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GAS CHROMATOGRAPHIC DETERMINATION OF NITROGEN-CONTAINING ANTICONVULSANT DRUGS AND OPIUM ALKALOIDS USING A THERMOAEROSOL DETECTOR

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SUMMARY

A simple and sensitive gas chromatographic method for determining nitrogen-containing anticonvulsant drugs (phenobarbital, hexamidine and diphenylhydantoin) and opium alkaloids (codeine, morphine, papaverin and narcotin) with a thermoaerosol detector is described. All the compounds were determined by using 0.1–0.2 ml of plasma and without their transformation into volatile derivatives. Packed glass columns and quartz capillary columns of short lengths were employed.

INTRODUCTION

Many clinical laboratories have to determine anticonvulsant drugs in the plasma and serum of epileptic patients. Gas chromatography (GC) being reliable and a highly sensitive with selective detectors, is the most promising tool for this task. Usually, the drugs are first transformed into volatile derivatives by means of appropriate derivatizing agents[1–4]. Inevitable losses and insufficient sensitivity of non-selective detectors mean that over 1 ml of plasma is required for the analysis.

The aim of present paper is to elaborate an highly sensitive method for GC determination of nitrogen-containing anticonvulsant drugs (phenobarbital, hexamidine, diphenylhydantoin) and opium alkaloids (codeine, morphine, papaverin, narcotin) in plasma without the need to transform them into volatile derivatives. We used a thermoaerosol detector which was selective towards nitrogen- and phosphorus-containing compounds. This detector[5] differs from other types of thermoionic detectors in that a salt of an alkali metal (caesium bromide) is driven into the hydrogen flame as an aerosol which has been preformed in a special generator. As a result the sensitivity of the detector is stabilized, which substantially increases its reliability and permits a decrease in the limit of concentrations detectable. Thus, only 0.1–0.2 ml of biological fluids are required for the analysis. Such a reduction in the necessary volume of plasma is very important for experimental studies of small animals and pediatrics. The direct determination of anticonvulsant drugs and opium alkaloids by means of GC without converting them into volatile derivatives can be performed with short columns and a small amount of liquid phase[6,7].

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EXPERIMENTAL

Apparatus

The gas chromatograph Chrustal 5002 was equipped with a thermoaerosol detector and glass columns (0.3 m × 4 mm I.D.). Columns were filled with Inertone NAW DMCS (Czechoslovakia), particle size 0.12–0.16 mm, containing 5% of SE-30. Column temperature programme: from 100 to 300°C at a rate of 5°C/min, then isothermal for 1 h without carrier gas (nitrogen); cooled to 30°C, nitrogen added, heated to 280°C; finally nitrogen passed for over 8 h.

The carrier gas flow-rate was 20 ml/min. Hydrogen was added to the detector at the rate of 30 ml/min. The air slow-rate was 200 ml/min. An auxiliary nitrogen flow into the aerosol generator was 300 ml/min. The column thermostat temperature during the determination of anticonvulsants was 260°C; temperature the evaporator, 280°C, and of the detector, 290°C. Opium alkaloids were determined in the temperature programming regime with an initial isothermal period (5 min) at 240°C a temperature increase of 35°C/min and a final temperature of 295°C. The temperatures of the evaporator and detector were 295°C.

Analysis of anticonvulsants was also performed with a gas chromatograph Biochrom I equipped with a flame ionization detector and a quartz capillary column (10 m × 0.25 mm I.D.) containing OV-17. The carrier gas was hydrogen from a Go-Mac (U.S.A.) electrolyser at a pressure of 1.1 kg/cm^2. The evaporator and detector temperatures, were 240°C. Column temperature programme isothermal for 4 min, 200 to 230°C at 6°C/min.

Reagents

All anticonvulsants and opium alkaloids were over >99.5% pure, except for diphenylhydantoin (85% in the samples). Sodium dihydrogenphosphate for buffer solutions and the solvents (ethanol, diethyl ether, *n*-butanol) were reagent grade.

