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Analytical Methods

Determination of biogenic diamines with a vaporisation derivatisation approach using solid-phase microextraction gas chromatography–mass spectrometry

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ABSTRACT

A gas-phase on-fibre derivatisation method for the determination of putrescine and cadaverine by gas chromatography/mass spectrometry using trifluoroacetylacetone (TFAA) has been studied and optimised. Small amounts (2 μl) of putrescine, cadaverine and TFAA standards were vaporised at high temperature in a 20 cm^3 closed SPME vial. The subsequent derivatives were recovered from the headspace of the vial using a PDMS/DVB fibre. The optimised mole ratio for [TFAA]/[Putrescine + Cadaverine] reaction was 22.3/1 with a derivatisation and extraction temperature of 120 °C and an extraction time of 20 min. The retention times for the derivatised putrescine and cadaverine were 20.5 and 22.2 min, respectively on a capillary column, CP-Sil 8CB; 30 m length × 0.25 mm i.d. × 0.25 μm film thickness. The correlation coefficients (R^2) of calibration curves for putrescine and cadaverine were 0.999 and 0.997, respectively over a range of sample masses of 20–350 ng, using nonadecane as an internal standard. Putrescine and cadaverine recoveries were determined to be 93.9% and 103.3%, respectively. The method was found to be a straightforward single step procedure that was unaffected by complex sample matrices and was successfully tested on samples of meat, vegetables and cheese.

1. Introduction

The biogenic amines, putrescine and cadaverine are aliphatic organic bases that are associated with metabolic processes in the cells of living organisms. They are essential for cell growth (Önal, 2007; Teti, Visalli, & McNair, 2002). Their participation in human cell growth and proliferation has been studied widely, particularly for their role in tumour growth (Moinard, Cynobera, & Bandt, 2005). The requirement for putrescine increases rapidly in growing tissues (neoplastic growth) (Pegg & Feith, 2007).

Biogenic amines are also important factors in food quality, and their presence in food is determined by the food-processing and microbial factors involved, especially in fish, cheese and meat products. These amines are mainly derived as a result of the microbial decarboxylation of amino acids (Önal, 2007). So, biogenic amines in food and meat products are related both to food spoilage and food safety. Considerably higher levels of biogenic amines have been observed in meat produced where hygiene is poorly controlled (Kalač, 2006). In vegetables biogenic amines are associated with spoilage due to poor storage (Moret, Smela, Populin, & Conte, 2005). The presence of biogenic amines in wines has also been studied extensively (Anli, Vural, Yilmaz, & Vural, 2004; Herbert, Cabrita, Ratola, Laureano, & Alves, 2005; Leitão, Marques, & San Romão, 2005; Marcobal, Polo, Martin-Alvarez, & Moreno-Arribas, 2005) where their formation is dependent on: the presence of specific micro-organisms, the levels of amino acids, the duration of fermentation phase, the period of contact with grape skin, the levels of sulphur dioxide, pH, and duration of wine contact with yeast residues (Leitão et al., 2005). Putrescine and cadaverine are important factors in food poisoning as they amplify the toxic effects of another biogenic amine, histamine (Önal, 2007). In general the concentrations of biogenic amines give an indication of the levels of microbiological contamination in food products (Shalaby, 1996) and hence act as reliable quality indicator.

Many standard chromatographic techniques have been described for the identification and quantification of biogenic amines (Teti et al., 2002). Most approaches use derivatisation to enhance the chromatography, and methods using trifluoroacetic anhydride (Jiang, 1990), trifluoroacetylacetone (Khuhawar, Memon, Jaipal, & Bhanger, 1999), pentafluoropropionic anhydride (Rattenbury, Lax, Blau, & Sandler, 1979), heptafluorobutyric anhydride (Fujihara, Nakashima, & Kurogochi, 1983), pentafluorobenzoyl chloride (Clements, Holt, Gordon, Todd, & Baker, 2004), pentafluorobenzaldehyde (Ngim, Ebeler, Lew, Crosby, & Wong, 2000) and pentafluoropropionyl anhydride (Choi, Kim, & Chung, 2000) have all been reported. In one of these studies a GC/FID technique using trifluoroacetylacetone enabled putrescine and cadaverine determination in

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serum of cancer patients (Khuhawar et al., 1999). The method exhibited good sensitivity with a linear calibration range 0–100 μg cm^{-3} along with the detection limits in the range 0.5–0.6 μg cm^{-3} for the two amines. Typically all the liquid-phase derivatisation methods (mentioned above) require a multi-step procedure including a solvent extraction stage. Such approaches are not amenable to automation or integration within instrumental systems. Further, interactions with the sample matrix tend to result in reactions producing interferences and artefacts in the analysis.

