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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF OPTICALLY ACTIVE AND RACEMIC EPHEDRINES BY DERIVATIZATION AND METAL CHELATES FORMATION

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Nickel chelate of dithiocarbamate derived from *d*- or *l*-ephedrine gave one peak in high-performance liquid chromatography while that from racemic ephedrine gave two peaks, the same one and additional one. By measurement of areas of these peaks, optically active and racemic ephedrines were determined.

The formation of chemical derivatives has been play an important role in high-performance liquid chromatography(HPLC) to improve detection sensitivity. It has been well known that the dithiocarbamates of the corresponding bases are formed almost instantaneously at room temperature in alkaline medium by the reaction of carbon disulfide on primary or secondary aliphatic amines[1]. The resulting dithiocarbamates coordinate with various metal ions as shown below.

$$R_1R_2NH + CS_2 + OH^- \rightleftharpoons R_1R_2NCSS^- + H_2O$$
$$nR_1R_2NCSS^- + M^{n+} \rightleftharpoons M(R_1R_2NCSS)_n \quad (1)$$

In the previous report[2], HPLC determination of various metal ions as diethyldithiocarbamate chelates has been described. The present report shows the applicability of metal complex formation of dithiocarbamate for optically active organic amine analysis.

An HPLC apparatus of our own construction was used[2,3]. A single-beamed spectrometer(Model Specta 20, Toshiba Beckmann Co. Ltd.) equipped with a flow cell was used as a detector. Data analysis of the chromatograms were carried out by "Chromatopak E-1A"(Shimazu Co. Ltd.) mini computor.

The reaction was carried out by the following way. To 1 cm^3 of each amine sample, 1 cm^3 of 10 mM nickel chloride solution in conc. aqueous ammomia and 5 cm^3 of chloroform containing 1 % of carbon disulfide were added. The mixed solution in a test tube equipped with a ground grass stopper was shaken vigorously for 20 sec to complete the reaction, and the greenish yellow chelate produced was extracted with chloroform. Thus complex formation and extraction were carried out simultaneously.

Fig. 1(a) and (b) show chromatograms of nickel(II) dithiocarbamate chelates

derived from *l*- and *dl*-ephedrine, respectively. *l*-Ephedrine exists in nature and has been used as antiathetic agents. Contrary to this, *d*-ephedrine is not effective for antiathetic purpose.

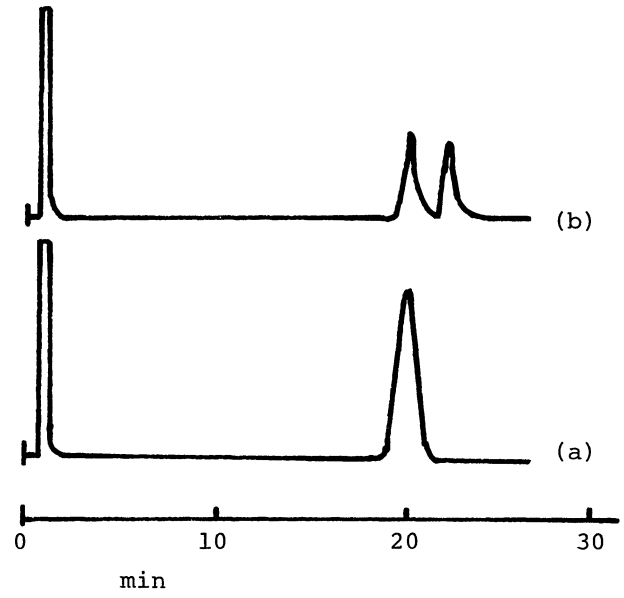

Fig. 1 chromatograms of nickel(II) dithiocarbamate chelates of *l*- and *dl*-ephedrine

(a) 0.2 mM *l*-ephedrine (b) 0.2 mM *dl*-ephedrine

Column: LiChrosorb SI 100 (4 mm x 25 cm) Eluent: hexane:isopropyl-acetate = 100:11.5(water saturated) Detector: 325 nm Flow rate: 2.5 cm^3/min Pressure: 110 kg/cm^2 Sample size: 50 μl

