 2 mL of conc. HCl was added and the solution was evaporated to completely dryness. The residue was further dried in vacuum oven for 24 h. The crude product was washed with EtOAc, dried and further purified by recrystallization from H_2O–ethanol yielded 0.1035 g of white powder (Fig. 3). Yield = 20.2%. Molecular formula: $C_4H_9Cl_2D_5N_2$. Molecular weight = 166.10. ^1H NMR (D_2O): δ 3.15–3.11 (2H, m, H-α), 2.09–2.05 (2H, t, J = 8 Hz, H-β); ^{13}C NMR (D_2O): δ 45.8 (C-α, s), 36.25 (C-γ, m), 23.4 (C-β, s). Positive ion ESI HRTOFMS: Calcd. for $[M+H]^+$ $C_4H_8D_5N_2$ 94.1387, found 94.1386. Isotope distribution: 0% d_0–d_3, 2% d_4, 98% d_5. Negative ion ESI LRMS/MS: Molecular formula (as NMPA-d_5-PFPA derivative): $C_{10}H_5O_2N_2D_5F_{10}$. Calcd. for $[M-H]^-$ $C_{10}H_4O_2N_2D_5F_{10}$ 384.1, found 384.1. MS/MS: 384.1 > 119.0, $[C_2F_5]^-$. 384.1 > 179.0, $[C_2F_5CON(CD_3)]^-$.

2.4. Sample preparation

To 1 g of blended tissue, I.S. was fortified at 100 μg/kg. The sample was equilibrated at room temperature for at least 30 min before hydrolysis. After addition of 10 mL of 4 M $KOH_{(aq)}$, the sample was heated at 105 °C for 2 h. The hydrolysate was cooled to room temperature before transferring into a 50 mL-centrifuge tube containing 7.5 ± 0.5 g of KOH pellets. The reaction mixture was mixed vigorously in a vortex mixer to dissolve the pellets and cooled in an ice bath immediately. It was then extracted with 10 mL of diethyl ether. After centrifugation at 2000 rpm for 5 min, the upper diethyl ether layer was transferred to a 50 mL centrifuge tube containing 0.5 mL of 1 M HCl. The reaction mixture was extracted with additional 10 mL of diethyl ether. After centrifugation the upper diethyl ether layer was transferred and combined in the 50 mL centrifuge tube. The solution was evaporated to ≤0.5 mL at room temperature with N_2. 1 mL n-hexane was added and shaked vigorously for 30 s. After centrifugation at 4000 rpm for 5 min, the upper hexane layer was discarded and the lower aqueous layer was evaporated to dryness at 40 °C with N_2. The residue was redissolved with 1 mL of dry EtOAc before derivatization.

2.5. Derivatization procedure

75 μL of perfluorocarboxylic acid anhydride (TFAA, PFPA or HFPA) was added to the sample extract. The sample was capped immediately and mixed by shaking vigorously on a vortex mixer for 1 min. The mixture was allowed to react at room temperature for 30 min. Then 1 mL of EtOAc was added. The reaction was quenched by washing with 2 mL of 1 M K_2HPO_4 (pH 7.4) followed by 1 mL of distilled water. The lower aqueous layer was discarded and small amount of anhydrous Na_2SO_4 was added. The solution was mixed vigorously and allowed to stand for 15 min. 1 mL of the EtOAc layer was transferred to another 15 mL centrifuge tube containing 1 mL acetonitrile. The solution was evaporated to ≤0.5 mL by N_2 stream at 40 °C. The residue remained was redissolved with acetonitrile to 1 mL. The sample was then analyzed with LC-QqQ-MS/MS and LC-IT-MS/MS.

2.6. LC–MS/MS conditions

2.6.1. LC-QqQ-MS/MS

LC conditions: Gradient elution was used in the analysis using mobile phase A – acetonitrile and mobile phase B – water with a flow rate of 300 μL/min. Analysis was performed with the following elution program: 20% A for 0 min, increasing to 90% A between 0 and 8 min. From 8 to 8.1 min, decreasing to 20% A and kept constant till 11 min. The injection volume was set to 5 μL. MS/MS conditions: Negative ion ESI; probe depth position = C; probe lateral position = 0; probe front-to-back position = 1; spray voltage = 3.0 kV; sheath gas pressure = 35 (arbitrary units); aux gas (nitrogen) pressure = 15 (arbitrary units); capillary heater temperature = 350 °C; collision gas (argon) pressure = 1.5 mTorr; scan widths = 1.0 m/z; scan times = 0.1 s; peak widths (FWHM) for Q1 quadrupole = 0.4 m/z. The MS/MS parameters were tuned for optimum sensitivity using the Thermo TSQ Tune Master (version 2.1.0). Instrument control and data acquisition were performed with the Xcalibur software (rev. 2.1). Data acquisition was performed in the selected reaction monitoring (SRM) mode.

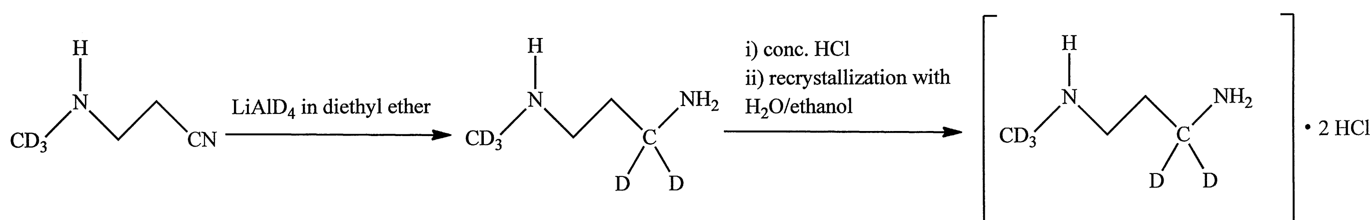

Fig. 3. Synthesis of N-methyl-d_3-3,3′-d_2-propane-1,3-diamine (NMPA-d_5).

2.6.2. LC-IT-MS/MS

While the number of transitions which can be monitored at the same time on IT-MS/MS is lower, a longer LC column and a larger range of eluent strength are used. LC conditions: Gradient elution was performed using mobile phase A – acetonitrile and mobile B – water with a flow rate of 200 μL/min. Analysis was started with the following elution program: 20% A for 2 min, increasing to 75% A between 2 and 32 min. From 32 to 33 min, decreasing to 20% A and kept constant till 40 min. The injection volume was set to 5 μL. MS/MS conditions: Negative ion ESI; probe depth position = C; probe lateral position = 0; probe front-to-back position = 0.59; spray voltage = 4.0 kV; sheath gas pressure = 40 (arbitrary units); aux gas (nitrogen) pressure = 20 (arbitrary units); capillary heater temperature = 300 °C; capillary voltage = 39 V; collision gas (helium) pressure = 1.2 mTorr; isolation width = 3.0 *m*/*z*; activation *Q* = 0.250; activation time = 30 ms. The MS/MS conditions were tuned for optimum sensitivity using the LCQ Tune (rev. 1.4). Instrument control and data acquisition were performed with the Xcalibur software (rev. 1.4). Data acquisition was performed in the MS/MS mode.

2.7. GC-EI-MS

GC conditions: Temperature programming was used. The following temperature program was employed: Initial 90 °C hold for 1 min; 15 °C/min to 200 °C; 25 °C/min to 300 °C and hold for 5 min. The injector temperature was 250 °C and all injections were made in the splitless mode. Column flow was held constant at 1 mL/min throughout the run. Ionization of the analyte was performed in EI mode at 70 eV. The ion source and analyser temperatures were held constant at 230 °C and 150 °C, respectively. The transfer line temperature was held at 280 °C. The mass selective detector was operated in full scan mode with scan range from 50 to 550 *m*/*z*. Injection volume was 2 μL.

