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Identification of Barbiturates by Computerized Mass Spectrometry

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Summary. A computer has been used for rapid and exact identification of mass spectra for the barbiturates presently on sale in Sweden. The compounds were isolated from blood, or liver from intoxicated persons in suicide cases and a small part of the chloroform extracts were analyzed on a gas chromatograph-mass spectrometer instrument. The mass spectra were recorded on a digital tape off line system and the tape was evaluated and processed by the computer. The amount of tissue or blood was in the magnitude of less than 50 mg in these cases.

Zusammenfassung. Es wurde ein Computer für eine schnelle und exakte Identifizierung der Massenspektren zum Nachweis von Barbituraten, die zur Zeit im Verkauf in Schweden angeboten werden, angewandt. Aus Blut- oder Leberproben der Personen, die Selbstmorde durch Vergiftung begangen haben, wurden Substanzen isoliert und eine geringe Menge der Chloroformextrakte mit Hilfe der Gaschromatografie-Massenspektrometrie(GC-MS)-Apparatur analysiert. Die Massenspektren wurden auf einem Digitalband aufgezeichnet und danach durch den Computer bewertet und wiedergegeben. Das Gewicht der Gewebe und des Blutes in den untersuchten Fällen lag unter 50 mg.

Key words: Barbiturates — Identification of Barbiturates — Mass Spectrometry, computerized.

In the last two decades an increasing number of barbiturates has been available, which has given rise to a great number of intoxication cases for verification by the forensic chemist [1]. The methods commonly used for identification of barbiturates found in tissue from intoxicated persons are: 1. UV-spectrometry which classifies the unknown drugs as barbiturates and does not specifically identify the compound. 2. Paper or thin-layer chromatography which permits separation of the substances in groups but not the individual compound and is a rather time-consuming method. 3. Gas chromatography, which allows a much better separation than the two other methods mentioned, has also severe limitations because of many natural occuring components in tissue giving peaks on the GC and this makes identification impossible by GC alone [2]. Barbiturates have also been studied using mass spectrometry [3]. A combined GC-MS which facilitates an immediate and accurate analysis and identification of all the eluated compounds from the GC has been used for identifying barbiturates [4, 5]. The use of computers for searching and identification of various drugs has also been reported [6]. In the future, when most hospitals employ the GC-MS instrument for rapid identification of drugs found in comatose patients it will be of primary importance that one and only one correct answer is provided by the computer. This paper describes

the identification of 14 of the barbiturates on sale in Sweden and a special computer program for searching among fixed mass numbers in the mass spectra. This part of the work is intended to cover most barbiturates used in suicidal cases.

Methods and Materials

The tissue (10 g of liver or 20 g of blood) in all cases was extracted with ethanol, centrifuged, evaporated to a small volume and separated from fat by cooling methods [7]. Following chloroform extraction at acidic pH, the organic layer was evaporated to 0.5 ml per sample and 2 μl were used for the analysis.

The 2 μl of chloroform solution thus equals 40 mg of liver or 80 mg of blood. Using micromethods only about 100 mg of blood (just as for a blood-sugar determination) should be necessary for an analysis when an overdose is suspected. It is an advantage to use micromethods when living people and especially small children are concerned. (See also discussion.)

The advantage of using an ethanol extraction before shaking with chloroform is that all metabolites can be found in the ethanol extract and further work will deal with the simultaneous determination of unaltered drug and its more abundant metabolites. For the toxicologist it is also of great importance when evaluating the data to know the relative amount of drug to metabolites. The mass spectrometer used was LKB 9000 with ion source temperature 270°C and with an electron beam energy of 70 eV. The samples were introduced via the gas chromatograph inlet in all cases. A 1% OV-17 column was used with helium as carrier gas, at a flow rate of about 30 ml/min. The temperature of the column was programmed up from 100°C at 10°C/min.

