DETERMINATION OF TRIETHANOLAMINE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH POST COLUMN REACTION

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SUMMARY

A new method for liquid chromatography with post column reaction is suggested for the separation and quantification of tertiary amines. A mixture of triethanolamine and N-ethyl diethanolamine was separated by a strong cation exchange column, followed by spectrophtometric detection of the blue colors generated from the reaction of each amine with the Folin-Ciocalteau reagent.

The tertiary amines were properly separated when an eluent of pH 9.5 containing 0.5M sodium nitrate was used. Under this condition, calibration curve of triethanolamine in 2–10mg/100ml concentration range was attained. Good results were obtained when cream and shampoo preparations containing known amount of triethanolamine were analysed accoring to this method.

In case the samples did not contain any other interfering reducing substances, the amine was quantitatively determined by the simple reaction of the samples with Folin-Ciocalteau reagent, and the subsequent spectrophotometric measurement.

I. INTRODUCTION

Triethanolamine (TEA) is an important raw material in cosmetics. It is used extensively, in combination with fatty acids, as emulsifiers for creams, lotions, shampoos and skin cleaners. The TEA soap is mild, with low alkalinity and exellent detergency.

TEA has been determined by paper chromatography (1), ion exchange chromatography (2), gas chromatography (3-5) and post column chromatography (6).

The post column chromatographic method was developed by Nakae et al. (6), who converted ethanolamines to chloramine derivertives with hypochlorite, followed by the subsquent reaction of chloramine derivertives with iodide. The resulting triiodide ion was measured spectrophotometrically at 350nm.

In the present study, however, the property that the Folin-Ciocalteau reagent (Folin reagent) (7) reduced by tertiary amine turns to blue was applied to the post column technic. The Folin reagent is a mixture of Mo (VI) and W (VI). When the reagent is reduced partially by the tertiary amines, its components turn to Mo (V, VI) (molibdenum blue) and W (V, VI) (tungsten blue) (8).

$$\underset{\text{Folin reagent}}{\underset{\text{yellow color}}{\text{Mo(VI), W(VI)}}} \xrightarrow{\text{reduction}} \underset{\text{blue color}}{\text{Mo (V, VI), W (V, VI)}}$$

In these experiments, tertiary amines were separated by strong cation exchange resin, colored by the Folin reagent, stabilized by alkaline solution, and detected spectrophotometrically at 658nm. According to this method, some cream and shampoo preparations containing known amount of TEA were analyzed with another tertiary amine (N-ethyl diethanolamine; NEDA) as the internal standard.

And also, in case of the samples not containing any other interfering reducing substances, they were directly reacted with the Folin reagent and stabilized with alkaline solution. The resulting blue color was measured with spectrophotometer, and thus TEA was analysed quantitatively by this simple method without time consuming HPLC separation.

2. EXPERIMENTAL

2-1. Instrumentation

Fig. 1 shows the schematic diagram of the High Performance Liquid Chromatography (HPLC) system used for these experiments. The pH 9.5 buffer solution of eluent 1 was eluted by pump 2 (Waters Associates, Model 510) at the

rate of 0.5 ml/min. The column 9 (Waters, aminoacid analysis column) was installed in a column heating oven (Waters, Model 79151) and the temperature was maintained at 62°C by a temperature contoller 8 (Waters, TCM).

The Folin solution 3 was contained in a pressure bottle (General Kinetic co. 200 CC) and flowed at 0.5ml/min by setting the helium gas 4 pressure to 3.3kg/cm. Since the reagent reacts with metals, the use of metallic pump should be avoided.

The reaction coils 15, 16 made of tefron tube (ID. 0.3mm, legth 1.5m per each) were immersed in a water bath 10 (Tokyo Rikakikai co. NE-1) at 80°C.

The sodium carbonate solution 5 flowed through pump 6 (Waters, Model 501) at 0.5ml/min. The blue color produced by the reaction was detected by a photometric detector 11 (Waters, Model 441, 658nm) which was linked to an integrater 12 (Hewlett Packard co. 3390A), and peak area was calculated.