Alternative approaches to these liquid-phase extraction and derivatisation approaches include the use of ion mobility spectrometry (IMS) and on-fibre derivatisation. The IMS approach was based on nonylamine doping with sample treatment with potassium hydroxide, and offers a fast screening methodology (Karpas, Tilman, Gdalevsky, & Lorber, 2002). On-fibre derivatisation with solid-phase microextraction (SPME) has been used to enhance derivatisation methods involving complex sample matrices (Dietz, Sanz, & Cámara, 2006; Stashenko & Martínez, 2004). A good example of such an approach described the simultaneous derivatisation and extraction of five amphetamine-like drugs in urine samples. The method used headspace SPME GC–MS with heptafluorobutyric anhydride and heptafluorobutyric chloride as derivatisation agents (Chia & Huang, 2005). The method exhibited good sensitivity with a linear calibration range 0.1–1000 ng cm^{-3} along with the detection limits in the range 0.016–0.193 ng cm^{-3} for the five test drugs.

The present study extends the concept of on-fibre derivatisation to the recovery of gas-phase derivatisation products, where the derivatisation and extraction of putrescine and cadaverine occurs simultaneously in the headspace of a sample vial using vaporised samples and derivatisation reagents at elevated temperature (90–120 °C). Such an approach uses small (μg) quantities of sample-extract and derivatising reagent in a gas-phase derivatisation reaction that in turn minimizes the effect of the sample matrix. Trifluoroacetylacetone (TFAA) was selected as the derivatisation reagent in this work, as the trifluoromethyl group enhances the volatility of derivatised molecules (Khuhawar et al., 1999) making it suitable to use in conjunction with GC–MS and GC–FID.

2. Experimental

Eight studies were undertaken to variously characterise the derivatives (Studies 1 and 2) optimised the stoichiometry of the reagents (Study 3), temperature (Study 4) and reaction time (Study 5). Further the effect of surface treatments within the vials were investigated (Study 6) before calibration (Study 7) and validation (Study 8) tests were undertaken. The final stage of this work programme applied the approach to samples of meat, cheese and vegetables.

2.1. Materials

1,4-Butanediamine (putrescine, Aldrich) 99% and 1,5-pentanediamine (cadaverine, Aldrich) 95%; 1, 1, 1-trifluoroacetylacetone (Fluka) ⩾98% and nonadecane ($C_{19}H_{40}$, Fluka) and analytical solvents methanol (BDH), ethanol (BDH) and dichloromethane (BDH) were used in this research. 65 μm polydimethylsiloxane divinylbenzene (PDMS/DVB) SPME fibres, 20 cm^3 glass vials and magnetic stainless steel screw caps with a hole and PTFE/silicone septa were purchased from Supelco.

2.2. Preliminary characterisation of derivatives

2.2.1. Liquid-phase derivatisation (Study 1)

Preliminary mass spectrometric characterisation of the derivatives was based on a liquid-phase derivatisation followed by GC–MS ion trap (Varian CP-3800 GC coupled to Saturn 2000 mass spectrometer) analysis. TFAA, putrescine and cadaverine stock solutions were prepared in 10 cm^3 volumetric flasks with ethanol at concentrations of 38.2 mg cm^{-3}, 8.77 mg cm^{-3} and 8.73 mg cm^{-3}, respectively. The derivatives were obtained by reacting a mixture containing 0.5 cm^3 of each putrescine and cadaverine stock solutions with 1.6 cm^3 of the TFAA in a 4 cm^3 reaction vial in triplicate. The vial was sealed and heated to 95 °C on a hot plate for 15 min. The vial was then left to cool down to room temperature and the mixture was diluted twenty-fold in ethanol before analysis on GC–MS using a 30 m 0.25 mm i.d. column with a 0.25 μm thick 5%phenyl–95%methyl stationary phase (Varian CPSil-8).The instrumental settings are summarized in Table 1.

