Journal of Chromatography, 547 (1991) 447–451
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 279

Short Communication

High-performance liquid chromatographic determination of low levels of primary and secondary amines in aqueous solutions including 2-amino-2-methylpropanol by pre-column derivatisation to sulphonamides

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(First received July 25th, 1990; revised manuscript received February 20th, 1991)

ABSTRACT

Pre-column derivatisation of primary and secondary amines to sulphonamides, by reaction with 5-dimethylamino-1-naphthalenesulphonyl chloride (dansyl chloride) has advantages over the corresponding derivatisation with 1,2-naphthoquinone-4-sulphonate (NQS) because the reaction is less susceptible to steric effects, and the resultant sulphonamides are readily extracted from the derivatising solution. A procedure employing a relatively high reaction temperature and chloroform extraction followed by reversed-phase high-performance liquid chromatography using ultraviolet detection at 340 nm provides a robust method for determining primary and secondary amines. The method was used to analyse samples of industrial waters containing various mixtures (0–2 m*M*) of ammonia, hexamethyleneimine, hydrazine, methylamine and piperidine, as well as 2-amino-2-methylpropanol and 1,6-hexanediamine that could not be analysed via NQS derivatisation.

INTRODUCTION

Previously we reported [1] a convenient high-performance liquid chromatographic (HPLC) method for determining low levels of primary and secondary amines in industrial waters based on pre-column derivatisation with 1,2-naphthoquinone-4-sulphonate (NQS). Although useful for some routine work it has been found to have some serious disadvantages. Derivatisation of hindered amines is slow, and derivatives with hydrophilic groups such as hydroxyl do not partition favourably with organic solvents to such an extent they may not be extracted from the aqueous derivatising solution. For example, it was not possible to analyse dilute aqueous solutions for

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2-amino-2-methylpropanol, an amine of particular interest. We therefore devised a derivatisation procedure that does not suffer these deficiencies. For practical reasons we wished to employ UV detection and use a fairly high wavelength to minimize possible background interference by strongly UV-absorbing contaminants that were often present. Of the possible derivatisation reagents 2,4-dinitrofluorobenzene was rejected because it causes contact hypersensitivity [2], and some of its derivatives are known to be very toxic [3]. In contrast formation of sulphonamides from 5-dimethylamino-1-naphthalenesulphonyl chloride (dansyl chloride) appeared not to present such potential problems, and fluorescence detection could be employed should extra sensitivity be required.

Derivatisation of amines by dansyl chloride to form sulphonamides was originally developed as a thin-layer chromatographic (TLC) technique by Seiler and Wiechmann [4] and has since been modified [5–7] for use with HPLC. In the reversed-phase procedure described here problems associated with NQS were overcome, and it has been successfully used for estimating a wide range of primary and secondary amines in industrial waters over an extended period.

EXPERIMENTAL

Equipment

A Waters 840 HPLC system equipped with 510 pumps, a WISP 710B autosampler, an M490 UV detector and an 840 data module was used throughout the work described here. The reversed-phase column was a 25 cm × 4.6 mm I.D., 5-μm Spherisorb ODS2 column supplied by HiChrom (Reading, UK).

Chemicals

HPLC-grade acetonitrile (Rathburns, UK), chloroform (Aldrich, UK) and water were used. Potassium carbonate came from BDH (Poole, UK), dansyl chloride and all other chemicals were of the highest purity available from Aldrich or Fluka.

Derivatisation

All calibration solutions were prepared using HPLC-grade water. Reactions were carried out in screw-top glass vials, capable of holding a minimum of 15 ml liquid. To the analyte solution (3 ml; amine concentration in the range 0.01–5 mM) were added aqueous potassium carbonate solution (0.5 ml; 0.7 M) and dansyl chloride solution in acetone (6 ml; 18.5 mM). Solutions were incubated at 55°C for 90 min. After cooling to 50°C (acetone boils at 56°C) aqueous proline (3 ml; 1.3 M) was added to each vial, and the samples were maintained at 55°C for a further 20 min to remove excess dansyl chloride by forming the ionised proline derivative. After cooling the solutions to 50°C, water (5 ml) was added, and the samples were allowed to cool to ambient temperature. Each solution was extracted with chloroform (2 ml), and the organic phase collected by filtering through silicone-treated phase separator filter papers (Whatman 1PS). The organic phase (1 ml) was diluted with acetonitrile (1 ml) and the solution used for HPLC analysis.

HPLC analysis methodology

An injection volume of 10 μl was used. The mobile phase was a mixture of

17.5 m*M* pH 7.2 phosphate buffer and acetonitrile. The phosphate buffer was prepared by titrating an aqueous solution of disodium hydrogenphosphate with orthophosphoric acid. This was preferred to titration with glacial acetic acid described by Hayman *et al.* [5], which was found to give a cloudy solution on standing for a day. When orthophosphoric acid was used no turbidity was observed. For optimal HPLC separation the flow-rates and solvent composition used depended on the amines to be separated, and typically the amount of aqueous buffer in the mobile phase was in the range 30–40% with flow-rates of 1.0–1.5 ml min^{-1}. The eluting peaks were monitored at 340 nm. Table I shows the conditions employed for analysis of ammonia with a variety of other amines including 2-amino-2-methylpropanol. Calibration graphs prepared using six to ten samples of pure amine solutions up to 2.0 m*M* (0.5 m*M* for ammonia) were linear with correlation coefficients better than 0.998.