Standard solutions

Phenobarbital (12.95 mg), hexamidine (11.55 mg) and diphenylhydantoin (14.80 mg) were dissolved in 9.16 ml of ethanol (solution A). A 5-ml volume of this solution was diluted in the solution of dry plasma to 50 ml (solution B). A standard solution B was used for the preparation of a series of grading solutions of anticonvulsants in plasma. Ethanol solutions of phenobarbital, hexamidine and diphenylhydantoin at a concentration of 10^{-4} g/ml were made up by dilution of 10–15 mg of each drug in 10 ml and by subsequent dilution of 1 ml to 10 ml in ethanol.

Solutions of codeine, morphine, papaverin and narcotin bases in *n*-butanol at concentrations of 10^{-4} g/ml were made in the same way.

Internal standard

Muriatic brevicollin [1-methyl-4-(1-methyl-2-pyrrolidinyl)-β-carboline), an alkaloid from *Carex brevicollis,* was used as an internal standard. It was added to samples as an ethanol solution (13.55 mg per 10.02 ml).

Extraction procedure

A 0.3 *M* aqueous solution of sodium dihydrogenphosphate (0.1 ml) and 4 μl

of internal standard solution were added to 0.2 ml of plasma sample, extracted twice with 1 ml of diethyl ether for 6 min and centrifuged at 3000 *g* for 7 min. The ether phase was removed by aspiration and decanted into a conical glass tube. It was taken to dryness under a stream of air by warming in a water-bath. The residue was dissolved in 20–50 ml of ethanol, and 1 μl of the resulting solution was injected into the evaporator of the gas chromatograph Chrustal 5002.

The sensitivity of the detectors was determined by injecting 1 μl of anticonvulsants in ethanol and of opium alkaloids in *n*-butanol (concentration 10^{-4} g/ml) into this gas chromatograph. With the gas chromatograph Biochrom I, 5 μl of anticonvulsants in ethanol were injected (concentration 10^{-3} g/ml).

RESULTS

The sensitivity of the detectors to anticonvulsants was compared by means of the signals of the Chrustal 5002 detector with the aerosol generator "on" and "off". In the latter position the detector is working as a flame ionization detector. The results are shown in Table I. Sensitivity parameters of the gas chromatograph Biochrom I for the separation of anticonvulsants on the quartz capillary column with flame ionization detection are shown in the same table.

The sensitivity was calculated by the formula[8]

$$A = hA_t a_{1/2} E/lm$$

where A = sensitivity in mV · s/mg, h = peak height in mm, $a_{1/2}$ = peak width at half its height, in s, A_t = attenuation of the "off" signal, l = width of the registering band in mm, m = sample weight in mg and E = registering scale range in mV.

The minimum detectable drug input rate, Q_{min} (g/s), was calculated by the formula[8]

$$Q_{min} = 2\,E/A$$

where, E = noise amplitude in mB. The minimum detectable drug input rate was recalculated for nitrogen by multiplying Q_{min} by the nitrogen weight content in the sample, $W_{(N)}$[9]:

$$Q_{min(N)} = Q_{min}\, W_{(N)}$$

where $W_{(N)}$ = (atomic weight of nitrogen in the substance)/(mol.wt. of the substance). Finally, the minimum detectable amount of drug in the sample was calculated by the formula[8]:

$$J_{min}\,(g) = Q_{min}\, a_{1/2}$$

A typical chromatogram of a plasma sample containing phenobarbital, hexamidine and diphenylhydantoin is shown in Fig. 1. Fig. 2 shows typical calibration curves for these compounds. Data on the verification of the method with solutions of known anticonvulsants concentrations are listed in the Table II.

Fig. 3 shows a chromatogram of the separation of anticonvulsants on a quartz capillary column with flame ionization detection.