Table 1
Summary of instrumental parameters used in this study

Study number	1	2, 6	3, 4, 5	7, 8
Injector (Varian 1177)				
Split ratio	50:1	10:1	5:1	Splitless
Temperature/°C	250	250	250	250
Injection volume/μl	1	n/a	n/a	n/a
Desorption time (in GC injector)/min	n/a	30	30	30
GC–MS				
Column flow/cm^3 min^{-1}	1	2	2	2
Start temperature/°C (time/min)	90 (2)	90 (2)	90 (2)	90 (2)
Temperature ramp rate/°C min^{-1}	10	10	10	10
Final temperature/°C (time/min)	250 (12)	250 (12)	250 (12)	250 (12)
Transfer line temperature/°C	240	240	240	240
Iontrap temperature	200	200	200	200
Ionization/eV	EI	EI	EI	EI
Scan range/*m/z*	40–450	40–400	40–400	40–400
CombiPal Autosampler				
PDMS/DVB fibre conditioning temperature/°C	n/a	250	250	250
PDMS/DVB fibre conditioning time/min	n/a	30	30	30
Agitator pre-extraction time/min	n/a	2	2	2
Pre-extraction agitator speed/rpm	n/a	750	750	750
Extraction agitation speed/rpm	n/a	500	500	500

The values in brackets indicate GC column oven temperature hold times (min).

2.2.2. General procedure for on-fibre derivatisation (Study 2)

Automation of the on-fibre derivatisation/extraction was achieved by using a CTC Analytics CombiPal autosampler coupled to a GC–MS ion trap. A general procedure for on-fibre derivatisation/extraction was developed by pipeting 2 μl each of TFAA, putrescine, cadaverine and nonadecane (internal standard) stock solutions (in ethanol) into a 20 cm^3 SPME vial. The vial was sealed with a magnetic stainless steel screw cap (with PTFE/silicone septum). On-fibre derivatisation and extraction occurred in the agitator of the autosampler and by inserting SPME (PDMS/DVB) fibre in the vial headspace. During extraction the agitator was heated to between 90 °C and 120 °C vaporising the vial's contents. Derivatisation occurred and the products were extracted into the fibre from the vapour phase. The extracted derivatives were then desorbed into the injector of the GC–MS system and subsequently analysed with a 30 m 0.25 mm i.d. column with a 0.25 μm thick 5%phenyl–95%methyl stationary phase (Varian CPSil-8). The instrument settings are summarized in Table 1.

2.3. Optimisation of on-fibre derivatisation method

The on-fibre derivatisation yield was optimised with respect to the stoichiometric ratios of the reagents, the derivatisation time, and temperature and the extraction temperature and time. The sequence of studies run to optimise the proposed protocol along with corresponding instrumental settings (parameters) are summarized in Table 1.

2.3.1. Effect of TFAA:amine stoichiometric ratio (Study 3)

The effect of varying TFAA levels on derivative yield was studied by monitoring the derivative yield obtained from constant concentrations of putrescine and cadaverine at different TFAA concentrations. The level of amines in the headspace vials was maintained at 15 nmol, comprised of putrescine (0.702 μg, 8 nmol) and cadaverine (0.698 μg, 7 nmol) with a 0.64 ng nonadecane internal standard. Six different TFFA concentrations: 12.74 μg (83 nmol), 25.5 μg (165 nmol), 38.22 μg (248 nmol), 51.0 μg (331 nmol), 63.70 μg (413 nmol), 76.44 μg (496 nmol) were used and each measurement was undertaken in triplicate. The derivatisation and extraction steps were same as described in general procedure above, see Table 1.

2.3.2. Effect of derivatisation/extraction temperature (Study 4) and time (Study 5)

The effects of derivatisation and extraction temperatures on derivative yield were investigated using the optimised TFAA/putrescine and cadaverine stoichiometric ratios determined in Study 3 (see Section 3; Study 3). TFAA (51 μg), putrescine (0.7 μg), cadaverine (0.7 μg) and nonadecane (0.64 ng) were used in each experiment, and five derivatisation/extraction temperatures were studied: 80 °C, 90 °C, 100 °C, 110 °C, 120 °C. The derivatisation/extraction time was 20 min using the general procedure described above and each experiment was run in triplicate, see Table 1. Derivatisation/extraction time dependencies were studied using the same quantities of reagents with a derivatisation/extraction temperature of 120 °C (see Section 3; Study 4). The general procedure described above was used and the experiments were run in triplicate, see Table 1.

2.3.3. Effect of glass surface on derivatives yield (Study 6)

The effect of glass surface treatments of the SPME vial on the putrescine and cadaverine derivative formation was studied by running derivatisation/extraction in both silanised and un-silanised vials following the general procedure for on-fibre derivatisation (Study 2 above). Silanisation was achieved by immersing glass vials in 10% (v/v) dimethyldichlorosilane solution (in toluene) for 30 min (Tsutsumi, Nishikawa, Katagi, & Tsuchihashi, 2003).

