Z. Rechtsmedizin 69, 52—61 (1971)

Gas Chromatography-Mass Spectrometry in Forensic Chemistry for Identification of Substances Isolated from Tissue

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Received March 17, 1971

Summary. The use of combined gas chromatography-mass spectrometry in forensic chemistry is demonstrated in 13 cases where various substances had to be isolated and identified, the substance was present in small amounts in some samples while in others the sample available was very small. In other instances a rapid and certain identification was important. The results are compared with data obtained by conventional procedures. The advantage of this method is discussed and it is emphasized that a collection of mass spectra of all common substances searched in forensic chemistry simplifies the indentification. Reference mass spectra of some drugs are given.

Zusammenfassung. Die Anwendung einer kombinierten gaschromatographischen und massenspektrophotometrischen Methode in der gerichtlichen Chemie wurde anhand von 13 untersuchten Fällen diskutiert. Verschiedene Substanzen, z.T. in kleinsten Mengen, wurden isoliert und identifiziert. Die erhaltenen Ergebnisse werden mit den Resultaten der konventionellen Methoden verglichen, zudem wird auf die Vorteile der GC-MS-Kopplung hingewiesen. Gleichzeitig werden einige Massenspektren wiedergegeben.

Eine Massenspektrensammlung von den in der gerichtlichen Chemie häufig anzutreffenden Substanzen ist für eine rasche und einfache Identifizierung erforderlich.

Key-Words: Forensic chemistry — Gas Chromatography-Mass Spectrometry — Methaqualon — Pethidine — Orphenadrine.

As drugs become more powerful and thus decrease the amount of substance necessary for serious or fatal intoxications, the methods used by the forensic chemist must facilitate the detection and identification of smaller and smaller amounts of substance. The number of drugs are also steadily increasing and therefore methods for screening and identifying must be simpler, and also more specific. Several works dealing with this subject have been published [1–4]. From these papers it is evident that mass spectrometry gives undoubtedly the most specific information in comparison to other analyzing methods. For this reason the identification of the analyzed sample can be rather simple in as much as the mass spectrum has been collected in the reference library. The method of searching and comparing an unknown mass spectrum with reference spectra can be accomplished using a computer with the known spectra on magnetic tape or from books having an index of mass spectral data [5].

Hitherto, gas chromatography-mass spectrometry has fundamentally been used in forensic chemistry to identify and to determine the structure of special compounds and metabolites and not in routine work. In this paper we describe

some cases, selected at random, where the sample sent to the laboratory was small or the concentration of the substance to be analyzed very low. Thus it would have taken a long time and a lot of work to examine the cases without using the GC-MS method, if possible at all. In some cases there was special need for an accurate and rapid identification. The results are summarized in the Table.

Methods

In addition to the conventional methods (yield of which is given in the references or in previously published works referred to in text) the substances were analyzed by LKB 9000 gas chromatograph-mass spectrometer. When sufficient amounts of sample were available, complete mass spectra were taken at the GC-peaks. For sample amounts in the nanogram range only selected mass numbers were studied using the AVA (Accelerating Voltage Alternator) unit, connected to the mass spectrometer [6]. If the desired mass numbers differed in mass more than 10% of the highest mass number, separate registrations were made.

The mass spectra were obtained at a constant accelerating voltage of 3500 V. The electron energy was normally 70 eV, but in some cases lowered to 15 eV. The temperature of the ion source was 270° C. The columns used were 1% SE-30 on Chromosorb W (80–100 mesh) 1.5 m × 2 mm i.d. and 5% Carbowax +5% KOH on Chromosorb G (80–100 mesh) 2 m × 2 mm i.d. The temperature of the injection port and the molecule separator was about 30° higher than the temperature of the column.

History, Analysing Methods and Results of Thirteen Cases of Poisoning

1. Two women were found in a car. The younger one was dead and the older one unconscious. A rubber tube was led into the car from the gas outlet. Murder was suspected. The dead woman suffered from epilepsy with severe psychical disturbances. She had been prescribed many different drugs by various doctors. The liver was ground and extracted with alcohol [7]. After centrifugation, a part of the alcohol extract was evaporated to dryness on a water-bath. The residue was dissolved in hot diluted hydrochloric acid and the fat removed by cooling and filtration [8]. The acid water solution was then extracted with chloroform. Part of the chloroform extract was shaken with 0.5 N NH_4OH and the UV-spectra of the ammonia solution was recorded. Another part of the chloroform extract was used for paper chromatography. Part of the chloroform extract was evaporated to a small volume and used for GC-MS.

