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Application of polarography to the development of a stability-indicating assay method for a new indole derivative and its tablet formulations

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A polarographic method has been developed for the determination of an antidepressant, 10-(*m*-chlorophenyl)-2,3,4,10-tetrahydropyrimido [1, 2a] indol-10-ol hydrochloride. The method is not subject to interference from the immediate precursor in the synthetic route of drug preparation or the products of thermal degradation of the active drug substance. The method may be used to monitor storage stability studies.

10-(*m*-Chlorophenyl)-2,3,4,10-tetrahydropyrimido [1,2a]indol-10-ol hydrochloride, I (Wy 23409) is an antidepressant drug (White, 1972, White & Black, 1972).

Of the various assay methods available, ultraviolet spectroscopy is limited by the fact that degradation products also absorb in the same region. Preliminary t.l.c. separation is too time consuming for routine analysis and application of g.l.c. methods suggested decomposition on the columns. A suitable polarographic method has been devised.

METHODS

Preliminary polarographic studies showed that precipitation of the drug base occurred at pH values higher than 6·8 and that reduction waves usually occurred at values above 1·4 V. The utilization of this potential range is facilitated by using tetra-alkylammonium ions as the supporting electrolytes at 2·5 V (Heyrovsky & Zuman, 1968) and in the present case tetrabutylammonium chloride (TBACl) proved best. The residual current of this supporting electrolyte is negligible when the concentration is between 0·005 and 0·025 M. Well shaped, smooth polarograms are obtained for the drug in appropriate concentration (see Fig. 1A). A solution of the drug in the TBACl is sufficiently stable, after storage overnight, to give identical polarograms to those originally obtained.

Fig. 1B shows that there is a marked increase in the height of the polarogram due to the drug with an increase in TBACl concentration above 0·025 M; subsequent polarograms were obtained using 0·01 M TBACl to dilute the sample solution. The baseline of the normal DC polarographic wave obtained using concentrations of TBACl between 0·005 and 0·01 M is linear from −1·0 to −1·65 V so the height of the wave is thus proportional to the concentration of the drug. A calibration curve, relating wave height to concentration of drug, gave a straight line passing through the origin.

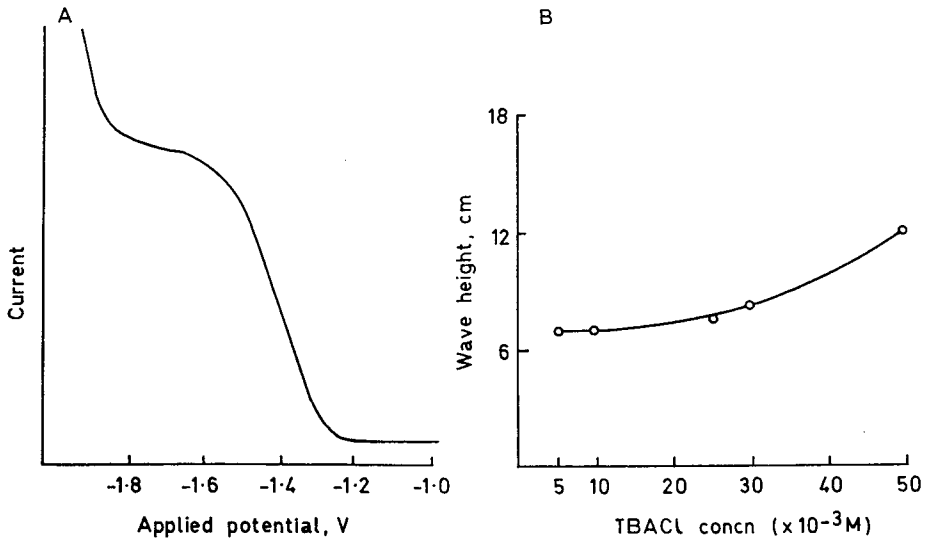


FIG. 1.A. Typical Cambridge DC Polarogram; drug concentration $1{\cdot}2 \times 10^{-4}$M in 0·01M aqueous TBACl. B. Effect of the concentration of TBACl on the wave height; drug concentration $1{\cdot}2 \times 10^{-4}$M.

