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# Determination of primary and secondary amines in foodstuffs using gas chromatography and chemiluminescence detection with a modified thermal energy analyser

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## ABSTRACT

A simple method is described for the determination of primary and secondary amines in foodstuffs by gas chromatography with a modified thermal energy analyser, operated in the nitrogen mode. Food samples were subjected to mineral oil vacuum distillation and the isolated amines were derivatized with benzenesulphonyl chloride to form the corresponding sulphonamides, which were fractionated to yield primary and secondary amine derivatives using a modified Hinsberg procedure. The detection limit for individual amines using a 10-g food sample was 10 $\mu$g/kg (ppb) and recoveries were in excess of 80%.

## INTRODUCTION

Amino compounds are important in the human diet and environment. Aliphatic primary and secondary amines are widely distributed in the environment as they are endogeneously synthesized and excreted by living organisms and synthesized in bulk as raw materials for both chemical and manufacturing industries. In foodstuffs, aliphatic amines have seldom been investigated [1–4], despite the fact that they can act as precursors to carcinogenic N-nitroso compounds [5], which occur in the human diet [6] and the environment [7]. N-Nitroso compounds can also be formed endogeneously from ingested amines and nitrate/nitrite [8]. The dietary exposure to nitrate is well documented [9]. However, until the dietary exposure to nitrosatable amines in foods is determined, it remains impossible to evaluate the potential risk of endogeneous formation of N-nitroso compounds.

Quantitative methods for the determination of amines involving either high-performance liquid chromatography (HPLC) or gas chromatography (GC) have several inherent problems related both to the difficulty in handling low-molecular-weight amines owing to their volatility and high water solubility, and to the necessity to form derivatives to enhance both the detection limit and chromatographic separation. Derivatization is required in order to increase the detection sensitivity for HPLC analysis using ultraviolet (UV) [10–13], fluorescence [14,15], electrochemical [16] and chemiluminescence excitation detection [17]. However, despite the increased sensi-

tivity of detection, amine derivative selectivity cannot be guaranteed during the analysis of complex matrices, limiting the practical use of HPLC for the determination of amines in foodstuffs and environmental and biological samples. The determination of underivatized amines by GC [18–20] results in amine adsorption (tailing) and possible decomposition on the column. These problems can be effectively solved by derivatization of amines to either benzenesulphonamide [21,22], dimethylthiophosphinicamide [23], p-toluenesulphonamide [4,24], trifluoroacetamide [1] or 2,4-dinitrofluorobenzene [25] derivatives in which the volatility of the amine is reduced and the hydrophobic derivative is readily extractable into organic solvents. Most GC methods using these derivatives employ flame photometric detection (FPD) [21–23], flame ionization detection (FID) [1,18–20] or GC–mass spectrometry with selected ion monitoring (GC–MS/SIM) [4,24] in which amines, or their derivatives, are determined in the presence of other potentially interfering analytes.

The thermal energy analyser (TEA) was originally designed for the specific detection of N-nitroso compounds [26], but after modification it can also be used in the nitrogen mode for the detection of nitrogen-containing organic compounds [27]. Despite the obvious advantages of this highly sensitive nitrogen-specific detection method, TEA detection has never been used for the routine determination of amines. We report here a simple method for the routine determination of amines in foodstuffs using mineral oil vacuum distillation and derivatization with benzenesulphonyl chloride (BSC) to form benzenesulphonamides, which are separated using a modified Hinsberg procedure prior to determination by GC with a modified TEA detector.

EXPERIMENTAL

*Reagents*

All solvent and reagents were of analytical-reagent grade and, with the exception of amines, were used without further purification. Amines were purified by fractional distillation. The internal amine standards (ethylpropylamine and *tert.*-butylamine) were prepared in 0.1 $M$ hydrochloric acid at a concentration of 150 $\mu$g/ml.

Standard benzenesulphonamines were prepared by condensation of BSC with the amine under alkaline conditions prior to acidification and extraction with hexane. The crude benzenesulphonamides were purified by double recrystallization or fractional distillation. Purity was confirmed by GC with FID, TEA and MS detection, ^{1}H NMR and determination of melting or boiling points. Standard mixtures of representative primary and secondary amine benzenesulphonamides (10 $\mu$g/ml) were prepared in isooctane.

