

A contribution to the chemical profiling of 3,4-methylenedioxymethamphetamine (MDMA) tablets

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Abstract

A profiling method for the identification of impurities found in seized 3,4-methylenedioxymethamphetamine (MDMA) tablets is presented. Impurities of interest are extracted from an alkaline solution (pH 12.8) by diethyl ether and submitted to gas chromatography (GC)–mass spectrometry (MS) analyses. Identification of impurities is performed by electron impact ionization (Ei) mass spectrometry and confirmation by positive chemical ionization (Ci+) MS or, when possible, MS/MS (MS^2). Repeat extractions of the same sample give an average relative standard deviation (R.S.D.) of less than 8% within the same day and 15% between days (results were obtained after normalization by the sum of peak areas, each one being acquired by selected ion monitoring (SIM)). Possible application toward batch comparison of samples is discussed. Chromatographic profiles are compared using the cosine function for evaluating similarity and/or dissimilarity among exhibits. © 2002 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: 3,4-Methylenedioxymetamphetamine (MDMA); Impurities; Gas chromatography; Mass spectrometry; Profiling

1. Introduction

The 3,4-methylenedioxymethamphetamine (MDMA) is a synthetic compound discovered in Germany in 1913 and patented by a pharmaceutical company in 1914 [1]. It was intended as an appetite suppressant but was never marketed. Illicit use of MDMA did not become popular until the late 1980s and early 1990s. Users of the drug say it produces positive feelings and empathy to others, but long term abuse seems to cause brain damage and impairments in visual and verbal memory [2,3]. Although the main chemical precursors of MDMA (isosafrrole, safrrole, piperonal, 3,4-methylenedioxyphenyl-2-propanone (MDP2P or PMK)) are controlled in the European Union, significant quantities of the drug in tablet or powder are still manufactured in clandestine laboratories operating throughout western Europe, primarily The Netherlands and Belgium. In France, seizures of MDMA tablets were multiplied by nine between 1997 and 1999, and they increased by 23% between 1999 and 2000 [4]. The global increase of

MDMA production in Europe is principally due to the advances in chemical and technology knowledge, the increased availability of basic chemicals and equipment, and to the easier access to literature on the subject, especially via on-line web sites. In order to fight against this increase, it would be useful for forensic chemists to know the synthesis routes used in the clandestine laboratories. In that aim, impurity profiling, i.e. physical and chemical characterization of seized drug materials, is a tool which can give information on the synthesis conditions used (reagents, catalysis, purification, etc.). Such information is helpful to regulatory authorities and particularly in programs for monitoring precursors. Moreover, based on their impurity profile, samples of seized drugs can be classified into related groups, or even batches, for both evidential and intelligence purposes [5]. Finally, identification of the impurities can be essential for the knowledge of their toxicity as they may have potential harmful effects on user [6].

The reasons for the presence of trace impurities in clandestinely manufactured drugs are manifold. Impurities may be generated as by-products during drug manufacture. They may already be present in the starting materials,

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reagents and/or solvents, and are carried over unchanged to the final product. On the contrary, these initial impurities may react and be transformed at each step of the global process. Finally, conditions of storage, such as exposure to light and/or heat, as well as addition of “cutting” compounds may alter the main drug and/or minor components thus producing new impurities. Therefore, to draw correct conclusions from similarities and differences between impurity profiles, the forensic chemist needs to know more about the chemistry of illicit drug manufacturing processes, more precisely, the generation and stability of impurities, the significance of individual impurities for a given synthesis route, and the extent of possible variation in impurity profiles of drugs synthesized via the same route. This information may be obtained by carrying out a thorough chemical analysis of samples of known manufacturing origin. For instance, in the case of illicitly prepared amphetamine or methamphetamine, the presence of “route specific” impurities have been used to determine the manufacturing process [7–14].

Concerning MDA and analogs, the main synthesis routes used for their production are already well known (leuckart, reductive amination, nitropropene, etc.) [15–23], even if unusual conditions are sometimes noticed [15–17]. Some studies of precursors, intermediates and reaction by-products have been performed, and identification of many trace level impurities reported [19–21,23–32]. Nevertheless, few articles deal with the profiling of MDMA seizures [20,33–35]. First one presents a profiling method of MDMA and 3,4-methylenedioxyethylamphetamine (MDEA) tablets and gives mass spectral data of trace level impurities [20]. A mass of tablet corresponding to 80 mg of pure MDMA was dissolved in 5 ml of phosphate buffer (pH 7) and impurities were extracted by diethyl ether containing heneicosane (C21) as internal standard and analyzed by gas chromatography (GC)–mass spectrometry (MS) according to the splitless mode. Other authors also used phosphate buffer (pH = 6 [35] or pH = 9 [33]) to dissolve MDMA powders but organic impurities were extracted, respectively by dichloromethane [35] and ethyl acetate [33]. In that last study, comparison between liquid–liquid extraction (LLE) and solid phase extraction (SPE) for the profiling of ecstasy tablets. Finally, head-space analysis of MDMA seizures showed to be an interesting means to determine common batch membership [34].

2. Materials and methods

2.1. Gas chromatography and mass spectrometry

All analyses were carried out on a Thermofinnigan GC trace 2000 gas chromatograph interfaced with an ion trap Polaris mass spectrometer. The 2 μ l of sample extract were injected according to the splitless mode using a Thermofinnigan AS 2000 autosampler. The column was a Supelco PTA5 capillary column (cross-linked poly 5% diphenyl/95% dimethyl-

siloxane); 30 m \times 0.32 mm (i.d.) \times 0.5 μ m film thickness. The oven temperature was programmed as follows: 50 $^{\circ}$ C for 1 min, 5 $^{\circ}$ C min $^{-1}$ to 150 $^{\circ}$ C for 12 min, and 15 $^{\circ}$ C min $^{-1}$ to 300 $^{\circ}$ C for 10 min. The injection port and transfer line temperatures were, respectively 280 and 275 $^{\circ}$ C. The ion source temperature was set at 200 $^{\circ}$ C. The helium carrier gas flow rate was fixed at 1 ml min $^{-1}$. The mass spectrometer was tuned for low-mass analysis and two kinds of ionization were performed: electron impact ionization (Ei) for detection and identification, positive chemical ionization (Ci+) for the confirmation of each impurity (reagent gas was methane, ion source pressure 70 mTorr). For the reproducibility evaluation or the profiling comparison, selected ion monitoring (SIM) was used on the most intense impurity mass fragments. In order to preserve MS filament life, the mass spectrometer was stopped during elution of the major compounds.

2.2. MDMA materials

Ten different MDMA samples (Ref1, Ref2, S1–S8) have been used in the experiments. Two samples (Ref1 and Ref2) were used for the overall reproducibility of the process. Samples S1–S3 were added to the first ones for the identification of all impurities detected on samples analyzed. Other samples (S4–S8) were added for impurities profiles comparison. Samples Ref1 and Ref2 consisted, respectively of 38 and 53% of MDMA hydrochloride diluted with lactose. The composition of the other samples used is reported in Table 1.

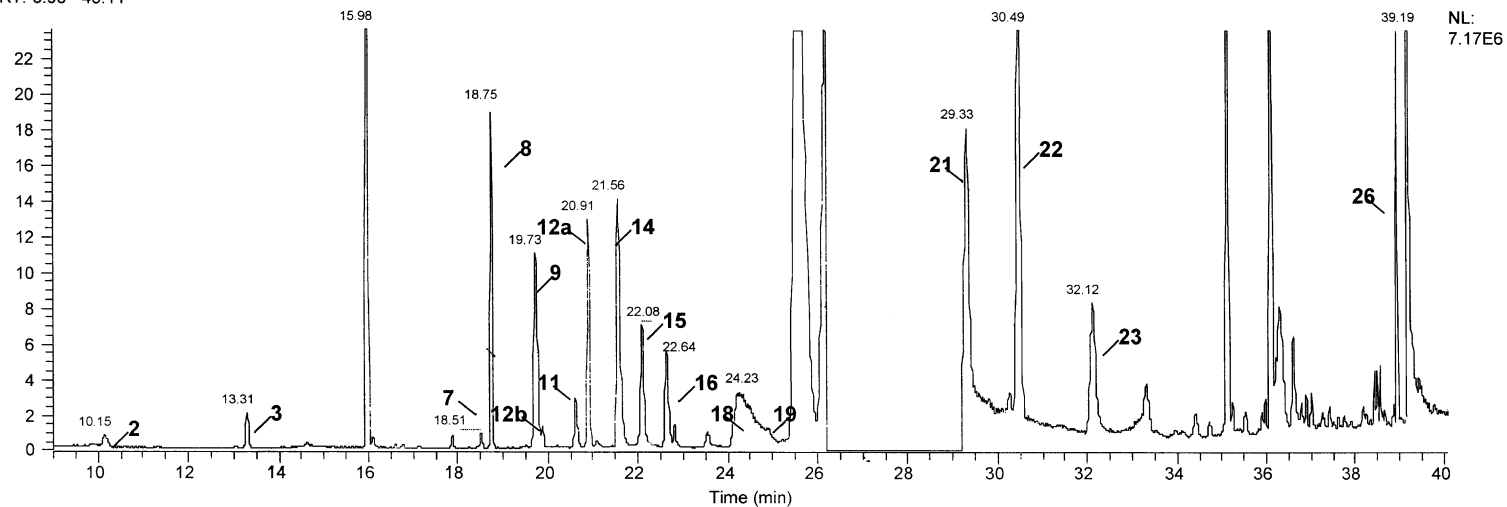
2.3. Standard extraction method

An amount of sample equivalent to 10 mg of pure MDMA hydrochloride was weighed and dissolved in 3 ml of distilled water. For MDMA phosphate sample (S5) an adjustment factor was applied. The solution was made basic (pH 12.8) with 200 μ l of 1 M sodium hydroxide and shaken for 10 min at 1800 rpm. The extraction was obtained adding 3 ml of diethylether and shaking for another 20 min. After

Table 1
MDMA materials used in the study

Sample	Active substances	Other substances
Ref1	38% MDMA HCl	Lactose
Ref2	53% MDMA HCl	Lactose
S1	35% MDMA HCl, ephedrine	Lactose
S2	25% MDMA HCl	Starch, lactose
S3	33% MDMA HCl	Lactose
S4	43% MDMA HCl	Starch, lactose, saccharose
S5	35% MDMA H ₂ PO ₄	Lactose
S6	25% MDMA HCl	Starch, lactose
S7	19% MDMA HCl	Lactose, saccharose
S8	31% MDMA HCl	Lactose

RT: 8.98 - 40.11



RT: 16.99 - 26.34

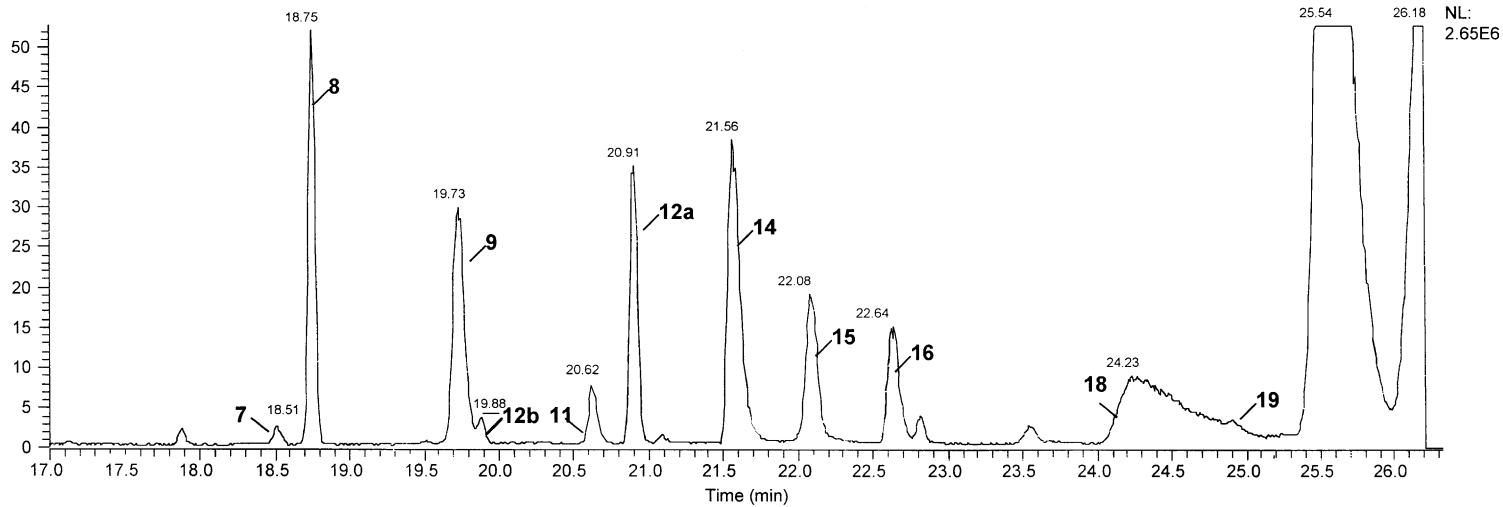


Fig. 1. Ei/full scan impurity profile of sample Ref1.

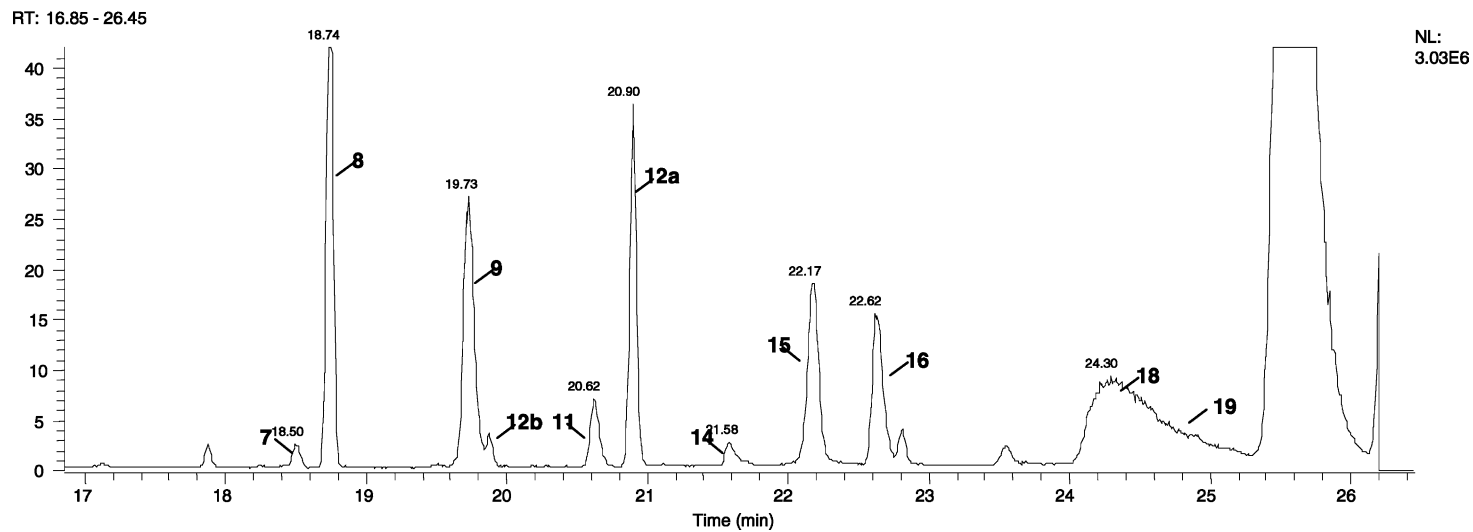
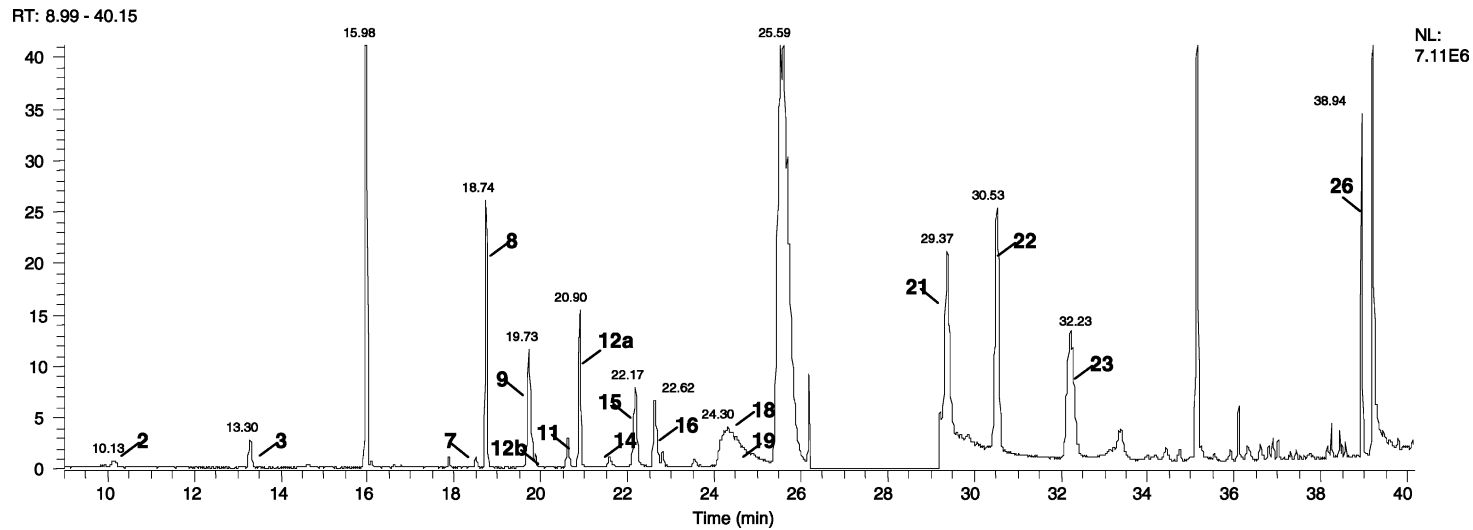


Fig. 2. Ei/full scan impurity profile of sample Ref2.

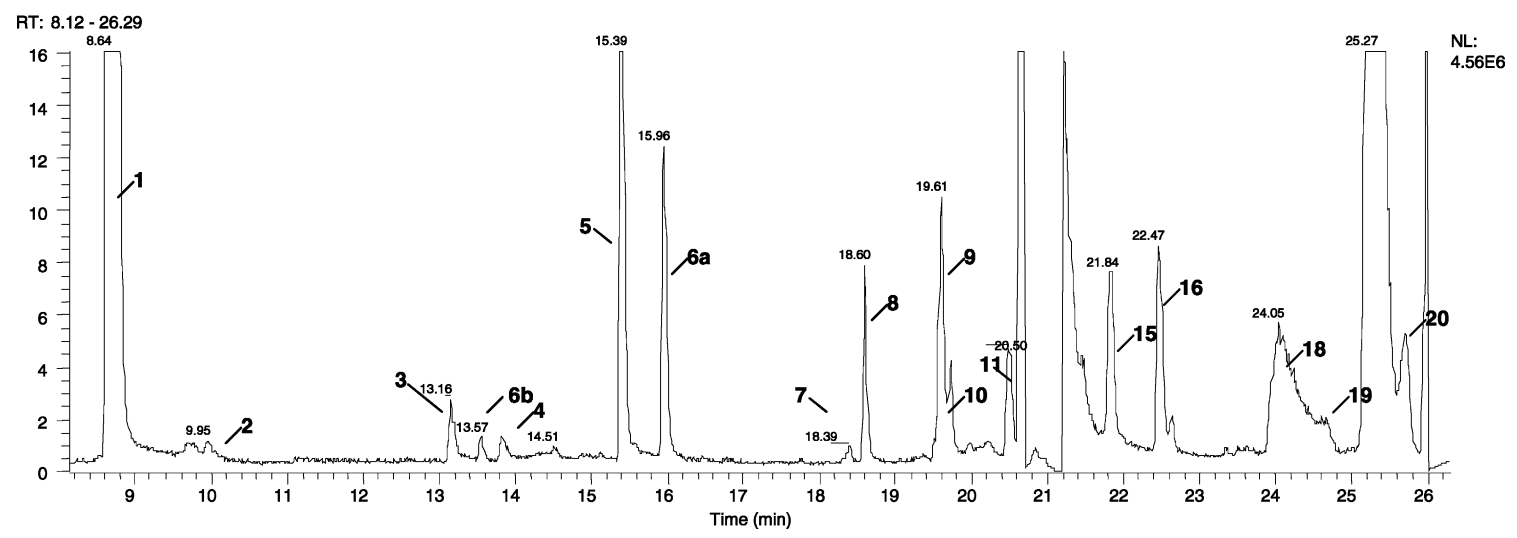
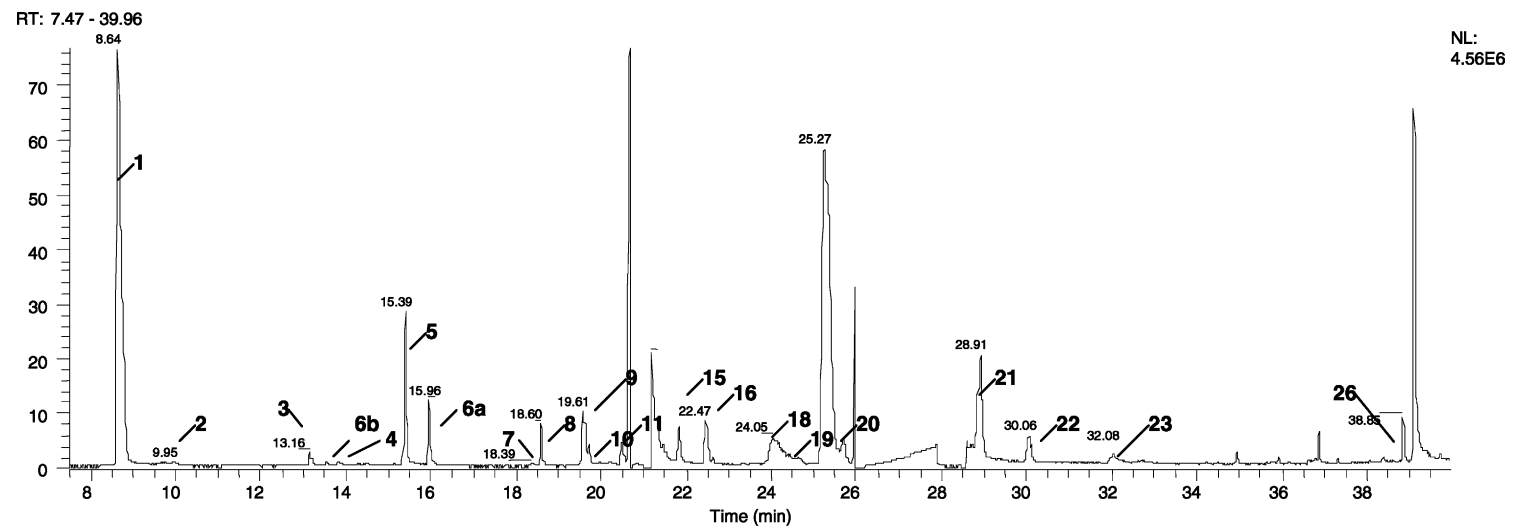


Fig. 3. Ei/full scan impurity profile of sample S1.