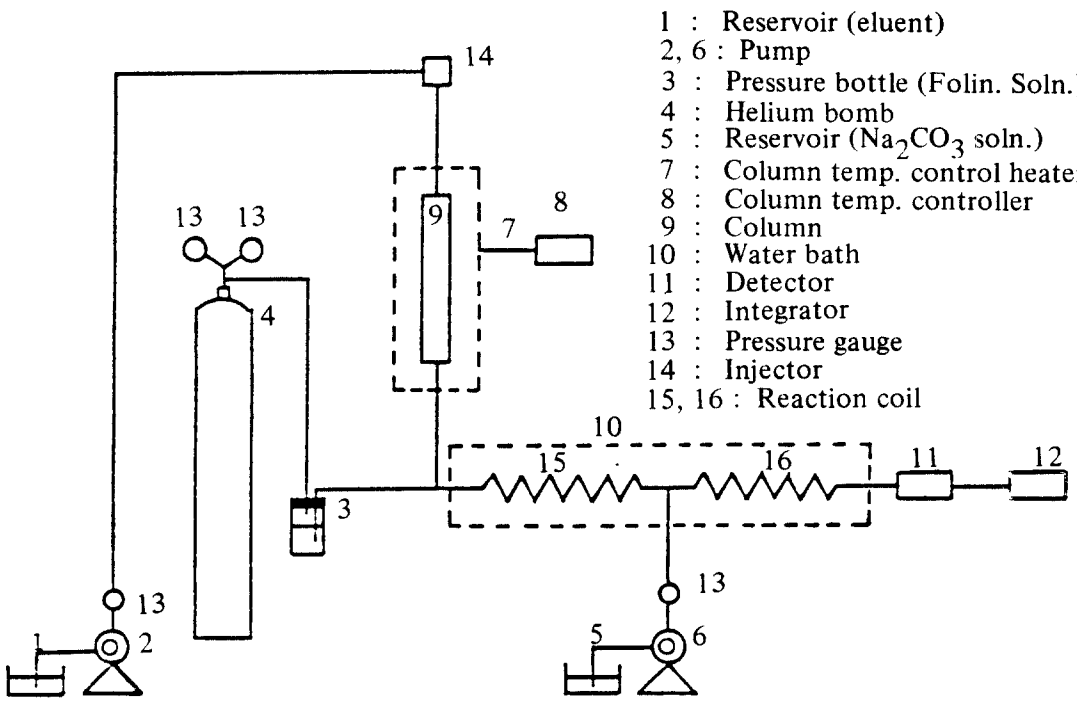

Fig. 1 Schematic diagram of the liquid chromatograph

2-2. Reagents

1) Eluent: 1.5g of boric acid and 51.0g of sodium nitrate were dissolved into 1 L volumetric flask with 950ml water. The pH was adjusted to 9.5 with 6N NaOH, and the resulting solution was diluted to the mark and filtered.
2) Folin reagent: 100.0g of potassium tungstate ($Na_2Wo_4 .2H_2 O$), and 25.0g of sodium molybdate ($Na_2 Mo_4 .2H_2 O$) were dissolved into 1.5L flask with 700ml of water. 50ml of phosphoric acid (85%) and 100ml of concentrated hydrochloric acid were added. The mixture was refluxed mildly for 10 hours, and cooled to room temprature. After the reflux condenser was removed, 50g of Lithium sulfate (Li_2SO_4), and 50ml of water were added and boiled for 15 minutes. The resulting solution was cooled and diluted with water to 1L and filtered.
3) Folin-Ciocalteau solution: The solution was prepared by diluting 50ml of Folin reagent to 1000ml with water.
4) $Na_2 CO_3$ solution: 20.0g of anhydrous sodium carbonate was weighed into 1L volumetric flask, and dissolved to volume with 0.1N sodium hydroxide solutin.
5) TEA stock solution: 1.00g of triethanolamine was weighed into 1L volumetric flask and dissolved to volume with water.
6) Internal standard stock solution: 1.00g of N-ethyl diethanolamine was weighed into 1L volumetric flask and, dissolved to volume with water.

2-3. Samples

The samples of creams and shampoos were prepared with the following formulations in Table I and II.

