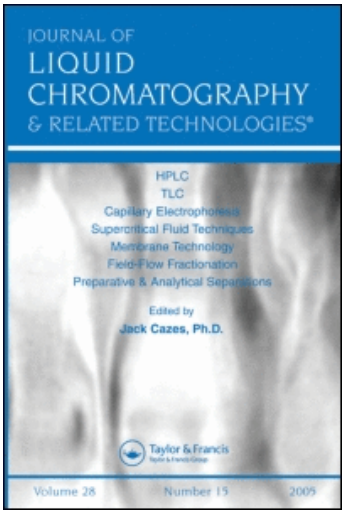


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# The Analysis of Enantiomeric and Diastereoisomeric Mixtures of Ephedrine and Pseudoephedrine Using Reversed-Phase High Performance Liquid Chromatography of Nickel Dithiocarbamate Complexes

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# THE ANALYSIS OF ENANTIOMERIC AND DIASTEREOISOMERIC MIXTURES OF EPHEDRINE AND PSEUDOEPHEDRINE USING REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF NICKEL DITHIOCARBAMATE COMPLEXES

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## ABSTRACT

Following their conversion to dithiocarbamate ligands and thence to nickel complexes, enantiomeric mixtures of ephedrine or pseudoephedrine may be separated and quantitated by reversed-phase High Performance Liquid Chromatography (HPLC) using ternary solvent mixtures. The solvent used to dissolve the complexes prior to injection was found to have a significant effect on the separation. In a similar manner, a mixture of the diastereoisomers ephedrine and pseudoephedrine was separated and quantitatively analysed using a binary solvent as the mobile phase. This separation was achieved both with prior formation of the nickel complexes and also with on-column formation using nickel(II) ions in the mobile phase. The analysis of diastereoisomeric contaminants in pharmaceutical products and raw materials containing ephedrine or pseudoephedrine is illustrated.

## INTRODUCTION

Dithiocarbamate complexes of nickel can readily undergo ligand exchange reactions to produce ternary, or mixed-ligand, complexes (1), according to the following equilibrium:

$$Ni(L_1)_2 + Ni(L_2)_2 \rightleftarrows NiL_1L_2$$



 0148-3919/830602-0311$3.50/0

$L_1$ and $L_2$ are bidentate dithiocarbamate ligands, $Ni(L_1)_2$ and $Ni(L_2)_2$ are binary complexes and $NiL_1L_2$ is a ternary complex. If this reaction mixture is analysed by normal-phase High Performance Liquid Chromatography (HPLC), three peaks are observed, with the ternary complex peak lying between the two binary complex peaks. When $L_1$ and $L_2$ are enantiomers, then the two binary complexes co-elute as a single peak, prior to the ternary complex peak.

Moriyasu and Hashimoto (2,3) have utilised these observations for the precise quantitation of enantiomeric impurities in amines, after conversion of the amines to dithiocarbamate ligands by reaction with carbon disulphide under alkaline conditions, and subsequent formation of nickel complexes. The HPLC separation of the binary and ternary nickel complexes was achieved using a water deactivated silica column, however this method has the serious disadvantage that long equilibration times are required to give reproducible results (4).



In this paper, we describe the use of reversed-phase HPLC for the analysis of enantiomeric and diastereoisomeric impurities in amines, using binary and ternary solvent mixtures. This approach is discussed both for the separation of nickel complexes formed prior to injection, and also for the separation of dithiocarbamate ligands using mobile phases containing nickel ions. In the latter method, dithiocarbamate binary and ternary nickel complexes are formed on the column. This procedure reduces the number of manipulative steps in the analysis. The reversed-phase HPLC approach provides the basis of a rapid, sensitive analytical method for the screening of enantiomeric and structurally related contaminants in pharmaceutical products containing amines such as ephedrine and pseudoephedrine. Such an analysis has hitherto proved very difficult (5). A brief survey of the application of the proposed method to the analysis of some pharmaceutical products and raw materials is also presented.

## EXPERIMENTAL

### Standards and Reagents

(i) Carbon disulphide-chloroform solution. Carbon disulphide (A.R.Grade, AJAX) was freshly distilled in all glass apparatus and made up to 1% v/v solution with redistilled chloroform.

(ii) Nickel-ammonia solution. 50 ml of 1% w/v of $NiCl_2 6H_2O$ in water was made up to 100 ml with 35% ammonia solution (ARISTAR, BDH).

