Journal of Chromatography, 432 (1988) 290–296
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4344

Note

Gas chromatographic quantification of underivatized amphetamine in whole blood

Blood stored in plastic containers – a source of error

ASBJØRG SOLBERG CHRISTOPHERSEN*, ELLEN DAHLIN and GRETE PETTERSEN

National Institute of Forensic Toxicology, P.O. Box 16, Gaustad, 0320 Oslo 3 (Norway)

(First received December 31st, 1987; revised manuscript received June 13th, 1988)

Few methods for the quantification of amphetamine in whole blood have been reported. The procedures have mainly been worked out for plasma or urine samples [1–3]. Other described methods include complicated extraction steps, often combined with derivatization [4–6].

At our institute, amphetamine is frequently found in blood and urine samples from motor drivers. A selective, sensitive and simple method for whole blood quantification is necessary to estimate the dose taken and the degree of intoxication. Our earlier amphetamine quantification method with back-extraction used diethyl ether as the organic phase throughout. The method was complicated and poorly reproducible [6]. This paper describes a modification and simplification of this method that gives improved precision.

EXPERIMENTAL

Chemicals

Amphetamine sulphate was purchased from Norsk Medisinaldepot (Oslo, Norway). Quinoline, which was used as an internal standard, was obtained from Fluka (Buchs, Switzerland). Sodium chloride, sodium fluoride, sodium oxalate, diethyl ether and toluene, all p.a. grade, were from E. Merck (Darmstadt, F.R.G.). Diisopropyl ether (pest. grade) and methanol (HPLC grade) were from Fisons (Loughborough, U.K.). Dimethyldichlorosilane was from Supelco (Bellefonte, PA, U.S.A.).

0378-4347/88/$03.50

Instrumentation

Two different systems were used.

(1) Varian 3300 gas chromatograph (Palo Alto, CA, U.S.A.) with a nitrogen–phosphorus sensitive detector operated in the nitrogen mode and a silanized glass column (180 cm × 2 mm I.D.) with GP 10% Apiezon L/2% potassium hydroxide on 80–100 mesh Chromosorb W AW (Supelco). The operating conditions were: injector temperature 230°C; detector temperature 245°C; isothermal oven temperature, 170°C; carrier gas, nitrogen (30 ml/min); detector gases, hydrogen (4.5 ml/min) and air (70 ml/min).

(2) HP 5890 gas chromatograph (Hewlett-Packard, Avondale, PA, U.S.A.) with a nitrogen-specific detector (nitrogen mode) and a capillary split/splitless injector. The silica inlet liner was cleaned and silanized as described below. As a rule, it was changed for every new series or when peak-tailing appeared. The capillary column was cross-linked methylsilicone (12.5 m × 0.2 mm I.D., 0.32 μm film thickness) from Hewlett-Packard. The operating parameters were: injector temperature, 250°C; detector temperature, 250°C; column temperature, 60°C at injection and for 30 s, then 10°C/min to 130°C and then 40°C/min to 250°C. Gas flow-rates were: helium, 1.3 ml/min (carrier gas) and 30 ml/min (make-up gas); hydrogen, 3.5 ml/min and air, 60 ml/min (detector gases). The samples were injected splitless and the splitter was reopened after 30 s.

Cleaning of inlet liner

The liner was treated for 10 min in Lipsol (Lip, Shipley, U.K.) in an ultrasonic bath and for 10 min in Deconex 25 (Borer Chem., Solothurn, Switzerland). Subsequently, it was rinsed with distilled water and methanol, and air-dried. Finally it was treated for 30 min in 3% dimethyldichlorosilane in toluene, rinsed in toluene and water and dried in air.

Reference whole blood

Whole blood used for standard samples was collected into glass containers with sodium fluoride and sodium oxalate [to make 1% (w/v) of each] and kept in glass containers at −20°C until use. Standard blood bank blood (from Blood Bank Ullevål Hospital, Oslo, Norway) collected in transfusion PVC plastic bags (Travenol, Halden, Norway) was also tested. After storage for one to fourteen days in the plastic bags in a refrigerator at +4°C, it was transferred to glass containers and stored at −20°C until use.

Authentic samples

Blood was collected in polystyrene vials with polythene caps (Sterilin, Feltham, U.K.), containing sodium fluoride and sodium oxalate [1% (w/v) of each]. The samples were sent unfrozen through the mail and thereafter kept frozen (−20°C) in the laboratory until analysed.

