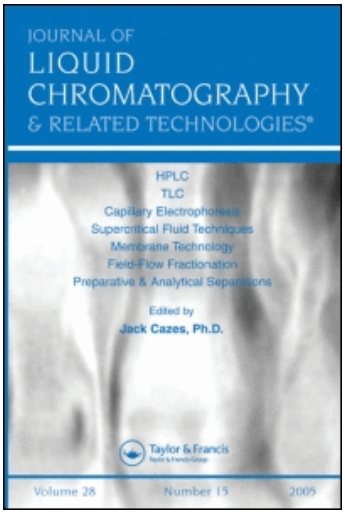

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Liquid Chromatographic and Spectral Methods for the Differentiation of 3,4-Methylenedioxymethamphetamine (MDMA) from Regioisomeric Phenethylamines

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LIQUID CHROMATOGRAPHIC AND SPECTRAL METHODS FOR THE DIFFERENTIATION OF 3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA) FROM REGIOISOMERIC PHENETHYLAMINES

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ABSTRACT

The analytical profiles are described for four amines, 3,4-methylenedioxymethamphetamine (MDMA) and three isomeric phenethylamines of MW = 193. These four amines all contain an identical 3,4-methylenedioxyphenyl moiety, thus the regioisomerism is within the carbon-carbon bond located alpha to the amine. Therefore these phenethylamines are regioisomeric within the imine fragment (m/z = 58) which is the base peak in the electron impact mass spectrum of MDMA. The ultraviolet absorption spectra for these compounds show the characteristic methylenedioxyphenyl group absorption bands in the 235 to 280 nm range. These amines may best be differentiated by chromatographic separation and are well resolved by liquid chromatographic techniques. The four regioisomeric amines were separated using an isocratic reversed-phase system consisting of a C_{18} stationary phase and an acidic (pH) mobile phase. The elution order under these conditions appears to parallel the length of the carbon chain attached to the aromatic ring.

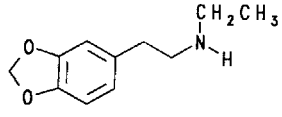
INTRODUCTION

The various N-substituted derivatives of 3,4-methylenedioxyamphetamine (MDA) have become popular drugs of abuse in recent years [1-3]. Structurally, MDA and its N-methyl derivative, MDMA (**3** in Chart I) resemble both methamphetamine and mescaline and are reported to act primarily as central nervous system stimulants that may be hallucinogenic in large doses [4,5]. MDMA is the most popular derivative of this series and is known by the street names "Ecstasy" or "XTC". MDMA is claimed to have a unique ability to facilitate interpersonal communication by reducing the anxiety or fear that normally accompanies the discussion of emotionally painful events [6].

The continued interest in drugs of the MDA-type is evidenced by numerous literature reports of new derivatives. Several "designer analogs" of MDA and MDMA , including the N-ethyl (MDEA) and N-hydroxy (NOHMDA) derivatives (Chart 1), have been encountered in forensic samples and these analogs are reported to have psychotomimetic activity in humans [7]. Also, the MDA homologue, 1-(3,4-methylenedioxyphenyl)-3-butanamine (HMDA) has been detected in clandestine drug samples [8]. Finally, Nichols et al. [9] recently reported the synthesis and unique pharmacological properties of alpha-ethyl analogs of MDA, the 1-(3,4-methylenedioxyphenyl)-2-butanamines. Members of this series have been classified as "entactogens" since they induce a pleasant state of introspection which facilitates the discussion of emotionally painful issues, without producing the profound sensory distortions typical of hallucinogens such as LSD.

Forensic analysis of controlled substances requires the use of a variety of analytical techniques to completely characterize

an individual compound and exclude the possibility of a sample consisting of a closely related analogue or homologue. The four compounds examined in this study are regioisomeric 3,4-methylenedioxyphenethylamines of molecular weight 193 containing identical 3,4-methylenedioxybenzyl moieties. Thus, these compounds represent a unique group of regioisomers which should yield essentially identical mass spectra. The compounds included (Chart I) are N-ethyl-1-(3,4-methylenedioxyphenyl)-2-ethanamine (**1**), N,N-dimethyl-1-(3,4- methylenedioxyphenyl)-2-ethanamine (**2**), N-methyl-1-(3,4-methylenedioxyphenyl)-2-propanamine (MDMA, **3**), 1-(3,4-methylenedioxyphenyl)-2-butanamine (**4**).



