

# Liquid Chromatographic and Mass Spectral Analysis of *N*-Substituted Analogues of 3,4-Methylenedioxyamphetamine

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## Abstract

The  $C_1$  to  $C_3$  *N*-alkyl, *N,N*-dimethyl, and *N*-hydroxy analogues of 3,4-methylenedioxyamphetamine (MDA) are identified by high performance liquid chromatographic (HPLC) and spectrometric techniques. The compounds are separated using reversed-phase procedures on  $C_{18}$  stationary phase with an acidic (pH 3) aqueous methanol mobile phase. The mass spectra of the compounds are distinctive and reference spectra are provided. The *N*-hydroxy derivative is unstable at high temperatures and decomposes to MDA and the oxime of 3,4-methylenedioxyphenyl-2-propanone.

## Introduction

3,4-Methylenedioxyamphetamine (MDA) and its *N*-methyl (MDMA), *N*-ethyl (MDEA), *N,N*-dimethyl, and *N*-hydroxy derivatives have been encountered by forensic laboratories in the United States and Canada. The pharmacological actions of 3,4-methylenedioxyamphetamine allow for its classification as an hallucinogen (1). Although it has other atypical effects, such as enhancing empathy and a low potential to produce severe sensory disruption, MDA has become a popular street drug primarily because of its enhancing effect on empathy (2). Methylation to yield the secondary amine 3,4-methylenedioxyamphetamine (MDMA) produces significant changes in the pharmacological properties. Methylation results in a shorter duration of action, a general decrease in potency, and elimination of the hallucinogenic properties. Nevertheless, the empathy-enhancing properties are retained and appear to be more pronounced in MDMA. MDMA is claimed to have unique properties in psychotherapy, reducing the anxiety that normally accompanies the discussion of emotionally unpleasant events (3). The recent appearance of MDMA on the street market sold as "Ecstasy" indicates the popularity and potential for abuse of this drug. Its popularity is probably due to its mild effects and its ability to facilitate interpersonal communication (4).

The increased availability of MDMA on the street and its potential to be neurotoxic in serotonergic pathways led to its

inclusion as a Schedule I drug. More recently, "designer drug" modifications of MDMA and MDA have produced the *N*-ethyl, *N,N*-dimethyl and *N*-hydroxy derivatives of MDA (5). Based upon these facts, reference samples of several low molecular weight *N*-substituted derivatives of MDA have been synthesized and identified by high-performance liquid chromatography (HPLC), UV, IR, and mass spectral (MS) methods.

## Experimental

### Reagents and chemicals

The *N*-alkyl analogs of 3,4-methylenedioxyamphetamine (MDA) were prepared according to previously described methods (6,7). The *N*-hydroxy derivative of MDA was prepared from 3,4-methylenedioxyphenyl-2-propanone via the intermediate oxime (8). The oxime was obtained in good yield using hydroxylamine hydrochloride and isolated as a crystalline solid. The oxime was reduced with sodium cyanoborohydride to yield the desired *N*-hydroxy MDA derivative. The product amines were converted to the corresponding hydrochloride salt using HCl in diethyl ether and recrystallized from ethanol-diethyl ether. 3,4-Methylenedioxyamphetamine hydrochloride and 3,4-methylenedioxy-methamphetamine hydrochloride were received from the Drug Enforcement Administration. 3,4-Methylenedioxyphenyl-2-propanone was obtained from Fluka Chemical Corp. Phosphate buffer (pH 3.0) was prepared by mixing 9.2 g monobasic sodium phosphate ( $\text{NaH}_2\text{PO}_4$ ) in 1 L double distilled water and adjusting to pH 3.0 with concentrated  $\text{H}_3\text{PO}_4$ . Methanol, acetonitrile, and triethylamine were HPLC-grade from J.T. Baker Chemical Co. All other chemicals were reagent-grade or better and used without further purification. Melting points were determined in open glass capillaries using a Thomas-Hoover melting point apparatus.

### Liquid chromatographic procedures

The liquid chromatograph consisted of a Waters Associates Model 6000A pump, U6K injector, Model 440 UV detector with dual wavelength accessory operated at 254 and 280 nm, and Houston Instruments OmniScribe dual pen recorder. The column was 30 cm  $\times$  3.9 mm i.d. packed with  $\mu$ Bondapak  $C_{18}$  (Waters Associates). The analytical column was preceded by a 7-cm  $\times$  2.1-mm i.d. guard column dry packed with CO:Pell ODS (Whatman). The mobile phase consisted of pH 3.0 phos-