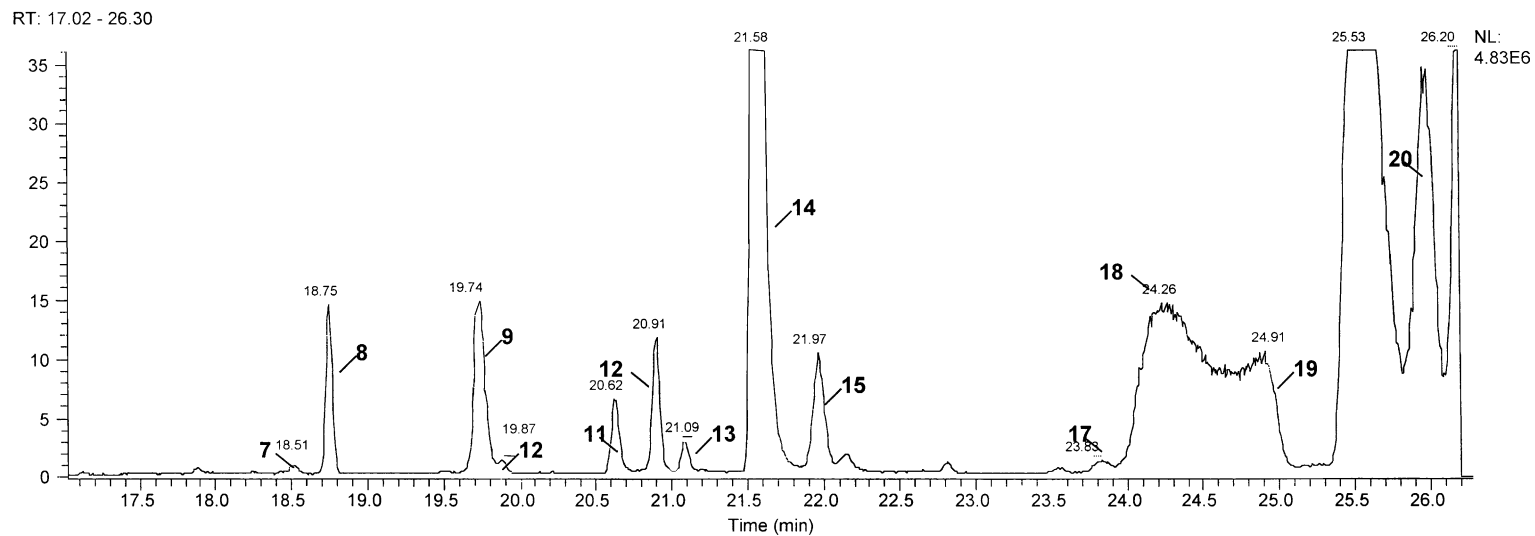
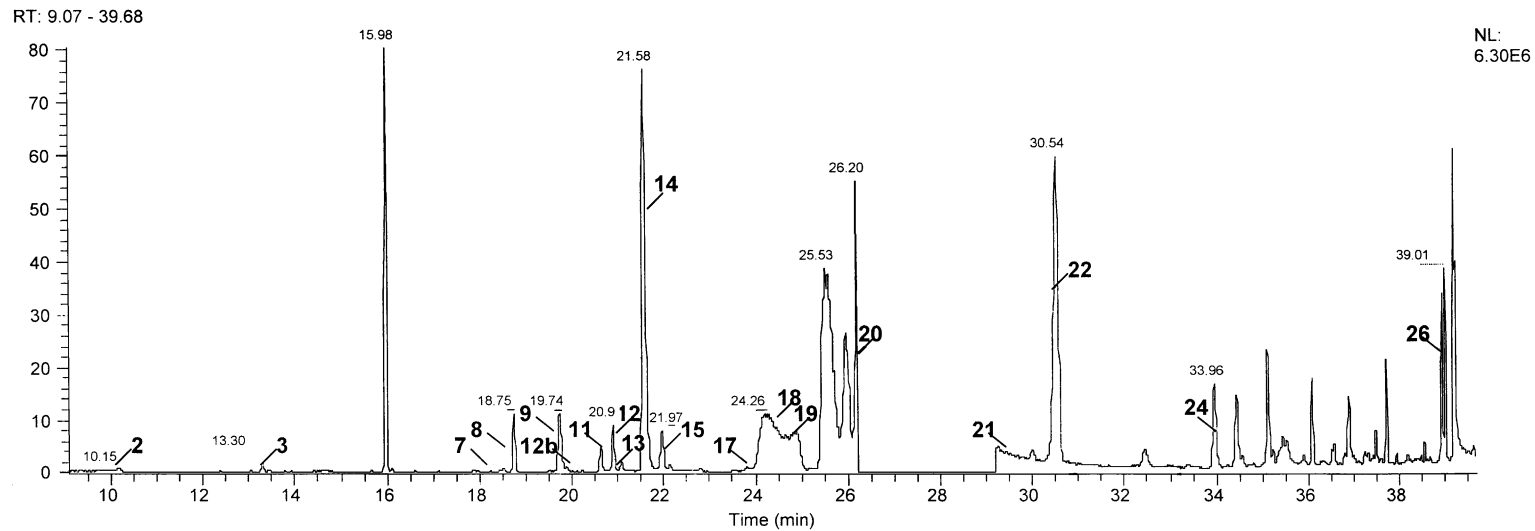


Fig. 4. Ei/full scan impurity profile of sample S2.

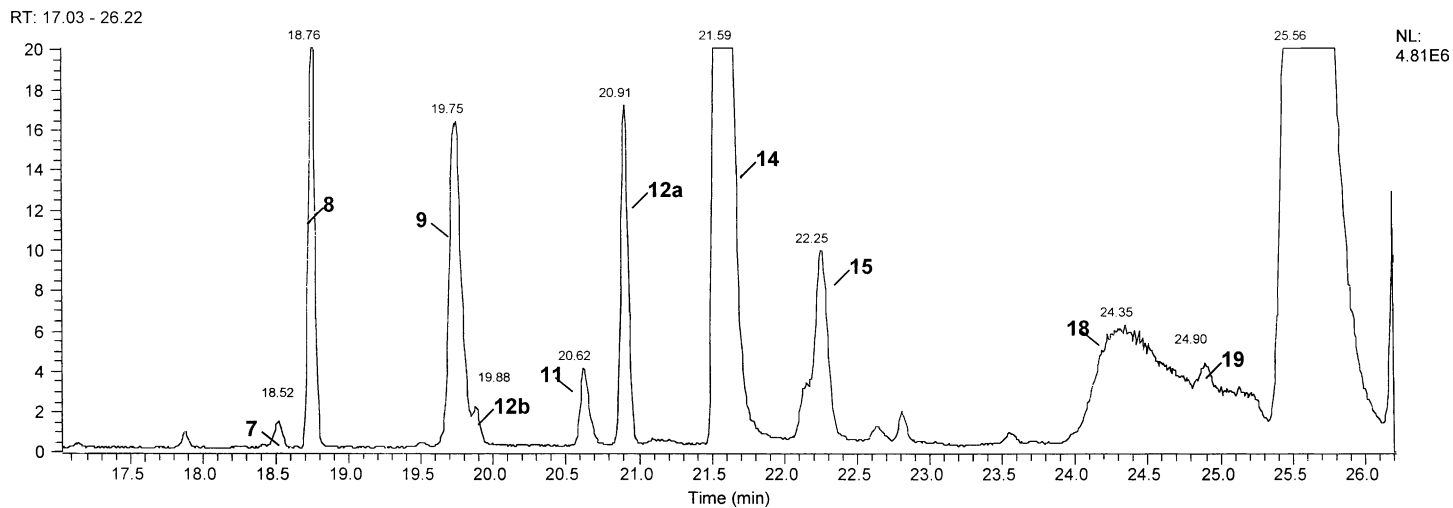
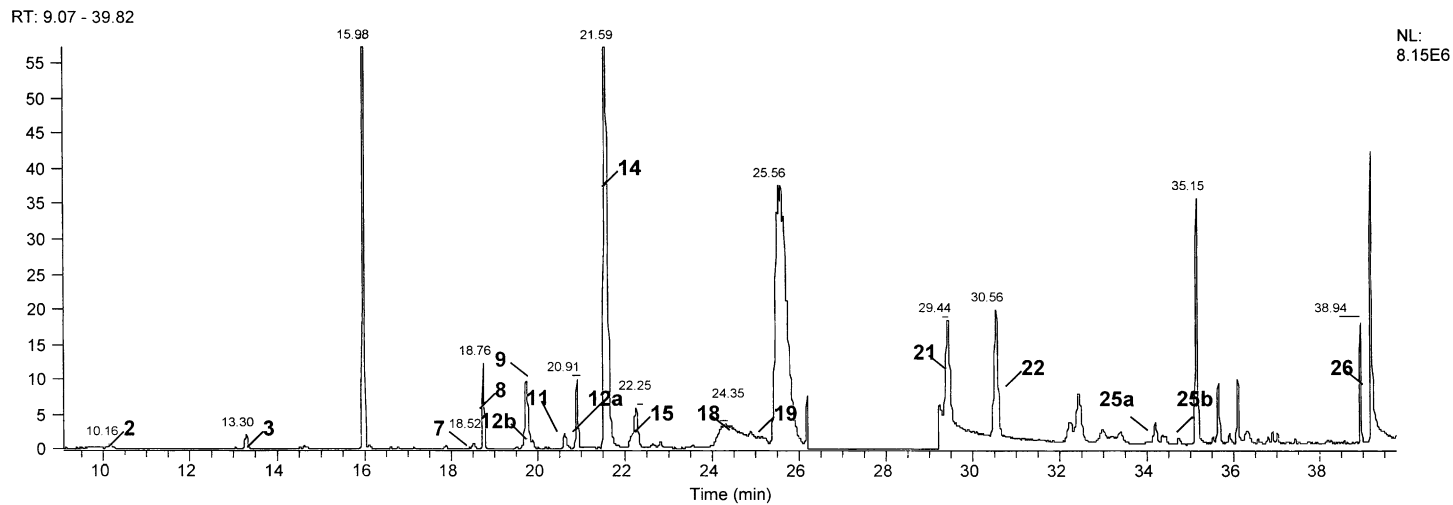


Fig. 5. Ei/full scan impurity profile of sample S3.

Table 2
Impurities found in MDMA samples

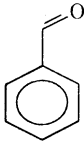
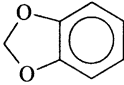
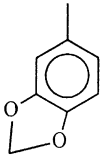
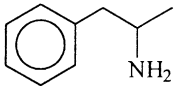
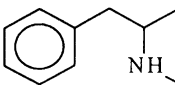
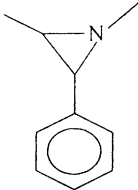
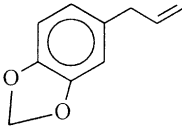
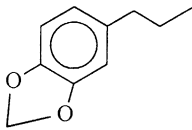
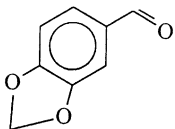
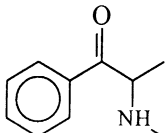
Impurity name	Chemical structure	Chemical origin	Ei mass spectral data	Peak number
Benzaldehyde: C ₇ H ₆ O MW106 CAS #100-52-7		Chemical precursor of ephedrine [36]	105/106, 77, 51	1
1,3-Benzodioxole: C ₇ H ₆ O ₂ MW122 CAS #274-09-9		Impurity present in safrole	121/122, 63/64	2
3,4-Methylenedioxytoluene: C ₈ H ₈ O ₂ MW136 CAS #7145-99-5		Impurity present in safrole, isosafrole [32] and piperonal	135/136, 78/77, 51	3
Amphetamine: C ₉ H ₁₃ N MW135 CAS #300-62-9		Contamination	44, 91	4
Methamphetamine: C ₁₀ H ₁₅ N MW149 CAS #537-46-2		Contamination	58, 91	5
1,2-Dimethyl-3-phenyl-aziridine: C ₁₀ H ₁₃ N MW147 CAS #68277-68-9		Methamphetamine impurity (ephedrine synthesis route) [7]	146, 105, 132, 42, 91	6a, 6b
Safrole: C ₁₀ H ₁₀ O ₂ MW162 CAS #94-59-7		Chemical precursor of MDMA	162, 104, 131, 77, 51	7
3,4-(Methylenedioxy)-phenylpropane: C ₁₀ H ₁₂ O ₂ MW164 CAS #94-58-6		Chemical reduction of safrole or isosafrole	135, 164, 77, 51	8
Piperonal: C ₈ H ₆ O ₃ MW150 CAS #120-57-0		Chemical precursor of MDMA or Impurity found after MDP2P synthesis from isosafrole	149/150, 121, 63, 91	9
2-Methylamino-1-phenyl-1-propanone: C ₁₀ H ₁₃ NO MW163 CAS #5650-44-2		Methamphetamine impurity (ephedrine synthesis route) Also known as methcathinone [7]	58, 77	10

Table 2 (Continued)

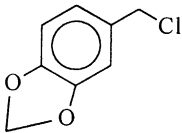
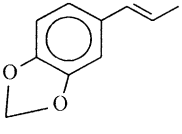
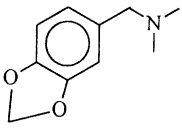
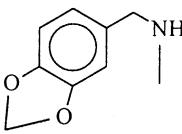
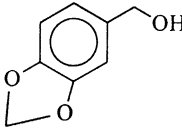
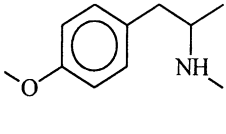
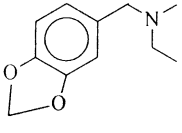
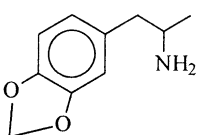
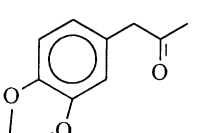
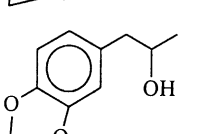
Impurity name	Chemical structure	Chemical origin	Ei mass spectral data	Peak number
Piperonyl chloride: C ₈ H ₇ ClO ₂ MW170 CAS #20850-43-5		Nucleophilic substitution of piperonyl alcohol by hydrochloric acid	135, 77, 51, 170	11
Isosafrole: C ₁₀ H ₁₀ O ₂ MW162 CAS #120-58-1		Chemical precursor of MDMA (<i>cis</i> and <i>trans</i> isomers forms)	162 , 104, 131, 77, 51	12a, 12b
3,4-Methylenedioxy-N,N-dimethylbenzylamine: C ₁₀ H ₁₃ NO ₂ MW179 CAS #58995-64-5		MDMA by-product (reductive amination of piperonal by dimethylamine, impurity present in methylamine)	135 , 58, 179, 77	13
3,4-Methylenedioxy-N-methylbenzylamine: C ₉ H ₁₁ NO ₂ MW165 CAS #15205-27-3		MDMA by-product (reductive amination of piperonal by methylamine) [21]	135/136 , 164/165, 44, 77	14
Piperonyl alcohol: C ₈ H ₈ O ₃ MW152 CAS #495-76-1		Chemical reduction of piperonal	152 , 135, 93, 65, 123	15
p-Methoxymethamphetamine: C ₁₁ H ₁₇ NO MW 179 CAS #22331-70-0		MDMA by-product (from 1-methoxy-4-(2-propenyl)-benzene [32], impurity present in safrole)	58 , 121, 78, 91	16
3,4-Methylenedioxy-N-ethyl-N-methylbenzylamine: C ₁₁ H ₁₅ NO ₂ MW193 CAS #90704-72-6		MDMA by-product (reductive amination of piperonal by ethyl-methylamine, impurity present in methylamine)	135 , 72, 193	17
3,4-Methylenedioxyamphetamine: C ₁₀ H ₁₃ NO ₂ MW179 CAS #4764-17-4		MDMA by-product (reductive amination of MDP2P by ammonia, impurity present in methylamine) [31]	44, 135/136 , 77	18
3,4-Methylenedioxyphenyl-2-propanone: C ₁₀ H ₁₀ O ₃ MW178 CAS #4676-39-5		MDMA intermediate or precursor	135 , 178, 77, 51, 43	19
3,4-Methylenedioxyphenyl-2-propanol: C ₁₀ H ₁₂ O ₃ MW180 CAS #6974-61-4		Chemical reduction of MDP2P	135/136 , 180, 77, 106, 51, 45	20

Table 2 (Continued)

Impurity name	Chemical structure	Chemical origin	Ei mass spectral data	Peak number
1,2-Methylenedioxy-4-(2-N-methyliminopropyl)benzene: C ₁₁ H ₁₃ NO ₂ MW191		MDMA intermediate (amination of MDP2P by methylamine) [31]	56 , 191, 135, 77	21
N,N-Dimethyl-(1,2-methylenedioxy)-4-(2-aminopropyl)benzene: C ₁₂ H ₁₇ NO ₂ MW207		MDMA by-product (reductive amination of MDP2P by dimethylamine, impurity present in methylamine) [31]	72 , 56, 44, 73, 58, 70	22
N-Methyl-1-[1,2-dimethoxy-4-(2-aminopropyl)benzene: C ₁₂ H ₁₉ NO ₂ MW209		MDMA by-product (from 1,2-dimethoxy-4-(2-propenyl)benzene [31,32], impurity present in safrole) [21]	58, 152 , 56	23
N-Ethyl-N-methyl-(1,2-methylenedioxy)-4-(2-aminopropyl)benzene: C ₁₃ H ₁₉ NO ₂ MW221		MDMA by-product (reductive amination of MDP2P by ethyl-methylamine, impurity present in methylamine) [31]	86 , 58, 87, 56, 44, 77	24
1-(3,4-Methylenedioxyphenyl)-2-propanone oxime: C ₁₀ H ₁₁ NO ₃ MW193 CAS #52271-42-8		Chemical reduction of a nitroisosafrrole [21]	135 , 193, 77, 51, 105, 118, 146	25a 25b
N-Methyl-(1,2-methylenedioxy)-4-(1-ethyl-2-aminopropyl)benzene: C ₁₃ H ₁₉ NO ₂ MW221		Unknown	58, 162 , 77, 135, 194	26

centrifugation, the organic layer was transferred to a conic tube and evaporated to dryness under monitoring conditions at room temperature (extracts were evaporated under a minimum nitrogen flow rate which was stopped right after complete evaporation to dryness). The 500 µl of diethylether were then added to the tube, shaken for a few seconds, and transferred to a micro-vial for profile analysis. In order to avoid possible impurity degradation, 2 µl of the extracts were injected the same day they were prepared.

3. Results and discussion

3.1. Identification of impurities in illicit MDMA tablets

Chromatographic profiles of samples Ref1, Ref2, S1, S2 and S3 are shown in Figs. 1–5, respectively. Table 2 gives

peak identity, chemical origin, and mass spectral data of the impurities found in the 10 different MDMA samples. Target ions used for the SIM mode analyses are bold typed in the table. This acquisition mode was used for a best accuracy on the reproducibility evaluation of the extraction method, and on the profiles comparison. For some impurities (peaks 6, 12 and 25) stereoisomers were detected, and noted “a” and “b”. Impurity peaks 12 and 14 were present in sample S1, but could not be detected due to the presence of ephedrine as a major component. They were identified after a previous derivatization of ephedrine by acetic anhydride. The Ei mass spectrum of all MDMA impurities is enclosed in Fig. 6.