The subsequent chemical analysis revealed that the blood from the younger woman contained 32% carbon monoxide. No alcohol was detected. In the liver about 12 mg% barbiturate according to ultra-violet spectrum was found, and in the blood about 4 mg%. Since the girl had been prescribed pheno-barbitone it was of great importance to make an accurate identification of the barbiturate. No certain conclusion could be drawn, from paper chromatograms, probably because so many metabolites of barbiturates and of other drugs were present. The first report given to the police was that the drug was Diminal Duplex® (aprobarbitone and vinbarbitone) according to paper chromatography. According to gas chromatography-mass spectrometry the main part of the barbiturate was phenobarbitone, run at a temperature of 155° C on a SE-30 column. Peaks of high intensity are m/e 77, 117, 146, 161, 174, 189, 204 and 232. Base peak is 204 and molecular peak is 232 [4].

2. A driver, 58 years old, drove his car on the wrong side of the road. He was apprehended by the police. No alcohol could be detected in the blood. Urine was not available. The blood was extracted with chloroform of about pH 3. The chloroform was evaporated and the residue freed from fat as described above. The chloroform was extracted with 0.5 N NH_4OH to get rid of acids and then a part of the chloroform was evaporated and the residue dissolved in 70% alkaline ethanol. Ultra-violet spectrum of blood extract suggested small amounts of methaqualone, about 0.3 mg%. The presence of methaqualone in the extract was proven by gas chromatography and mass spectrometry. An SE-30 column was used at a temperature of 195° C and a complete spectrum was recorded. From the reference mass spectrum of methaqualone (Fig. 1a) it can be seen that the analyzed sample (Fig. 1b) has the same cracking

pattern in the high mass region with the same characteristic peaks and similar retention time on the gas chromatogram. Consequently the compound was identified as methaqualone.

3. A four-month old child was found dead in bed. Upon autopsy old cracks in the skull were found. Alcohol extraction was performed as described in case 1. Part of the alcohol extract was evaporated and the residue dissolved in 50% KOH and hydrolysed on a boiling water-bath. The solution was cooled and extracted with diethylether and reextracted into sulphuric acid. The acid was made alkaline and extracted with chloroform. A part of the chloroform was evaporated and the residue was dissolved in 70% alkaline ethanol.

A part of the chloroform was used for the UV-spectrum, another part was chromatographed on a thin-layer plate (methanol: 25% ammonia, 100:1.5, spraying reagent Dragendorff). The rest of the chloroform extract was used for GC-MS, SE-30 column at 180°C. The chemical analysis of the liver revealed about 1 mg% of amitriptyline. By the mass spectrum the compound was identified as amitriptyline. Fig. 2a shows the reference mass spectrum of amitriptyline run on the GC-MS under normal conditions (ion source 270°C, electron energy 70 eV). This mass spectrum was difficult to use for the identification of amitriptyline, due to the β-cleavage of the amine group, which gave cause to an enormous peak at m/e 58, and that all other peaks were less than 3% relative intensity. The molecular ion at M = 277 was of very low intensity but the peak at m/e 275 (M-2) was several times higher. Fig. 2b shows a reference mass spectrum of amitriptyline where the sample was directly introduced into the ion source which was kept at 100°C and the probe was held at about 30°C. In this case the molecular ion M = 277 was obtained but still rather low in comparison to m/e 58. The peaks from m/e 61 were enlarged ten times in the drawing.

4. A 47 year-old female was found dead. Due to certain circumstances murder was suspected and it was of great importance to make an accurate identification of the type of drug in the liver. By chemical analysis 0.14% alcohol was found in the blood and 0.32% in the urine, and traces of chlordiazepoxide (< 0.02 mg%) in the urine. The same extraction method for the liver was used as for case 3. By ultra-violet spectrometry about 7 mg% of a tricyclic amine of amitriptyline type was detected in the liver. According to thin-layer and gas chromatography it was nortriptyline, which was confirmed by mass spectrometry. An SE-30 column was used at a temperature of 180°C. Fig. 3a shows the reference mass spectrum of nortriptyline run on the GC-MS instrument under normal conditions. This spectrum was enlarged ten times from m/e 47. As distinguished from amitriptyline the spectrum showed the molecular ion M = 263 under these circumstances. When this substance was run with an electron energy of 15 eV only the molecular ion and a few fragments could be detected as shown in Fig. 3b.