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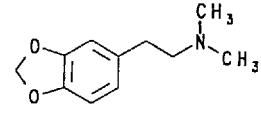

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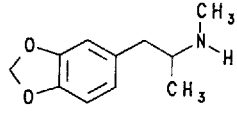

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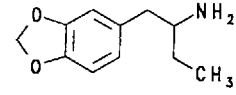

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EXPERIMENTAL

Instrumentation. The liquid chromatograph consisted of a Laboratory Data Control Constametric 3000 pump, 3100 Spectromonitor UV detector operated at 280 nm, CI 4100 Integrator and a Rheodyne 7125 Injector. Infrared spectra were recorded on a Perkin-Elmer Model 1710 Fourier transform infrared (FTIR) spectrophotometer. Ultraviolet spectra were recorded on a Shimadzu Instruments Model

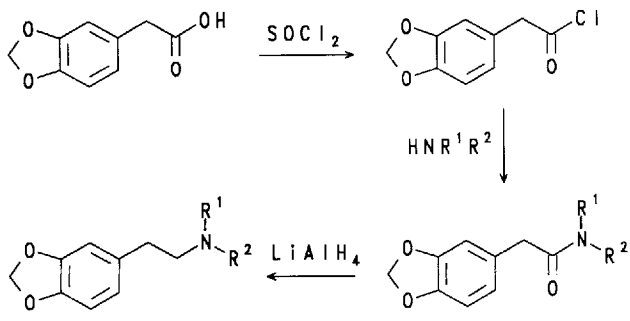
UV-160 spectrophotometer. Nuclear magnetic resonance spectra (1H) were determined using a Varian EM-360 60 MHz spectrometer.

The electron impact (EI) mass spectra were obtained using a Hewlett-Packard 5970B mass selective detector. The ionization voltage was 70 eV and the source temperature was 220° C. The individual amine hydrochlorides were dissolved in methanol (1 mg/mL) and 0.5 uL introduced into the mass spectrometer via a gas chromatograph equipped with a 12 m X 0.31 mm i.d. fused silica column with a 0.52 um thickness of OV-1. The column temperature was programmed from 70° C to 150° C at a rate of 15° C/min and from 150° C to 250° C at a rate of 25° C/min. The split ratio for the GC was 10:1 and all sample components eluted within approximately 7 minutes.

Liquid Chromatographic Procedures. The analytical column was 30 cm X 3.9 mm i.d. packed with uBondapak C_{18} (Waters Associates). The analytical column was preceded by a 7 cm X 2.1 mm i.d. guard column packed with CO:Pell ODS (Whatman). The amine hydrochlorides (1 mg/mL) were dissolved in HPLC grade methanol and separated using a mobile phase of pH 3.0 phosphate buffer, methanol, acetonitrile and triethylamine (600:100:25:1). The pH 3.0 phosphate buffer was prepared by mixing 9.2 g monobasic sodium phosphate (NaH_2PO_4) in 1 L of double-distilled water and adjusting the pH to 3.0 with H_3PO_4. The mobile phase flow rate was 1.5 mL/min and the detector was operated at 0.2 AUFS. A 15 uL aliquot of each amine solution was injected into the liquid chromatograph.

Synthesis of the Regiosiomeric Amines. The appropriate amine (ethylamine or dimethylamine, 20 mmoles) was added dropwise to a

