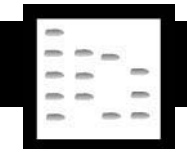
# AMINES: GAS CHROMATOGRAPHY



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Aliphatic and aromatic mono-, di- and polyamines are naturally occurring compounds formed as metabolic products in microorganisms, plants and animals, in which the principal routes of amine formation include the decarboxylation of amino acids, amination of carbonyl compounds and degradation of nitrogen-containing compounds. Accordingly, amines are important indicators of a wide variety of biochemical, clinical, toxicological and fermentation processes. Amines are also widely used as raw materials or as intermediates in the manufacture of industrial chemicals, e.g. pesticides, medicines, dyestuffs, rubbers, polymers, surfactants, cosmetics and corrosion inhibitors. Many of them are discharged into the atmosphere and water from anthropogenic sources such as foods, cattle feeds, livestock buildings, waste incineration, sewage treatment, automobile exhaust, cigarette smoke and various industries. Furthermore, many amines have an unpleasant smell and are hazardous to health as sensitizers and irritants to the skin, eye, mucous membranes and respiratory tract. Some amines are also suspected to be allergenic, mutagenic or carcinogenic substances due to their adsorption in living tissue. Amines are not only toxic of themselves but can also become toxic *N*-nitrosamines through chemical reactions with nitrosating agents such as nitrite or nitrate.

Gas chromatography (GC) has been widely used for amine analysis because of its inherent advantages of simplicity, high resolving power, high sensitivity, short analysis time and low cost. In addition, a wide variety of detectors can be used: nitrogen–phosphorus (NPD), electrolytic conductivity (ELCD) and chemiluminescent (CLD) detectors offer increased selectivity for specific amines. Furthermore, the combined technique of GC-mass spectrometry (MS) can provide structural information for the unequivocal identification of amines. Sub-nanogram detection limits can be achieved using these detectors. However, GC separation of free amines at very low concentrations generally has inherent problems related to the difficulty in handling low molecular mass amines because of their high water solubility, high volatility and ready oxidation under chromatographic conditions. Furthermore, amines tend to be strongly adsorbed and decomposed on the columns and give tailing peaks, ghosting phenomena and low detector response. The adsorption tendency in the analytical system, i.e. in sample vessels, injector, glass wool and GC column, is in the order primary > secondary > tertiary amines, and tailing becomes increasingly severe as the basicity of the amines increases. In addition, it is generally more difficult to chromatograph aliphatic than aromatic amines.

A common method of overcoming these problems is to convert such polar compounds to relatively nonpolar derivatives more suitable for GC analysis. A number of derivatives such as acyl, silyl, dinitrophenyl, permethyl, Schiff base, carbamate, sulfonamide and phosphonamide compounds have been used for this purpose.

Another successful approach has been to employ less reactive column packing materials to reduce the interaction with solutes, for example, the use of porous polymers and the deactivation of supports by treatment with alkali. Wall-coated (WCOT), support-coated (SCOT) and porous-layer (PLOT) open tubular capillary columns, which minimize column–solute interactions, have also been used for this purpose. Free amines can be analysed after addition of alkali, either by direct injection or by headspace sampling, or they can be extracted into an organic solvent before analysis. Direct or headspace analysis of samples minimizes sample preparation, thereby reducing the possibility of contamination. Solid-phase microextraction (SPME), with integrated sampling, extraction, concentration and sample introduction in a single step, has recently been used for amine analysis by coupling with GC.

This article is concerned with the general aspects of direct GC separation of underivatized aliphatic and aromatic amines, and various characteristics with respect to columns are considered in more detail below.

## Column Development

Packed-column GC is generally simpler to set up than capillary GC, because of the ability to apply the stationary phase easily to the solid support and modify it appropriately to the particular analysis

required. Deactivation of the glass surface can be effected using a suitable silylating reagent to limit the effect of adsorption on the wall of the column. However, the general difficulty in the chromatography lies in absorptivity on the solid support leading to tailing. The adsorption of amines by the support material has been attributed to the presence of free silanol groups on the silica surface participating in hydrogen bonding with the free electron pair of the nitrogen atom of the amine. Simple treatment with KOH reduces the adsorption to a minimum, allowing good peak shape and optimum performance.

Glass and fused silica capillary columns have also been used for the analysis of free amines. The inherent strength and flexibility of fused silica make it easier to use and less fragile than glass capillary columns. Furthermore, fused silica provides a more inert surface for improved performance and less adsorption. The analysis of free amines on packed columns has now largely been replaced by analysis on fused silica capillary columns that are commercially available with a range of stationary phases. The packed and capillary GC columns reported in the past 30 years for amine analysis are summarized in **Tables 1** and **2**.

## Packed Columns

Three types of packing can be used to separate amines: graphitized carbon coated with a stationary phase and deactivated, coated and uncoated porous polymers, and conventional columns packed with a deactivated diatomaceous earth coated with a stationary phase. The columns are usually deactivated with KOH, trimethylchlorosilane (TMCS) or ammonia in the carrier gas. Carbopack graphitized carbon and porous polymer packings are well suited for separating $C_1$–$C_{10}$ compounds, but retention times for larger molecules are excessive; deactivated and coated conventional packings are better suited to the analysis of higher molecular weight amines.