3.2. Ci+/MS–MS confirmation of impurities

In addition to Ei identification (Table 2, Fig. 6), each impurity was confirmed by Ci+/MS–MS analyses. Some

RT: 10.01 AV: 1 SB: 59 9.55-9.71, 10.42-10.78 NL: 4.98E3

T: + c Full ms [40.00-650.00]

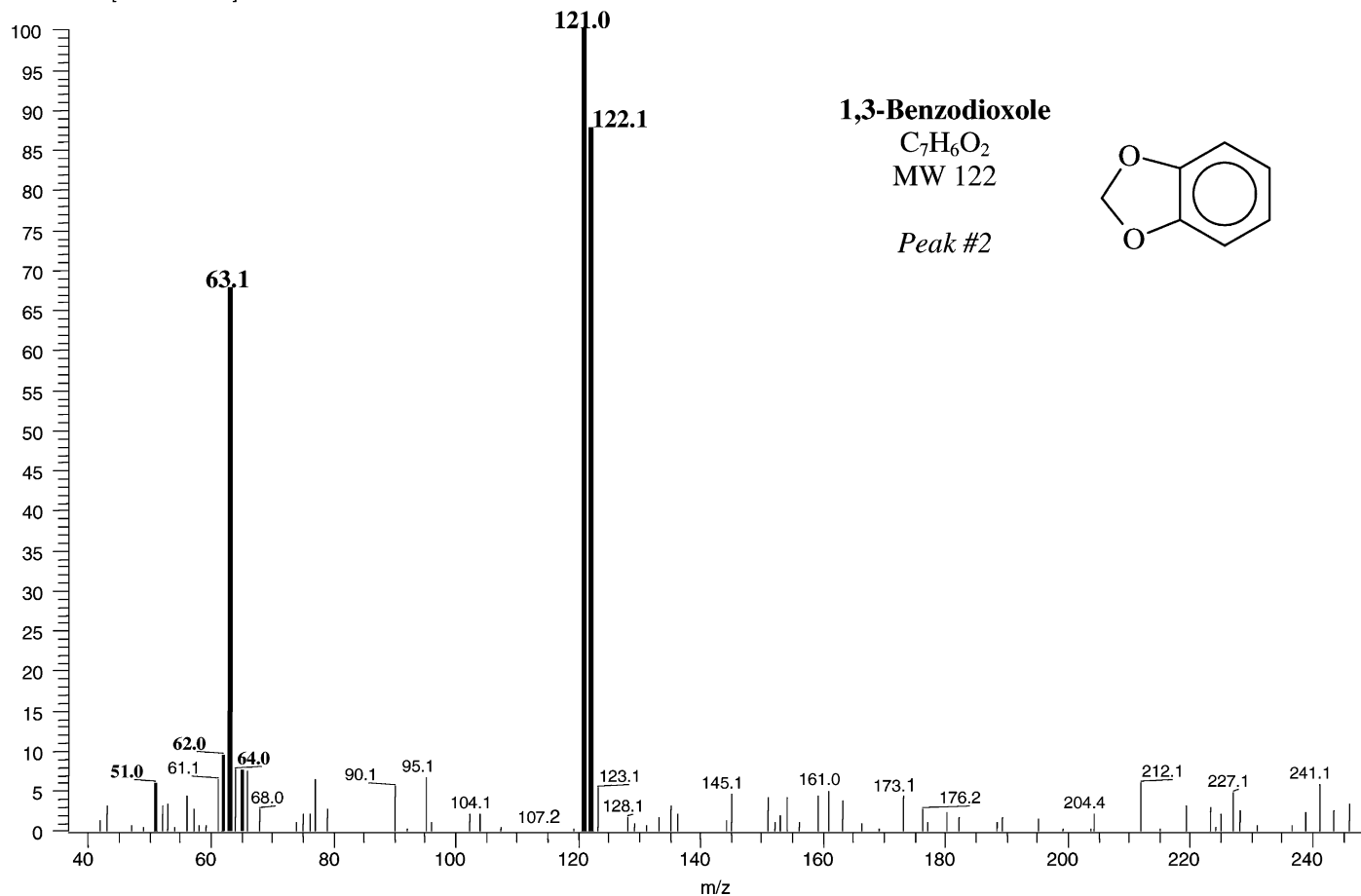


Fig. 6. Electron ionization mass spectrum of impurity peaks 2, 3, 7, 8, 9, 11-26.

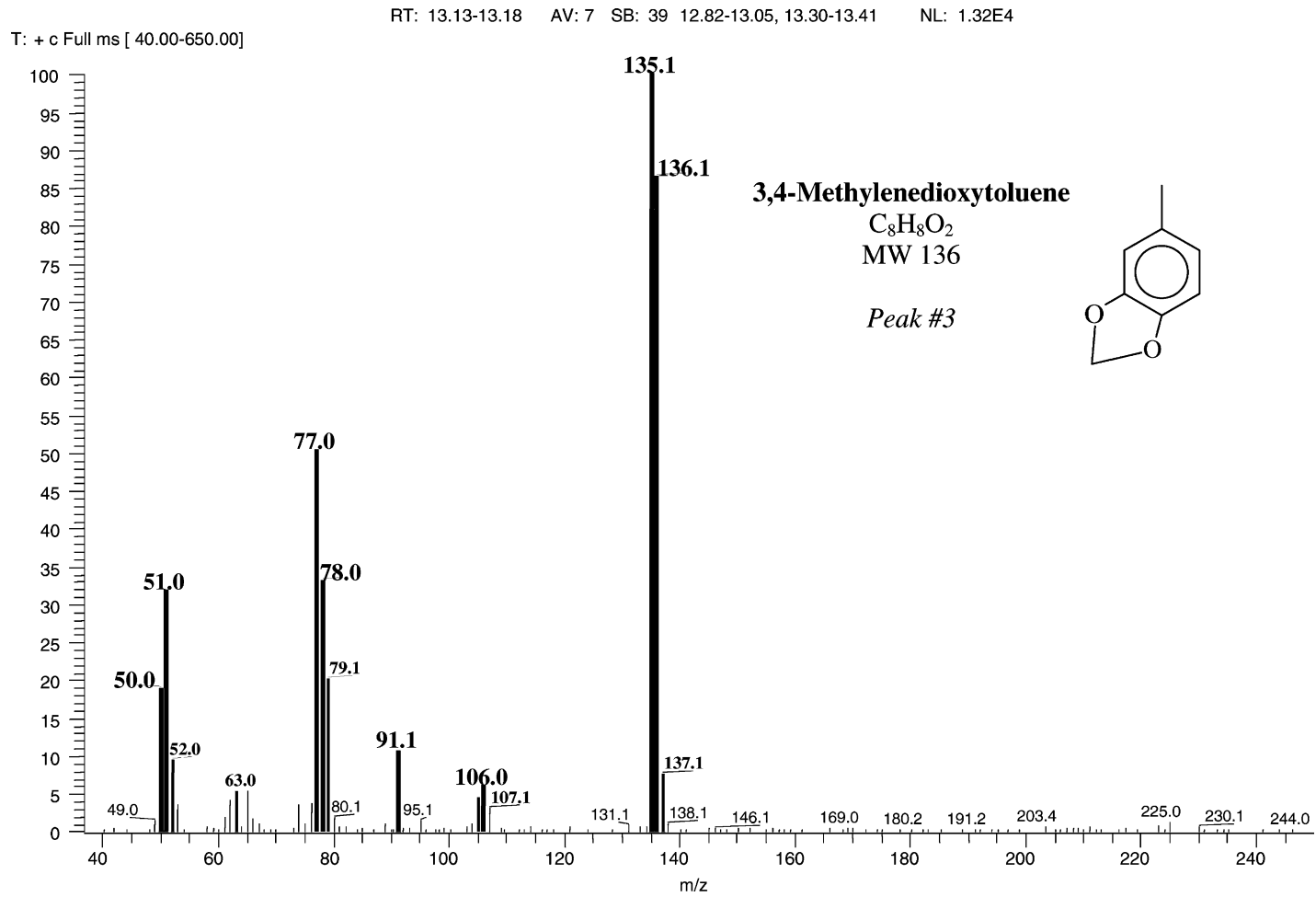


Fig. 6. (Continued).

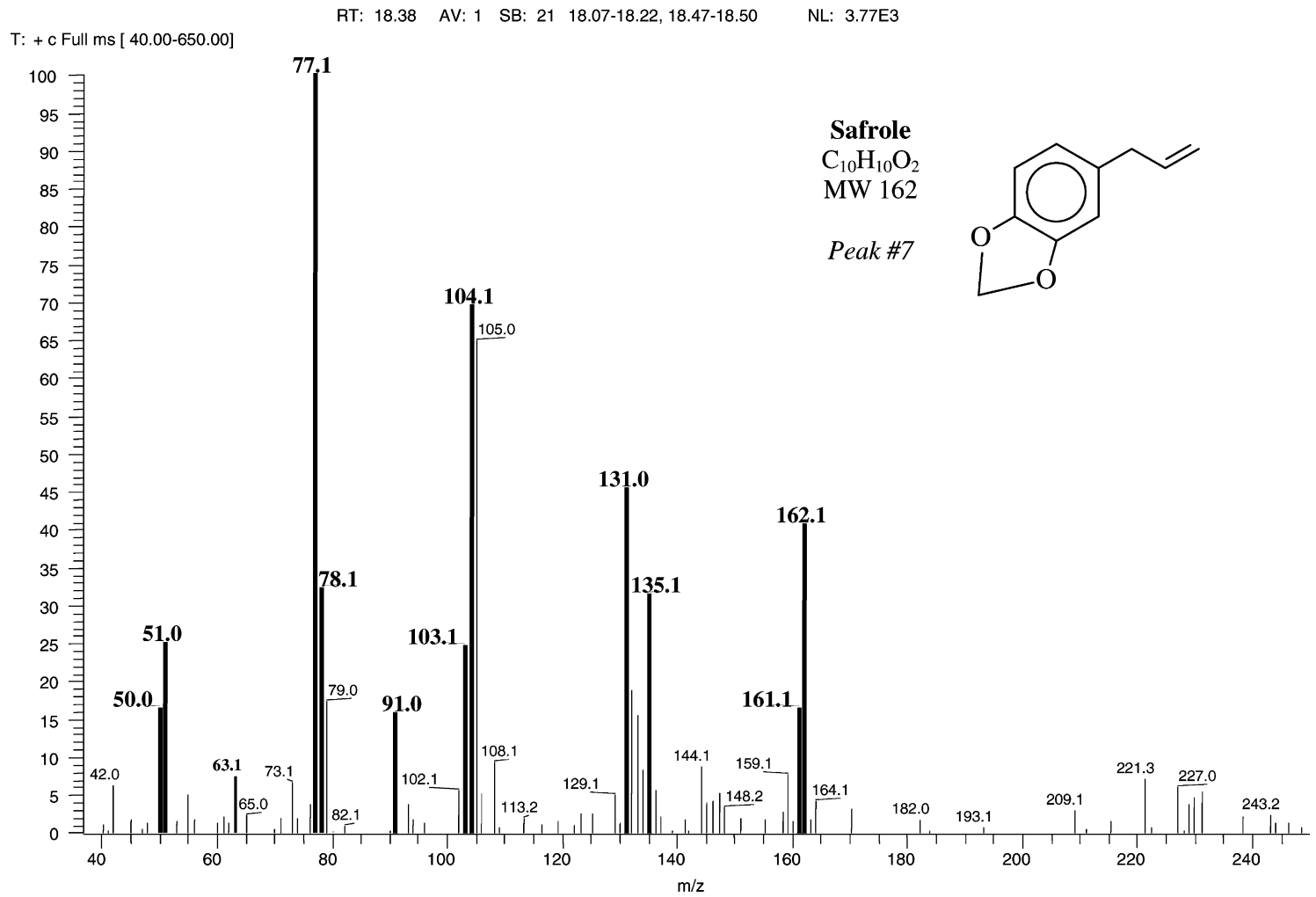


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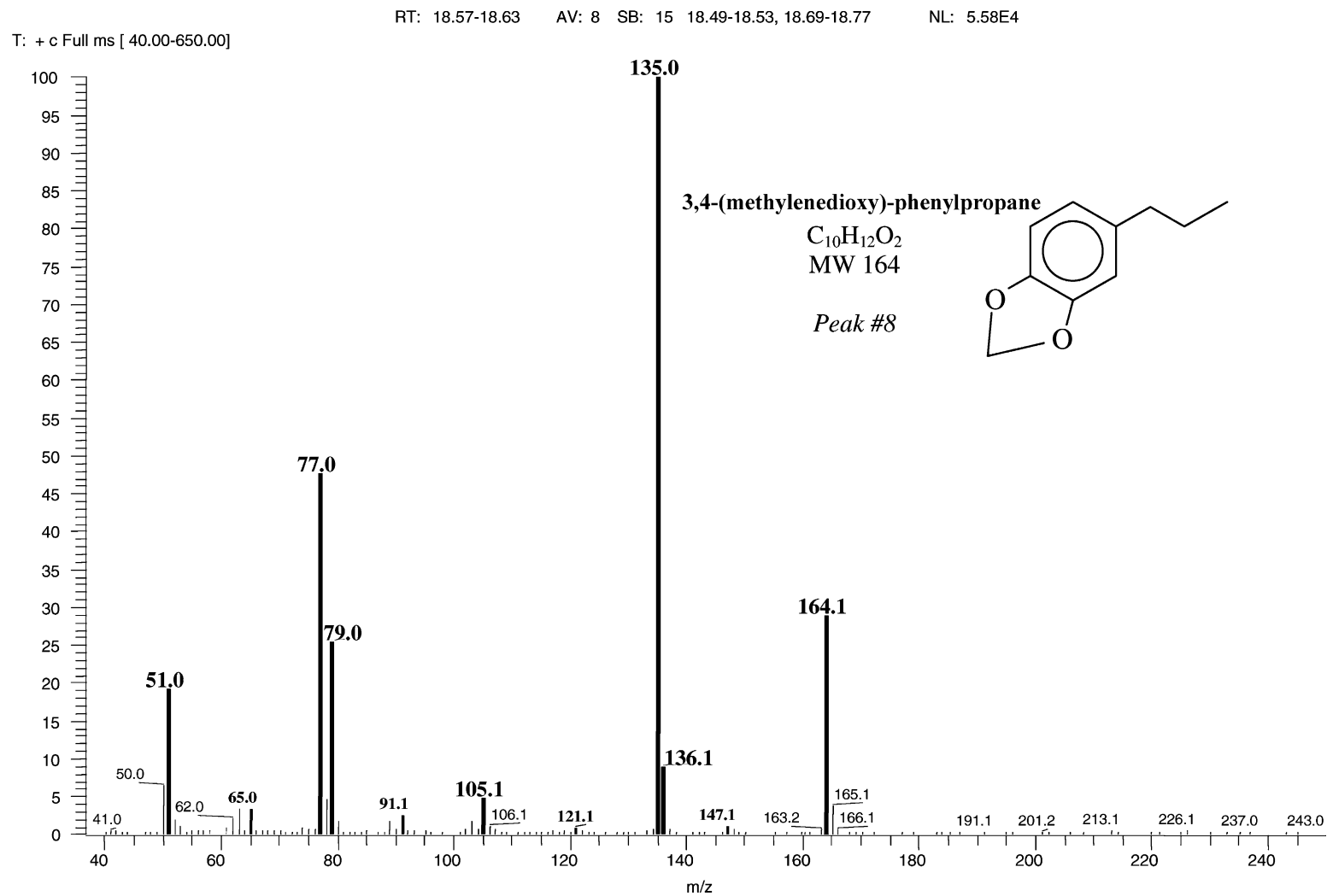


Fig. 6. (Continued).

RT: 19.58-19.62 AV: 6 SB: 11 19.44-19.49, 19.70-19.73 NL: 7.93E4

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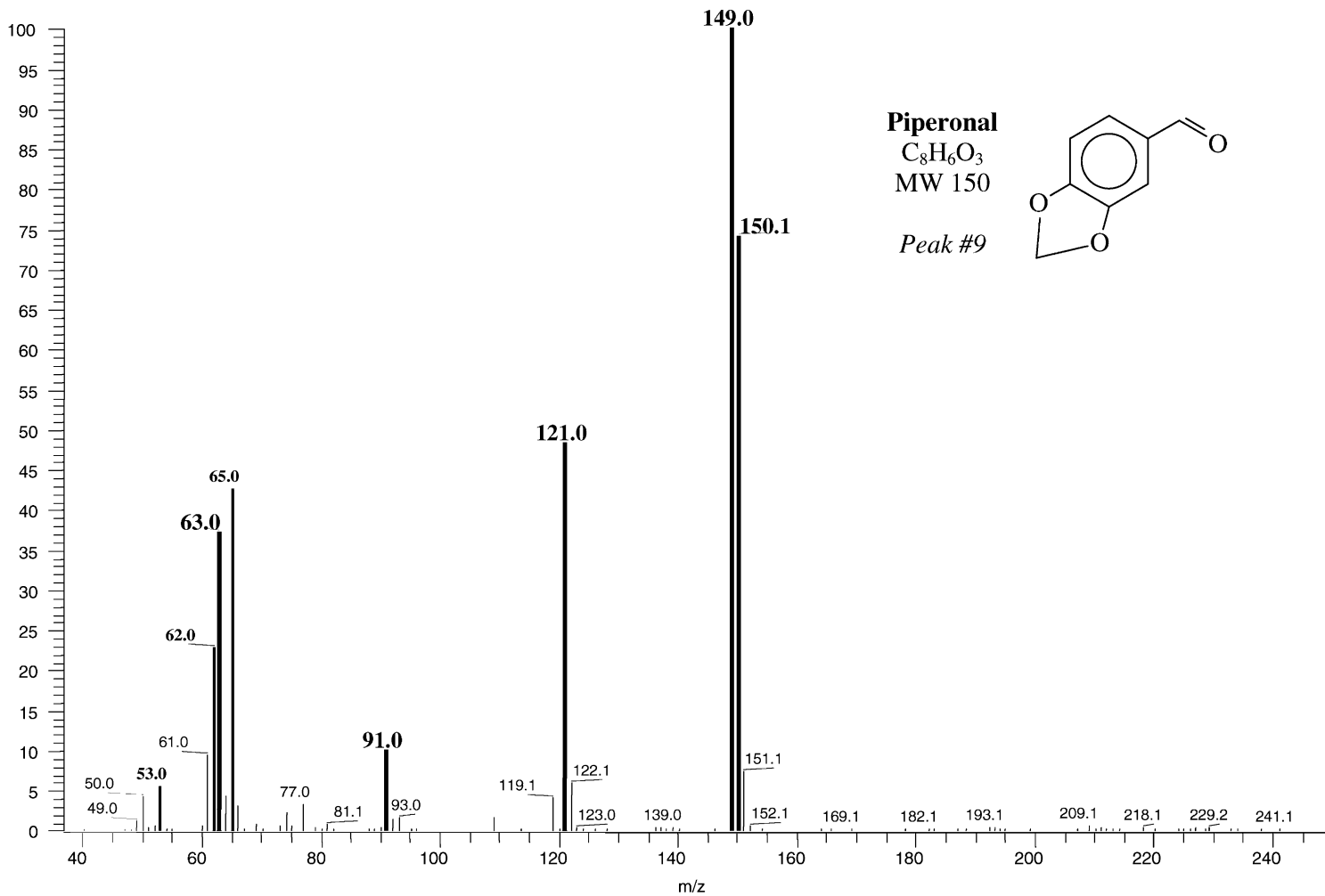


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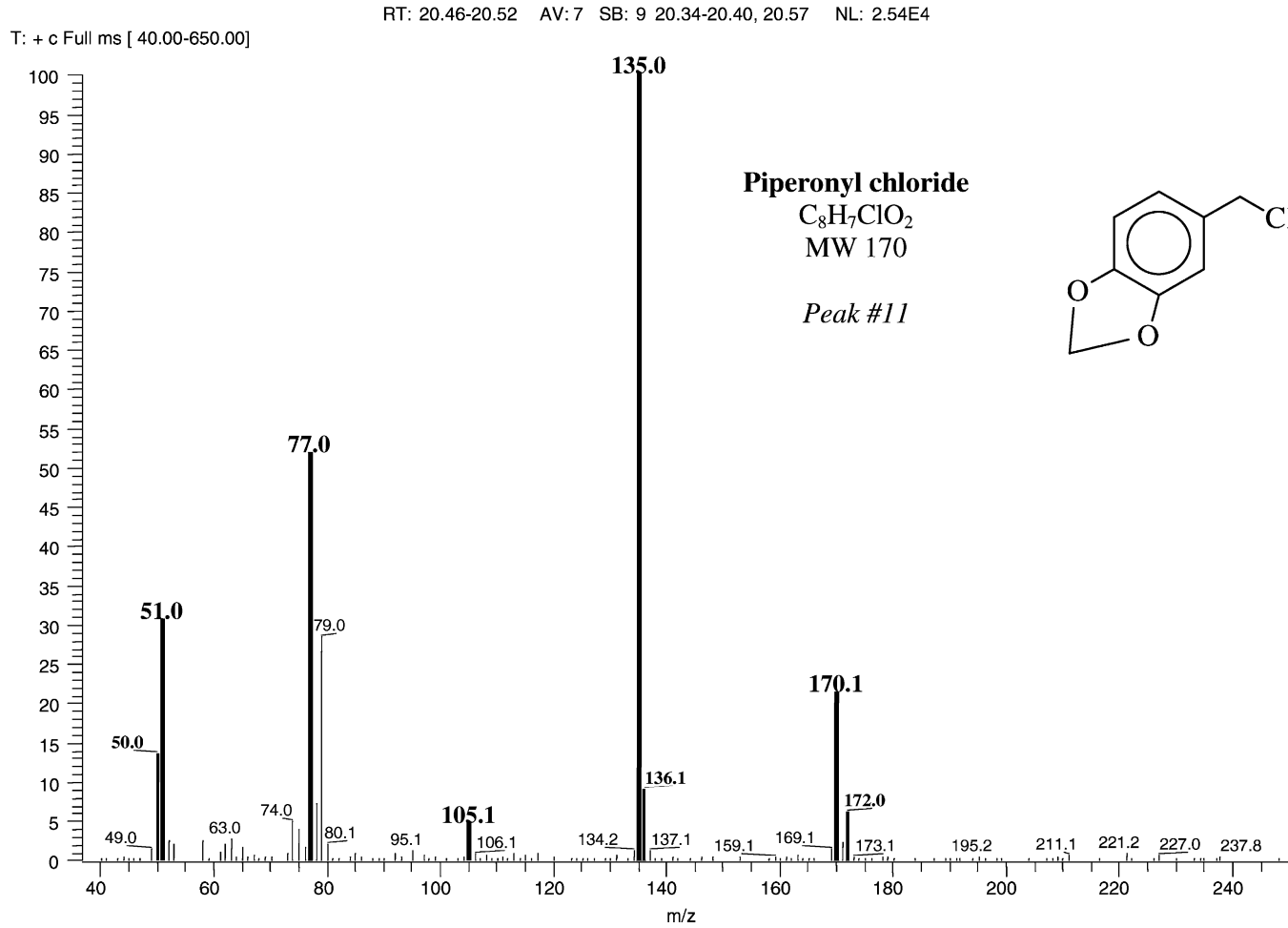


Fig. 6. (Continued).