(iii) Aromatic amine solutions. (±) ephedrine hydrochloride, (-) pseudoephedrine hydrochloride, and (+) pseudoephedrine hydrochloride were obtained from Sigma Chemical Company (USA). (-) ephedrine was obtained from Fluka (Switzerland). These materials were shown to be free from contaminants by GCMS analysis and by compositional data from microanalysis. Test solutions containing approximately 1.0 mg/ml were accurately made up in methanol.

(iv) Tablets and raw materials containing ephedrine and pseudoephedrine. Raw materials containing pseudoephedrine and ephedrine were donated by various pharmaceutical manufacturers in Australia. Single ingredient tablets with declared potencies of 15, 30 or 60 mg of amine were purchased over the counter.



### Synthesis of Dithiocarbamate Complexes

To 1.0 ml of aromatic amine solution, 2 ml of nickel-ammonia solution were added and the mixture extracted with carbon disulphide-chloroform (5 ml; 1% v/v). The chloroform layer was then washed with distilled water (3 x 2 ml) and dried over anhydrous sodium sulphate. The filtered chloroform layer was evaporated to dryness under a steady stream of nitrogen to remove excess carbon disulphide which may produce extraneous chromatographic peaks, and made up to an appropriate volume in methanol or acetonitrile. The nickel complex prepared in this way was shown

to give only one chromatographic peak by HPLC. The identity of each complex was confirmed by Desorption Chemical Ionisation Mass Spectrometry (6).

## Analysis of Ephedrine and Pseudoephedrine in Pharmaceutical Formulations

Twenty randomly selected tablets from a single batch were crushed and an accurately weighed portion of powder equivalent to the average weight of a single tablet was taken and dissolved in water in a volumetric flask with the aid of an ultrasonic bath. The sample was diluted to a suitable volume to give a concentration of 1 mg/ml, after which the solution was filtered and an aliquot equivalent to 10 mg of drug was made alkaline with 2M NaOH and extracted with 20 ml of chloroform. The organic layer was passed through a column of anhydrous sodium sulphate and 10 ml was evaporated to dryness under a stream of nitrogen. The nickel complex was then formed by the method described in the previous section. The final solution was made up to a concentration of 0.1 mg/ml in methanol and 5-10 µl of the solution was injected with the HPLC detector set at 0.2 AUFS.



For the raw materials, appropriate dilutions were made to give the concentration described above and the analysis performed using the same procedure as for the tablets.

Quantitations were made using chromatographic peak heights and all assays were performed in triplicate. For most samples, two determinations at two different sensitivity levels were required: the first was performed at the most sensitive attenuation position of the detector to establish whether any contaminant was present, after which a less sensitive attenuation was used for the determination of the active ingredient in the sample.

## HPLC Instrumentation and Procedure

The liquid chromatograph consisted of Waters Associates (Milford Ma) Model M6000 solvent pump, Model U6K injector, Model M440 UV

detector and QD 15 Hitachi Recorder. The column used was a 15 cm x 4.6 mm ID Ultrasphere (Altex Scientific Inc.,Berkeley,Ca) $C_{18}$ column, with a mean particle diameter of 5 μm. The detector was operated at 313 nm with a sensitivity setting of 0.2 AUFS and all separations were carried out at 20°C using a mobile phase flow rate of 1.5 ml $min^{-1}$.

Methanol and triethylamine were of Analytical Grade and were distilled in all glass apparatus. Acetonitrile (HPLC Grade) was purchased from Waters Associates. The exact ingredients of the mobile phases used are given in the captions to the figures. Mobile phases were aspirated through 0.7μm glass microfibre paper filters (GF/F Whatman), degassed in an ultrasonic bath and allowed to equilibrate to ambient temperature before used.



## RESULTS AND DISCUSSION

### Analysis of Mixtures of Enantiomers

A mixture containing both enantiomers of ephedrine or pseudoephedrine was quantitatively analysed by conversion of these amines to dithiocarbamate ligands and thence to nickel complexes, with subsequent separation using reversed-phase HPLC. Typical chromatograms are shown in Fig. 1; these separations are similar to that previously reported for normal-phase HPLC using water deactivated silica columns (2,3). In each chromatogram, the symmetrical binary complexes derived from each enantiomer coeluted as the first peak, which was separated from the peak due to the ternary complex containing ligands derived from both enantiomers. To simplify reference to these complexes, they will be identified as follows: $Ni[CS_2:(+)eph]_2$ is the complex containing two ligand molecules, both of which are dithiocarbamate ligands derived from (+) ephedrine; similarly $Ni[CS_2:(+)eph][CS_2:(-)eph]$ is the ternary complex containing dithiocarbamate ligands derived from (+) ephedrine and (-) ephedrine. Pseudoephedrine will be abbreviated to pse.