2.8. Method validation

In this study, LC with tandem-in-space mass spectrometer (QqQ-MS/MS) and tandem-in-time mass spectrometer (IT-MS/MS) were employed and compared. In addition, isotopically labeled I.S. and analogous I.S. were used and compared. The method was validated and evaluated in terms of the following parameters: conversion of parent drugs to NMPA, hydrolysis conditions, matrix effect, linearity, limit of detection (LOD) and limit of quantification (LOQ), selectivity, recovery and repeatability.

2.8.1. Conversion of parent drugs to NMPA and hydrolysis conditions

Conversion of MOTL and PYTL to NMPA by alkaline hydrolysis was evaluated by fortifying the parent drugs (MOTL and PYTL) at 100 μg/kg NMPA equivalent into bovine muscle prior to alkaline hydrolysis. After reflux at 105 °C for 16 h, the sample was extracted, derivatized with PFPA and analyzed by negative ion ESI LC-QqQ-MS/MS. The conversion to NMPA under the hydrolysis conditions described above was calculated. In addition, hydrolysis conditions with critical effects on the extent of conversion such as time and temperature were also evaluated.

2.8.2. Matrix effect (ME)

ME is common in LC-ESI-MS/MS due to competition of charge carriers between analytes and co-eluting compounds during the ionization process. We adopted the procedure described by Matuszewski et al. to evaluate the ME [21]. Two sets of solutions were prepared and analyzed. Solutions set A were neat standard solution prepared in acetonitrile while solutions set B were extracts of 5 different blank bovine muscle samples fortified at the same level (after the extraction). The ME can then be calculated according to Eq. (1):

$$\mathrm{ME}\,(\%) = \frac{B}{A} \times 100 \quad (1)$$

where

A = average response from solutions set A (standard solution prepared in acetonitrile);
B = average response from solutions set B (sample extract spiked after extraction).

ME of NMPA, NMPA-d_5 and NEPA were studied and evaluated in pure solvent (acetonitrile) and matrix solution (bovine muscle extract) by LC-QqQ-MS/MS and LC-IT-MS/MS.

2.8.3. Linearity

The linearity of NMPA was evaluated from 10 to 1000 μg/L (7 calibration points). Each calibration level was fortified with mixed I.S. containing NMPA-d_5 and NEPA at 50 μg/L. The standard solutions were then derivatized and redissolved in acetonitrile and bovine muscle matrix solution. The calibration solutions were then analyzed by LC-QqQ-MS/MS and LC-IT-MS/MS.

In addition to single level evaluation of ME, comparison of the difference of the slopes of multi-level solvent-based (acetonitrile) and matrix-matched calibration curves was another way to evaluate the ME. Calibration curves were constructed with NMPA-d_5 and NEPA as I.S. for comparison. The slopes obtained for solvent-based calibration curve and matrix-matched calibration curve were compared statistically with student's *t*-test (Eqs. (2) and (3)) [22,23]:

$$t_{(n-4)} = \frac{b_1 - b_2}{s_{b_1-b_2}} \quad (2)$$

$$s_{b1-b_2} = \left(s_{b1}^2 + s_{b2}^2\right)^{1/2} \quad (3)$$

where b_1 and b_2 are the slopes of solvent-based calibration and matrix-matched calibration curves, respectively. s_{b1-b2} = standard error of the difference between the two slopes and s_{b1} and s_{b2} are the standard errors of the slopes of solvent-based and matrix-matched calibration curves, respectively. $t_{(n-4)}$ = student's *t* value on degree of freedom (*df*) = (n−4) and n = total number of determinations.

When the tabulated t-value is larger than the critical t-value ($n-4$), there is significant difference between two slopes and significant ME is suspected.

2.8.4. LOD and LOQ

LOD and LOQ were calculated according to Eqs. (4) and (5), respectively:

$$LOD = \frac{3.3\sigma}{S} \quad (4)$$

$$LOQ = \frac{10\sigma}{S} \quad (5)$$

where σ is the standard deviation of the responses of at least seven blank muscle samples in respective matrix and S is the slope of the calibration curve [24].

2.8.5. Selectivity

Selectivity was evaluated by analyzing different blank bovine muscle samples and bovine muscle samples fortified with various structurally related diamines such as 1,3-PDA, 1,4-BDA, 1,5-PDA, NEPA and NIPA at 500 μg/kg each.

2.8.6. Recovery

Recovery of the method was evaluated by fortifying NMPA and the parent drugs into bovine muscle. Recovery of NMPA in bovine muscle was determined experimentally using fortified blank matrix at three levels ($N \geq 7$ for each level): 0.5 × MRL (50 μg/kg), MRL (100 μg/kg) and 2 × MRL (200 μg/kg). Similarly, bovine muscles fortified with MOTL or PYTL at MRL ($N \geq 7$) were prepared. The samples were then hydrolyzed and analyzed using the procedures described above. The amount of NMPA was calculated against calibration curve prepared in acetonitrile using internal standard calibration. Recovery of NMPA calculated according to Eq. (6):

$$\text{Recovery}\,(\%) = \frac{C_x - C_y}{C_f} \times 100 \quad (6)$$

%Recovery of MOTL or PYTL (expressed in term of NMPA) was calculated according to Eq. (7):

$$\text{Recovery}\,(\%, \text{as NMPA}) = \frac{C_x - C_y}{C_z \times f} \times 100\% \quad (7)$$

where C_x = measured analyte concentration by internal standard calibration, C_y = analyte concentration in sample, C_f = fortified NMPA concentration, C_z = fortified MOTL or PYTL concentration and f = conversion factor (0.43 for PYTL and 0.40 for MOTL).

2.8.7. Repeatability

Fortified blank bovine muscle samples at three levels ($N \geq 7$ for each level): 0.5 × MRL (50 μg/kg), MRL (100 μg/kg) and 2 × MRL (200 μg/kg) were prepared. The samples were analyzed by different instruments with same operator. The relative standard deviation (RSD) was calculated.

3. Results and discussion

3.1. Method development

3.1.1. Sample preparation

Analytical methods reported by Lynch et al. for the determination of MOTL and PYTL residues as NMPA involved basic hydrolysis, extraction of the NMPA into toluene, clean-up by back extraction, derivatization with 4-fluoro-3-nitrobenzotrifluoride and further clean-up of the derivative by thin layer chromatography (TLC) prior to analysis by either GC-FID, GC-ECD or GC-EI-MS [8–10]. We have evaluated the extraction efficiency of NMPA from the hydrolysate using different solvents like toluene, dichloromethane and diethyl ether. Both toluene and diethyl ether showed similar extraction efficiency (absolute recovery >50%) while DCM could only recover <10% of NMPA. Since apparent recovery was used throughout the study, diethyl ether was used as the extraction solvent to facilitate faster evaporation during the pre-concentration step. By replacing 4-fluoro-3-nitrobenzotrifluoride with PFPA as the derivatization reagent, the derivatization conditions could be reduced from 16 h at 55 °C to 30 min at room temperature.

3.1.2. Selection of instrumentation

Perfluorocarboxylic acid anhydrides such as PFPA and HFBA are commonly employed as derivatization reagents for the analysis of amines with GC-ECD and GC-EI-MS or GC-NCI-MS [25–29]. Apart from GC analysis, perfluorocarboxylic acid anhydride derivative of amine can also be analyzed by LC-UV [13,14] and LC–MS [15,16]. In our method development, we found that selective and sensitive detection of NMPA-PFPA derivative could be achieved by negative ion ESI LC-MS/MS. Similar approach has been reported for the analysis of amines in biogenic samples as pentafluoropropionic acid anhydride derivatives by LC–MS/MS [17]. With the use of more sophisticated and selective instrument like LC–MS/MS, sensitive and selective analysis of NMPA could be achieved without prior clean-up of the derivative.