The mass spectra were recorded by a magnetic tape off line system and the tape was processed and evaluated by an IBM 1800 computer. The computer has a cycle time of 2 μs and a 24 K core storage of 16-bit words. It is equipped with two 512 K words disk storages, a line printer and a 60 kc nine-track magnetic tape unit. 43 fixed mass numbers were used in the process of identifying the 14 barbiturates. The mass spectra for comparison were taken from our reference library which also contained about a hundred spectra of various other drugs.

Results and Discussion

Fig. 1 shows the chromatogram of blood sample containing 2.4 μg allobarbitone which corresponds to a 2.4 mg%. In this case the barbiturate component is of high intensity as compared to other unidentified peaks in the chromatogram and could be studied at a five times lower concentration with good results. By using a multiple ion detector the amount of substance could be minimized to a few nanograms. This, however, is not a critical point in this work since in all the intoxicated cases the available amounts of substance is adequate for a good spectrum. For a quantitative estimation the metabolites must be included, but for the positive identification it is more practical to rely on the unchanged drugs.

Fig. 2 shows a mixture of the studied barbiturates. By using a 3% OV-17 column only 2 of the compounds could be completely separated. The other compounds exist as overlapped peaks. Mass spectra were taken of all peaks in the chromatogram and recorded on magnetic tape.

Since some of the mass spectra of barbiturates are rather similar, it was of great importance to extract the characteristic peaks from each spectra in such a way that the greatest surety of identification was obtained.

From earlier experiments, identifying unknown compounds by computer searching of reference mass spectra using the 6 highest peaks in each spectrum, unsatisfactory results were obtained in comparison to other methods [8]. The reason for this was when different mass spectrometers and different inlet systems

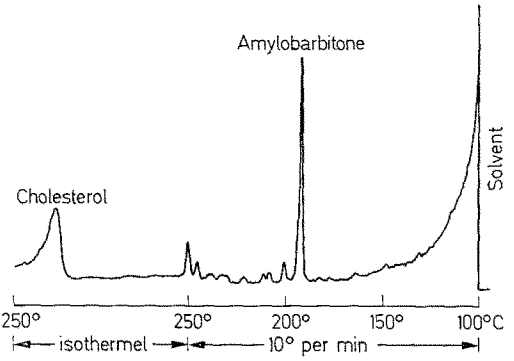

Fig. 1. Chromatogram of a blood sample shows an amount of 2.4 μg Allobarbitone obtained with a 1% OV-17 column at a temperature of 210°C

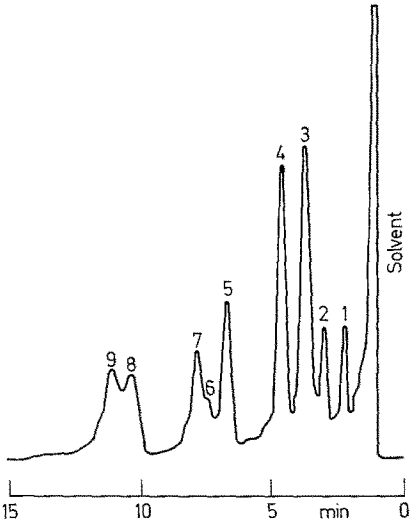

Fig. 2. Gas chromatogram of barbiturates obtained with a 3% OV-17 column using the GC-MS instrument. Peak No. 1, Barbitone, No. 2, Allobarbitone, Aprobarbitone, No. 3, Nealbarbitone, Idobutal, Amylbarbitone, Pentobarbitone, No. 4, Quinalbarbitone, Vinbarbitone, No. 5, Hexabarbitone, No. 6, Brallobarbital, No. 7, Methylphenobarbitone, No. 8, Cyclobarbitone, No. 9, Phenobarbitone

were used the mass spectra often showed a difference in intensity although the cracking pattern was rather similar. In order to avoid the effects of these variations only fixed mass numbers are used for identification of barbiturates. Mass numbers of base peaks and other characteristic fragments of the barbiturates are given in Table 1. They can be divided into four groups:

A. 3 spectra (1—3) with base peaks at the same mass number m/e 156 and the intensity of almost all other peaks rather similar except m/e 112, 183 and 198.