Separation of the binary and ternary nickel dithiocarbamate complexes shown in Fig. 1 was achieved using a ternary solvent

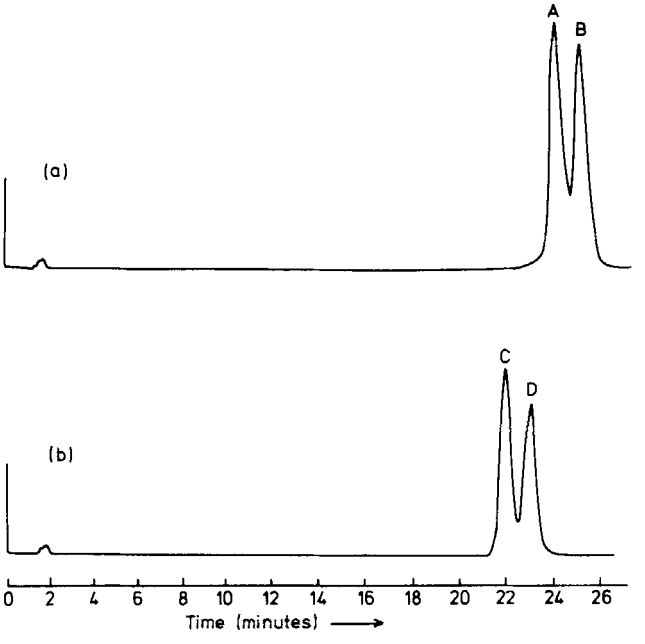


FIGURE 1. Analysis of a mixture of enantiomers of ephedrine [Fig. 1(a)] or pseudoephedrine [Fig. 1(b)].

Mobile Phase: 0.2% (v/v) triethylamine in 25:40:35 (v/v) $CH_3OH$:$CH_3CN$:$H_2O$. Flow rate 1.5 ml/min.

Peak Identities: See text for key to abbreviations
A, unresolved $Ni[CS_2:(+)eph]_2$ and $Ni[CS_2:(-)eph]_2$;
B, $Ni[CS_2:(+)eph][CS_2:(-)eph]$; C, unresolved $Ni[CS_2:(+)pse]_2$ and $Ni[CS_2:(-)pse]_2$; D, $Ni[CS_2:(+)pse][CS_2:(-)pse]$.

system. The seven experiment optimisation procedure of Glajch and co-workers (7) was applied to determine the composition of a suitable isocratic solvent system. A mobile phase containing 25:40:35 (v/v) methanol:acetonitrile:water was found to give optimum resolution, and a small amount of triethylamine was added to further improve the separation, because our previous experiences (6) have indicated that this solvent has a highly selective interaction with the dithiocarbamate complexes under study.

The solvent used to dissolve the complexes prior to injection was found to have a significant influence on the separation achieved by reversed-phase HPLC. When chloroform was used, poor separation and diffuse peak shape resulted; on the other hand, methanol and acetonitrile gave better peak shape and resolution, with the latter solvent giving optimum results. This effect was probably due to a change in the composition of the adsorbed layer of organic modifier (from the mobile phase) on the stationary phase surface. Adsorption of organic modifiers onto reversed-phase columns has been reported previously (8) and it has been proposed that this adsorbed layer can be partially displaced by solute or other molecules (9). In the present case, it is likely that injection of a chloroform solution caused a change in the adsorbed layer of methanol and acetonitrile (from the mobile phase), thereby influencing the ability of the column to resolve the closely related nickel complexes.



The formula proposed by Moriyasu and Hashimoto (2,3) for calculation of the composition of an enantiomeric mixture of amines using normal phase HPLC is equally applicable to reversed-phase HPLC. The reversed-phase method however has the advantage that no lengthy column equilibration time was required for reproducible results, as was the case for the normal-phase method. We have found excellent agreement for analyses of racemic mixtures using both methods.