3.1.3. Selection of derivatization reagents and their chromatographic behaviors

To explore the possibility of using other perfluorocarboxylic acid anhydrides as the derivatization reagent, TFAA and HFPA were also tested and evaluated by LC-QqQ-MS/MS. Chromatographic behavior of TFAA, PFPA and HFPA derivatives of NMPA, NEPA and NIPA on C18 column was evaluated (Fig. 4). All derivatives were well separated on a C18 column. PFPA was selected as the derivatization reagent throughout the study because well separation of the PFPA derivatives was obtained on the C18 column within a reasonable analytical run cycle.

3.1.4. Evaluation of interfering peaks from matrix

1,4-BDA (also known as putrescine) and 1,5-PDA (also known as cadaverine) were found in blank bovine muscle samples before alkaline hydrolysis (Fig. 5). Putrescine and cadaverine have been suggested as an index of acceptability in fresh meat because their concentrations increased prior to spoilage and correlated well with the microbial load [30,31]. Previous studies showed that putresine and cadaverine could be found in fresh and cooked pork and beef meat [31,32]. The level of putrescine and cadaverine increased slightly after alkaline hydrolysis. It was possible that they were formed by decarboxylation of the free amino acids at refluxing conditions [33]. The presence of these aliphatic diamines was confirmed by comparing the ion ratios of two SRM and the relative retention time (RRT) in LC-QqQ-MS/MS with respect to the authentic standards according to requirements stipulated in EU directive 2002/657 EC [34] (Table 2).

In addition, the presence of these two diamines was further confirmed by GC-EI-MS analysis of their PFPA derivatives in full scan mode. The relative abundances of four selected intense fragment ions and retention time (RT) were compared with respect to the authentic standards according to requirements stipulated in EU directive 2002/657 EC (Table 2).

3.1.5. Chromatographic separations

Standard mixtures prepared in acetonitrile and bovine muscle extract containing 1,3-PDA, 1,4-BDA, 1,5-PDA, NEPA and NIPA, NMPA and NMPA-d_5 were analyzed on a Kinetex C18 column (2.6 μm, 50 mm × 2.1 mm) by LC-QqQ-MS/MS. The interfering peak 1,5-PDA was eluted closely to NMPA. The resolutions (R_s) of the

Table 2
Confirmation of putrescine and cadaverine in hydrolyzed bovine muscle extract by LC-QqQ-MS/MS and GC-EI-MS according to EU directive 2002/657 EC.

Analyte[a]	Sample name	Technique							
		LC-QqQ-MS/MS				GC-EI-MS			
		SRM and relative intensity (% of base peak)[d]	% Deviation	RRT[e]	% Deviation	Ions and relative intensity (% of base peak)[f]	% Deviation	RT	% Deviation
1,4-BDA (Putrescine)	Standard in ACN	379 > 259[b], 379 > 119[c] (20.6)[d]	–	0.895	–	176(100)	–	6.56	–
						119(51.2)	–		
						217(33.6)	–		
						261(7.5)	–		
	Blank bovine muscle	379 > 259[b], 379 > 119[c] (19.6)[d]	−4.9	0.894	−0.03	176(100)	–	6.53	0.46
						119(48.4)	5.62		
						217(34.6)	−2.96		
						261(7.9)	−5.56		
	Bovine muscle matrix-matched standard	379 > 259[b], 379 > 119[c] (18.1)[d]	−12	0.898	0.32	–	–	–	–
1,5-PDA (Cadaverine)	Standard in ACN	393 > 273[b], 393 > 119[c] (38.0)[d]	–	0.963	–	176(100)	–	7.30	–
						119(55.7)	–		
						100(50.5)	–		
						218(19.9)	–		
	Blank bovine muscle	393 > 273[b], 393 > 119[c] (37.2)[d]	−2.1	0.962	−0.01	176(100)	–	7.29	0.14
						119(56.8)	−1.94		
						100(49.7)	1.52		
						218(20.2)	−1.35		
	Bovine muscle matrix-matched standard	393 > 273[b], 393 > 119[c] (41.4)[d]	8.9	0.966	0.32	–	–	–	–

[a] Analyte was determined as PFPA derivatives.
[b] SRM 1 (for quantitation).
[c] SRM 2 (for qualification).
[d] Numbers in parentheses are relative intensities to SRM 1 (base peak).
[e] RRT was calculated as the ratio of the retention time of the analyte divided by the retention time of NMPA-d_5.
[f] Numbers in parentheses are relative intensities to m/z 176 (base peak).

Table 3
Equations, square of coefficients of determination (r^2), calculated t-values of solvent-based and matrix-matched calibration curves and ME.

Technique	LC-QqQ-MS/MS									LC-IT-MS/MS								
	NMPA (NMPA-d_5 as I.S.)			NMPA (NEPA as I.S.)			ME (%)[c]			NMPA (NMPA-d_5 as I.S.)			NMPA (NEPA as I.S.)			ME (%)[c]		
Matrix	Equation[a]	r^2	t-value[b]	Equation[a]	r^2	t-value[b]	NMPA	NMPA-d_5	NEPA	Equation[a]	r^2	t-value[b]	Equation[a]	r^2	t-value[b]	NMPA	NMPA-d_5	NEPA
Acetonitrile	$y = 0.0160x - 0.0199$	0.999	–	$y = 0.0178x + 0.0150$	0.978	–	–	–	–	$y = 0.02087x - 0.0323$	0.995	–	$y = 0.01612x - 0.2798$	0.934	–	–	–	–
Bovine muscle	$y = 0.0160x - 0.0361$	0.997	0.12	$y = 0.0179x + 0.0131$	0.967	0.11	117(13)	120 (13)	98 (9.6)	$y = 0.02190x - 0.0634$	0.986	1.43	$y = 0.0200x - 0.3648$	0.924	2.58	115(5.9)	122 (11.5)	89 (8.5)

[a] Linear regression equations of the calibration curves were expressed in the form of $y = mx + c$ (weighing factor = $1/x$ for QqQ-MS/MS and IT-MS/MS), where y = area of analyte divided by the area of the internal standard, x = concentration of analyte, m = slope of the regression line, c = intercept of the regression line, and r^2 = square of coefficient of determination.
[b] t-tests were performed by comparing the slopes of the calibration curves acquired within the same day. Critical t-value = 2.11, α = 0.05 with degrees of freedom.
[c] For ME, no. of determination = 5 in all cases. Numbers in the parentheses were the %RSD.

Table 4a
Recovery and repeatability of MOTL and PYTL under various hydrolysis conditions by LC-QqQ-MS/MS.

