Z. Rechtsmedizin 81, 25–30 (1978)

Zeitschrift für
Rechtsmedizin

A Micromethod for the Isolation of Drugs from Blood Using Amberlite XAD-2

H. J. Schlicht and H. P. Gelbke

Institut für Rechtsmedizin der Universität, Voßstraße 2, D-6900 Heidelberg, Federal Republic of Germany

Summary. The extraction of drugs from small blood samples (1 ml or less) for subsequent quantitative determination is described. Isolation was carried out by adsorption of the drugs to Amberlite XAD-2 resin utilizing a batch procedure that enabled the simultaneous extraction of up to 200 samples in approx. 5 hours. A new desorption technique yielded extracts of high purity that could be used directly for gas chromatographic or radioimmunological determinations, even if hemolyzed or putrid blood was to be examined. The following 26 substances were quantitated after addition to postmortem blood specimens at concentrations of 1–10 μg/ml: tilidine, diphenhydramine, dibenzepine, imipramine, chlorpromazine, amphetamine, pentazocine, phenacetin, methaqualone, meprobamate, parathion, diazepam, digoxin, β-methyldigoxin, carbromal, glutethimide, amobarbital, pentobarbital, cyclobarbital, phenobarbital, d,phenylhydantoin, carbutamide, tolbutamide, glycodiazin, tolazamide and chlorpropamide. Thereby recoveries of 60–100 % could be achieved. The reproducibility of the procedure was satisfactory as demonstrated by coefficients of variation of 3.7–8 %.

Key words: Amberlite XAD-2, Isolation of Drugs – Drugs, Isolation in Blood

Zusammenfassung. Es wird die Extraktion von Arzneistoffen aus kleinen Blutproben (1 ml oder weniger) für die nachfolgende quantitative Bestimmung beschrieben. Die Isolierung der Substanzen erfolgte durch Adsorption an ein Kunstharz (Amberlite XAD-2) mittels eines Batch-Verfahrens, das die Aufarbeitung von bis zu 200 Proben in ungefähr 5 Stunden ermöglichte. Die Anwendung einer neuartigen Desorptionstechnik führte selbst bei zersetzten Blutproben zu sehr sauberen Extrakten, die direkt für gaschromatographische oder radioimmunologische Bestimmungen verwendet werden konnten. Nach Zusatz zu Leichenblut in Konzentrationen von 1–10 μg/ml wurden die folgenden 26 Wirkstoffe quantitativ bestimmt: Tilidin, Diphenhydramin, Dibenzepin, Imipramin, Chlorpromazin, Amphetamin, Pentazocin, Phenacetin, Methaqualon, Meprobamat, Parathion, Diazepam, Digoxin, β-Methyldigoxin, Carbromal, Glutethimid, Amobarbital, Pentobarbital, Cyclobarbital, Phenobarbital, Diphenylhydantoin, Carbutamid, Tolbutamid, Glycodiazin, Tolazamid und Chlorpropamid. Dabei konnten Wiederfindungen von 60–100 % erreicht werden. Die Reproduzierbarkeit war bei Variationskoeffizienten von 3,7–8 % zufriedenstellend.

Schlüsselwörter: Amberlite XAD-2, Isolierung von Arzneistoffen – Arzneistoffe, Isolierung im Blut

The problem of drug consumption during the last years resulted in a significant increase of the amount of samples for toxicological examination. In this context, the evaluation of the degree of impairment by drugs is highly important, especially in cases of traffic violations or crimes. As generally accepted, only blood levels render a rational basis for the estimation of drug effects [e.g., 1], but often the small amounts of blood available prevent systematic routine investigations. With the advent of highly sensitive analytical procedures (e.g., RIA, GC, GC-MS) the measurement even of those amounts of drugs, that are to be expected in 1 ml of blood or less, presents no problem today. On the other hand, most of the isolation procedures generally used are unsatisfactory with respect to practicability, recovery and purity of the final extracts.

Several authors have described the application of Amberlite XAD-2, an unpolar styrene-divinylbenzene copolymer, to the extraction of drugs from blood [2–10]. These investigations prompted the elaboration of the micromethod presented here for serial determinations in daily routine work.