Table I. Formulation of cream (unit: g)

Raw Material \ Sample No.	#1	#2	#3	#3	#5
Stearic acid	2.0	2.0	2.0	2.0	2.0
Cetostearyl alcohol	3.0	3.0	3.0	3.0	3.0
Liquid paraffin	10.0	10.0	10.0	10.0	10.0
Silicone oil	0.5	0.5	0.5	0.5	0.5
Polysorbate 60	1.0	1.0	1.0	1.0	1.0
Sorbitan stearate	0.2	0.2	0.2	0.2	0.2
Triethanolamine	1.2	1.0	0.8	0.6	0.6
Ascorbic acid magnesium phosphate	-	-	-	-	0.5
Water	to100	to100	to100	to100	to100

Table II. Formulation of shampoo (unit: g)

Raw Material \ Sample No.	#1	#2	#3	#4
Fatty acid diethanol amine	5.0	5.0	5.0	5.0
Sodium lauryl ether sulfate (30%)	30.0	30.0	30.0	30.0
Sodium lauryl sulfate (30%)	20.0	20.0	20.0	20.0
Triethanolamine lauryl sulfate (30%)	3.0	4.0	5.0	6.0
Propylene glycol	1.0	1.0	1.0	1.0
Water	to100	to100	to100	to100

2-4. Procedure

1) Preparation of TEA-standard solution: 2,4,6,8,10ml of the TEA stock

solution were pipetted into each 100ml volumetric flask. 4ml of internal standard solution was added to each flask and made to volume with water.

2) Preparation of samples solution: 0.50g of creams and 1.00g of shampoos were weighed into each 100ml volumetric flask, 4ml of internal standard solution was added to each flask and diluted to the mark with water.
3) Preparation of calibration curve: exactly 20ul of each TEA standard solution was injected into the HPLC. The areas of TEA and NEDA peaks were calculated for each standard solution. The peak area (P.A.) factor for each concentration were calculated by the followed equation.

$$\text{P.A. factor} = \frac{\text{Area of TEA peak}}{\text{Area of NEDA peak}}$$

Calibration curve was made from the concentrations and the P.A. factors.

4) Analysis of sample: exactly 20ul of each sample solution was injected. The resulting peak areas, and thus, the P.A. factors were calculated. The TEA contents were determined from the calibration curve.

2-5. A simple method by UV-spectrophotometer

1) Preparation of sample solution: 0.50g of cream and 1.00g of shampoo were weighed into each 100ml volumetric flask, and made to volume with water.
2) Preparation of standard solution: 1,2,3,4,5,6,7ml of TEA stock solution were pipetted accurately into each 100ml volumetric flask and diluted with water to the mark.
3) Procedure: Each 2ml of sample and standard solutions were transfered into 10ml test tube, and 1ml of Folin reagent and 5ml of sodium carbonate solution 2% Na_2CO_3 in 0.2N NaOH) were added to each test tube. After mixing well, the test tubes were placed in a 60°C water bath for 30 minutes, and cooled to room temperature. The solutions were centrifuged at 3000 rpm for 10 minutes and the supernatants were filtered through filter paper (Whatman No. 2). The absorbances were measured in 1 x 1cm quartz cell at 650nm. The calibration curve

3. RESULTS & DISCUSSION

3-1. Conditions for separation

When the separation of tertiary amine is regarded as an ion exchange phenomenon by the ion exchange resin, the distribution coefficient (D) of the tertiary amine is represented by the following equation (2)

$$D = \frac{K_{ex}\ [Na^+]_r}{[Na^+]\left[\frac{K_a}{[H^+]} + 1\right]}$$

K_{ex} : the ion exchange equilibrium constant of the tertiary amine
K_a : the acid dissociation constant of the tertiary amine
$[Na^+]$, $[Na^+]_r$: the concentrations of sodium ion in eluent, and in resin

From the above equation, it is obvious that the concentration of sodium ion and pH affect significantly upon the separation of the tertiary amines.

To examine the effect of pH on the separation, pH of the eluent was increased from 7.7 to 10.4 by 0.3, while the concentration of sodium nitrate wasfixed at 0.5M. The examination showed that the retention times decreased with the increase of pH. Therefore, the capacity factor which was calculated from the retention time data also decreased with the increasing pH as shown in Fig. 2. However, in these experiments, the pH of the eluent was arbitrarily fixed at 9.50.

To investigate the effect of the concentration of sodium nitrate in the eluent, the retention times of the tertiary amines were examined on varing concentration of sodium nitrate from 0.1M to 0.6M, while the pH of the eluent was fixed at 9.5. As a result, the retention times of TEA showed small variation, but those of NEDA reduced significantly with the increase of the sodium nitrate concentration. The variation of capacity factor K'over the concentration is depicted at Fig.3.

With the results obtained thus far, in these experiments, the pH of the eluent was set at 9.5 and the sodium nitrate concentration at 0.5M. This condition showed good separation and rather small time consumption.