Following preliminary observations the surface treatment study was expanded to include the effect of aprotic conditions on derivative formation. This was examined by carrying out derivatisation and extraction with putrescine, cadaverine and TFAA reagents prepared in dichloromethane (aprotic solvent) both in silanised and un-silanised vials. In both of these studies the vapour phase masses of TFAA, putrescine and cadaverine in the 20 cm^3 closed SPME vials were 25.5 μg, 1.05 μg and 1.04 μg, respectively. The internal standard (nonadecane) was not used in this study to eliminate interference from any additional compound. The derivatisation/extraction temperature was set at 90 °C for 20 min. The GC–MS instrument settings were kept same as mentioned in Study 2 above, see Table 1.

2.3.4. Calibration curve and validation (Studies 7 and 8)

Calibration curves were generated over the analyte mass range 20–350 ng using five putrescine or cadaverine standards, all run in triplicate. Since these standards were at lower concentrations than the optimisation study, the mass of TFAA (25.5 μg) was adjusted to maintain the optimum mole ratio. Nonadecane (0.32 ng) was added to each vial. The derivatisation/extraction was carried out at optimised conditions: 120 °C for 20 min. The remaining steps were same as described in general procedure above, see Table 1.

The accuracy of the method was assessed by estimating the recovery percentage

$$\%R = 100 \times \left[\frac{C_0}{C_S}\right] \quad (1)$$

between found (C_0) and known (C_S) amounts of putrescine and cadaverine using standards at two amine levels; 70 ng and 330 ng run in triplicate.

The robustness of the approach was further tested by repeating the calibration experiments on a GC–FID (Varian CP-3800 GC coupled to flame ionization detector with CTC Analytics CombiPal autosampler) with an analyte mass range of 20–200 ng with TFAA (25.5 μg) and nonadecane (6.0 ng) in each experiment. The subsequent procedural steps were same as described in the GC–MS study above, with each concentration run in triplicate. The accuracy (in terms of recovery percentage) of this method was determined against both amine standards at 30 ng.

2.4. Sample preparation

Samples of salmon, chicken breast, minced beef, matured cheddar cheese and frozen green peas were purchased from a local supermarket and stored at 4 °C in a refrigerator. Five grams of each sample were divided into small pieces (<5 mm diameter) with a stainless knife and placed into a 20 cm^3 glass vial. 10 cm^3 of 80% methanol: 20% distilled water was added (Antoine et al., 2002) and the vials were sealed and agitated for 5 min. The vials were then opened, warmed (50 °C) and stirred for 15 min. After cooling, the evaporative losses of the extracting solvent from the vial were replenished and the vial sealed once more and allowed to equilibrate for 5 min. A portion, 4 μl, of the supernatant extract was injected into a 20 cm^3 SPME vial and to this 2 μl of TFAA (25.5 μg) and 2 μl of nonadecane (0.32 or 6.0 ng) solutions were added, The derivatisation/extraction took place at the optimised conditions: 120 °C for 20 min using procedure described in calibration studies, see Table 1.

The effect of storage on the levels of putrescine and cadaverine in the samples of salmon and chicken breast were assessed using GC–MS. Fish extracts were obtained on 24, 48 and 96 h after purchase, chicken was assessed at 48 and 96 h. The beef, cheese and peas samples were extracted 48 h after purchase and analysed using GC–FID.

3. Results and discussion

3.1. Evaluation of the derivatisation procedure (Studies 1 and 2)

Study 1 tested the feasibility of eliminating water and using non-aqueous solvents in the derivatisation process in order to remove the time consuming steps of solvent extraction. Fig. 1 shows the chromatogram of the putrescine and cadaverine derivatives obtained by treating a mixture of putrescine and cadaverine standards with TFAA in ethanol. The chromatogram shows principal peaks appearing at 20.8 and 22.5 min for the putrescine and cadaverine derivatives, respectively. The mass spectra have been examined under positive-ion electron ionization (+EI) and positive-ion chemical ionization (+CI) modes using the ion trap instrument with a scan range *m/z* 40–450.

Replacing the aqueous-based multi-step derivatisation approach described previously (Khuhawar et al., 1999) in favour of a simultaneously trifluoroacetylacetone derivatisation and extraction yielded unexpected and interesting mass spectral data. The molecular formulae of the reported putrescine and cadaverine derivatives were $C_{14}H_{18}N_2O_2F_6$ and $C_{15}H_{20}N_2O_2F_6$, respectively.