5. A 29-year-old male was found dead. Ultra-violet absorption of the liver extract could be due to orphenadrine. Extraction was carried out as described for case 3. The GC-MS confirmed the presence of orphenadrine and according to ultra-violet spectrometry and gas chromatography the concentration was calculated to about 20 mg%. (The stomach contained about 0.65 g orphenadrine). The column used was SE-30 at 180°C. Fig. 4a shows the reference mass spectrum of orphenadrine, which gave only a few peaks of high intensity. The base peak m/e 58 was the same fragment as in amitriptyline, and other characteristic peaks used for identification work, were found between m/e 160 and m/e 185. The molecular ion was non-detectable and even for a lower electron voltage of 12 eV the ion intensity did not increase. In Fig. 4b the mass spectrum of the liver extract gives evidence for the assumption of orphenadrine content.

6. An automobile was driven by a 39 year-old male in a very queer manner. This was reported to the police. A blood sample was destilled and the volatile was trapped in cold ethanol and quantitative determination was done by a modified Fujiwara method [9]. The subsequent analysis of the destillate of the blood by gas chromatography showed a peak on the recorder which according to standards was equal to about 0.5 mg% trichloroethylene in the blood. This method has a low specificity and confirmative identification was carried out by the GC-MS method on the ethanol solution. Since only a small amount of sample was available the two most characteristic peaks m/e 95 (M-Cl) and m/e 130 (M) were studied by the AVA unit. The Carbowax column was used at a temperature of 40°C. The intensity ratios agree with the reference mass spectrum of trichloroethylene [5]. (The blood also contained 4.4 mg% trichloroacetic acid. The urine contained 76 mg% trichloroacetic acid).

7. A 14 year-old boy was accustomed to staying away from school periodically. While visiting a bakers shop, he suddenly died. Upon autopsy aspiration of stomach content was found. Blood and brain were destilled and the destillate was extracted with n-hexane. Fujiwara's test gave a colour indicating the presence of more than one clorinated carbon. The n-hexan extract was used for GC-MS. Two GC-peaks were obtained and by mass spectrometry these peaks were identified as trichloroethylene and chloroform (about 2 mg% chloroform and less than 0.4 mg% trichloroethylene in the blood. The brain contained about 1.3 mg% chlorinated hydrocarbons calculated as chloroform). The Carbowax column was used at a temperature of 55°C and complete mass spectra were taken. The boy must thus somehow have had access to chloroform.

8. Some children found a number of glass ampuls containing a coloured solution in a sand box. Soon afterwards other ampuls were also found in different places in the surrounding. The police wanted to know if the content was dangerous and if the inhabitants in the area should be alarmed as so many children were found playing with the ampuls.

A rapid analysis was to be performed. The solution was volatile, and used directly for GC-MS. Because the SE-30 column was already in the gas chromatograph of the mass spectrometer it was used, at room temperature. In a few minutes the solution was shown to contain dichloromethane. The mass spectrum was identical to the reference spectrum [5]. Later on the colour in the solution was found to be a methyl orange indicator.

In the following five cases the extraction methods were as follows:

The blood or urine was extracted at about pH 8.5 with chloroform; bicarbonate buffer was used to correct the pH. The chloroform was reextracted into 0.1 N H_2SO_4. This extract was used for ultra-violet spectrometry. The sulphuric acid was alkalized and extracted with diethylether. Gas chromatography, and if there was enough material, also thin-layer chromatography were carried out. The ether extract was used for GC-MS. Alfes and Clasing have described a method using thin-layer chromatography and mass spectrometry, for determination of dansylated methamphetamine (dansyl = 1-diemethylamino-naphtalene-5-sulfonyl) [10].

9. A 22 year-old student, who had behaved strangely, was killed by a truck. He was suspected to have thrown himself in front of the truck. In the blood was found, by gas chromatography, a peak with the same retention time as amphetamine, the amount corresponding to about 17 μg per 100 ml of blood. The concentration in the sample was too small to be identified by our usual method (colour reaction on thin-layer chromatogram). By GC-MS the blood extract was proven to contain amphetamine. The small amount present made it necessary to use the AVA unit, and three characteristic peaks at m/e 44, 91 and 120 were recorded. The intensity ratio of these peaks were identical to the same peaks in the reference mass spectrum of amphetamine.

