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Determination and Identification of Sympathomimetic Amines in Blood Samples from Drivers by a Combination of Gas Chromatography and Mass Spectrometry

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Summary. Gas chromatographic and mass spectrometric data concerning the analysis for certain sympathomimetic amines in blood samples from drivers are presented. In all cases where gas chromatography gave peaks at the correct retention times for amphetamine or phenmetrazine respectively, mass spectrometry confirmed the presence of the corresponding drugs in the extracts from blood. Drug-free blood samples gave neither gas chromatographic nor mass spectrometric responses at the retention times characteristic for amphetamine or phenmetrazine.

Computer-drawn mass spectra of phenmetrazine are shown in Fig. 2. Analytical data obtained from twelve drivers and one hospital patient are collected in the table.

Key-Words: Amines, in blood samples — Amphetamine — Phenmetrazin.

Zusammenfassung. Blutproben von Kraftfahrern, die sympathomimetische aromatische Amine zu sich genommen hatten, zeigten gaschromatographische „Zacken" mit den Retentionszeiten von Amphetamin oder Phenmetrazin (Preludin®). Eine Reihe solcher Fälle wurden mit Hilfe einer kombinierten gaschromatographisch-massenspektrometrischen Methodik untersucht. In sämtlichen Fällen konnte die vermutete Identität der beiden Amine massenspektrometrisch einwandfrei bestätigt werden. Blutproben von drogenfreien Personen, auf exakt gleiche Weise untersucht, gaben weder im Gaschromatogramm noch im Massenspektrum irgendwelchen Ausschlag.

Massenspektren von Phenmetrazin, rein und nach Isolierung aus Blutproben, wurden durch einen Computer berechnet und gezeichnet. Abb. 2 zeigt die Resultate.

Quantitative Analysen und einwandfreie Identifizierung durch Gaschromatographie und Massenspektroskopie sind noch bei Mengen von ca. 1 µg Amphetamin oder Phenmetrazin in Blutproben ausführbar.

Qualitative und quantitative Resultate der Untersuchung von 12 Kraftfahrern und einem Patienten sind in der Tabelle zusammengestellt.

The abuse of stimulating drugs of the amphetamine group has become a social and legal problem in Sweden, as in other industrialized countries.

It is not difficult to detect these drugs in urine samples from persons who have taken large amounts, usually by injection. Quantitative analyses can be carried out by gas chromatography [1, 2] and in some cases by ultraviolet spectrophotometry, and identification can be aided by thin-layer chromatography using color reactions [3].

Beckett and coworkers [1] have published methods for detecting this group of drugs by gas chromatography. Small doses (10—25 mg) of amphetamine or metamphetamine, taken by persons who are not used to these drugs, have been reported to leave the blood stream rapidly, usually within not more than a few minutes [4, 5], so that they could not be found later on. However, in more recent studies Campbell [6] has reported that 4—5 μg of amphetamine per 100 ml of blood could be detected 1.5—2 hours after administration of 10—15 mg of the drug. Even after 8—10 hours about 0.2 μg per 100 ml could be found. No quantitative data seem to have been published on the fate of phenmetrazine, though both Eberhardt and Debackere [7] and Heyndrickx and De Leenheer [8] could demonstrate the presence of this drug in the urine of volunteers.

After the injection of large doses by drug addicts (100—200 mg, injected several times a day, are not uncommon amounts in severe cases) we found it quite possible to detect amphetamine [9] and phenmetrazine in blood samples which were taken several hours later.

Narcotic addicts are frequently caught driving a car while under the influence of stimulating drugs, or — more often — while suffering from abstinence symptoms such as fatigue and irritability. In 1969 alone, around 50 such cases were registered and samples sent to the Laboratory of Forensic Chemistry. In several instances, urine specimens were not available. Many of the drivers who have taken these drugs are dehydrated and have difficulty in providing urine specimens. Besides, it has previously been widely believed that the stimulating amines could only be detected in the urine (which was therefore withheld) and not in the blood (which often was allowed to be taken without protests).

