plates that have 96 wells containing discs or packed beds of sorbent particles arranged in an 8-row × 12-column rectangular matrix. Although the plates can be processed manually, instrumentation is preferred for processing liquids through the SPE plates. The advent of high throughput sample preparation formats, in combination with the specificity, sensitivity and speed of LC/MS/MS analytical techniques, have created a superior combination for the analytical chemist to meet the demands for faster sample processing and data generation to support the drug development process.

Conclusion

The future of drug bioanalysis using SPE is promising as more and more laboratories increase the usage of LC/MS/MS instrumentation and justify the need for high throughput SPE. LC/MS/MS instrumentation is now common in pharmaceutical laboratories and clinical laboratories are slowly beginning to demonstrate utility for mass spectrometry for certain drug classes, e.g. tacrolimus (FK506, an immunosuppressant). The 96-well plate format is finding its way into many bioanalytical applications; when coupled with automation, there is currently no faster sample preparation method. Individual SPE cartridges will continue to have a role in sample preparation, but 96-well plates will proliferate in pharmaceutical applications. More bioanalytical applications will adopt smaller sorbent mass products, as the productivity gains from reduced solvent volume are realized, especially using automation. As the sorbent mass in plates decreases, the number of applications that demonstrate elimination of the evaporation step by using small elution volumes of mobile phase compatible solution for direct injection will increase.

See also: **II / Extraction:** Solid-Phase Extraction. **III / Drugs and Metabolites:** Liquid Chromatography – Mass Spectrometry. **Solid-Phase Extraction with Discs.**

Further Reading

Brewer E and Henion J (1998) Minireview. Atmospheric pressure ionization LC/MS/MS techniques for drug disposition studies. *Journal of Pharmaceutical Science* **87**(4): 395–402.

Hartmann C, Smeyers-Verbeke J, Massart DL and McDowall RD (1998) Review: Validation of bioanalytical chromatographic methods. *Journal of Pharmaceutical and Biomedical Analysis* **17**: 193–218.

Hoja H, Marquet P, Verneuil B *et al.* (1997) Applications of liquid chromatography-mass spectrometry in analytical toxicology: a review. *Journal of Analytical Toxicology* **21**: 116–126.

Lensmeyer GL, Darcey BA and Wiebe DA (1991) Application of a novel form of solid phase sorbent (Empore membrane) to the isolation of tricyclic antidepressants from blood. *Journal of Chromatographic Science* **29**: 444–449.

Lensmeyer GL, Onsager C, Carlson IH and Wiebe DA (1995) Use of particle-loaded membranes to extract steroids for HPLC analyses. Improved analyte stability and detection. *Journal of Chromatography A* **691**: 239–246.

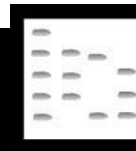
McDowall RD, Doyle E, Murkitt GS and Picot VS (1989) Review: Sample preparation for the HPLC analysis of drugs in biological fluids. *Journal of Pharmaceutical and Biomedical Analysis* **7**(9): 1087–1096.

Wells DA (1999) 96-Well plate products for solid-phase extraction. *LC/GC* 17(7): 600–610.

Wong SHY (1989) Review: Advances in liquid chromatography and related methodologies for therapeutic drug monitoring. *Journal of Pharmaceutical Biomedical Analysis* **7**(9): 1011–1032.

Wu Y, Lily Y-T, Henion JD and Krol GJ (1996) Determination of LTE_4 in human urine by liquid chromatography coupled with ionspray tandem mass spectrometry. *Journal of Mass Spectrometry* **31**: 987–993.

BIOGENIC AMINES: GAS CHROMATOGRAPHY



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Introduction

The term 'biogenic amine' was proposed by Guggenheim in 1940 in order to define the low-molecular-weight organic bases, produced by the decarboxylation of amino acids, that possess biological activity. Biogenic amines are receiving increasing interest because they are often present in foods, such as cheese, meat, and fish where they are used as a useful indicator of spoilage and markers of food quality. They occur naturally in the central nervous system, where they play an important role as neurotransmitters. Their presence in metabolic pathways in health and disease has been studied because of their biological activity as reported by Parvez *et al.*, and they are

Table 1 GC of biogenic amines with FID detection

Analyte	*Chromatographic column*	*Carrier, type and flow rate*	*Injector temperature and program*	*Matrix*	*Sample treatment*	*Reference*
Aliphatic monoamines, aliphatic polyamines, catecholamines, indolyl amines, imidazoyl amines (57 amines)	Dual fused silica capillary DB-5 DB-17, length 30 m, i.d. 0.25 mm, film thickness 0.11 μm	He	260°C, 260°C → 4°C min^{-1} → 280°C	Biological fluids	Two-phase isobutyloxy carbonylation, SPE purification and derivatization with *N*-methyl-*N*-(butyldimethylsilyl)-trifluoroacetamide	Kim *et al.* (1997)
Histamine, tyramine, putrescine, cadaverine, adrenaline, noradrenaline, tryptamine, dopamine, agmatine, spermine, spermidine, phenylethylamine	Fused silica capillary SE-54, length 25 m, i.d. 0.31 mm, film thickness 0.52 μm	He, 50 ml min^{-1}	310°C, 125°C → 10°C min^{-1} → 300°C	Foods	Liquid–liquid extraction, SPE purification, derivatization with trifluoroacetic anhydride	Laleye *et al.* (1987)
Cadaverine, 1,3-diaminopropane, putreanine, isoputreanine, putrescine, spermine, spermidine	Fused silica capillary cross-linked methyl siloxane deactivated by silanization, length 35 m, i.d. 0.20 mm, i.d. 0.20 mm, i.d. 0.20 mm, film thickness 0.11 μm	He, 0.5 ml min^{-1}	300°C, 120°C → 7°C min^{-1} → 260°C	Biological fluids	SPE purification, derivatization with heptafluorobutyric anhydride	Muskiet *et al.* (1984)
1,3-Diaminopropane, putrescine, cadaverine, 3-aminopropylcadaverine, spermidine, *sym*-homospermidine, *sym*-norspermidine, spermine, *sym*-norspermine, thermospermine, canavalamine	0.5% KT-300 on Uniport HP packing, length 0.5 m, i.d. 3 mm	N_2, 80 ml min^{-1}	285°C, 130°C → 10°C min^{-1} → 280°C	Plants	Ion exchange resin separation, derivatization with ethylchloroformate	Yamamoto *et al.* (1984)
Adrenaline, dopamine, noradrenaline, 5-hydroxytryptamine	5% OV-17 on Chromosorb W HP 80-100 mesh packing, length 1 m, i.d. 3 mm	N_2, 36 ml min^{-1}	265°C isothermal	Biological tissues	Derivatization with pentafluorobenzoyl chloride and pyridine in acetonitrile	Bock and Waser (1981)