**Graphitized carbon packings** Graphitized carbon packings are generally used for free amine analysis after coating with a stationary phase. Sterling FT-G and Vulcan, sold by Supelco as Carbopack A and Carbopack B, respectively, have been used for the analysis of $C_1$–$C_{16}$ aliphatic amines with suitable amounts of KOH and polyethylene glycol (PEG), e.g. PEG 20M and PEG-1500. A 4.8% PEG 20M/0.3% KOH on Carbopack B column is recommended for the analysis of $C_1$–$C_4$ aliphatic amines in aqueous solution at nanogram level. This column offers complete separation of the $C_2$–$C_3$ amine isomers and is less affected by water than the other packed columns. However, the preparation of the column seems to be difficult for routine analysis. A 1.5% UCON 50-HB-2000 on Carbopack B packing deactivated with 0.8% KOH has also been used to separate a mixture of aliphatic, aromatic and cyclic amines, and rapid separation of nine amines without ghosting was obtained by temperature programming and treatment of glass wool in the column ends with dimethylchlorosilane (DMCS). On the other hand, 4% Carbowax 20M on 0.8% KOH-deactivated Carbopack B packing has

**Table 1** Packed columns for analysis of free amines

| *Column packing* | *Type* | *Length (m)* | *Amine* | *Detection* |
|---|---|---|---|---|
| 1.3% PEG 20M/0.3% KOH on Sterling FT-G | GC | 2.0 | AL | FID |
| 0.5% PEG-1500/0.2% KOH on Sterling FT-G | GC | 1.4 | AL | FID |
| 4% PEG 20M/0.8% KOH on Vulcan | GC | 1.4 | AL | FID |
| 4.8% PEG 20M/0.3% KOH on Carbopack B | GC | 1.8 | AL | FID |
| 1.5% UCON 50-HP/0.8% KOH on Carbopack B | GC | 1.83 | AL, AR | FID |
| 4% Carbowax 20M/0.8% KOH on Carbopack B | GC | 1.7–3.75 | AL | FID, NPD |
| Tenax GC | PP | 1.52 | AL | FID |
| Chromosorb 103 | PP | 1.5–3.3 | AL | FID, NPD |
| Chromosorb 102/5% TMCS/5% KOH | PP | 2.0 | AL | NPD |
| 5% Squalene/2% KOH on Chromosorb 103 or 104 | PP | 3.0 | AL | CLD |
| 4% Carbowax 20M/1% KOH on Corning glass | PC | 1.8 | PO | FID |
| 10% Carbowax 20M/2% KOH on Chromosorb W AW | PC | 1.5–1.9 | AL | FID, NPD |
| 5% PEG-1000/0.5% $Na_3PO_4$ on Chromosorb G | PC | 2.0 | AL | SID |
| 5% PEG-HT/1% KOH on Umiport HP | PC | 2.0 | AR | FID |
| 3% SP-2250 on Supelcoport | PC | 1.83 | AR | NPD |
| 5% SP-2401-DB on Supelcoport | PC | 1.83 | AR | NPD |
| 1.5% SP-2250/1.95% SP-2401 on Supelcoport | PC | 1.83 | AR | NPD |
| 3% Silar 5CP on Supelcoport | PC | 1.83 | AR | NPD |

GC, Graphitized carbon; PP, porous polymer; PC, partition column; AL, aliphatic amine; AR, aromatic amine; PO, polyamine; FID, flame ionization detection; NPD, nitrogen–phosphorus detection; surface ionization detection; CLD, chemiluminescence detection.

**Table 2** Capillary columns for analysis of free amines

| *Column* | | | *Amine* | *Detection* |
|---|---|---|---|---|
| *Stationary phase* | *Type* | *Length (m)* | | |
| 10% PEG 400 | WCOT/G | 99 | AR | FID |
| 5% PEG 400/2% KOH | WCOT/G | 40 | AR | FID |
| Supelcowax 10 | WCOT/G | 10 | AL, AR | FID |
| SP-2250 | WCOT/G | 30 | AR | NPD |
| SP-2100 | WCOT/G | 30 | AR | NPD |
| Carbowax 20M | WCOT/G,F | 25–37 | AR | FID, NPD, MSD |
| SE-54 | WCOT/G,F | 30 | AR, DR | FID, NPD |
| SE-52 | WCOT/G,F | 30 | AR | NPD, ELCD, PID |
| SE-30 | WCOT/G,F | 30 | AR | FID, NPD |
| CAM | WCOT/F | 30 | AL | FID |
| HP-20M | WCOT/F | 25 | AR | FID |
| Carbowax Amine | WCOT/F | 30 | AL, AR | FID |
| PoraPLOT Amines | PLOT/F | 25 | AL | FID, ELCD |
| CP-Sil-19CB | WCOT/F | 10 | AL, AR | FID |
| DB-35ms | WCOT/F | 25 | AR | FID |
| DB-5ms | WCOT/F | 30 | AL, AR | FID, MSD |
| HP-5 | WCOT/F | 25–30 | DR | FID, MSD |
| HP-101 | WCOT/F | 25 | AL, AR | FID |
| HP-1 | WCOT/F | 10–30 | AL, AR | FID, NPD |
| DB-1 | WCOT/F | 30 | AL, DR | FID, MSD |
| OV-1 | WCOT/F | 25 | DR | MSD |
| SBP-1 | WCOT/F | 30 | AL, AR | FID |
| CBJ-17 | WCOT/F | 30 | DR | NPD |