*Sample distillation and derivatization*

The food sample was homogenized and a representative sample (10–20 g) was removed and placed in a 100-ml distillation flask together with glycerine (10 ml), 2 $M$ sodium hydroxide solution (2 ml) and distilled water (5–15 ml, depending on the water content of the sample). The sample was spiked with two internal standards (150 $\mu$g each of *tert.*-butylamine and ethylpropylamine in 1 ml of 0.1 $M$ hydrochloric acid). The distillation flask was fitted with a short distillation bridge and connected to a 100-ml distillation receiver fitted with a side-arm which was connected to a vacuum pump. The distillation receiver was charged with 1 $M$ hydrochloric acid (5 ml) and

cooled to $-80°C$ in a Dewar flask containing acetone–solid carbon dioxide. The food sample was distilled under vacuum (8 mmHg) for 20 min at ambient temperature and then heated at 100°C for 40 min. On completion of the distillation, the heating mantle was switched off and the system left to cool for 20 min before venting and removal of the distillation receiver. The acidic distillate, containing the amine hydrochloride salts, was removed and corrected to a total volume of 30 ml with distilled water. Aliquots (2 ml) were removed and placed in a 15-ml screw-topped Pyrex glass reaction vial containing 10 $M$ sodium hydroxide solution (1 ml) and BSC (0.4 ml). The reaction vial was then heated at 105°C for 60 min with occasional shaking.

The benzenesulphonamides were isolated using a modified Hinsberg separation as follows. The alkaline reaction mixture was extracted with hexane (4 × 4 ml), the combined hexane fractions were washed by back-extraction with 1 $M$ sodium hydroxide solution (2 ml) and concentrated under a stream of nitrogen to yield a final concentrate of 1 ml of hexane containing the secondary amine fraction derivatized as benzenesulphonamides.

The aqueous alkaline wash fraction was added to the original derivatization mixture and made acidic by careful addition of 10 $M$ hydrochloric acid (1 ml). The acidic mixture containing primary amine derivatives was extracted with diethyl ether (3 × 3 ml) and the ether fractions were dried by filtration through a short column containing anhydrous sodium sulphate prior to concentration to dryness under a stream of nitrogen. The residue was taken up in 10% dichloromethane in hexane (1 ml) for analysis.

*Sample analysis by gas chromatography using a thermal energy analyser in the nitrogen mode*

Sample aliquots (10 $\mu$l) were analysed on a gas chromatograph (Hewlett-Packard Model 5880A) using a 3 m × 2 mm I.D. glass column packed with 10% OV-101 on Volaspher A2 (120–140 mesh). Helium was used as the carrier gas at a flow-rate of 18 ml/min and the injection port temperature was maintained at 250°C. For the primary amine derivatives the oven was programmed directly from 210 to 224°C at 0.5°C/min and then to 280°C at 2°C/min and maintained at 280°C for 10 min. The retention times for standard primary amine benzenesulphonamides were methylamine 9.7, ethylamine 10.9, *tert.*-butylamine (internal standard) 12.9, propylamine 14.0, isobutylamine 15.9, *n*-butylamine 18.4, isopentylamine 21.5, pentylamine 24.2, hexylamine 31.3 and phenethylamine 49.6 min.

For the secondary amine derivatives the oven was programmed directly from 210 to 222°C at 0.5°C/min and then to 250°C at 5°C/min and maintained at 250°C for 10 min. The retention times for standard secondary amine benzenesulphonamides were dimethylamine 9.4, methylethylamine 11.3, diethylamine 13.3, ethylpropylamine (internal standard) 16.2, dipropylamine 20.3, pyrrolidine 21.3, morpholine 23.3, piperidine 25.4, dibutylamine 29.7 and methylbenzylamine 37.9 min. The limit of detection for both primary and secondary amines was 10 $\mu$g/kg foodstuff.

For detection, a Model 502 thermal energy analyser (Thermo Electron, Waltham, MA, U.S.A.) was modified for use in the nitrogen mode as shown in Fig. 1. The column outlet from the gas chromatograph was connected to the pyrolyser tube with a metal T-piece through which oxygen, at a flow-rate of 5 ml/min, was mixed with the carrier gas prior to passing through the pyrolyser tube. The pyrolyser tube
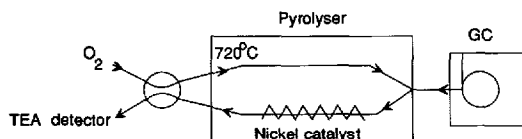
Fig. 1. GC with thermal energy analyser modified for use in the nitrogen mode.

contained a tightly packed nickel gauze which acted as a catalyst for pyrolysis of nitrogen-containing compounds to NO radicals at a pyrolyser temperature of 720°C. The pyrolyser was connected to the reaction chamber of the TEA detector via a short PTFE tube and an on-line CTR gas stream filter (Thermo Electron). Thermal pyrolysis of the N–NO bond in N-nitroso compounds at 450°C or pyrolysis of amines at 720°C with a nickel catalyst produces NO radicals which react with ozone in the reaction chamber of the TEA detector to produce $NO_2^*$ in an electronically excited state. Decay of $NO_2^*$ back to the ground state results in photon emission which is detected and quantified using a photomultiplier tube (chemiluminescence detection). Quantification was effected by peak-area integration using a Trilab 3000 integrator (Trivector Computer Systems).