As shown in Fig. 1(b) when racemate was used, two peaks appeared on a chromatogram. The peak area of the former(S_1) was equal to that of the latter(S_2) within an experimental error($S_1/S_2 = 1.00 \pm 0.03$ for 4 measurements). Total peak area ($S = S_1 + S_2$) was equal to peak area of *l*-ephedrine appeared in Fig. 1(a). Dithiocarbamate chelate derived from *d*-ephedrine gave one peak whose retention time was identicical to that derived from *l*-ephedrine. These phenomena were explained as follows. When racemic mixture exists in solution, different metal chelates shown by the following equilibrium are formed.

$$MD_2 + ML_2 \rightleftharpoons 2MDL \qquad (2)$$

Here, D and L indicate dithiocarbamates derived from *d*- and *l*-ephedrine, respectively. When metal chelates are not so labile and disproportionation of ternary complex during chromatography can be neglected, three peaks corresponding to each species shown in Eq.(2) should appear on chromatograms. Since MD_2 and ML_2 are enantiomers to each other and their chemical properties except optical activity should be identical, they will not be separated. On the other hand, ML_2(or MD_2) and MDL are diastereomers to each other, and might be separated. The former peak in Fig. 1(b), therefore, should be attributed to the mixture of MD_2 and ML_2, and the latter MDL. The ratio of *d*- and *l*-isomers is determined by the following way. Taking the initial concentrations of *d*- and *l*-isomers to be d_0 and l_0, concentrations of MD_2 and ML_2 are $d_0 - r/2$ and $l_0 - r/2$, respectively, where r is the concentration of MDL. If ternary complex formation shown in Eq.(2) is controlled by a statistical factor[3] ($K = [MD_2][ML_2]/[MDL]^2 = 0.25$), it can be derived that $r = 2d_0l_0/(d_0 + l_0)$. The ratio, S_2/S, is calculated to be

$$S_2/S = S_2/(S_1 + S_2) = [MDL]/([MD_2] + [ML_2] + [MDL]) = r/(l_0 + d_0)$$
$$= 2d_0l_0/(d_0 + l_0)^2 = 2p(1 - p) = -2(p - 1/2)^2 + 1/2 \quad (3)$$

where $p = d_0/(d_0 + l_0)$. Fig. 2 shows parabola plot of Eq.(3) with different ratio of *d*- and *l*-isomers at a definite total ephedrine content. Total peak area(S) was constant within an experimental error (<2 %). Both experimental and calculated values agreed well as shown in Fig. 2. Thus total amount and optical purity of *dl*-mixture can be determined.

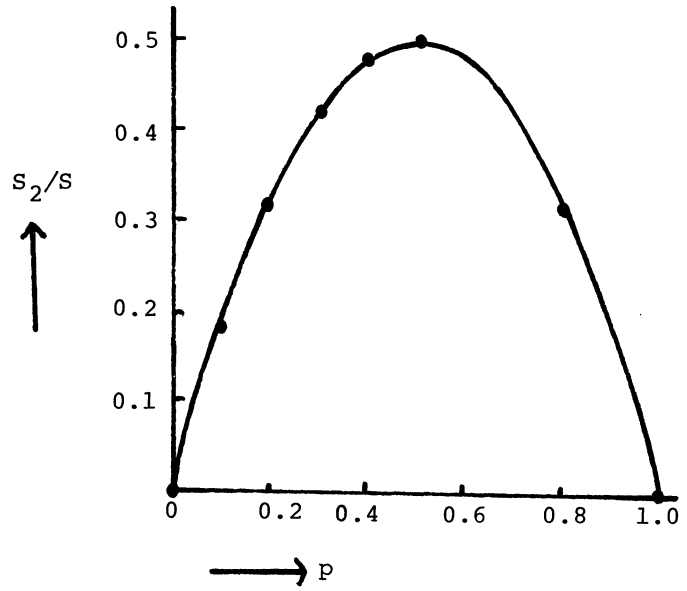

Fig. 2 Plot of Eq.(3) at various *d*- and *l*-ephedrine ratio

Total content of ephedrine was 0.1 mM. Other experimental conditions were similar to those shown in Fig. 1.

