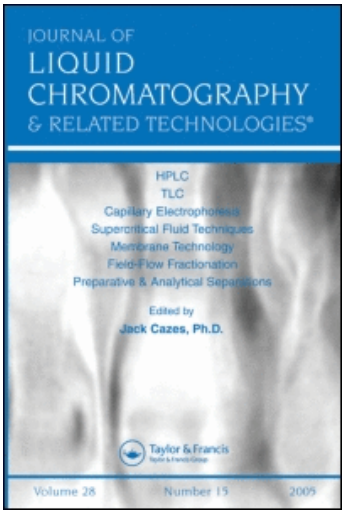

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DETERMINATION OF CHIRAL DIAMINES BY LC-DAD AND LC WITH POLARIMETRIC DETECTION

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ABSTRACT

The chromatographic method for the separation of racemic aminoacetonitriles and diamines, the intermediates in the synthesis of N^1-alkyl-1-arylethylenediamines derivatives, possessing biological activity, was elaborated on. The results of pharmacological testing were acquired so far only for racemic mixtures, so that a method, which would allow the control of purity, is required. The DNBPG chiral stationary phase (*R*-3,5-Dinitrobenzoyl-phenylglycine) was used for the enantioseparation of derivatives of aminoacetonitriles and diamines. Using a polarimetric detector the optical rotation of the separated enantiomers was determined.

The effect of various substituents on the retention and enantioselectivity was investigated. The potential possibility of

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using a DAD detector in characterisation of the separated enantiomers and checking their purity was also investigated.

INTRODUCTION

Different biological activity of particular enantiomers of chiral substances on a living organism was the main reason for the development of chiral separation methods. They are mostly based on the chromatography with utilisation of TLC, GC, and HPLC techniques. In the HPLC method, the separation is frequently achieved by the application of chiral stationary phases (CSPs).

Elaboration of separation and identification methods for the enantiomers of drugs is necessary, e.g., the Food and Drug Administration (USA) requires full descriptions of a racemic drug, which includes identity, molecular structure, identification of all chiral centers, and enantiomers ratio, although 50 : 50, is by definition for a racemate.[1] In this regard, HPLC with chiral (enantioselective) stationary phase is extremely useful.

Many different methods of detection were used for the identification of enantiomers. Some usually used in chromatography detectors were utilized, like UV-VIS, NMR, or special detectors like a circular dichroism detector.[2–6] The absolute configurations of the enantiomers of five O-aryl-O-alkyl-*N*-alkyl-phosphoramido-thioates, after their separation on Pirkle model column (OA-4700), were determined by circular dichroism (CD) spectroscopy.[7] Circular dichroism based detection for monitoring chiral HPLC was applied by Kudo and co-workers.[8] Vibration circular dichroism was used by Tran and co-workers[9] for the chiral detection of the (*R*) and (*S*)-2,2,2-trifluoro-1-(9-antryl)-ethanol and (*R*)- and (*S*)-benzoin.

Pirkle and Selness[10] applied intra- and intermolecular 1H [^{1}H]-nuclear Overhauser effects to investigate interactions between analytes and CSP. The absolute configurations of the enantiomers have been established by a combination of HPLC and NMR methods by Pirkle and Liu.[11]

Polarimetric detector has also been frequently used in the separation of chiral substances. Lloyd and co-workers described the utility of polarimeter based on a near-infrared semiconductor diode laser.[12] A new polarization photometric detector for chiral LC was constructed and tested by Hamasaki and co-workers.[13]

Combined utilisation of UV and polarimetric LC detectors for the enantiomers of transpermethrinic acid pentafluorobenzyl ester was demonstrated by Boehme and co-workers.[14]

Utility of the LC/MS tandem technique in chiral separations of several β-blokers was shown by McCullagh et al.[15]

Horvath et al. investigated bonding of mandelic acid on the Pirkle type CSP by Raman spectroscopy.[16]

Different strategies for the quantification of partially coeluting optical isomers based on the use of different treatments of the chromatographic ultraviolet signals, was shown by Verdu'-Andred et al.[17]

A diode array detector was also used for confirmation that the investigated substances are chiral. Analysis of the optical isomers of fenvaletrate was investigated by Papadopoulou-Mourkidou.[18,19]

The chiral separation of lorazepam in plasma was achieved on a chiral column with a UV and DAD detector by Kanazawa et al.[20] who also showed that chiroptical detection was a useful tool for the pharmacokinetic study of chiral drugs.