stirred solution of 3,4-methylenedioxyphenacetyl chloride (10 mmoles) in chloroform (50 mL) and the mixture stirred at room temperature for 1 hour. The mixture was then stirred at reflux for ca. 15 minutes and the solvent evaporated under reduced pressure to yield an oil. The oil was partitioned between 20% potassium carbonate (50 mL) and chloroform (50 mL) and the chloroform layer separated. The chloroform solution was then washed with 10% HCl (50 mL) and evaporated under reduced pressure to yield the intermediate amide. A solution of the amide in THF (40 mL) was added dropwise to a suspension of lithium aluminum hydride (1 g) in THF (10 mL) stirred under a nitrogen atmosphere. After the addition was complete, the mixture was stirred at reflux overnight. The mixture was then cooled to room temperature, filtered and the filtrate solvent evaporated under reduced pressure to yield the crude amines as oils. The oils were partitioned between 10% HCl (50 mL) and chloroform (50 mL) and the aqueous layer separated and made basic (pH 12) with aqueous sodium hydroxide. The aqueous base suspension was extracted with chloroform (50 mL) and the chloroform removed under reduced pressure to yield the product amines in free base form. Treatment of the bases with etheral HCl afforded the N-ethyl- (**1**) and N,N-dimethyl-1-(3,4-methylenedioxyphenyl)-2-ethanamines (**2**) as the hydrochloride salts. The sample of 1-(3,4-methylenedioxyphenyl)-2-butanamine (**4**) was prepared from piperonal as described previosuly [10]. The structures of the products were confirmed by IR (KBr) and ^{1}H-NMR (deuterated DMSO). The purity of the products was established by GC-MS and the liquid chromatographic analysis. MDMA was synthesized as reported earlier[3].

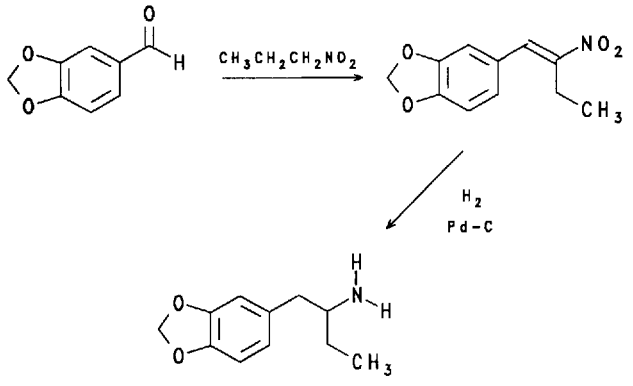


Scheme 1. Synthesis of N-ethyl- and N,N-dimethyl-1-(3,4-methylenedioxyphenyl)-2-ethanamines.

RESULTS AND DISCUSSION

The four amines examined in this study each contain a 3,4-methylenedioxyphenethylamine fragment and have a molecular weight of 193. 3,4-Methylenedioxymethamphetamine (MDMA) is a popular drug of abuse and its current street drug form is referred to as "XTC". The N-ethyl- and N,N-dimethyl-1-(3,4-methylenedioxyphenyl)-2-ethanamines and the 1-(3,4-methylenedioxyphenyl)-2-butanamine are MDMA isomers having a common 3,4-methylenedioxyphenethylamine skeleton.

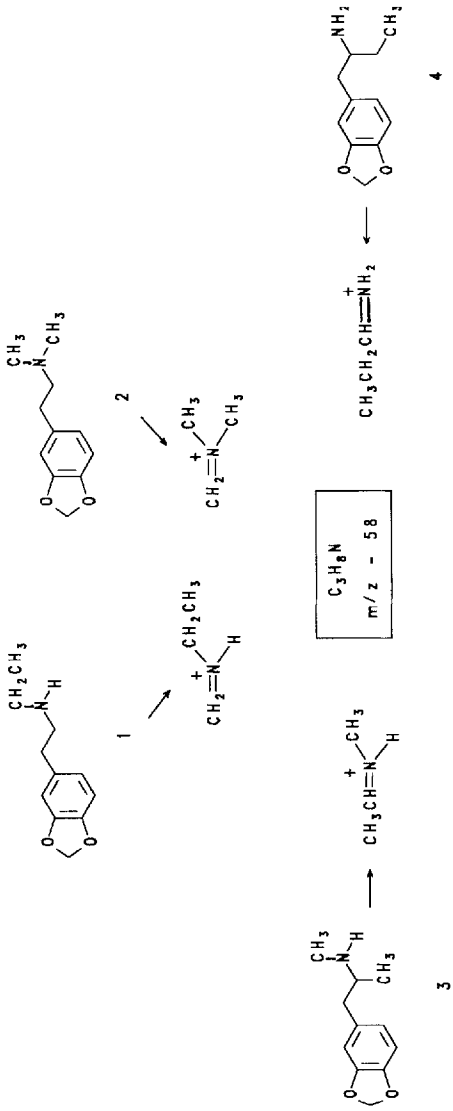
The N-ethyl and N,N-dimethyl-1-(3,4-methylenedioxyphenyl)-2-ethanamines were prepared according to the method outlined in Scheme 1. Treatment of 3,4-methylenedioxyphenacetyl chloride with ethylamine or dimethylamine gave the N-ethyl and N,N-dimethyl 3,4-methylenedioxyphenylacetamides, respectively. The amides were reduced with lithium aluminum hydride to yield the desired phenethylamine products. The sample of 1-(3,4-methylenedioxy-