Hydrolysis conditions		Fortified analyte	
Time (h)	Temperature (°C)	MOTL % Recovery (as NMPA)	PYTL % Recovery (as NMPA)
2	105	91.2 (10.9)	92.7 (2.3)
4	105	92.5 (5.5)	97.4 (0.9)
16	105	91.0 (1.8)	101.4 (4.2)
0	Room temp	0.5 (22.2)	0.4 (28.6)
2	Room temp	1.4 (28.6)	2.8 (3.6)
16	Room temp	4.9 (16.3)	8.8 (0)

Remarks: Numbers in parentheses were the relative percentage deviations (%RPD).

closely eluting peaks 1,5-PDA and NMPA in acetonitrile and bovine muscle extract on a Kinetex C18 column were between 0.22 and 0.27. To achieve better separation, we looked for LC column with different stationary phase. Fluorinated phenyl stationary phases generally offer alterative selectivity over traditional alkyl phases. In addition to the dispersive interactions found in the commonly used C18 and C8 column, dipole interactions, π–π interactions, charge transfer and ion-exchange interactions contributed the unique selectivity of perfluorophenyl columns [35–37]. Pentafluorophenyl (PFP) column is suitable for the separation of difficult-to-resolve mixtures of halogenated compounds and non-halogenated compounds containing polar functional groups. As all PFPA derivatives in the study contained two perfluoro alkyl group and two polar amide groups, PFP column may show better selectivity or resolution for these compounds. The R_s of the closely eluting peaks 1,5-PDA and NMPA on a Kinetex PFP column (2.6 μm, 50 mm × 2.1 mm) were between 0.37 and 0.44. With similar particle size and column dimension, the chromatographic separation of the closely eluting peaks 1,5-PDA and NMPA in terms of R_s on the Kinetex PFP column is nearly double to that on the Kinetex C18 column. Therefore, Kinetex PFP column was used throughout this study. Chromatograms of blank bovine muscle and bovine muscle fortified with mixed diamines on Kinetex PFP column were shown in Fig. 5.

3.1.6. ME: isotopically labeled I.S. vs. structural analogous I.S.

ME of NMPA was evaluated and compared with that of NMPA-d_5 (an isotopically labeled analog) and NEPA (a structurally related analog). Mean ME was calculated (n = 5) and compared statistically using student's t-test (Table 3). ME > 100% indicates matrix enhancement while ME < 100% means matrix suppression. Matrix enhancement was observed for NMPA and NMPA-d_5 whereas matrix suppression was observed for NEPA acquired on both QqQ-MS/MS spectrometer and IT-MS/MS spectrometer. To evaluate whether NMPA-d_5 or NEPA was more suitable as internal standard for the quantitative analysis of NMPA in bovine muscle, the mean values of ME for NMPA-d_5 and NEPA were compared statistically with that of NMPA by student's t-test ($t_{critical}$ = 2.31 at confidence level (CL) = 95%, degree of freedom = 8). When $t_{exp} < t_{critical}$ or $p > 0.05$, there is no significant difference at CL = 95% and vice versa.

The mean ME of NMPA and NMPA-d_5 were not statistically different in bovine muscle on QqQ-MS/MS and IT-MS/MS at CL = 95% (p = 0.733 and 0.289, respectively). This indicated that NMPA-d_5 was a suitable internal standard for NMPA analysis since the matrix effect could be adequately compensated. On the other hand, the mean ME of NMPA and NEPA were statistically different at CL = 95% in bovine muscle on both instruments (p = 0.0251 and 0.0008, respectively) which implied that they were suffered from different degree of matrix enhancement/suppression on both type of mass spectrometers. Apparently, NEPA was not an appropriate internal

Table 4b
Recovery and repeatability of NMPA, MOTL and PYTL in bovine muscles by LC-QqQ-MS/MS and LC-IT-MS/MS.

Fortified analyte	Spike level (μg/kg)	LC-QqQ-MS/MS			LC-IT-MS/MS		
		Mean recovery (%)	RSD (%)	No. of determination (n)	Mean recovery (%)	RSD (%)	No. of determination (n)
NMPA	50 (0.5 MRL)	90.8	5.5	9	93.6	10.4	8
	100 (MRL)	98.8	6.1	9	96.5	6.7	7
	200 (2 MRL)	96.8	6.9	10	96.0	9.6	11
MOTL[a]	100 (MRL)	96.8	8.3	12	89.7	6.5	11
PYTL[a]	100 (MRL)	92.6	8.8	8	90.2	6.4	8

[a] Remark: MOTL and PYTL were fortified at 100 μg/kg equivalent of NMPA.

Table 5a
SRM and product ions assignment for PFPA derivatives of selected N-alkyl-1,3-propanediamines [R′(R)NCH$_2$CH$_2$C(R″)$_2$N(H)R′] by QqQ-MS/MS and IT-MS/MS.

Analyte	R	R′	R″	Precursor ion (m/z)	Product ion 1 (m/z)	Assignment	Product ion 2 (m/z)	Assignment	Relative intensity (product ion 1/product ion 2)	
									QqQ-MS/MS	IT-MS/MS
NMPA-PFPA	CH_3	C_2F_5CO	H	379	176	$[C_2F_5CON(CH_3)]^-$	119	$[C_2F_5]^-$	5.1	54.8
NMPA-d$_5$-PFPA	CD_3	C_2F_5CO	D	384	179	$[C_2F_5CON(CD_3)]^-$	119	$[C_2F_5]^-$	5.2	62.9
NEPA-PFPA	C_2H_5	C_2F_5CO	H	393	190	$[C_2F_5CON(C_2H_5)]^-$	119	$[C_2F_5]^-$	2.3	74.7
NIPA-PFPA	$CH(CH_3)_2$	C_2F_5CO	H	407	204	$[C_2F_5CON(CH(CH_3)_2)]^-$	119	$[C_2F_5]^-$	8.2	91.7

standard for NMPA analysis. Therefore, NMPA-d$_5$ was used as the internal standard throughout the study unless otherwise stated.

3.2. Conversion of parent drugs to NMPA and hydrolysis conditions

The extent of conversion of MOTL and PYTL to NMPA under different hydrolysis conditions (temperature and time) was investigated. The results were summarized in Table 4a. MOTL and PYTL were quantitatively converted to NMPA at refluxing conditions for 16–18 h according to previous report [9,10]. Hydrolysis time could be reduced to 2 h without significant effect on the extent of conversion. The effect of heating on the hydrolysis was also evaluated. At ambient temperature for up to 16 h, less than 10% of NMPA was found when the parent drugs was kept in 4 M KOH$_{(aq)}$. Quantitative conversion to NMPA was achieved by performing alkaline hydrolysis at 105 °C for at least 2 h.

3.3. Comparison of mass spectra acquired on QqQ-MS/MS and IT-MS/MS

With either QqQ-MS/MS or IT-MS/MS operated in negative ion ESI mode, intense signal of deprotonated molecular ions $[M-H]^-$ were observed at m/z 379 and 384 for NMPA-PFPA and NMPA-d$_5$-PFPA, respectively. Two product ions were observed for NMPA-PFPA and NMPA-d$_5$-PFPA (Fig. 6) in their product ion scan spectra. In IT-MS/MS, in addition to the common product ion m/z 119, intense characteristic product ions m/z 176 and 179 corresponding to $[C_2F_5CON(CH_3)]^-$ and $[C_2F_5CON(CD_3)]^-$, respectively, were observed. The relative intensities of product ion 1 ($[C_2F_5CON(R)]^-$, R = alkyl) to product ion 2 ($[C_2F_5]^-$) for NMPA-PFPA and NMPA-d$_5$-PFPA were 54.8 and 62.9, respectively (Table 5a). On the contrary, m/z 119 was observed as the major and most intense fragment ion in QqQ-MS/MS. Characteristic product ions m/z 176 and m/z 179 were only observed in relatively low intensity. The relative intensities of product ion 1 to product ion 2 for NMPA-PFPA and NMPA-d$_5$-PFPA were 5.1 and 5.2, respectively (Table 5a). Proposed identity of the product ions for NMPA and NMPA-d$_5$ was illustrated in Fig. 6. While CID MS/MS spectra acquired on QqQ-MS/MS and IT-MS/MS are generally similar, there were cases that the spectra differed significantly [38–40].