A new kind of chromatography (hydrophilic interaction chromatography—HILIC) with Evaporative Light Scattering Detection (ELSD) to investigate chiral polar compounds was used by Risley and Strege.[21]

Tkaczynski and Matosiuk[22] obtained products of condensation of *N*-alkyl-arylethylenediamines (both imidazoline derivatives and fused heterocyclic systems containing this ring), which were racemic mixtures. It was found that these new compounds possessed a significant activity in the Central Nervous System, e.g., analgesic, antiepileptic, and serotonergic action.[23] Syntheses of racemates of these derivatives were briefly described earlier.[22]

The aim of our study was to elaborate on a chromatographic method, which allows controlling the enantiomeric purity of diamines, intermediates in the reaction course, during the separation, by formation of their diastereomeric salts.

We also wanted to confirm the utility of a DAD detector combined with a polarimetric detector, in investigations of chiral substances of potential pharmacological activity. Another purpose was to estimate how the selectivity and retention of chromatographed enantiomers are influenced by the hydrophobic properties of the hydrocarbon chain (chain length) of the alkyl moiety.

EXPERIMENTAL

Apparatus

Liquid chromatography was performed with a modular liquid chromatograph Hitachi-Merck [Darmstadt, Germany] equipped with a diode array detector and a Rheodyne injector model 7125 (Cotati, CA, USA) with a 20 μL loop. The column dead-time (t_m) was calculated from the retention time of toluene.

A PU 4010 Philips pump with Chiralyser (IBZ Messtechnik GmbH, Hannover, Germany) equipped with a polarimetric detector, (kindly provided by the Medical Centre of Postgraduate Education, Pharmaceutical Studies, Biopharmacy Department, Bydgoszcz), was used for the determination of the optical rotation of enantiomers.

Detection was accomplished at 254 nm or by tandem ultraviolet and polarimetric detectors operating at 254 nm and 589 nm, respectively.

Chromatography was carried out at ambient temperature. Injection volume was 10 μL of ethanol solution of investigated substances.

Chemicals

2-propanol and *n*-hexane of spectral purity grade were obtained from PPH Odczynniki Chemiczne S.A. (Gliwice, Poland).

Derivatives of N^1-alkyl-1-arylethylenediamines (their chemical structures are given in Table 1) were obtained from the Department of Synthesis and Technology of Drugs, Medical University of Lublin, and were dissolved in eluent in a concentration of 0.5 mg/mL. The solutions of investigated racemates were stable during chromatographic analysis; no precipitation or additional peaks were observed. All substances were stored in a cool place.

The amino group in diamine was treated with benzoyl chloride to give the corresponding dibenzoyl derivatives shown in Table 1, by the general method described in.[24]

A column packed with the stationary phase: (+) (*R*)-3,5-Dinitrobenzoyl-phenylglycine (DNBPG), Hichrom; (Berkshire, United Kingdom); (25×0.4 cm, $d_p = 5$ μm) was used.

RESULTS AND DISCUSSION

The potentially pharmacologically active compounds, derivatives (IV and V) of N^1-alkyl-1-arylethylenediamines (III) can be obtained according to the following scheme (Scheme 1).

