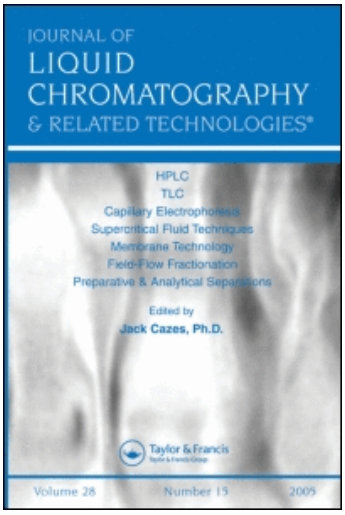

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Reversed Phase Liquid Chromatographic Separation of Lysergic Acid Diethylamide (LSD) and Lysergic Acid Methylpropylamide (LAMPA)

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REVERSED PHASE LIQUID CHROMATOGRAPHIC SEPARATION OF LYSERGIC ACID DIETHYL-AMIDE (LSD) AND LYSERGIC ACID METHYL-PROPYLAMIDE (LAMPA)

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Abstract

A fast and convenient procedure is described for the HPLC separation of LSD and LAMPA. These compounds are separated by a reversed phase (C18) procedure using a binary solvent system of methanol and pH 3 phosphate buffer. Under these conditions all compounds are eluted in a retention volume of 27 mL or less. This procedure allows a forensic sample to be identified as LSD and to eliminate LAMPA as a possibility.

Introduction

Lysergic acid diethylamide (LSD) and lysergic acid methylpropylamide (LAMPA) have very similar analytical profiles by many standard techniques. These

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two compounds are reported[1] to exhibit nearly identical infrared and mass spectra. Thus, these analytical procedures do not allow for a differentiation between LSD and LAMPA. The identification of a forensic drug sample as LSD requires analytical methods which distinguish it from LAMPA.

Several GLC procedures are reported for the analysis of lysergic acid derivatives[2] although many suffer from the thermal instability of this class of compounds. Capillary column GC procedures[1] produce less decomposition of lysergic acid derivatives than packed columns.

A number of solvent systems have been developed for TLC separation of lysergic acid derivatives. These methods are useful only for sample screening and cannot differentiate between LSD and LAMPA. An extensive study on the reversed phase liquid chromatographic properties of a series of lysergic acid derivatives has been reported by McDonald et al.[3] The method was useful for the separation of 18 lysergic acid derivatives, however, LAMPA was not included in the study.

Relative retention data for LSD and LAMPA using an ion-pairing chromatographic procedure has been reported.[4] The method developed in the present study allows for the separation of LSD and LAMPA via a fast and convenient HPLC procedure.

Experimental

General: Melting points were determined in open glass capillaries using a Thomas-Hoover melting point apparatus. All ^1H NMR spectra were measured in $CDCl_3$ solution using a Varian T-60A spectrometer with an internal standard of tetramethylsilane. IR spectra were recorded on a Perkin-Elmer model 1500 Fourier transform infrared spectrophotometer.

Chromatographic Procedures: The liquid chromatograph consisted of a Waters Associates (Milford, MA) model 6000A pump, U6K injector, 440 UV detector with dual wavelength accessory and a Houston Instruments (Austin, TX) Omniscribe

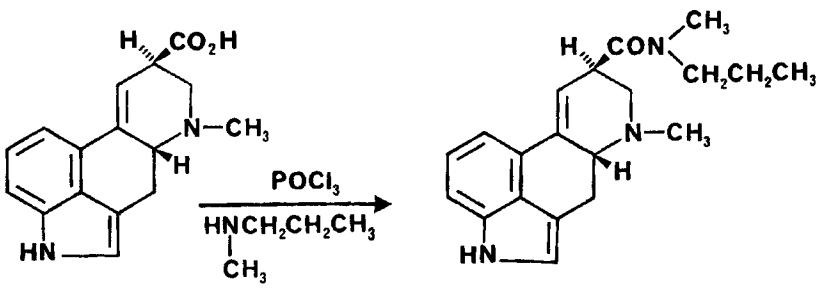
dual pen recorder. The column was 30 cm x 3.9 mm id packed with u Bondapak C_{18} (Waters Associates) and the mobile phase consisted of pH3 phosphate buffer and methanol (2:1). The mobile phase flow rate was 1.5 mL/min and the UV absorbance detector was operated at 0.2 AUFS. Sample solutions for analysis were prepared in methanol and separations were accomplished at ambient temperature.