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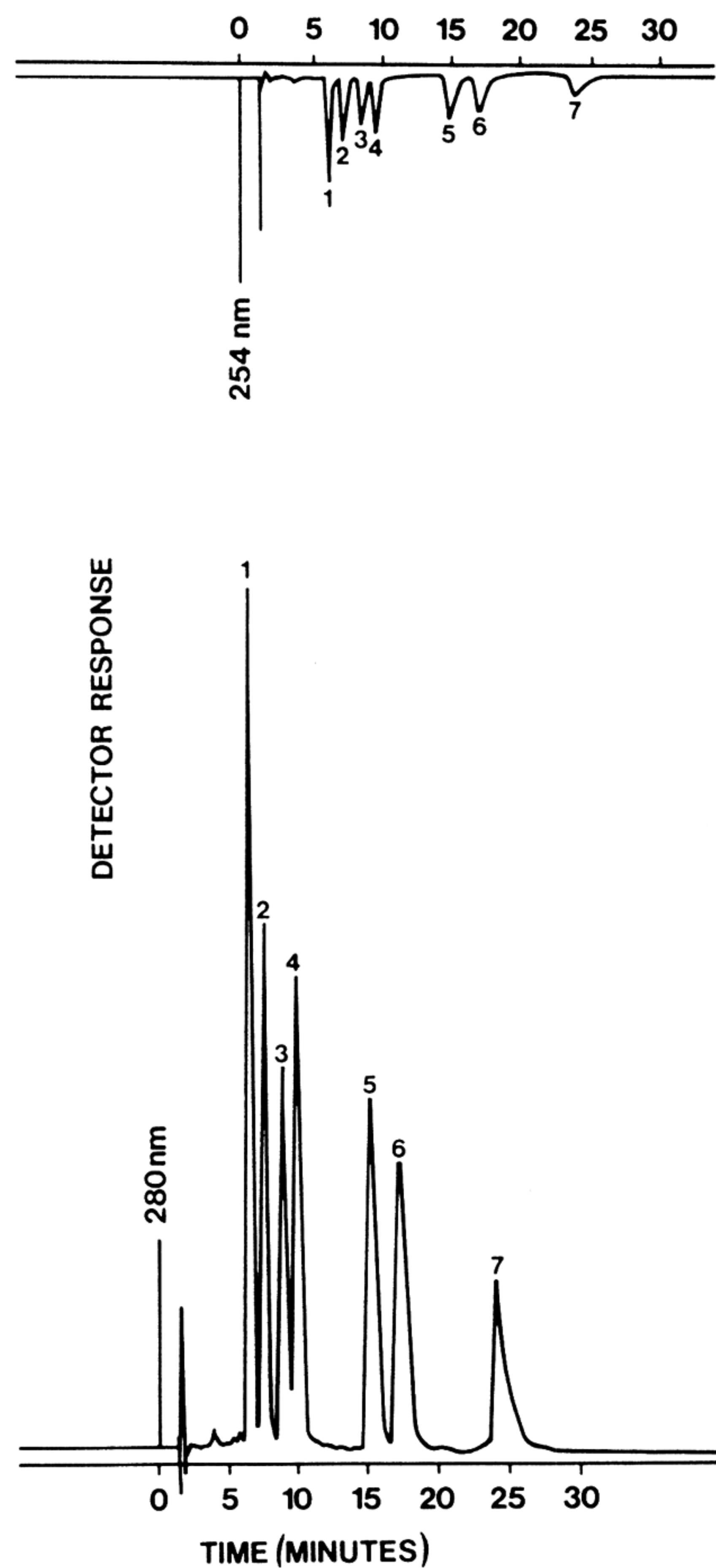


phate buffer/methanol/acetonitrile/triethylamine (600:100:25:1) (solvent system 1) or pH 3.0 phosphate buffer/methanol (650:100) (solvent system 2). The mobile phase flow rate was 1.5 mL/min and the UV absorbance detector was operated at 0.2 AUFS. Sample solutions for analysis were prepared in methanol (1 mg/mL) and all separations were accomplished at ambient temperature.

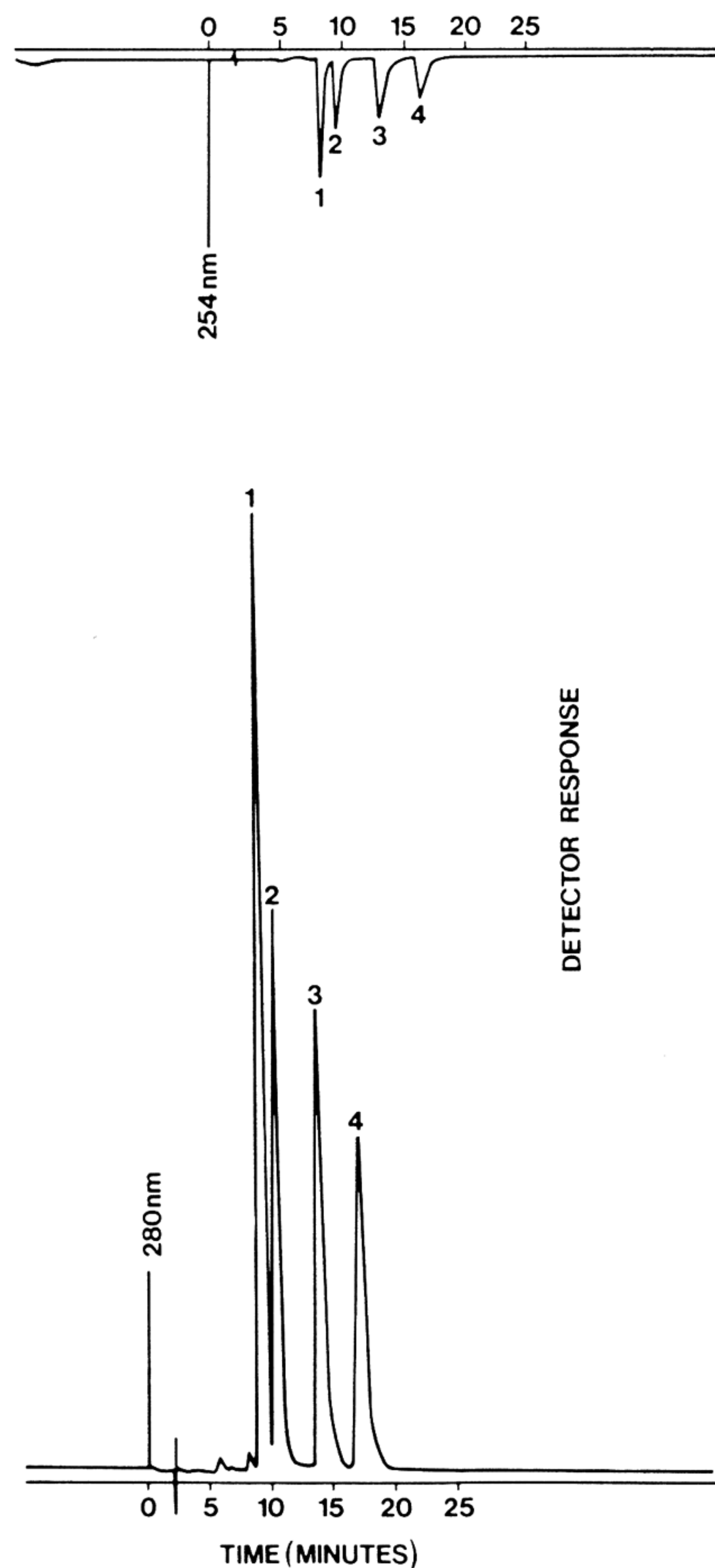
#### Mass spectroscopic procedures

The electron impact mass spectra were obtained using a

Hewlett-Packard 5970B mass selective detector. The ionization voltage was 70eV and the source temperature was 280°C. The individual samples were dissolved in methanol (1 mg/mL) and 0.5  $\mu$ L introduced into the mass spectrometer via the gas chromatograph equipped with a 12-m  $\times$  0.31-mm i.d. fused-silica column with a 0.52- $\mu$ m 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 of the GC was 10:1. All samples had eluted after approximately 8 min. Solid probe electron impact mass spectra (70 eV) were recorded on a VG 70-70.