Pharmacological investigations of the final racemic products of condensation of N^1-alkyl-1-arylethylenediamines (IV and V) were successful.[22] In the next stage it is necessary to carry out pharmacological investigation using individual enantiomers. The separation of the racemate of N^1-alkyl-1-arylethylenediamines in the micro scale, by formation of the diastereomers with chiral acids, is not easy owing to their rather weak basicity. It seems much easier to separate in this way, the diamine (III) (Scheme 1) and to use pure enantiomers in the synthesis of the final derivatives. The identity and purity of N^1-alkyl-1-arylethylenediamines was earlier confirmed by NMR investigation.[22–24] Elaboration of a chromatographic method was necessary for the control of the enantiomeric purity of the diamine, during the separation by formation of the diastereomeric salts, and for further pharmacological investigations.

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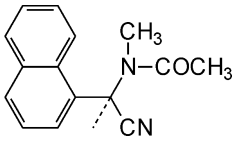
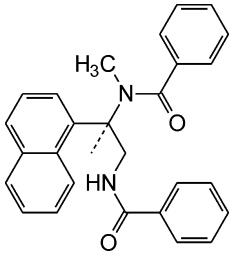
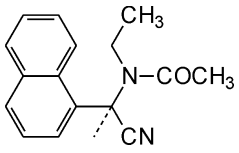
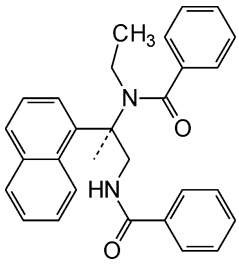
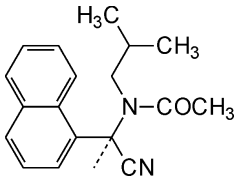
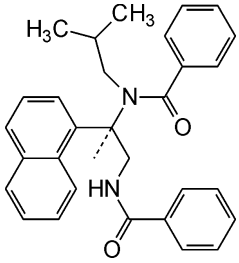
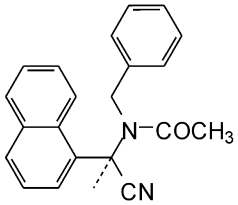
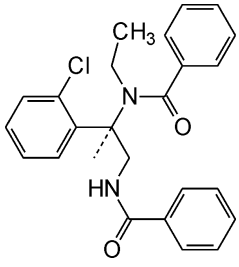
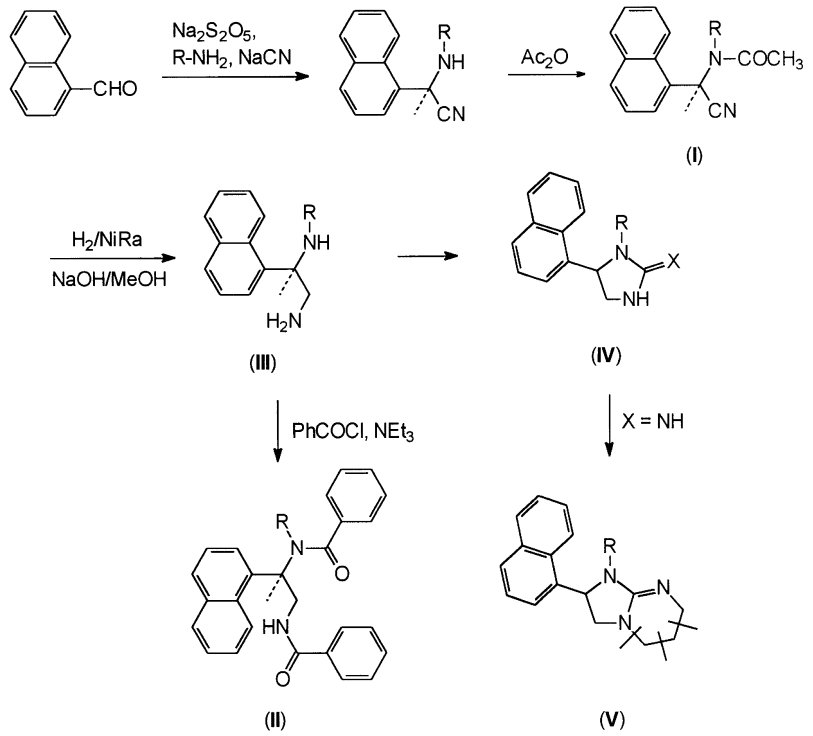
Table 1. The Chemical Structure of Investigated Compounds

Substance	Structure	Substance	Structure
A		E	
B		F	
C		G	
D		N	



Scheme 1. Synthesis and reaction of N^1-alkyl-1-arylethylenediamines.