It is important to note that the main mass fragments reported and those obtained in Study 1 are the same with comparable relative intensities. The difference between the previously published spectra and Study 1's mass spectra was the molecular ions. Positive-ion electron ionization (+EI) mass spectra indicated that the molecular ions $[M]^{+\cdot}$ for the putrescine and cadaverine derivatives

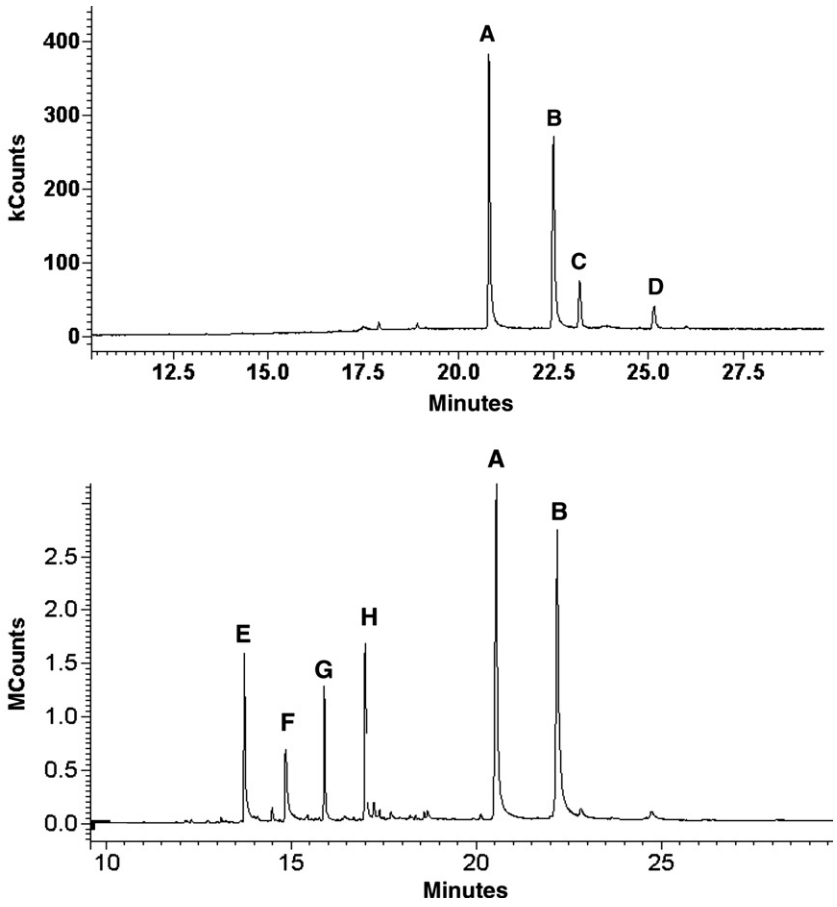

Fig. 1. Top (Study 1): total ion GC–MS traces for putrescine (A) and cadaverine (B) principal derivatives along with side products (C and D) obtained from the liquid-phase with on-column masses of 13.9 ng and 12.3 ng, respectively. Bottom (Study 2): Total ion GC–MS traces for putrescine (A) and cadaverine (B) principal derivatives along with side products (E–H) obtained from the gas-phase derivatisation of putrescine (1.1 μg) and cadaverine (1.0 μg) with TFAA (25.5 μg) in 20 cm^3 SPME vial keeping GC injector split ratio 10:1.

were *m/z* 414 and *m/z* 428, respectively, whereas previously (Khuhawar et al., 1999) the molecular ions were assigned to *m/z* 360 and *m/z* 374. The chemical ionization (+CI) mass spectra indicated that the principal species for the putrescine derivative was *m/z* 361, $[M + H]^+$, along with a lower intensity ion at *m/z* 415, corresponding to $[M + 54]H^+$. Similarly, for the cadaverine derivative the principal species was observed at *m/z* 375, $[M + H]^+$, with a lower intensity ion *m/z* 429, corresponding to $[M + 54]H^+$. Supporting studies undertaken under the same conditions with 1,2-diaminoethane ($M_r = 60$; $C_2H_8N_2$) and 1,3-diaminopropane ($M_r = 74$; $C_3H_{10}N_2$) yielded $[M + 54]^{+\cdot}$ under +EI conditions and $[M + H]^+$ with a lower intensity ion corresponding to $[M + 54]H^+$ under +CI conditions.

Along with the principal GC peak, less intense side product peaks were also observed, see Fig. 1 (top). The retention times of these side products were 23.2 and 25.1 min for putrescine and cadaverine, respectively and their main mass fragments were *m/z* 69, 84, 98, 110, 138, 168, 188, 207, 228 and *m/z* 69, 84, 98, 110, 140, 188, 194, 242. Their presence did not interfere with the chromatography and reproducibility of the principal putrescine and cadaverine derivatives.