**Figure 1.** Reversed-phase LC separation of MDA and *N*-substituted derivatives. Peaks: 1=MDA; 2=*N*-methyl MDA; 3=*N,N*-dimethyl MDA; 4=*N*-ethyl MDA; 5=*N*-isopropyl MDA; 6=*N-n*-propyl MDA; 7=*N*-hydroxy MDA. Solvent system 1.



**Figure 2.** Reversed-phase LC separation of MDA, *N*-methyl MDA, *N*-ethyl MDA, and *N*-hydroxy MDA. Peaks: 1=MDA; 2=*N*-methyl MDA; 3=*N*-ethyl MDA; 4=*N*-hydroxy MDA. Solvent system 2.



### Infrared spectroscopic procedures

IR spectra were recorded on a Perkin-Elmer Model 1600 Fourier transform infrared spectrophotometer (FTIR) equipped with a Hewlett-Packard ColorPro printer via the KBr disk technique.

### Results and Discussion

Previous studies have shown that the 3,4-methylenedioxyphenyl group is a strong chromophore in the UV range. The two major UV absorption bands are at 285 and 235 nm. The absorptivity is slightly higher at 285 nm; however, the molar absorptivity at both maxima is approximately  $3.5 \times 10^3$  L/mole-cm.

The LC separation of MDA, the  $C_1$  to  $C_3$   $N$ -alkyl MDAs, and  $N$ -hydroxy MDA are shown in Figure 1. This reversed-phase separation was achieved on a  $C_{18}$  stationary phase and a mobile phase of pH 3 phosphate buffer, acetonitrile, methanol, and triethylamine. The triethylamine is necessary on some stationary phases to prevent peak tailing for basic compounds. The chromatogram in Figure 1 was obtained using dual wavelength UV detection at 254 and 280 nm, producing large peak ratios as these wavelengths are very close to the absorbance minima and maxima, respectively. For the  $N$ -alkyl MDA derivatives, reten-

tion on the  $C_{18}$  hydrocarbon stationary phase increases with the size of the alkyl chain on the nitrogen. The  $N,N$ -dimethyl derivative elutes before  $N$ -ethyl, and the branched isopropyl elutes before the  $N$ - $n$ -propyl MDA. Under the conditions used for this separation the  $N$ -hydroxy derivative has a higher  $k'$  value than any of the  $N$ -alkyl derivatives. Although this compound might be expected to be more polar than the  $N$ -alkyl derivatives, the high retention may reflect the lower basicity of the  $N$ -hydroxy amine. Thus, at the pH of the mobile phase the protonation of the  $N$ -hydroxy derivative may be less complete than the more basic  $N$ -alkyl MDAs. Evidence in support of this hypothesis was derived from studies where retention times were investigated as a function of mobile phase pH (10). As the pH of the mobile phase is decreased from 6 to 3, the retention time for the  $N$ -hydroxy compound decreases, while the retention times for the  $N$ -alkyl MDAs increase slightly.

In a recent report, Braun et al. (8) described a series of  $N$ -substituted MDAs and outlined their pharmacological properties in humans. From a large series of compounds, psychotomimetic properties in humans was observed in only four: MDA,  $N$ -methyl,  $N$ -ethyl, and  $N$ -hydroxy MDA. These results probably account for the appearance of the  $N$ -hydroxy derivative in the clandestine market. The four compounds having human psychotomimetic activity were separated on a  $C_{18}$  stationary phase using pH 3 phosphate buffer and methanol. The results in Figure 2 show that these four compounds can be easily separated in a relatively short period of time. Thus, the most likely compounds to appear in forensic samples can be analyzed