Reproduced with permission by Kim KR *et al.* (1997).

reported to be responsible for diseases related to their biological activity.

Muskiet *et al.* investigated a comparison of the popular high performance liquid chromatographic (HPLC) methods, whose chief advantage is a reduced sample pre-treatment, and other chromatographic techniques, such as gas chromatography, for the separation and determination of biogenic amines. In chromatographic methods, two main steps are necessary: (a) the separation of amines from the matrix and (b) their determination. Pre-column derivatization is very often required for selectivity and sensitivity enhancement.

Sample Preparation

The first step is the most critical in terms of obtaining an adequate recovery for amines, because of the

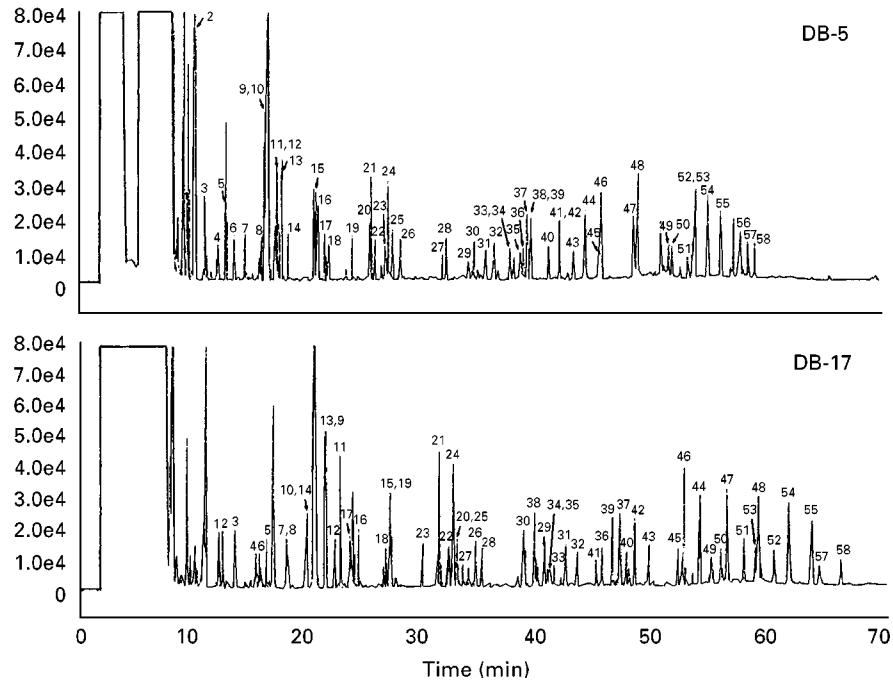

Figure 1 Dual chromatograms of amines as *N(O)*-isoBOC, *O*-TBDMS derivatives separated on DB-5 and DB-17 (both 30 m × 0.25 mm I.D., 0.11 μm film thickness) dual-capillary column system. GC conditions are described in the text. Peaks: 1 = ethylmethylamine; 2 = *tert*-butylamine; 3 = diethylamine; 4 = *sec*.-butylamine; 5 = isobutylamine; 6 = diisopropylamine; 7 = *n*-butylamine; 8 = dipropylamine; 9 = pyrrolidine; 10 = isoamylamine; 11 = morpholine; 12 = piperidine; 13 = *n*-amylamine; 14 = diisobutylamine; 15 = thiazolidine; 16 = *n*-hexylamine; 17 = dibutylamine; 18 = cyclohexylamine; 19 = *n*-heptylamine; 20 = diphenylamine; 21 = *o*-toluidine; 22 = benzylamine; 23 = *n*-octylamine; 24 = *m*-toluidine; 25 = *p*-toluidine; 26 = β-phenethylamine; 27 = dihexylamine; 28 = *n*-decylamine; 29 = 2,4-dichlorobenzylamine; 30 = dicyclohexylamine; 31 = 1,3-diaminopropane; 32 = 3,4-dichlorobenzylamine; 33 = β-hydroxyphenethylamine; 34 = norephedrine; 35 = ephedrine; 36 = putrescine; 37 = 3,4-dimethoxyphenethylamine; 38 = diethanolamine-1; 39 = dibenzylamine; 40 = cadaverine; 41 = diethanolamine-2; 42 = histamine; 43 = 1,6-diaminohexane; 44 = tryptamine; 45 = 1,7-diaminoheptane; 46 = tyramine; 47 = 3-methoxytyramine; 48 = 5-methoxytryptamine; 49 = synephrine; 50 = octopamine; 51 = metanephrine; 52 = 3,4-dihydroxybenzylamine; 53 = normetanephrine; 54 = dopamine; 55 = spermidine; 56 = serotonin; 57 = epinephrine; 58 = norepinephrine. (Reproduced with permission from Kim KR *et al*. (1997).)

complexity of the matrices usually considered. All matrices pose different problems depending on their complexity so many clean-up techniques have been proposed making use of different extraction procedures, i.e. liquid–liquid, solid-phase extraction (SPE), etc. and a variety of solvents (perchloric, trichloroacetic, and hydrochloric acids and/or both polar and non-polar organic solvents).