QqQ mass spectrometer and IT mass spectrometer are both tandem mass spectrometers but operated in different principles. During the MS/MS process in QqQ mass spectrometer, the first step is the selection and isolation of the precursor ion in the first mass analyser (Q1). Collision-induced dissociation (CID) occurs in the collision cell (q2) which is filled with inert gas such as Ar or N_2. The product ions are then sorted according to their m/z ratio in the second mass analyser (Q3) and recorded by the detector. The ions undergo multiple collisions inside the q2 of QqQ mass spectrometer. Once the product ions are formed they are reactivated and undergo further fragmentation [41,42]. In IT mass spectrometer (like the LCQ Deca XP used in our study), helium is used as collision gas and the MS/MS is carried out successively inside the same physical space but separately in time [42,43]. Fragmentation within an IT instrument occurs solely by excitation of the precursor ion (usually corresponding to 1–4 m/z units or up to 20 m/z units when using wide band excitation mode). Once the precursor ion is fragmented, the result product ions no longer resonate with the MS/MS isolation window. The product ions will cool back down to the centre of the trap via collisions with helium buffer gas atoms (the so-called "ion cooling" process) [43]. Hence the product ions mostly will not undergo secondary or tertiary fragmentation (Fig. 7) [44].

Several PFPA derivatives of N-alkyl-1,3-propanediamines were prepared and investigated (Table 5a). The intensity of the product ion 1 was low in all cases by QqQ-MS/MS. On the contrary, intense signals of the product ion 1 were observed in IT-MS/MS (Table 5a).

Table 5b
Relative intensity of the SRM of NMPA-PFPA derivative (379 > 176/379 > 119) by QqQ-MS/MS under different collision gas pressures and collision energies.

Trial	Collision energy (V)	Collision gas pressure (mTorr)	Relative intensity (379 > 176/379 > 119)
1	17[a]	1.5[a]	5.1
2	12	1.5	4.6
3	7	1.5	3.7
4	17	1.3	5.0
5	12	1.3	4.6
6	7	1.3	3.9
7	17	1.1	4.8
8	12	1.1	4.4
9	7	1.1	4.0

[a] Optimum collision energy = 17 V and collision gas pressure = 1.5 mTorr were obtained by optimization using the Thermo TSQ Tune Master through pump infusion of 1 μg/mL standard solution of NMPA into the ESI source at 10 μL/min with 200 μL/min mobile phase.

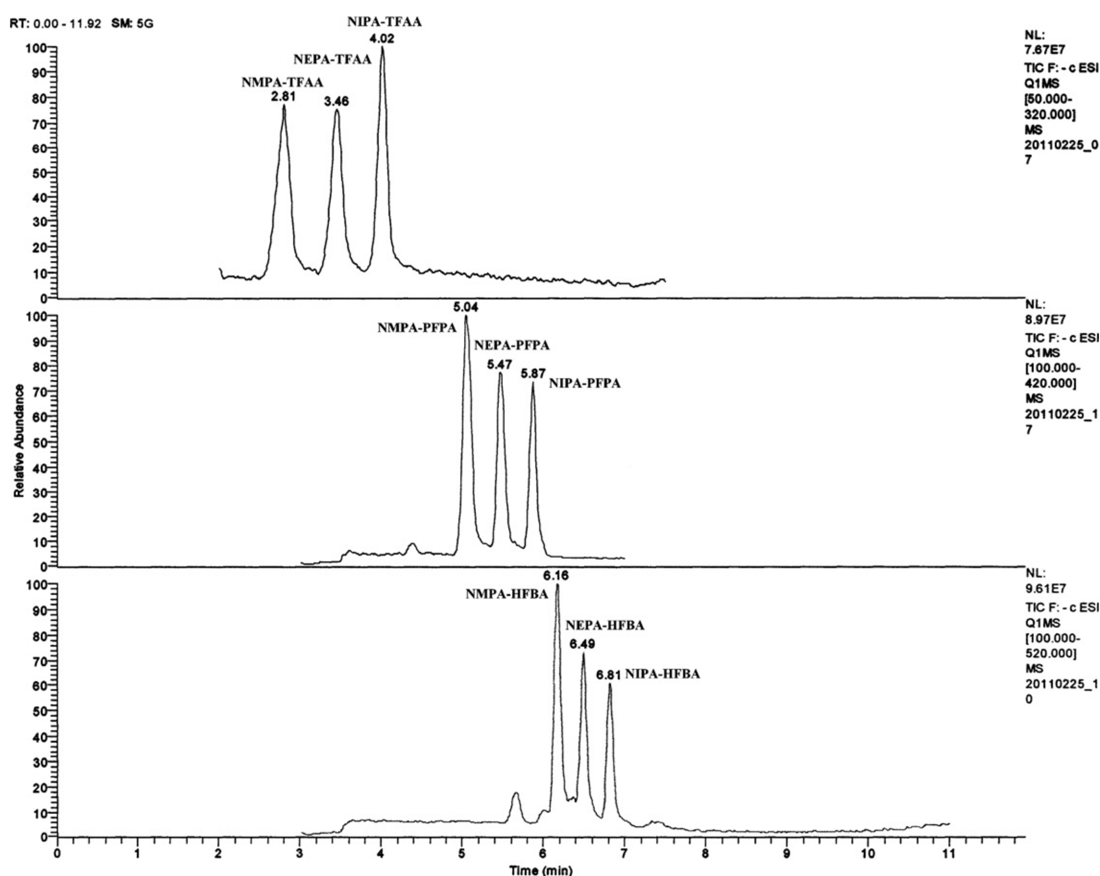

Fig. 4. Full scan TIC chromatogram of TFAA, PFPA and HFBA derivatives of NMPA, NEPA and NIPA by LC-QqQ-MS/MS.

Multiple fragmentations may occur in QqQ-MS/MS which resulted in further fragmentation of m/z 176 and m/z 179 to m/z 119. This postulate was further examined by isolating the product ion1 of NMPA-PFPA, NEPA-PFPA and NIPA-PFPA for MS^3 determination by IT mass spectrometer. Further fragmentation of product ion 1 produced m/z 119 as major ion (which was corresponding to the $[C_2F_5]^-$ fragment). In addition, attempts have been tried to optimize the intensity of product ion 1 in QqQ-MS/MS by manually adjusting the parameters such as collision gas pressure and collision energy. The results were shown in Table 5b. The intensity of product ion 1 was affected slightly by varying the collision gas pressure and/or collision energy. Further fragmentation of production ion 1 cannot be controlled by varying the collision gas pressure and/or collision energy. Similarly, the response ratio of product ion 1 to product ion 2 was not sensitive to the value of collision energy and activation time in IT-MS/MS. In general, the difference of the MS/MS spectra observed in our study between a QqQ-MS/MS and IT-MS/MS was attributed to factors such as: (i) different collision gases were used (Ar or N_2 for QqQ and He for IT) and (ii) differences in their CID fragmentation mechanisms.

3.4. Linearity

Linearity was accessed by at least triplicate injections of the calibration solutions. The slopes of acetonitrile-based and matrix-matched calibration curve were compared statistically with two different I.S. (i.e. NMPA-d_5 and NEPA) using student's t-tests (according to Eqs. (2) and (3)). The results were tabulated in Table 3. When the calculated t-value was larger than the critical t-value, difference between two slopes was considered to be significant and significant ME was suspected. With NMPA-d_5 as the I.S., the difference between two slopes was not statistically different which indicated no considerable ME or the ME was adequately compensated. The acetonitrile-based and matrix-matched calibration curves acquired on QqQ-MS/MS spectrometer were linear over the range of 0–1000 ng/mL with the $r^2 \geq 0.999$ and 0.997, respectively. With the same set of solutions acquired on IT-MS/MS spectrometer, the r^2 of solution and bovine muscle matrix-matched calibration curve over the same calibration range were 0.995 and 0.986, respectively. With NEPA as the I.S., the difference between two slopes was not statistically different on QqQ-MS/MS but statistically different on IT-MS/MS. In addition, with NEPA as the I.S., the r^2 of acetonitrile-based and matrix-matched calibration curves acquired on both mass spectrometers ranged from 0.924 to 0.978. This was in good agreement with the single-level ME evaluation described above that NEPA exhibited significantly different ionization suppression/enhancement during negative ion ESI LC-MS/MS analysis compared to NMPA. With NMPA-d_5 as the I.S., the linearity of matrix-matched calibration curve on QqQ-MS/MS ($r^2 = 0.997$) was better than that on IT-MS/MS ($r^2 = 0.986$).