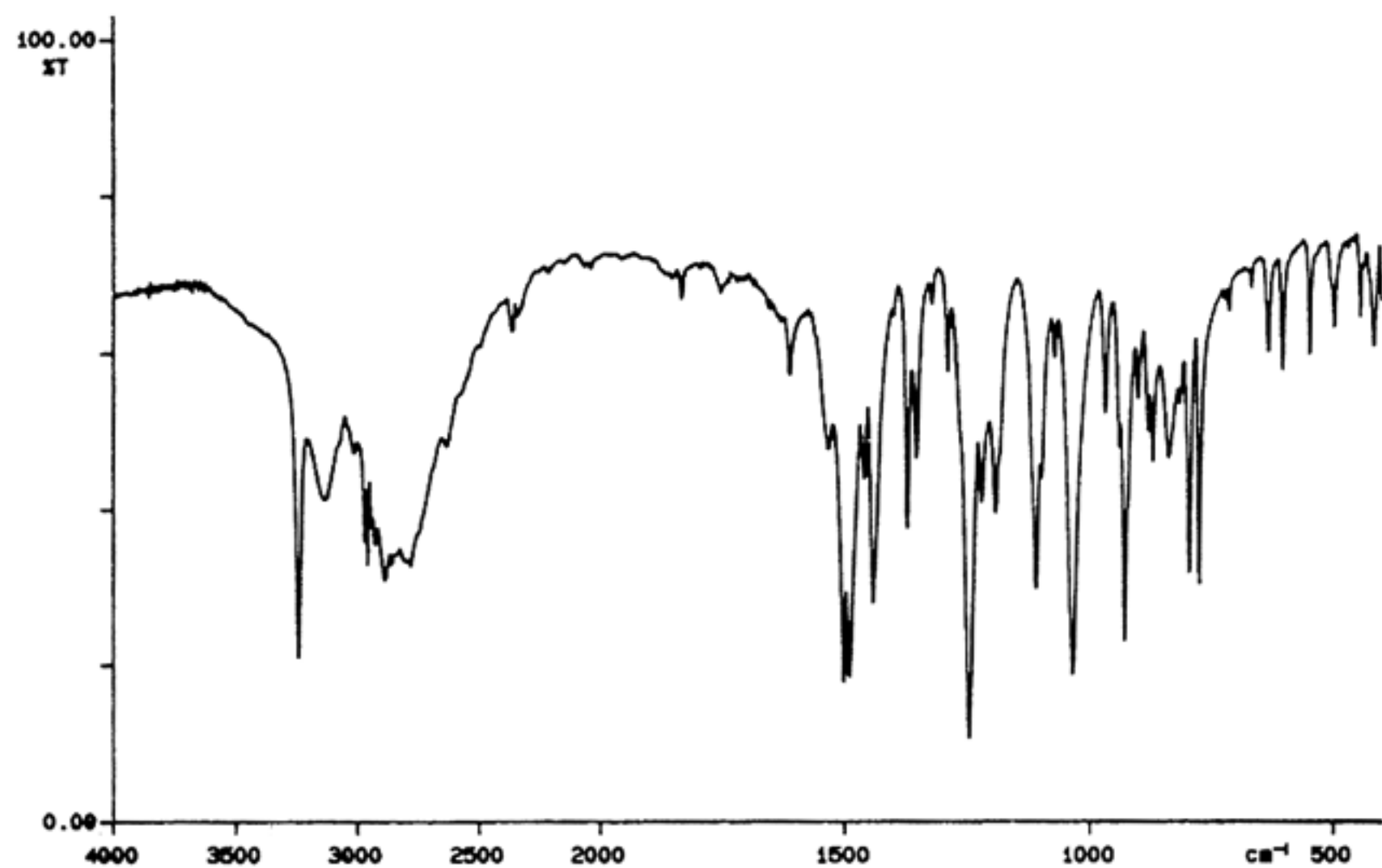


Figure 3. Infrared spectrum of  $N$ -hydroxy MDA.