— calculated
● observed

Since absorption coefficient of nickel diethyldithiocarbamate is high, (log ε = 4.57 at 325 nm[4]), this chelate is sometimes used for colorimetric determination of nickel. Absorption coefficient of nickel chelate derived from *l*-ephedrine at 325 nm was similar to that of nickel diethyldithiocarbamate chelates. Since ephedrine itself only shows weak absorption bands in UV range (log ε = 2.31 at 254 nm[5]), after this derivatization detection sensitivity should become higher than 90 times considering two ligand molecules coordinate with nickel ion. The calibration graph of *l*-ephedrine was linear from 50 ng to 2 μg. At the smaller amount, calibration graph deviated slightly from a linear plot and peak areas were smaller than the values predicted by a linear plot. The detection limit was 5 ng by the present apparatus under the condition shown in Fig. 1(a).

When various similar amines are coexisted, chromatograms will be complicated because of the formation of various ternary complexes. In such case, peaks of various ternary complexes formed by two different kinds of amines should appear on chromatograms. Therefore, for the determination of individual amines, pretreatment of the sample, for example TLC, will be required.

Chromatogram patterns will be dissimilar when in place of nickel ion other metal ion which forms very labile complexes is used. In such case, as soon as

each chelate is separated, ternary complex should disproportionate into two different binary complexes in the column promptly ($2MAB \longrightarrow MA_2 + MB_2$). Therefore, peaks of ternary complexes should not appear on chromatograms. From the measurement of peak areas of binary complexes, determination of individual amines will be achieved. Fig. 3(a) and (b) show chromatograms of copper chelate derived from *l*-ephedrine in the absence(a) and presence(b) of other amines. Only peaks of binary complexes corresponding to each amine appeared on chromatograms, provided that initial flow rate of the pump was set to be so low that disproportionation of the ternary complex in the column may proceed completely. In this case metal chelate derived from *l*- and *dl*-ephedrine gave

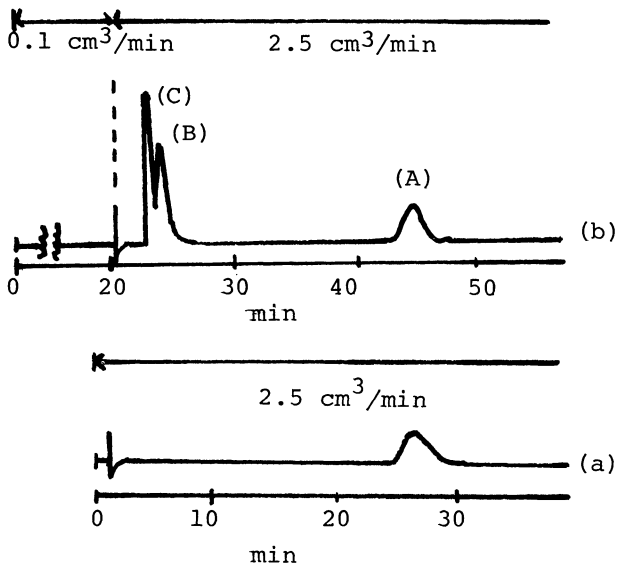

Fig. 3 Chromatograms of copper(II) dithiocarbamate chelates of *l*-ephedrine in the absence and presence of other amines

(a) 0.2 mM *l*-ephedrine (b) 0.2 mM *l*-ephedrine(A) + 0.2 mM pyrrolidine (B) + 0.2 mM piperidine(C)
Column: LiChrosorb SI 100
Eluent: hexane:isopropylacetate = 100:8 (water saturated)
Detector: 440 nm
Flow rate: from 0.10 to 2.5 cm^3/min (flow rate gradient)
Sample size: 50 µl

similar chromatograms.

The present method will be applicable to other optically active amines and will be useful especially for the determination of optical purity of these amines.

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