The 54 amu discrepancy between these observations and previous reports for this reaction merits further elucidation and will be the subject of future work. Preliminary investigations of this phenomenon appear to suggest the formation of a cyclic adduct involving three water molecules. The detailed mass spectrometric study required to test such a hypothesis was beyond the scope of this study. Nevertheless, the quantitation in this study was based upon total ion current (TIC) measurements, in which both analytes generated stable chromatographic and mass spectral responses throughout this study. The discrepancy was judged not to materially affect the analytical outcomes of this work.

Removing the liquid-phase and vaporising the reagents (Study 2) resulted in a more complicated mixture of products, Fig. 1 (bottom). The mass spectra for the peaks at 20.5 and 22.2 min indicated that these were the same putrescine and cadaverine derivatives as obtained previously in Study 1. The additional peaks between 13.7 and 17.0 min (E–H) were not observed in liquid-phase derivatisation and blank gas-phase (SPME) chromatograms. The main mass fragments of these additional peaks along with relative intensities (%) are; E: 69(20%), 70(68%), 84(28%), 96(10%), 110(26%), 138(29%), 184(15%), 225(100%), F: 69(17%), 84(100%), 96(9%), 110(8%), 152(12%), 194(10%), 239(46%), G: 69(31%), 84(14%), 96(10%), 126(21%), 166(66%), 194(17%), 251(100%), 320(17%), H: 69(33%), 96(9%), 126(18%), 180(24%), 208(11%), 247(33%), 265(100%), 334(12%). It is interesting to note that all additional peaks E–H had several mass fragments that were also present in putrescine and cadaverine derivatives. This indicates the additional peaks might be derivatives produced from half or partial derivatisation of putrescine and cadaverine molecules. These side products were stable as they were produced in all the gas-phase derivatisation reactions (Studies 2–8). However, their presence did not interfere with the chromatography of the principal putrescine and cadaverine derivatives during the optimisation and calibration/validation

studies. The reproducibility of the derivatisation process was assessed by repeating each derivatisation. On each occasion the chromatography and associated mass spectra were identical.

3.2. The effect of the TFAA: analyte stoichiometric ratio (Study 3)

The derivative yield, and hence the sensitivity of the method, was effected significantly by the TFAA/amine ratio Over the range of TFAA/amine ratios, 5.6 to 22.3, the derivative yield increased 5.5 times for putrescine and 5.9 times for cadaverine. Above a ratio of 22.3 the yield of derivative decreased. The optimum mole ratio [TFAA]/[Put + Cad] was found to be 22.3:1.0. This optimum was significantly higher than the theoretical ratio of 2:1, for increasing the TFAA concentration increased the reaction-rate of the derivatisation processes pushing the reaction further towards completion during reaction and extraction processes. Accounting for the reduction in yield beyond this ratio was not so straight forward. The reduction in the observed yield may have been due to inhibition of the reaction, perhaps due to adsorption of the TFAA onto active sites in the glass vial, or from competitive adsorption of TFFA into the SPME fibre reducing the amount of derivative recovered.

3.3. Temperature and time effects (Studies 4 and 5)

The temperature of the derivatisation and extraction procedure had the same effect for both analytes. Increasing the temperature from 80 °C to 120 °C resulted in an 11.3-fold and 17.6-fold increase in putrescine and for cadaverine yields, respectively for a 20 min reaction and extraction time. The trends indicated that increasing the temperature above 120 °C would result in further increases in the yields of derivatives. In this study, 120 °C was the maximum temperature that the vial oven could be operated at, so this temperature was used in all subsequent studies.

The effect of increasing the derivatisation and extraction time was studied at optimised conditions of temperature and stochiometric ratio (120 °C and 22.3:1.0). The recovery of the derivatives increases to a maximum at approximately 20 min, after which the yield reduces. Such a time profile is consistent with the operation of two opposing mechanisms. The production of the derivatives and the loss of derivative though absorption and permeation into the septum of the vial. A derivatisation and extraction time of 20 min was selected for the subsequent analyses.

3.4. Effect of glass surface on derivatives yield (Study 6)

The reaction between TFAA and the biogenic amines has previously been reported for aqueous conditions (Khuhawar et al., 1999) and the proposed reaction mechanisms invoke labile protons. Consequently, the possible role of the silanol functional groups on the interior surfaces of the vial in the formation of the derivative was investigated. The study compared putrescine and cadaverine derivative yields obtained from silanised and un-silanised vials with ethanol and dichloromethane as solvents, see Table 2. The effect of surface treatment and solvent was marked. The highest derivative peak area (6.28×10^6 counts s) was obtained from ethanol with untreated vials. The yields followed a clear trend that indicates that the presence of proton functionality within the experimental system was important and followed the trend: untreated with ethanol > silanised with ethanol > untreated with dichloromethane > silanised with dichloromethane. These observations indicate that the derivatisation mechanism within the vial was probably not wholly confined to the gas-phase and that a reaction pathway involving an adsorbed intermediate may be a significant factor in this approach. Untreated, that is non-silanised vials were used in all the other studies.