Scheme 2. Synthesis of 1-(3,4-methylenedioxyphenyl)-2-butanamine.

phenyl)-2-butanamine was prepared via reduction of an intermediate nitrostyrene according to Scheme 2.

These isomeric amines were prepared to examine the specificity of analytical methods for the identification of MDMA. While these isomers clearly yield very different proton magnetic resonance spectra, this is not a very common technique employed for the small amounts of material often associated with forensic samples. Certainly NMR methods would not be directly useful for the analysis of drugs from biological samples. These amines were chosen for their isomeric relationship to MDMA, all having the same molecular weight and the predicted similarity in their mass spectra. The EI fragmentation of the 3,4-methylenedioxyphenethylamines of molecular weight 193 should yield a propylimine major fragment (base peak) of m/z = 58. Thus, these four isomers are uniquely similar in that all the structural variation is on the nitrogen atom or the alpha-carbon of the phenethylamine moiety. This isomeric relationship produces the m/z = 58 via the



Scheme 3. Electron impact fragmentation pathway for the isomeric 1-(3,4-methylenedioxyphenyl)amines.

amine-dominated alpha-cleavage fragmentation (Scheme 3) which eliminates the 3,4-methylenedioxy benzyl radical (mass = 135) in each compound. Other isomeric 3,4-methylenedioxyphenethyl amines of molecular weight 193 would require substitution on the beta-carbon or the aromatic ring and thus yield a base peak of m/z = 44 (ethylimine) or m/z = 30 (methylimine) and a substituted 3,4-methylenedioxy benzyl radical from an analogous alpha-cleavage reaction. Since mass spectrometry is often the method of choice or the mandated method for confirmation of drug identity, these compounds represent a unique challenge for the specificity of analytical methods in forensic analysis and related drug screening methods.

The predicted fragmentation to yield m/z = 58 in each of these amines is confirmed by the mass spectra in Figure 1. The predominate feature in these spectra is the base peak at m/z = 58 as predicted in Scheme 3. The mass spectra in Figure 1 were obtained by GC-MS analysis using an OV-1 capillary column with temperature programming. Under these chromatographic conditions the four amines eluted within a 0.5 minute window from 7.1 to 7.6 minutes. The similar retention properties of these amines on this stationary phase again points to the potential for confusion in the analysis of these materials. A substance producing the appropriate mass spectrum for MDMA at approximately the same retention time could lead to the incorrect identification of these structural analogs as MDMA.

It would be most difficult to differentiate these compounds based on their UV absorption spectra since the shape of the curves in acid and base, the relative absorbance, the various absorption maxima as well as the wavelengths of maximum absorp-