In many of these cases, gas chromatographic analysis of the blood samples showed a very small peak with the correct retention time for either phenmetrazine or amphetamine. Increasing the sensitivity of the apparatus was of little value, due to the low signal to noise ratio. The analytical procedure was simple: The blood was made alkaline (with conc. ammonia or bicarbonate) and was extracted with chloroform or ether. The organic layer was shaken with 5.0 ml of 0.1 N sulfuric acid, whereupon aliquots of the aqueous layer were again extracted at alkaline pH, this time with 0.5—1.0 ml of ether. For quantitative work, 10—20 μg of nicotine was added to the aqueous layer as an internal standard. The ether extracts were dried over anhydrous sodium sulfate, and aliquots were injected into the gas chromatograph. Fig. 1 shows a typical recording [9a].

The total amount of the amines present in the blood samples (routinely about 10 ml) was too small for thin-layer chromatography, and in many cases even for analysis by a second gas chromatographic method, such as derivative gas chromatography [1, 10, 11, 12]. Frequently, not more than 1—2 μg of drug was available. Thus, stringent identification of the drugs — essential in many legal cases — could not be carried out if no urine specimens were available.

At this point we decided to investigate mass spectrometry as a tool for identification of the minute amounts of amines available from blood samples. Using the same extraction procedure as outlined above, aliquots of the final ether extract were evaporated to smaller volumes (when necessary) and applied to the combined gas chromatograph — mass spectrometer LKB 9000A.

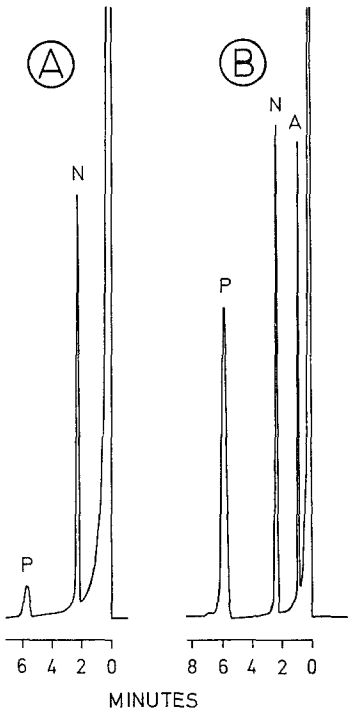


Fig. 1. A) The gas chromatographic trace from an extract of blood, obtained from a driver, 24 years old, and free from ethanol. 11 ml of blood and about 2 g of sodium bicarbonate were extracted with 3 × 100 ml of chloroform. The organic layer was shaken with 5 ml of 0.1 N sulfuric acid. 2.5 ml of the aqueous layer was made alkaline with sodium hydroxide, 70 μg of nicotine (as the sulfate) was added and the solution extracted with 1.0 ml of ether. The extract was dried over anhydrous sodium sulfate. 5 μl of the ether extract was injected into the gas chromatograph. The glass column (2 m × 1/4″ i.d.) was filled with a mixture of 90% AW-DMCS Chromosorb G (80—100 mesh), 5% Carbowax 20 M, and 5% potassium hydroxide. Column temperature 160°. Retention time for phenmetrazine 5.5 minutes. The concentration of phenmetrazine (free base) was calculated to have been 2.0 μg per ml of blood, or about 60 μg in the injected sample. B) The gas chromatographic trace from an extract of standard substances. 70 μg of nicotine (as the sulfate, internal standard), 25 μg of amphetamine (as the sulfate), and 100 μg of phenmetrazine (as the chloride) in alkaline solution were extracted with 1.0 ml of ether. 5 μl of the dried extract were injected into the gas chromatograph under the same experimental conditions as described above. Abbreviations: *A* amphetamine, *N* nicotine, *P* phenmetrazine

In some cases, whole mass spectra were recorded and fed into a computer, which evaluated them, printed out the numerical results, subtracted the background and produced an appropriate graph [13]. Mass spectra of amphetamine have previously been published [1, 14], but to our knowledge not any spectra of phenmetrazine. Fig. 2 shows the mass spectra of pure phenmetrazine, and of the drug after isolation by two different gas chromatographic techniques. The details of these spectra will be discussed below.