Table 2
Peak areas (10^6 counts s) for putrescine and cadaverine derivatives obtained from silanised and un-treated vials with and without ethanol and dichloromethane (DCM)

Compound	Untreated ethanol	Silanised ethanol	Untreated DCM	Silanised DCM
Putrescine	6.28 (0.394)	2.36 (0.166)	1.38 (0.165)	0.71 (0.167)
Cadaverine	4.89 (0.207)	1.09 (0.235)	1.24 (0.176)	0.46 (0.169)

The 95% confidence limits are given in brackets based on triplicate measurements.

3.5. Calibration and Validation (Studies 7 and 8)

Calibration curves were obtained from five putrescine and cadaverine standards ranging from 20 ng to 350 ng with nonadecane as an internal standard. Each standard was run in triplicate, see Fig. 2. The response was non-linear, reflecting a more complex derivatisation reaction, although linear-regression analysis provided an approximate response model within the limit of experimental accuracy. A second-order least-squares regression could also be used to model the data, with greater accuracy. The response functions obtained for GC–MS and GC–FID were essentially the same with correlation coefficients (R^2) of 0.999 and 0.994 for putrescine and 0.997 and 0.999 for cadaverine, respectively. Such second-order polynomial approach has also been reported to accommodate non-linear calibration data obtained by a GC–MS study of VOCs in water (Lavagnini, Favaro, & Magno, 2004). The ultimate limits of detection were not determined in this study. The sensitivity and signal to noise characteristics of the responses observed were judged to be satisfactory for the analysis and presumptive testing of food (meat) types and biological matrices.

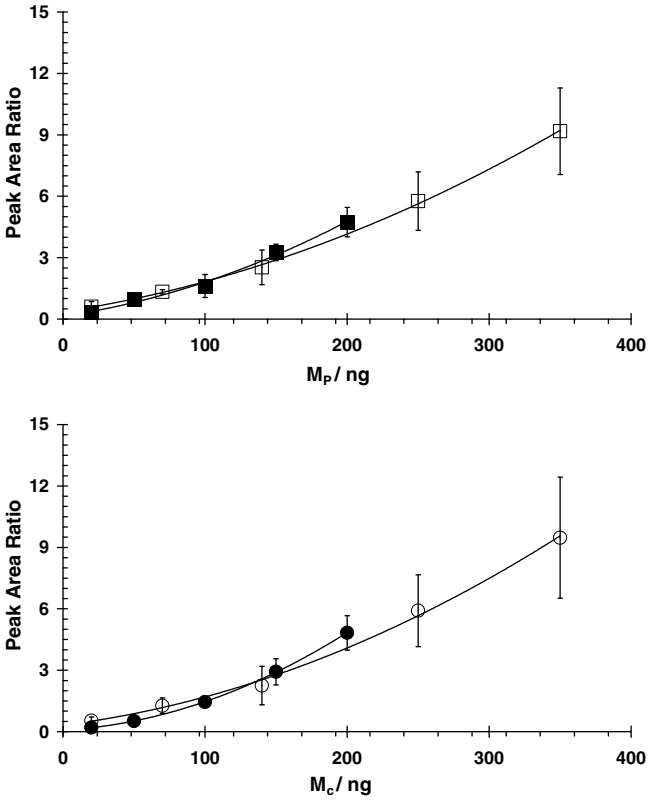

Fig. 2. Response curves, expressed as the derivative/nonadecane internal standard ratio for GC–FID (Solid symbols) and GC–MS (open symbols) as the mass of putrescine, Mp, (top) and cadaverine, Mc, (bottom) were increased from 20 ng to 350 ng. These calibrations were run at 120 °C from a reaction and extraction time of 20 min.

Table 3
Levels of biogenic amine determined in demonstration tests with GC–MS ($n = 2$), and GC–FID ($n = 3$)

Sample	Instrument	[Putrescine]/mg kg^{-1} (S)	[Cadaverine]/mg kg^{-1} (S)
Chicken day 2	GC–MS	68 (7)	55 (3)
Chicken day 7	GC–MS	133 (3)	144 (4)
Fish day 1	GC–MS	35 (5)	26 (3)
Fish day 2	GC–MS	41 (4)	129 (14)
Fish day 4	GC–MS	68 (1)	182 (1)
Beef day 2	GC–FID	29 (1)	18 (2)
Green peas	GC–FID	9 (1)	13 (2)
Matured cheese	GC–FID	38 (3)	22 (3)

The standard deviations on the estimated values is given in brackets.

