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Estimation of barbiturates by quantitative thin-layer chromatography

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A method for separating and analysing quantitatively, mixtures of barbiturates by thin-layer chromatography is described. The barbiturates are eluted from the silica gel and coupled with mercury which is then estimated by dithizone. The method can be used to assay commercial pharmaceutical preparations.

NUMEROUS chromatographic methods have been used to analyse and identify barbiturate mixtures. Paper chromatography has been widely employed in separations, with analysis times ranging from 4 to 12 hr (Neil & Payton, 1961). Phenobarbitone has been separated from other barbiturates and barbiturates isolated from common analgesics by column partition chromatography (Sabatino, 1954). More recently, gas chromatography has been utilised to separate and identify individual barbiturates and has allowed a quantitative study to be made of their metabolism and excretion in man (Svendsen & Brochmann-Hanssen, 1962). Although rapid, this latter method is still relatively sophisticated and requires expensive apparatus.

Thin-layer chromatography has been used by Cochin & Daly (1963) to identify 16 commercially available barbiturates after their extraction from urine into methylene chloride, and Machata (1960) separated various barbiturates from common pharmaceutical mixtures. A simple method for the identification of barbiturate mixtures when present in blood was devised by Petzold, Camp & Kirch (1963), a procedure which was especially useful in differentiating between amylobarbitone and pentobarbitone. Besides being rapid, the degree of resolution achieved by thin-layer chromatography is usually high and it was thought that a quantitative assay procedure using this technique would be of value. We report the application of such a technique to barbiturate mixtures and to preparations containing barbiturates commercially available in Canada.

Experimental

PREPARATION OF PLATES

Mix 25 g of silica gel with 60 ml of 0·1 N sodium hydroxide and spread the resulting slurry over five 20 × 20 cm glass plates to give a thickness of approximately 0·25 mm. Activate the plates at 110° for 1 hr before use.

REAGENTS

Dithizone solution. Dissolve 150 mg of dithizone in 1000 ml of chloroform. Store the solution in a refrigerator and protect from light.

Mercuric chloride solution. Dissolve 300 mg mercuric chloride in 1 ml of 0·1 N hydrochloric acid and dilute to 100 ml with distilled water.

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Mercuric nitrate solution. Dissolve 1·5 g mercuric nitrate in 5 ml of 2 N nitric acid and dilute to 100 ml.

Buffer solution pH 8·0. Mix 50 ml of 0·2 N potassium dihydrogen phosphate with 46·8 ml of 0·2 N sodium hydroxide and dilute to 200 ml with distilled water.

Barbiturate standards. Prepare a solution containing 20 mg/ml of the relevant barbiturate in 95% ethanol.

Solvent system 1. Mix together isopropanol, chloroform and 25% ammonium hydroxide (60:30:10 ml). Shake well.

Solvent system 2. Water saturated isopropyl ether.

GENERAL PROCEDURE

Samples of the barbiturate standards and of the barbiturate mixtures (in ethanol) were applied in 5 μl and 10 μl quantities to the silica gel plates from self-filling lambda pipettes to give spot sizes of 6–8 mm.

For commercial preparations, three or four tablets were crushed and extracted with 10 ml of 0·1 N sodium hydroxide. The filtrate was acidified with 2 ml N hydrochloric acid and the barbiturates extracted with two 4 ml portions of chloroform which were later diluted to 10 ml exactly. A similar procedure was followed for capsules.

Ten μl of these chloroform extracts was then applied to the thin-layer plates: spot sizes were again 6–8 mm. The plates were placed in tanks which had been equilibrated overnight with the appropriate solvent system and allowed to develop about 15 cm from the starting line. The plates were then removed from the tanks, air-dried, and sprayed with 1·5% aqueous mercuric nitrate solution. The barbiturates appeared as white spots on a pale yellow background. On drying, the relevant areas of silica gel were removed from the plates by an apparatus composed of a small inverted chromatographic tube, the end of which was attached to a vacuum line. The other end was sealed with a rubber stopper through which was placed a right-angled piece of glass tubing (7 mm ext. diam.). The silica gel was sucked through this glass tubing into the column from which the barbiturates could be easily eluted.

The barbiturates were extracted by allowing 30 ml water to filter through the silica gel slowly. To this filtrate, 2 ml of the mercuric chloride solution was added, followed by 2 ml of the phosphate buffer. The resulting opalescent solution was then extracted with one 10 ml portion of chloroform followed by two further 15 ml portions. Two ml of dithizone solution was added, and the solution diluted to 100 ml. The solution was shaken vigorously and allowed to stand for at least 2 hr after which the barbiturates were estimated by measuring the extinction at 475 mμ and comparing with an appropriate standard.

