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A Specific G. C. Method for the Determination of Carbamazepine in Blood

KAREN WORM
Institute of Forensic Medicine, Department of Forensic Chemistry
University of Copenhagen (Denmark)

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Eine spezifische GC Methode für die Bestimmung von Carbamazepin im Blut

Summary: Carbamazepine is extracted with dichloromethane from whole blood. After a rinsing procedure the residue is gaschromatographed partly in methanol, where two peaks occur, and partly in a methylating reagent, where the same two peaks are seen, the heights of these now being reversed. This reversal is used as a means of identification. Recovery was found to be 94 ± 12 %. Blood analysis from 12 patients being treated with carbamazepine gave values from 0.6 - 6.5 mg/kg.

Zusammenfassung: Carbamazepin wird mittels Dichlormethan aus Vollblut extrahiert. Nach Reinigung wird der Rest zuerst in Methanol gaschromatographiert, wo 2 Peaks auftreten, und danach in einem Methylierungsreagens, wo die gleichen 2 Peaks beobachtet werden, deren Höhen aber jetzt umgekehrt sind. Diese Änderung der Peakhöhen wird zur Identifikation von Carbamazepin benutzt. Wiedergefunden wurden 94 ± 12%. Blutanalysen von 12 Patienten die mit Carbamazepin behandelt worden waren, ergaben Werte von 0.6 - 6.5 mg/kg.

Key word: **Carbamazepine, determination in blood**

The anticonvulsant compound carbamazepine Tegretol (R) often has to be determined in small volumes of blood taken from traffic offenders. Mainly following the method described by ROGER *et al.* (1973) this determination can be performed satisfactorily from a forensic point of view by adding a special gaschromatographic procedure for further identification of the compound. By increasing the gaschromatographic run from 4 to 9 minutes, phenobarbital, primidone, and diphenylhydantoin may be determined by the same procedure.

Several methods are available for the determination of carbamazepine. GARDNER-THORPE *et al.* (1971, 1972) and LARSEN *et al.* (1969) both make a direct extraction combined with GLC without any cleaning of the extract. The specificity in such a procedure cannot be considered satisfactorily for forensic use, where you may have to analyse blood samples partly from traffic offenders, who notoriously may be considerable consumers of many different drugs, and partly from corpses, where putrefaction-products may occur, which may also interfere

with a gaschromatographic determination of a drug. An extraction combined with a rinsing by hexane is used by KUPFERBERG (1972), FRIEL and GREEN (1973) and ROGER *et al.* (1973). The two first mentioned split up the extract into an acidic fraction and a fraction containing carbamazepine before the GLC, while the method described by ROGER and coworkers only yields one fraction. Because of this we have chosen the last mentioned method, and introduced a modification of the gaschromatographic quantitation, which essentially increases the specificity of the method. This is achieved by injecting into the gaschromatograph aliquots of the residue before and after its methylation, thereby producing a peak pattern, which is unique for carbamazepine.

EXPERIMENTAL

Extraction: To 2.0 g of blood is added 12.0 ml of dichloromethane, and the mixture is shaken for 10 minutes. After centrifugation the dichloromethane layer is filtered, and 9.0 ml of the filtrate is evaporated at 60°C under a stream of nitrogen. The residue is dissolved in 1 ml of methanol and 4 ml of 0.5 N HCl is added. This solution is washed twice by shaking it for 2 minutes with 10 ml of n-hexane, centrifugation and careful aspiration of the hexane layers. To the aqueous solution is then added 12.0 ml of dichloromethane, the mixture is shaken for 2 minutes, and after centrifugation 10.0 ml of the organic layer is evaporated at 60°C under a stream of nitrogen. The residue is first dissolved in 100 µl of methanol and gaschromatographed. Then the methanol is evaporated at 60°C under a stream of nitrogen, and to the residue is added 100 µl of tetramethylammonium hydroxide, 25 % in methanol, and this solution is also gaschromatographed.

Gaschromatography: A Pye gaschromatograph model 104 with double flame ionization detector was used, equipped with glass columns 5 feet long, internal diameter 1/4 inch and filled with 3 % OV-17 on Chrom.W, AW, DMCS-treated, 80/100 mesh. Oven 224°C, detector 300°C, and injection site 235°C when chromatographing the methanolic solutions, and 350°C when chromatographing the methylated solutions.

RESULTS AND DISCUSSION

Using the method described above and adding from 5 to 60 µg of carbamazepine to 2 g of blood the recovery was 94 % ± 12 %, average from 10 experiments.

In Fig. 1 is shown the change in peak heights by the two different gaschromatographic procedures. Using the reversal of the peak height ratios as a means of identification of carbamazepine, we naturally wanted the identity of the peaks established. This was done by mass spectrometry[1]. In the methanolic solution peak no. 1 was found to be iminostilbene, and peak no. 2 to be the parent compound. Methylation by tetramethylammonium hydroxide gave mostly

[1] Courtesy of Dr. Ron Skinner, Finnigan Instr. Ltd.