RT: 20.71 AV: 1 SB: 21 20.61, 20.91-21.07 NL: 1.86E5

T: + c Full ms [40.00-650.00]

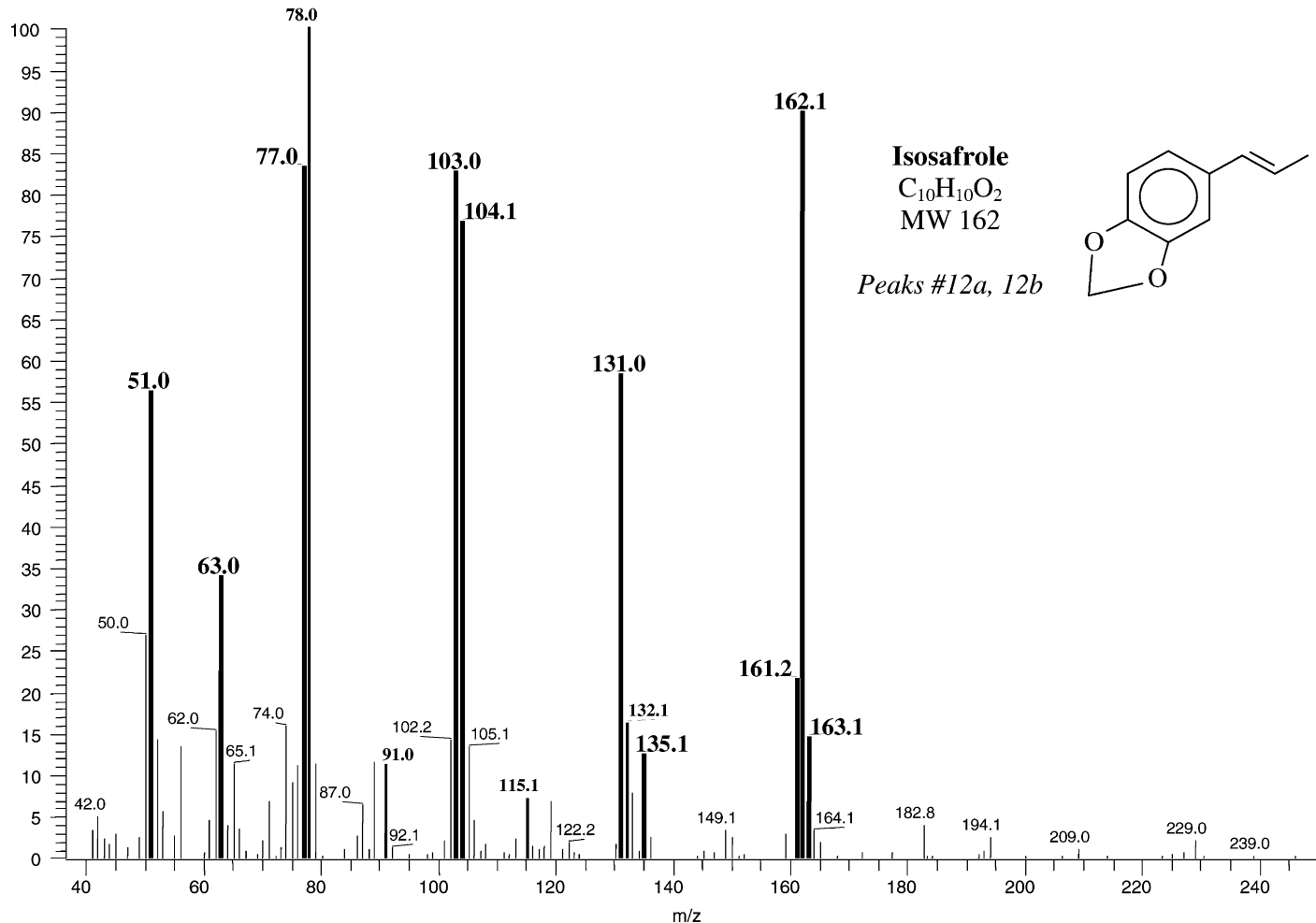


Fig. 6. (Continued).

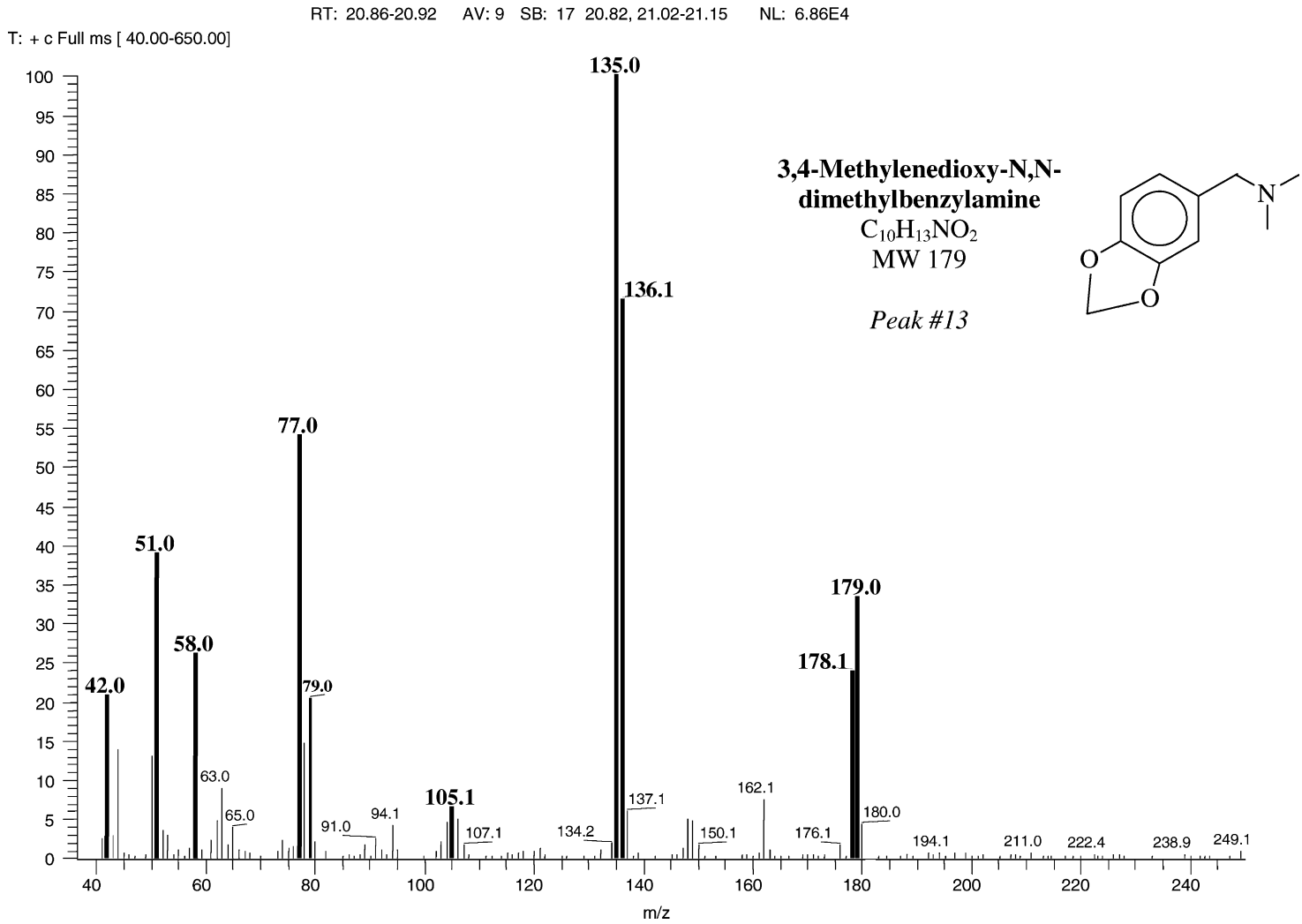


Fig. 6. (Continued).

RT: 21.34 AV: 1 SB: 45 21.11-21.24, 21.60-21.83 NL: 2.58E6

T: + c Full ms [40.00-650.00]

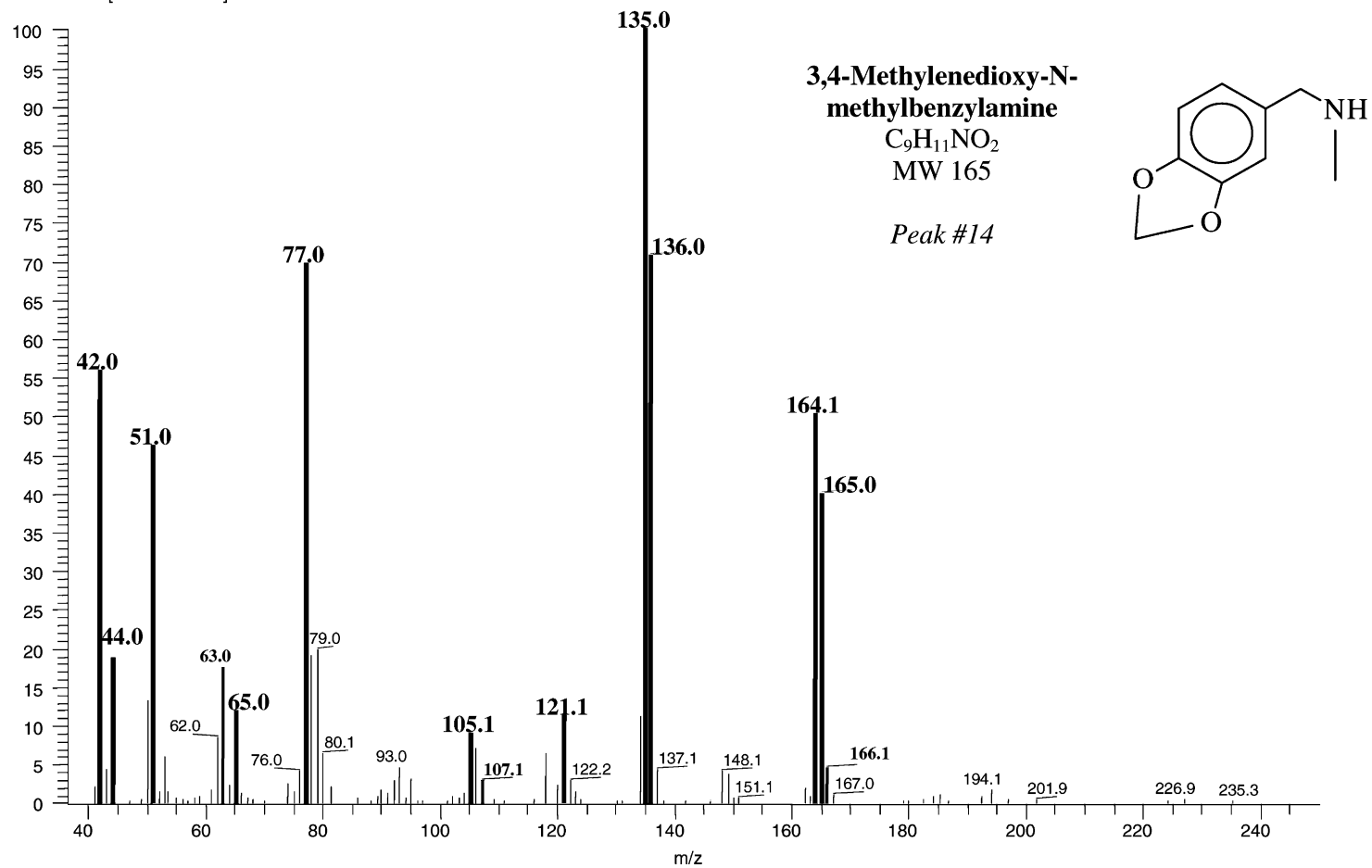


Fig. 6. (Continued).

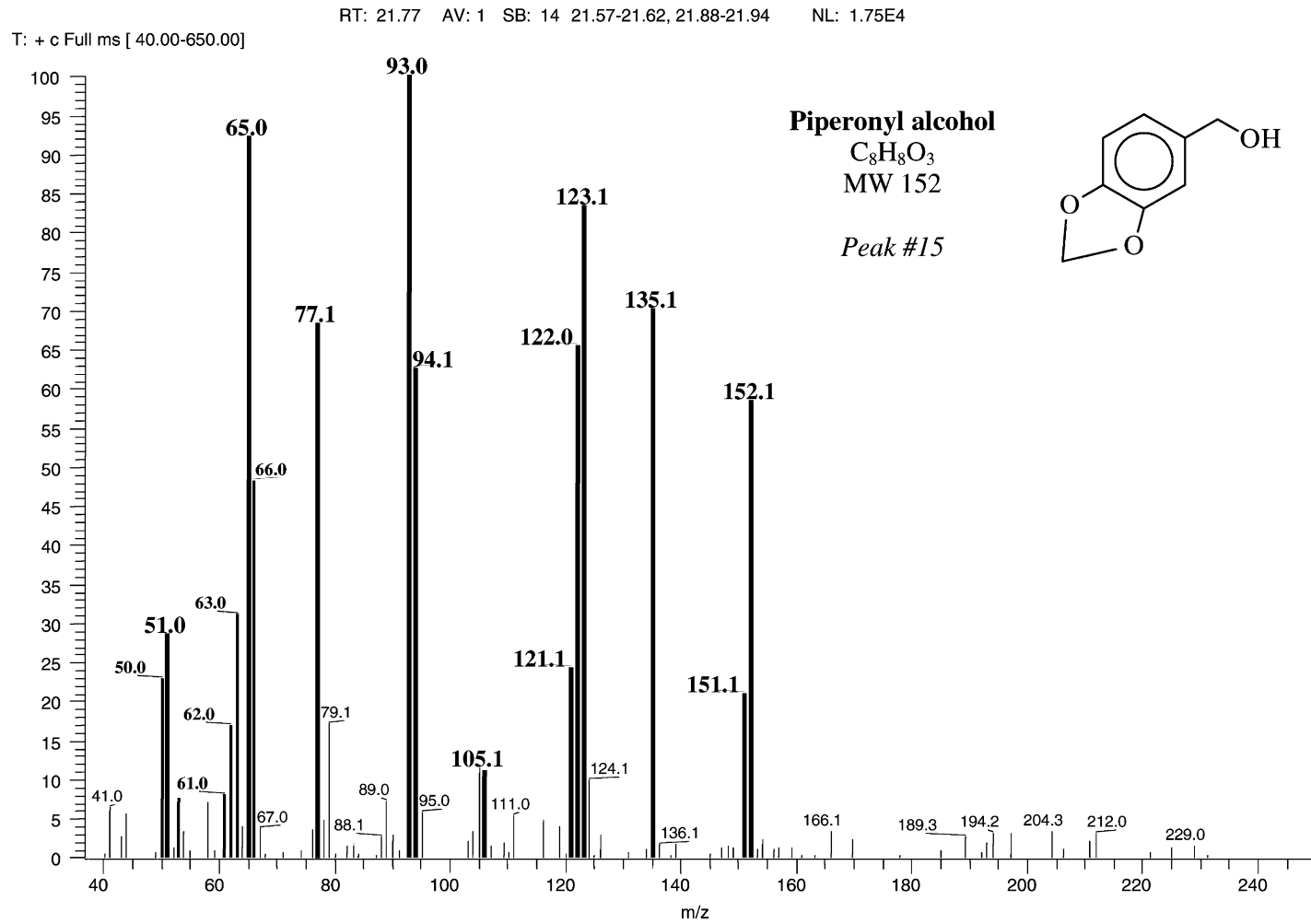


Fig. 6. (Continued).

RT: 22.50 AV: 1 SB: 20 22.25-22.35, 22.72-22.78 NL: 1.01E5

T: + c Full ms [40.00-650.00]

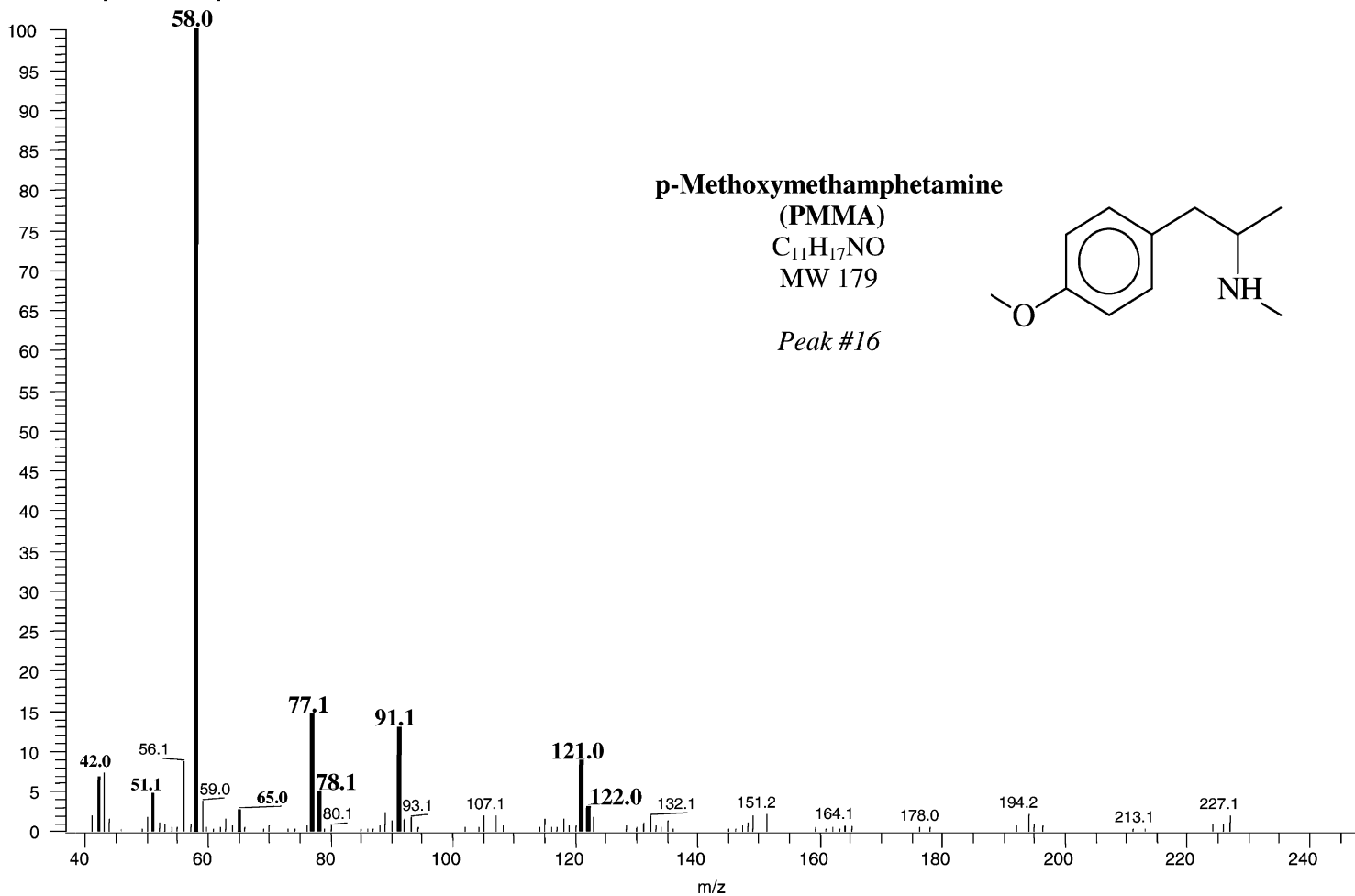


Fig. 6. (Continued).