Materials and Methods

Amberlite XAD-2 resin (Serva, Heidelberg, GFR; particle size 50–100 μ) was purified by continuous Soxhlet-extraction with ethyl acetate for at least 6 h. Afterwards the resin was washed twice with acetone and 4 times with water. All solvents and reagents were of p.a.-grade and were used as supplied. Radioimmunological determinations of digoxin and β-methyldigoxin were carried out with a commercial digoxin kit (NEN, Mass.). The ^{14}C-diazepam was generously donated by the Hoffmann-La Roche A. G. (Grenzach-Wyhlen, G. F. R.) and had a radiochemical purity of better than 98 % as checked by radio thin-layer chromatography. Scintillation counting was carried out with a Berthold BF 5000 using Unisolve 1.

Extraction procedure A (for basic and neutral substances): 1 ml of blood was pipetted into a polypropylene tube (ϕ 10 mm). 1 ml of an Amberlite XAD-2 slurry in 5 % aqueous ammonia (1 g/10 ml) was added and the suspension was shaken for 20 min. After centrifugation (3 min, 4000 rpm) the supernatant was discarded and the residue was twice washed with 2 ml of an 5 % aqueous ammonia solution by shaking for 5–10 min, centrifugation and decantation. Then 1 ml of the 5 % aqueous ammonia solution and 1.5 ml of ethyl acetate were added to the resin. After shaking for another 15 min the layers were separated by centrifugation (5 min, 4000 rpm). The ethyl acetate was evaporated and the dry material was used for quantitative determinations by radioimmunoassay or gas chromatography.

Extraction procedure B (barbiturates): Adsorption of the drugs to the resin and subsequent washing was carried out according to procedure A, using acetate buffer (pH 4.6, 0.2 molar) instead of the ammonia solution. The prepurified resin thus obtained was suspended in 2 ml of ether and shaken for 15 min for desorption of the drugs. After centrifugation (5 min, 4000 rpm) the solvent was transferred into another polypropylene tube and evaporated under a stream of nitrogen at room temp. The residue was partitioned between 1 ml of the buffer and 2 ml of ether. The ether was evaporated and the residue was used for gas chromatography.

Procedure C (other acidic drugs): The procedure C corresponds to procedure A using acetate buffer (pH 4.6, 0.2 molar) instead of the ammonia solution.

Gas chromatography. Perkin Elmer F 22 gas chromatographs equipped with FID, ECD and nitrogen-phosphorous sensitive detector were used under the following conditions: carrier gas 40 ml of N_2/min, ECD purge gas 70 ml/min of argon-methane (95:5), detector temp 320°C, injection port temp 30°C above the column temp, glass columns (200 x 0.3 cm); stationary phase: 3 % of SP 2250 DA or 3 % of SP 2250 DB on Supelcoport 100/120 (Supelco Inc., Pens.) or 3 % OV-17 or 3 % SE 30 on Chromosorb W AW-DMCS 80/100.

Derivatization Procedures

Amphetamine: Acetylation with approx. 100 μl of acetic acid anhydride-pyridine (1:1) 30 min at room temp. The solution was evaporated and the residue dissolved in methanol.

Carbromal: Reaction with dimethylformamide dimethylacetal 30 min at 100°C. The reagent was evaporated at room temp and the residue dissolved in methanol.

