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The polarographic determination of chlorpromazine and chlorpromazine sulphoxide

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Cathodic polarography of solutions containing between 0·1 and 5·0 μg/ml of both chlorpromazine and its sulphoxide enables the sulphoxide to be determined directly. Reductive polarography of the solution after bromination gives an additive wave from which the chlorpromazine is determined by subtraction. The method has been applied to urine, interfering substances being removed by ion-exchange on Amberlite resins.

CHLORPROMAZINE, although not reduced at the dropping mercury cathode, can be determined by reductive polarography after bromination (Porter, 1964). This method has been adapted to the determination of chlorpromazine and its detoxication product chlorpromazine sulphoxide when present together in solution. The sulphoxide is directly reducible and neither bromination nor the presence of chlorpromazine quantitatively affects its calibration graph.

Chlorpromazine and chlorpromazine sulphoxide, on bromination under the conditions described, each give a reducible derivative. The two bromination derivatives are polarographically and chromatographically indistinguishable and have identical ultraviolet spectra. In mixtures, therefore, the sulphoxide is reducible before bromination; after bromination an additive wave is obtained from which the chlorpromazine concentration is obtained by subtraction of the sulphoxide wave.

Determinations were made on urine containing low concentrations of added chlorpromazine and sulphoxide, and on urine from volunteers who had taken chlorpromazine orally. Quantitative differentiation was possible between reducible and non-reducible excreted forms of the drug.

Experimental and results

APPARATUS

A Southern Analytical K 1000 polarograph was used. Determinations were made at 25° using a mercury pool anode, a start potential of −0·5 V and the derivative circuit. All peak current readings were expressed in graticule units taken at, or corrected to, an instrument sensitivity of 0·004.

CALIBRATION GRAPHS

Chlorpromazine sulphoxide. Twenty-five ml quantities of solutions containing up to 5 μg/ml of base were prepared in 0·5N hydrochloric acid. One ml amounts of these solutions were reductively polarographed after flushing with nitrogen (3 min), and a peak current-concentration graph constructed.

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Chlorpromazine. Solutions of the base were prepared as for the sulphoxide. Each 25 ml quantity was treated with two drops of saturated bromine water, shaken, stood for 1 min and reductively polarographed after flushing with nitrogen (3 min). The peak current-concentration graph was then plotted.

Brominated sulphoxide. Solutions of sulphoxide when brominated as described for chlorpromazine gave a calibration graph identical with that of the unbrominated sulphoxide.

Peak voltages. Untreated sulphoxide, brominated sulphoxide and brominated chlorpromazine all gave cathodic waves whose peak voltages were about −0·75 V.

ANALYSIS OF SOLUTIONS CONTAINING BOTH CHLORPROMAZINE AND CHLORPROMAZINE SULPHOXIDE

Twenty-five ml quantities of solutions containing 4 ml of deionised normal urine and up to 5 μg/ml of each base were prepared in 0·5N hydrochloric acid. Each solution was polarographed as described for the preparation of the sulphoxide calibration graph. The peak current was recorded and the sulphoxide content calculated. One drop of bromine water was added to the solution in the cell and the determination completed as for chlorpromazine. The additive peak current was recorded. Subtraction of the sulphoxide peak current from this, and reference to the chlorpromazine calibration graph, gave the chlorpromazine concentration. Results are summarised in Table 1.

TABLE 1. ANALYSIS OF SOLUTIONS CONTAINING CHLORPROMAZINE AND CHLORPROMAZINE SULPHOXIDE

Substance	No. of determinations	Range of concentrations polarographed (μg/ml)	Standard deviation (μg/ml)
Chlorpromazine	13	0·1 to 0·5	0·030
	25	0·5 to 1·0	0·041
	40	1·0 to 3·0	0·099
	8	3·0 to 5·0	0·115
Chlorpromazine sulphoxide ..	17	0·5 to 1·0	0·042
	33	1·0 to 3·0	0·105
	36	3·0 to 5·0	0·114

Application to urine. Varying amounts of chlorpromazine and its sulphoxide were added to normal urine samples to give concentrations of up to 125 μg/ml of each base. Urine (up to 4 ml) accurately measured, was applied to a 10 × 1 cm column of Amberlite resin IRA 400 (Cl) The column was washed with water and the water-white eluate and washings were collected in a 25 ml flask containing 2·5 ml of 5N hydrochloric acid and made up to the mark. A suitable quantity of solution was transferred to a polarographic cell and the determination completed as described.

