A SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF PHENINDIONE

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A spectrophotometric method for the estimation of phenindione in pharmaceutical preparations and body fluids and tissues is described. It has a greater sensitivity than the existing British Pharmacopoeia method. Advantage has been taken of the solubility of the drug in toluene and the sensitivity of measurement has been found to be enhanced by the addition of alcoholic potassium hydroxide solution. This method accurately estimates 1–2 mg. of the substance as opposed to the 300 mg. required for the B.P. method. Further, as little as 2·5 μg./ml. of the substance can be detected in biological fluids after eliminating interfering substances.

FEW methods are available for the estimation of phenindione in pharmaceutical preparations. The B.P. method, developed by Sharp (1955), involves bromination followed by iodometric titration. But this requires at least 150 mg. of the substance. A spectrophotometric method, in which the substance is dissolved in aqueous potassium hydroxide solution and the extinction measured at 279 mμ, has also been described (Council on Pharmacy and Chemistry of the American Medical Association, 1953). This again is not very sensitive, and cannot be used for the estimation of the drug in biological fluids.

EXPERIMENTAL

A Beckman Spectrophotometer Model Du with 1 cm. standard silica cells was used. Of several solvents initially investigated, toluene was considered to be the most suitable, as although it was found to be less sensitive than some of the other solvents spectrophotometrically, it extracted phenindione from aqueous medium after acidification.

The sensitivity of measurement was found to be greatly increased if a mixture of toluene and 0·05N alcoholic potassium hydroxide was used. This gives a maximum extinction at 288 mμ. Taking known concentrations of the compound in 2 ml. of toluene and adding 3 ml. of 0·05N alcoholic potassium hydroxide solution and measuring the extinction at 288 mμ gave a linear relation.

Conc./μg./ml.	1	2	4	6	8	10	15	20
Extinction	0·032	0·060	0·104	0·155	0·202	0·256	0·365	0·480

Estimation in Commercial Samples

Tablets of phenindione were weighed, powdered, and extracted in a mortar with toluene and the extract filtered, and diluted to a final strength of 10 to 20 μg./ml. Phenindione as powder, was dissolved directly in toluene and the concentration adjusted to the same level.

The results of the estimations are: 10 mg. of powder gave a recovery of 10·2 mg.; three 50 mg. samples from tablets gave recoveries of 51·2, 51·5 and 51·5.

It can be seen that binding material present in the tablets did not interfere with the analysis which showed an error of 2–3 per cent.

The method was extended to the estimation of phenindione in biological materials.

Estimation of Phenindione in Tissues and Body Fluids

Phenindione can be extracted from aqueous medium with toluene after acidification. The optimum pH at which quantitative recovery can be obtained was found to lie between pH 1 and 2.

Proteins are removed with trichloroacetic acid at this optimum pH, but as interfering substances cannot be completely eliminated by the above treatment, the toluene extract must be further extracted with aqueous potassium hydroxide which removes phenindione quantitatively. After acidification of the alkaline solution the drug was re-extracted with toluene for final estimation (Table I).

TABLE I

SHOWING RECOVERY PER CENT OF PHENINDIONE AFTER TREATMENT WITH TRICHLOROACETIC ACID, TOLUENE AND AQUEOUS POTASSIUM HYDROXIDE

Phenindione content μg.	Phenindione detected μg.	Deviation per cent
200	205	+2·5
400	385	−3·5
500	510	+2·0
750	720	−4·0
1000	950	−5·0

From these observations it will be seen that treatment with acid and alkali followed by toluene after each did not interfere with the estimation, which had an error of ±5 per cent.

Estimation of Phenindione in Liver Homogenates and Blood

The homogenised liver from freshly killed rats, or human serum, was used. Known quantities of the drug were added to samples and the final concentration adjusted to 10 μg./ml.

3–5 ml. of the samples were taken in a centrifuge tube and diluted to 10 ml. with water. 2·0 ml. of 10 per cent trichloroacetic acid was added and mixed thoroughly to precipitate the proteins. 5 ml. of toluene was then added, and the tube shaken for 2 min., then centrifuged. From the toluene layer 2·5 ml. was transferred to a second centrifuge tube containing 2·5 ml. of 0·1N aqueous potassium hydroxide solution. This tube was shaken thoroughly for 1 min. and centrifuged. The toluene layer was discarded and 2 ml. of alkaline extract were transferred to a third centrifuge tube, to which 0·5 ml. of 1 per cent hydrochloric acid was added. The pH of the solution was then adjusted to 1–2. To the mixture, 5 ml. of toluene was added and the tube shaken for 1 min. After centrifugation 2 ml. of the toluene layer solution were transferred to a test tube.

3 ml. of 0·05N alcoholic potassium hydroxide was added and mixed, and the estimation was made at 288 mμ, keeping the slit width at 0·88 mm. The findings are shown in Table II.

TABLE II

RECOVERY PER CENT OF KNOWN QUANTITIES OF PHENINDIONE FROM LIVER HOMOGENATE AND BLOOD SERUM

Liver homogenate			Serum		
Phenindione added μg.	Phenindione detected μg.	Deviation per cent	Phenindione added μg.	Phenindione detected μg.	Deviation per cent
100	102	+2·0	100	97	−3·0
200	210	+5·0	200	210	+5·0
300	290	−3·3	300	315	+5·0
400	380	−5·0	400	385	−3·75

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