WCOT, Wall-coated; PLOT, porous layer; G, glass; F, fused silica; AL, aliphatic amine; AR, aromatic amine; DR, basic drug; FID, flame ionization detection; NPD, nitrogen–phosphorus detection; ELCD, electrolytic conductivity detection; MSD, mass selective detection; PID, photoionization detection.

been specifically developed for monitoring low molecular weight aliphatic amines at p.p.m. levels in water. Heterocyclic amines can also be separated on this packing, but aromatic amines exhibit excessively long retention times. By using this column and an NPD, low molecular weight amines in sea water were determined. In order to reduce the appearance of ghost peaks, 15% ammonia solution was injected on to the hot (150–200°C) column after each sample run. As shown in **Figure 1**, 23 amines were separated within 25 min (Figure 1A), and 12 amines were selectively detected in sea water (Figure 1B).

A general characteristic of Carbopack-based columns is that sample components are separated by carbon number and are eluted in the order $C_1 \rightarrow C_2 \rightarrow C_3$, and so forth. This is seen in the separation of methylamine, dimethylamine, trimethylamine and ethylamine. Both $C_2$ amines (dimethylamine and ethylamine) are eluted before the $C_3$ amine (trimethylamine). On these packings, it is necessary to use small samples to prevent tailing due to overloading. Furthermore, the column must be conditioned by injecting a number of relatively large amounts of water when analysing amines in aqueous solution. This treatment converts any $K_2CO_3$ in the column to KOH, making the column more basic and improving its inertness for amines. Acidic compounds in the sample are irreversibly adsorbed by the KOH. In addition, a certain amount of stationary phase is hydrolytically decomposed and appears as a water peak in the chromatogram when water passes through the column. Therefore, conditioning is needed to clean the column and minimize the water peak when standards and sample are subsequently injected. These packed columns should not be exposed to air, since the packing will adsorb carbon dioxide and lose its deactivation. Furthermore, the water used should be distilled or deionized, and freshly boiled to remove $CO_2$.

**Porous polymer packings** Porous polymers possessing large surface area are often used as column packing in GC without coating with a stationary phase. Tenax GC and Chromosorb 103 were specifically developed to separate low molecular weight aliphatic amines. Although Chromosorb 103 proved inconsistent and difficult to handle, and tended to expand on heating, leaving gaps in the column upon cooling, these effects could be minimized by paying scrupulous attention to packing. By using Chromosorb 103,

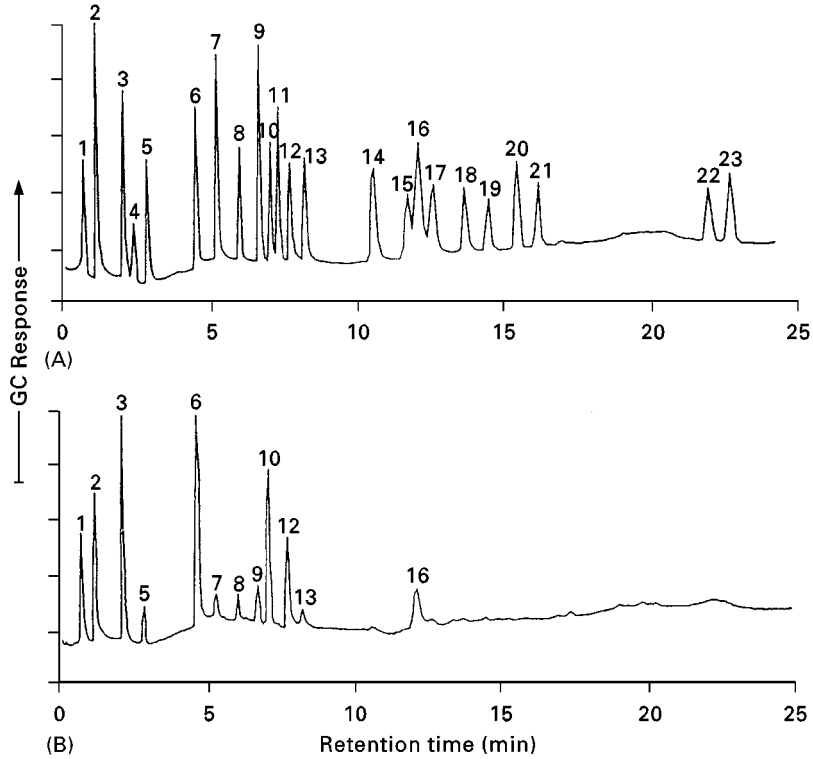


**Figure 1** (A) Amine standards and (B) a sea water sample. GC conditions: packed column, 4% Carbowax 20 M and 0.8% KOH on Carbopack B (2 m × 2.5 mm i.d. glass); column temperature, initially hold at 85°C for 2.5 min, increase to 150°C at 32°C $min^{-1}$ for 6 min and then to 220°C at 10°C $min^{-1}$; injector and detector temperatures, 200 and 220°C, respectively; He carrier gas flow rate, 22 mL $min^{-1}$; detector, NPD. Peaks: 1, ammonia; 2, monomethylamine; 3, dimethylamine; 4, ethylamine; 5, trimethylamine; 6, 2-propylamine; 7, 1-propylamine; 8, *tert*-butylamine; 9, diethylamine; 10, *sec*-butylamine; 11, 2-butylamine; 12, pyrrolidine; 13, 1-butylamine; 14, piperidine; 15, triethylamine; 16, pyridine; 17, 2-amylamine; 18, 1-amylamine; 19, pyrrole; 20, dipropylamine; 21, cyclohexylamine; 22, tripropylamine; 23, dibutylamine. (Reproduced with permission from Yang *et al.* (1993) *Analytical Chemistry* 65: 572.)