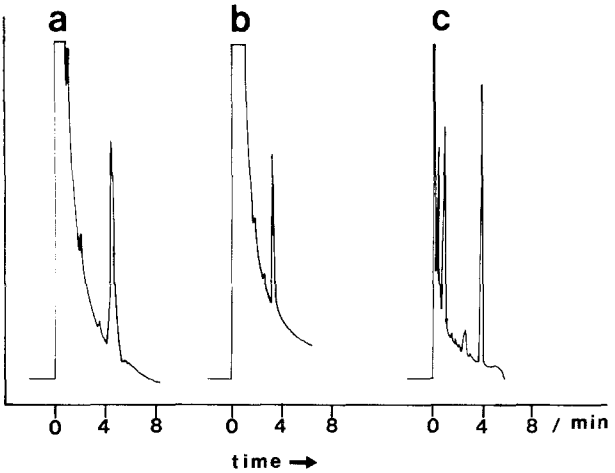

Fig. 1. Gas chromatograms obtained for tilidine (a), glutethimide (b), and diphenylhydantoine (c) after extraction from blood with Amberlite XAD-2. a 1 μg of tilidine/ml; GLC: SE 30, 180°C, nitrogen sensitive detector. b 10 μg of glutethimide/ml; GLC: SE 30, 180°C, nitrogen sensitive detector. c 2 μg of diphenylhydantoine/ml; GLC: methylation, OV-17, 245°C, nitrogen specific detector

Diphenylhydantoine and Glycodiazin: Methylation by reaction with 200 μl of an ethereal diazomethane solution for 20 min at room temp. After evaporation of the solution the residue was dissolved in methanol.

Tolbutamide, carbutamide, tolazamide, and chlorpropamide were reacted to the methyl-trifluoroacetyl derivatives according to Braselton et al. [11].

For calculation of the recoveries postmortem blood specimens were spiked with known amounts of drugs (1–10 μg/ml) and extracted as described above. After gas chromatography the peak heights of the drugs recovered were related in % to those obtained with known reference solutions. Quantitation of digoxin and β-methyldigoxin was carried out by radioimmunoassay.

Results

Recoveries of the drugs examined after addition to postmortem blood specimens varied between 60–100 %. Details and analytical conditions are given in Table 1. The purity of the extracts finally obtained is demonstrated by the gas chromatograms in Fig. 1. Peaks corresponding to any of the drugs were not observed if blood was extracted by the procedure described here without prior addition of drugs. The reproducibility of the procedure was determined for 9 different drugs by extracting and subsequent quantitation of 10 samples in parallel. The coefficients of variation were found to be between 3.7–8 % (Tab. 2). Recoveries did not depend on the absolute amounts of the drugs for concentrations between 1–100 μg/ml as checked for methaqualone, tilidine, and pentobarbital.

Discussion

The use of the Amberlite XAD-2 resin for isolation of drugs allowed to avoid the drawbacks of conventional solvent extraction or protein precipitation methods as there are significant substance losses, formation of emulsion, the need of large amounts of solvents and often time-consuming further purification steps. Application of the resin enables analysis of hemolyzed or putrid blood without additional problems. As compared to the column chromatographic XAD-2 extraction procedures described

Table 1. Recovery of drugs from blood using Amberlite XAD-2. 1 ml of blood was extracted with the resin as described in the experimental section. The recoveries given are the mean of four determinations

Substance	Concentration (μg/ml)	GLC-conditions	Amount of solvent/ aliquot injected [c]	Extraction procedure	Recovery
Amphetamine [a]	2	SE 30, 180°C, PND	50/2	A	95 %
Chlorpromazine	0.5	SE 30, 235°C, PND	50/2	A	83 %
Dibenzepine	1	SE 30, 240°C, PND	50/2	A	98 %
Diphenhydramine	1	SP 2250 DB, 200°C, PND	50/2	A	90 %
Imipramine	0.5	SE 30, 215°C, PND	50/2	A	90 %
Pentazocine	1	SE 30, 225°C, PND	50/2	A	96 %
Tilidine	1	SE 30, 180°C, PND	50/2	A	100 %
Carbromal [a]	5	SE 30, 140°C, PND	50/2	A	98 %
Diazepam	0.2	OV 17, 250°C, ECD	50/2	A	85 %
Digoxin [b]	0.002	-	-	A	60 %
β-Methyldigoxin [b]	0.002	-	-	A	80 %
Glutethimide	10	SE 30, 180°C, PND	50/2	A	85 %
Meprobamate	10	SE 30, 180°C, FID	50/2	A	90 %
Methaqualone	1	SE 30, 200°C, PND	50/2	A	92 %
Parathion	1	SE 30, 200°C, PND	100/1	A	73 %
Phenacetin	1	SP 2250 DB, 200 °C, PND	50/2	A	70 %
Amobarbital	5	SP 2250 DA, 200°C, PND	50/2	B	75 %
Carbutamide	10	SE 30, 230°C, ECD	500/2	C	81 %
Chlorpropamide [a]	10	SE 30, 205°C, ECD	500/2	C	88 %
Cyclobarbital	5	SP 2250 DA, 240°C, PND	50/2	B	100 %
Diphenylhydantoine [a]	2	OV 17, 245°C, PND	50/2	C	95 %
Glycodiazin [a]	10	SE 30, 250°C, PND	500/2	C	70 %
Pentobarbital	5	SP 2250 DA, 200°C, PND	50/2	B	98 %
Phenobarbital	5	SP 2250 DA, 240°C, PND	50/2	B	95 %
Tolazamide [a]	10	SE 30, 245°C, ECD	500/2	C	84 %
Tolbutamide [a]	10	SE 30, 205°C, ECD	500/2	C	70 %