Synthesis of d-N-Methyl-N-n-propyllysergamide (LAMPA)

Solutions of $POCl_3$ (0.1 mL, 1.1 mmole) in $CHCl_3$ (5.0 mL) and N-methyl-N-n-propylamine (350 mg, 4.8 mmole) in $CHCl_3$ were added simultaneously from separate addition funnels over a 3 minute period to a solution of d-lysergic acid (158 mg, 0.59 mmole) in $CHCl_3$ (15 mL) stirred at reflux under dry nitrogen. After the addition was complete, the reaction mixture was stirred at reflux for 10 minutes and then cooled to room temperature. The reaction solution was then diluted to 50 mL with $CHCl_3$, and the $CHCl_3$ solution washed successively with 1N NaOH (2 x 50 mL) and H_2O (50 mL). The $CHCl_3$ solution was then dried over $MgSO_4$. Filtration followed by evaporation of the filtrate solvent under reduced pressure at 50°C yielded the product as a brown oil; IR (neat): 1640 cm^{-1} (C = O); ^{1}H-NMR ($CDCl_3$) : 0.90 (t, J = 6Hz, 3H0, 1.15 - 1.75 (m centered at 1.45, 2H), 2.60 (s, 3H), 2.80 (s, 3H), 2.6 to 3.2 (complex m, 7H), 6.4 (s, 1H), 6.9 (s, 1H), 7.1 - 7.3 (m, 3H).

Results and Discussion

Numerous methods have been reported for the analysis of lysergic acid diethylamide (LSD) in forensic samples. Methods which allow differentiation between LSD and lysergic acid methyl-n-propylamide (LAMPA) are less common. LSD and LAMPA are reported to exhibit nearly identical infrared and mass spectra as well as having the same molecular weight. A common problem in forensic analysis is the question of whether a sample identified as LSD could actually be LAMPA.



Scheme I

In this study a simple reversed-phase liquid chromatographic procedure is developed for the separation of LSD and LAMPA. An analytical sample of LAMPA was prepared from lysergic acid by in situ generation of the acid chloride followed by treatment with N-methyl-N-n-propylamine (Scheme I). The product was isolated as the oily free base and used in the chromatographic procedures. The reversed-phase separation of these two similar compounds was accomplished using an octadecyl-stationary phase (C18) and a mobile phase of pH 3 phosphate buffer and methanol (2 + 1). Figures I and II illustrate the separation of these compounds using a 30 cm u-Bondapak column. LSD and LAMPA are well resolved under these conditions and are easily separated from lysergic acid.

The more polar lysergic acid has a much lower k'-value than either of the amides. LSD shows a lower k' than LAMPA under these reversed phase conditions suggesting that the n-propyl-group is responsible for a stronger hydrophobic interaction than the diethyl-side chains even though the total number of carbons attached to nitrogen is the same in both compounds. This same elution order for LSD and LAMPA was observed by Lurie[4] using a reversed phase procedure with the hydrophobic ion-pairing agent 1-heptane sulfonate. The separation illustrated in Figures 1 and 2 was accomplished with phosphate as the only counterion in the mobile phase. The low pH of the mobile phase should protonate the nitrogen of the tetrahydropyridine ring. However, the low basicity of