In other cases, a different technique was used. The molecular ion, or 2 to 3 characteristic ion fragments were focused on the collector slit during the recording of the gas chromatographic analysis [15]. It is possible to switch

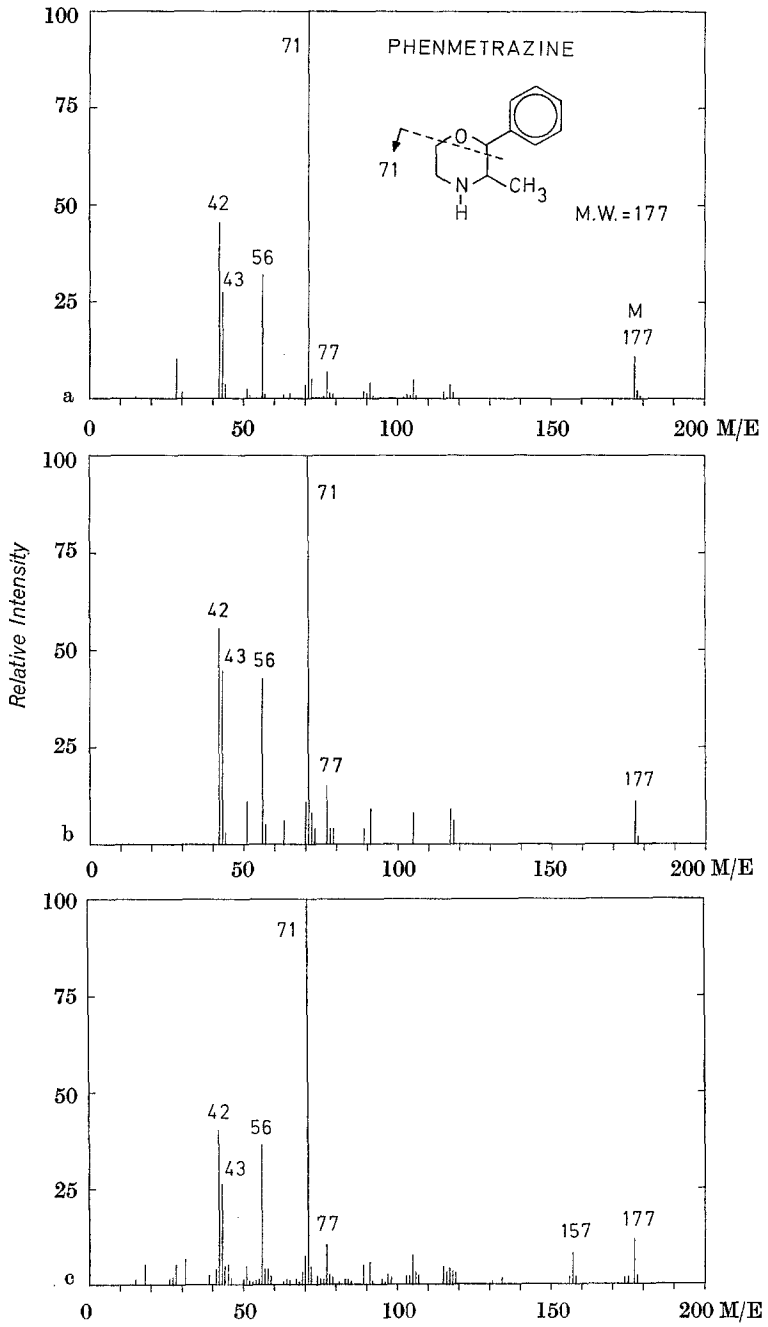
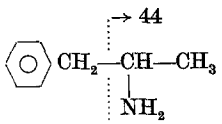