[a] Derivatization according to the experimental section

[b] Quantitation by radioimmunoassay

[c] For example: 50/2 means, the residue finally obtained was dissolved in 50μl solvent and 2 μl were injected into the gas-chromatograph

Table 2. Reproducibility of the extraction procedure

Drug	Concentration (μg/ml)	Detection by	CV
Chlorpropamide [a]	10	GLC	4.2 %
Diazepam	0.2	GLC	3.6 %
Digoxin	0.002	radioisotope counting	3.8 %
Glycodiazin [a]	10	GLC	8 %
Methaqualone	2	GLC	3.7 %
Pentobarbital	5	GLC	4.4 %
Tilidine	2	GLC	8.9 %
Tolazamide [a]	10	GLC	5.4 %
Tolbutamide [b]	10	GLC	4.2 %

CV = Coeffizient of variation calculated from 10 parallel measurements
[a] Tolbutamide was used as internal standard
[b] Chlorpropamide was used as internal standard

previously [3–6], the batch procedure elaborated here needs definitely smaller amounts of blood (1 ml or less) and reagents resulting in a high practicability (200 samples can be extracted within approx. 5 h by one technican). Thus, with respect to cost and time, this method is very useful for serial determinations and daily routine work. In this connection a note of Missen [9] should be mentioned who compared an Amberlite XAD-2 batch procedure with charcoal and celite for the isolation of diazepam and nitrazepam from blood.

Optimal recoveries were obtained by careful selection of the extraction conditions. A decrease of the recovery was found if acidic drugs were adsorbed to the resin from alkaline media or basic drugs from acidic media, an effect already attributed by other authors [6, 12–17] to be due to the unpolar structure of the resin which preferably adsorbs unpolar unionisized compounds. Therefore, the drugs were divided into two groups, one containing the basic and neutral drugs, which were adsorbed from alkaline blood, and another containing the acidic ones, which were adsorbed from slightly acidic blood.

When comparing different solvents and techniques for desorption, best results were obtained when the resin was suspended in a buffer of pH 4.6 (acidic drugs) or aqueous ammonia (basic and neutral drugs) and subsequently extracted with ethyl acetate. Only for barbiturates, higher recoveries and purities of the final extracts were observed when the resin was directly extracted with ether.

Apart from the barbiturates, after evaporation of the solvent the extracts could be used directly for gas chromatography. Barbiturates had to be subjected to an additional solvent extraction step prior to gas chromatography to guarantee sufficient purity. Combined with the high sensitivity and specificity of the electron capture and the phosphorous-nitrogen sensitive detector it was possible to determine even therapeutic drug levels in 1 ml blood samples.

For radioimmunological purposes some special advantages should be stressed: Radioimmunoassays with ^{14}C and ^{3}H tracer compounds cannot be directly applied to blood due to serious quenching problems. Radioimmunological results obtained when serum is directly used for the assay often show unsatisfactory reliability due to a high

unspecific protein binding. Both of these problems can be solved by prepurification of the material using the procedure presented here. For serial determinations of diazepam in blood and serum this extraction procedure was extensively studied and found to be the method of choice for radioimmunological screenings [18].