*Amine confirmation using capillary gas chromatography–mass spectrometry*

Sample aliquots (3 μl) were analysed on a gas chromatograph (Hewlett-Packard Model 5890A) using a 12 m × 0.2 mm I.D. U-1 fused-silica capillary column (Hewlett-Packard). Helium was used as the carrier gas (2 ml/min) and sample injection was made in the splitless mode with an automatic sample injector (Hewlett-Packard Model 7673). For both primary and secondary amine derivatives, the oven was programmed directly from 70 to 300°C at 10°C/min and maintained at 300°C for 10 min. The capillary column was interfaced with a mass-selective detector (Hewlett-Packard Model 5970) operated either in the scanning mode (range 40–400 u) or programmed for selective ion monitoring (SIM). The ionizing voltage was 70 eV. The retention times for standard primary amine benzenesulphonamides were methylamine 7.1, ethylamine 7.5, *tert.*-butylamine (internal standard) 8.0, propylamine 8.4, isobutylamine 8.8, *n*-butylamine 9.3, isopentylamine 9.9, pentylamine 10.3 and hexylamine 11.2 min. The retention times for standard secondary amine benzene-sulphonamides were dimethylamine 7.8, methylethylamine 8.6, diethylamine 9.3, ethylpropylamine (internal standard) 10.3, dipropylamine 11.0, pyrrolidine 11.2, morpholine 11.6, piperidine 12.9 and dibutylamine 14.4 min.

RESULTS AND DISCUSSION

Derivatization of amines as benzenesulphonamides proved to be a reliable and versatile method for the GC determination of amines using the TEA detector. Using a reagent blank, recovery experiments (determined five times) at a spiking concentration of 10 and 100 μg amine/kg (ppb) gave recoveries of secondary amine benzenesulphonamides of the order of 80–90% with little cross-contamination from primary amine benzenesulphonamides; traces of longer chain ($>C_5$) primary amines occurred occasionally (at less than 5% carryover) in the secondary amine fraction.

Almost quantitative recovery (90–100%) of primary amine benzenesulphonamides was achieved. In the presence of a food matrix, no change in the recoveries was apparent and other food components did not interfere with the analytical procedure.

The standard mixtures were based on a representative selection of primary and secondary amines known to occur in foodstuffs [1]. Similarly, the two internal standards (ethylpropylamine and *tert.*-butylamine) were selected for their known absence from foodstuffs. We have so far analysed over 200 food samples during which we regularly checked for the presence of these two amines in samples analysed without addition of the internal standards. We have never detected their presence, or the presence of other amines with identical retention times. Representative chromatograms obtained using TEA detection for the primary and secondary amine benzenesulphonamide fractions of standard compounds and a typical food sample (chocolate powder) are shown in Figs. 2 and 3, respectively.