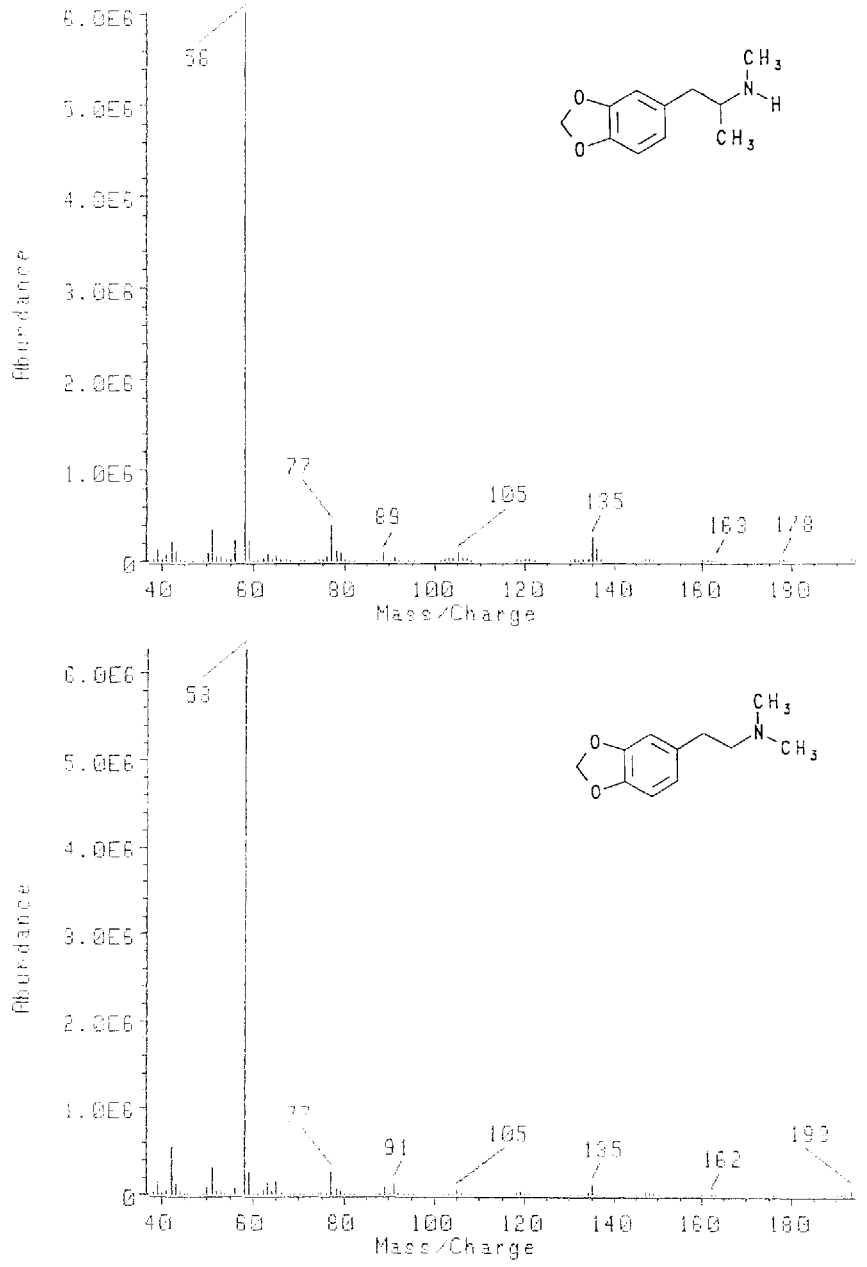


Figure 1. Mass spectra for the four isomeric 1-(3,4-methylenedioxyphenyl)amines.