TABLE I

SENSITIVITIES AND MINIMUM DETECTABLE AMOUNTS OF ANTICONVULSANT DRUGS

Drug	*Biochrom I, flame ionization detector*			*Chrystal 5002, thermoaerosol detector*						
				Without aerosol			*With the aerosol generator switched on*			
	$A \cdot 10^{-4}$ *(mV · s/mg)*	$Q_{min} \cdot 10^{10}$ *(g/s)*	$J_{min} \cdot 10^{8}$ *(g)*	$A \cdot 10^{-3}$ *(mV · s/mg)*	$Q_{min} \cdot 10^{9}$ *(g/s)*	$J_{min} \cdot 10^{8}$ *(g)*	$A \cdot 10^{-6}$ *(mV · s/mg)*	$Q_{min} \cdot 10^{12}$ *(g/s)*	$Q_{min(N)} \cdot 10^{13}$ *(g/s)*	$J_{min} \cdot 10^{11}$ *(g)*
Phenobarbital	1.8	5.6	0.98	4.5	2.2	2.3	1.6	6.4	7.8	4.5
Hexamidine	2.1	4.8	1.2	9.4	1.1	1.6	2.9	3.5	4.4	4.2
Diphenylhydantoin	1.6	6.3	1.1	1.1	3.1	2.3	0.84	11.7	13.0	25.3

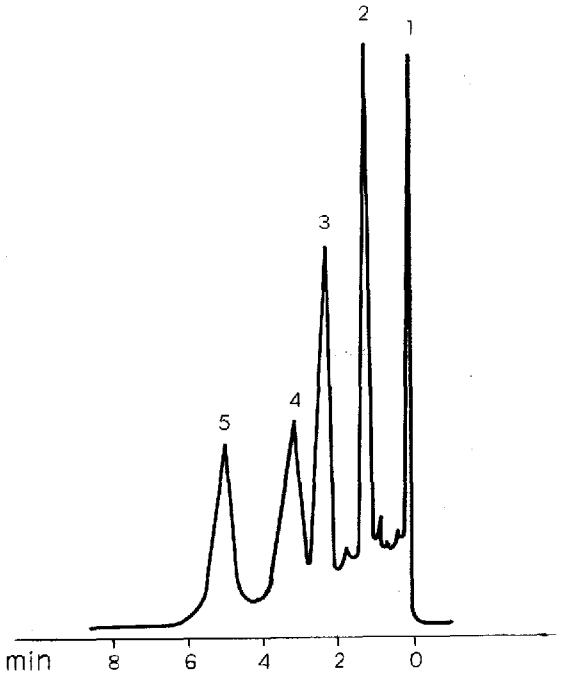

Fig. 1. Chromatogram of a mixture of anticonvulsants extracted from plasma. Thermoaerosol detector. Column (0.3 m) packed with 5% SE-30 on Inertone NAW DMCS. Peaks: 1 = solvent; 2 = phenobarbital; 3 = hexamidine; 4 = diphenylhydantoin; 5 = brevicollin (internal standard).