B. 4 spectra (4—7) with base peaks at the same mass number m/e 167 or 168 and with several characteristic peaks at the same mass numbers.

C. 5 spectra (8—12) with base peaks at different mass numbers. All other peaks have intensity of less than 50% of the base peak.

D. 2 spectra (13—14) with base peaks at the same mass number m/e 207. All other peaks have an intensity of less than 50% of the base peak, but they are characteristic for each spectrum.

Since only 14 compounds were to be studied it was possible to increase the accuracy in the identification process. By working with fixed mass numbers from each compound, or a compiled total of 43 mass numbers and to some extent using the intensity of these fragments to differentiate between compounds, the unknown compound could be classified as a known barbiturate or not with an accuracy of close to 100%. The choice of fixed mass numbers varies for the different barbiturates depending upon whether or not the spectra are easy to identify. The chosen characteristic peaks in each mass spectrum of barbiturates are given different percentage values. If these peaks in an unknown mass spectra are identical with the same mass numbers in a mass spectrum in the reference library the similarity is set to 100%. When these are peaks which do not satisfy established requirements the similarity will be less than 100%. Only similarities over 60% are printed out.

Fig. 3 shows a typical example of 2 mass spectra of compounds with quite similar cracking patterns except for small differences in intensity at m/e 43, 71, 183 and 198. Of these peaks have high priority been given to the peaks m/e 183 and 198 in the searching program.

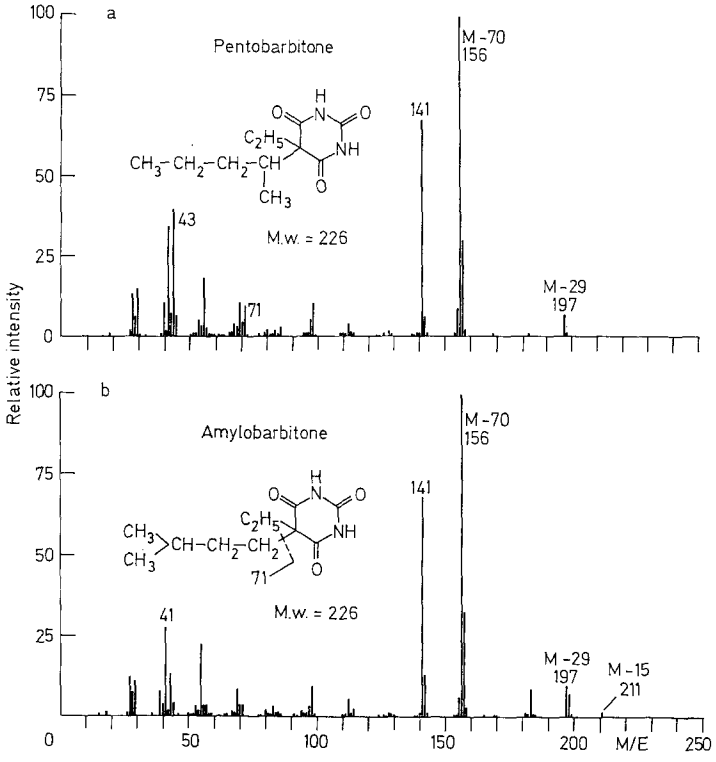

Fig. 3a and b. Mass spectra of Pentobarbitone (a), Amylobarbitone (b)

Table 1 shows the mass number used in the identification process and the computer flow diagram is given in Fig. 4. To test the identification method the Aldermaston magnetic tape with about 9000 mass spectra and our own 1300 reference spectra with drugs and barbiturates included, were compared with the mass spectra of the 14 barbiturates studied. The results show that each mass spectrum of the barbiturates was identified by the computer with only one correct answer for each unknown compound.