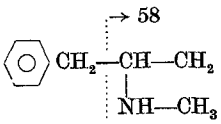
Fig. 2a—c. Mass spectra obtained using the combined gas chromatograph mass spectrometer. a Reference mass spectrum of phenmetrazine. Column: 1% SE-30, 115° C. Retention time approximately 4 min. b Mass spectrum of a fraction from a blood sample from a driver. Column: 1% SE-30, 115° C. Retention time approximately 4 min. c Mass spectrum of a fraction from a blood sample of a driver. Column: 5% Carbowax + 5% KOH, 180° C. Retention time approximately 8 min. Cf. the text for details

between three different mass numbers by means of the accelerating voltage alternating unit used in combination with the LKB 9000A. However, the focused ions must have mass numbers which differ not more than 30 per cent from the lowest mass number used. In the case of phenmetrazine the molecular peak (m/e 177) alone was chosen for this purpose, since this peak is very characteristic. For amphetamine, strong peaks at m/e 91 and 120 were selected and recorded individually. In some cases, the characteristic peak m/e 44 was also recorded as an additional check.

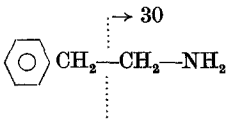
In addition to phenmetrazine and amphetamine, methylamphetamine and the amphetamine homologue 2-phenylethylamine were also subjected to gas chromatography combined with mass spectrometry. These amines have retention times that differ not very greatly from amphetamine [9] and could conceivably interfere with identification of amphetamine itself. The mass spectra obtained for these other amines exhibited characteristic peaks, allowing for a clear differentiation between them. Characteristic ion fragments found were m/e 58 for methylamphetamine, and m/e 30 for 2-phenyl-ethylamine. The corresponding fragmentation patterns are the following (cf. also Beckett *et al.* [1]):



amphetamine



methylamphetamine



2-phenylethylamine

In all instances where a gas chromatographic peak with the correct retention time for phenmetrazine or amphetamine had earlier been observed, a positive response from the mass spectrometer was obtained. In cases where psychostimulants of the amphetamine type were known to be absent in a blood sample, no peak in the gas chromatogram and no response in the mass spectrometer could be observed. This fact makes it probable that gas chromatographic peaks observed at the correct position for these two stimulants after the extraction procedure described above are genuine, and not due to possible unknown components normally occurring in blood.

An inspection of Fig. 2 shows that the reference mass spectrum of phenmetrazine and the spectrum obtained from blood when using a 1% SE-30 gas chromatographic column are very similar. When the Carbowax column was used, an additional peak with m/e 157 appears and this peak is also found in the extracts from blood containing amphetamine, when the mass spectrum was taken at the retention time for phenmetrazine. Blank tests with blood samples devoid of sympathomimetic amines were run in the identical way as the analyses described above. In these instances, the peak at m/e 157 was absent. This indicates that the blood samples containing phenmetrazine or amphetamine also contain a compound of unknown structure, which — under the gas chromatographic conditions used — had a retention time very similar to that of phenmetrazine. By using a column with 1% SE-30 on Chromosorb W (80—100 mesh) it was possible to separate the unknown compound from phenmetrazine itself.

Some of the results obtained with blood samples from drivers are collected in the table. The table shows the concentrations of the amines in the blood and

Table. *Determination and identification of amphetamine and phenmetrazine in blood (and urine) with mass spectrometry, as well as some results of the medical checkup per-*