In the present paper, the chiral resolution of the intermediates (I, II) using LC with chiral stationary phase of the Pirkle type is described. The (*R*)-3,5-Dinitrobenzoyl-phenylglycine was chosen as the chiral stationary phase (CSP).

In Table 1, the structures of the investigated compounds are shown. The samples can be divided into two groups; the first group contains aminoacetonitriles (A, B, C, D) and the second group derivatives of ethylenediamines (E, F, and G). For each investigated pair of the enantiomers, the retention factors [*k*], and the enantiomers' separation factor [α], and the optical rotation sign of the first eluted enantiomer were determined and are presented in Table 2.

All these data were obtained for the mobile phase containing 15% v/v of 2-propanol in *n*-hexane.

Examination of the optical rotation data in Table 2 indicates that for the three acetonitrile derivatives (compounds A, B, and C), the first eluted peaks possess a negative sign of rotation, but not so for the fourth acetonitrile derivative (compound D), for which the first eluted enantiomer has a positive sign.

Table 2. The Chromatographic Data from the Resolution of Investigated Compounds

Substance	Retention Factor k_1	Retention Factor k_2	The Sign of the Optical Rotation of the First Eluted Enantiomer	Separation Factor, α
A	4.83	5.16	(−)	1.07
B	4.32	4.69	(−)	1.09
C	3.20	3.47	(−)	1.09
D	4.39	4.54	(+)	1.03
E	4.84	5.07	(+)	1.05
F	4.30	4.82	(+)	1.12
N	3.46	3.68	(+)	1.06

It is known that the addition of the benzoyl substituent to the chiral compound, close to the chiral center, may function as an additional interactive site. It is seen in Table 2, that the π-basic moiety in compound D, which replaced the alkyl chain in compounds A, B, and C, is involved in a charge-transfer complex with the complementary π-acid 3,5-dinitrobenzoyl moiety of the DNBPG CSP. Thus, changing of the optical rotation sign for sample D probably results from π–π interaction, the more so as the retention of compound D is also slightly increased in comparison to compounds B and C.

Another interesting phenomenon is the opposite elution order of chiral diamines as amide derivatives (+ before − for compounds E, F, and G). Probably, two π-basic benzene rings in every diamine derivative interact with the π-acid moiety on CSP and cause a reversal of the elution order, indicating that charge–transfer interaction can contribute to the chiral recognition.

Considering Table 2, and making a comparison of separation factors α between two compounds, e.g., A and B or A and C, it is seen that enantioselectivity increases with the elongation of the alkyl chain length. The same can be said about compounds E and F, where the addition of one methylene group increases the selectivity factor from $\alpha = 1.05$ to $\alpha = 1.12$ and decreases the retention factors for both enantiomers of compound F. These results indicate that steric hindrance close to the chiral centre is, in this case, advantageous for chiral recognition. It should be noted that if the alkyl substituent increases too much (e.g., C_4H_9 in compound G), the chiral recognition decreases.

Since the absolute configurations of the investigated compounds are presently unknown, their identity remains to be described by optical rotation, (−) and (+). Another possibility is to use the DAD detector, which allows comparison of UV spectra for pair of separated enantiomers.

