Journal of Chromatography, 333 (1985) 220–224
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 17 924

Note

Ion-pair high-performance liquid chromatographic determination of dansylated aliphatic polyamines with fluorescence and ultraviolet detection

MAJ-LEN HENRIKS-ECKERMAN* and TERTTU LAIJOKI
Turku Regional Institute of Occupational Health, Hämeenkatu 10, SF-20500 Turku 50 (Finland)
(First received April 29th, 1985; revised manuscript received May 28th, 1985)

The lower aliphatic polyamines [*e.g.* diethylenetriamine (DETA) and triethylenetetramine (TETA)] are the most commonly used aliphatic amine hardeners for epoxy resins. The polyamines are skin irritants, and they may cause sensitization leading to allergic dermatitis and asthma[1]. Users of epoxy resins can be exposed to polyamines as vapours, as aerosols or as dusts. Thus, there is a need to determine trace amounts of polyamines in air and in products.

Mainly low-molecular-weight aliphatic polyamines have been analysed by both gas chromatography (GC) and high-performance liquid chromatography (HPLC), usually as derivatives[2–6]. A very sensitive GC method using permethylation and a nitrogen-sensitive detector has been applied to ethylenediamine (EDA), DETA and TETA in water or acidic solutions[3]. In the HPLC applications, different types of derivatization reagent *e.g.* salicylaldehyde, *m*-toluoyl chloride, and 1-naphthyl isothiocyanate) have been used[4–6]. EDA has also been analysed by isotachophoresis without derivatization[7].

The fluorescent reagent dansyl chloride has been successfully applied to trace analysis of diamines and polyamines in urine[8]. Dansylation is usually carried out in a mixture of acetone and aqueous sodium hydrogen carbonate[9], which would make it suitable for analysis of polyamines in both air and product samples. Of the aliphatic polyamine hardeners, only EDA has been analysed as its dansyl chloride derivative[10]. The method was developed for biological samples[10]. In this paper, we describe the application of the dansyl chloride reagent to trace analysis of acidic solutions of both low- and high-molecular-weight aliphatic polyamines. The ion-pair HPLC method was applied to the following amines: EDA, DETA, TETA and tetraethylenepentamine (TEPA), using both fluorescence and UV detection.

EXPERIMENTAL

Apparatus

A Varian 5000 Series liquid chromatograph equipped with a Valco loop injector and a Spherisorb 5 ODS reversed-phase column (250 × 4.6 mm I.D.) was used for the chromatographic separations of the dansylated polyamines. Two different detectors were used. A Perkin Elmer 2000 fluorescence spectrophotometer with ex-

0021-9673/85/$03.30

citation filters and a variable emission wavelength was used for spectrofluorometric detection. A Pye Unicam LC-UV detector with a variable wavelength was used for UV detection. The peak areas were measured with a Shimadzu C-R1B Chromatopac integrator.

Chemicals

The ion-pair mobile phase was a mixture of acetonitrile (HPLC grade, Oriola) and distilled, Millipore-purified water, containing 0.005 *M* 1-pentanesulphonic acid sodium salt (Fisons Scientific Apparatus). The pH was adjusted to 3.5 with acetic acid (Merck, p.a.). The following aliphatic polyamines (Fluka) were used to prepare standard solutions: ethylenediamine (99.5% purity), diethylenetriamine (95% purity), triethylenetetramine (95% purity) and tetraethylenepentamine (85% purity). The standards were dissolved in 0.1 *M* hydrochloric acid (Merck, p.a.). Dansyl chloride (5-dimethylaminonaphthalene-1-sulphonyl chloride, Pierce) was used to derivatize the polyamines. All chemicals used in the derivatization and extraction procedures were commercially available and of analytical reagent grade. The water was distilled and then purified through the Millipore system.

Derivatization procedure

Dansylated polyamines were prepared as follows. A 1-ml volume of polyamine standard solution was pipetted into a 15-ml test-tube with a screw-cap. The solution was neutralized and saturated with sodium hydrogen carbonate, and 3 ml of 10 mg/ml dansyl chloride in acetone were added and thoroughly mixed. The tube was kept in a water-bath at 55°C for 2 h. The sample was then allowed to cool to room temperature. Water (4 ml) was added and allowed to react at 55°C for 1 h to make the residual reagent water-soluble. After cooling to room temperature, the dansylated polyamines were extracted into 2 ml of ethyl acetate. The sample was thoroughly mixed and then allowed to stand for at least 1 h before analysis.