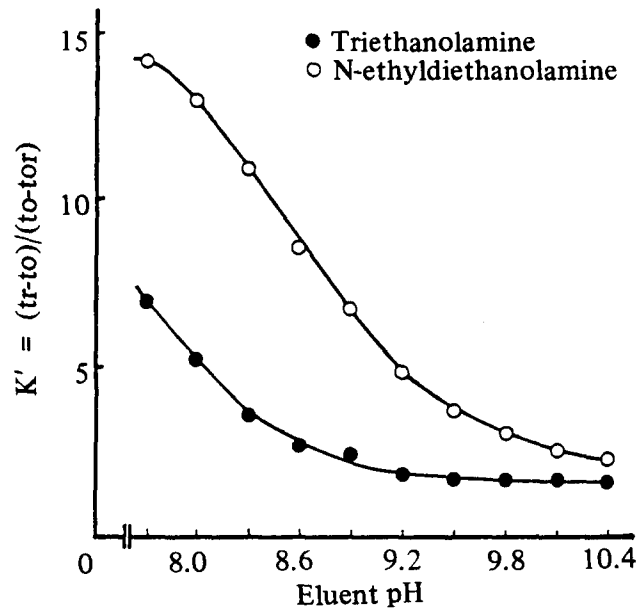

Fig. 2 Effect of eluent pH on the separation of tertiaryamines.

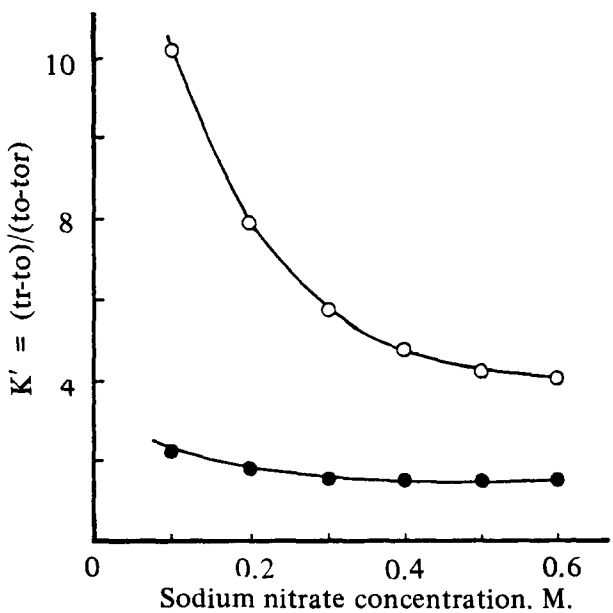

Fig. 3. Effect of sodium nitrate concentration on the separation of tertiaryamines
- ● Triethanolamine
- ○ N-ethyldiethanolamine

3-2. Effect of reaction temperature

The separation conditions of the eluent for tertiary amines had been fixed at pH of 9.5 and the concentration of sodium nitrate at 0.5M, and the reaction conditions were examined. When the water bath containing the reaction coil increased from 50°C to 90°C by 10°C, the peak intensities of TEA and NEDA incerased with increasing temperature, and showed rather mild increment near 80°C.

Therefore the temperature of the reaction coils was set to 80°C. Fig.4 shows the relationship between reaction temperature and peak area.