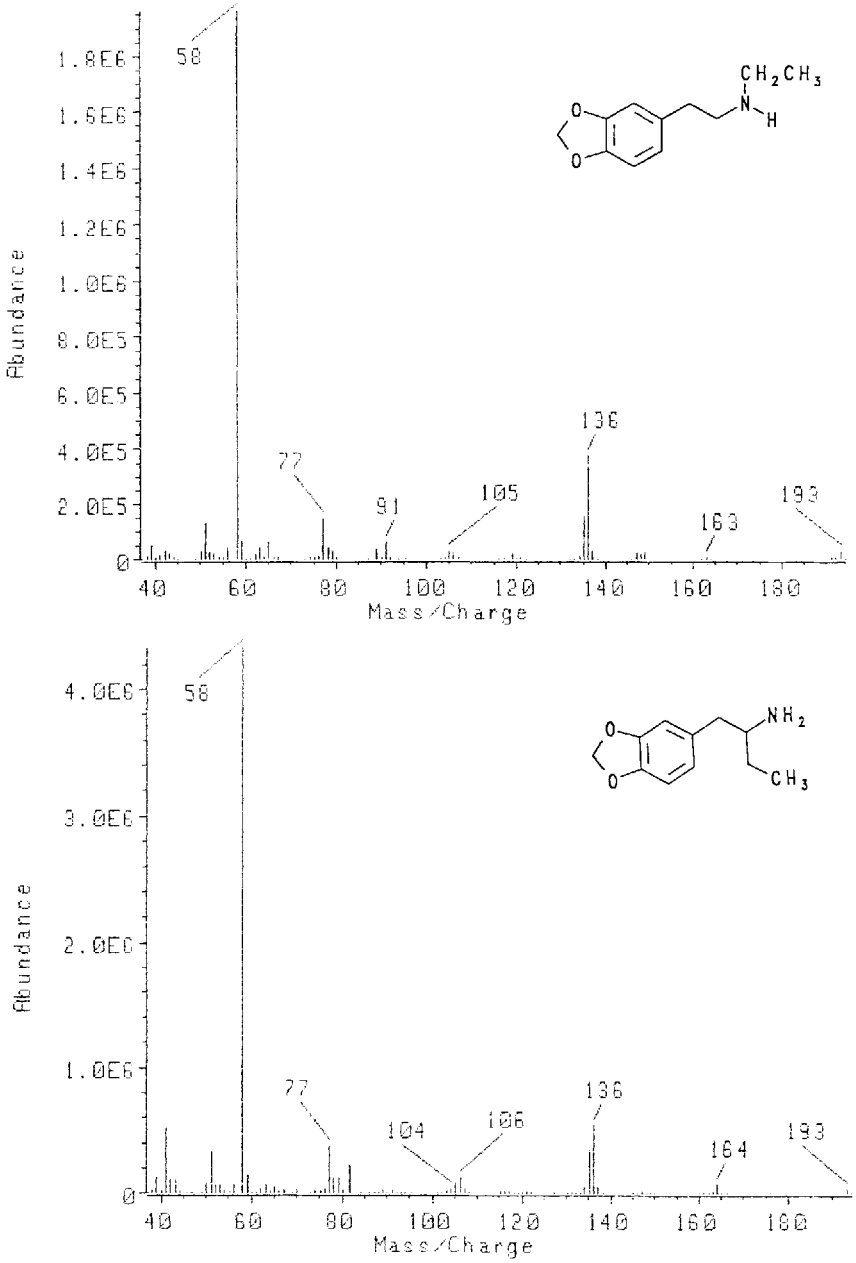


Figure 1 (continued).

tion are all very similar. The UV spectra are characteristic of 3,4-methylenedioxyphenethylamines in general and these very similar regioisomers would be expected to display electronic spectra characteristic of the common molecular fragment.

The infrared absorption spectra for MDMA and N-ethyl-1-(3,4-methylenedioxyphenyl)-2-ethanamine are shown in Figure 2. While some slight differences are obvious in the position of absorption bands and relative intensities, these spectra are also very similar. Variations from sample to sample and time to time are often observed for the infrared analysis of low molecular weight amines. These variations are the result of crystalline structure, degree of hydration and other related factors.

The liquid chromatographic separation of the four amines is shown in Figure 3. The amines were separated on a C_{18} stationary phase using an acidic (pH 3) mobile phase consisting of phosphate buffer, methanol, acetonitrile and triethylamine (600:100:25:1). Under these conditions the amines would exist predominantly in the protonated form. The N,N-dimethyl-1-(3,4-methylenedioxyphenyl)-2-ethanamine has the lowest capacity factor under these conditions followed closely by the N-ethyl derivative. MDMA elutes third in the sequence and 1-(3,4-methylenedioxyphenyl)-2-butanamine has the highest capacity factor. These four amines elute over a 9 minute window in this system with retentions between 7 and 16 minutes. MDMA (peak 3) is well resolved from both of the N-substituted 1-(3,4-methylene-dioxyphenyl)-2-ethanamines (peaks 1 and 2) and the primary amine having a four carbon chain attached to the aromatic ring. The elution order of the compounds parallels the length of the carbon chain attached to the methylenedioxyphenyl ring with the C_2 (phenethyl) compounds