Liquid chromatographic separation and detection

An ion-pair mobile phase consisting of 72% acetonitrile was used to separate dansylated EDA and DETA from each other and from the reagent peaks. Dansylated TETA and TEPA were separated by an ion-pair mobile phase consisting of 90% acetonitrile. The flow-rate was 1.5 ml/min. A 10-μl volume of the upper ethyl acetate layer was injected for spectrofluorometric detection at an excitation wavelength of 341 nm and at an emission wavelength of 520 nm. For UV detection, 1 ml of the ethyl acetate layer was transferred to another tube and evaporated in a rotary evaporator. The dansylated polyamines were redissolved in the ion-pair mobile phase (72% or 90% acetonitrile, respectively) and 50 μl were injected for analysis. The peaks were monitored at 365 nm.

RESULTS AND DISCUSSION

Separation

The dansylated polyamines were separated in two subsequent runs with two different acetonitrile concentrations: EDA and DETA were separated within 11 min and TETA and TEPA within 9 min (Fig. 1). The content of acetonitrile in the mobile

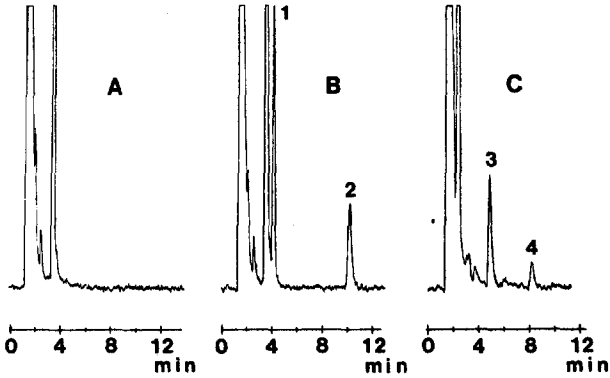

Fig. 1. Separation of dansylated aliphatic polyamine standards (each 1 μg/ml in 0.1 *M* hydrochloric acid). Peaks: 1 = ethylenediamine; 2 = diethylenetriamine; 3 = triethylenetetramine; 4 = tetraethylenepentamine. Fluorescence detector, 341 nm excitation and 520 nm emission; injection volume 10 μl; column, 250 × 4.6 mm I.D. Spherisorb 5 ODS; ion-pair mobile phase containing 72% acetonitrile (A and B) or 90% acetonitrile (C); flow-rate, 1.5 ml/min. A = blank.

phase is either 70% or 90% in order to achieve a good and rapid separation of the polyamine of interest: it should not exceed 90% because of the insolubility of the ion-pair reagent 1-pentanesulphonic acid in pure acetonitrile. The amount of the ion-pair reagent in the mobile phase was not optimized. Without the ion-pair reagent, all polyamines except EDA tailed badly, and the sensitivity of the method decreased.

Detection and linearity

Fluorescence detection of dansylated amines is about ten times more sensitive than the direct UV analysis[11]. In this study, the UV detector was as sensitive as the fluorescence detector, if the injected amount was five times the amount injected for the fluorescence detection (Fig. 1 and 2). For sensitive UV detection, ethyl acetate should be replaced with the mobile phase, otherwise the peaks would broaden too much. The sensitivity of the fluorescence detection may also be enhanced in the same

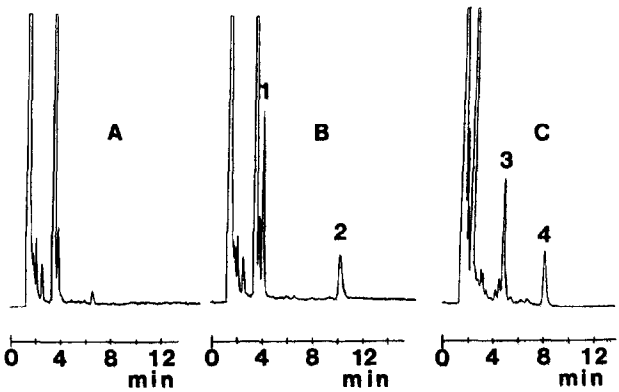

Fig. 2. Separation of dansylated aliphatic polyamine standards (each 1 μg/ml in 0.1 *M* hydrochloric acid). UV detection at 365 nm. Injection volume, 50 μl. Other details as in Fig. 1.

way. The excitation and emission maxima of different amines may vary to some extent. In this study, the wavelengths were chosen from the maximum wavelength areas, and they were the same for all polyamines. For the most sensitive detection, the optimum wavelength should be determined separately for each amine of interest. The linearity between the concentration of the injected amount and both the fluorescence and UV response was excellent for all polyamines in the 1–30 μg/ml range ($r^2 \geq 0.993$, four different concentrations).