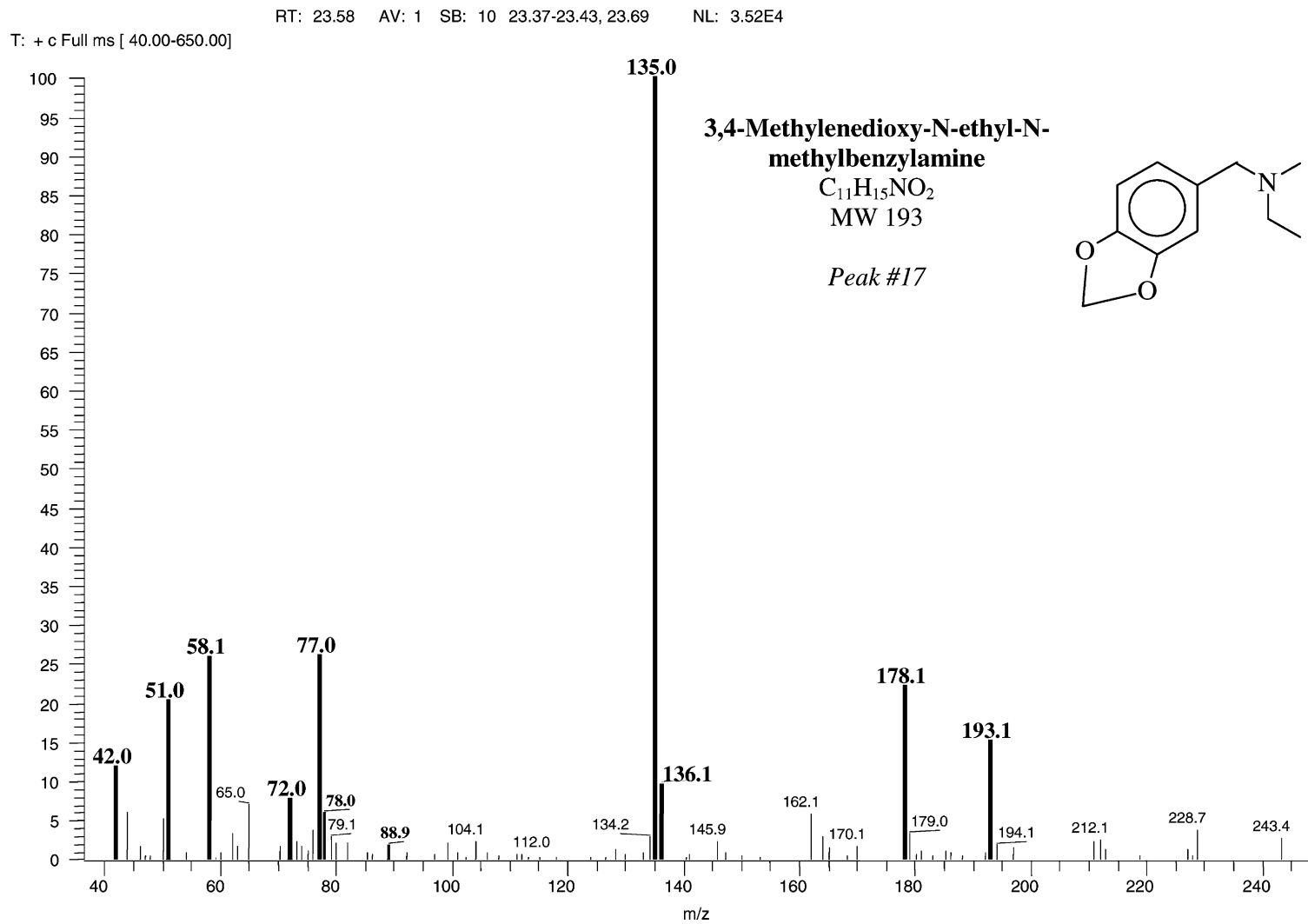


Fig. 6. (Continued).

RT: 23.94-24.18 AV: 32 SB: 9 23.70-23.77, 24.68 NL: 7.56E4

T: + c Full ms [40.00-650.00]

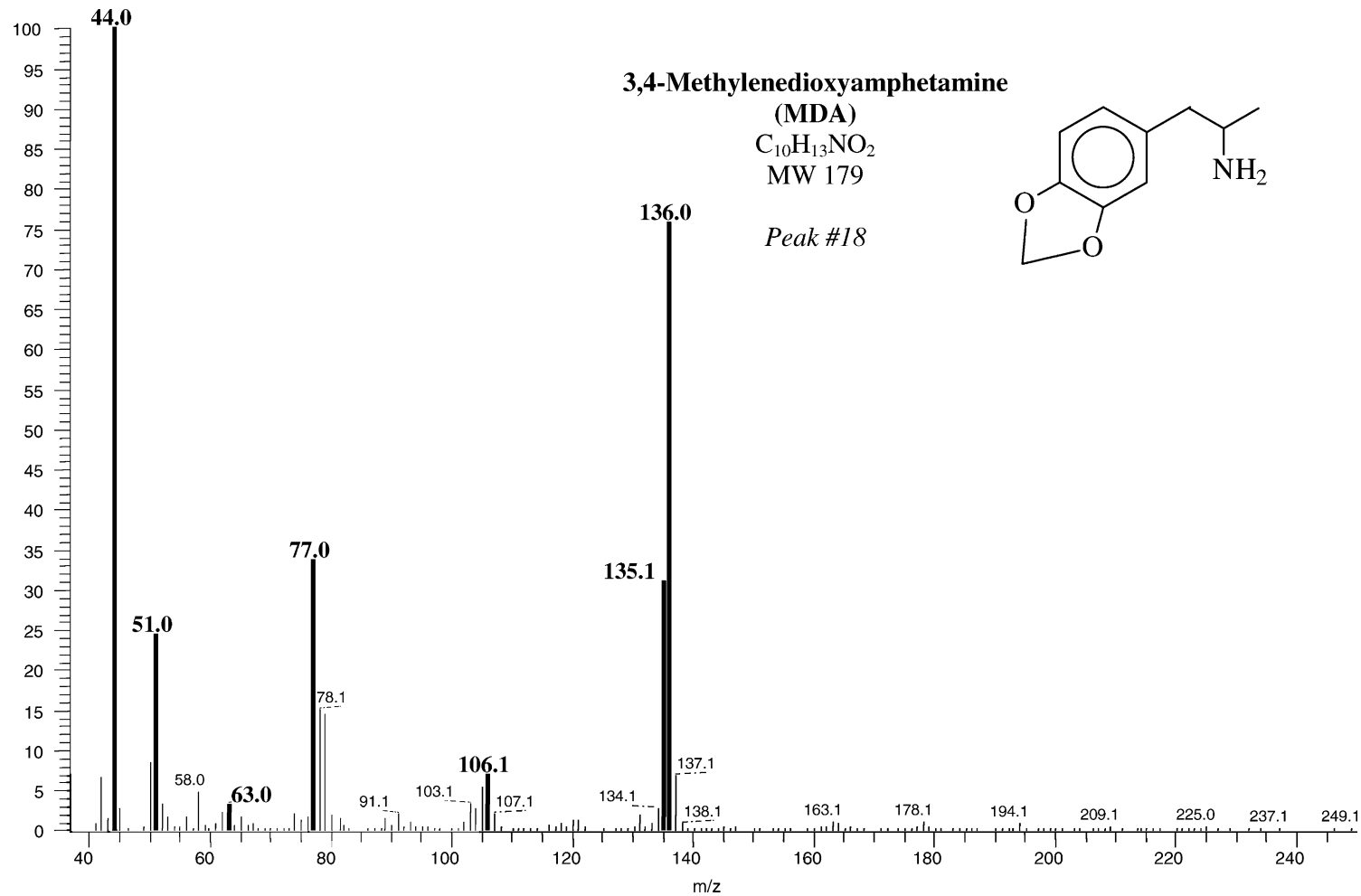


Fig. 6. (Continued).

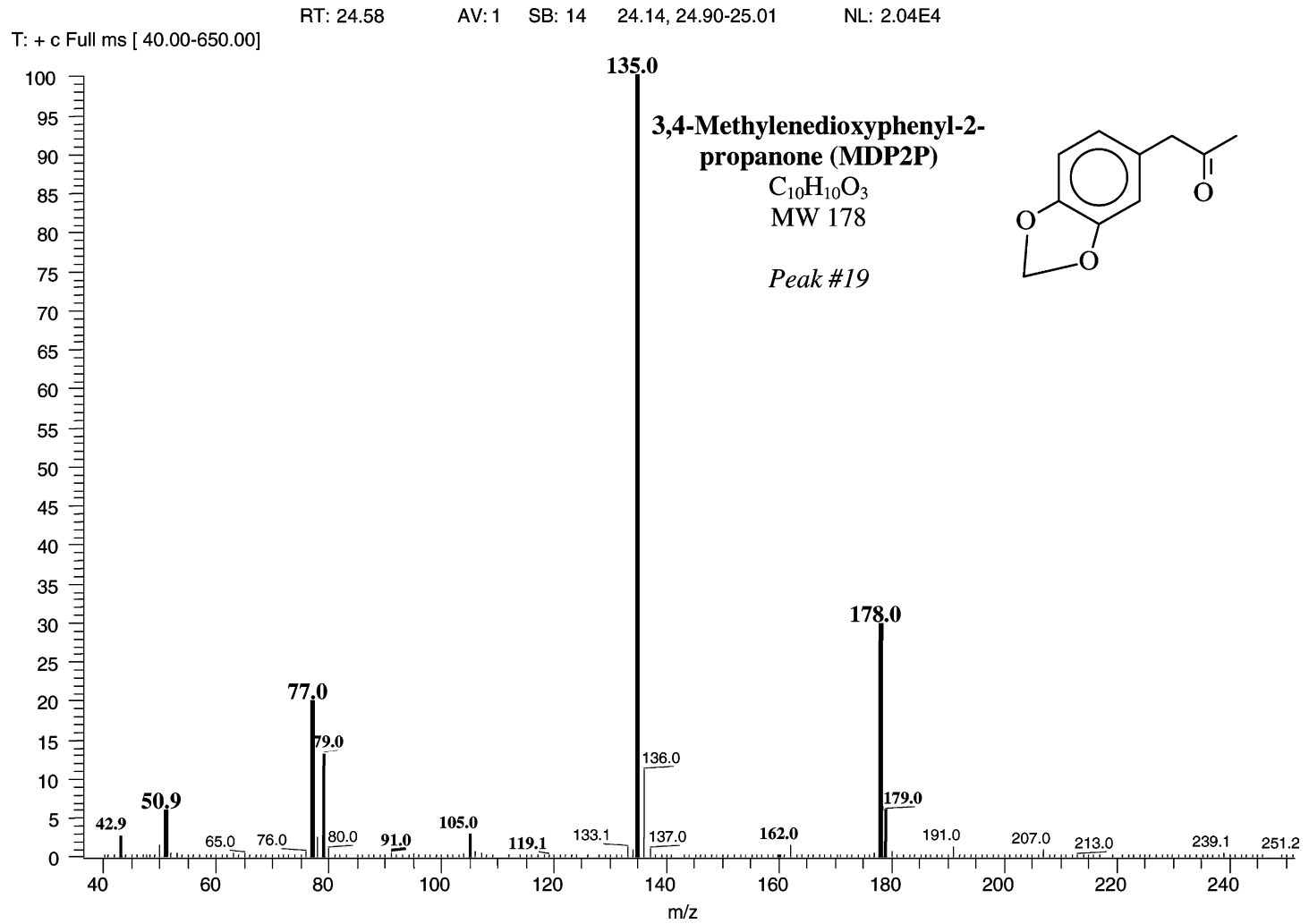


Fig. 6. (Continued).

RT: 25.97 AV: 1 SB: 28 25.74-25.88, 26.06-26.14 NL: 2.47E5

T: + c Full ms [40.00-650.00]

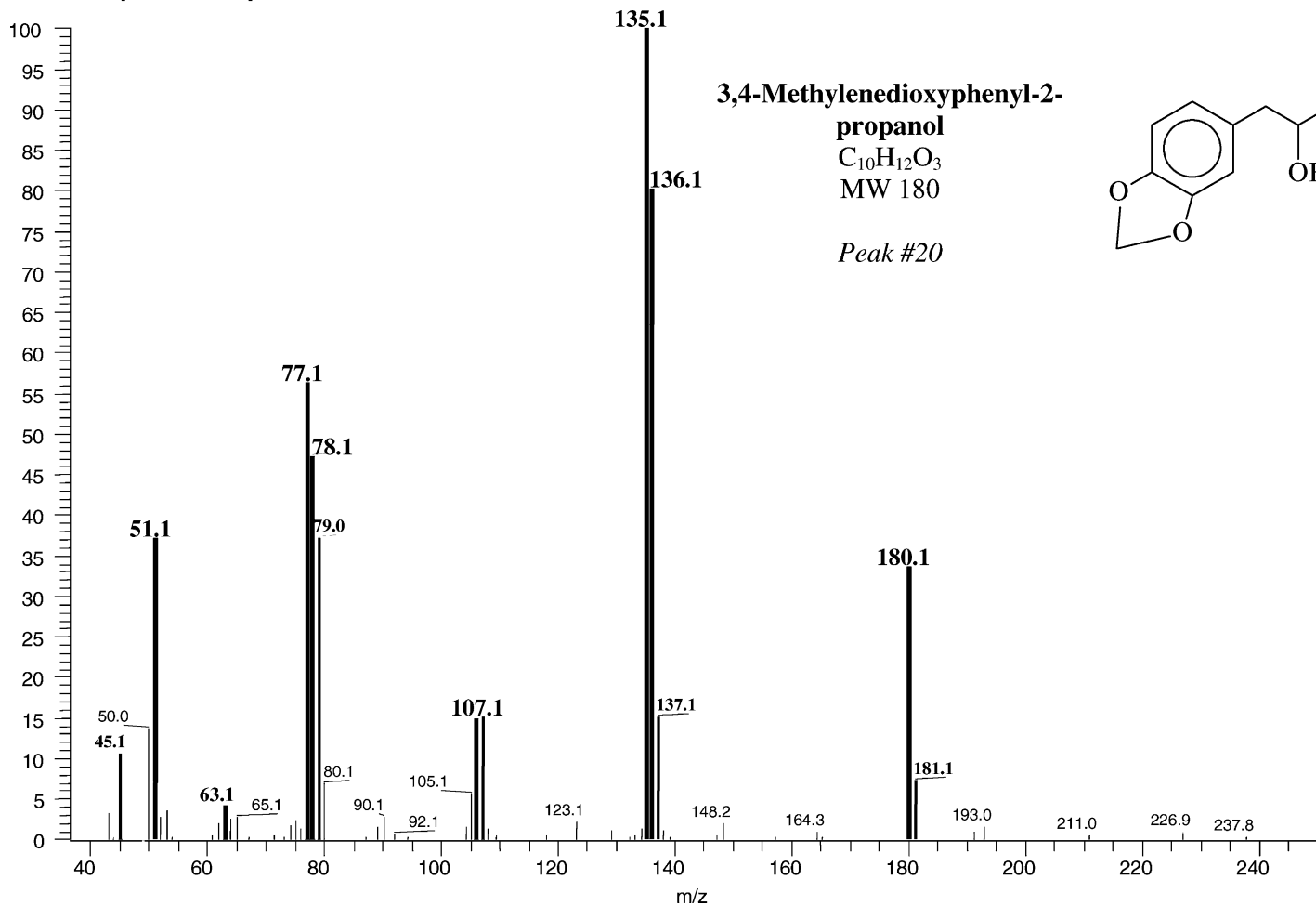


Fig. 6. (Continued).

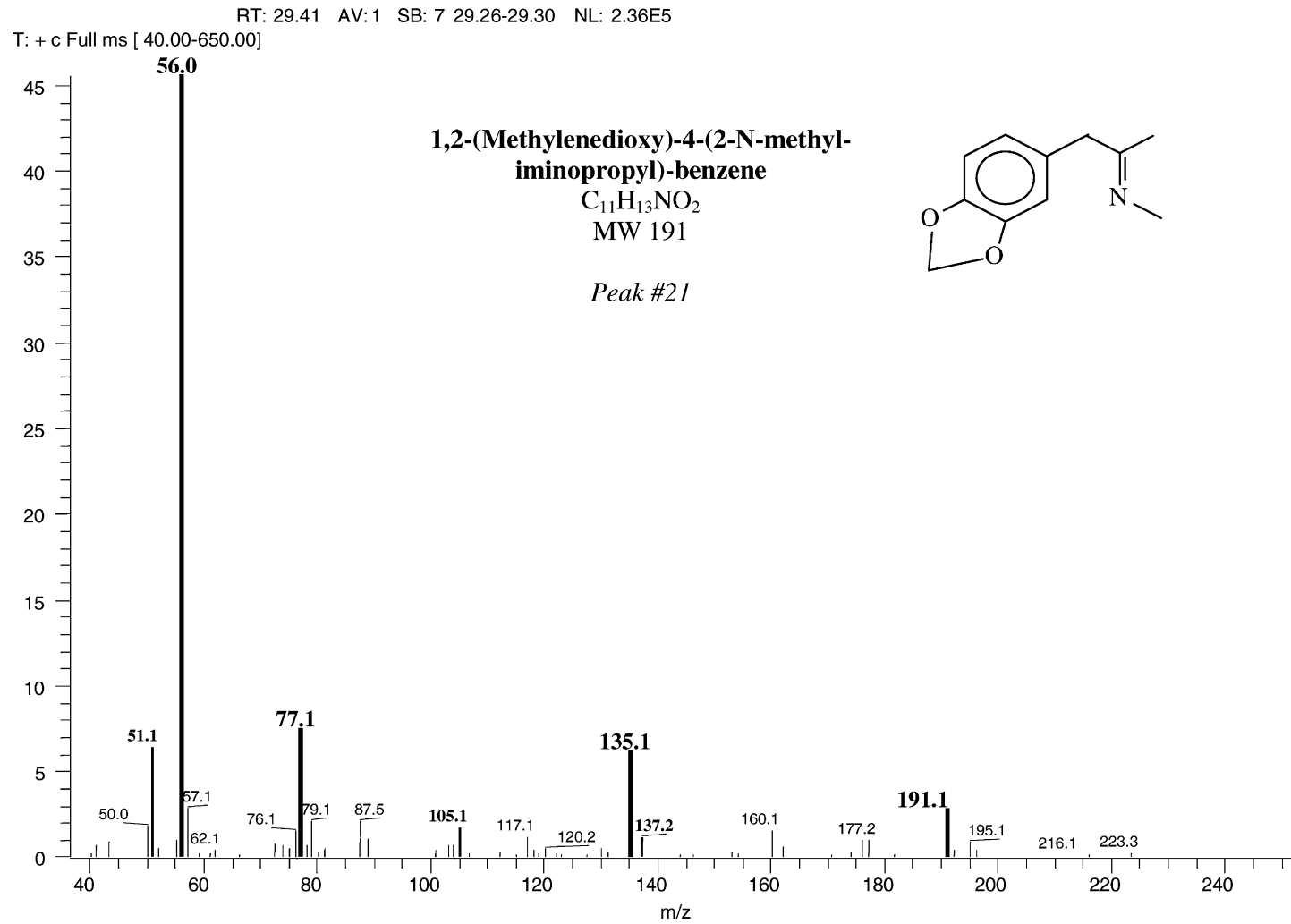


Fig. 6. (Continued).