The searching results show that all the compounds were correctly identified and other compounds had a similarity of less than 60%. Other groups of drugs are under mass spectrometric study and these will later be included in our reference library of mass spectra.

Conclusion

The advantage of computer searching among mass spectra of only groups of compounds as compared to a more general method in which all different types of compounds are included is that the accuracy of identification is distinctively increased. This is of importance if the GC-MS method is used by non-technical persons and when rapid and exact identification of toxic compounds is the objective.

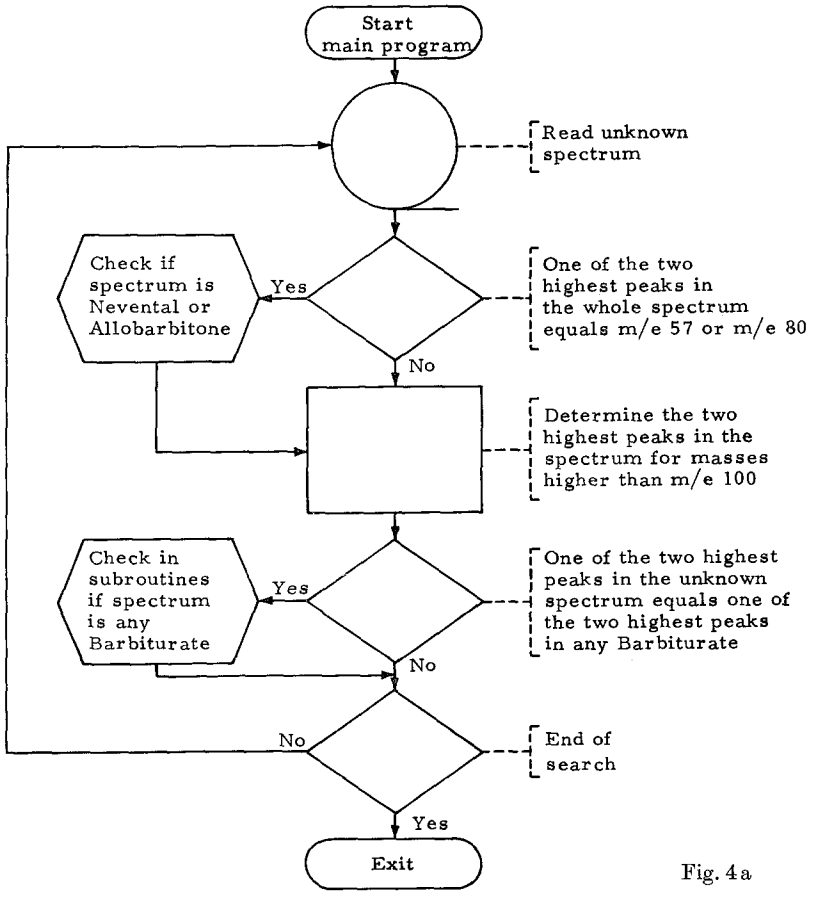

Fig. 4a

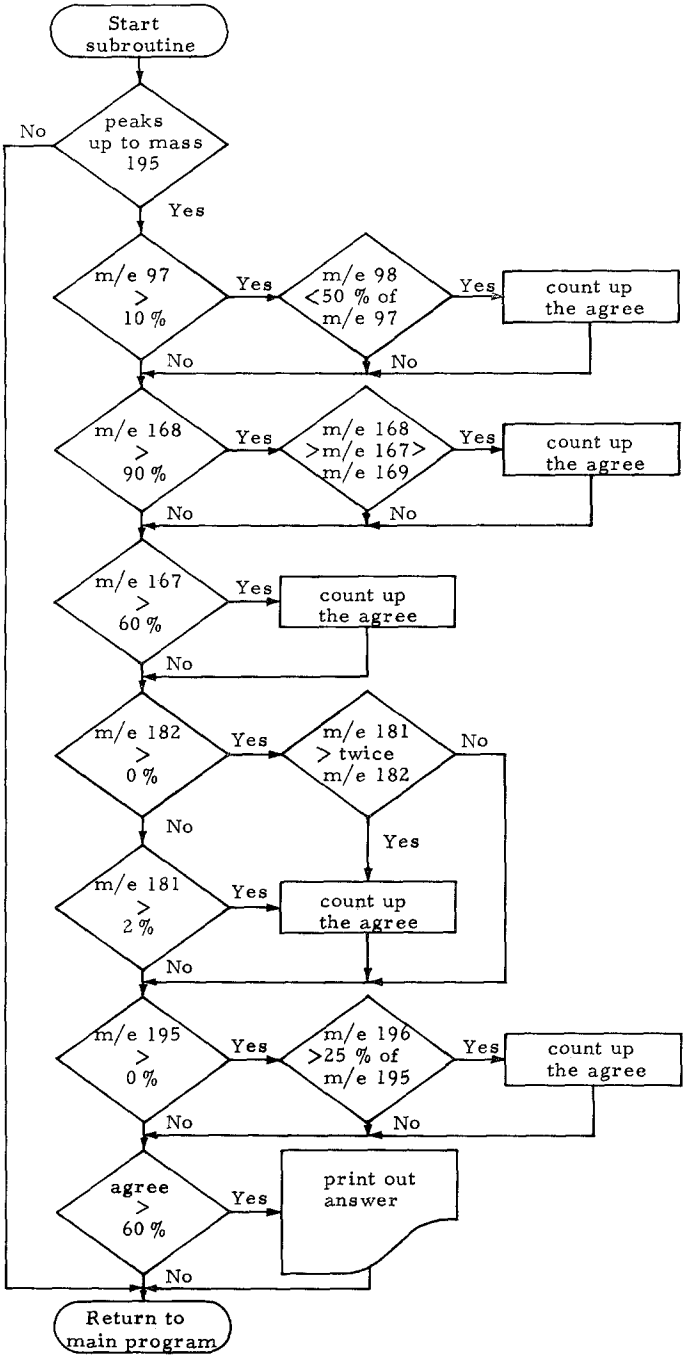

Fig. 4b

Fig. 4a and b. Flow chart over the program. a The main program for identification of barbiturates. b Subroutine for identification of Quinalbarbitone. The countings up of the agree are weighted, so more important peaks have higher weight than others