10. A 17 year-old girl had previously used narcotics. It was suspected that she had begun to use narcotics again. A very small sample was sent to us. The blood extract gave at the gas chromatogram a peak with exactly the same retention time as amphetamine, corresponding to about 0.3 mg%. According to mass spectrometry the peak was not amphetamine. The Carbowax column was used at a temperature of 140°C. Two peaks were found, but they have not yet been identified. They both have the base peak at m/e 75 and probably contain chlorine. The retention times were 0.4 and 0.5 relative to nicotine.

Peaks of high intensity were for GC-peak one m/e 47, 75, 103, 185 and 187, for GC-peak two m/e 47, 75, 103, 145, 147, 219 and 221. Judging from the intensity ratios of the assumed molecular peaks (185 and 187, 219 and 221) they could contain two and three chlorine atoms respectively.

11. A 26 year-old male shot himself. He was a known narcotic user to the police. As a woman was present in the apartment during the suicide a chemical analysis was performed. According to the story the man should have injected amphetamine immediately death. The ampuls found in the apartment contained amphetamine. In the blood 0.04% alcohol was found. According to gas chromatography and thin-layer chromatography 5 mg% phenmetrazine was found in the urine, 0.8 mg% in the blood and 0.7 mg% in the liver. On the gas chromatogram of the blood extract was obtained an additional peak with practically the

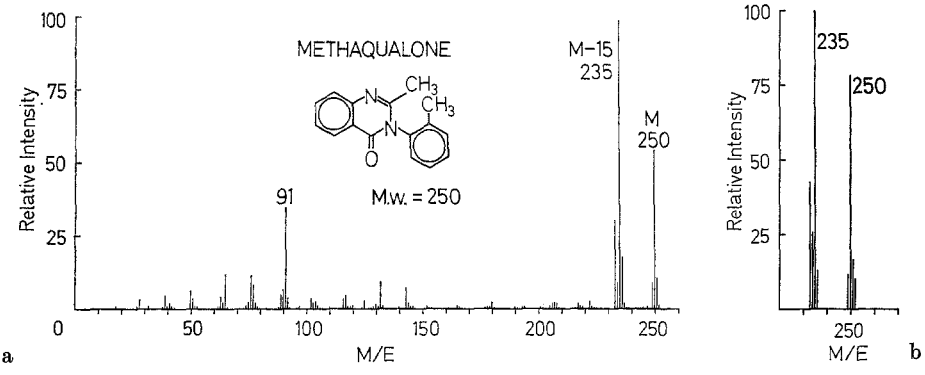

Fig. 1. a Reference mass spectrum of methaqualone. b The high mass region of a mass spectrum from blood extract in case 2. Both spectra were run using an SE-30 column at 195° C

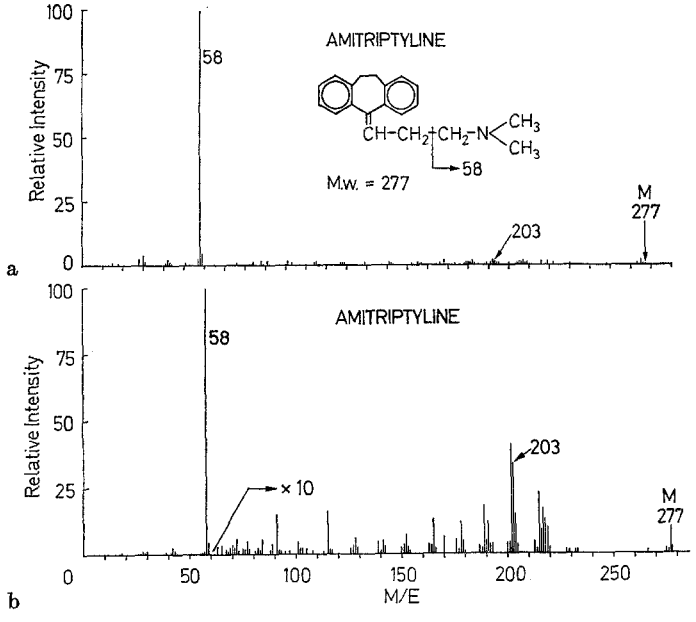

Fig. 2a and b. Reference mass spectra of amitripyline. See case 3. a An SE-30 column at 180° C was used. The ion source was kept at 270° C. b The direct inlet was used at 30° C. The ion source was kept at 100° C. All peaks from m/e 61 were enlarged ten times

same retention time as amphetamine. By GC-MS using the Carbowax column at 150° C this peak was shown to be 2-phenylethylamine, with the characteristic peaks at m/e 30, 91 (M-30) and 121 (M). Fig. 5 shows a reference mass spectrum of 2-phenylethylamine. To the story must be added that the suicide was not reported to the police until two days later, so some putrefaction of the blood had probably taken place.