TABLE I

TYPICAL EXAMPLES OF SOLVENT COMPOSITIONS AND FLOW-RATES FOR HPLC SEPARATION OF SULPHONAMIDE AND ITS N-SUBSTITUTED DERIVATIVES

Parent amines	Solvent composition (acetonitrile–buffer)	Flow-rate (ml min^{-1})
NH_3/hydrazine	60:40	1.0
NH_3/2-amino-2-methylpropanol	60:40	1.0
NH_3/2-amino-2-methylpropanol/piperidine	70:30	1.3
NH_3/2-amino-2-methylpropanol/morpholine	65:35	1.0
NH_3/2-amino-2-methylpropanol/hexamethyleneimine/1,6-hexanediamine	62:38	1.5

RESULTS AND DISCUSSION

In order to reduce derivatisation times and optimise conversion a relatively high reaction temperature (55°C for 90 min) was chosen. Temperatures much in excess of this are detrimental, possibly due to side-reactions between the co-solvent acetone and the analyte amine. After derivatisation proline was reacted with the excess dansyl chloride that otherwise would cause interference in the HPLC analysis. Of the organic solvents used to extract, and if necessary concentrate the sulphonamide, chloroform was chosen for routine work because it separated readily from the aqueous layer and was more selective than commonly employed ethyl acetate [5,6,8]. Moreover, chloroform is less likely than ethyl acetate or aromatic solvents [4–8] to hamper UV detection. Extraction with chloroform combined with the use of phase-separating filters made the extraction process straightforward.

Monitoring eluting peaks due to dansylated derivatives at 340 nm minimised interference by acetone encountered by others [8] as well as by highly absorbing species in the original samples, and afforded detection limits (twice the average baseline noise) of less than 10 μM for all the amines analysed. Relative standard deviations for six repeat determinations were generally better than 5%. If required higher sensitivity can be obtained by using a lower detection wavelength (monitoring at 254 nm rather than

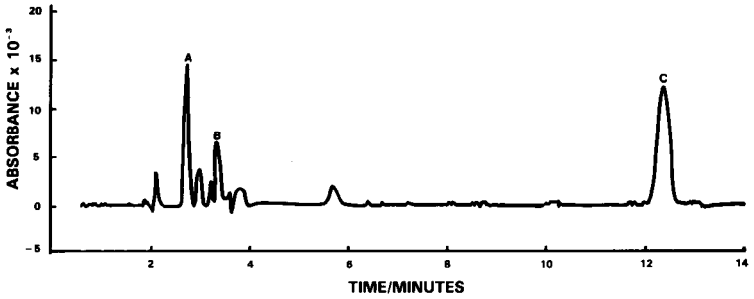

Fig. 1. Chromatogram showing the separation of three amines in an industrial liquor: (A) ammonia, 346 μM; (B) 2-amino-2-methylpropanol, 232 μM; (C) hexamethyleneimine, 237 μM. Chromatographic conditions: mobile phase, acetonitrile–17.5 mM pH 7.2 phosphate buffer (62:38); flow-rate, 1.5 ml min^{-1}; column, Spherisorb ODS2, 5 μm (25 cm × 4.6 mm I.D.), injection size, 10 μl; monitoring wavelength, 340 nm.

at 340 nm increased sensitivity five-fold), but baseline interference was more common. Alternatively, a fluorescence [9] detector could be used.

Dansyl chloride itself has negligible solubility in water, so it was added to the derivatisation mixture as an acetone solution, and surprisingly the amount of acetone used was found to determine the linearity range of the method. This was particularly important for 1,6-hexanediamine where, when 3 ml of a 18.5 mM solution of dansyl chloride in acetone was used, the linearity range was only 0–0.25 mM, whereas, when 6 ml of a 9.25 mM derivatising solution was used, the linearity range increased more than an order of magnitude. Similar patterns were observed with other amines: for example, the linear range with hexamethyleneimine increased from 0–1 mM to 0–10 mM under the same conditions. This may be a consequence of higher chloroform solubility of sulphonamides in the presence of acetone (some was extracted into the chloroform layer), or acetone may assist transferring the sulphonamides from the aqueous phase to the organic phase. However, tests with dansylamide showed it is readily and completely extracted into chloroform in the absence of acetone, suggesting other effects may be operating. It is known that acetone inhibits first-order neutral hydrolysis of some sulphonyl chlorides [10], but insufficient data are available about second-order reactions to comment meaningfully about solvent effects in kinetic terms; we did not pursue this point experimentally.

There was no problem in estimating ammonia [11] in the presence of any of the amines we examined, and methylamine and hydrazine could similarly be analysed. In addition 1,6-hexanediamine and 2-amino-2-methylpropanol, which could not be determined via NQS derivatisation, were readily estimated and Fig. 1 is a typical routine chromatogram showing the separation of ammonia, hexamethyleneimine and 2-amino-2-methylpropanol in an industrial liquor.

CONCLUSIONS

The reversed-phase HPLC procedure reported here involving pre-column sulphonamide derivatisation is a reliable and sensitive method for the determination of a wide range of primary and secondary amines. Problems with NQS derivatives of

sterically hindered and/or hydroxyl-containing amines were successfully circumvented by forming sulphonamide derivatives. Nucleophilic substitution at sulphur in dansyl chloride appears at least as facile and less susceptible to steric effects than at the carbon centre of NQS. For the hydrophilic amine 2-amino-2-methylpropanol, which is sterically crowded around the nitrogen, it provides a ready means for its routine analysis. The NQS methodology failed to detect it mainly because the derivative is too hydrophilic to extract into non-polar solvent [1]. Linearity of the calibration graphs with UV detection was excellent and enabled less than 10 μM amine to be measured. Low-molecular-weight amines, including ammonia, can be analysed in the presence of a variety of other amines and, if necessary, significantly lower detection levels could no doubt be achieved using more sensitive detection systems.

ACKNOWLEDGEMENT

A.U.S. thanks ICI Chemicals & Polymers Ltd. for a research studentship.

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