3.5. LOD and LOQ

At least 9 blank bovine muscle samples were analyzed in three consecutive days to estimate the LOD and LOQ. The LOD and LOQ of LC-QqQ-MS/MS were 0.42 and 1.39 μg/kg, respectively. The LOD and LOQ of LC-IT-MS/MS (0.63 and 2.09 μg/kg, respectively) were higher than that of LC-QqQ-MS/MS. In general, the estimated LOQs of both methods were far below the required action level (i.e. MRL).

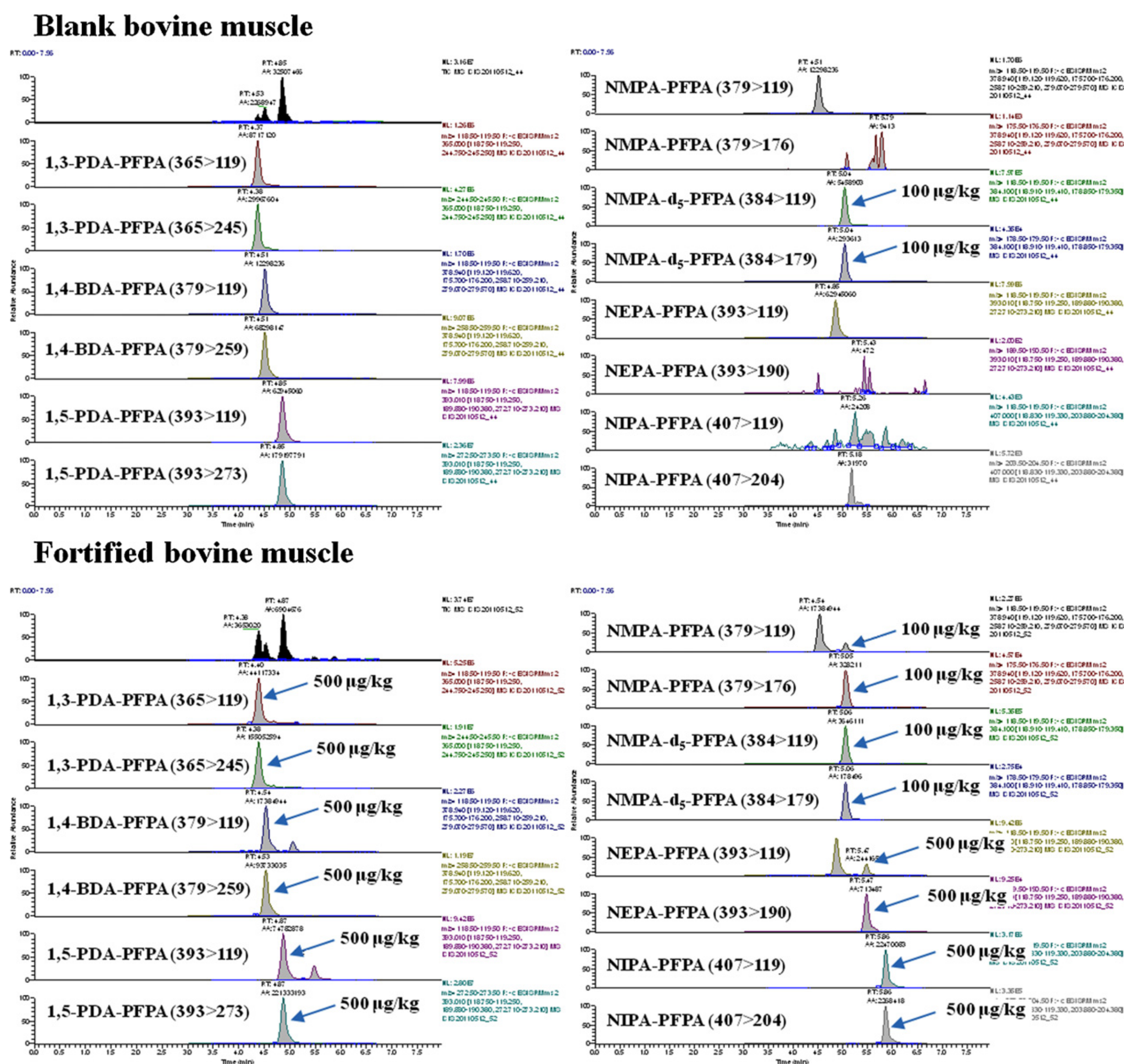

Fig. 5. LC-QqQ-MS/MS chromatogram of blank bovine muscle sample after PFPA deriviatization (upper). LC-QqQ-MS/MS chromatogram of bovine muscle fortified with various diamines after PFPA deriviatization (lower).

3.6. Selectivity

Selectivity studies were performed by analyzing blank bovine muscle samples and bovine muscle samples fortified with NMPA in the presence of various structurally related diamines including 1,3-PDA, 1,4-BDA, 1,5-PDA, NEPA and NIPA. The resulting sample extracts were derivatized and analyzed by LC-QqQ-MS/MS and LC-IT-MS/MS. No interference was observed in both methods.

3.7. Recovery and repeatability

Average recovery and repeatability were determined by analyzing fortified blank muscle samples at three different levels (0.5 MRL, MRL and 2 MRL). It is commonly believed that QqQ-MS/MS generally provided higher precision and better linearity and hence robustness compared to IT-MS/MS [38,39]. To compare the results from two different mass spectrometers, the samples were analyzed by LC-QqQ-MS/MS and LC-IT-MS/MS. The results were summarized in Table 4b. For LC-QqQ-MS/MS, the RSD at all spike levels was less than 7% with recovery between 91 and 97%. With LC-IT-MS/MS, the RSD at all spike levels was less than 11% with recovery between 94 and 97%. In addition to the recovery studies of the marker residue, the recovery of the parent drugs (MOTL and PYTL) in bovine muscles at 100 μg/kg NMPA equivalent was evaluated. MOTL and PYTL were quantitatively converted to NMPA in fortified bovine muscles through alkaline hydrolysis. With LC-QqQ-MS/MS, average recoveries of MOTL and PYTL in bovine muscles (reported as NMPA equivalent) were 96.8 and 92.6%, respectively. With IT-MS/MS, average recoveries of MOTL and PYTL in bovine muscles (reported as NMPA equivalent) were 89.7 and 90.2%, respectively. Repeatability of both methods was less than 10%.