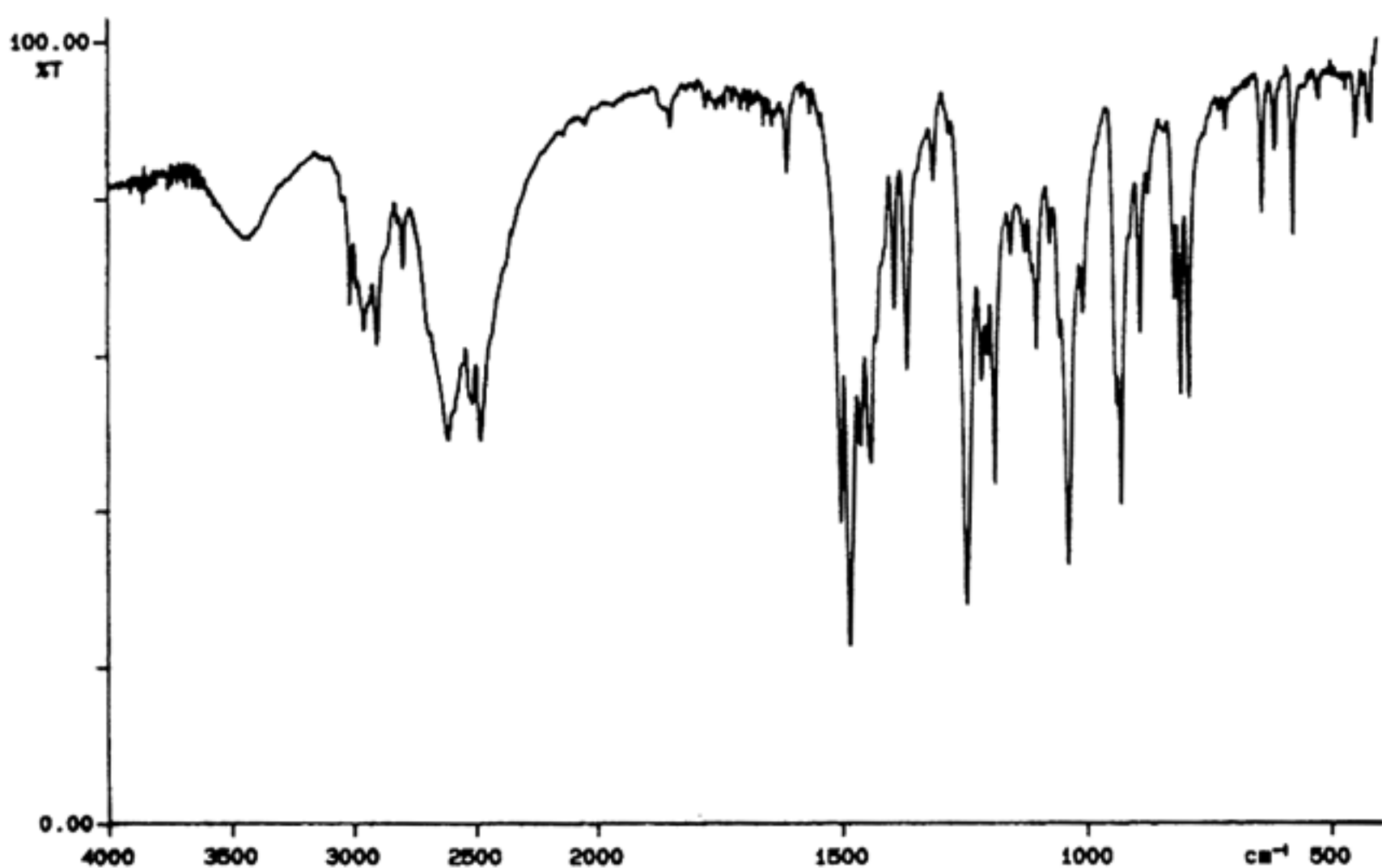
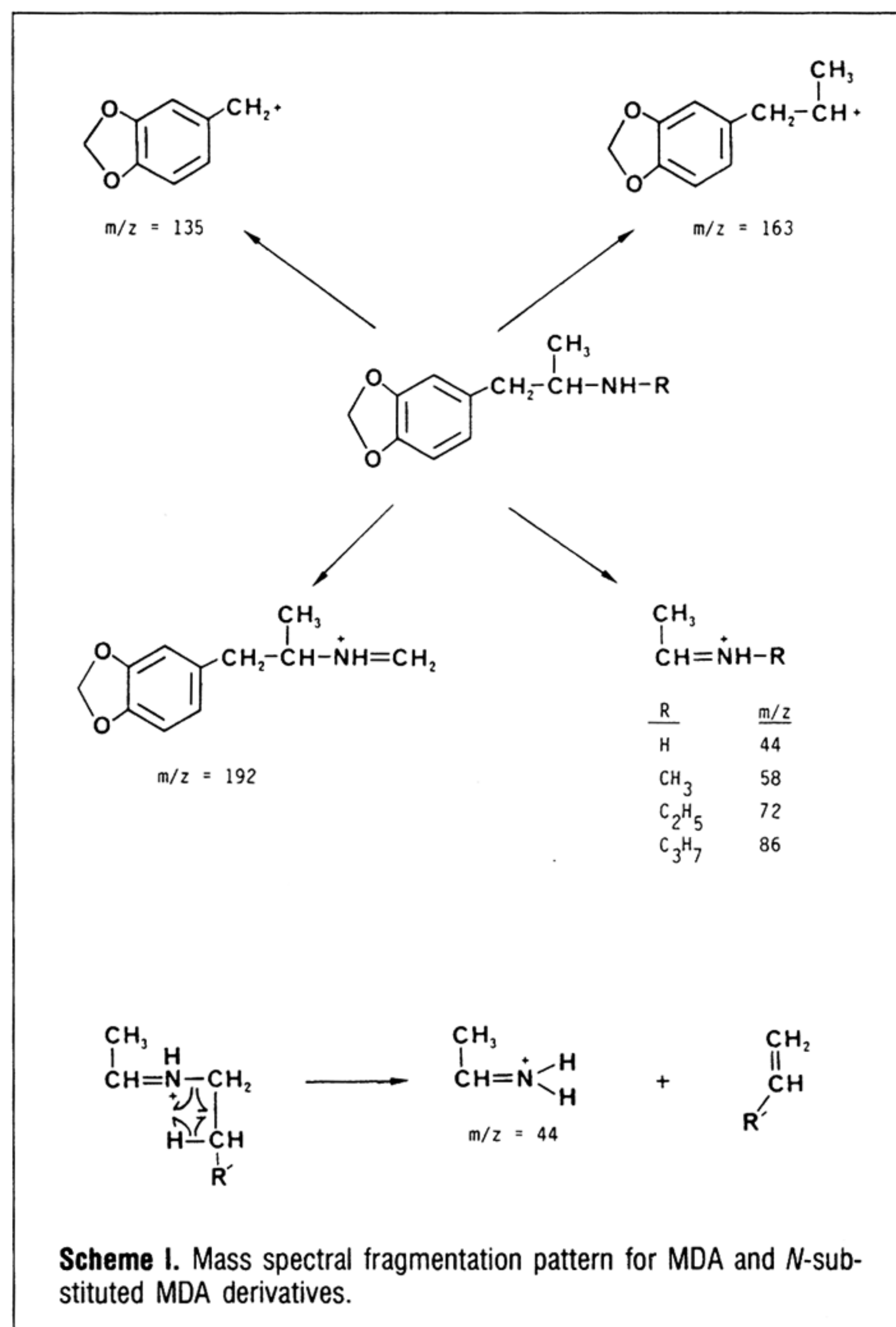
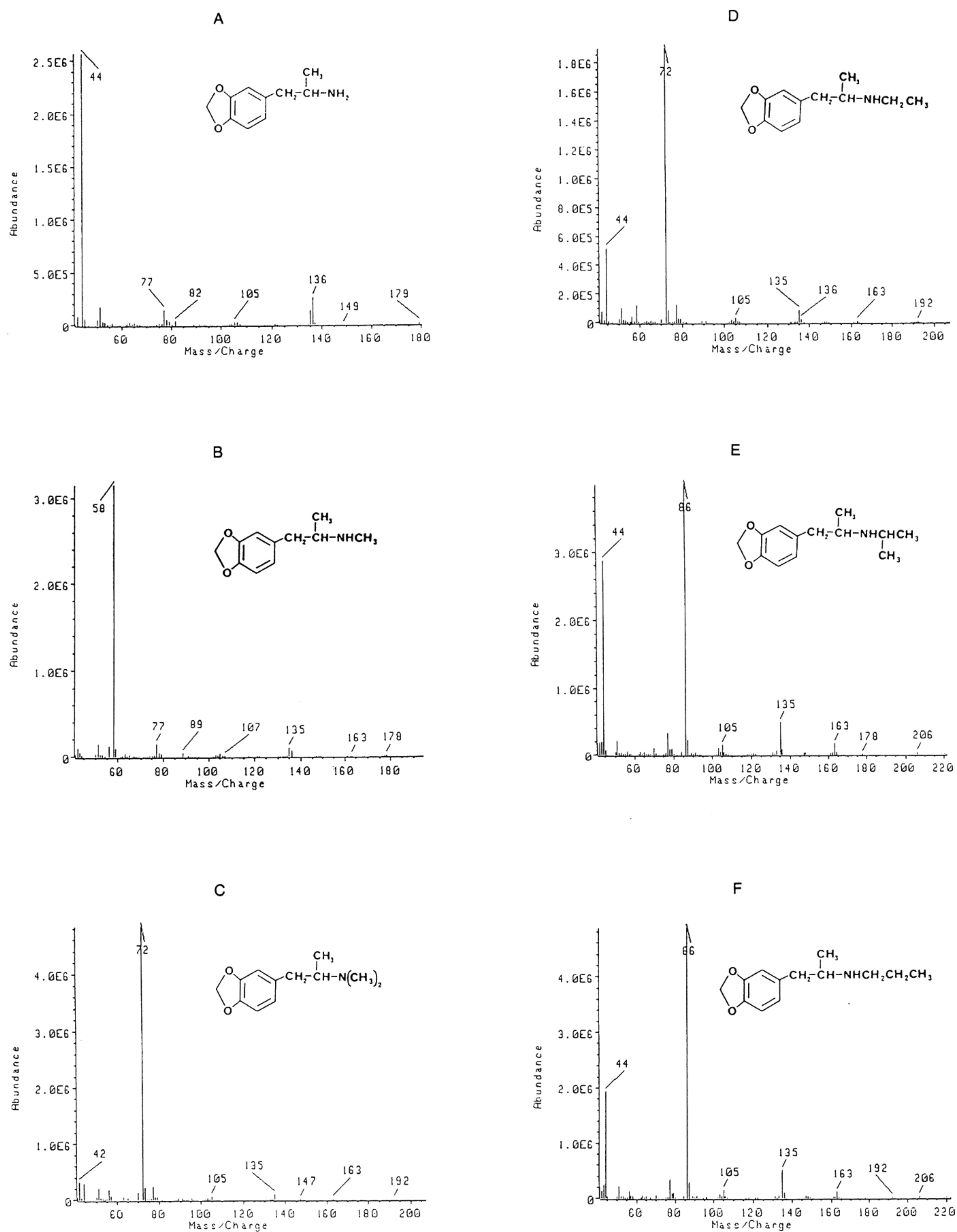