Results from three determinations are in Table 2. Twelve analyses gave recoveries between 91 and 106% for chlorpromazine and between 90 and 102% for the sulphoxide.

TABLE 2. ANALYSIS OF NORMAL URINE SAMPLES CONTAINING ADDED CHLORPROMAZINE AND CHLORPROMAZINE SULPHOXIDE

Sample	1	2	3
Volume analysed (ml)	2·0	0·5	1·0
Content (μg/ml) chlorpromazine sulphoxide	35·0	65·1	21·0
chlorpromazine	12·8	65·1	10·5
Peak current before bromination (graticule units)	3·10	1·50	0·90
Peak current after bromination (graticule units)	4·35	3·18	1·45
Difference	1·25	1·68	0·55
Recovery % chlorpromazine sulphoxide	97	101	95
chlorpromazine	96	101	105

Application to urine containing chlorpromazine metabolites. 100 mg of chlorpromazine hydrochloride was taken orally by each of two volunteers before retiring. Urine was collected in fractions as voided over the next 24 hr and each fraction was analysed. Results are in Table 3.

TABLE 3. DETERMINATION OF REDUCIBLE AND NON-REDUCIBLE METABOLITES IN URINE AFTER A SINGLE 100 MG DOSE OF CHLORPROMAZINE HYDROCHLORIDE

Sample	Fraction	Amount excreted (mg)	
		Reducible metabolites calculated as sulphoxide	Non-reducible metabolites calculated as chlorpromazine
1.	1	2·6	3·0
	2	0·4	0·6
	3	0·4	0·9
	4	0·2	1·2
	5	0·2	1·0
	6	0·0	0·8
		3·8	7·5
2.	1	2·7	2·1
	2	1·2	1·2
	3	0·3	0·7
	4	0·2	0·5
	5	0·1	0·4
		4·5	4·9

DETECTION AND REMOVAL OF INTERFERENCE

In the determinations on normal urine, samples from twenty-three adult male and seven adult female volunteers were examined. Two of the females were pregnant and two provided samples during menstruation.

Four ml quantities were diluted to 25 ml, the solution containing sufficient hydrochloric acid to render it 0·5N. On polarographing, a number of waves of varying amplitude were seen in the range −0·5 to −0·9 V. Bromination produced a further wave in this voltage range.

Deionisation [IRA 400 (Cl)] of urine samples from 29 of the volunteers before preparing the 25 ml of solution resulted in no polarographic wave being seen in the region −0·5 to −0·85 V before or after bromination.

Since after deionisation of the urine samples no interfering wave occurred within 0·1 V of the peak voltage of the phenothiazine compounds

(−0·75 V), interference with the determination of added chlorpromazine or sulphoxide, or both, was neither anticipated nor encountered in these 29 cases.

One sample (J.B.) on deionisation showed a wave at −0·79 V which interfered with the determination of added sulphoxide giving results up to 20% low, while the determination of chlorpromazine was unaffected. The interfering wave could be demonstrated consistently in successive urine samples, varying only in amplitude from time to time.

Using this sample (J.B.), containing added chlorpromazine and sulphoxide, a method adapted from an ion-exchange procedure of Forrest, Wechsler & Sperco (1963) was devised to detect and eliminate interference.

With an approximate knowledge of the total phenothiazine content obtained by the method already described, a definite weight of cation exchange resin was used to remove the phenothiazines from a suitable volume of the sample, these being subsequently eluted with N sodium hydroxide solution and determined.

The full procedure for a sample (J.B.) containing 4·0 μg/ml of chlorpromazine and 10·0 μg/ml of sulphoxide is described below.