In the second step, account must first be taken of the amine pre-column derivatization; this is often, if not always, necessary for enhancing the analyte response and selectivity. The other aim of pre-column derivatization is to obtain derivatives, which are both volatile and sufficiently stable for subsequent analyses. Gas chromatographic analysis of the amines requires one or more appropriate derivatization procedures to block active protons in amino and other polar groups and acylation, silylation, benzoylation, sulfonylation, phosphorylation and alkyloxycarbonylation have been used. Baker *et al*. have given an example of a complete study of the derivatization process in biogenic amines determination.

Chromatography

Separations are mostly performed on capillary columns whose length ranges from 15 to 30 m. The silica capillary columns are coated with different film thickness of methylsiloxane, methylphenylsiloxane, or cyanopropylphenylsiloxane polymers or polyethylene glycols. Only a few examples in the past reported the use of packed columns. Both isothermal and programmed temperature conditions are used.

Detection

Among the different detectors used in gas chromatography for biogenic amine analysis, the four detectors most commonly used are: flame ionization detector (FID), electron-capture detector (ECD), nitrogen–phosphorus detector (NPD) and mass spectrometry (MS).

Flame Ionization Detector (FID)

Some biogenic amines such as aliphatic mono-, di- and polyamines do not exhibit structural features that

permit their specific detection, in this case a non-specific detector such as flame ionization (FID) can be used, but the lack of sensitivity and/or selectivity may require the use of other detectors coupled with FID as shown in **Table 1**.

Bock and Waser acylated, via a single-step reaction, some biogenic amines for their specific and quantitative gas chromatographic assay. The retention times were found to be inversely proportional to their molecular weights and also to the number of the pentafluorobenzoyl groups. FID and ECD detector responses were compared; FID was selected for nanogram range and ECD for picogram range determinations, in order to optimize linearity and accuracy. The structure of the derivatives was confirmed by GC–MS technique.

Yamamoto *et al*. proposed a routine method for the determination of polyamines as their *N*-ethyloxycarbonyl derivatives. The sample preparation is simple and reproducible and the derivatives are stable. Also in this case, the identity of the derivatives was confirmed by GC–MS technique.

Muskiet *et al*. prepared extracts of acid-hydrolysed biological fluids by pre-purification with silica gel to give recoveries of better than 90%. The isolated compounds were derivatized and simultaneously determined by a capillary gas chromatographic method. FID and NPD detector responses were compared showing that NPD is both more selective and sensitive than FID.

Laleye *et al*. developed trifluoroacetylation for quantifying biogenic amines on a microgram scale in foods.

One of the most recent and notable applications of FID detector is the determination of 57 amines described in the paper of Kim *et al*. In their work they demonstrated the wide applicability of the FID supported by the simultaneous use of two capillary columns of different polarity, such as DB-5 and DB-17 for resolving all the considered amines, as shown in **Figure 1**. The proposed method is based on two-phase isobutyloxycarbonylation with a pH shift. The resulting derivatives are separated by SPE and the remaining hydroxyl groups are derivatized for dual capillary column gas chromatographic analysis. Response is linear in the 0.2–12-ppm range.

Electron-Capture Detector ECD

Examples of the use of the electron-capture detection (ECD) are given in **Table 2**.

Staruskiewicz and Bond developed a procedure for the quantitative determination of putrescine and cadaverine in foods. The amines were extracted with methanol and a dry residue of their hydrochloride salts was prepared. The salts were derivatized and both the reagent excess and reaction by-products were removed by means of an alumina column, minimizing in this way the solvent fronts and interfering extraneous peaks, some of which adversely affected the ECD response. The recovery of diamines was within the range 97 to 102%. Gas chromatographic separation was performed and the retention time for the derivatives of putrescine and cadaverine was found to be 4.3 and 5.7 min, respectively. When

Table 2 GC of biogenic amines with ECD detection

Analyte	*Chromatographic column*	*Carrier, type and flow rate*	*Injector temperature and program*	*Matrix*	*Sample treatment*	*Reference*
Histamine, *tele*-methylhistamine, *m-, p*-tyramine, 3-methoxytyramine, putrescine, cadaverine, tryptamine, spermine, spermidine, 2-phenylethylamine, 5-hydroxytryptamine	Fused silica capillary SE-54, length 15 m, i.d. 0.25 mm	He, 2 mL min^{-1}	250°C, 105°C → 25°C min^{-1} → 240°C	Foods	Liquid–liquid extraction, derivatization with pentafluorobenzoyl chloride–benzene–acetonitrile	Baker *et al.* (1987)
Cadaverine, putrescine, spermine, spermidine	3% OV-17 on Chromosorb W HP packing length 1.5 m, i.d. 3 mm	He, 60 mL min^{-1}	120°C → 15°C min^{-1} → 280°C	Biological fluids	SPE purification, derivatization with heptafluorobutyric anhydride	Fujihara *et al.* (1983)
Cadaverine, putrescine	3% OV-225 on Gas Chrom Q 100-120 mesh packing, length 1.8 m, i.d. 2 mm	He, 28 mL min^{-1}	200°C, 180°C isothermal	Foods	Liquid–liquid extraction, derivatization with pentafluoropropionic anhydride, SPE purification	Staruszkiewicz and Bond (1981)