Figure 4. Infrared spectrum of  $N,N$ -dimethyl MDA.

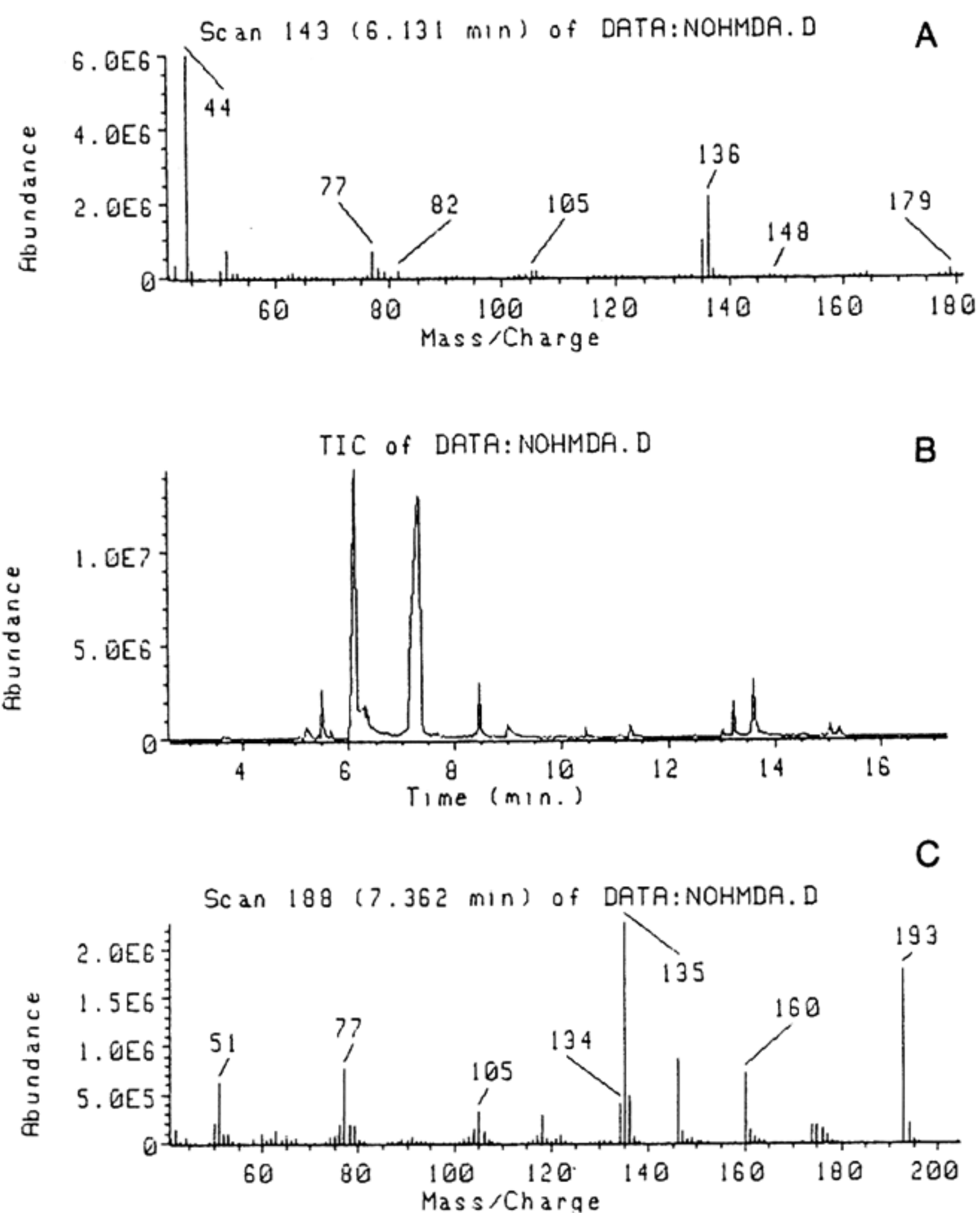


Scheme I. Mass spectral fragmentation pattern for MDA and  $N$ -substituted MDA derivatives.