RT: 30.56-30.75 AV: 25 SB: 87 30.30-30.53, 30.87-31.34 NL: 5.39E5

T: + c Full ms [40.00-650.00]

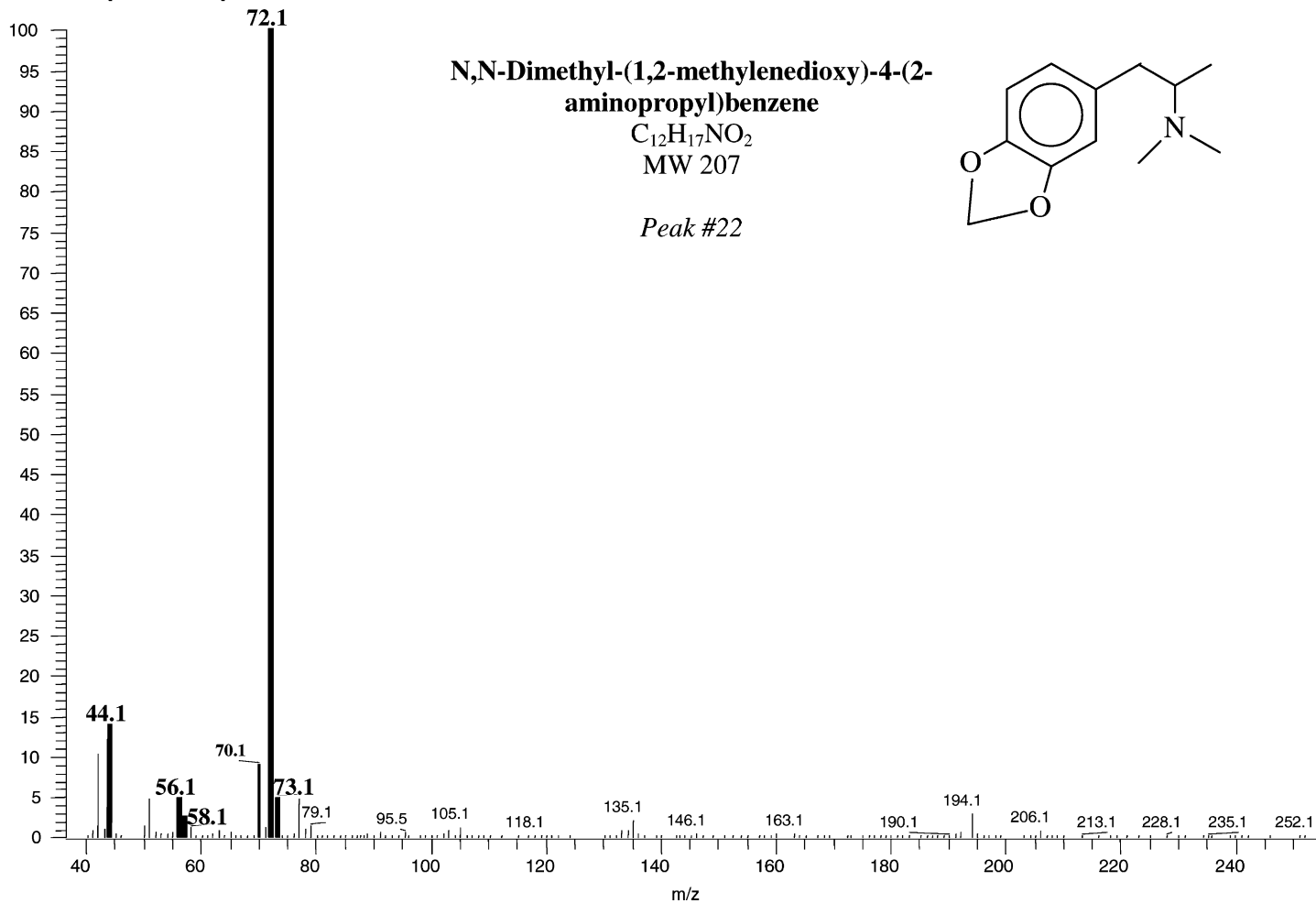


Fig. 6. (Continued).

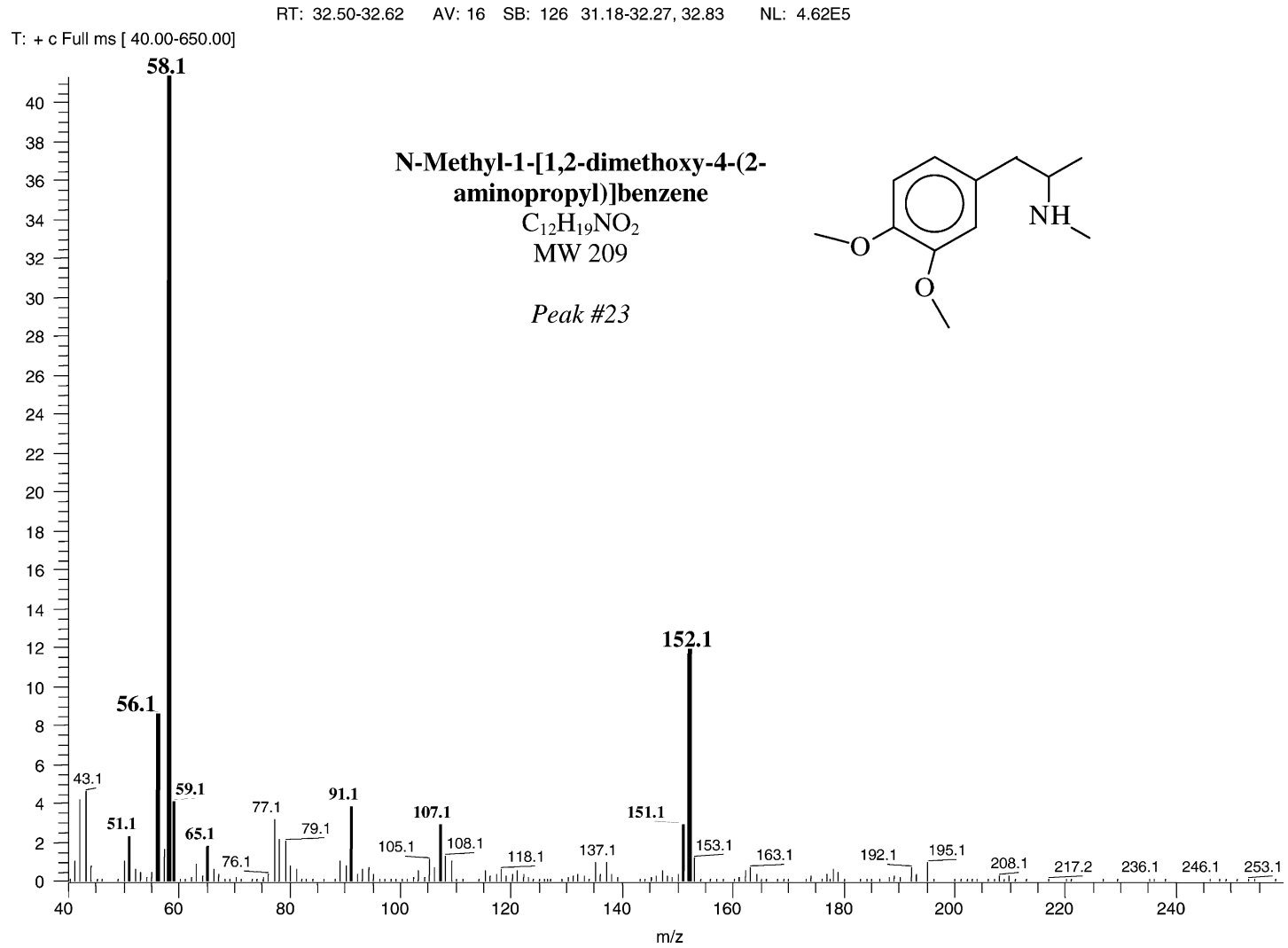


Fig. 6. (Continued).

RT: 33.97 AV: 1 SB: 71 33.53-33.91, 34.09-34.33 NL: 4.46E5

T: + c Full ms [40.00-650.00]

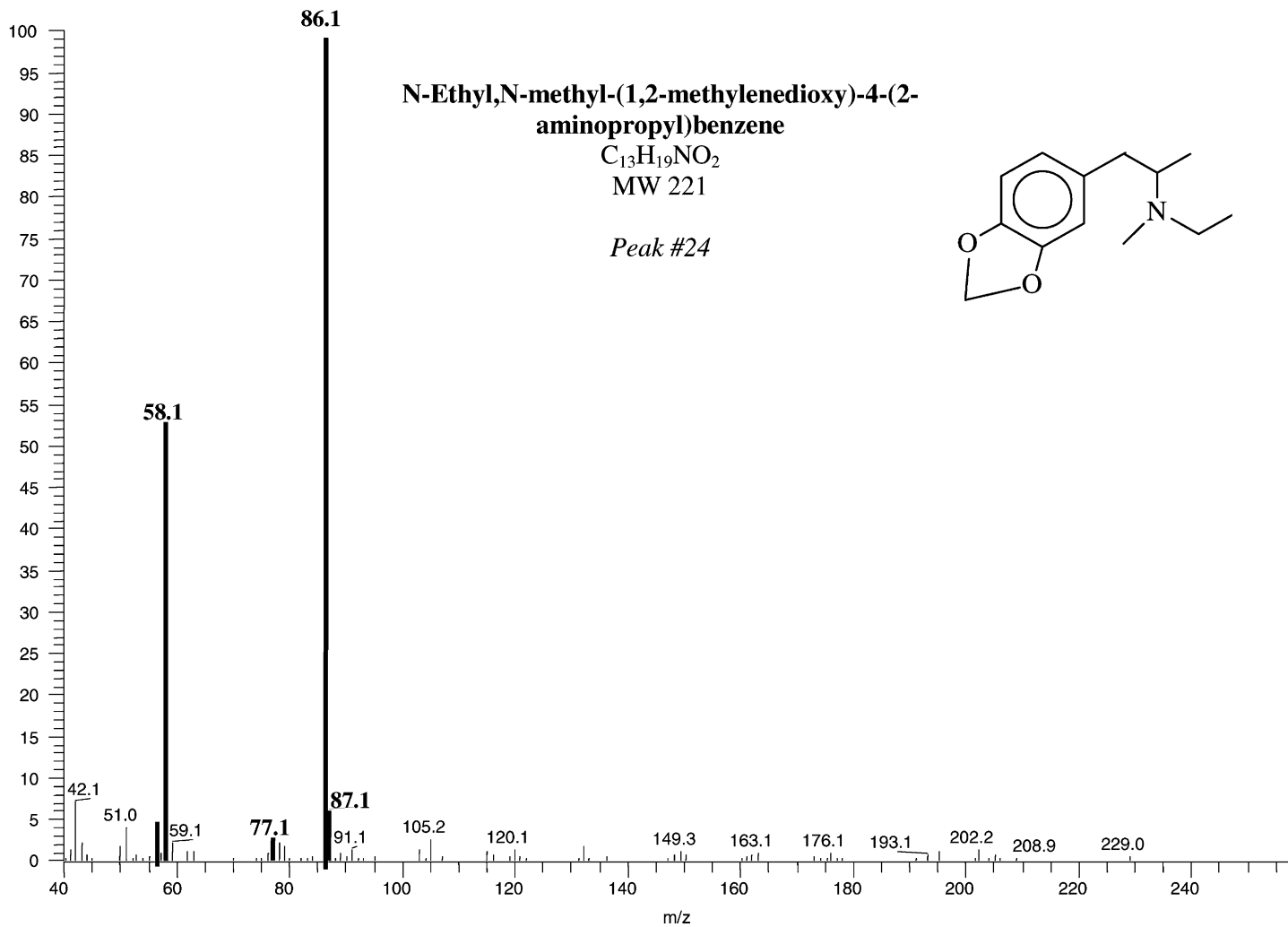


Fig. 6. (Continued).

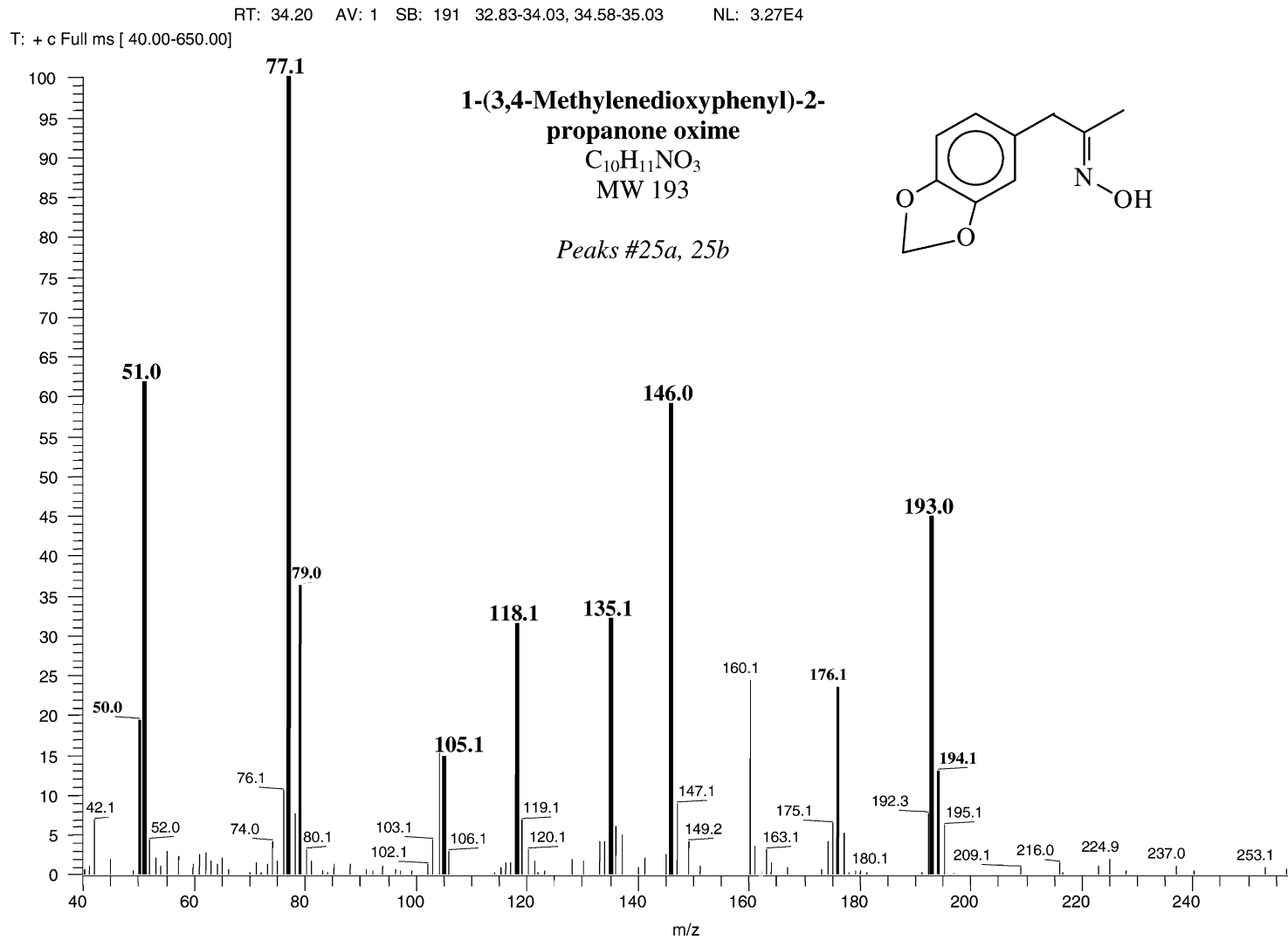


Fig. 6. (Continued).

RT: 38.93-38.96 AV: 5 SB: 77 38.22-38.89, 39.00 NL: 4.80E5

T: + c Full ms [40.00-650.00]

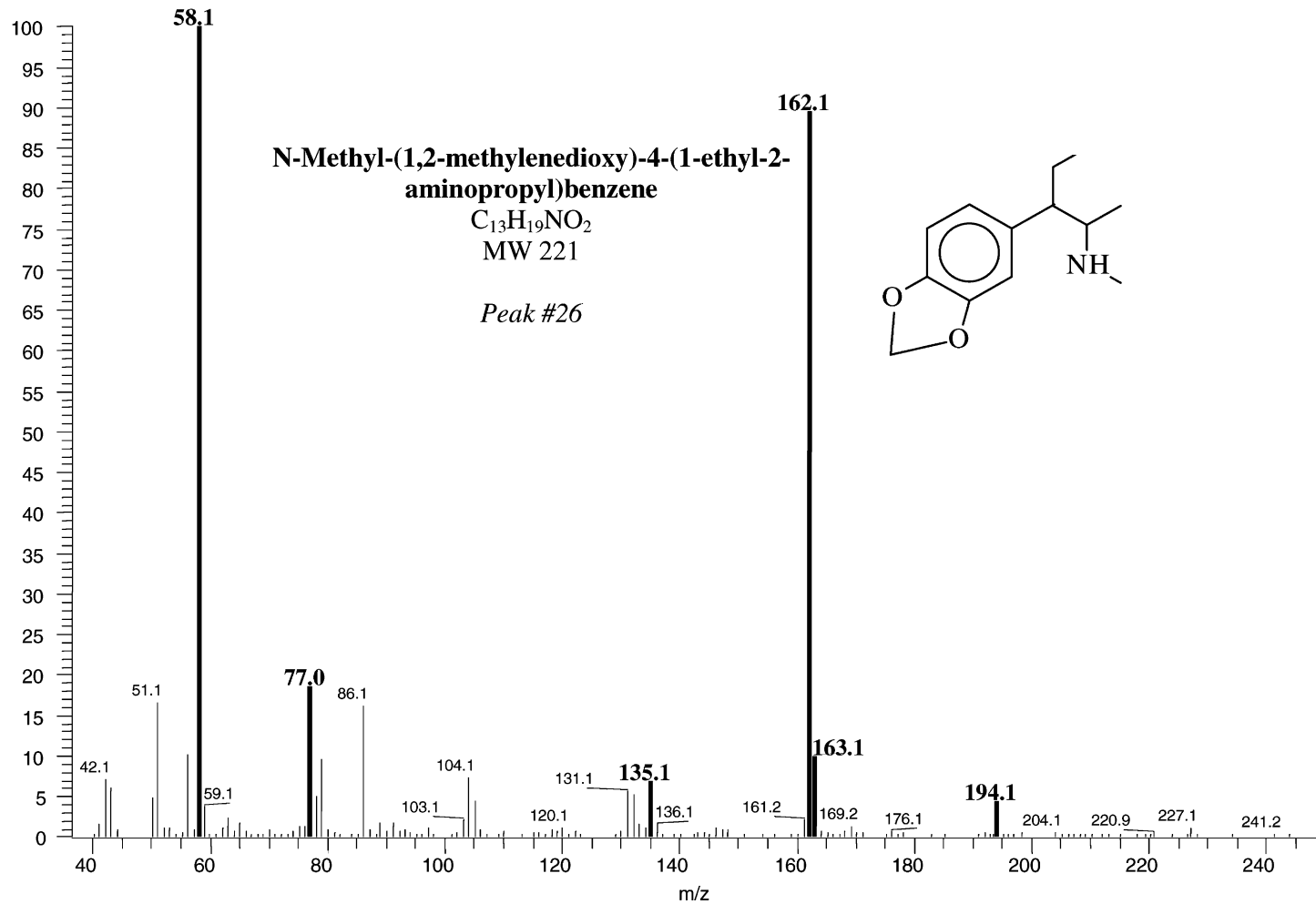


Fig. 6. (Continued).

RT: 10.12-10.14 AV:4 SB: 403 8.97-10.06, 10.34-11.93 NL: 2.71E2
F: + c SRM ms2 122.00@2.00 [40.00-250.00]

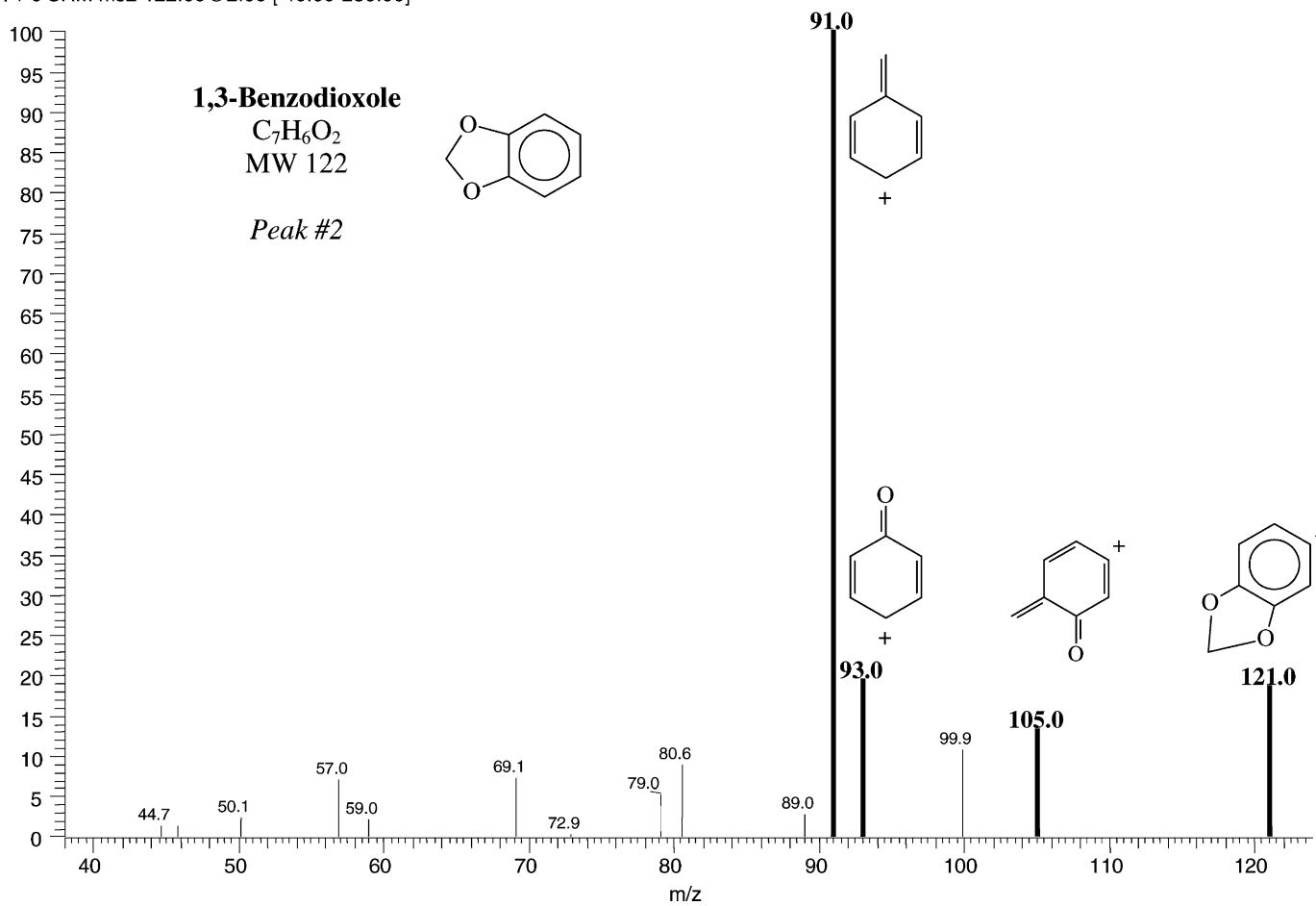


Fig. 7. Peak #2-Ci+/MS-MS confirmation on M+ molecular ion ($m/z = 122$).