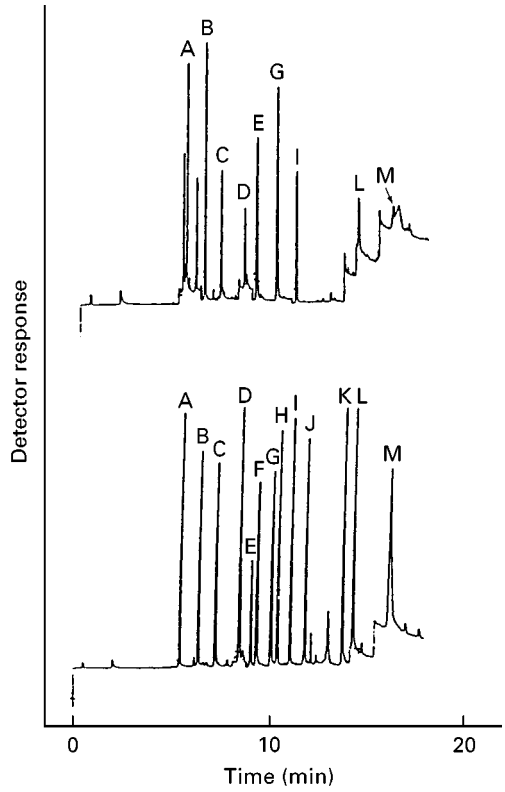

Figure 2 Gas chromatogram of extracts of bioactive amines from (top) cheese sample and (bottom) a solution of authentic standards. Extraction and derivatization were as described in the text. Derivatives of 2-phenylethylamine (A); 2-(4-chlorophenyl)ethylamine, internal standard (B); *tele*-methylhistamine (C); histamine (D); putrescine (E); tryptamine (F); cadaverine (G); *m*-tyramine (H); *p*-tyramine (I); 3-methoxytyramine (J); 5-hydroxytryptamine (K); spermidine (L); and spermine (M). Attenuation changes were programmed in to ensure that all peaks remained on scale for the purpose of illustration. (Reproduced with permission from Baker GB *et al.* (1987).)

compared to a nitrogen-specific detector, the ECD had greater sensitivity. Less than 1 ppm of diamine in the food can be quantitated with a coefficient of variation lower than 3.0%.

Fujihara *et al.* developed a sensitive and selective method for the estimation of polyamines in biological fluids after their separation from other compounds and derivatization. These derivatives are well resolved within 15 min with detection limit of 0.1 pmol for putrescine and cadaverine and 0.02 pmol for spermine and spermidine.

Baker *et al.* proposed a method based on extractive derivatization of the amines with a perfluoroacylating agent under basic aqueous conditions. Polyamine recovery was virtually quantitative, whilst for the other amines the recoveries ranged from 70 to 85%. Derivatives showed good chromatographic properties and ECD was used for enhancing sensitivity of biogenic amines in food samples. The derivative structures were confirmed by GC–MS. The detection limits (peak/noise ratio = 2) of the different types ranged between 5 and 20 pg on-column and calibration curves were linear over two orders of magnitude.

A typical chromatogram obtained with this technique is shown in **Figure 2**, that is relative to the determination of perfluoroacetylderivatives in foods. The method of Baker *et al.* resulted in a reference paper for all later authors using the ECD.

Nitrogen–Phosphorus Detector (NPD)

Another very specific detector for amines is the nitrogen–phosphorus detector (NPD). The most recent papers relative to the use of this detector for biogenic amine determination are reported in **Table 3**.

One of the first comparisons between FID and another detector (NPD) appeared in the paper of Muskiet *et al.* These authors prepared extracts of acid-hydrolysed biological fluids by pre-purification with silica gel. The isolated compounds were derivatized and simultaneously determined by capillary gas chromatography with nitrogen–phosphorus detection. Their comparison between typical chromatograms obtained with FID and NPD is shown by an example in **Figure 3**.

Perez-Martin *et al.* also compared FID and NPD results, but contrary to Muskiet *et al.* they considered both detector responses as equivalent. These authors treated seafoods with trichloroacetic acid and then extracted the resulting solution with benzene under highly basic conditions. Retention times are 1.03 min for dimethylamine and 1.35 min for trimethylamine.

Hessels *et al.* developed a capillary gas chromatographic method with NPD for the determination of the *N*′-acetylspermidine catabolite in biological fluids and compared it with the GC–MS method of Van den Berg *et al.*, shown in **Table 4**. The results were within a relative standard deviation of 3%.

One of the most recent applications of NPD for polyamines profiling is the work of Dorhout *et al.* that used capillary gas chromatography with NPD for the determination of polyamines, *N*-acetylated polyamines and the polyamine analogues *N*′,*N*″-bis(ethyl)norspermine and 1,19-bis(ethylamino)-5,10,15-triazanonadecane in biological samples. The suggested method provided for the use of four internal standards and pre-purification steps comprising deproteinization and isolation on silica at pH 9.0 with 70–90% recoveries depending on polyamine type. The precision is better than 7%, while the detection limits (peak/noise ratio = 2) of the different components ranged from 0.4 to 0.7 pmol. **Figure 4** shows a typical chromatogram obtained with the method described in the Dorhout work.

Table 3 GC of biogenic amines with NPD detection

Analyte	*Chromatographic column*	*Carrier, type and flow rate*	*Injector temperature and program*	*Matrix*	*Sample treatment*	*Reference*
Putrescine, cadaverine, spermine, spermidine, 1,3-diaminopropane	Fused silica capillary methyl silicone phase, length 37.5 m, i.d. 0.20 mm, film thickness 0.11 μm	He, 0.6 mL min^{-1}	260°C, 120°C → 5°C min^{-1} → 260°C → 2°C min^{-1} → 280°C	Biological tissues	Deproteinization, SPE purification and derivatization with heptafluorobutyric anhydride	Dorhout *et al.* (1997)
Polyamines, *N*′-acetylspermidine	Fused silica capillary HP 17, length 25 m, i.d. 0.20 mm, film thickness 0.17 μm		260°C, 180°C → 3°C min^{-1} → 270°C	Biological fluids	Alkalization, liquid–liquid extraction and SPE purification	Hessels *et al.* (1991)
Trimethylamine, dimethylamine	4% Carbowax 20M + 0.8% KOH on Carbopack B and Chromosorb 103 packing, length 1.8 m, i.d. 2 mm	N_2, 25 mL min^{-1}	250°C, 115°C isothermal	Seafoods	Liquid–liquid extraction	Perez-Martin *et al.* (1987)
Cadaverine, 1,3-diaminopropane, putreanine, isoputreanine, putrescine, spermine, spermidine	Fused silica capillary cross-linked methyl silicone and siloxane deactivated, length 35 m, i.d. 0.20 mm, film thickness 0.11 μm	He, 0.5 mL min^{-1}	300°C, 120°C → 7°C min^{-1} → 260°C	Biological fluids	SPE purification, derivatization with heptafluorobutyric anhydride	Muskiet *et al.* (1984)

Reproduced with permission from Muskiet FAJ *et al.* (1984).