## Analysis of Diastereoisomeric Mixtures

A mixture containing two diastereoisomers, such as ephedrine and pseudoephedrine, can also be separated by reversed-phase HPLC using binary solvent mixtures as the mobile phase. Such a separation is shown in Fig. 2, where the three peaks in the chromatogram can be assigned to the two binary complexes $Ni[CS_2:(-)eph]_2$ and $Ni[CS_2:(-)pse]_2$ and to the ternary complex $Ni[CS_2:(-)eph][CS_2:(-)pse]$. Again, this chromatogram is similar to that obtainable with normal-phase HPLC (2,3). The relative peak heights in Fig. 2 may be used to quantitatively

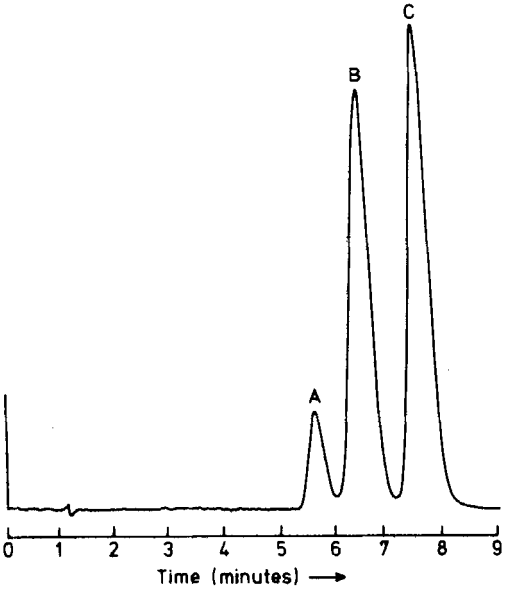


FIGURE 2. Analysis of a mixture of the diastereoisomers (-) ephedrine and (-) pseudoephedrine by prior formation of their nickel dithiocarbamate complexes.

Mobile Phase: 70:30 (v/v) $CH_3OH:H_2O$, flow rate 1.5 ml/min.

Peak Identities: See text for key to abbreviations
A, $Ni[CS_2:(-)pse]_2$; B, $Ni[CS_2:(-)pse][CS_2:(-)eph]$;
C, $Ni[CS_2:(-)eph]_2$.

determine the composition of the original mixture of diastereoisomers. Table 1 compares the results obtained using this method with those obtained using GCMS analysis (6) of a series of standard mixtures of ephedrine and pseudoephedrine.

The accuracy of the reversed phase HPLC method was somewhat poorer than the GCMS method and best results were obtained with mixtures containing a minor percentage of one diastereoisomer. This situation is likely to occur in the analysis of diastereoisomeric contaminants in pharmaceutical samples.

TABLE 1

Analysis of Standard Mixtures of the Diastereoisomers Eephedrine and Pseudoephedrine by the Proposed Reversed-Phase HPLC Method and by GCMS

| % Ephedrine in standard diastereoisomeric mixtures | | |
|---|---|---|
| Actual (%) | by HPLC* (%) | by GCMS (%) |
| 100 | 100 | 101 |
| 82.3 | 87.4 | 83.0 |
| 62.6 | 69.0 | 64.4 |
| 41.2 | 38.0 | 41.6 |
| 21.0 | 22.6 | 22.0 |
| 0.0 | 0.0 | 0.5 |

* Results shown are the average of two runs. For the HPLC method, the maximum range obtained is 2.5% and for GCMS, 1.2%. The HPLC results were calculated using the following formulae applied to chromatograms similar to that given in Fig. 2

If peak 3 > peak 1, then

$$\% \text{ Ephedrine} = 100[0.5 + \sqrt{0.5(0.5 - H_2/\sum_{i=1}^{3} Hi)}]$$

If peak 3 < peak 1, then

$$\% \text{ Ephedrine} = 100[0.5 - \sqrt{0.5(0.5 - H_2/\sum_{i=1}^{3} Hi)}]$$

where H is the height of respective nickel complex peak.

A brief survey was conducted for the presence of diastereoisomeric contamination in ephedrine and pseudoephedrine tablet formulations and pharmaceutical raw materials. A binary mixture of solvents was used for the mobile phase, therefore the resultant chromatogram did not provide information on the presence of optical isomers in the active ingredient of the formulation. A ternary solvent mobile phase would be necessary to elucidate this information (see preceding section). The results are given in Table 2.

It is noteworthy that no interference in the assay method was detected for the sugars and binding compounds present in the tablet formulations. Only peaks corresponding to the active ingredient and the diastereoisomeric contaminant (if present)

TABLE 2

Analysis of Diastereoisomeric Contaminants in Pharmaceutical Formulations and Raw Materials Containing Ephedrine or Pseudoephedrine.