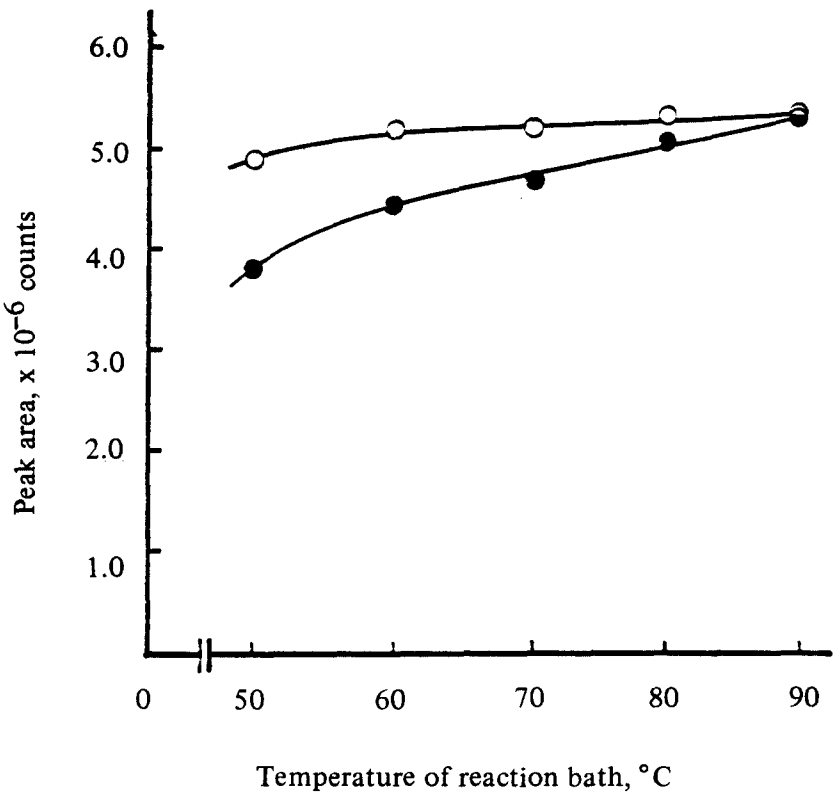

Fig. 4 Effect of temperature on the detection of tertiaryamines

● Triethanolamine
○ N-ethyldiethanolamine

3-3. Assay

20ul of each TEA standard solution was injected into HPLC and peak areas were obtained. Linear calibration curve was obtained in the concentration range of 2-10mg TEA/100ml. Therefore, the amount of TEA could be determined from the calibration curve. Fig. 5 shows the curve of P.A. factor vs concentration of TEA.

The amounts of TEA in cream obtained from the assay were 98.8-103.7 % of theoretical value, and in shampoo, 98.9-101.5%. In case of cream #5, 0.5% of ascorbic acid magnesium phosphate was contained as an interfering substance. However, since it was separated from TEA by the column, it didn't interfere the assay. Fig. 6 shows the typical chromatogram of cream #5. The actual analysis of TEA using the post column reaction appeared to be feasible from the fact above. Table III shows the result.