RT: 20.87-20.89 AV: 3 NL: 2.77E1

T: + c SRM ms2 180.00@1.00 [40.00-250.00]

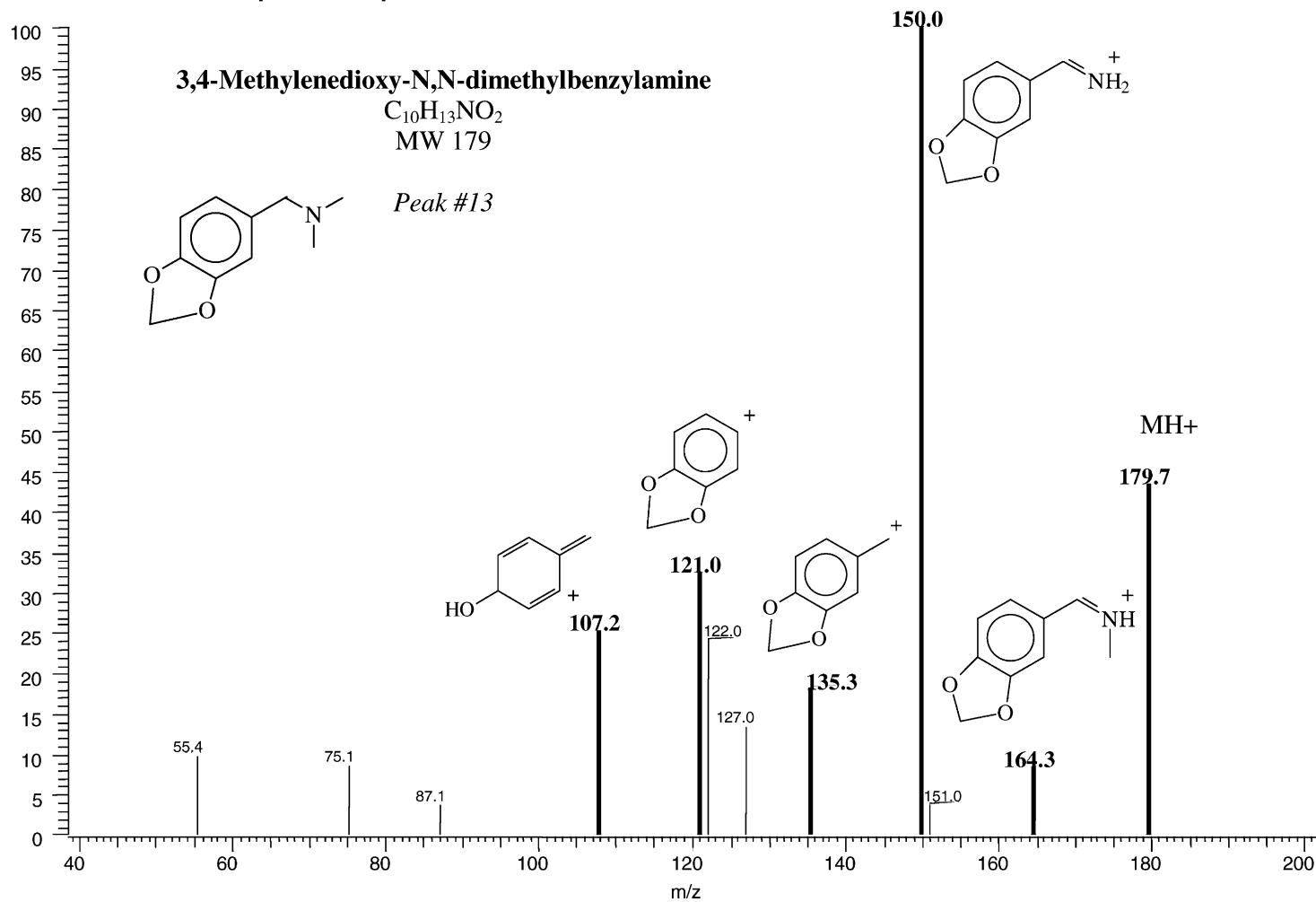
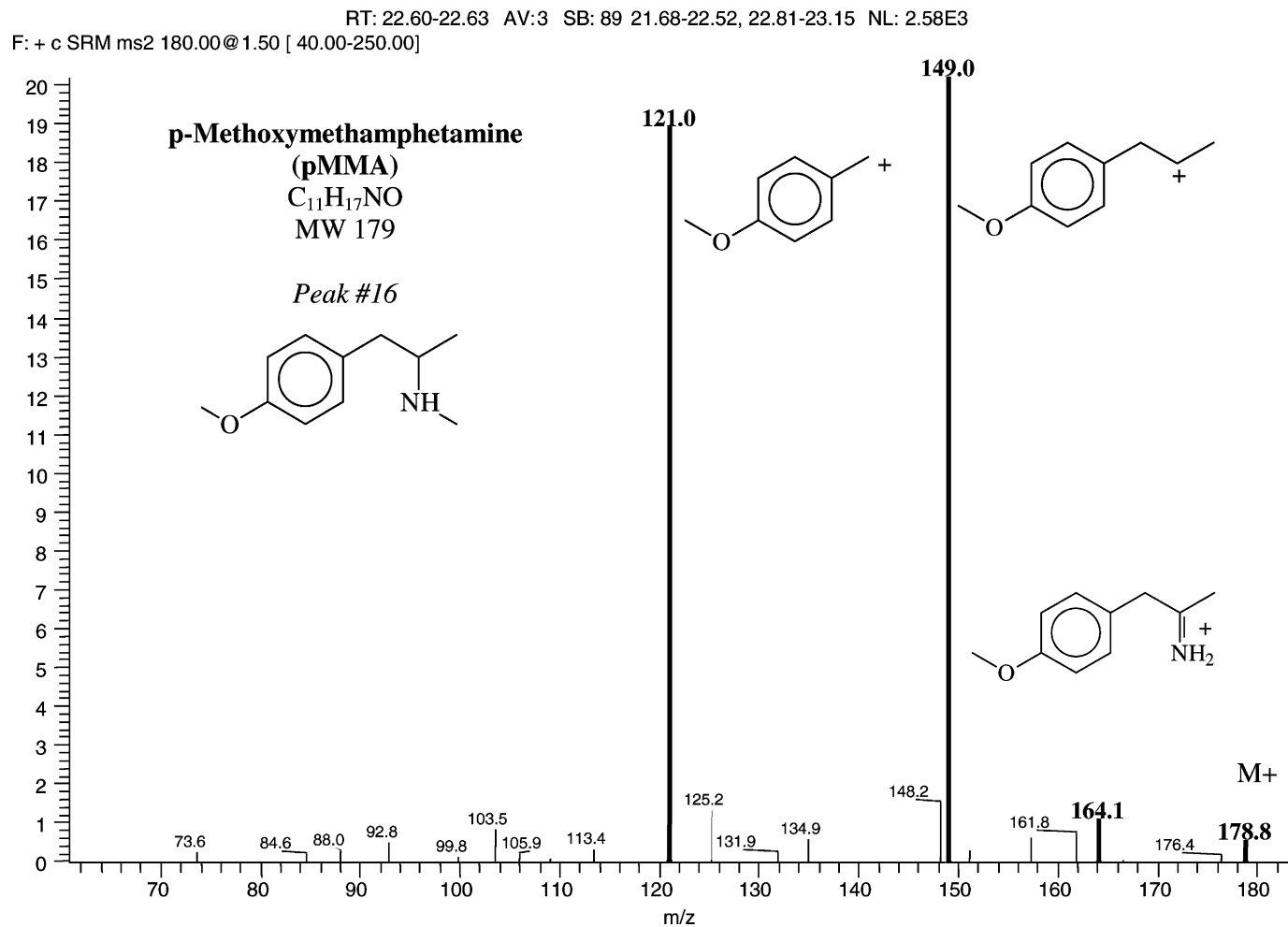


Fig. 8. Peak #13-Ci+/MS-MS confirmation on MH+ molecular ion ($m/z = 180$).

Fig. 9. Peak #16-Ci+/MS-MS confirmation on MH+ molecular ion ($m/z = 180$).

RT: 31.77-31.92 AV:23 SB: 229 30.81-31.67, 31.92-33.22 NL: 1.14E3
F: + c SRM ms2 210.00@1.00 [40.00-250.00]

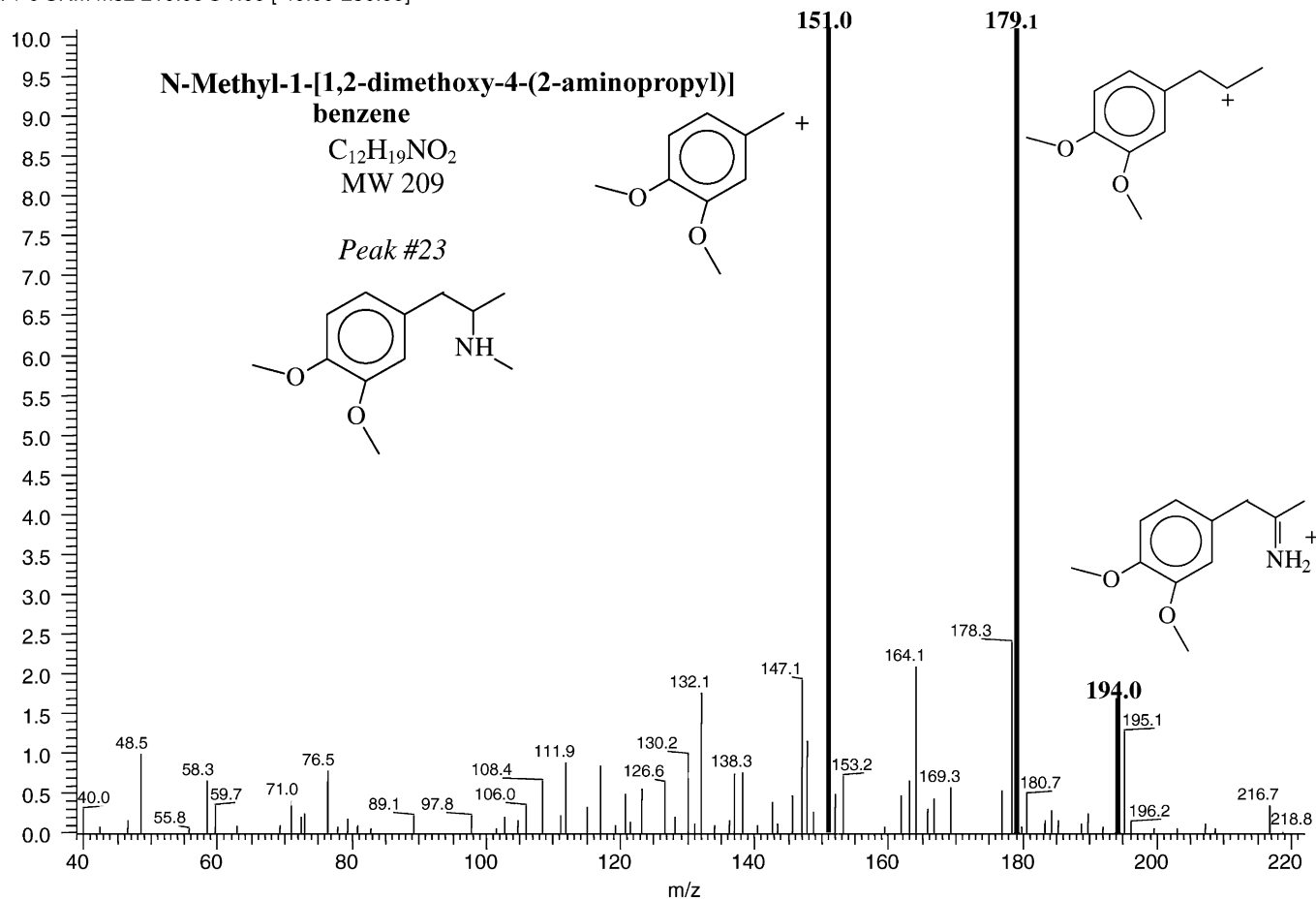
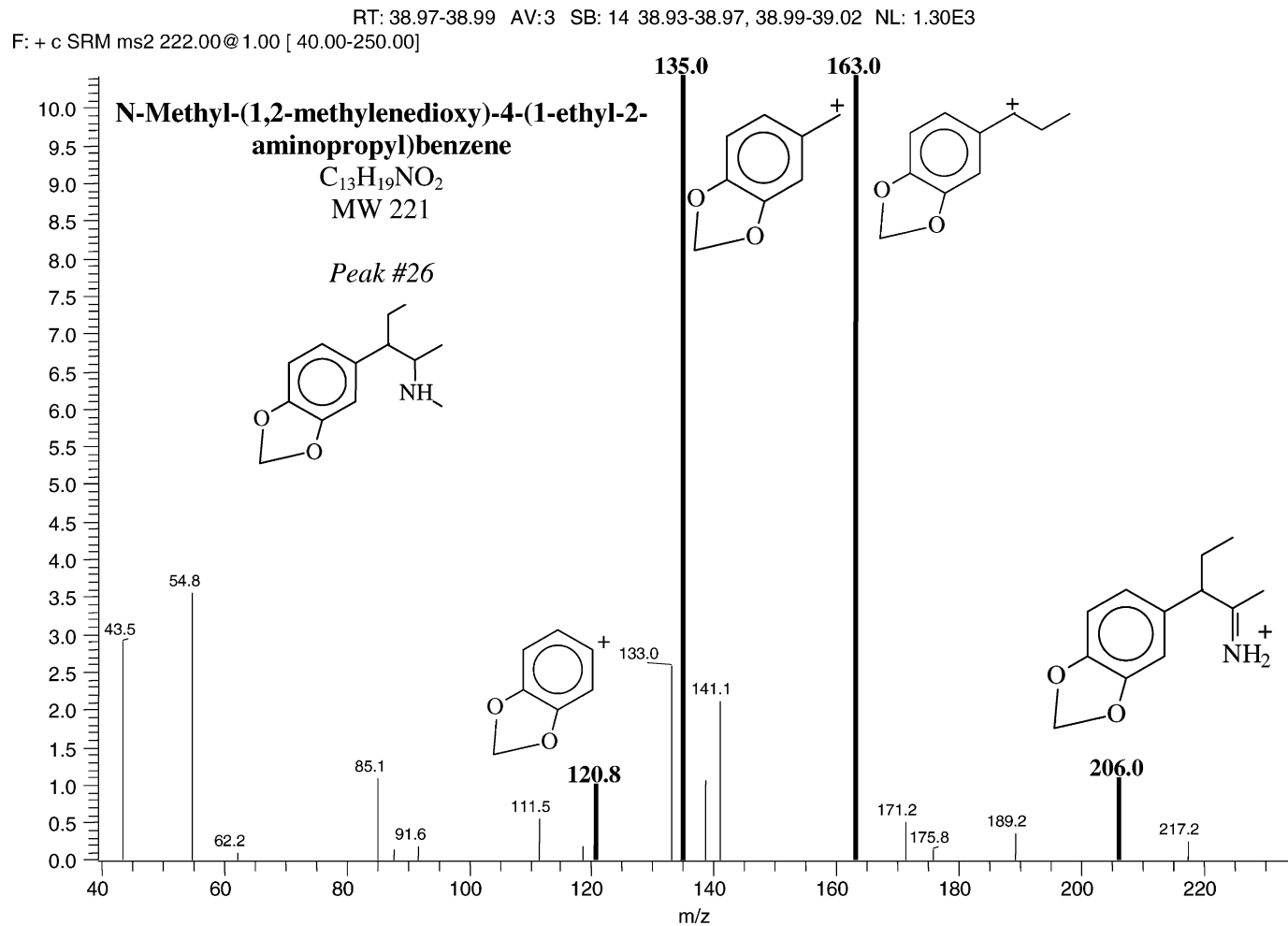


Fig. 10. Peak #23-Ci+/MS-MS confirmation on MH+ molecular ion ($m/z = 210$).

Fig. 11. Peak #26-Ci+/MS-MS confirmation on MH^+ molecular ion ($m/z = 222$).

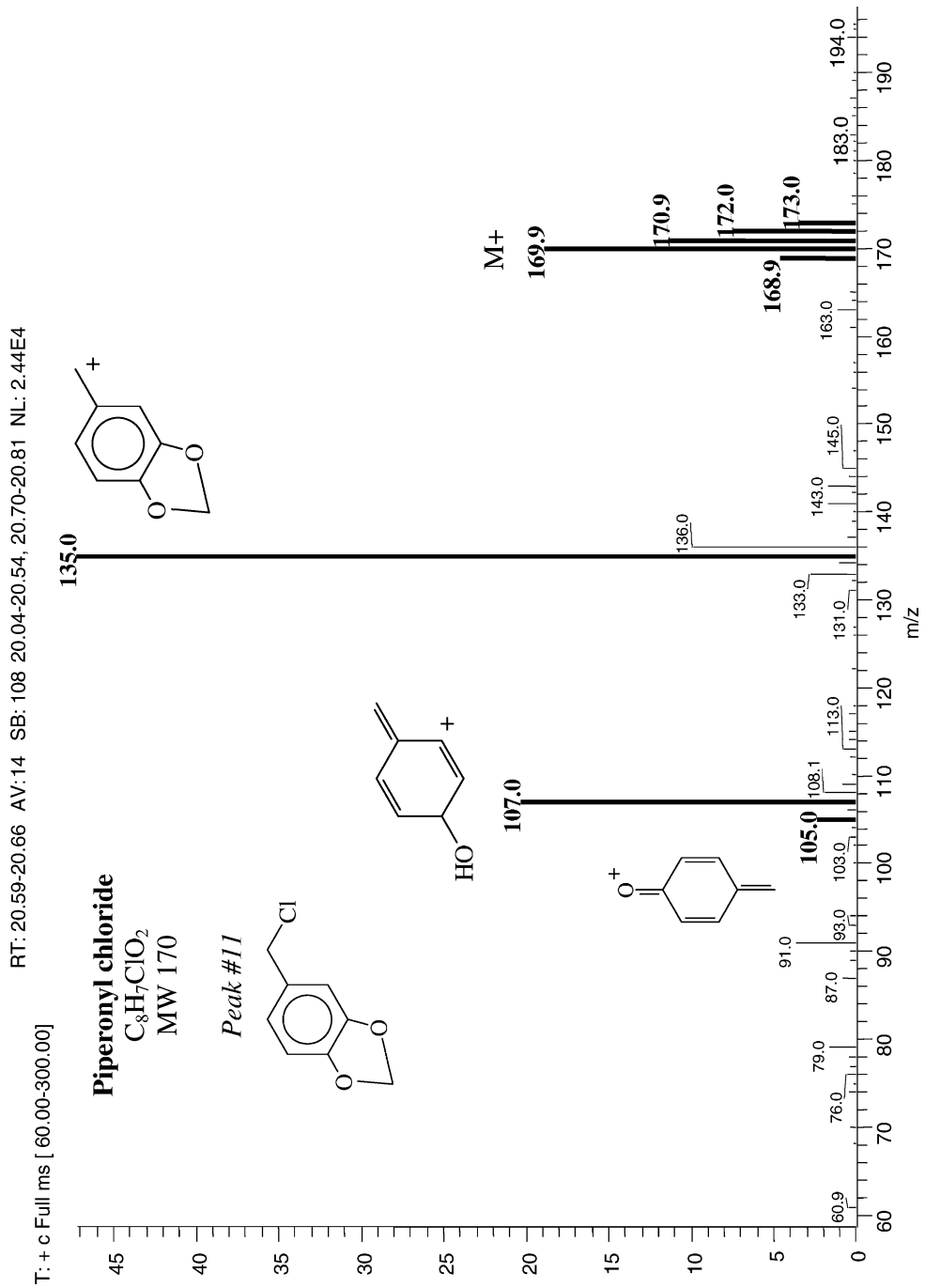


Fig. 12. Peak #11-Ci+/full scan mass spectrum.