Mass Spectrometry (MS)

The actual trend in biogenic amine gas chromatographic analysis is to establish a complete profile of all the important amines by using a single method, thus the widest used detector is mass spectrometry (MS) (Table 4). This technique was formerly used in conjunction with other detectors for confirmation purposes only.

The advantage of MS over other detection systems is based on the possibility of identifying compounds with a high degree of specificity. This can be realized using high resolution capillary gas chromatography combined with the monitoring of characteristic ions in the mass spectrum of a compound (GC–MS). GC–MS can afford characteristic ratios of ion intensities or the m/z value of a particular ion. These parameters may be changed in a predictable manner by derivatization processes in order to provide additional proof of identity.

Duncan *et al.* compared the results obtained using both HPLC with electrochemical detection and GC–MS in the single-ion monitoring mode (SIM) when applied to dopamine and norepinephrine (noradrenaline) determination, after a single-step alumina extraction, in a selection of food and beverage samples. The results provided an opportunity for qualitative and quantitative comparison of these two techniques and discussion of their respective merits and limitations.

The specificity associated with SIM is responsible for a significant reduction in the complexity of data obtained when using a GC–MS system. The use of appropriate deuterated internal standards eliminates the influence of the original sample matrix, minimizing sample pre-treatment, and allows precise multicomponent quantitation at low levels.

Davis *et al.* preferred to use GC–MS in the multiple-ion detection mode (MID) after derivatization with two different reagent systems and precipitation of protein with sulfosalicylic acid prior to extraction, in order to analyse a large number of biogenic amines and their metabolites in a single run.

Van den Berg *et al.* described the identification of a metabolite of spermidine by GC–MS in biological fluids of normal healthy humans and cancer patients. The quantification was based on stable isotope dilution mass fragmentography monitored at m/z 185 and 188.

Fujihara *et al.* measured putrescine metabolism in biological fluids. Putrescine was decomposed by oxidative deamination to form ammonia that was