11 aliphatic amines were isothermally separated without the ghosting observed with alkali-washed support packings. The use of longer columns resulted in increased analysis time that could not be reduced with a higher final temperature owing to excessive column bleed. Amines tail on other porous polymers, but performance can be improved by coating them with a stationary phase and TMCS. By using 5% squalene/2% KOH on Chromosorb 103 or 104 and GC-CLD, low molecular weight aliphatic amines have been determined. A column packed with Chromosorb 102 treated with 5% TMCS and coated with KOH has been used to determine methylamines in biological materials at low concentration by headspace GC-NPD. Use of headspace sampling avoids the possibility of interference from other water-soluble biological substances.

**Partition columns** A partition column consists of a support, generally a diatomaceous material, coated with a stationary phase. However, the support tends to interact with active analytes, such as amines, causing the peaks to tail, unless it is made strongly basic by adding KOH or an amine or by using an amine as the stationary phase. This alkaline deactivation of diatomaceous supports appears to be more effective than silanization for the analysis of amines. The major disadvantage of alkali-washed packings lies in the thermal instability of the liquid phases that prevents temperature-programmed analysis. Generally, Chromosorb W (white and light weight) and Chromosorb P (pink) supports are used as support materials; polytetrafluoroethylene supports are widely regarded as very inert, but they do not appear to be especially inert to amines. The stationary phase must be compatible with the basic material. Polyglycols, such as Carbowax and certain hydrocarbons, have been used successfully with basic materials.

Although a 10% Carbowax 20M/2% KOH on Chromosorb W AW packing was used for separating aliphatic mono- and diamines, many of the higher boiling anilines apparently did not elute from the column. Other PEG packings, such as PEG-1000 on Chromosorb G and PEG-HT on Uniport HP, have

also been used for the analysis of aliphatic and aromatic amines. Di- and polyamines in tissue samples were analysed using Corning glass beads coated with 4% Carbowax and 1% KOH. This column gave a good separation and a nearly complete recovery of these amines.

Aromatic amines are generally less basic than aliphatic amines and consequently present less of an adsorption problem. Although they can be separated on the highly basic columns described above, the analysis is generally carried out with a silicone stationary phase on an acid-washed, dimethylchlorosilane (AW-DMCS) treated support. The SP-2250 and SP-2401-DB packings were specially developed for separating amines at low concentrations. When analyte concentrations are very low, even AW-DMCS treatment is inadequate, and it is necessary to use a specially deactivated column or derivatize the analytes. The 3% SP-2250 column partially resolved all the anilines, but some peak tailing was evident, especially for aniline. The 5% SP-2401-DB (containing KOH) and 1.5% SP-2250/1.95% SP-2401 gave no improvement in peak shape, in spite of the greater polarity, compared to SP-2250. In addition, several compounds were not resolved on these columns. The Silar 5CP column partially resolved all the anilines except for 3- and 4-chloroanilines, and gave good peak shape for all the compounds. As shown in **Figure 2**, the best separation of 19 anilines was obtained using a 3% SP-2250 on Supelcoport.