Standard samples

Stock standards of amphetamine (4 μmol/ml) and quinoline (internal standard, I.S.) (15 μmol/l) were prepared in methanol and diluted in distilled water

to make working standard solutions. The concentrations used were 0.1 and 0.01 μmol/ml for amphetamine and 0.01 μmol/l for quinoline.

A calibration curve in the concentration range 0.5–8 μmol/ml was prepared by spiking blood with aliquots of amphetamine working standard solutions. Only blood stored in glass containers was used.

Extraction

To 1 ml of whole blood were added 1 ml of distilled water, 200 μl of quinoline solution (0.01 μmol/l), 300 μl of 5 *M* sodium hydroxide and 6 ml of diethyl ether. After shaking for 10 min and centrifugation, the organic phase was transferred to a new glass tube, washed with 2 ml of distilled water, centrifuged and transferred to a tapered glass tube. Then 30 μl of 6 *M* hydrochloric acid were added and the diethyl ether phase was evaporated under nitrogen (30°C). Next 100 μl of diisopropyl ether, 20–25 mg of solid sodium chloride and 50 μl of 5 *M* sodium hydroxide were added. After brief vortexing, 1 μl of organic phase was injected into the gas chromatograph.

Precision and inter-assay variation

Whole blood was spiked with amphetamine to concentrations of 0.5, 1, 2, 3 and 5 μmol/l. On the same day ten replicates were analysed from the different batches for calculation of the precision of the analysis. The remainder of the batches was dispensed into polystyrene vials, kept at −20°C and used for calculation of the inter-assay variation.

RESULTS AND DISCUSSION

The extracts were clean with no interfering peaks in both chromatographic systems (Figs. 1A and 2A). The calibration graph was linear from 0.5 to 8 μmol/l, with a regression coefficient of 0.998.

Figs. 1B and 2B show chromatograms from the packed and the capillary column system, respectively. Satisfactory chromatograms were obtained for amphetamine without derivatization in both systems. Methylamphetamine was separated from amphetamine on both columns and could be quantified by the same method.

The detection limit was 0.2 and 0.1 μmol/l for the packed and capillary column systems, respectively. This is sufficient for quantitative purposes (Figs. 1C and 2C).

Table I shows precision data from the two different systems. Diisopropyl ether, which is used in the last step, improved the reproducibility compared with diethyl ether. Intra-assay variability of less than 6% was obtained with diisopropyl ether (packed column system), compared with more than 20% with diethyl ether. Diisopropyl ether has a lower solubility in water than diethyl ether, and equilibrium is reached more readily. This is an improvement on the previous method [6]. Diethyl ether is more favourable in the first extraction step because of its higher volatility.

Table II shows recovery values for amphetamine and the I.S.

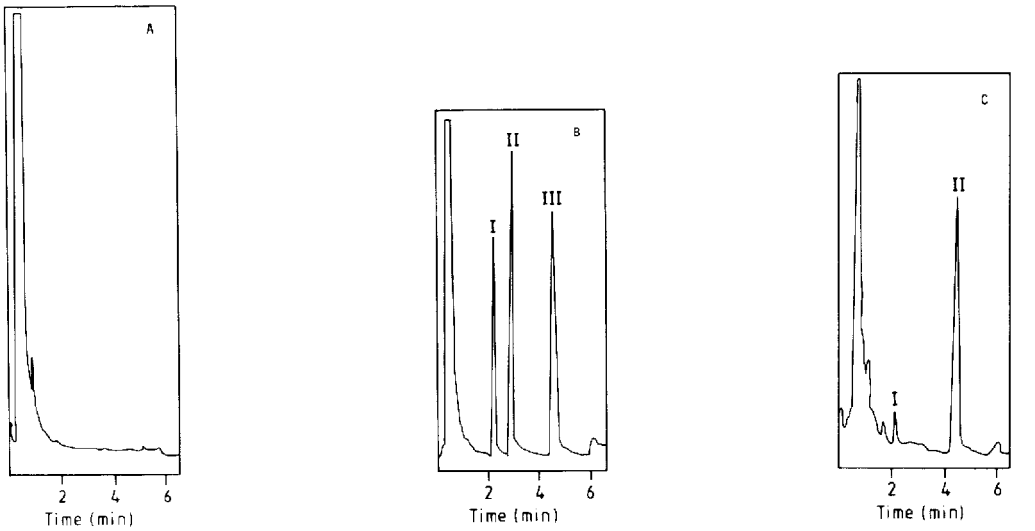

Fig. 1. Packed column chromatograms of (A) blank whole blood extract; (B) whole blood extract spiked with 2 μmol/l amphetamine (I), 2 μmol/l methylamphetamine (II) and 2 μmol/l quinoline (III); (C) whole blood extract spiked with 0.2 μmol/l amphetamine (I) and 2 μmol/l quinoline (II). Chromatographic conditions as in text.