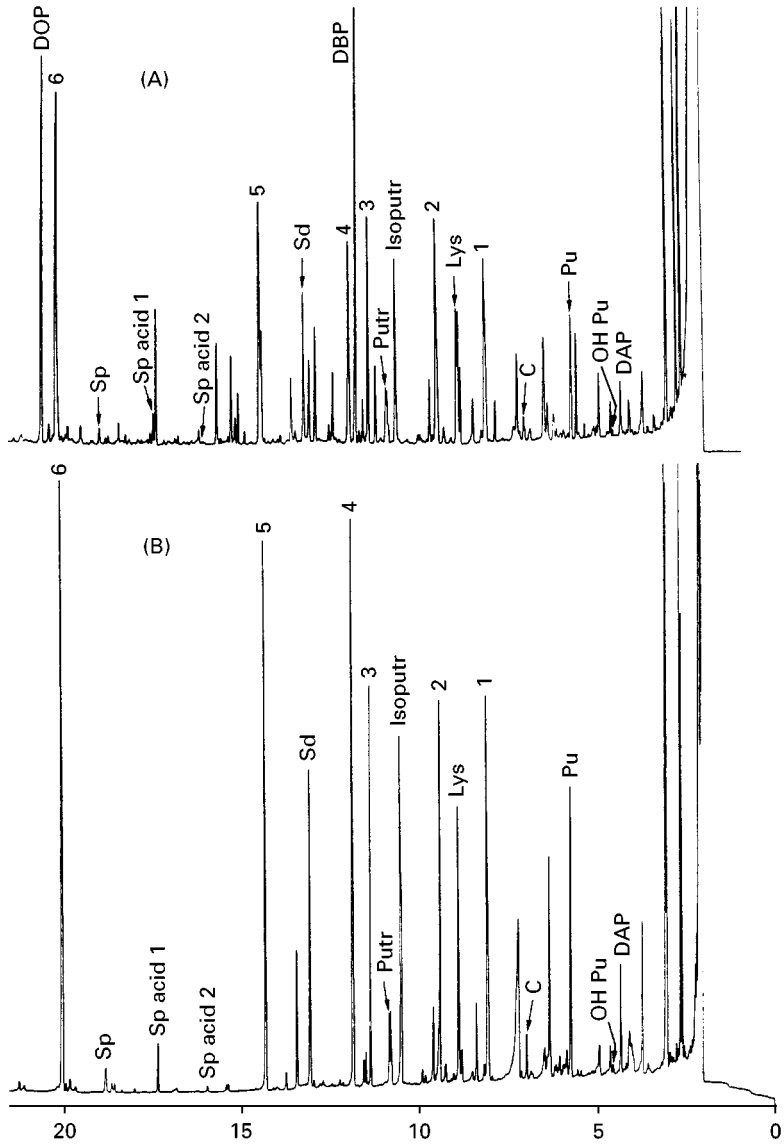

Figure 3 Comparison between typical chromatograms obtained with flame-ionization (A) and nitrogen–phosphorus (B) detectors after capillary gas chromatography of methyl-heptafluorobutyryl-derivatized extracts of acid-hydrolyzed urine from a normal man. *Abbreviations:* DAP, 1,3-diaminopropane; OH Pu, 2-hydroxyputrescine; Pu, putrescine; C, cadaverine; 1, 1,6-diaminohexane; Lys, lysine; 2, 1,7-diaminoheptane; *Isoputr*, isoputreanine; Putr, putreanine; 3, *N^1-methylisoputreanine*; DBP, *dibutylphthalate (plasticizer, tentatively identified by gas chromatography-mass spectrometry); 4, bis(3-aminopropyl)amine; Sd, spermidine; 5, N-(3-aminopropyl)-1,5-diaminopentane; Sp acid 2, N, N′*-bis(2-carboxyethyl)-1,4-diaminobutane; Sp acid 1, *N*-(3-aminopropyl)-*N′*-(2-carboxyethyl)-1,4-diaminobutane; Sp, spermine; 6, *N, N′*-bis(3-aminopropyl)-1,5-diaminopentane; DOP, dioctylphthalate (plasticizer). 1 through 6 are added internal standards. Time axis, minutes. (Reproduced, with permission, from Muskiet FAJ, van den Bergh GA, Kingma AW, Fremouw-Ottenvangers DC and Halie R (1984) Total polyamines and their non-α amino acid metabolites simultaneously determined in urine by capillary gas chromatography, with nitrogen–phosphorus detector; and some clinical applications. *Clinical Chemistry* 30: 687.)

determined by GC–MS. Its quantification was based on mass fragmentography synchronously monitored at m/z 195, 211 and 212. The method is inferior to isotope mass spectrometry, but its speed, reproducibility (better than 0.5%) and sensitivity are superior.

Shafi *et al*. applied GC–MS in the negative ion chemical ionization mode (NICI) for the determination of biogenic amines. After derivatizing the amines and separating them by solvent extraction, they hydrolysed the phenolic esters and converted the free hydroxyl groups to trimethylsilyl esters and analysed these derivatives by GC–MS–NICI. The molecular ion of these derivatives (together with the isotope peaks) carried more than 60% of the ion current, which made the method highly specific and gave a potential limit of detection below the picogram level.

This technique has the advantage that the silylating reagent may be changed in order to shift both the m/z

Table 4 GC–MS of biogenic amines

Analyte	*Chromatographic column*	*Carrier, type and flow rate*	*Injector temperature and program*	*Matrix*	*Sample treatment*	*Reference*
p-Tyramine, *p*-octopamine, *p*-synephrine, dopamine, noradrenaline, adrenaline, 5-hydroxytryptamine	Fused silica capillary HP 1, length 12.5 or, 25 m, i.d. 0.20 mm, film thickness 0.2 μm	He, 40 mL min^{-1}	250°C, 100°C → 10°C min^{-1} → 300°C	Biological tissues	Liquid–liquid extraction and derivatization with 5-ditrifluoromethylbenzoyl chloride and isopropyl dimethylsilyl-*N*-methyltrifluoroacetamide	Shafi (1995)
Indole ethylamines	Fused silica capillary OV 1, length 12 m, i.d. 0.20 mm, film thickness 0.33 μm		260°C, 100°C → 40°C min^{-1} → 300°C	Biological fluids	Formaldehyde addition, hyrdrolysis, derivatization with methyl chloroformate, liquid–liquid extraction and SPE purification	Musshoff *et al.* (1993)
Phenolamines, catecholamines, 5-hydroxytryptamine, phenylalanine, tyrosine, dopamine, *p*-tyramine, *p*-synephrine, *p*-octopamine, adrenaline, noradrenaline	Fused silica capillary SGE BP1, length 12 m, i.d. 0.20 mm, film thickness 0.25 μm	He	250°C, 100°C → 10°C min^{-1} → 300°C	Biological tissues	Liquid–liquid extraction and derivatization with butyldimethylsilyl chloride and ditri-fluoromethylbenzyl bromide	MacFarlane *et al.* (1991)
p-tyramine, *m*-, *p*-synephrine, *m*- *p*-octopamine, dopamine, 5-hydroxytryptamine, noradrenaline, adrenaline, dihydroxybenzylamine	Fused silica capillary HP1, length 12.5 m or 25 m, i.d. 0.20 mm	He	250°C, 100°C → 10°C min^{-1} → 300°C	Biological tissues	Extraction–derivatization with difluoromethylbenzoyl chloride, liquid–liquid extraction, derivatization with bistrimethyl silylacetamide (or *t*-butyldimethylsilyl chloride and imidazole) SPE purification	Shafi *et al.* (1989)
Putrescine	3% OV-1 on Chromosorb W AW DMCS 80–100 mesh packing, length 2 m, i.d. 3 mm	He, 60 mL min^{-1}	280°C, 280°C isothermal	Biological fluids	Derivatization of the ammonia resulting from metabolilsm with pentafluorobenzoyl chloride	Fujihara *et al.* (1986)
Spermidine	Fused silica capillary CP-Sil-19, length 25 m, i.d. 0.32 mm, film thickness 0.20 μm	He, 1 mL min^{-1}	250°C, 200°C → 10°C min^{-1} → 260°C	Biological fluids	Liquid–liquid extraction, derivatization with acetyl chloride	Van den Bergh *et al.* (1986)
Dopamine, adrenaline, noradrenaline, phenylethylamine, phenylethanolamine, tryptamine, *m*-, *p*-tyramine, *m*-, *p*-octopamine	Fused silica capillary DB-1, length 60 m, i.d. 0.32 mm	He	140°C → 10°C min^{-1} → 240°C	Biological fluids	Deproteination and derivatization with methanolic hydrogen chloride (or trifluoroethanol) and pentafluoropropionic anhydride	Davis *et al.* (1986)
Dopamine, norepinephrine (noradrenaline)	3% OV-17 packing, length 0.7–1 m	He, 30 mL min^{-1}	222°C, 146°C → 200°C	Foods, beverages	SPE purification, derivatization with trifluoroacetic anhydride and trifluoroethanol	Duncan *et al.* (1984)