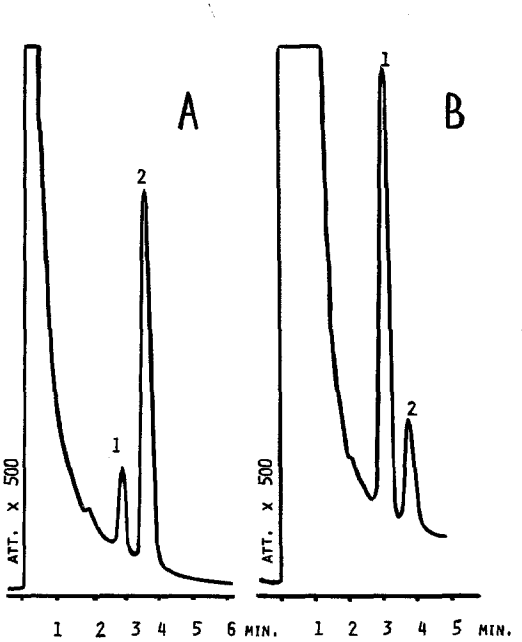

Fig. 1

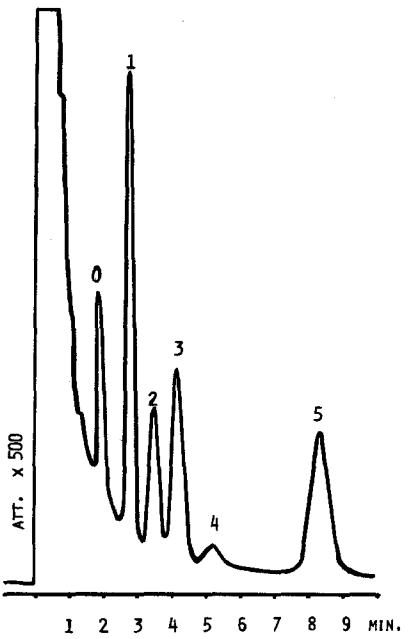

Fig. 2

Fig. 1. A. 0.25 µg carbamazepine in methanol. Peak height ratio $\frac{\text{peak 1}}{\text{peak 2}} = 0.2$
B. 0.25 µg carbamazepine in tetramethylammonium hydroxide 25 % in methanol. Peak height ratio $\frac{\text{peak 1}}{\text{peak 2}} = 4.3$

Fig. 2. 0 Phenobarbital, 1 (+2) carbamazepine, 3 primidone, 5 (+4) diphenylhydantoin, 0.25 µg of each in tetramethylammonium hydroxide, 25 % in methanol

methylated iminostilbene (peak 1, Fig. 1 B). FRIGERIO *et al.* (1973) have studied by mass spectrometry the degradation of carbamazepine when injected as a methanolic solution into an OV-17 column. They found a large peak due to carbamazepine plus two minor peaks identified as iminostilbene and 9-methylacridine.With trimethyl-phenylammonium hydroxide as a methylating agent ROGER *et al.* (1973) identified by mass spectrometry a major peak as iminostilbene, and a minor peak as methyliminostilbene.

The shift in peak heights can easily be seen down to 0.5 mg/kg blood of carbamazepine. There is good agreement between the concentrations of carbamazepine calculated from peak no. 2 (methanolic solution) and from peak no. 1 (methylated solution), confer Fig. 1 A and B.

If the gaschromatographic run after methylation is prolonged from 4 to 9 minutes, phenobarbital, primidone and diphenylhydantoin may als be determined (Fig. 2). The last three mentioned compounds will not be seen or will only

Table 1. *Concentrations of carbamazepine (and diphenylhydantoin and phenobarbital) in blood from 12 patients treated with carbamazepine and a combination of carbamazepine and other antiepileptic drugs. Where more results from the same person are listed, the blood samples were taken on different days during the drug administration*

Patient No.	mg/kg blood		
		Other anticonvulsants determined by the same method.	
	Carbamazepine	Diphenylhydantoin	Phenobarbital
1	0.6	0	0
2	1.8 2.4 2.2 3.6	2.9 2.6 1.8 0.6	9.8 13.1 11.4 7.7
3	2.6 3.0	6.7 6.4	0 0
4	3.2	8.7	0
5	3.8	0	0
6	3.7	8.0	0
7	3.4	10.4	0
8	4.4	0	0
9	4.3	0	0
10	5.7 5.0	14.5 8.5	0 0
11	5.8	0	0
12	6.5	0	0

be seen as small peaks during the methanolic gaschromatographic run under the described conditions. This behaviour, low or no peaks in the methanolic solution, and high, narrow peaks in the methylated solution, serves as a means of identification of the above mentioned antiepileptics.

In order to test the specificity of our procedure, 21 barbiturates, 4 carbamides, 4 carbamates, 6 benzodiazepines, a tricyclic antidepressant (amitriptyline) and a tranquillizer of the phenothiazine-group (promazine) were gaschromatographed in the methylating agent under the described conditions. None

of the tested compounds gave peaks with the same retention time as carbamazepine.

To evalutate the method and to get an impression of the therapeutical concentration level, blood samples from 12 patients, all under treatment with carbamazepine, were analysed by the described method. In Table 1 the results of the blood analysis are listed in the order of increasing concentrations of carbamazepine. The therapeutic level for carbamazepine was found to be from 4 - 6 mg/kg. When other anticonvulsants were administered at the same time, the concentration level of carbamazepine was lower, due to a dosage reduction of carbamazepine.

The results are in fairly good agreement with what is generally considered to be the therapeutic level of carbamazepine. GARDNER-THORPE *et al.* (1972) mention 0 - 10 mg/l, and KUPFERBERG (1972) 2- 13 mg/l plasma. Also the concentrations of diphenylhydantoin found seem to be coherent with what other authors have found (MC GEE (1970) 2 - 40 mg/l, KUPFERBERG (1972) 1 - 20 mg/l, SOLOW and GREEN (1972) 10 - 20 mg/l, and GARDNER-THORPE *et al.* (1972) 0 - 40 mg/l).

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Karen WORM
Inst. of Forensic Medicine
University of Copenhagen
Juliane Maries Vej 16
DK-2100 Copenhagen