12. Three cases of queer car driving. Only blood was available. No alcohol was found and sympathomimetic amines were suspected. Small amounts of blood were sent to the lab, extracted and analyzed by gas chromatography. Very small peaks, which could correspond

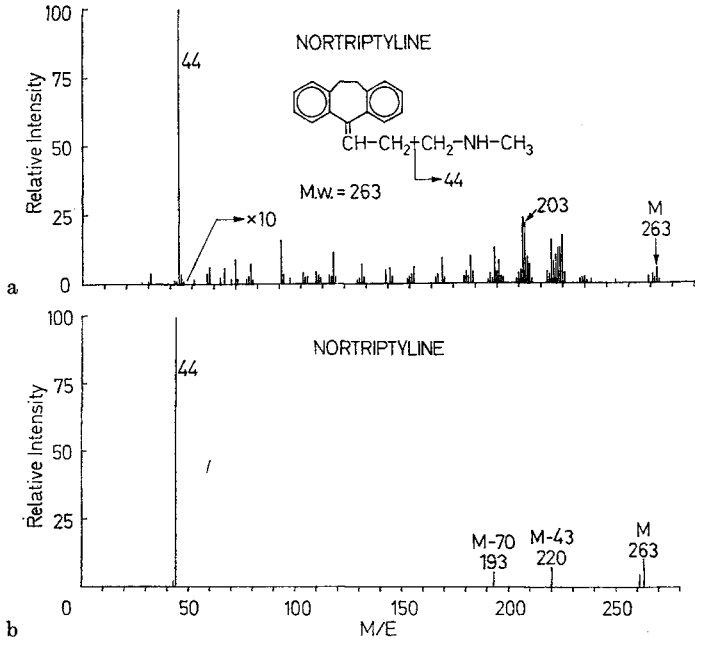

Fig. 3a and b. Reference mass spectra of nortriptyline run on an SE-30 column at 180°C. See case 4. a The ionization potential was 70 eV. All peaks from m/e 47 were enlarged ten times. b The ionization potential was 15 eV

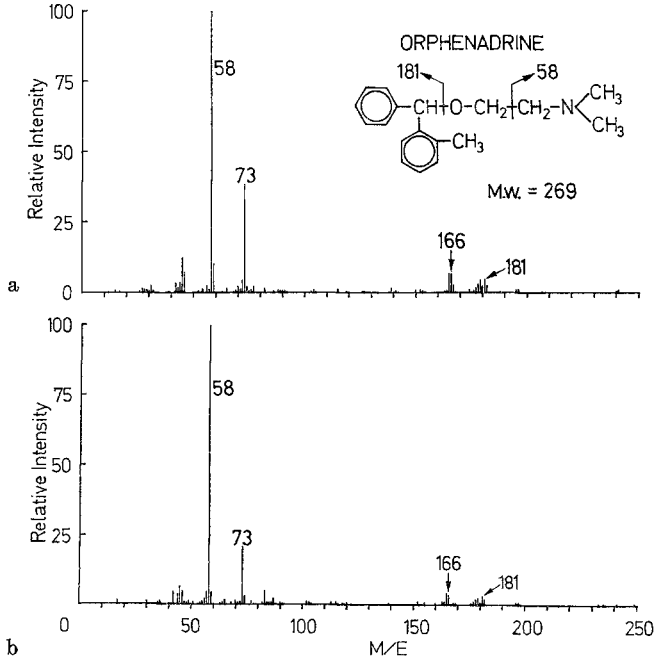

Fig. 4. a Reference mass spectrum of orphenadrine. b Mass spectrum from liver extract in case 5. Both spectra were run using an SE-30 column at 180°C