4. Potential applications

4.1. Long-chain N-alkyl-1,3-propanediamines based anticorrosive and antifouling formulation

Long-chain N-alkyl-1,3-propanediamines can be used as anticorrosive and antifouling formulation for water-steam circuit of energy system in the power industry [29,45]. In order to understand

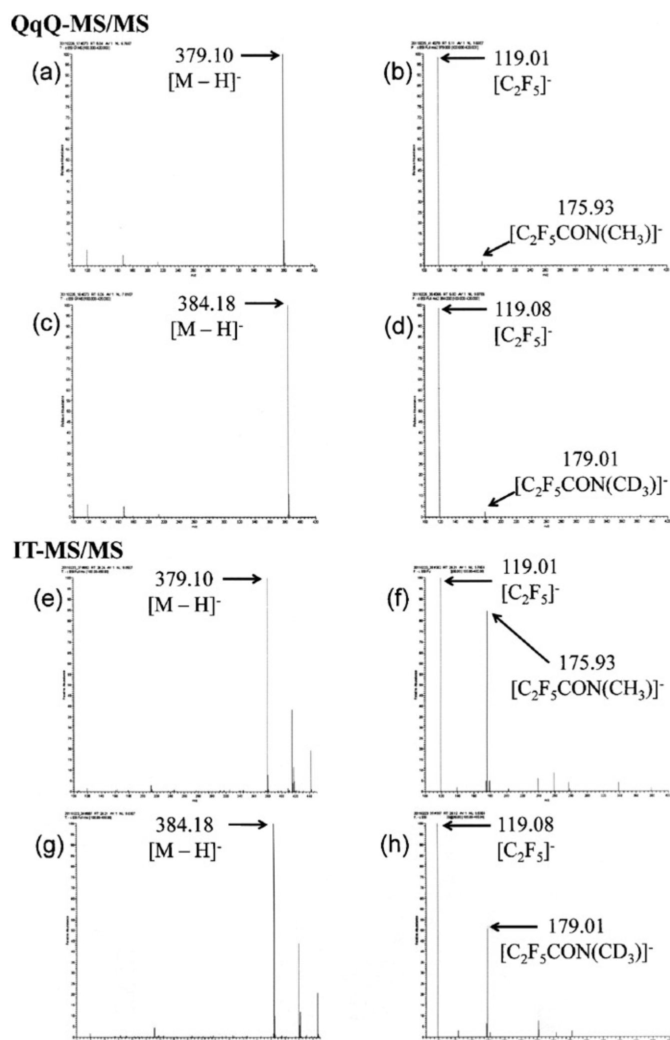

Fig. 6. Spectra (a)–(d) were collected on QqQ tandem mass spectrometer and spectra (e)–(h) were collected on IT mass spectrometer. (a) and (e) Negative ion ESI full scan spectra of NMPA-PFPA, (b) and (f) negative ion ESI MS/MS spectra of NMPA-PFPA, (c) and (g) negative ion ESI full scan spectra of NMPA-d_5-PFPA, (d) and (h) negative ion ESI MS/MS spectra of NMPA-d_5-PFPA.

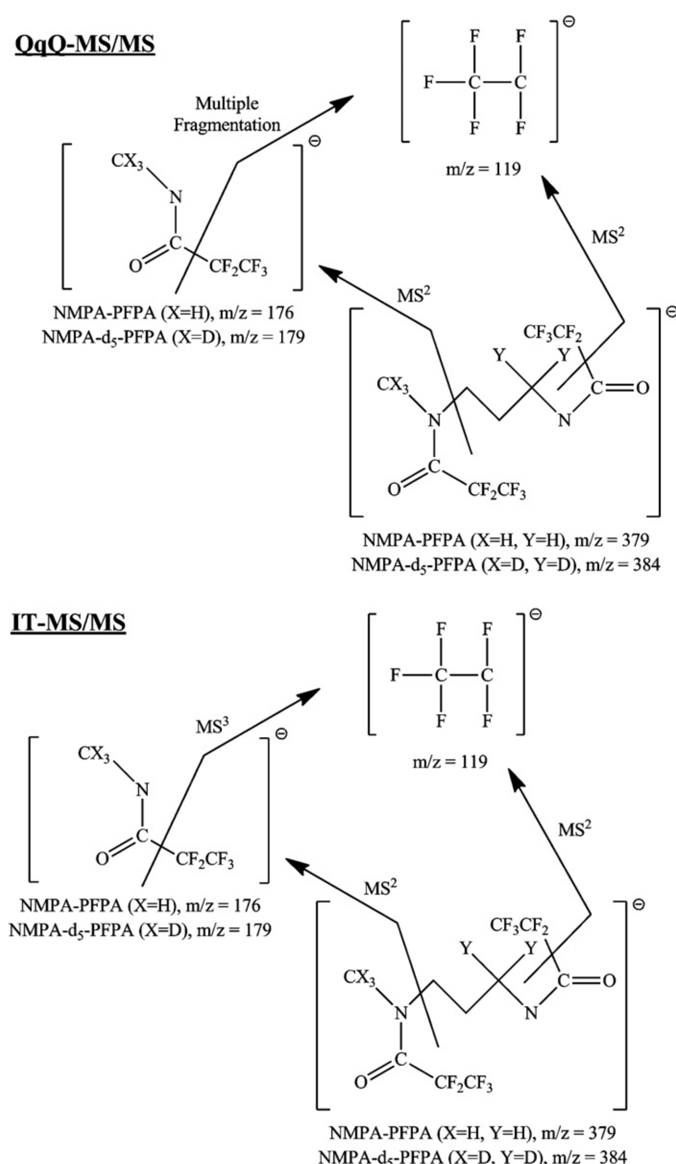

Fig. 7. Proposed fragmentation of NMPA-PFPA and NMPA-d_5-PFPA by LC-QqQ-MS/MS and LC-IT-MS/MS.

the processes during its technical use and its ecological and economical impact, there is a need to development sensitive and selective method to characterize these diamines in the formulation, water samples from the boiler water, superheated steam and condensate from the power plant. Analytical methods were reported to determine long-chain N-alkyl-1,3-propanediamines by GC-EI-MS or GC-NCI-MS after TFAA derivatization [29,45]. However, accurate quantitation of these compounds may encounter inlet discrimination by GC analysis. LC–MS/MS provides a good alternative for this type of analysis.

4.2. Platinum and N-alkyl-1,3-propanediamines based antitumor drugs

Antitumor drugs based on platinum complexes derived from N-alkyl-1,3-propanediamine ligands containing different carbon chain length were reported [46]. A correlation between the carbon chain length of the N-alkyl group and the cytotoxic activity was found. Analytical method for accurate determination and characterization of these ligands may be desirable. Our proposed LC–MS/MS method is not limited to NMPA analysis in food sample but may also applicable to analysis and characterization of different N-alkyl-1,3-propanediamines in various matrices.

5. Conclusions

Analysis of polar short-chain aliphatic amine in hydrolysed protein extract is a difficult issue since amino acids or peptides are formed as a result of protein hydrolysis which may impose interference on the detection. The most recent reported method for the analysis of NMPA in animal tissues involved derivatization at 55 °C for 16 h and required prior purification of the derivative on TLC before GC-ECD or GC-NCI-MS analysis. In addition, the analytical techniques associated with these methods do not provide enough identification information for confirmation according to the requirements stipulated in EU directive 2002/657 EC [34]. We reported herein a rapid, sensitive and selective method for the analysis of short-chain aliphatic amine NMPA in bovine muscle after alkaline hydrolysis utilizing PFPA derivatization and negative ion ESI LC-MS/MS detection. It is noteworthy that the newly developed method exhibited several advantages over the previous

methods: (i) simple and rapid (ii) high sensitivity and selectivity (iii) provide confirmative result. PFPA derivatization is simple and rapid and could be completed at room temperature within 30 min. The derivatized sample, without further purification by preparative chromatography, can be analyzed directly by LC–MS/MS. To put it another way, PFPA derivatization can be viewed as the attachment of ion pair reagents "covalently" onto the NMPA which resulted in reduced analytical background interference, enhanced sensitivity in negative ion ESI MS/MS analysis and improved retention on reversed phase LC. Two SRM were monitored in LC–MS/MS which contributed totally four identification points for confirmation purpose [34]. The isotopically labeled NMPA, NMPA-d_5, was synthesized as I.S. to monitor and compensate factors affecting the method precision such as hydrolysis, extraction, derivatization and matrix effect. Results also demonstrated the advantages of isotoptically labeled I.S. over analogous I.S. by LC–MS/MS. The performances of LC-QqQ-MS/MS and LC-IT-MS/MS are comparable even though the LOD, LOQ and linearity acquired on QqQ-MS/MS were slightly superior. The analytical method was well validated and the method performance was satisfactory. In conclusion, the reported LC–MS/MS method is useful for routine monitoring of MOTL- and PYTL-drug related residues (as NMPA) in food sample of animal-origin and could also be further extended to analysis and identification of different N-alkyl-1,3-propane diamines or N-alkyl diamines in other complex sample matrices.