In previous studies using BSC derivatives of secondary amines, Hamano and co-workers [21,22] reported the use of 2% OV-1, 3% OV-1 and 3.5% SE-30 on Chromosorb W as column packing materials. However, in our studies these packing materials resulted in unacceptable peak tailing of primary amine derivatives. To permit the separation of both primary and secondary amine benzenesulphonamides on the same column, 10% OV-101 on Volasphere A2 (120–140 mesh) was used, which resulted in good resolution of secondary amine derivatives and greatly reduced the tailing of primary amine benzenesulphonamides.
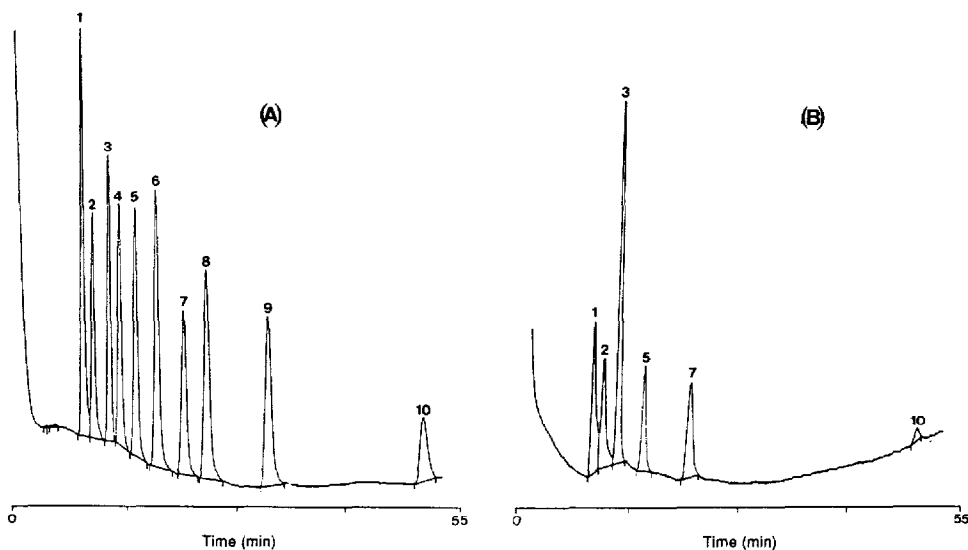


Fig. 2. Gas chromatograms of primary amine BSC derivatives on a 3 m × 2 mm I.D. glass column packed with 10% OV-101 on Volasphere A2, carrier gas helium (18 ml/min), injection port temperature 250°C, oven programmed from 210 to 224°C at 0.5°C/min and then to 280°C at 2°C/min with TEA detection. Amount injected, 5 μl, corresponding to 50 ng of each standard compound. (A) Standard mixture; (B) chocolate powder. Peaks: 1 = methylamine; 2 = ethylamine; 3 = *tert.*-butylamine (internal standard); 4 = propyl-amine; 5 = isobutylamine; 6 = *n*-butylamine; 7 = isopentylamine; 8 = pentylamine; 9 = hexylamine; 10 = phenethylamine.
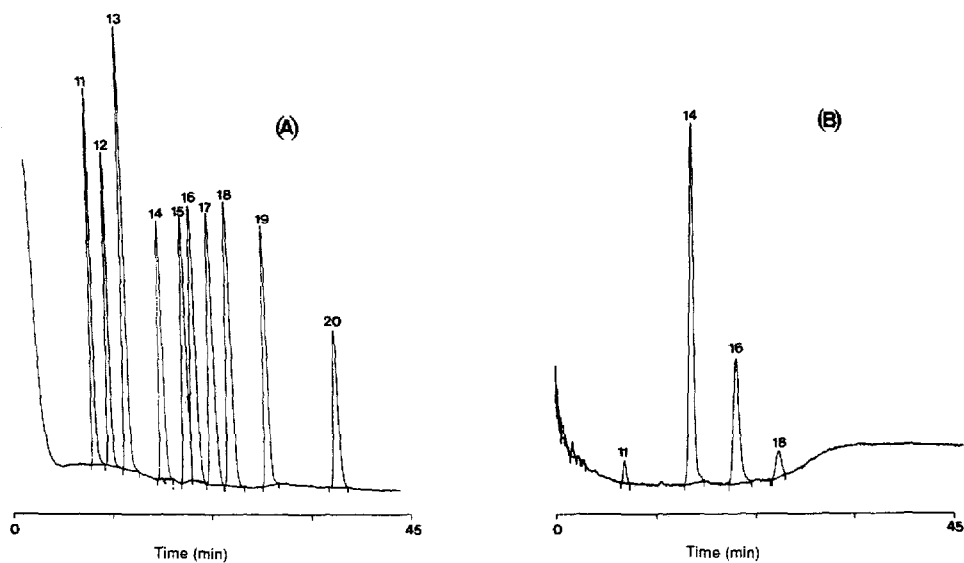
Fig. 3. Gas chromatograms of secondary amine BSC derivatives on a 3 m × 2 mm I.D. glass column packed with 10% OV-101 on Volasphere A2, carrier gas helium (18 ml/min), injection port temperature 250°C, oven programmed from 210 to 222°C at 0.5°C/min and then to 280°C at 5°C/min with TEA detection. Amount injected, 5 µl, corresponding to 50 ng of each standard compound. (A) Standard mixture; (B) chocolate powder. Peaks: 11 = dimethylamine; 12 = methylethylamine; 13 = diethylamine; 14 = ethylpropylamine (internal standard); 15 = dipropylamine; 16 = pyrrolidine; 17 = morpholine; 18 = piperidine; 19 = dibutylamine; 20 = methylbenzylamine.