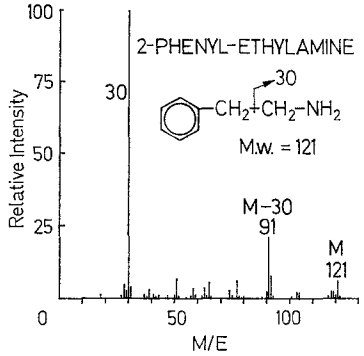

Fig. 5. Reference mass spectrum of 2-phenyl ethylamine run on a Carbowax column at 150° C. See case 11

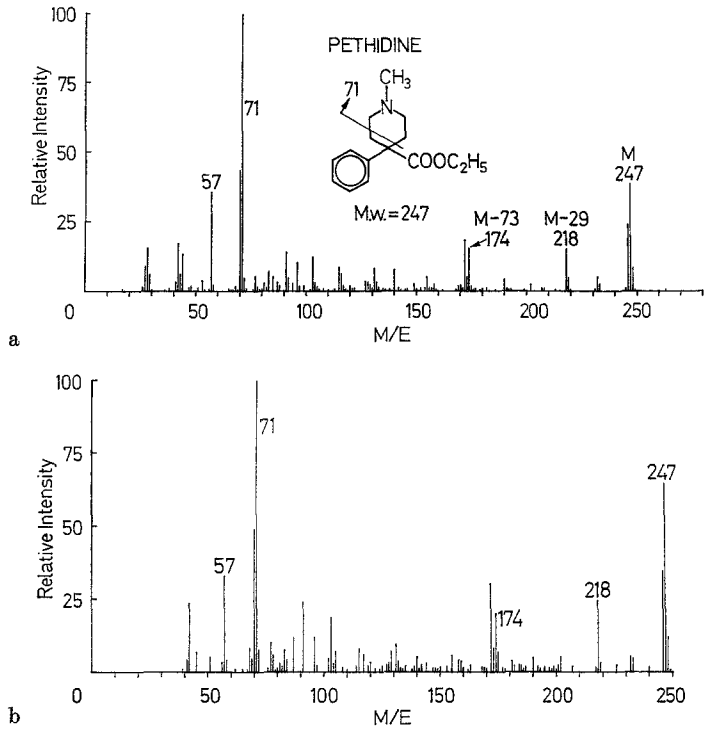

Fig. 6. a Reference mass spectrum of pethidine. b Mass spectrum of urine extract in case 13. Both spectra were run using a Carbowax column at 185° C

to phenmetrazine were obtained. By GC-MS the peaks were shown to do so. The samples were injected on the Carbowax column at 190° C, and the molecular ion in the phenmetrazine spectrum (m/e 177) was registered using the AVA [3].

13. A urine sample was sent to the lab to be tested for central-stimulating amines. A peak in the gas chromatogram indicated the presence of phenmetrazine. Much further on the recording there was another peak. As the results were of some importance mass spectra of the two peaks were taken. The one corresponding to phenmetrazine was another substance

Table. *A summary of the results from the thirteen cases as described in the text*

No.	Material	Conventional methods	Concentration	GC-MS results	Column temperature °C	Additional results
1.	liver	UV, PC	12 mg-%	phenobarbitone	SE-30, 155	32% CO
	blood	UV, PC	4 mg-%	phenobarbitone	—	—
2.	blood	UV, GLC	0,3 mg-%	methaqualone	SE-30, 195	—
3.	liver	UV, TLC, GLC	1 mg-%	amitriptyline	SE-30, 180	—
4.	blood	—	—	—	—	0,14% alcohol
	urine	—	—	—	—	0,32% alcohol, chlordiazepoxid $< 0{,}02$ mg-%
	liver	UV, TLC	7 mg-%	nortriptyline	SE-30, 180	—
5.	liver	UV, GLC	20 mg-%	orphenadrine	SE-30, 180	—
	stomach	UV	total amount 0,65 g	orphenadrine	—	—
6.	blood	Fujiwara, GLC	0,5 mg-%	trichloroethylene	Carbowax, 40	chlorinated hydrocarbons calculated as trichloroacetic acid 4,4 mg-%
	urine	—	—	—	—	d:o 76 mg-%
7.	blood	Fujiwara	2 mg-%	chloroform	Carbowax, 55	—
			$< 0{,}4$ mg-%	trichloroethylene		
	brain	Fujiwara	ε 1,3 mg-%	chloroform and trichloroethylene	—	—
8.	ampul	—	—	dichloromethane	SE-30, 30	indicator methyl orange
9.	blood	GLC	17 µg/100 ml	amphetamine	Carbowax, 150	—
10.	blood	GLC	—	unidentified (expected amphetamine)	Carbowax, 140	(the unidentified peak, corresponds to 0.3 mg-% amphetamine)
11.	blood	GLC	—	2-phenylethylamine	Carbowax, 150	0,8 mg-% phenmetrazine 0,04% ethanol
	urine	—	—	—	—	5 mg-% phenmetrazine
	liver	—	—	—	—	0,7 mg-% phenmetrazine
12 a)	blood	GLC	traces	phenmetrazine	Carbowax, 190	—
b)	blood	GLC	traces	phenmetrazine	Carbowax, 190	—
c)	blood	GLC	traces	phenmetrazine	Carbowax, 190	—
13.	blood	GLC	0,8 mg-%	pethidine[a]	Carbowax, 185	—