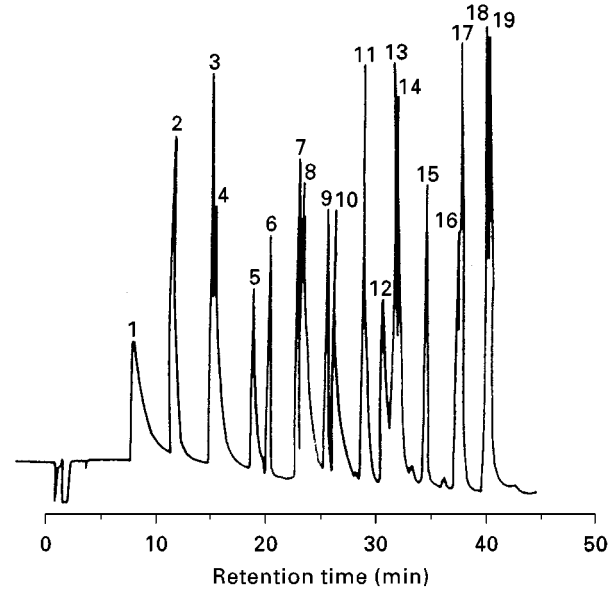


**Figure 2** Aniline compounds on packed column. GC conditions: column, 3% SP-2250 on Supelcoport (1.83 m × 2 mm i.d. glass); column temperature, 4°C $min^{-1}$ from 80 to 230°C; injector and detector temperatures, 250 and 300°C, respectively; He carrier gas flow rate, 30 mL $min^{-1}$; detector, NPD. Peaks: 1, aniline; 2, 2-chloroaniline; 3, 3-chloroaniline; 4,4-chloroaniline; 5, 4-bromoaniline; 6, 3,4-dichloroaniline; 7, 2,4,6-trichloroaniline; 8, 2-nitroaniline; 9, 2,4,5-trichloroaniline; 10, 3-nitroaniline; 11, 4-chloro-2-nitroaniline; 12, 4-nitroaniline; 13, 2,6-dichloro-2-nitroaniline; 14, 2-chloro-4-nitroaniline; 15, 2-bromo-6-chloro-4-nitroaniline; 16, 2,6-dibromo-4-nitroaniline; 17, 2-chloro-4,6-dinitroaniline; 18, 2,4-dinitroaniline; 19, 2-bromo-4,6-dinitroaniline. (Reproduced with permission from Riggin *et al.* (1983) *Analytical Chemistry* 55: 1862.)

### Capillary Columns

Capillary columns offer a significant improvement in separation, in comparison to conventional packed columns, and have been used for the separation of complex mixtures and components closely related chemically and physiologically. As shown in **Table 2**, various glass and fused silica capillary columns have been used for free amine analysis. Fused silica capillary columns provide strength, flexibility and a more inert surface for improved performance and less adsorption. Cross-linked or bonded-phase columns can be washed with solvents, prolonging their lifetime. Furthermore, the advance of commercially available cross-linked and bonded-phase capillary columns and precise temperature-controlled GC ovens has meant that the retention times are extremely reproducible. This is critical when using automated date-handling equipment for identification and quantification. Typically, 10–30 m long columns, coated with either nonpolar or polar stationary phases, have been used for amine analysis. Many of the phases used today are specifically manufactured by the column supplier, and give excellent performance, low bleed and high efficiency. However, there is the drawback that a column from one supplier may not give the same separation as the nominally equivalent column from another supplier.

**Glass capillary columns** In early work, glass capillaries were employed for the separation of aromatic amines using alkaline PEG as the stationary phase. Although a disadvantage of this phase is its tendency to deteriorate at temperatures slightly above 200°C, it has been used for the separation of methylanilines and methylpyridines in coal-tar light oil. Carbowax 20M columns have been used for the determination of airborne aromatic amines with an NPD. The necessary inertness of glass capillary columns may be achieved by deactivation with octamethylcyclotetrasiloxane (OMCTS). The glass or fused silica columns were silanized using OMCTS and trifluoropropyl(methyl)cyclosiloxane, and coated with various phases (SE-30, SE-52, SE-54). Test mixtures containing about 1 ng of such difficult substances as primary mono- and diaminoalkanes gave symmetrical peaks on some of these phases. As shown in