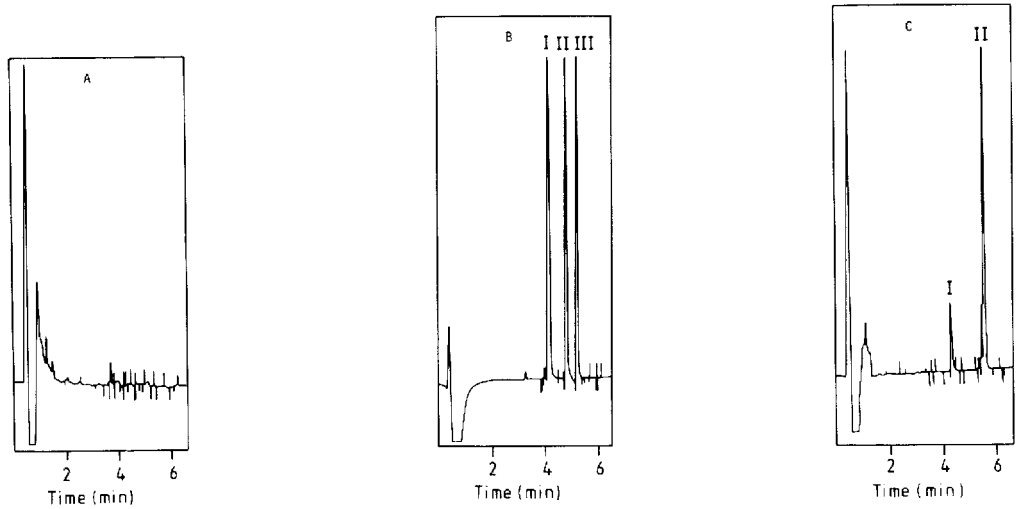

Fig. 2. Capillary column chromatograms of (A) blank whole blood extract; (B) mixture of amphetamine (I), methylamphetamine (II) and quinoline (III), 50 pmol of each injected; (C) whole blood extract spiked with 0.1 μmol/l amphetamine (I) and 2 μmol/l quinoline (II). Chromatographic conditions as in text.

The addition of sodium chloride is also an improvement, as the amphetamine recovery value is increased; without this addition the recovery was only 65%.

Compared with the previously described method [6] the extraction procedure has been simplified, as the first time-consuming back-extraction step into sulphuric acid has been omitted. This makes the handling of large sample numbers

TABLE I

PRECISION DATA FOR AMPHETAMINE QUANTIFICATION IN TWO GAS CHROMATOGRAPHIC SYSTEMS

$n = 10$ for all values.

Solvent	Amphetamine added (μmol/l)	Coefficient of variation (%)	
		Packed column	Capillary column
Diisopropyl ether	0.5	–	7.5
	1.0	6.1	–
	2.0	5.8	–
	3.0	4.0	6.6
	5.0	5.6	–
Diethyl ether	2.0	>20	

TABLE II

RECOVERY VALUES FROM WHOLE BLOOD STORED IN DIFFERENT CONTAINERS

Compound	Recovery (%)	Concentration (μmol/l)	Blood storage	Method
Amphetamine	91	2	Glass container	Described method
	95	5		
Quinoline	85	2	Glass container	Described method
Amphetamine	65	2	Glass container	Without sodium chloride
Amphetamine	55–65	2	Blood collected in PVC plastic bags	Described method
Amphetamine	>90	2	Collected and stored in polystyrene vials	Described method

more efficient. Most routine samples are now analysed on the packed column system, which gives the shortest analysis time, best precision (Table I) and most stable gas chromatographic conditions. The capillary column system is used in the routine for samples with special separation problems caused by the presence of other drugs or metabolites. Good chromatographic properties and a clean inlet liner are necessary for the analysis of underivatized amphetamine on the capillary column to avoid peak-tailing. It may be difficult to keep the capillary system stable over time to obtain satisfactory results.

In forensic toxicology more than one method is necessary for verification. In our laboratory, derivatization of amphetamine with trifluoroacetic anhydride is used for verification of blood samples [6]. When urine samples are also available,

they are screened for amphetamine by enzyme multiplied immunoassay (EMIT), followed by thin-layer chromatographic verification [6].