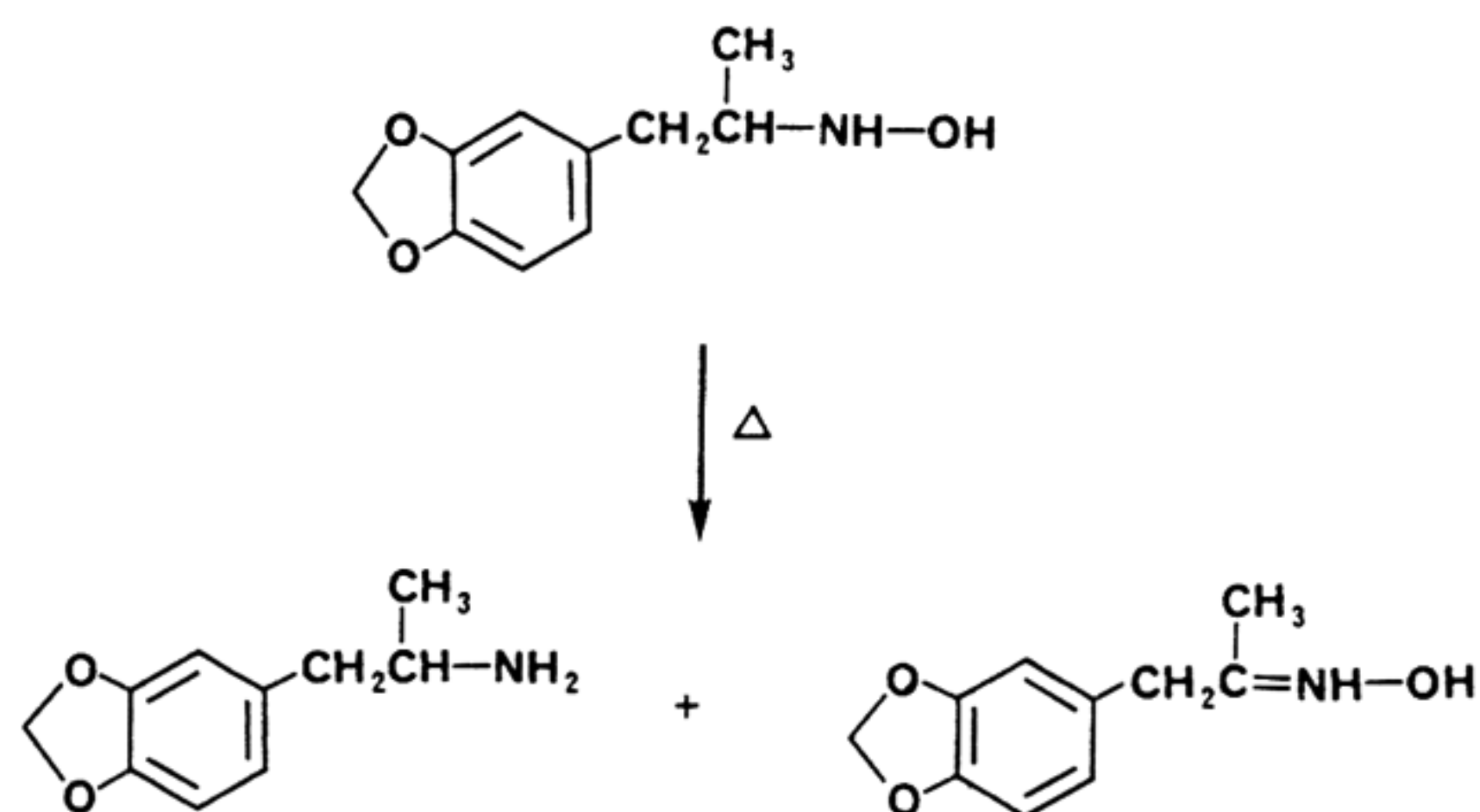


**Figure 5.** Electron impact mass spectra of MDA and *N*-substituted derivatives. Spectra: A=MDA; B=*N*-methyl MDA; C=*N,N*-dimethyl MDA; D=*N*-ethyl MDA; E=*N*-isopropyl MDA; F=*N*-propyl MDA.





**Figure 6.** GC/MS analysis of *N*-hydroxy MDA. A=mass spectrum of MDA (peak 1); B=chromatogram resulting from *N*-hydroxy MDA (peak 1, 6.131 min=MDA; peak 2, 7.362 min=oxime of 3,4-methylenedioxyphenyl-2-propanone); C=mass spectrum of the oxime.