Four ml of sample were analysed as described under *Application to urine*. The results gave 8 μg/ml for sulphoxide and 3·8 μg/ml for chlorpromazine. Concurrently with this determination a further 4 ml of sample was passed through two vertical ion-exchange columns in series, the upper one being a 5 × 1 cm column of Amberlite IRC 50 (H) analytical grade and the lower a 10 × 1 cm column of Amberlite IRA 400 (Cl). The eluate and washings were collected in a 25 ml flask containing 2·5 ml of 5N hydrochloric acid and made up to the mark. The polarogram of this solution showed only the interference wave at −0·79 V.

A fresh 4 ml portion of sample was shaken (5 min) with 0·5 g of Amberlite IRC 50 (H) resin in a stoppered cylinder, tapered at one end and having a sintered glass filter fused into the taper. The supernatant liquid was removed and the resin washed with water, liquid and washings being passed through a 10 × 1 cm column of IRA 400 (Cl) resin into a 25 ml flask containing 2·5 ml of 5N hydrochloric acid. No polarographic wave due to sulphoxide or chlorpromazine was seen in this solution either before or after bromination, indicating complete adsorption of the phenothiazines on the IRC 50 (H) resin. Although the interfering wave at −0·79 V was still apparent, the procedure was found to be a more rapid and reliable indication of complete phenothiazine adsorption than use of a colorimetric test.

The IRC 50 (H) resin remaining in the cylinder was shaken (30 min) with 5 ml of N sodium hydroxide solution to extract adsorbed phenothiazines. The residual liquid was filtered into a 25 ml flask containing 3 ml of 5N hydrochloric acid, the resin washed with water and the solution made up to the mark with washings. Polarography of this solution gave results for chlorpromazine and chlorpromazine sulphoxide content in precise agreement with theory.

Working with 0·5 g of resin in each case, and on volumes from 10 ml down to 4 ml, analyses of 12 portions of the urine sample (J.B.) containing

concentrations of added chlorpromazine and sulphoxide between 2·0 μg/ml and 10·0 μg/ml gave recoveries between 95 and 100%.

Determination on the urine (J.B.) after the donor had taken a single oral dose of 50 mg of chlorpromazine hydrochloride gave a total phenothiazine recovery of about 10% in 24 hr.

Discussion

Each five point calibration graph can be prepared in about 30 min; a single determination on urine in the absence of interference takes 15 min. The longer procedure requires an hour for a single determination and about 2 hr for four concurrent determinations.

Calibrations were unaffected by up to 4 ml of deionised [IRA 400(Cl)] normal urine and by such quantities of sodium chloride as were introduced after eluting the IRC 50 (H) resin with sodium hydroxide.

Both resins were used as supplied but whereas the IRA 400 (Cl) was regenerated with saturated sodium chloride solution it was found advisable to use fresh IRC 50 (H) for each determination. Blank determinations on eluates from both resins showed no polarographic waves from either resin, before or after bromination.

Fels, Kaufman & Karczmar (1958) reported instability of chlorpromazine in N sodium hydroxide solution as evidenced by spectrophotometric determinations. This was not found in trial experiments where chlorpromazine was adsorbed on IRC 50 (H) resin, eluted with N sodium hydroxide solution and polarographed in acid medium.

Since ion-exchange would be expected to account for all soluble chlorpromazine metabolites (Forrest & others, 1963), ether extraction (Flanagan, Lin, Novick, Rondish, Bocher & Van Loon, 1952) followed by polarography will differentiate not only between oxidised and non-oxidised derivatives but also polar and non-polar excreted forms of the drug.

The slope and linearity of the calibration graphs depend on the electrolyte, capillary characteristics and instrument parameters. Variation of the start potential caused much variation in peak height for a given concentration. This effect was noted by R. C. Rooney & D. L. Jones (private communication) and is thought to be caused by some of the current being due to catalytic hydrogen discharge. Determinations throughout, therefore, were made with the start potential constant at −0·5 V.

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