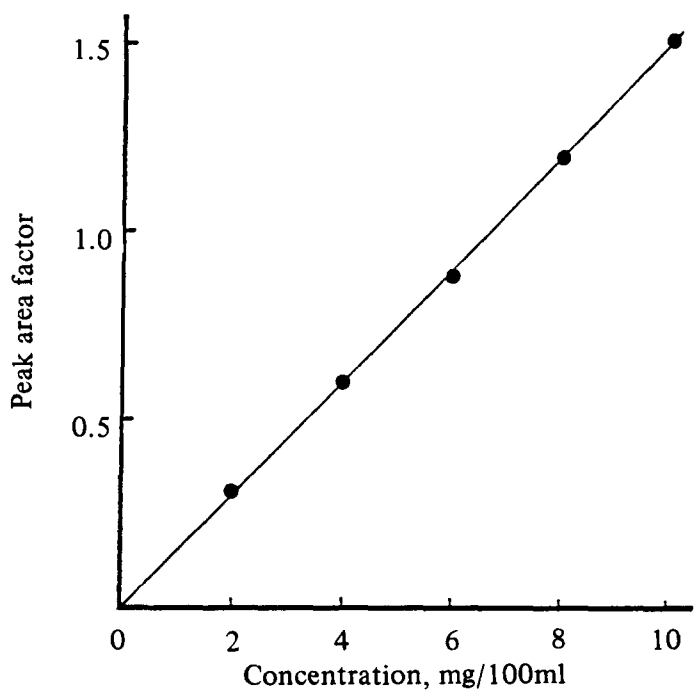

Fig. 5 Calibration curve for triethanolamine

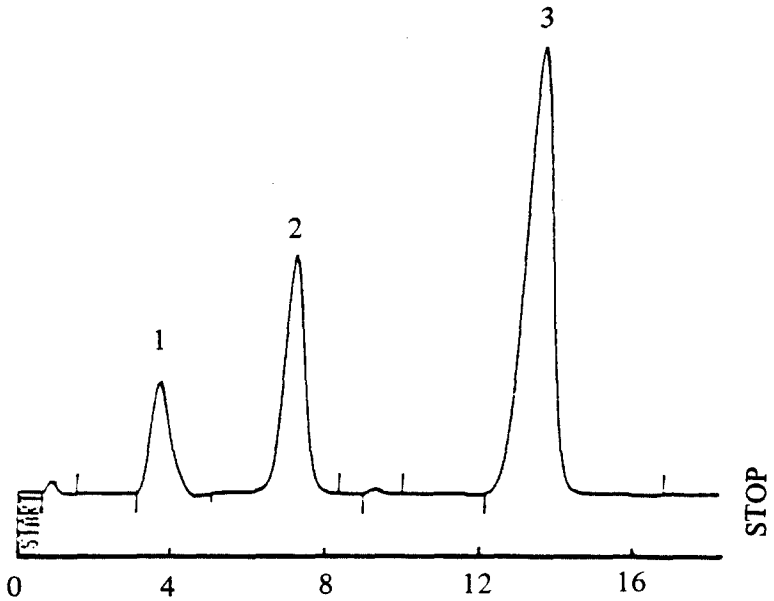

Fig. 6 Typical chromatogram of sample
1. Ascorbic acid magnesium phosphate
2. Triethanolamine
3. N-ethyldiethanolamine

Table III. Determination of triethanolamine in cream and shampoo

Sample		Present (mg/100ml)	Found (mg/100ml)	Recovery (%)
Cream	No.1	6.00	5.97	99.5
Cream	No.2	5.00	4.94	98.8
Cream	No.3	4.00	4.07	101.8
Cream	No.4	3.00	3.11	103.7
Cream	No.5	3.00	3.04	101.3
Shampoo	No.1	3.23	3.28	101.5
Shampoo	No.2	4.31	4.32	100.2
Shampoo	No.3	5.38	5.40	100.4
Shampoo	No.4	6.46	6.39	98.9

3-4. Consideration over simple method

By far only the post column method was discussed for the assay of TEA incream and shampoo. When there were no reducing compounds other than TEA, the applicability of the direct reaction using Folin reagent without HPLC separation was examined. The linear calibration curve was obtained in the range of 1-7mg TEA/100ml, as shown at Fig. 7. The results of TEA analysis in the samples are shown at Table IV. The recovery range of TEA in the creams was 95.8-103.3%. In shampoos, 99.4-102.1%.

In case of cream #5 which contains ascorbic acid magnesium phosphate, the result was 117.3%. Since ascorbic acid also reduced the Folin reagent, the amount obtained from cream #5 was more than the theoretical amount.

When the blue color solution was scanned over the UV-Visible region, the λ max of the reduced folin reagent was 765 nm. However, in the actual experiment, the wavelength for the experiments was arbitarily set to 650 nm. Fig. 8 shows the visible range spectrum of the reduced Folin reagent.