Discussion

According to Truter (1963), one of the main sources of error with elution techniques in thin-layer chromatography is the elution of impurities from the adsorbent. These impurities absorb ultraviolet radiant energy,

particularly from 250–380 mμ, and so interfere with the spectrophotometric assay of many drugs. This was confirmed in the present technique, since attempts to estimate the barbiturates directly in the ultraviolet region following elution from the silica gel gave recoveries ranging as high as 600%. In an attempt to eliminate the need for colorimetric assays

TABLE 1. QUANTITATIVE RESULTS ON BARBITURATE MIXTURES

Drugs	Amount added mg	Number of determinations	Recovered %*	Solvent system	Rf values
Single drugs—					
Amylobarbitone	0·10	4	102·5 ± 1·3	1	0·86
Secobarbitone	0·10	4	102·2 ± 2·1	1	0·83
Barbitone	0·10	8	97·0 ± 2·5	1	0·72
Phenobarbitone	0·10	8	99·8 ± 1·5	1	0·60
Simulated mixtures—					
Secobarbitone	0·20	4	103·0 ± 2·0	1	0·83
Barbitone	0·20	4	100·0 ± 2·5	1	0·72
Phenobarbitone	0·20	4	100·0 ± 2·1	1	0·60
Amylobarbitone	0·20	4	100·0 ± 2·6	1	0·86
Barbitone	0·20	4	101·0 ± 2·2	1	0·72
Phenobarbitone	0·20	4	102·2 ± 2·0	1	0·60
Butabarbital	0·20	12	95·0 ± 4·6	2	0·48
Pentobarbitone	0·20	12	97·0 ± 4·1	2	0·56
Phenobarbitone	0·20	12	100·0 ± 5·2	2	0·37
Secobarbitone	0·20	12	96·0 ± 4·4	2	0·63

* "Recovered %" is expressed as the mean of all determinations ± one standard deviation.

the silica gel was extracted with chloroform, water, decinormal sodium hydroxide and acetone to remove all the absorbing materials before spreading the plates. This proved unsuccessful as recoveries still showed gross overestimation up to 400%. Similarly, using an extract from the silica gel as a blank and treating this under the same conditions as the sample gave results which were very erratic. Interference by impurities still occurred despite these precautions.

The method reported here has been adapted from that of Björling, Berggren & Willman-Johnson (1959), who reported that as little as 0·1 μg/ml of barbiturate could be detected in solution. The present method was found to be quantitative when the amount of the barbiturate applied to the plate was in the range of 50–250 μg. When quantities smaller than 50 μg were applied recoveries were high, and for quantities larger than 250 μg recoveries were low. The orange-coloured solution which results in the mercury-dithizone assay was found to have an absorption peak at 475 mμ. Repeated scanning of the solutions revealed no absorbance maxima in the region reported by Björling & others (1959). By coupling the barbiturates with mercury and subsequently estimating the mercury with dithizone, the wavelength used in the determination of the barbiturates is shifted from 258 to 475 mμ and errors resulting from any absorbing substances in the silica gel are eliminated. Recoveries from simulated mixtures as well as from pharmaceutical preparations are shown in Tables 1 and 2 respectively.

Attempts to estimate phenacetin and aspirin in some of the mixtures analysed were unsuccessful since no satisfactory colorimetric procedure

was found for these drugs. Consequently it was not possible to shift the absorption peaks from the region of 260 mμ.

It was found necessary to prepare the silica gel with decinormal sodium hydroxide, which gave the slurry an almost neutral pH of 6·7 as opposed to a pH of 5·4 when prepared with water. Barbiturates did not separate with sufficient sharpness to allow their removal from plates prepared with water. Despite the use of several solvent systems, no separation of secobarbitone and amylobarbitone was achieved under any set of conditions.

TABLE 2. QUANTITATIVE RESULTS ON BARBITURATES IN PHARMACEUTICALS

	Labelled strength mg	Number of determinations	Recovered %*	Solvent system	Rf value
Luminal—					
Phenobarbitone	100	14	97·6 ± 2·1	2	0·37
Twin-Barb (Tablets)—					
Sod. Secobarbitone ..	65	13	96·3 ± 3·2	2	0·63
Butabarbital	50	13	92·0 ± 1·7	2	0·48
Twin-Barb (Capsules)—					
Sod. Secobarbitone.. ..	65	8	107·1 ± 1·6	2	0·63
Butabarbital	50	12	98·2 ± 1·3	2	0·48
Tri-Barb—					
Sod. Secobarbitone ..	45	12	95·6 ± 2·4	2	0·63
Butabarbital	30	12	101·3 ± 1·1	2	0·48
Phenobarbitone	45	12	98·1 ± 1·8	2	0·56
Multi-Barb—					
Sod. Secobarbitone ..	150	8	93·6 ± 4·8	2	0·63
Sod. Pentobarbitone ..	150	8	93·1 ± 2·9	2	0·56
Sod. Butabarbital ..	48	8	96·9 ± 5·1	2	0·48
Phenobarbitone	48	8	95·4 ± 3·4	2	0·37
Somnol—					
Hexobarbitone	50	8	94·7 ± 2·7	1	0·85
Phenobarbitone	25	9	99·3 ± 3·3	1	0·60
Sedadrops—					
Phenobarbitone	120	4	97·1 ± 3·4	2	0·37
Sod. Pentobarbitone ..	80	4	94·6 ± 3·8	2	0·56

* "Recovered %" is expressed as the mean of all determinations ± one standard deviation.

Tablet or capsule excipients present in the preliminary extraction do not interfere with the separation of the barbiturates. Similarly, some colouring material present in the Sedadrops elixir did not affect the assay and no preliminary extraction proved necessary.

It would appear that the method could be adapted readily to the quantitative analysis of barbiturates in blood and urine.

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