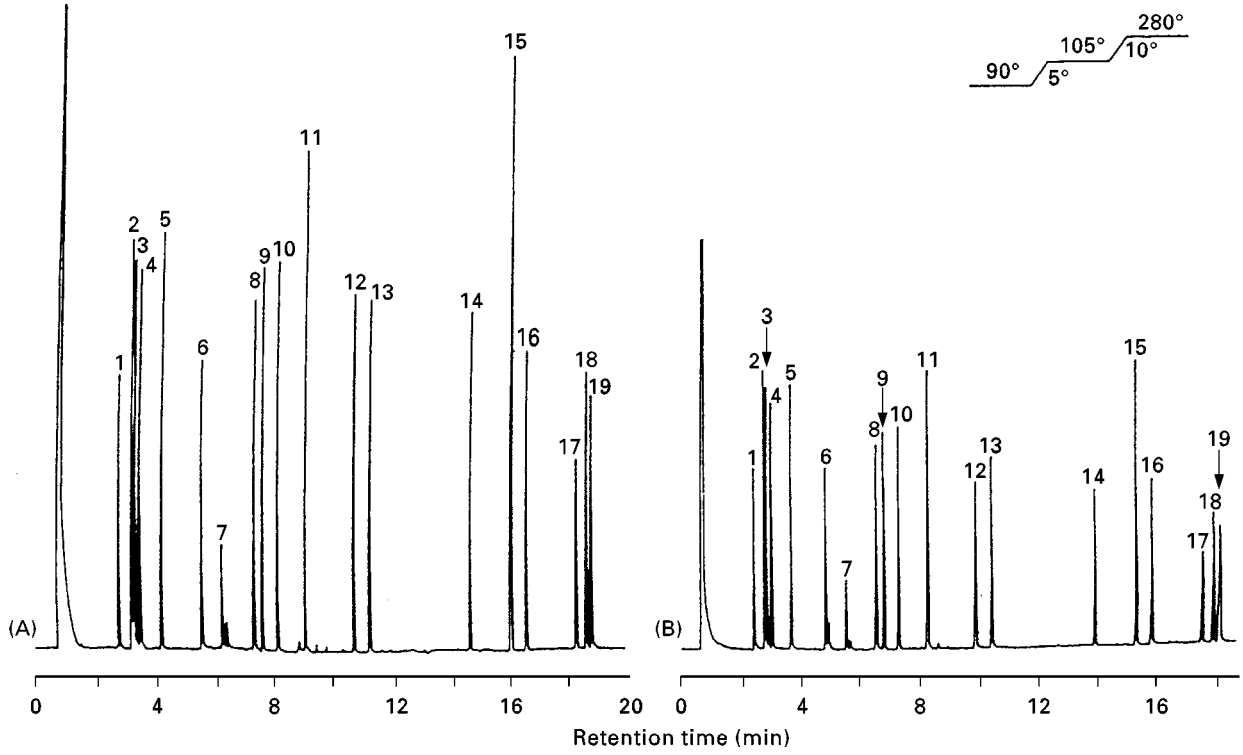


**Figure 3** Drug standard mixtures on (A) AR glass and (B) fused silica capillary columns coated with SE-54 and with flame ionization detector. Temperature programmes are shown within the figure. Peaks: 1, amphetamine; 2, phentermine; 3, propylhexedrine; 4, methamphetamine; 5, ethylamphetamine; 6, propylamphetamine; 7, ephedrine; 8, phenmetrazine; 9, phendimetrazine; 10, amfepramone; 11, benzocaine; 12, phenacetin; 13, methyl phenidate; 14, procaine; 15, methaqualone; 16, cocaine; 17, codeine; 18, ethylmorphine; 19, morphine. (Reproduced with permission from Blomberg *et al. Journal of Chromatography* 239 (1982) 51).

**Figure 3**, the separation of some underivatized drugs is equally good on alkali-resistant (AR) glass and fused silica capillaries, although alkali-resistant (AR) glass has a basic character that can be reduced by careful leaching.

On the other hand, interesting results dealing with the separation of free amines and other nitrogen compounds were reported in glass capillary columns with stationary phases polymerized *in situ*.

**Fused silica capillary columns** For the analysis of amines, capillary columns with a nonimmobilized PEG-type stationary phase have been specially prepared and are commercially available. For the analysis of volatile amines, aromatic and heterocyclic amines and other amino substances, CAM, CP-Wax, HP-20M, Carbowax 20M and Carbowax Amine capillary columns have been recommended. These columns are generally deactivated with KOH to elute basic compounds with good peak shapes and responses. Three types of fused silica capillary columns, Supelcowax 10 (PEG), CP-Sil-19CB (methylphenylcyanopropylsilicone) and HP-1 (methylsilicone) have also been used for the separation of aliphatic and aromatic amines. Ammonia as a carrier gas can drastically affect the retention factors and improve the peak symmetry for aliphatic amines. A porous polymer fused silica capillary column, PoraPLOT Amine, has been used to separate very volatile amines. By using this column and ELCD, $C_1$–$C_6$ amines in aqueous and methanolic solution were analysed. The separation of aniline and its halogen and nitrogen derivatives in waste water were evaluated using several glass and fused silica capillary columns of polysiloxane type (SE-30, SE-52, SE-54, SP-2100) and NPD. Each of the capillary columns gave excellent peak shape for all the anilines, but failed to resolve at least one compound pair (e.g. the SE-30 completely resolved 3- and 4-chloroaniline that co-eluted on SE-54, but failed to resolve the 2,6-dibromo-4-nitroaniline and 2,4-dinitroaniline which were completely resolved on SE-54). **Figure 4** shows a chromatogram of an aniline mixture on an SE-54 fused silica column. The NPD sensitivities for many anilines are substantially better with the SE-54 capillary column (Figure 4) than with the 3% SP-2250 packed column (Figure 2), primarily because less peak tailing is observed at low concentration. Interestingly, the fused silica and glass capillary SE-54 columns gave different elution patterns for the various anilines. Using both SE-54 and SE-30

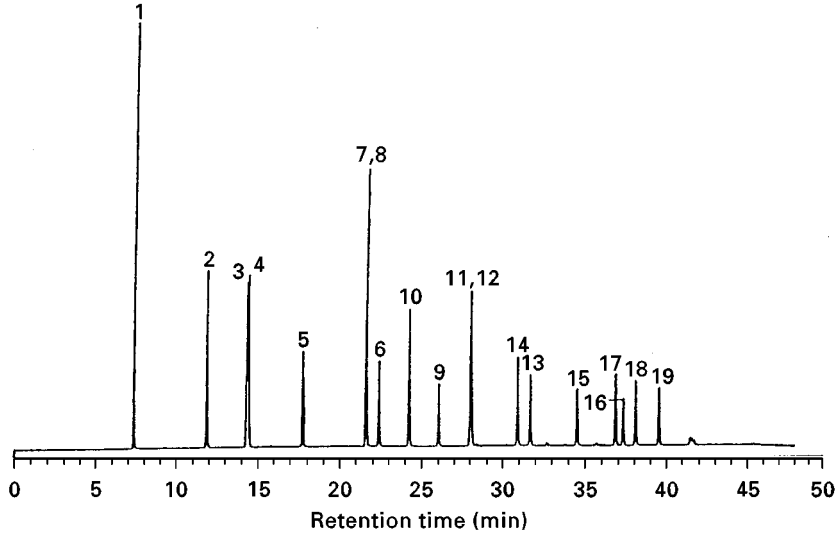


**Figure 4** Aniline compounds on fused silica capillary column. GC column, SE-54 (30 m × 0.25 mm i.d.); He carrier gas flow rate, 30 cm $s^{-1}$; split ratio, 10 : 1. Other conditions and peak numbers are the same as Figure 2. (Reproduced with permission from Yang *et al.* (1993) *Analytical Chemistry* 65: 572.)