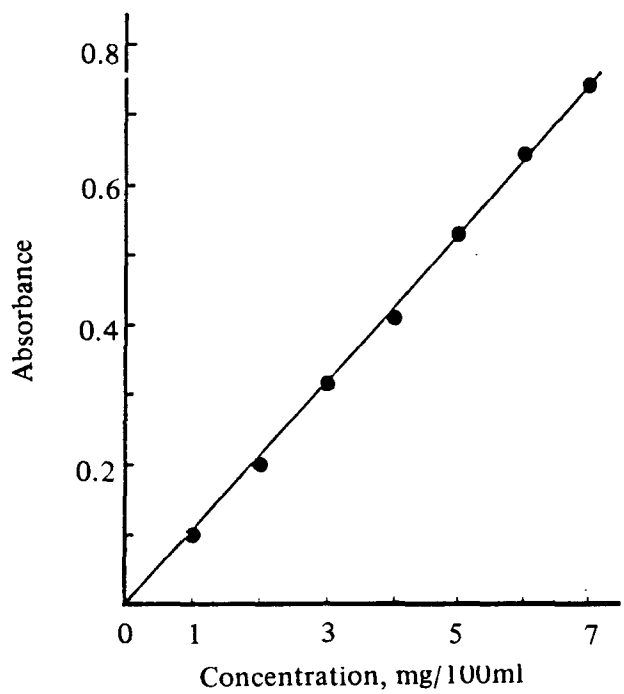

Fig. 7 Calibration curve for triethanolamine

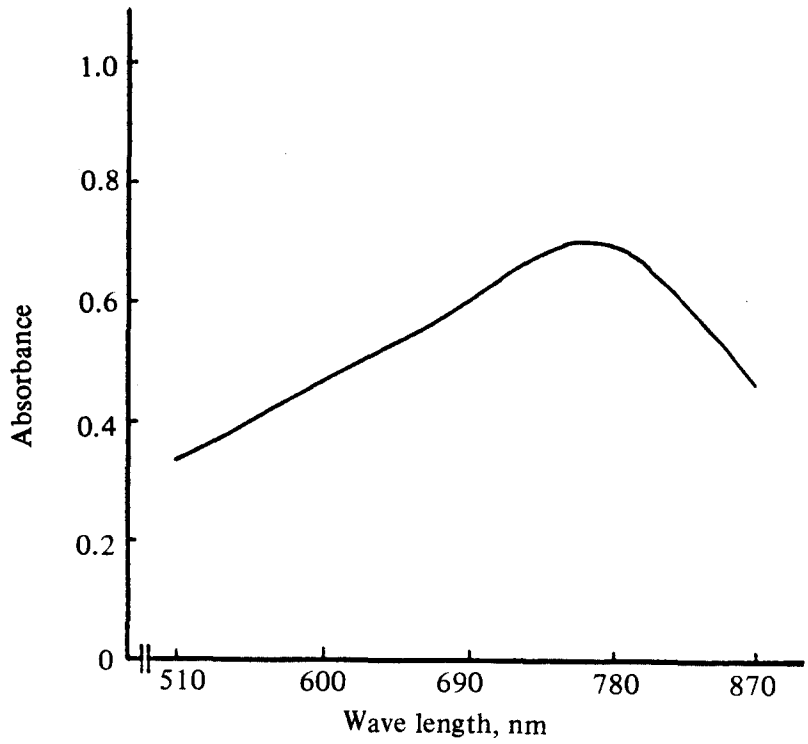

Fig. 8 Typical photospectrum of reduced Folin-Ciocalteau reagent

Table IV. Determination of triethanolamine in cream and shampoo.

Sample		Present (mg/100ml)	Found (mg/100ml)	Recovery (%)
Cream	No.1	6.00	5.75	95.8
Cream	No.2	5.00	4.90	98.0
Cream	No.3	4.00	3.85	96.3
Cream	No.4	3.00	3.10	103.3
Cream	No.5	3.00	3.52	117.3
Shampoo	No.1	3.23	3.21	99.4
Shampoo	No.2	4.31	4.40	102.1
Shampoo	No.3	5.38	5.42	100.7
Shampoo	No.4	6.46	6.54	101.2

As Table IV shows, TEA content in cream and shampoo was determined with ease. This kind of simple method appears to be an easy method without the separation using HPLC. In case there are no other reducing materials in the sample, this method can be easily applied to quality control in factory.

4. CONCLUSION

TEA was separated and determined by high performance ion exchange chromatography with a post column reaction method. The post column reaction for the detection of the tertiary amines in the column effluent involved the reduction of Folin reagent by the amines and the stabilization of the reduced reagent with an alkaline solution. The spectrometric detection of the reduced Folin reagent eventually leaded to a fairly good result for the assay of TEA in the cream and shampoo products.

Moreover, in case there were not any other interfering materials like the reducing agents, TEA was easily determined by the direct reaction with Folin reagent without HPLC separation.

In this study, the quantification of TEA as a tertiary amine was mentioned only. But trimethyl amine, triethyl amine, triisopropyl amine, etc. also showed good separation and response. Therefore, the post column method can be applied to any other tertiary amines that can be separated by an ion exchange column.

REFERENCES

1. E. Heinerth and J. Pollerberg, Fette-Seifen-Anstri chmittel, 61, 376 (1959)
2. Y. Yoshino and H. Kinoshita, Nippon Kagaku Kaishi, 86, 405 (1965)
3. L.E. Brydia and H.E. Persinger, Anal. Chem. 39, No. 11, 1319 (1967)
4. R. Pickos, K. Kobylczyk, and J. Grzybowski, Anal. Chem. 47, 7, 1157 (1975)
5. D. Valdez, J. Chromatogr. Sci., 23, 128 (1985)
6. A. Nakae, K. Mansho, and K. Tsuji, Bunseki Kagaku, 30, 353 (1981)

7. O.H. Lowry, N.J. Resenbrough, A.L. Farr, and R.J. Randall, J. Bio. Chem., 193, 265 (1951)
8. F.A. Cotton and G. wilkonson, Advanced Inorganic Chemistry, 4th ed., Johnwiley & Sons, N.Y., (1980)