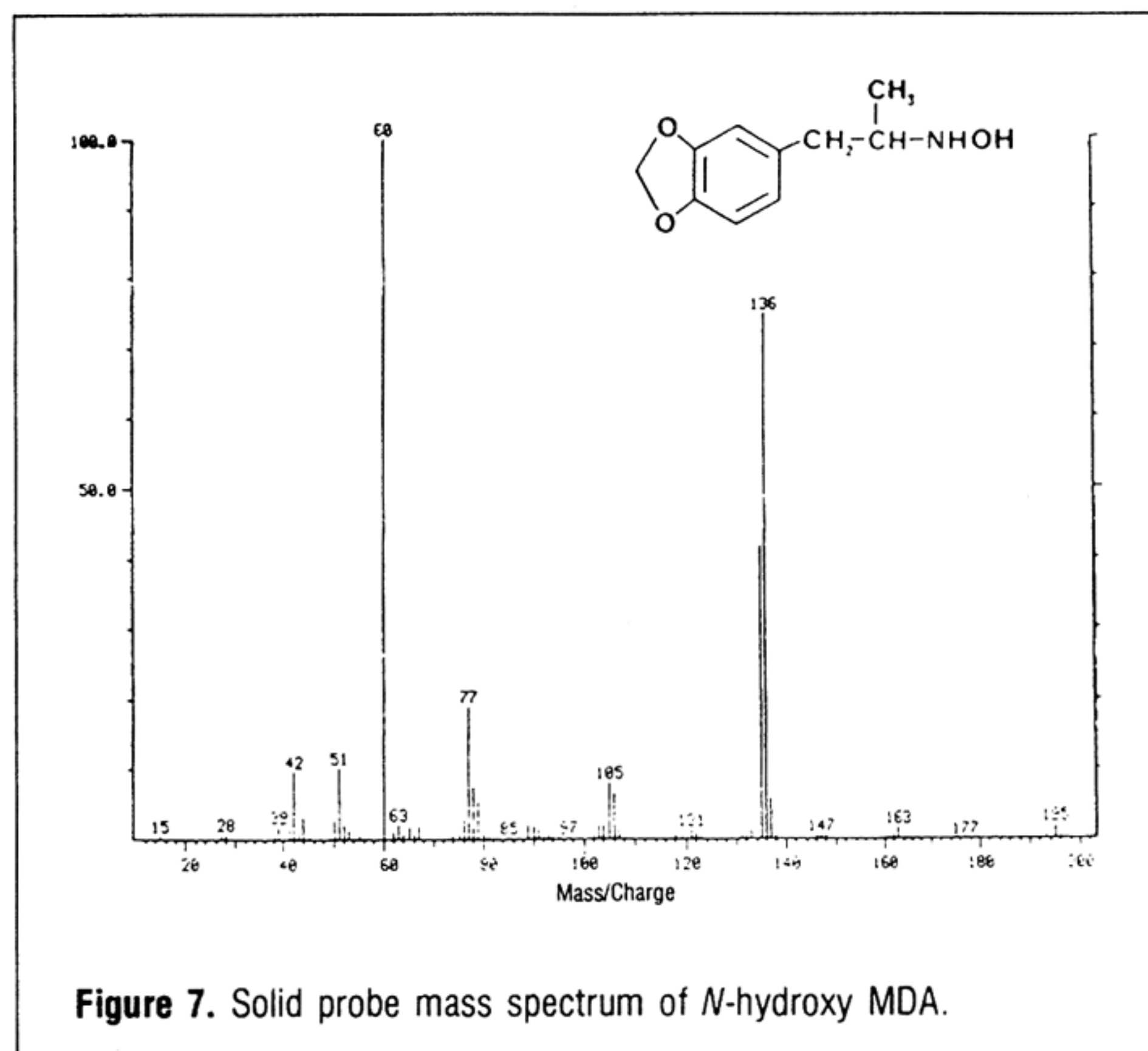


**Scheme II.** Decomposition of the *N*-hydroxy derivative to yield MDA and the oxime.

rapidly using this chromatographic system.

The infrared spectrum of *N*-hydroxy-MDA is shown in Figure 3, and the infrared spectrum of *N,N*-dimethyl-MDA hydrochloride is shown in Figure 4. Because these samples have been identified by forensic laboratories they are included for comparative purposes. The infrared spectra of MDA, MDMA, and the *N*-ethyl analogs are similar and are published elsewhere (9).

The mass spectra of these amines are characteristic and can be used for specific identification. Figure 5, A through F shows the mass spectra for MDA and the *N*-alkyl MDA derivatives. These compounds show only a low abundance of molecular ion and undergo amine dominated fragmentation. The base peak in these spectra results from the loss of the 3,4-methylenedioxy



**Figure 7.** Solid probe mass spectrum of *N*-hydroxy MDA.

benzyl radical yielding  $m/z$  44 for MDA, 58 for MDMA, etc., as shown in Scheme I. For those amines having an *N*-substituent of at least two carbons in length, the base peak containing the *N*-alkyl group can rearrange to yield the 44 ion. For example, compare the spectra for *N,N*-dimethyl MDA (Figure 5C) with that of *N*-ethyl MDA (Figure 5D). The 44 ion is produced from the 4-centered rearrangement reaction for *N*-ethyl, but not for the *N,N*-dimethyl MDA derivative.

The spectra in Figure 5 were obtained by gas chromatographic/mass spectrometric (GC/MS) analysis of the standard compounds. Figure 6 shows the analysis of the *N*-hydroxy MDA derivative under the same conditions. The resulting chromatogram (Figure 6B) shows two well-resolved peaks separated by a little more than 1 min. The first peak at a little more than 6 min matches the retention time and the spectrum for MDA (Figure 6A), while the second peak at more than 7 min matches the retention characteristics and mass spectrum of the oxime of 3,4-methylenedioxyphenyl-2-propanone (Figure 6C). Thus, the *N*-hydroxy derivative appears to have decomposed in the injection port of the GC to yield MDA and the oxime, as shown in Scheme II. Figure 7 is the mass spectrum of *N*-hydroxy MDA determined by solid probe inlet. This spectrum shows characteristics similar to those for the *N*-alkyl MDA derivatives, a low abundance of molecular ion, and a base peak from the loss of the 3,4-methylenedioxybenzyl radical (i.e., fragmentation as in Scheme I with  $R = OH$ ). Thus, the *N*-hydroxy MDA derivative is thermally unstable and GC methods for its analysis will suffer from this fact. Although *N*-hydroxy MDA is unstable under some aqueous solution conditions (10), LC analysis without the accompanying decomposition is possible. The peak assigned as the *N*-hydroxy derivative in Figures 1 and 2 is the undecomposed parent compound and not the oxime. The oxime has a higher  $k'$  than any of the compounds separated under these conditions; the retention time for the oxime is more than 40 min.

## Conclusion

The reported methods describe the separation of the most likely designer-drug modifications of MDA using reversed-phase LC. The *N*-hydroxy MDA derivative is unstable yielding MDA

and the oxime at high temperatures. The mass spectra of MDA and the *N*-substituted derivatives can be used to specifically identify these compounds.

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