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Note

Gas chromatographic procedure for the simultaneous determination of five common antidiabetic drugs in blood

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For many years, chlorpropamide, tolbutamide, carbutamide, tolazamide and glycodiazin have been successfully applied to the treatment of diabetes mellitus. As these compounds can induce fatal hypoglycemia[1,2] and also interact with alcohol in man, they are of interest in forensic, traffic and insurance medicine, and analytical methods for quantitation of these drugs in biological material, especially in blood, should be available to the forensic toxicologist.

Procedures so far used for blood-level measurements of these substances[3–5] generally allow the determination of only one compound. This is a serious drawback if blood specimens have to be examined for an antidiabetic drug, the nature of which is unknown. We have developed a method that enables simultaneous quantitation of all five agents in one blood sample.

MATERIALS AND METHODS

Amberlite XAD-2 resin (Serva, Heidelberg, G.F.R.; particle size 50–100 μm) was purified by continuous Soxhlet extraction with ethyl acetate for at least 6 h. The resin was then washed twice with acetone and four times with water. All solvents and reagents were of analytical grade and were used as supplied.

Blood (0.1–0.5 ml) was pipetted into a polypropylene tube (10 mm I.D.). A volume of 1 ml of an Amberlite XAD-2 slurry in 0.2 *M* acetate buffer of pH 4.6 (1 g/10 ml) was added and the suspension was shaken for 20 min. After centrifugation (3 min, 2200 *g*), the supernatant was discarded and the resin was twice washed with 2 ml of the buffer by shaking for 5–10 min, centrifugation and decantation. Then 1 ml of the buffer and 1.5 ml of ethyl acetate were added to the resin and, after shaking for another 15 min, the phases were separated by centrifugation (3 min, 2200 *g*). An aliquot of the ethyl acetate extract containing *ca.* 0.1–2 μg of the drug was evaporated under a stream of nitrogen. The residue was treated with diazomethane and trifluoroacetic acid anhydride, as described by Braselton *et al.*[6], and then screened for the five compounds by gas chromatography.

Quantitative determinations were carried out only after identification of one of the antidiabetic drugs in a blood sample. For these measurements further aliquots of the ethyl acetate extracts were evaporated and derivatized after addition of an appropriate amount of internal standard (chlorpropamide or tolbutamide). For ref-

erence purposes a standard curve was constructed by addition of known amounts of each drug to drug-free blood specimens and subsequent treatment in parallel with the unknown samples. For the final evaluation the quotient of internal standard and substance peak heights of the unknown sample was compared with a reference value.

For gas chromatographic analysis a Perkin-Elmer F-22 gas chromatograph with a Ni-63 electron-capture detector (ECD) was used under the following conditions: helium carrier gas flow-rate, 40 ml/min; ECD purge gas: argon–methane (95:5), at a flow-rate of 70 ml/min; detector temp., 320°; injector temperature, 250°; glass columns, 200 × 0.3 cm I.D.; stationary phases, 3% SE-30, 5% SE-30 or 2% OV-17 on Chromosorb W AW DMCS (80–100 mesh). For the oven temperature the following programmes were selected:

3% SE-30: 1 min at 195°, 10°/min to 250°, 5 min at 250°
5% SE-30: 1 min at 220°, 10°/min to 280°, 5 min at 280°
2% OV-17: 1 min at 210°, 10°/min to 250°, 5 min at 250°

Mass spectrometry was carried out with a Hewlett-Packard 5992-A gas chromatograph–mass spectrometer at 70 eV.

RESULTS

Table I lists the recoveries of the extraction procedure, the detection limits, the reproducibility and the retention times of the compounds on different stationary phases. The sensitivity of the procedure is also demonstrated by the gas chromatogram (Fig. 1). For elucidation of the structure, the new derivatives of glycodiazin (Fig. 2a) and carbutamide (Fig. 2b) were subjected to gas chromatography–mass spectrometry. The fragmentation patterns were as follows.

Derivative of glycodiazin (Fig. 2a)

Mol. peak (*m/e* 323, 1.6%); expulsion of SO_2 and subsequent loss of a hydrogen atom (*m/e* 258, 100%); expulsion of SO_2 and loss of $-(CH_2)_2-O-CH_3$ (*m/e* 200, 23%); fragments $-(CH_2)_2-O-CH_3$ (*m/e* 59, 14%) and C_6H_5 (*m/e* 77, 20%).