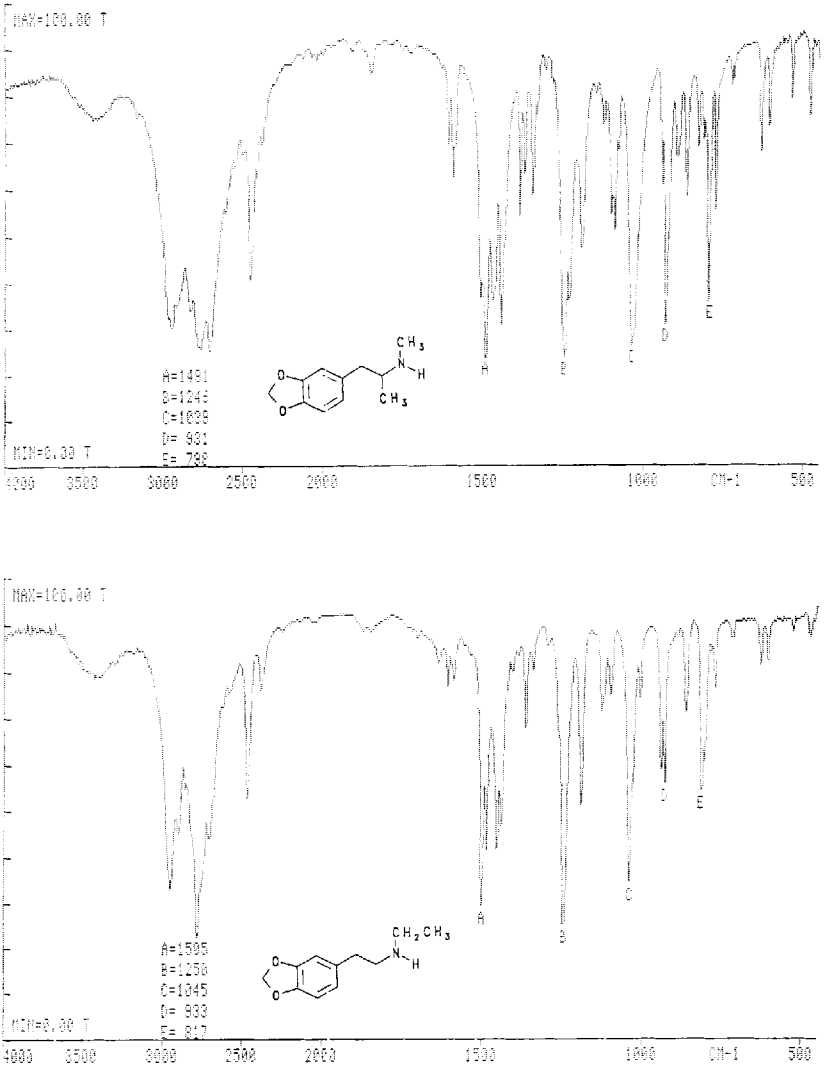


Figure 2. FT-IR spectra for the hydrochloride salts of MDMA (A) and N-ethyl-1-(3,4-methylenedioxyphenyl)-2-ethanamine (B).

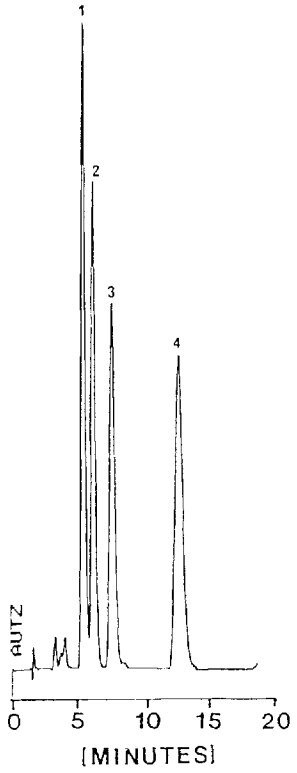


Figure 3. Liquid chromatographic separation of the isomeric (3,4-methylenedioxyphenyl)amines. Peaks: 1 = N,N-dimethyl-1-(3,4-methylenedioxyphenyl), 2 = N-ethyl-1-(3,4-methylenedioxyphenyl), 3 = N-methyl-(3,4-methylenedioxyphenyl)-2-propanamine (MDMA), 4 = 1-(3,4-methylenedioxyphenyl)-2-butanamine. u-Bondapak C_{18} column and a mobile phase of pH 3 phosphate buffer, methanol, acetonitrile and triethylamine (600:100:25:1).

eluting before the C_3 compound (MDMA) which elutes prior to the C_4 compound. This chromatographic separation easily allows the differentiation of MDMA from the other three isomers, even though many of their analytical profiles are quite similar.

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