Stability of polyamines

The standard solutions of the polyamines were made up in 0.1 *M* hydrochloric acid, since amines in air can be collected in 0.1 *M* hydrochloric acid (or 0.05 *M* sulphuric acid) for analysis[12]. Solutions of EDA and DETA in 0.1 *M* hydrochloric acid can be stored at +5°C for two months without any noticeable changes in the chromatograms. Solutions of TETA and TEPA in 0.1 *M* hydrochloric acid stored at +5°C decomposed slowly to give smaller peaks and also extra peaks in the chromatograms. Thus, TETA and TEPA standard solutions more than one month old should not be used. The dansyl chloride derivatives of polyamines in the ethyl acetate layer can be stored for one week at +5°C without any noticeable changes in the chromatograms.

Precision of the derivatization procedure

The precision of the derivatization procedure improved with increasing polyamine concentrations (Table I). At concentrations of 10 μg/ml or more the precision was satisfactory. The use of long-chain aliphatic diamines as internal standards was also tested, but the overall precision of the method did not improve. Therefore, concentrations of 10 μg/ml or more of external standards should be used to achieve satisfactory quantitative results.

Advantages and disadvantages of the method

Advantages. The derivatization method can easily be applied to acetone-soluble polyamines in epoxy products. Large sets of polyamine samples can conveniently be derivatized at the same time, since all reaction steps are carried out in the same test-tube. The method is sensitive: *e.g.* 10 μg/ml of DETA corresponds to the Finnish

TABLE I

RELATIVE STANDARD DEVIATION (%) OF PEAK AREAS OF DANSYLATED POLYAMINES

Fluorescence detection and UV detection (values in parentheses): for details, see text. Number of derivatizations, $n = 4$.

Polyamine	*Concentration in standard solutions (μg/ml)*			
	1	*2*	*10*	*30*
EDA	3 (3)	9 (9)	1 (4)	3 (1)
DETA	29 (29)	15 (16)	7 (8)	4 (3)
TETA	20 (25)	21 (27)	13 (5)	7 (9)
TEPA	67 (50)	7 (–)	11 (7)	9 (8)

threshold limit value of 4 mg/m³ (8 h) when 25 l of air is pumped through 10 ml of the absorber liquid.

Disadvantages. Except for EDA, the precision of the method is not very good at the 1–2 μg/ml level. The derivatization procedure is time-consuming.

Applications

We have used this method to determine trace amounts of aliphatic di- and polyamines in work-place air. The amine vapours were collected in mineral acidic water according to standard procedures[12].

ACKNOWLEDGEMENTS

The authors are grateful to Mrs Tuike Nenonen for skilful technical assistance. This work was supported by a grant from the National Board of Labour Protection.

REFERENCES

1 A. Thorgersson, *Acta Dermatovener.*, 58 (1978) 332.
2 *Manual of Analytical Methods, Vol. 4, Method No. 276,* Dept. Health, Education and Welfare, National Institute of Occupational Safety and Health, Cincinnati, OH, 1978.
3 M. Dalene, T. Lundh and L. Mathiasson, *J. Chromatogr.*, 322 (1985) 169.
4 P. Bauer and M. Richter, *J. Chromatogr.*, 206 (1981) 343.
5 E. C. M. Chen and R. A. Farquharsson, *J. Chromatogr.*, 178 (1979) 358.
6 K. Andersson, C. Hallgren, J.-O. Levin and C-A. Nilsson, *Amer. Ind. Hyg. Ass. J.*, 46 (1985) 225.
7 L. Hansèn, B. Kristiansson and J. Sollenberg, *Scand. J. Work Environ. Health*, 10 (1984) 95.
8 N. D. Brown, R. B. Sweet, J. A. Kintzios, H. O. Cox and B. D. Doctor, *J. Chromatogr.*, 164 (1979) 35.
9 J. F. Lawrence, *Organic Trace Analysis by Liquid Chromatography*, Academic Press, New York, 1981, 167.
10 S.-C. Chu, M. J. Tallant and R. S. H. Yang, *J. Chromatogr.*, 280 (1983) 394.
11 J. F. Lawrence and R. Leduc, *J. Chromatogr.*, 152 (1978) 507.
12 *Manual of Analytical Methods, Vol. 3, Methods No. S143 and S147,* Dept. Health, Education and Welfare, National Institute of Occupational Safety and Health, Cincinnati, OH, 1977.