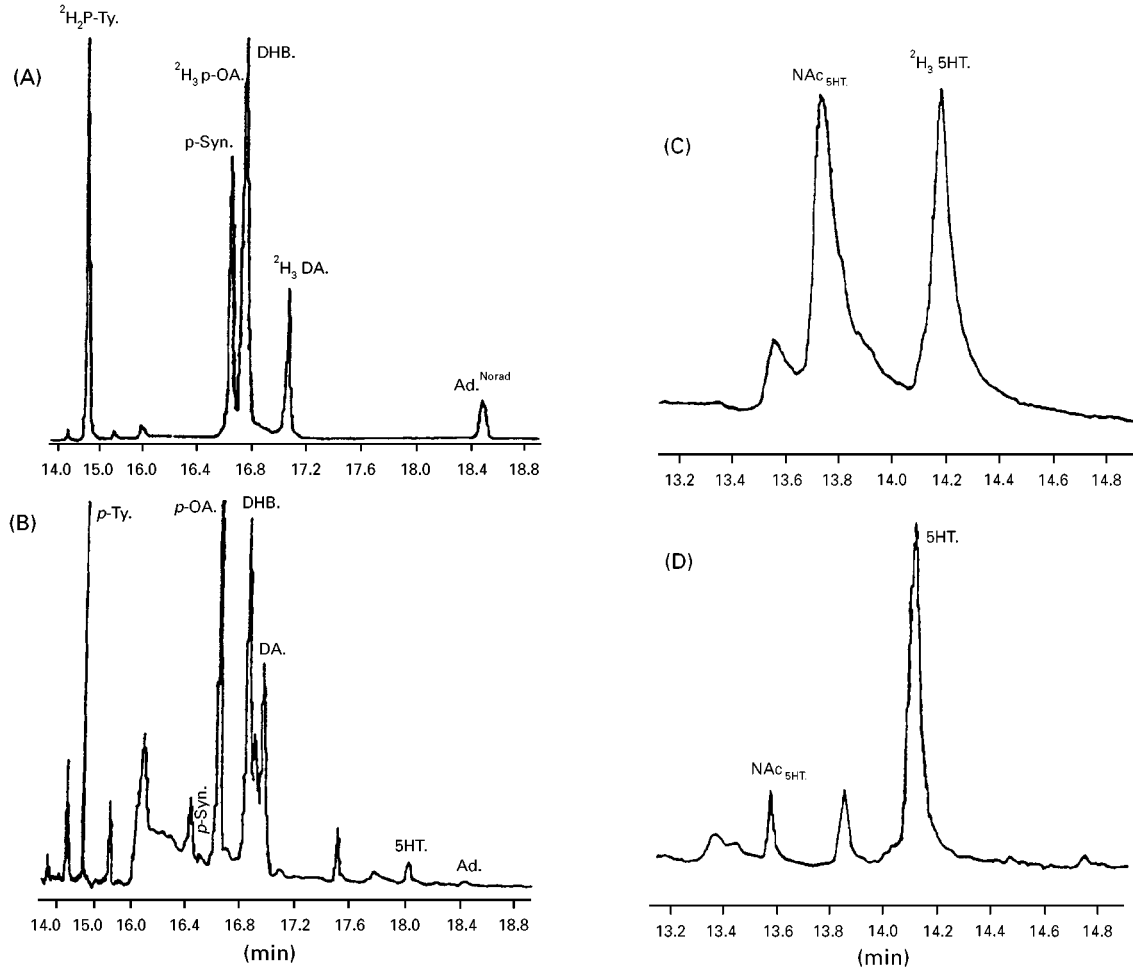

Figure 4 (A) NICI SIM trace of DTFMB-IPDMS derivatives of deuteriated and undeuteriated biogenic amines (each 20 ng) and (B) the corresponding endogenous biogenic amines from a single brain of American cockroach after addition of deuteriated internal standards (20 ng). (C) NICI SIM trace of Pr-PFP derivatives of deuteriated and undeuteriated biogenic amines (each 20 ng) and (D) the corresponding endogenous biogenic amines from a single brain of American cockroach after addition of deuteriated internal standards (20 ng). (Reproduced, with permission, from Shafi N (1995) Identification and quantitative determination of biogenic amines and their metabolites by gas chromatography negative ion chemical ionisation mass spectrometry (GC–NICIMS) *Journal of the Chemical Society of Pakistan* 17: 103.)

value of the molecular ion and the retention time of the derivative to ensure that its identification is unequivocal. In the same laboratories MacFarlane *et al.* used a similar procedure, but they state that the ion current was generally carried by four or five large ions.

Musshoff *et al.* derivatized the compounds formed by reaction of formaldehyde with biogenic amines in biological fluids in order to avoid the simultaneous formation of these compounds via condensation. These derivatization products are stable and can be well purified from most of the interfering matrix compounds by liquid–liquid and solid-phase extraction. They have good GC–MS properties, therefore the retention times, together with the diagnostic mass fragments (at least three) and the specific ion ratios, can be used for separation and identification.

Shafi summarized the use of different derivatizing agents, whose scope was shifting both the m/z value and the retention time in order to ensure the unequivocal identification of amines (**Figure 5**). In each case, the principal ion in the mass spectrum was the molecular ion, which carried almost all of the ion current under negative ion chemical ionization conditions; the sensitivity of this method of derivatization was 1 pg of biogenic amine on-column.

The possibility of easy determination of biogenic amines by other chromatographic techniques, such as HPLC, has reduced the interest in updating the existing gas chromatographic methods.

See also: **II/Chromatography: Gas:** Column Technology; Derivatization; Detectors: General (Flame Ionization

Detectors and Thermal Conductivity Detectors); Detectors: Mass Spectrometry; Detectors: Selective. **Extraction:** Solid-Phase Extraction.