The choice of BSC derivatives for amine analysis by GC has two major advantages over other commonly used derivatization techniques. The identification of unknown compounds is simplified, as fractionation using a modified Hinsberg method immediately allows the distinction between primary and secondary amines. Further, benzenesulphonamides have very characteristic mass spectral fragmentation patterns at ion masses of $m/z$ 77 and 141 relating to the structure of the derivatization reagent, which can be easily located using GC–MS/SIM for gaining structural information on the suspected amine parent molecule.

Changing from packed to capillary columns resulted in an even better separation of secondary amine derivatives while tailing of primary amine benzenesulphonamides still occurred, as shown in Fig. 4. However, for routine analysis of a large number of food samples the advantages gained from superior peak resolution using capillary columns are outweighed by the disadvantages of reduced column life and the problems incurred on interfacing capillary columns with the TEA pyrolyser [28,29].

The advantages of the TEA detector for the determination of nitrogen-containing compounds are immediately apparent on comparison of the detection limit for other reported methods for amines. Using the reported method, analysis of a 10-g food sample allows detection down to 10 µg amine/kg (ppb) foodstuff (signal-to-noise ratio = 3). Other methods give detection limits of 4 µg amine/kg foodstuff (100 g foodstuff, as p-toluenesulphonamides, with FID) [4] and 5–50 µg/kg (50–500 g foodstuff, as trifluoroacetamides, with FID) [1].
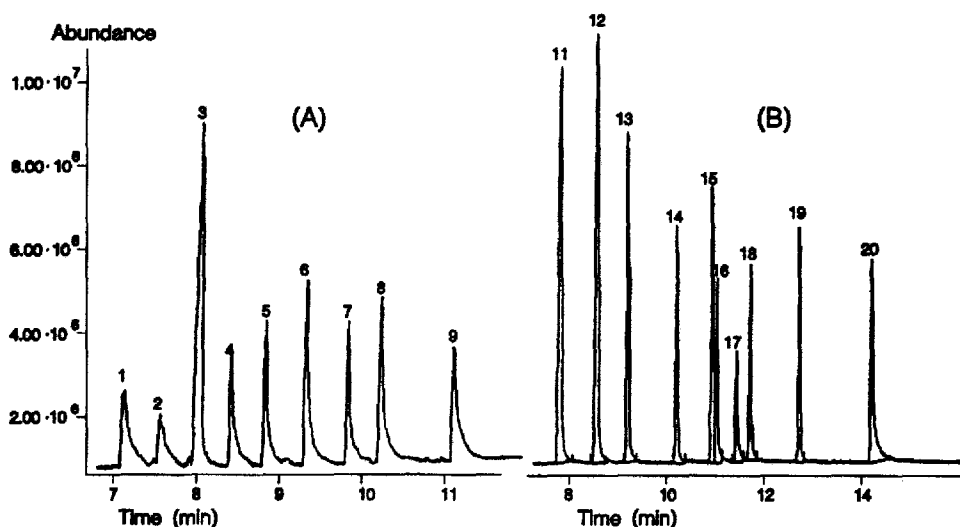
Fig. 4. Gas chromatograms of (A) primary amine and (B) secondary amine BSC derivatives on a 12 m × 0.2 mm I.D. U-1 fused-silica column, carrier gas helium (2 ml/min), oven programmed from 70 to 300°C at 10°C/min with MS detection (scanning mode 40–400 u). Splitless 2-$\mu$l injection corresponding to 20 ng of each standard compound. Peaks as in Figs. 3 and 4.

The use of BSC derivatives for the determination of secondary amines in foods using FPD [23] and mixtures of primary and secondary amines in water and sediments using GC–MS/SIM [24] has been reported to give similar detection limits to the present method.

The use of sulphur-containing BSC derivatives results in a gradual partial poisoning of the nickel catalyst in the pyrolysis tube of the modified TEA detector. Under normal operating conditions, the nickel gauze catalyst requires replacement after about 200 sample injections if the detection limit is to be maintained.

We have recently conducted a food survey using the above methodology for the determination of primary and secondary amines (results to be reported). The proposed method has proved robust and reliable for routine analysis of foodstuffs and with little modification can be used for the determination of amines in biological samples (*e.g.*, urine, faeces, gastric juice and plasma) and environmental samples (*e.g.*, tobacco smoke).

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