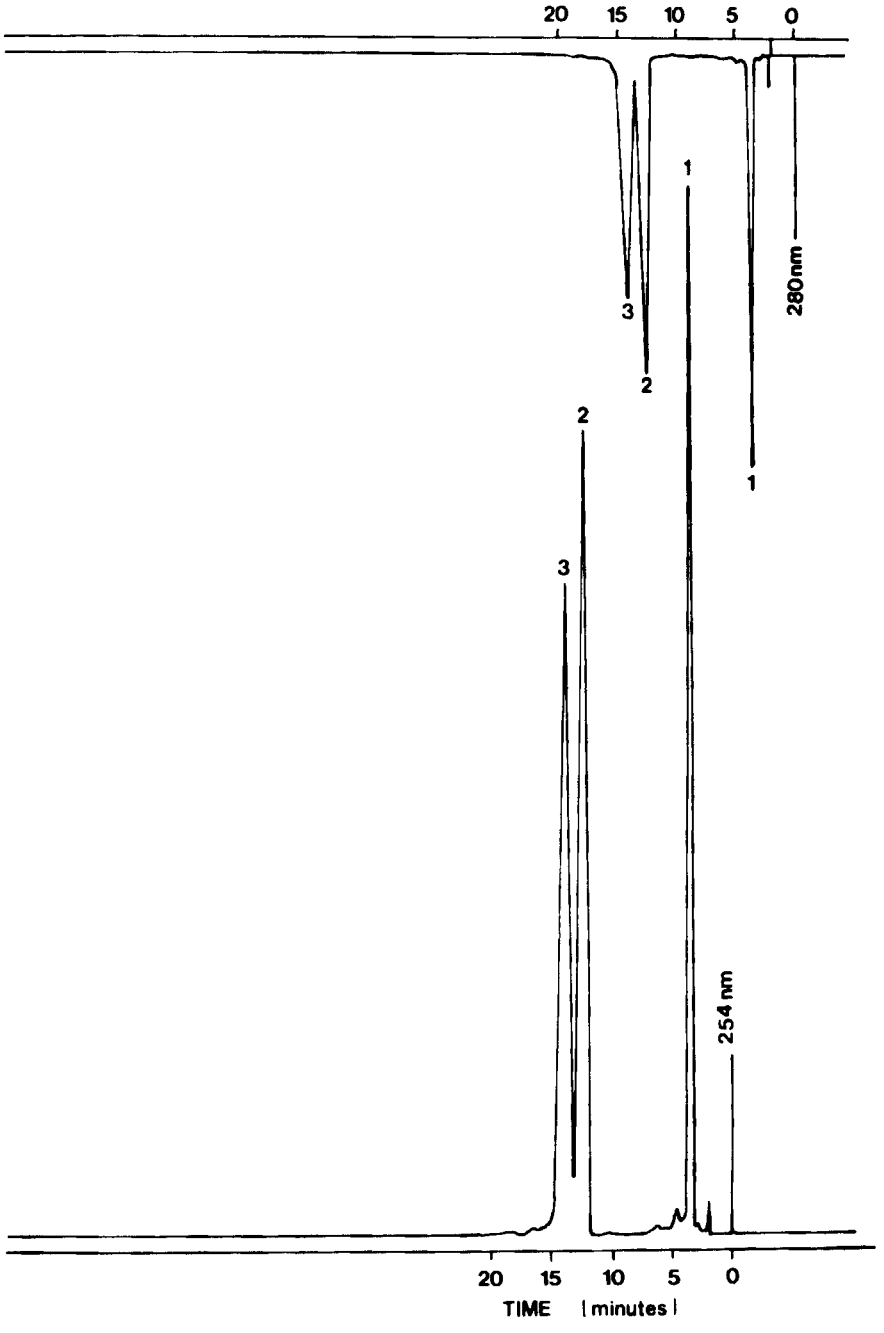


Figure 1

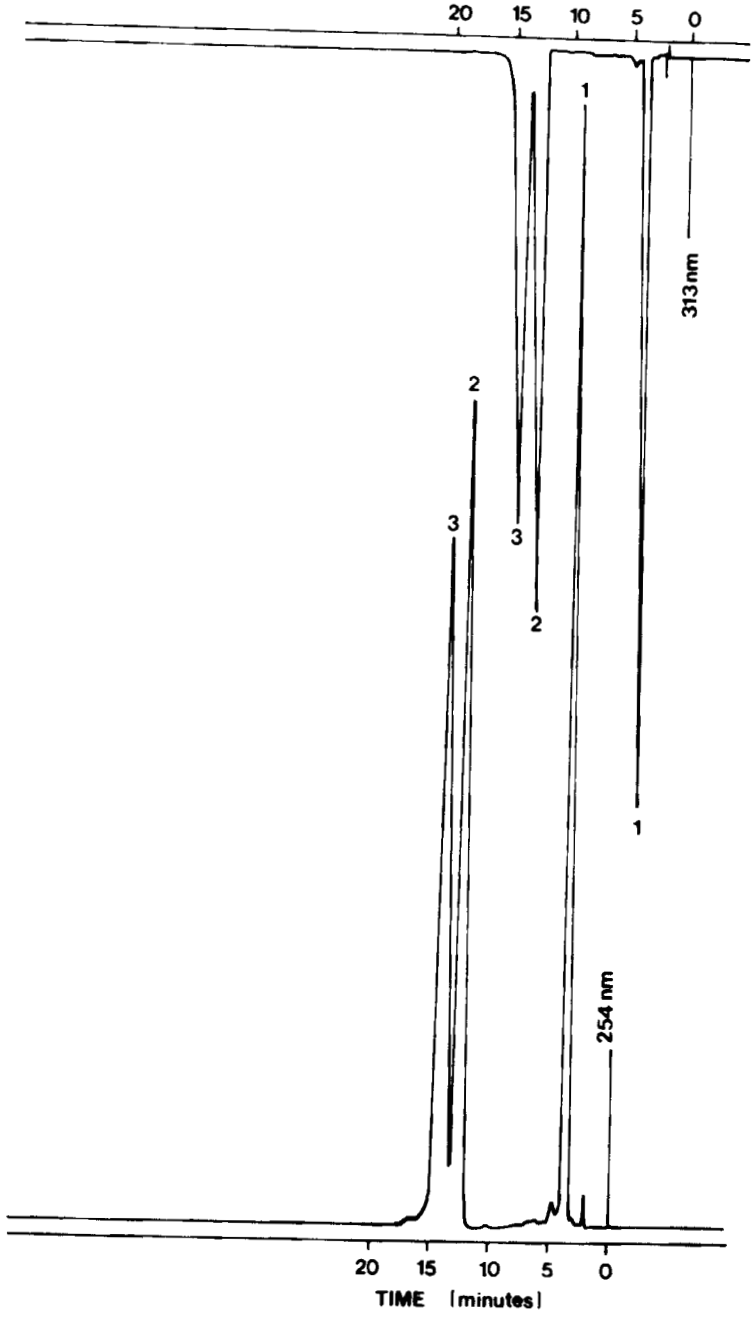


Figure 2

Table 1

Absorbance Ratios for Lysergic Acid Derivatives

Compound	A_{254}/A_{280}	A_{254}/A_{313}
Lysergic acid	2.54	1.49
LSD	2.54	1.48
LAMPA	2.71	1.46

the indole nitrogen would preclude protonation under the mobile phase conditions. Thus, the separation is achieved under conditions producing the monocation as the predominate species in sclution. Protonation decreases the k'-values for the elution of these compounds and allows the separation to be achieved in a reasonable analysis time. The elution volume for peak 3 (LAMPA) is approximately 27 mL.

The chromatograms in Figures 1 and 2 were obtained under identical chromatographic conditions. The only difference is the wavelengths used for dual wavelength detection in the two chromatograms. Figure 1 was obtained by monitoring at 254 and 280 nm while Figure 2 was produced by monitoring at 254 and 313 nm. The ratio of absorbances at both sets of wavelengths are quite similar for all three compounds as would be expected. The indole ring system present in all three compounds is the major chromophore. Table 1 shows the absorbance ratios for these compounds determined in this study. Absorbance ratios are a means to gain more structural information about an eluite than just retention data. Absorbance ratios can be considered a "two-point UV curve" and can be used to determine the identity of drugs having similar elution characteristics. In the case of LSD and LAMPA the absorbance ratios show the electronic spectra of these compounds to be very similar. Thus, the combination of HPLC elution characteristics and absorbance ratios in this method allow for the differentiation of LSD and LAMPA in forensic samples.

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