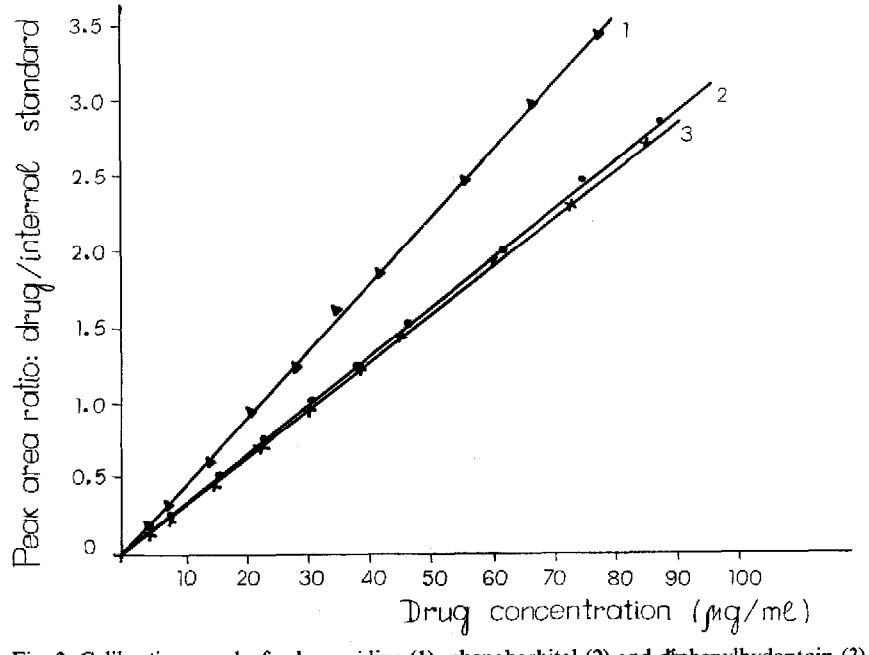

Fig. 2. Calibration graphs for hexamidine (1), phenobarbital (2) and diphenylhydantoin (3).

TABLE II

ACCURACY AND REPRODUCIBILITY OF THE GC DETERMINATION OF ANTICONVULSANT DRUGS IN PLASMA SAMPLES

$E_{0.95}$ = half of the width of the 95% confidence interval.

Drug	*Amount (μg/ml)*		*n*	*S.D.*	*Relative S.D.*	$E_{0.95}$ *(μg/ml)*
	Injected	*Found*				
Phenobarbital	12.45	12.76	5	0.43	3.37	0.56
Hexamidine	11.11	11.48		0.64	5.57	0.81
Diphenylhydantoin	12.10	12.48		0.54	4.33	0.70
Phenobarbital	24.90	24.44	6	1.25	5.02	1.34
Hexamidine	22.21	22.05		1.02	4.62	1.11
Diphenylhydantoin	24.20	25.61		1.43	5.58	1.52
Phenobarbital	37.35	35.01	5	2.85	8.14	3.61
Hexamidine	33.32	31.70		2.40	7.57	3.03
Diphenylhydantoin	36.30	35.53		3.20	9.01	4.03

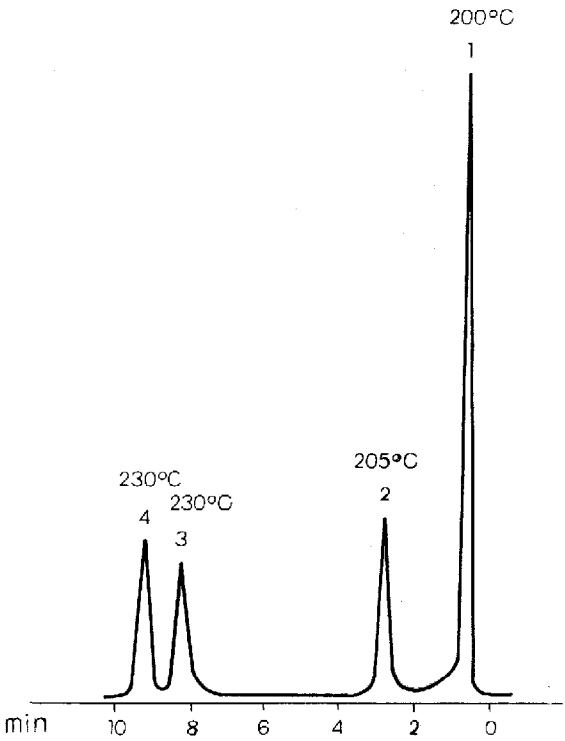

Fig. 3. Chromatogram of an ethanol (1) solution of a mixture of anticonvulsant drugs: phenobarbital (2); hexamidine (3); diphenylhydantoin (4). Flame ionization detector. Quartz capillary column (10 m) containing OV-17.