The UV spectrum of the pure compound is usually a very powerful proof of identity for most compounds. Two enantiomers of the racemate should have the same values of molar absorptivity, maxima of wavelengths, and shapes of spectrum. The DAD detector confirms the identity only for satisfactorily resolved enantiomers ($R_s > 1.0$). The chromatographic separation of the enantiomers of compounds C (Table 2) on DNBPG column is shown in Fig. 1. As can be seen from the chromatogram (Fig. 1), both selectivity ($\alpha = 1.086$) and resolution ($R_s = 1.24$) are quite good; the three-dimensional chromatogram was taken (Fig. 2). It can be seen that the coloured UV spectra of both resolved enantiomers of substance C have an identical shape in a wide region of wavelengths.

The chromatographic method of the separation of enantiomers reported here is presently being applied for monitoring degree of separation of diamine enantiomers (compound *N*— the most interesting from the pharmacological point of view) on micropreparative scale. As is seen in Fig. 4, after the first step of formation of diastereoisomers and crystallisation the optical purity for the

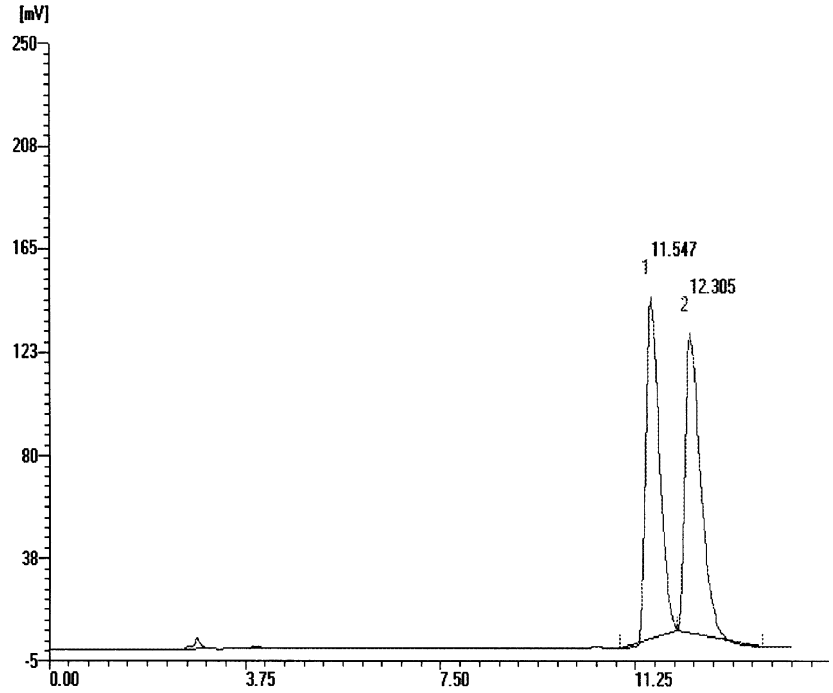


Figure 1. Chromatographic resolution of the racemate of substance C. Chromatographic conditions as in Table 2.