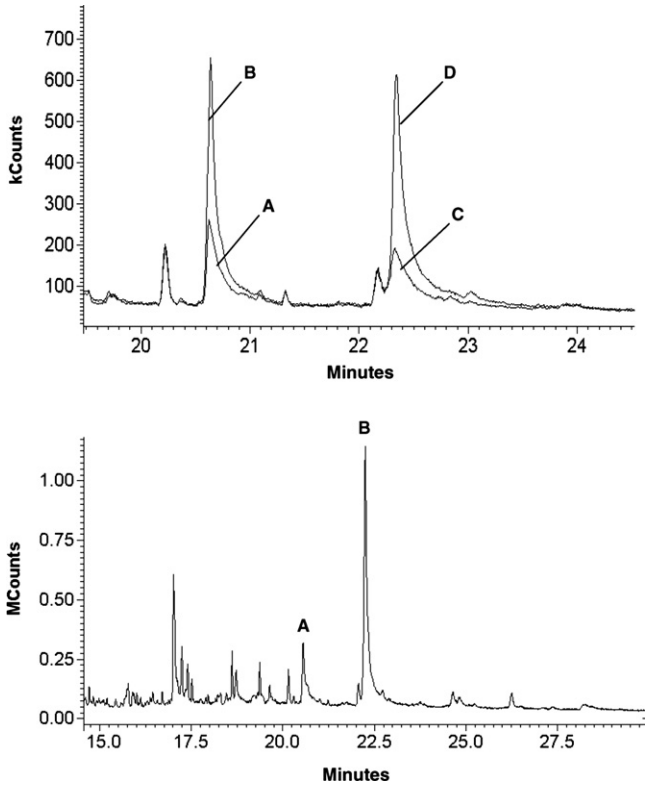

Fig. 3. Examples of detail from the GC–MS total ion chromatograms obtained during the demonstration analyses. Showing: top putrescine (A and B) and cadaverine (C and D) present in chicken sample at 2 day and 7 day, respectively. Bottom putrescine A and cadaverine B detected in fish sample at day 4.

The accuracy of this approach was estimated in terms of percentage recovery obtained by analysing putrescine and cadaverine standards at the lower and upper parts of the response ranges (see Section 2.4 above). The recoveries observed were in the range 93.9–103.3% indicating a satisfactory level of accuracy over the selected range of study. The student t test also confirmed the validity of the developed method as all the calculated t values are less than the reported (Christian, 2004) value $t = 4.303$ for 2° of freedom $(N - 1)$ and 95% confidence level. No significant difference was observed in the accuracy between the GC–MS and GC–FID platforms. The calibration ranges corresponded to liquid-phase concentrations of 5 μg cm^{-3}–88 μg cm^{-3}.

3.6. Samples

The calibration functions (see Fig. 2) of the GC–MS were used to estimate the levels of biogenic amines recovered from chicken and salmon samples used in demonstration tests. The masses obtained were then expressed as mg of recovered amines per kg of sample, see Table 3, and the levels of biogenic amines observed in these demonstration tests were found to be comparable with those reported previously (Kalač & Krausová, 2005). The values for the standard deviation indicated that the precision of this approach was satisfactory. The trend of increasing levels of biogenic amines with increasing storage time was also clear from these data. In the chicken sample the levels increased 1.95 times for putrescine and 2.6 times for cadaverine over the storage of 2–7 days, see Fig. 3 whereas, the level of cadaverine in fish sample increased more markedly, 6.9 times over the storage of 1–4 days, see Table 3. Examination of the chromatography showed that the biogenic amine peaks were not subject to significant matrix interferences and that quantification of the biogenic amine was a feasible proposition. The quantitation of putrescine and cadaverine in beef, green peas and matured cheese samples was also attempted with GC–FID with satisfactory results; see Table 3.

4. Conclusion

A new derivatisation method for putrescine and cadaverine analysis using μl quantities of vapourised trifluoroacetylacetone (derivatisation agent) and methanol:water extracts with derivative recovery by SPME fibre (PDMS/DVS) extraction offers significant time and sensitivity enhancements over established methods by eliminating multi-step extraction techniques with organic solvents. The derivatisation and extraction occurred simultaneously in an SPME vial maintained at 120 °C over a 20 min period. This approach reduced matrix interferences in complex samples with analyte masses as low as 20 ng. The potential importance of the surface treatments and solvents used this approach were an interesting and potentially useful observation. The incorporation of this derivatisation chemistry into sample introduction systems for direct reading instruments for use in clinics and environmental health applications is a logical extension of this research.

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