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## DETERMINATION OF TERTIARY ALIPHATIC AMINES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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### SUMMARY

A high-performance liquid chromatographic method using on-line derivatization is described for the determination of tertiary aliphatic amines. The amines are separated on a column packed with Nucleosil 5N(CH<sub>3</sub>)<sub>2</sub> resin, and are detected by post-column derivatization based on a reaction with a colour reagent consisting of an acetic anhydride solution of citric acid. The reaction, which gives a wine red colour, is specific and primary and secondary amines do not react or interfere. The calibration graph is linear over the range 0.05–0.5% for aliphatic mono-, di- and trialkylamines and lower limit of determination is 0.01%.

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### INTRODUCTION

The detection and determination of amines are concern not only in biochemical but also in industrial analysis. Highly sensitive and specific chromatographic determinations of primary and secondary amines are easy to perform because commercial derivatization reagents are available. However, there have been only a few reports on the determination of tertiary amines. Gübitz *et al.*<sup>1</sup> used 2-naphthyl chloroformate as a derivatization reagent, and determined trace levels of tertiary amines with fluorescence detection. Although the fluorescence derivatives of tertiary amines show good sensitivity, the derivatization reaction is slow (over 60 min).

On the other hand, identification methods and spot tests for tertiary amines using an acetic anhydride solution of citric acid<sup>2</sup>, or *cis*-aconitic anhydride<sup>3</sup> have been reported. However, there is no report on the liquid chromatographic determination of tertiary amines using these reagents.

This paper describes the on-line high-performance liquid chromatographic (HPLC) determination of tertiary aliphatic amines using an acetic anhydride solution of citric acid as a colour reagent. Tertiary amines are separated according to the differences in the substituted alkyl groups under normal-phase conditions employing a separation column packed with Nucleosil 5N(CH<sub>3</sub>)<sub>2</sub> resin and hexane-chloroform-ethyl acetate as the mobile phase, and are detected by a post-column derivatization technique.

## EXPERIMENTAL

### *Reagents*

The amines used were tridecylamine, tridodecylamine, octadecylhexadecylmethylamine, didodecylmethylamine, dodecyltrimethylamine, octadecylmethylamine, dodecylmethylamine, hexadecylamine and dodecylamine. Tridecylamine was obtained from Wako (Osaka, Japan); the other amines were synthesized in our laboratories. Quaternary ammonium salts (dodecyl- and octadecyltrimethylammonium chloride) were purchased from Tokyo Kasei (Tokyo, Japan). All reagents used in liquid chromatography were of analytical-reagent grade.

### *HPLC apparatus and procedure*

The HPLC separation of tertiary aliphatic amines was carried out with an instrument consisting of an HPLC pumping system (Trirotar; Jasco, Japan) and an ultraviolet-visible detector (Uvidec 100 III; Jasco). The separation column was a 250 × 4 mm I.D. stainless-steel column packed with Nucleosil 5N(CH<sub>3</sub>)<sub>2</sub> (Macherey, Nagel & Co., G.F.R.) by the balanced density method. A Milton Roy high-pressure pump (NSP 800 3u; Nihonseimitsu, Japan) was used for the delivery of the colour reagent. PTFE tubing (10 × 0.25 mm) was used as the reaction coil in a temperature-controlled oil bath (Thermo Minder H-80; Taiyo Kogyo, Japan). Hexane-chloroform-ethyl acetate (80:10:10) was used as the mobile phase at a flow-rate of 2.0 ml/min. The colouring reagent was a 1.0% solution of citric acid in acetic anhydride at a flow-rate of 1.0 ml/min. The reaction temperature was 120°C and the wavelength of detection was 550 nm. Sample injection was carried out with a six-port high-pressure valve (VL-611; Jasco) and the injection volume was 10 µl.

## RESULTS AND DISCUSSION

There were two problems with respect to the separation system and the colour detection system. As the colour reaction between tertiary amines and an acetic anhydride solution of citric acid is subject to interference by water<sup>2</sup>, the aqueous reversed-phase separation mode should be avoided. Methanol, ethanol and acetonitrile had the same effect as water. The normal-phase was therefore preferred to the reversed-phase mode for the separation and detection of tertiary amines. The other problem was the difficulty of mixing the colour reagent with the mobile phase. When mobile phase was hexane alone, mixing between the mobile phase and the colour reagent was incomplete and baseline drift occurred. However, satisfactory results were obtained by using hexane-chloroform-ethyl acetate (80:10:10) as the mobile phase.

A typical chromatogram showing the separation of tertiary aliphatic amines is shown in Fig. 1. Tertiary amines elute in the order trialkylamines, dialkylamines and monoalkylamines. Trialkylamines and dialkylamines eluted as sharp peaks but monoalkylamines showed tailing, owing to adsorption on the stationary phase. Tertiary amines were separated from each other according to the differences in their substituted alkyl groups but not the alkyl chain distribution. The retention times of the tertiary amines were not affected by the number of carbon atoms in the alkyl group, e.g., tridecylamine had the same retention time as tridodecylamine.

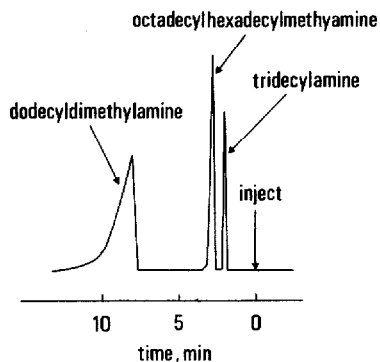


Fig. 1. Typical chromatogram of tertiary aliphatic amines.