Inter-assay variation

The inter-assay variation for amphetamine was calculated to be 10.8% (1 μmol/l) and 10% (3 μmol/l), respectively, from the same spiked sample analysed with twelve (1.0 μmol/l) and eleven (3.0 μmol/l) different series on the packed column system.

Stability tests

The amphetamine concentration was stable in spiked and real samples stored for several months at −20°C. As shown in Table III, real samples reanalysed after storage for up to one year at −20°C showed no significant concentration loss. To test the sample containers, whole blood without amphetamine was stored for one week at room temperature in the vials used for real samples. No interfering peaks were obtained after extraction and chromatography in the system described above. We have previously reported that natural rubber septa used to seal blood-sampling vials may contaminate the sample and interfere with the amphetamine analysis [6]. Rubber septa are therefore not accepted for sample vials.

Blood bank blood

For standard and control samples, blood bank whole blood collected in transfusion PVC plastic bags (citrated whole human blood) was originally used. Occasionally, non-linear calibration graphs, low values of the slope and very poor precision were obtained with this blood, resulting in poor accuracy for samples.

Fig. 3 shows an example of two different calibration graphs where blood used for standard samples has been collected and stored in different containers. Unacceptably different values for the same sample will result from the two different

TABLE III

AMPHETAMINE CONCENTRATIONS IN BLOOD SAMPLES STORED FOR DIFFERENT TIME INTERVALS AT −20°C

Result from first analysis (μmol/l)	Result from second analysis (μmol/l)	Storage time (months)	Type of sample
3.1	3.5	4	Spiked
3.4	3.3	3	Spiked
2.9	3.2	4	Spiked
1.1	0.9	4	Spiked
3.4	3.2	4	Spiked
2.9	2.7	3	Spiked
1.0	1.0	3	Spiked
2.3	2.1	11	Authentic
2.1	2.3	11	Authentic
1.1	1.0	4	Authentic
1.5	1.7	12	Authentic

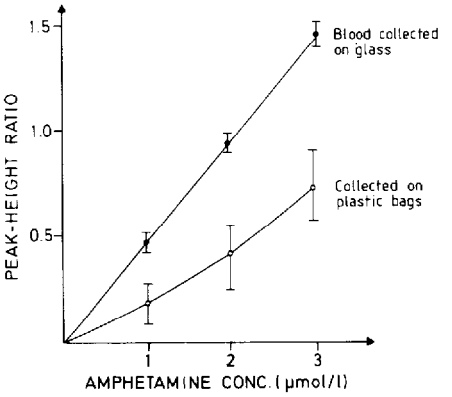

Fig. 3. Examples of two different amphetamine calibration graphs from whole blood collected in PVC plastic bags and whole blood collected in glass containers. The vertical bars indicate the variation at each concentration.

graphs. Blank samples from blood collected in transfusion bags showed no interfering peaks and no other peaks that were not seen in blood collected in glass containers.

The amphetamine recovery obtained from some of these plastic-bag blood batches varied from 55% to ca. 65%, while the I.S. recovery showed a very small variation (Table II). Recovery values for blood collected directly in the polystyrene containers used for the real samples were more than 90%, the same as for blood collected in glass containers.

The problems with the blood bank blood may be caused by diffusion of chemicals from the plastic into the blood and reaction with amphetamine. The possible new product might not be extracted or might have chromatographic properties that made it undetectable in our systems. These problems are avoided when blood used for standards was collected and stored in glass containers (with sodium fluoride and sodium oxalate, which are also used for real samples). It must be emphasized that control samples are important to follow the inter-assay variation and to avoid problems with blood that gives a low recovery of amphetamine.

REFERENCES

1 K. Shimosato, M. Tomita and I. Ijiri, Arch. Toxicol., 59 (1986) 135-140.
2 C.M. Kuhn and S.M. Shanberg, J. Pharmacol. Exp. Ther., 207 (1978) 544-554.
3 M. Endo, H. Imamichi, M. Moriyasu and Y. Hashimoto, J. Chromatogr., 196 (1980) 334-336.
4 B.M. Farrell and T.M. Jefferies, J. Chromatogr., 272 (1983) 111-128.
5 K. Hara, T. Nagata and K. Kimura, Z. Rechtsmed., 96 (1986) 93-104.
6 A.S. Christophersen, A. Bugge, E. Dahlin, J. Mørland and G. Wethe, J. Anal. Toxicol., 12 (1988) 147-149.