[a] An other peak with approximate retention time like phenmetrazine was investigated and found not to be phenmetrazine, but could not be identified.

not yet identified. The other peak was identified as being pethidine (0.8 mg%). At 185°C on the Carbowax column, the unknown peak had a retention time of 1.8, and pethidine a retentio time of 4.0 relative to nicotine.

Fig. 6a shows that the base peak in the reference spectrum of pethidine arises from cleavage of the methyl-piperidyl part of the molecule. The peak at m/e 57 comes from loss of a methylene group from the base peak at m/e 71. The peak at m/e 174 corresponds to the loss of the ester group from the molecule. The loss of the ethyl group gives rise to a peak at m/e 218. The molecular ion at m/e 247 is about 40% as compared to the base peak. Fig. 6b shows the mass spectrum of the urine extract.

Discussion

From the described cases of intoxication it is shown that the combined GC-MS method can be used for identification of quite different analytical problems. Almost all types of drugs or other similar compounds can usually be run by the GC-MS instrument. Compounds impossible to run on the gas chromatograph can in some cases be analyzed using the direct inlet of the mass spectrometer. If suitable derivatives are made, they can be run on the GC-MS. The mass spectrometric studies of several types of drugs have shown that the cracking patterns of molecules are, in general, characteristic for each one. There are however exceptions to this rule. From the reference mass spectra available it is for instance indicated that when a side chain contains an amine group, a very high peak is obtained for a β-cleavage. Mass spectra of such compounds normally exhibit a small or non-detectable molecular ion.

Fig. 2 shows mass spectra where the intensity of m/e 58 is extremely high as compared to the other peaks in the spectrum. Fig. 3 shows a similar cracking pattern with a very intense peak at m/e 44. There are however, groups of small peaks which are very characteristic for each compound. Even if it is difficult to show the structure of all these fragments they can be used for identification of drugs. If the electron energy is decreased from 70 eV to about 12 to 15 eV the relative intensity of the fragments change in such a way that most of the peaks decrease except the molecule ion. An example of this method is shown in Fig. 3b. To obtain a high intensity of the molecular ion these and similar compounds should be run on a gas chromatograph combined with a mass spectrometer with chemical ionization [11]. In the case of amitriptyline and nortriptyline small peaks of M-2 and M-1 are observed along with the molecular ion M. It seems evident that M-2 is a thermal degradation product, which is dependent upon the operating temperature of the ion source and introduction line and M-1 fragments are often seen in mass spectra from amino compounds. Methaqualone and six metabolite isomers have been studied by other scientists [12,13]. There are however several metabolites which show different molecular weights and a separate study of this subject is under examination.

In case 10 it was suspected that amphetamine should be indicated, but instead, two mass spectra of unknown compounds were recorded. These compounds must be studied further before an exact structure determination can be made. This and other identification problems show that it is of importance to develop a library of reference mass spectra of all available drugs and toxi-compounds, which will reduce the time required for identification. When only small amounts of sample are available, great demands are made upon the accuracy of the analyzing method. On the other hand, when methods become so sensitive that a few nano-

grams can be detected and identified, it is of utmost importance to be extremely careful. The samples are easily contaminated when working with such small amounts. Many substances are excreted through the skin and thus, simply handling of the sample could cause contamination. Reagents used for analysis could also contain tiny amounts of the particular substance. People arriving from countries where the use of certain narcotics is legal, could still have ng of the drug in their urine many days after having left the country.

Acknowledgement. This work has been supported partly by the Swedish Medical Research Council (project No. B71-13X-171-07C).

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