The concentration of citric acid in the colour reagent affected the detection sensitivity. As mentioned before, water in the reaction system interfered with the colour formation, and anhydrous citric acid was therefore used. Fig. 2 shows the relationship between citric acid concentration and the peak area of tridecylamine. The peak area increased in proportion to the concentration of citric acid and showed maximum sensitivity at a concentration of 1.0%. At a higher concentration of citric acid the sensitivity decreased and the baseline became unstable. The optimum concentration of citric acid in the colour reagent was therefore 1.0%.

The selectivity of the colour reagent for primary, secondary and tertiary amines was examined using tridodecylamine, dodecylmethylamine and dodecylamine. Amounts of 10 mg of each amine were placed in a screw-capped vial and 1 ml of the colour reagent was added, followed by heating at 120°C for 10 min. Only tridode-

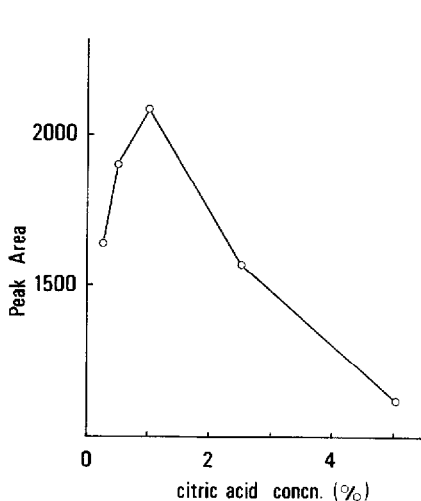


Fig. 2. Relationship between citric acid concentration and peak area.

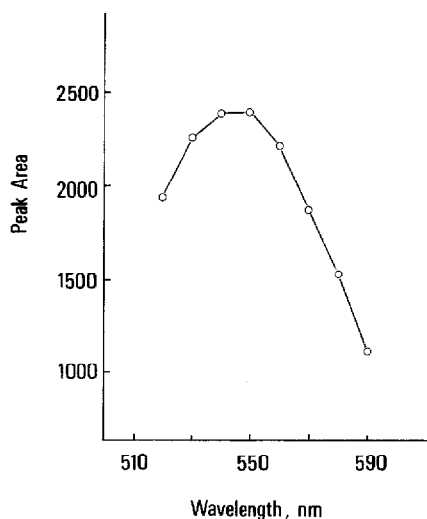


Fig. 3. Spectrum of reacted tridecylamine.

cylamine gave a colour (red). The same procedure was performed using tertiary mono-, di- and trialkylamines, and they also gave a red colour. The colour reagent is therefore specific for tertiary amines and does not react with primary and secondary amines.

The detection sensitivity was affected by the reaction temperature: the higher reaction temperature, the larger was the peak area obtained. However, at temperatures over 120°C, baseline drift occurred and the signal-to-noise ratio decreased. The requirement for a high reaction temperature owing to the slow reaction rate was considered. Using different lengths of the reaction coil (5, 10 and 15 m), the peak area of tertiary amines was measured. A slight increase in peak area was observed when the longest reaction coil was used. A longer reaction coil (over 15 m) will give more sensitive results, but it will cause peak broadening and a decrease in resolution. We selected a reaction temperature of 120°C and a length of reaction coil was 10 m. To obtain reproducible data, the reaction temperature was controlled at  $120 \pm 0.2^\circ\text{C}$  using a thermostated reaction bath.

Fig. 3 shows the spectrum of reacted tridecylamine. Maximum absorption was observed at 550 nm, while the wavelength of maximum absorption shifted slightly with other coloured tertiary amines such as didodecylmethylamine and dodecyl-dimethylamine, but the decrease in absorbance at 550 nm was small.

The linearity of the calibration graph was investigated by preparing various concentrations of tertiary aliphatic amines (tridecylamine, octadecylhexadecylamine and dodecyl-dimethylamine). Each amine showed good linearity over the range 0.5–0.05%, and the lower limit of determination was 0.01%.

Table I shows the tertiary amine contents, recoveries and reproducibilities obtained by measurements on various tertiary amines, secondary amines and quaternary ammonium salts. The concentration of each sample was 20% and the injection volume was 10  $\mu\text{l}$ . The recoveries of tertiary amines from secondary amines and quaternary ammonium salts were calculated by addition of known amounts of tridecylamine. The average recoveries (three determinations) showed that the tertiary amines in these samples were recovered quantitatively.

The reproducibility of determination was investigated using standard tride-

TABLE I  
MEASUREMENTS OF TERTIARY AMINE CONTENTS, RECOVERIES AND REPRODUCIBILITIES USING VARIOUS SAMPLES

<i>Sample</i>	<i>Tertiary amine content</i>	<i>Recovery (%)</i>	<i>Relative standard deviation (%)</i>
Didodecylamine	Less than 0.05%	93.5	—
Diocetadecylmethylamine	Less than 0.05%	95.1	—
Hexadecylamine	Less than 0.05%	99.8	—
Dodecyltrimethyl-ammonium chloride	Less than 0.05%	98.2	—
Octadecyltrimethyl-ammonium chloride	Less than 0.05%	97.3	—
Tridecylamine	—	—	2.4
Dodecyl-dimethylamine	—	—	2.1

cylamine and dodecyldimethylamine. The examination was repeated ten times and relative standard deviations were 2.4% for tridecylamine and 2.1% for decyldimethylamine (Table I). These results were considered to be satisfactory.

As the proposed method is specific for the detection of tertiary amines, it can be applied to the determination of tertiary aliphatic amines as by-products in primary and secondary amines and quaternary ammonium salts. The determination of tertiary amines in various secondary amines and quaternary ammonium salts was performed, and the results are given in Table I. None of the samples contained more than 0.05% of tertiary amines.

#### REFERENCES

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- 3 M. Palumba, *Farmaco*, 3 (1948) 675.