Results are given as percentage ± standard deviation. Each value represents the mean of three individual assay results. The results are calculated with respect to the labelled content of the drug in each tablet; raw products are assumed to be 100% pure. Each standard deviation is estimated from the range of three results.

| Sample | Ingredient Type | % Contaminant | % Active Ingredient |
|---|---|---|---|
| A | Ephedrine | n.d. | 99.8 ± 4.2 |
| B* | Ephedrine | n.d. | 98.9 ± 3.6 |
| C | Ephedrine | n.d. | 92.2 ± 4.3 |
| D* | Ephedrine | 6.0 ± 0.05 | 95.9 ± 1.2 |
| E | Ephedrine | n.d. | 96.9 ± 2.8 |
| F* | Ephedrine | n.d. | 102.0 ± 2.4 |
| G | Ephedrine | 3.9 ± 0.04 | 95.0 ± 1.2 |
| H | Pseudoephedrine | n.d. | 98.0 ± 3.2 |
| I | Pseudoephedrine | n.d. | 99.7 ± 1.6 |
| J* | Pseudoephedrine | n.d. | 101.6 ± 2.4 |
| K* | Pseudoephedrine | n.d. | 99.4 ± 1.3 |

n.d. indicates negligible detection
* indicates that the sample is a raw product



were observed, since the derivatisation procedure used was specific for primary and secondary amines. The sensitivity of this method, with which analyses at the sub μg/ml level presented no difficulty, compared favourably with that given by Barkan and co-workers (5) for their method. Linear calibration plots were obtained with ephedrine and pseudoephedrine for injected amounts of solute in the range 100 to 500 ng, with correlation coefficients better than 0.95.

## Mobile Phases Containing $Ni^{2+}$ Ions

One of the advantages of the use of reversed-phase HPLC for the analysis of metal complexes is that the separation system can

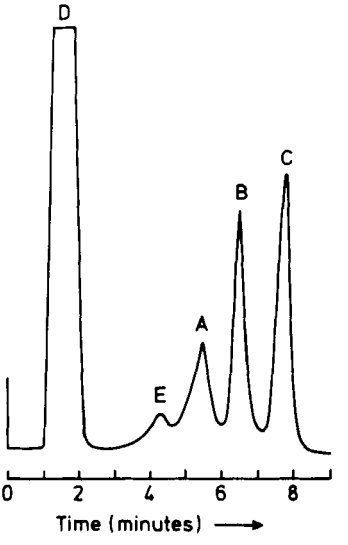


FIGURE 3. Analysis of a mixture of the diastereoisomers (-) ephedrine and (-) pseudoephedrine using on-column formation of nickel dithiocarbamate complexes.

Mobile Phase: 70:30 (v/v) $CH_3OH$:0.2% (w/v) aqueous $NiCl_2.6H_2O$. Flow rate 1.5 ml/min.

Peak Identities: A,B,C as for Fig. 2; D, excess carbon disulphide; E, solvent impurity.



be readily modified by addition of a metal ion to the mobile phase. When $Ni^{2+}$ was added to the mobile phase and a mixture of diastereoisomeric dithiocarbamate ligands (derived from ephedrine and pseudoephedrine) was injected, the chromatogram shown in Fig. 3 resulted. The large first peak was due to excess carbon disulphide remaining after derivatisation of the amine durgs, and a small peak due to solvent impurity was also observed. The remaining peaks were assigned to binary and ternary nickel complexes formed during the migration of the dithiocarbamate ligands through the reversed-phase column.

This method of separation was more simple than the use of prior formation of nickel complexes and forms the basis of a rapid

and sensitive method for the detection of diastereoisomeric contaminants in primary and secondary amines. It is noteworthy that the relative peak heights obtained with the in-situ complex formation method were somewhat dependent on the mobile phase flow rate, indicating that differences in lability probably exist between the different complexes. This variation in relative peak heights was not observed when the nickel complexes were formed prior to injection.

## CONCLUSIONS

Enantiomeric and diastereoisomeric amines may be separated and quantitated after their conversion to nickel dithiocarbamate complexes, using reversed-phase HPLC. This method gives similar results to those obtained using the previously reported normal-phase method, however the former procedure does not require extensive column equilibration times for reproducible results. In addition, the reversed-phase method can be modified to allow in-situ formation of the nickel complexes, thereby eliminating some of the manipulative steps. The proposed method was successfully applied to the analysis of diastereoisomeric contaminants in pharmaceutical products and raw materials containing ephedrine or pseudoephedrine.



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