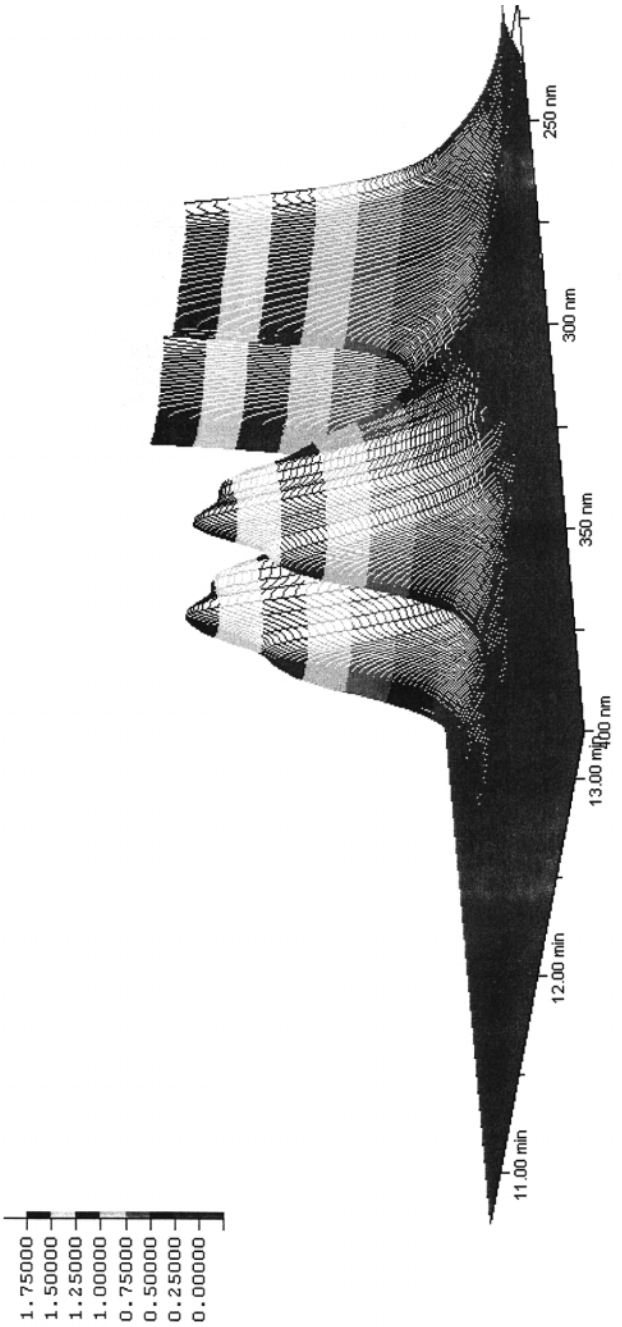


Figure 2. Confirmation of identity for two enantiomers of substance C obtained using a DAD detector. Chromatographic conditions as in Table 2.

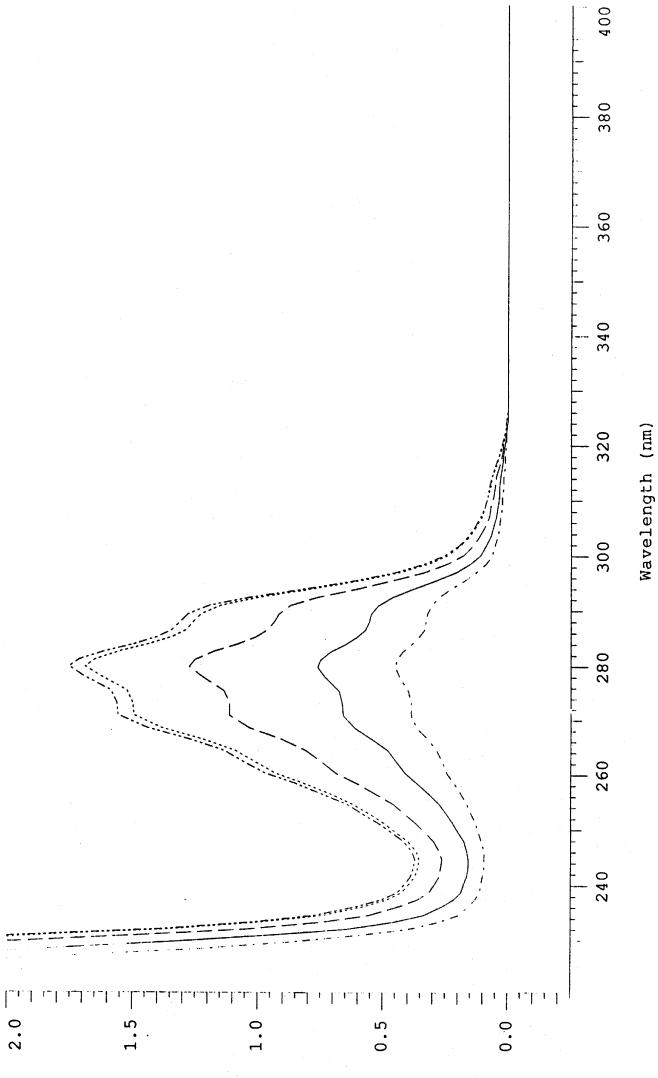


Figure 3. Determination of the purity of enantiomers of substance C using DAD detector by comparison of spectra at different parts of the peaks.