TABLE I

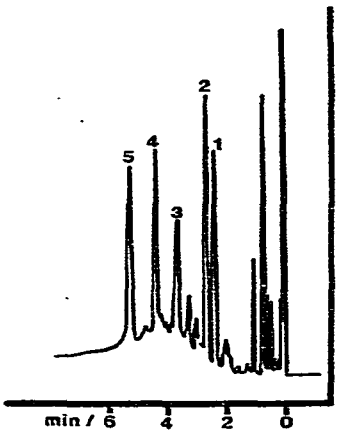
ANALYTICAL DATA FOR THE REPORTED PROCEDURE

Substance	*RE**	*Sensitivity (ng/ml of blood)*	*CV***	*Retention time (min)****		
				3% SE-30	*5% SE-30*	*2% OV-17*
Chlorpropamide	88%	250	4.2%	3.4	4.6	3.2
Tolbutamide	70%	250	4.2%	4.0	5.2	3.6
Carbutamide	81%	3000	26.0%	6.0	7.2	5.6
Tolazamide	84%	500	5.4%	6.8	8.8	6.8
Glycodiazin	70%	1500	8.0%	8.0	10.4	9.2

* RE = recovery of the extraction procedure. Mean of five determinations. Concentration: 10 μg/ml of blood.

** CV = coefficient of variation for the reproducibility of serial determinations. $N = 10$. Concentration: 10 μg/ml of blood.

*** GC conditions are given under Materials and methods.



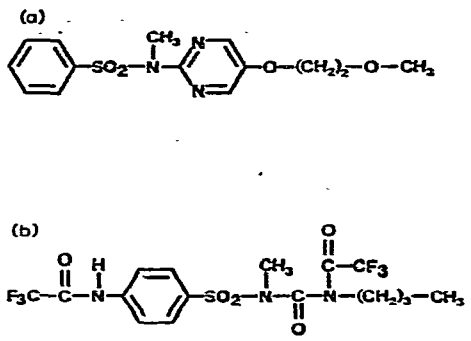


Fig. 1. Gas chromatographic separation of the five antidiabetic agents after methylation and trifluoroacetylation. The following amounts were added to 0.5 ml of blood and determined by gas chromatography on 5% SE-30 as described in the experimental section: chlorpropamide (1), 500 ng; tolbutamide (2), 500 ng; carbutamide (3), 3 μg; tolazamide (4), 1 μg; glycodiazin (5), 3 μg.

Fig. 2. Structures of the glycodiazin (a) and carbutamide (b) derivative.

Derivative of carbutamide (Fig. 2b)

The fragmentation was analogous to that of other methyl trifluoroacetyl sulphonylurea derivatives described previously[8]. Fragments corresponding to those described in that paper were, *e.g.*, *m/e* 209, 100%; *m/e* 188, 73%; *m/e* 252, 55%; *m/e* 282, 30%; *m/e* 413, 5%.

DISCUSSION

The extracts obtained by the Amberlite XAD-2 procedure described here could be used for quantitative measurements without further purification even if haemolized or putrid blood samples were examined. After extraction of drug-free blood specimens no peaks interfering with the five antidiabetic agents have been observed. In this connection it should be mentioned that this extraction method, sometimes slightly modified, has also been successfully applied to the isolation of other drugs from blood or serum[7].

As the drugs examined are unstable under gas chromatographic (GC) conditions[6] they have to be derivatized to obtain reliable results. A procedure for the formation of thermally stable derivatives of chlorpropamide, tolbutamide and tolazamide has been reported[6]. In the present investigation this method was successfully extended to carbutamide and glycodiazin, the derivatives of which exhibited excellent GC properties. Thus a procedure was developed that enabled the simultaneous determination of chlorpropamide, tolbutamide, carbutamide, tolazamide and glycodiazin.

As shown in Table I (apart from carbutamide) a good reproducibility was achieved for the procedure if chlorpropamide or tolbutamide were used as internal standard. The lower reproducibility for carbutamide might be due to the amino group, which is acetylated during derivatization. Thus, the yield of the carbutamide deriv-

ative depends on the trifluoroacetylation of two different nitrogen atoms of the molecule, in contrast to the internal standards, which are acetylated at only one nitrogen atom. Therefore variations of the yield of the carbutamide trifluoroacetic acid derivative cannot be exactly compensated by using chlorpropamide or tolbutamide as internal standard. Nevertheless, the most important question for the forensic toxicologist, whether or not a significant dose of carbutamide was incorporated, can still be answered.

The sensitivity and applicability of the procedure were tested by analysing 0.5-ml blood specimens of diabetic patients who had received a single therapeutic dose of one of these drugs. The measured blood levels corresponded well to those reported in the literature, and it was possible to detect tolazamide and glycodiazin at least 12 h, and chlorpropamide, tolbutamide and carbutamide at least 24 h, after intake[9].

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