Further Reading

Baker GB, Coutts RT and Holt A (1994) Derivatization with acetic anhydride: applications to the analyses of biogenic amines and psychiatric drugs by gas chromatography and mass spectrometry. *Journal of Pharmacological and Toxicological Methods* 31: 141.

Baker GB, Wong JTF, Coutts RT and Pasutto FM (1987) Simultaneous extraction and quantitation of several bioactive amines in cheese and chocolate. *Journal of Chromatography* 392: 317.

Bock UEG and Waser PG (1981) Gas chromatographic determination of some biogenic amines as their pentafluorobenzoyl derivatives in the picogram range and its applicability to biological materials. *Journal of Chromatography* 213: 413.

Davis BA, Durden DA and Boulton AA (1986) Simultaneous analysis of twelve biogenic amine metabolites in plasma, cerebrospinal fluid and urine by capillary column gas chromatography–high-resolution mass spectrometry with selected-ion monitoring. *Journal of Chromatography B* 374: 227.

Dorhout B, Kingma AW, de Hoog E and Muskiet FAJ (1997) Simultaneous determination of polyamines *N*-acetylated polyamines and the polyamine analogues BE-3-3-3 and BE-4-4-4-4 by capillary gas chromatography with nitrogen–phosphorus detection. *Journal of Chromatography B* 700: 23

Duncan MW, Smythe GA, Nicholson MV and Clezy PS (1984) Comparison of high-performance liquid chromatography with electrochemical detection and gas chromatography–mass fragmentography for the assay of salsolinol, dopamine and dopamine metabolites in food and beverage samples. *Journal of Chromatography B* 336: 199.

Fujihara S, Nakashima T and Kurogochi Y (1983) Determination of polyamines in human blood by electron-capture gas–liquid chromatography. *Journal of Chromatography B* 277: 53.

Fujihara S, Nakashima T and Kurogochi Y (1986) Determination of $^{15}NH_3$ by gas chromatography–mass spectrometry. Application to the measurement of putrescine oxidation by human plasma. *Journal of Chromatography B* 383: 271.

Guggenheim M (1940) *Die biogenen Amine*. Basel: Karger.

Halász A, Baráth A, Simon-Sarkadi L and Holzapfel W (1994) Biogenic amines and their production by microorganisms in food. *Trends in Food Science and Technology* 5: 42.

Hassels J, Kingma AW, Sturkenboom MCJM, Elzinga H, van den Bergh GA and Muskiet FAJ (1991) Gas chromatographic determination of *N*-acetylisoputreanine-γ-lactam, a unique catabolite of N^1-acetylspermidine. *Journal of Chromatography B* 563: 1.

Kim KR, Paik MJ, Kim JH, Dong SW and Jeong DH (1997) Rapid gas chromatographic profiling and screening of biologically active amines. *Jounal of Pharmaceutical and Biochemical Analysis* 15: 1309.

Laleye LC, Simard RE, Gosselin C, Lee BH and Giroux RN (1987) Assessment of Cheddar cheese quality by chromatographic analysis of free amino acids and biogenic amines. *Journal of Food Science* 52: 303.

MacFarlane RG, Midgley JM and Watson DG (1991) Biogenic amines: their occurrence, biosynthesis and metabolism in the locust, *Schistocerca gregaria*, by gas chromatography–negative-ion chemical ionisation mass spectrometry. *Journal of Chromatograpy B* 562: 585.

Muskiet FAJ, van den Bergh GA, Kingma AW, Fremouw-Ottenvangers DC and Halie R (1984) Total polyamines and their non-α-amino acid metabolites simulatneously determined in urine by capillary gas chromatography, with nitrogen–phosphorus detector; and some clinical applications. *Clinical Chemistry* 30: 687.

Muskiet FAJ, Dorhout B, van den Bergh GA and Hessels J (1995) Investigation of polyamine metabolism by high-perfomance liquid chromatographic and gas chromatographic profiling methods. *Journal of Chromatography B* 667: 189.

Musshoff F, Daldrup TH and Bonte W (1993) Gas chromatographic–mass spectrometric screening procedure for the identification of formaldehyde-derived tetrahydro-β-carbolines in human urine. *Journal of Chromatography B* 614: 1.

Parvez S, Nagatsu T and Parvez H (ed.) (1983) *Methods of Biogenic Amine Research*. Amsterdam: Elsevier.

Perez-Martin RI, Franco JM, Molist P and Gallardo JM (1987) Gas chromatographic method for the determination of volatile amines in seafoods. *International Journal of Food Science and Technology* 22: 509.

Shafi N (1995) Identification and quantitative determination of biogenic amines and their metabolites by gas chromatography negative ion chemical ionisation mass spectrometry (GC–NICIMS). *Journal of the Chemical Society of Pakistan* 17: 103.

Shafi N, Midgley JM, Watson DG, Smail GA, Strang R and MacFarlane RG (1989) Analysis of biogenic amines in the brain of the American cockroach (*Periplaneta americana*) by gas chromatography negative ion chemical ionisation mass spectrometry. *Journal of Chromatography B* 490: 9.

Staruszkiewicz WF Jr and Bond JF (1981) Gas chromatographic determination of cadaverine, putrescine and histamine in foods. *Journal of the Association of Official Analytical Chemists* 64: 584.

Van den Berg GA, Kingma AW, Elzinga H and Muskiet FAJ (1986) Determination of *N*-(3-acetamidopropyl) pyrrolidin-2-one, a metabolite of spermidine, in urine by isotope dilution mass fragmentography. *Journal of Chromatography B* 383: 251.

Yamamoto S, Iwado A, Hashimoto Y, Aoyama Y and Makita M (1984) Gas chromatography–mass spectrometry of polyamines as their *N*-ethyloxycarbonyl derivatives and identification of *sym*-homospermidine and *sym*-norspermine in mosses and ferns. *Journal of Chromatography* 303: 99.