P = phenmetrazine, A = amphetamine, GLC = gas liquid chromato-

Case No.	Sample B = blood U = urine	Type amine of	mg amines per 100 ml according to GLC		Approx. amount injected into MS	Peaks recorded (m/e) by MS	Remarks
			blood	urine			
1	9 ml B	P	0.05	—	10 ng	177	narcotic addict (patient)
2	10 ml B	P	0.1	5.6	30 ng	177	—
3	9 ml B	P	trace	—	30 ng	177	car theft
4	11 ml B	P	0.1	—	40 ng	177	car theft
5	11 ml B	P	0.2	—	70 ng	complete spectrum	—
6	9 ml B	P	0.3	14	several μg	complete spectrum	car theft
7		P	0.4	29	several μg	complete spectrum	car theft (hepatitis)
8	25 ml U	P	—	27	1 μg	complete spectrum	—
9	11 ml B	A	0.02		10 ng	44, 91, 120	—
10	10 ml B	A	0.02	4.8	10 ng	44, 91, 120	trace of A found in a syringe
11	9 ml B	A	0.02	12	5 ng	44, 91, 120	car theft (hepatitis)
12	9 ml B	A	0.08	6.3	20 ng	91, 120,	two amines
		+P	trace	0.5	5 ng (?)	177	present
13	10 ml B	A	trace	1.4	5 ng (?)	91, 120,	two amines
		+P	trace	17	20 ng (?)	177	present
14	10 ml B	neg.	0	0	—	(177)	blank run

the urine (if available), the mass spectroscopic data obtained from blood analysis, and finally, some observations made by the police surgeon during the physical examination of the drivers in question.

Typical levels of phenmetrazine and amphetamine in the urine of 105 drug abusers were found to lie between 4 and 20 mg of drug per 100 ml of sample, though values of more than 50 mg per 100 ml have been observed [9]. The values for urine in the table are thus in the normal range. The concentrations in the blood were found to range between 0.02 and 0.4 mg of amine per 100 ml of sample and are 4 to 100 times larger than those reported by Campbell [6] for amphetamine in volunteers.

In 15 cases (cf. ref. 9 and the table) gas chromatography gave two peaks, at the retention times of amphetamine and of phenmetrazine respectively. It was uncertain if these two peaks really represented the two drugs in question or if one of them was due to a parent drug and the other to one of its metabolites with the same retention time as the other parent drug. The question could now be decided by the use of mass spectrometry. In two cases chosen at random

samples from 12 drivers and one hospital patient with the help of gas chromatography combined formed on the drivers. The analytical procedures used are explained in the text

graphy, MS = mass spectrometer. Also: u.t.i. = under the influence.

Age of driver (years)	Results of the medical check-up				
	status of eyes	Romberg's test (closed eyes)	finger to finger test	general behaviour	conclusions of the physician
—	not reported				
20	normal	unsteady	unsteady	calm	u.t.i. of drugs ? (uncertain)
17	bloodshot	refuses these tests		in high spirits	u.t.i. of drugs
21	glazed	unsteady	steady	excited	u.t.i. of drugs
24	glazed	unsteady	unsteady	resentful	u.t.i. of drugs
28	normal	unsteady	unsteady	chatty	u.t.i. of drugs
22	bloodshot	unsteady	unsteady	sluggish	u.t.i. of drugs
32	bloodshot	unsteady	unsteady	rattled	u.t.i. of drugs
27	bloodshot	steady	steady	restless	abstinence symptoms after abuse
23	glazed	unsteady	steady	natural	u.t.i. of drugs
30	bloodshot	—	unsteady	somewhat sluggish	exhaustion after drug abuse
26	glazed	very unsteady	unsteady	sluggish	u.t.i. of drugs
20	bloodshot	unsteady	unsteady	sluggish	u.t.i. of drugs
—	autopsy case with reasonable assurance of absence of sympathomimetic amines (suicide with barbiturates)				

(nr. 12 and 13 in the table) amphetamine and phenmetrazine could be separated and identified in the extracts from the same person's blood.

The police investigation and the medical check-up gave relatively uniform results. The age of the drivers, who were all male, ranged between 17 and 32 years. Five out of the twelve were driving stolen cars. A few of the individuals seemed to have been in the stimulated state of drug action (nr. 3, 4 and possibly 6) but the majority already showed abstinence symptoms (bloodshot eyes, sluggish behaviour). Only one or two of them passed the balance tests and showed relatively normal behaviour. The examining physicians considered them all to be under the influence of drugs. The correlation between abuse of stimulating drugs and clinical symptoms is much more straightforward than in the case of alcohol.