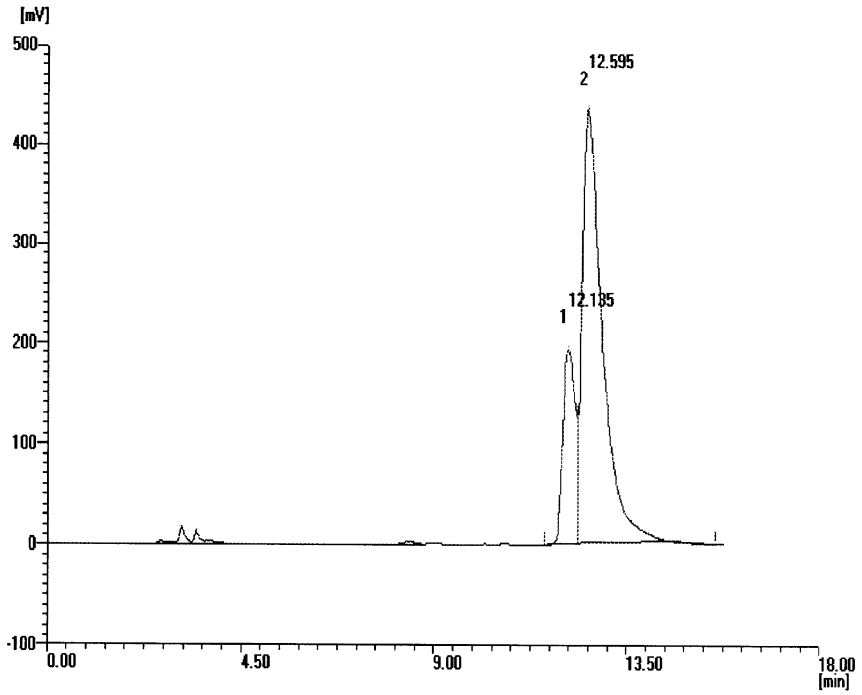


Figure 4. Chromatographic resolution obtained for the enantiomers of compound *N* after the first step of micropreparative separation by formation of diastereoisomers with chiral acid. Chromatographic condition as in Table 2.

stronger retained enantiomer is about 60%. The optical purity was next increased with continued recrystallization.

Confirmation of the purity of the enantiomers of substance C is demonstrated in Fig. 3. It was carried out by the determination of UV spectra at several specific wavelengths in the different parts of the eluted enantiomer peak (on the slopes and the top of the peaks). The spectra obtained for the different retention times correspond to different enantiomeric concentrations of substance C and have almost identical shapes. The same experiments for confirmation of the purity were done with racemate and partly resolved substance N on preparative scale.

CONCLUSIONS

The enantiomers of some of *N*-alkyl-1-arylethylenediamines derivatives have been chromatographically separated on THE DNBPG column. From the

results of separations observed, it is likely that the enantiomers of a wide variety of substituted *N*-alkyl-1-arylethylenediamines derivatives can be resolved by the DNBPG column.

Application of the elaborated chromatographic system, combined with DAD detector to the separation and identification of resolved enantiomers, are demonstrated.

The proposed method can be extremely efficient for compounds with two chiral centers, where two of four enantiomers have the same sign of optical rotation that cannot be distinguished by a polarimetric detector.

Searching for more selective chromatographic system, which would allow determination, e.g., 0.1% of one of the enantiomers in the presence of the other, is in progress.

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