Table 1. *Characteristic mass numbers used for identification of barbiturates*

No.	Compound[a]	Base peak	Other characteristic peaks	M. W.
1.	Pentobarbitone	156	97, 98, 112, 183, 197, 198	226
2.	Amylobarbitone	156	97, 98, 112, 181, 182, 183, 197, 198	226
3.	Barbitone	156	97, 98, 112, 183, 197, 198	184
4.	Aprobarbitone	167	57, 80, 97, 98, 168, 169, 181, 182, 195, 196	210
5.	Idobutal	167	80, 97, 98, 168, 169, 181, 182, 195, 196	224
6.	Allobarbitone	167	80, 81, 165, 166, 168, 193, 208	208
7.	Quinalbarbitone	168	97, 98, 167, 169, 181, 182, 195, 196	238
8.	Nealbarbitone	57	167, 180, 181, 182, 223	238
9.	Phenobarbitone	204	117, 118, 146, 161, 232	232
10.	Methylphenobarbitone	218	117, 118, 146, 246	246
11.	Hexobarbitone	221	79, 80, 81, 155, 157, 236	236
12.	Vinbarbitone	195	67, 69, 135, 141, 152	224
13.	Cyclobarbitone	207	79, 80, 81, 141, 157	236
14.	Brallobarbital	207	81, 124, 165, 167, 208	286

[a] Clarke, E. G. C., Isolation and Identification of Drugs. The Pharmaceutical press 1969.

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