In none of the 12 drivers was there alcohol found in the blood[1].

1 Änggård et al. [16] has recently determined amphetamine levels in the serum of narcotic addicts with the help of derivative gas chromatography.

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References

1. Beckett, A. H., Tucker, G. T., Moffat, A. C.: Routine detection and identification in urine of stimulants and other drugs some of which may be used to modify performance in sport. J. Pharm. Pharmacol. **19**, 273—294 (1967).
2. Zwol, D. E. van: Gas-chromatographic separation and identification of some sympathomimetic amines used in anorexigenic drugs. J. Chromatog. **24**, 26—31 (1966).
3. Schubert, B.: Identification and metabolism of some doping substances in horses. VMD thesis. Acta vet. scand., Suppl. **21** (1967).
4. Beckett, A. H., Rowland, M.: Urinary excretion kinetics of amphetamine in man. J. Pharm. Pharmacol. **17**, 628—639 (1965).
5. — — Urinary excretion kinetics of methylamphetamine in man. J. Pharm. Pharmacol. **17**, Suppl. 109 (1965).
6. Campbell, D. B.: A method for the measurement of therapeutic levels of (+)-amphetamine in human plasma. J. Pharm. Pharmacol. **21**, 129—131 (1969).
7. Eberhardt, H., Debackere, M.: Der Nachweis von zentral stimulierenden Substanzen in reiner Form und nach Körperpassage mit der Dünnschichtchromatographie. Arzneimittel-Forsch. **15**, 929—930 (1965).
8. Heyndrickx, A., Leenheer, A. de: Toxicological analysis of weckamines (amphetamine, pervitin, preludin and ritalin) in pharmaceutical compounds and urine of persons suspected from doping. J. Pharm. (Belg.) **1967**, 109—126.
9. Bonnichsen, R., Maehly, A. C., Åqvist, S.: Arzneimittel und Fahrtüchtigkeit. II. Mitt. Zentralstimulierende Amine und aromatische Kohlenwasserstoffe. Blutalkohol **6**, 245—255 (1969).

9a. Petrovics, J.: To be published.

10. Hall, C. R., Cordova, V., Rieders, F.: Urinary excretion of amphetamine in man following oral administration of amphetamine, methylamphetamine and phenmetrazine as determined by a rapid gas chromatographic method. Pharmacologist **7**, 148 (1965).
11. Brandenberger, H., Hellbach, E.: Über die gas-chromatographische Bestimmung von Amphetamin als (1-Phenyl-isopropyl)-isothiocyanat. Helv. chim. Acta **50**, 958—962 (1967).
12. Gunne, L. M., Galland, L.: Stereoselective metabolism of amphetamine. Biochem. Pharmacol. **16**, 1374—1377 (1967).
13. Hedfjäll, B., Jansson, P.-Å., Mårde, Y., Ryhage, R., Wikstöm, S.: Data acquisition and evalutating systems for a low resolution mass spectrometer. J. Sci. Instrum., Ser. II, **2**, 1031—1036 (1969).
14. Mattson, H. W.: Determining molecular structure. Int. Sci. and Tech., Feb. 1963, p. 22—31; through Eisdorfer, I. B., Warren, R. J., Zarembo, J. E.: Spectroscopy of amines of pharmaceutical interest. J. Pharm. Sci. **57**, 195—217 (1968).
15. Sweeley, C. C., Elliott, W. H., Fries, I., Ryhage, R.: Mass Spectrometric determination of unresolved components in gas chromatographic effluents. Analyt. Chem. **38**, 1549—1553 (1966).
16. Änggård, E., Gunne, L.-M., Jönsson, L. E., Niklasson, F.: Personal communication, 1970.

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