The method presented here is time-saving and enables the isolation of a wide range of drugs from one ml or less of blood with high recovery and purity. It proved to be a good tool for the daily routine work of clinical or forensic toxicologists.

References

1. Garriot, J. C., Di Maio, V. J. M., Zumwalt, R. E., Petty, C. S.: Incidence of drugs and alcohol in fatally injured motor vehicle drivers. J. forens. Sci. **22**, 283–389 (1977)
2. Missen, A. W., Lewin, J. F.: Non-ionic resin extraction of drugs in blood. Clin. Chim. Acta **53**, 389–390 (1974)
3. Pranitis, P. A. F., Milzoff, J. R., Stolman, A.: Extraction of drugs from biofluids and tissues with XAD-2 resin. J. forens. Sci. **19**, 917–926 (1974)
4. Ibrahim, G., Andryauskas, S., Bastos, M. L.: Application of Amberlite XAD-2 resin for general toxicological analysis. J. Chromatog. **108**, 107–116 (1975)
5. Machata, G., Vycudilik, W.: Isolierung von Drogen aus Blut mit makroretikulären Adsorberharzen. Arch. Toxicol. **33**, 115–122 (1975)
6. Pranitis, P. A. F., Stolman, A.: The differential elution of drugs from XAD-2 resin. J. forens. Sci. **20**, 726–730 (1975)
7. Vycudilik, W.: Isolation of drugs with macroreticular resins. Determination of Phentermine in blood. J. Chromatog. **111**, 439–442 (1975)
8. Dupeyron, J. P., Quattroccki, F., Fabiani, P.: Recherche et dosage de toxiques dans le sang et l'urine par chromatographie sur résine d'Amberlite XAD-2. Dérivés terpéniques volatils. Ann. Fals. Exp. Chim. **69**, 229–246 (1976)
9. Missen, A. W.: Procedures compared for extracting benzodiazepines from blood. Clin. Chem. **22**, 927–928 (1976)
10. Gelbke, H. P., Grell, T. H., Schmidt, Gg.: Isolation of Drugs from Blood by column chromatography on Amberlite XAD-2. Arch. Toxicol. (In press)
11. Braselton, W. E., Ashline, H. C., Bransome, E. D.: Formation of thermally stable derivatives for determination of Tolbutamide and Chlorpropamide by gas chromatography. Anal. Letters **8**, 301–313 (1975)
12. Bastos, M. L., Jukofsky, D., Saffer, E., Chedekel, M., Mulé, S. J.: Modifications of the XAD-2 resin column method for the extraction of drugs of abuse from human urine. J. Chromatog. **71**, 549–553 (1972)
13. Kullberg, M. P., Miller, W. L., McGowan, F. J., Doctor, E. P.: Studies on the single extraction of amphetamine and phenobarbital from urine using XAD-2 resin. Biochem. Med. **7**, 323–335 (1973)
14. Miller, W. L., Kullberg, M. P., Banning, M. E., Brown, L. D., Doctor, E. P.: Studies on the quantitative extraction of morphine from urine using nonionic XAD-2 resin. Biochem. Med. **7**, 145–158 (1973)
15. Roerig, D. L., Lewand, D., Mueller, M., Wang, R. I. H.: Methods of identification and confirmation of abusive drugs in human urine. J. Chromatog. **110**, 349–359 (1975)
16. Delbeke, F. T., Debackere, M.: Detection of sympathomimetic central nervous stimulants with special reference to doping. I. Comparative study of a conventional extraction procedure and adsorption chromatography using XAD-2 resin. J. Chromatog. **133**, 214–217 (1977)
17. Pietrzy, D. J., Chu, C. H.: Amberlite XAD copolymers in reversed phase gravity flow and high pressure liquid chromatography. Anal. Chem. **49**, 757–764 (1977)
18. Gelbke, H. P., Schlicht, H. J., Schmidt, Gg.: Radioimmunological screening and gas chromatographic identification of diazepam in blood and serum. Arch. Toxicol. **38**, 295–305 (1977)

Received October 20, 1977