MS–MS spectra for unusual or newly found impurities are shown in Figs. 7–11 with their fragment ions. It is important to point out that this confirmation was performed with a low $Ci+$ gas pressure in order to have enough sensitivity for the detection of each molecular ion. Therefore, it explains the presence, for some impurities, of $M+$ ion instead of $MH+$ ion. Nevertheless, MS–MS confirmation of peaks 7, 11, 12b, 17, 24, 25a, 25b, was not possible, due to the low amount of the corresponding $MH+$ (or $M+$) ion. For these impurities only $Ci+$ /full scan confirmation of the molecular ion was performed. As an example, $Ci+$ /full scan mass spectrum of impurity peak 11 with its fragment ions is given in Fig. 12.

3.3. Chemical origin for some characteristic impurities

The exact synthesis route used for the MDMA tablets seems rather difficult to determine. This difficulty is essentially due to the possibility of different origins for a same impurity, i.e. the overlap between impurities generated by different routes. For instance, MD2P2, i.e. peak 19, could be produced either from isosafrole (peaks 12a and 12b), through the intermediate isosafrole glycol [28], or piperonal (peak 9), through the intermediate β nitroisosafrole (nitropropene route) [21]. As isosafrole and piperonal are present in all samples, it is difficult to determine which one was used as a precursor, even if a specific impurity of the nitropropene route, 1-(3,4-methylenedioxyphenyl)-2-propanone oxime (peaks 25a and 25b) was identified in sample S3, pointing out the use of piperonal.

Anyhow, the primary precursor for all MDMA samples was safrole (peak 7), probably extracted from sassafras oil, as it is present in all profiles. However again, it is not possible to know exactly if isosafrole, as well as piperonal and MDP2P, was produced in the clandestine laboratory or purchased from an industrial source.

For all tablets, the key intermediate in MDMA synthesis was MDP2P, which was converted into MDMA via reductive amination (Fig. 13). Thus, 1,2-(methylenedioxy)-4-(2-*N*-methyliminopropyl)-benzene (peak 21) is the result of MDP2P amination [21,23,34]. Moreover, several impurities are MDMA by-products could be obtained via reductive amination of MDP2P and piperonal with trace level impurities present in methylamine (peaks 13, 17, 18, 22, 24), (Figs. 14 and 15). Reductive amination of piperonal with methylamine was also observed giving 3,4-methylenedioxy-*N*-methylbenzylamine (peak 14; Fig. 15).

Finally, *p*-methoxymethamphetamine (PMMA; peak 16) seems to be a by-product of MDMA synthesis. As a matter of fact, PMMA and *N*-methyl-1-[1,2-dimethoxy-4-(2-aminopropyl)]benzene (peak 23) are both end-products of trace level impurities present in safrole and which were modified during the whole process (Fig. 16). On the contrary, the presence of peaks 1, 4, 5, 6 and 10 in sample S1 is linked to methamphetamine contamination. Such amphetamine or methamphetamine contamination, which was also detected

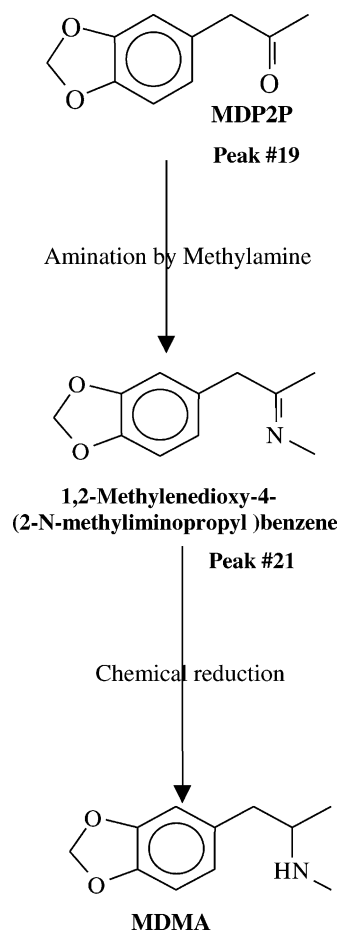


Fig. 13. MDMA synthesis from MDP2P by reductive amination.

in other MDMA samples analyzed in our laboratory, is probably due to fast and/or bad cleaning of the glassware.

3.4. Overall reproducibility of the method

Results were expressed giving relative standard deviation (R.S.D.) of each peak area, acquired with SIM mode and after normalization, i.e. dividing all areas in a run by the peak sum. Peaks used for this reproducibility study of samples Ref1 and Ref2 are peaks #2, 3, 8, 9, 11, 12a, 14, 15, 16, 21, 22 and 26. The same peaks were used for the profiles comparison. It is important to point out that the chromatographic separation from surrounding peaks was good, thanks to the SIM acquisition mode, and that the highest values obtained depend more on the low amount of the corresponding impurities.

3.4.1. Gas chromatography repeatability

Five injections of the same extract from sample Ref1 gave a minimum R.S.D. of 1.3% to a maximum of 10.9%, the mean value being 5.7%. The same study on sample Ref2

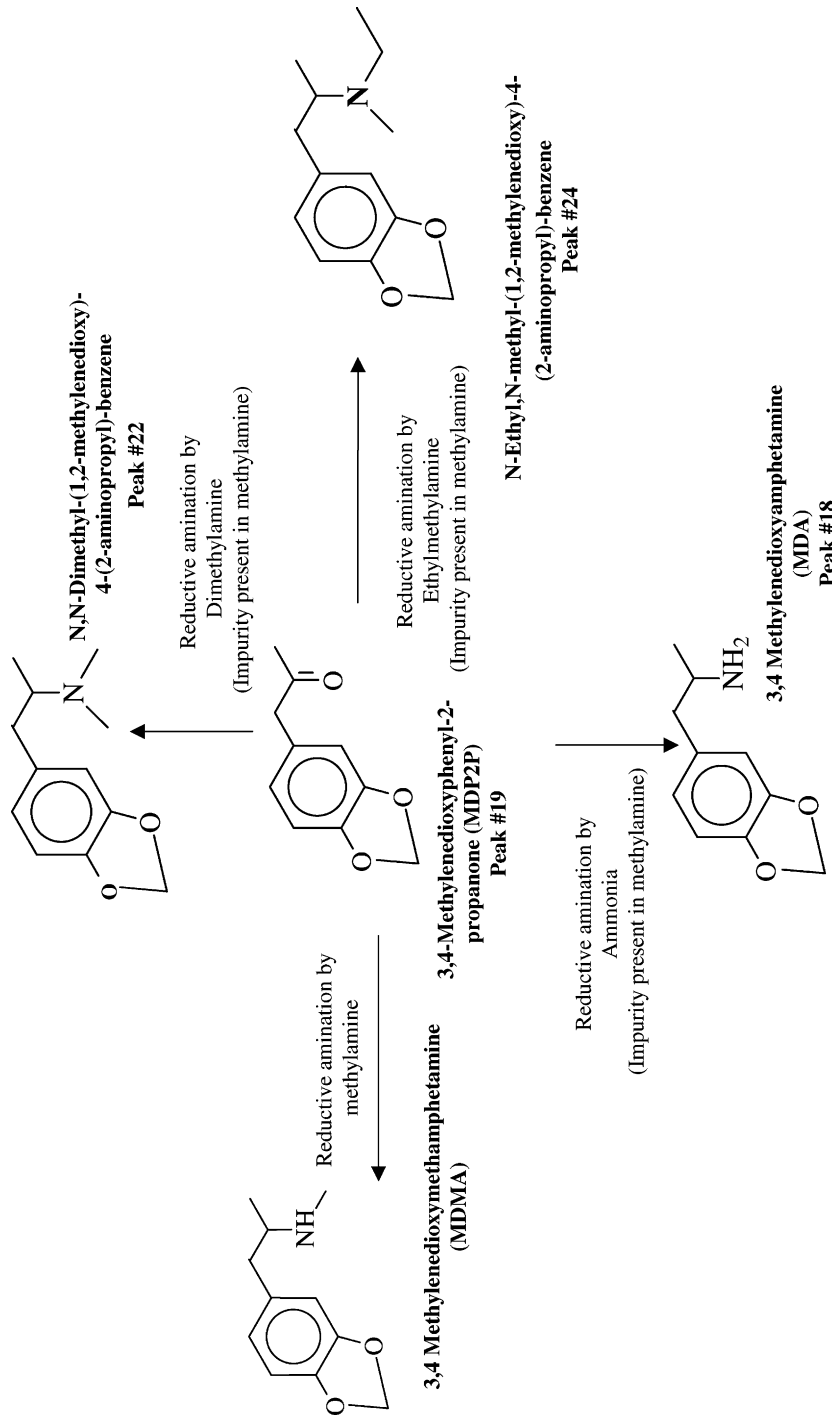


Fig. 14. MDMA by-products obtained from MDP2P.

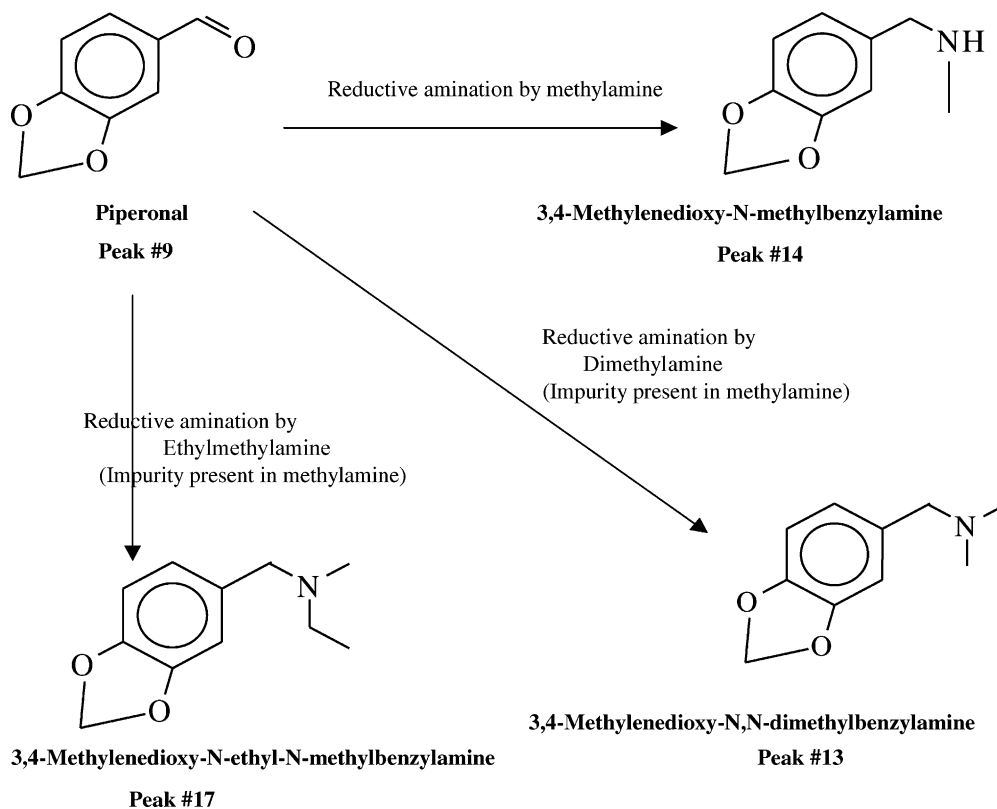


Fig. 15. MDMA by-products obtained from piperonal.

gave a minimum R.S.D. of 3.9% to a maximum of 12.2%, the mean value being 7.6%.

3.4.2. Overall reproducibility (extraction and gas chromatography)

3.4.2.1. Within batch reproducibility. Five different extractions were made from samples Ref1 and Ref2 and analyzed the same day. The R.S.D. for Ref1 sample varied from 1.8 to 13.0% with an average of 7.5%, and similar results were obtained for Ref2 sample with a minimum R.S.D. of 2.9% to a maximum of 15.1% and an average

of 7.3%. Comparing these average values with the ones obtained in the study of GC repeatability shows that the overall variation is linked to the chromatographic step and almost not to the extraction one (Table 3).

3.4.2.2. Between batch reproducibility. Three extractions were made every day from samples Ref1 and Ref2 during four consecutive days. If we consider sample Ref1, the R.S.D. varied from 6.9 to 25.6% depending on the impurity, with an average value of 13.6%. For Ref2 sample, values varied from 6.6 to 20.9% with an average of 13.4%. Between batch reproducibility shows an increase

Table 3

Average relative standard deviation calculated for 12 peaks (2, 3, 8, 9, 11, 12a, 14, 15, 16, 21, 22 and 26) from samples Ref1 and Ref2. Normalization was made with the sum of peak areas acquired with SIM mode

Sample	Analysis	Average of relative standard deviation after normalization (%)
Ref1	Five extractions analyzed the same day	7.5
	Three extractions by day during 4 consecutive days	13.6
Ref2	Five extractions analyzed the same day	7.3
	Three extractions by day during 4 consecutive days	13.4

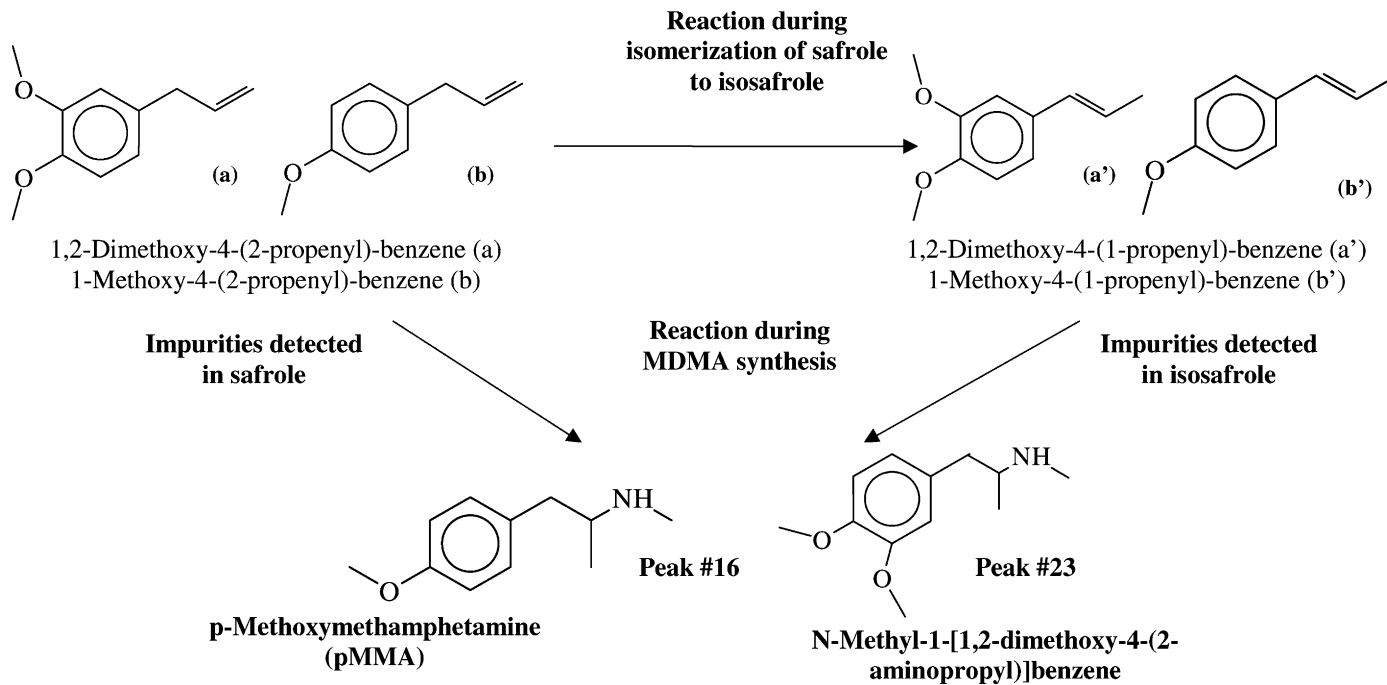


Fig. 16. Safrole/isosafrole impurities modified during MDMA synthesis.

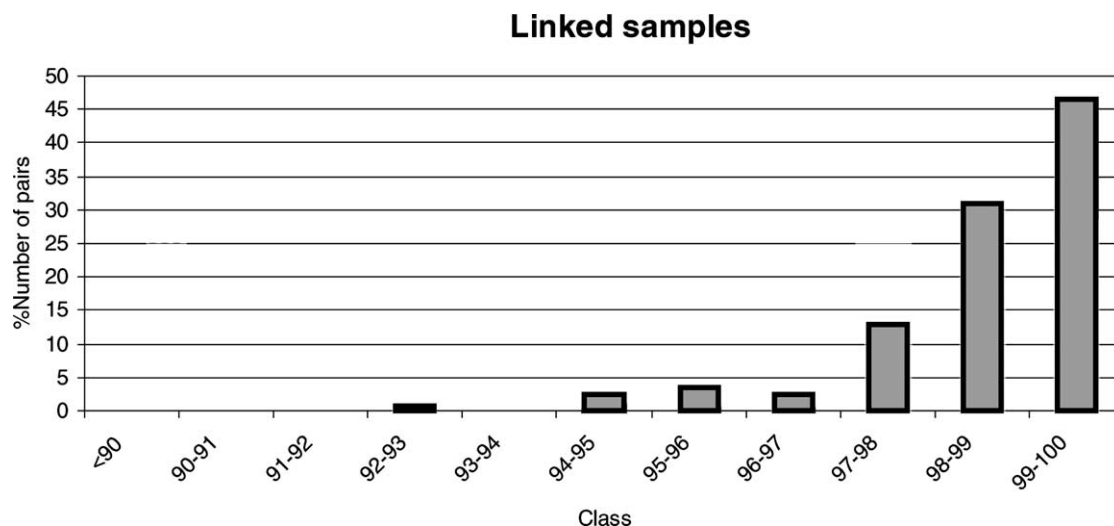


Fig. 17. Cosine function values for linked samples.

of R.S.D. Some parameters need to be optimized, such as the use of diethylether as extraction solvent.

4. Impurity profiles comparison for nine different MDMA tablets

In addition to samples Ref1, Ref2, S2 and S3, the profiling method was applied to five other MDMA samples (S4–S8). Sample S1 was not considered during this study due to the presence of methamphetamine impurities masking some target peaks. In order to compare the chromatographic profiles, the cosine function was used [37]. The cosine function is a statistical tool which can be used to compare two chromatograms. A chromatogram could be assimilated to a vector with n components, each one corresponding to the area of an impurity peak.

The correlation value C is defined by the following equation:

$$C = 100 \times \frac{\left[\sum_{i=1}^{12} A_i B_i \right]^2}{\sum_{i=1}^{12} A_i^2 \sum_{i=1}^{12} B_i^2}$$

Table 4

Cosine function values for unlinked samples

Sample	Ref2	S2	S3	S4	S5	S6	S7	S8
Ref1	49.5	72.7	84.8	60.9	34.3	26.7	48.1	46.0
Ref2	#	41.7	76.5	87.3	62.1	53.3	89.2	90.2
S2	#	#	82.6	42.8	62.8	28.1	39.8	43.4
S3	#	#	#	75.1	60.8	39.4	72.2	76.6
S4	#	#	#	#	49.2	73.8	95.1	81.5
S5	#	#	#	#	#	49.1	51.6	53.0
S6	#	#	#	#	#	#	70.8	45.8
S7	#	#	#	#	#	#	#	92.0

A_i and B_i are the respective area of peak i in chromatograms A and B . C is a number with no unit, varying between 0 and 100, self-normalizing and independent of vector length (sample size).

The cosine function values were first calculated for linked samples (overall reproducibility study) and plotted in Fig. 17. This histogram shows that the correlation values vary from 92.6 to 99.9, more than 75% of the values being above 98. The other 36 values in Table 4 were then calculated for each between-seizures (unlinked samples) comparison. These values range from 26.7 to 95.1, more than 90% of the values being below 90.

Therefore, there is an overlap of values between samples that are known to match and samples that are known to be from different sources. Establishing a particular correlation value, for instance 98, above which an unknown comparison could be unequivocally identified as a match seems to be too early. It is necessary to apply the profiling method to more MDMA samples to fix a threshold. Nevertheless, addition of peaks #20, 23, 24, which were not considered in this study, to the calculation of the cosine function will certainly improve the discrimination.

5. Conclusion

This study proposed a profiling method for the identification of organic impurities present in MDMA tablets. New impurities such as PMMA, and 3,4-methylenedioxy- N,N -dimethylbenzylamine were detected and confirmed by positive chemical ionization and MS/MS. The synthesis route allowing the formation of these new impurities was also described. Comparison of chromatographic profiles using the cosine function was made and discussed. Correlation values within and between samples were computed to

quantify the degree to which each chromatogram matched each of the others. Results showed a good potential of the method to discriminate MDMA samples, even if it was not optimized and requires some improvements.

Acknowledgements

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