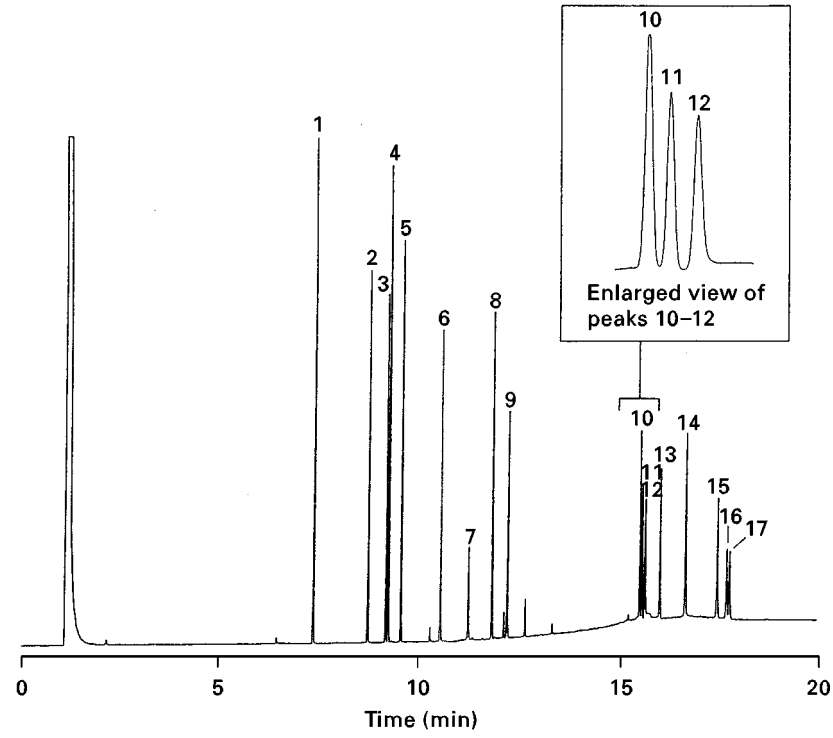


**Figure 5** Aromatic amines on fused silica capillary column. GC conditions: column, DB-35ms (25 m × 0.20 mm i.d. glass); column temperature, initially hold at 50°C for 2 min, increase to 340°C at 20°C $min^{-1}$ and then hold at 340°C for 10 min; injector and detector temperatures, 280 and 320°C, respectively; He carrier gas flow rate, 35 $cm^{-1}$; splitless injection; detector, NPD. Peaks: 1, *o*-toluidine; 2, 4-chloroaniline; 3, 2-methoxy-5-methylaniline; 4, 2,4,5-trimethylaniline; 5, 4-chloro-2-methylaniline; 6, 2,4-diaminotoluene; 7, 2,4-diaminoanisole; 8, 2-aminonaphthalene; 9, 2-methyl-5-nitroaniline; 10, 4,4′-oxydianiline; 11, 4,4′-methylenedianiline; 12, benzidine; 13, 2-aminoazotoluene; 14, *o*-tolidine; 15, 4,4′-thiodianiline; 16, 3,3′-dimethoxybenzidine; 17, 3,3′-dichlorobenzidine. (Reproduced with permission from Catalog and Technical Reference, C407, J & W Scientific, California.)

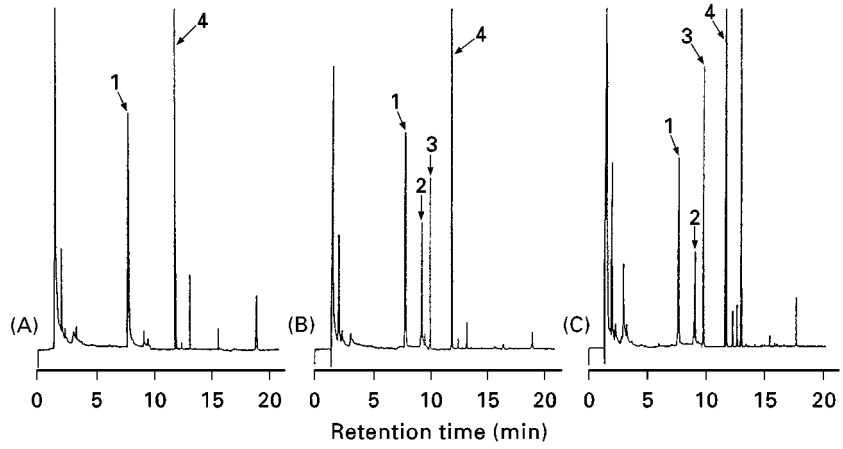


**Figure 6** Chromatograms obtained from hair samples. (A) Normal hair; (B) normal hair with standard amphetamines added; (C) abuser's hair. GC conditions: column, CBJ-17 (30 m × 0.53 mm i.d. fused-silica, Shimadzu); column temperature, initially hold at 100°C for 5 min, increase to 220°C at 10°C $min^{-1}$ and then hold at 220°C for 3 min; injector and detector temperatures, 220°C; He carrier gas flow rate, 4 mL $min^{-1}$; split ratio, 2 : 1; detector, NPD. Peaks: 1, α-phenethylamine (internal standard); 2, amphetamine; 3, methamphetamine; 4, *N*-propyl-β-phenethylamine (internal standard).

fused silica capillary columns, all 19 anilines can be resolved.