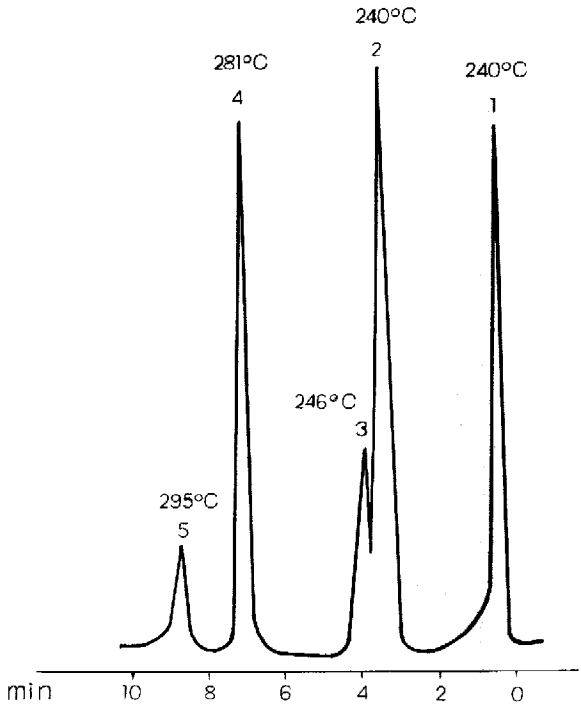

Fig. 4. Chromatogram of a *n*-butanol (1) solution of opium alkaloids: codeine (2); morphine (3); papaverin (4); narcotin (5). Thermoaerosol detector. Column as in Fig. 1.

A chromatogram of an opium alkaloids mixture obtained with the thermoaerosol detector is shown in Fig. 4; sensitivity parameters, calculated for the isothermal regime at 290°C, are given in Table III.

DISCUSSION

The sensitivity data for samples of anticonvulsants and opium alkaloids (Tables I and II) show, that the operation of the Chrustal 5002 chromatograph in the thermoionic detection regime, while turning on the aerosol generator, permits a 1000-fold increase in the sensitivity of determination of nitrogen-containing drugs

TABLE III

SENSITIVITY OF THERMOAERASOL DETECTOR AND MINIMUM DETECTABLE AMOUNTS OF OPIUM ALKALOIDS

Alkaloid	$A \cdot 10^{-6}$ (*mV · s/mg*)	$Q_{min} \cdot 10^{12}$ (*g/s*)	$Q_{min(N)} \cdot 10^{13}$ (*g/s*)	$J_{min} \cdot 10^{10}$ (*g*)
Codeine	2.0	5.0	2.4	0.5
Morphine	1.1	9.4	4.6	1.2
Papaverin	1.8	5.7	2.3	1.1
Narcotin	0.45	22.4	7.7	8.8

and a corresponding reduction in the minimum detectable amount of the drug to 10^{-10}–10^{-11} g in a sample. The sensitivity of the thermoaesosol detector is high enough for quantitation of such drugs both in official preparations and in biological samples, at the therapeutic concentrations used in medical practice, with sample volumes of 0.1–0.5 ml.

Figs.1, 3 and 4 demonstrate the possibility of determing anticonvulsants and opium alkaloids under the described conditions without converting them into volatile derivatives. This is achieved with the aid of relatively short packed (0.3 m) and quartz capillary (10 m) columns. The short columns provide a suitable separation of the drugs in 10 min, which is important in clinical practice. The preparation of the sample for GC is simple and not time-consuming.

The data in Table II show that the anticonvulsants can be determined at therapeutic concentrations, the reproducibility being 5–6%. Discrepancies between the introduced and calculated concentrations of the components do not exceed the standard deviations and are, therefore, not statistically significant. The mean error for three parallel determinations does not exceed 5.5%. Thus, the method described yields unbiassed information on the actual amount of anticonvulsant drugs in the plasma.

Taking into account the fact that all experiments on the determination of anticonvulsant drugs in plasma with the thermoaerosol detector were performed by dividing the output signal by a factor of 16, there is a possibility of reducing the sample volume to 0.1 ml without changing the working parameters of the chromatograph.

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