Acknowledgements

The authors would like to acknowledge Ms. Shirley Sau-Ling Lai, Ms. Hoi-Sze Yeung, Ms. Janet Ting-Fong Lau, Mr. George Fai Wong for their technical assistance. Special thanks are given to Mr. Andy Cheok-Man Chan for his assistance in HRMS measurement. The authors would also like to express their deepest gratitude to Dr. Chau-Ming Lau, Government Chemist and Dr. Della Wai-Mei Sin, Assistant Government Chemist of the Government Laboratory of the HKSAR, for their continuous support. The contents of this paper do not necessarily reflect the views of the Government of the HKSAR, nor does mention of trade names or commercial products constitute endorsement or recommendations of use.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2012.02.060.

References

[1] 2377/90 EEC Regulation, Official Journal of the European Communities L224 (1990) 1.
[2] 21 CFR 556.425, Code of Federal Regulations – Title 21: Food and Drugs, 2005.
[3] 21 CFR 556.560, Code of Federal Regulations – Title 21: Food and Drugs, 2005.
[4] S.S.C. Tai, N. Cargile, C.J. Barnes, P. Kijak, J. Assoc. Off. Anal. Chem. 73 (1990) 883.
[5] J.K. Faulkner, S.K. Figdor, A.M. Monro, M. Schach von Wittenau, D.A. Stopher, B.A. Wood, J. Sci. Food Agric. 23 (1972) 79.
[6] S.K. Figdor, M. Schach von Wittenau, M.J. Lynch, J. Assoc. Off. Anal. Chem. 61 (1978) 1228.
[7] M.J. Lynch, F.R. Mosher, D.M. Burnett, T.J. Newby, J. Agric. Food Chem. 35 (1987) 351.
[8] M.J. Lynch, S.R. Bartolucci, J. Assoc. Off. Anal. Chem. 65 (1982) 640.
[9] M.J. Lynch, D.M. Burnett, F.R. Mosher, M.E. Dimmock, S.R. Bartolucci, J. Assoc. Off. Anal. Chem. 69 (1986) 646.
[10] M.J. Lynch, S.R. Bartolucci, J. Assoc. Off. Anal. Chem. 65 (1982) 227.
[11] P. McCormack, P.J. Worsfold, M. Gledhill, Anal. Chem. 75 (2003) 2647.
[12] M. Cherlet, S.D. Baere, P.D. Backer, J. Mass Spectrom. 42 (2007) 647.
[13] P. Brunmark, P. Persson, G. Skarping, J. Chromatogr. 579 (1992) 350.
[14] A. Tiljander, G. Skarping, J. Chromatogr. 511 (1990) 185.
[15] G. Skarping, M. Dalene, P. Brunmark, Chromatographia 39 (1994) 619.
[16] G. Skarping, M. Dalene, H. Tinnerberg, Analyst 119 (1994) 2051.
[17] Å. Marand, D. Karlsson, M. Dalene, G. Skarping, Analyst 129 (2004) 522.
[18] Å. Marand, D. Karlsson, M. Dalene, G. Skarping, Anal. Chim. Acta 510 (2004) 109.
[19] C.H. Lindh, M. Littorin, Å. Amilon, B.A.G. Jönsson, Rapid Commun. Mass Spectrom. 21 (2007) 536.
[20] M.R. Häkkinen, T.A. Keinänen, A.R. Khomutov, S. Auriola, J. Weisell, L. Alhonen, J. Jänne, J. Vepsäläinen, Tetrahedron 65 (2009) 547.
[21] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 75 (2003) 3019.
[22] S. Fraselle, V. Derop, J.M. Degroodt, J.V. Loco, Anal. Chim. Acta 586 (2007) 383.
[23] D.L. Massart, B.G.M. Vandeginste, L.M.C. Buydens, S. De Jong, P.J. Lewi, J. Smeyers-Verbeke, Handbook of Chemometrics and Qualimetrics: Part A, Elsevier, 1997.
[24] U.S. Food and Drug Administration, Validation of Analytical Procedures: Methodology, Guidance for Industry No. 64, vol. 4, 1998, p. 1.
[25] B.A. Davis, D.A. Durden, Biomed. Mass Spectrom. 14 (1987) 197.
[26] M. Longo, A. Cavallaro, J. Chromatogr. A 753 (1996) 91.
[27] Official Methods of Analysis, 17th ed., AOAC International, Gaithersburg, MD, 2000 (Method 996.07).
[28] P.L. Rogers, W.F. Staruszkiewicz, R.A. Benner, J. AOAC Int. 86 (2003) 1172.
[29] P. Kusch, G. Knupp, M. Kozupa, M. Majchrzak, Chromatographia 70 (2009) 875.
[30] C. Ruiz-Capillas, F. Jiménez-Colmenero, Crit. Rev. Food Sci. Nutr. 44 (2004) 489.
[31] F. Galgano, F. Favati, M. Bonadio, V. Lorusso, P. Romano, Food Res. Int. 42 (2009) 1147.
[32] T. Hernádez-Jover, M. Izquierdo-Pulido, M. Teresa Veciana-Nogués, A. Mariné-Font, M. Carmen Vidal-Carou, J. Agric. Food Chem. 45 (1997) 2098.
[33] M. Fountoulakis, H.W. Lahm, J. Chromatogr. A 826 (1998) 109.
[34] European Commission 2002/657/EC, Off. J. Eur. Commun. L221 (2002) 8.
[35] M. Reta, P.W. Carr, P.C. Sadek, S.C. Rutan, Anal. Chem. 71 (1999) 3484.
[36] M.R. Euerby, A.P. McKeown, P. Peterson, J. Sep. Sci. 26 (2003) 295.
[37] J. Nichthauser, P. Stepnowski, J. Chromatogr. Sci. 47 (2009) 247.
[38] G. Bartolucci, G. Pieraccini, Fabio Villanelli, G. Moneti, A. Triolo, Rapid Commun. Mass Spectrom. 14 (2000) 967.
[39] C. Soler, J. Manes, Y. Picó, J. Chromatogr. A 1067 (2005) 115.
[40] A.A. Garrido Frenich, P. Plaza-Bolaños, J.L. Martínez Vidal, J. Chromatogr. A 1203 (2008) 229.
[41] J.V. Johnson, R.A. Yost, Anal. Chem. 62 (1990) 2162.
[42] G. Hopfgartner, in: K.T. Wanner, G. Höfner (Eds.), Mass Spectrometry in Medicinal Chemistry, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, 2007, p. 3.
[43] R.E. March, in: R.A. Meyers (Ed.), Encyclopedia of Analytical Chemistry, John Wiley & Sons Ltd, Chichester, 2000, p. 11848.
[44] G. Hopfgartner, E. Varesio, V. Tschäppät, C. Grivet, E. Bourgogne, L.A. Leuthold, J. Mass Spectrom. 39 (2004) 845.
[45] P. Kusch, G. Knupp, M. Hergarten, M. Kozupa, M. Majchrzak, Int. J. Mass Spectrom. 263 (2007) 45.
[46] H. Silva, C.V. Barra, C.F. da Costa, M.V. de Almeida, E.T. César, J.N. Silveira, A. Garnier-Suillerot, F.C.S. de Paula, E.C. Pereira-Maia, A.P.S. Fontes, J. Inorg. Biochem. 102 (2008) 767.