A polysiloxane capillary column specially designed for the analysis of basic compounds using new deactivation technologies has been developed. This proprietary deactivation provides both the inertness (basicity) and surface energies required to coat a 5% diphenyl/95% dimethylpolysiloxane stationary phase successfully. Using this column, $C_3$–$C_{10}$ primary amines can be separated as symmetrical peaks. This column allows lower limits of detection for basic compounds such as substituted anilines and benzidines. Since the column is virtually identical in polarity to the widely used ordinary columns with the same stationary phase, it can be directly substituted and run under the same temperature conditions. DB-5ms and DB-35ms columns certified for use with MS have been developed for the analysis of aliphatic and aromatic amines. These columns have very low bleed characteristics and excellent inertness. As shown in **Figure 5**, 17 aromatic amines were completely separated using a DB-35ms column.

Lower aliphatic tertiary amines in environmental samples were analysed by headspace GC with a mass selective detector (MSD) using a polymethylsiloxane column. The SPME method has gained popularity as a solvent-free, reliable and flexible tool for sampling a variety of volatile and semi-volatile compounds. By combining SPME with GC, these compounds can be simply and rapidly extracted, concentrated and introduced into the GC system. Using headspace SPME and GC-MSD on polysiloxane-type fused silica capillary columns such as DB-1, OV-1, SPB-1, HP-1 and HP-5, amphetamine, methamphetamine and related stimulants in urine can be analysed at the ng $mL^{-1}$ level. Recently, headspace SPME and GC-NPD using a slightly polar capillary column CBJ-17 (**Figure 6**) has developed as a method for determining amphetamines in human hair.

## Future Prospects

Much of the early work on the separation of free amines was done with columns packed with PEG and KOH on diatomaceous earths. Although this approach was reasonably successful, the analysis of free amines on packed columns has now largely been replaced by analysis on fused silica capillary columns. Application of capillary columns is expected to increase as further developments in these columns, e.g. shorter inactive columns with smaller internal diameters giving ultra-high column efficiency and speed, higher temperature phases and exterior coating for the fused silica tubing, permit the analysis of both high temperature and highly volatile amines. Furthermore, simple, rapid and automatic analysis of free amines in various samples will be achieved by combination with convenient sample preparation techniques such as SPME.

*See also:* **II/Chromatography: Gas:** Column Chromatography; Detectors: Selective.

## Further Reading

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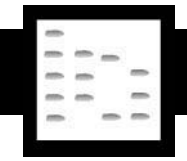
# AMINO ACIDS



## Gas Chromatography

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During the 1950s and 1960s, significant progress was made in the development of automated amino acid analysers based on separation by ion exchange. However, such instruments were dedicated to the task of amino acid analysis and were of limited application to the analysis of other types of compounds. Furthermore, they were expensive. During the same period, gas chromatography (GC) was being rapidly developed following the demonstration in 1952 by James and Martin that fatty acids could be assayed by GC. There followed a vast expansion in the application of GC to the analysis of other types of compounds. Amino acids were a logical target. In the intervening years, methods have been developed for assaying amino acids in protein hydrolysates and physiological fluids, and for determining the proportions of amino acid enantiomers in racemic mixtures. Some landmark developments are listed in **Table 1**.

### Proteic and Physiological Amino Acids

#### Derivative Development

Amino acids are not sufficiently volatile or stable at the temperatures required for analysis by GC. Thus, they must be converted to derivatives having the desired characteristics. It was to be no simple task to derivatize or mask the several functional groups in even the 20 proteic amino acids. Carboxy, amino, hydroxy and sulfhydryl groups all need to be converted to eliminate internal zwitterionic charges and hydrogen bonding, and thus increase the volatility of the derivatives. It was thought in those early years that the molecular mass also required to be reduced but it was later realized that this was not an absolute requirement. As new reagents became available, it was found that volatility could be significantly increased while increasing the derivative mass. Apart from the multiplicity of functional groups, it is also necessary that each group should be quantitatively converted.

The first report of amino acid analysis by gas liquid chromatography was published in 1956. Hunter, Dimick and Corse oxidized isoleucine and leucine with ninhydrin to form volatile aldehydes. These were resolved using a 10 ft long silicone oil–celite column operated isothermally at 69°C. Peaks were detected at about 44 and 48 min (**Figure 1**). The aldehydes were generated using 2–5 mg of each amino acid. Either of the leucines could be assayed in the presence of 10-fold quantities of the other. However, only about eight simple amino acids yield volatile aldehydes.

From this simple but momentous beginning, there followed, in the next two decades, a proliferation of reaction schemes to prepare stable, volatile amino acid derivatives. Various oxidation, hydrocracking, pyrolysis and reduction reactions were explored but significant progress was to evolve from those procedures which focused on substituting the exchangeable protons of the reactive groups. In 1957, Bayer, Reuther and Born separated glutamic acid, leucine, methionine, norleucine, norvaline, phenylalanine, sarcosine and valine methyl esters on a silicone oil–sodium caproate packing. The use of an acyl ester constituted the first report of a key component in