The optimum concentration range for the drug substance using the Cambridge polarograph (pen recording) coupled with an external Servoscribe type recorder is $1{\cdot}2 - 1{\cdot}7$, 10^{-4} M.

Method

Reagents. A fresh 0·01 M aqueous solution of tetrabutylammonium chloride (Eastman Kodak) prepared each day.

Apparatus. Polarograph—Cambridge or similar pen recording type of polarograph. Potentiometric recorder—Servoscribe model No. RE511.20 fitted with a 50Ω resistor across the terminals. Electrode-H-Type electrode for an external electrode with a 2-cm agar/KCl bridge.

Preparation of standard solution. Accurately weigh 50 mg of reference standard of the drug and quantitatively transfer to a 250 ml graduated flask using distilled water. Shake vigorously for 30 min to dissolve and dilute to the mark with distilled water. Pipette a 10 ml aliquot of the resulting solution into a 50 ml graduated flask, dilute to the mark with supporting electrolyte and mix well. This solution is about $1{\cdot}2 \times 10^{-4}$ M with reference to the drug substance.

Preparation of sample solution. Weigh 20 tablets and calculate the average weight per tablet, then finely powder the tablets and reserve in a stoppered container. Transfer an accurately weighed sample equivalent to 50 mg of the drug substance to a 250 ml graduated flask using distilled water. Shake vigorously for 30 min to extract the active ingredient. Dilute to volume with distilled water and mix well. Centrifuge and pipette 10 ml of the supernatant solution into a 50 ml graduated flask, dilute to the mark with supporting electrolyte and mix well.

Procedure. The procedure is identical for both the sample and the standard. Transfer an appropriate volume of solution to the sample compartment of the polarographic cell. Lower the mercury dropping electrode and glass hood until the

bottom of the hood is just below the surface of the water in the constant temperature water bath. Place the saturated calomel electrode in the electrode compartment. Half fill the gas saturator with the supporting electrolyte connect to the cell and bubble nitrogen freely through the solution for at least 5 min. Record the polarogram in the usual way using the external Servoscribe recorder. Measure the height of the polarographic wave of both the sample and the standard.

Calculation. Calculate the drug content per tablet as follows:

$$\frac{\text{H sample}}{\text{H standard}} \times \frac{\text{W standard}}{\text{W sample}} \times \frac{298{\cdot}771^{*}}{335{\cdot}228} \times \text{Average weight of tablet (mg)}$$

=mg (base) per tablet. Where H sample = polarographic height of sample. H standard = polarographic height of standard. W sample = weight of sample. W standard = weight of standard.

* $\frac{\text{Molecular weight of the free base}}{\text{Molecular weight of the hydrochloride}}$

RESULTS AND DISCUSSION

Comparison with other methods

Samples of tablets made from two formulations have been analysed by the polarographic method and the results compared with those obtained by direct ultraviolet spectroscopy and by quantitative thin layer chromatography. The t.l.c. method involves separation of the main spot from impurities, collection and extraction of the main spot with solvent followed by determination by ultraviolet spectroscopy. The results, shown in Table 1, obtained by polarography are in good agreement with the results obtained by the other two methods.

Table 1. *Comparison of methods.*

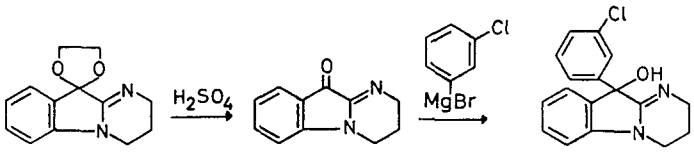
Formulation	Nominal strength mg(base)/ tablet	Determined mg(base)/tablet		
		Direct ultraviolet	t.l.c.	Polarography
I	10	9·74	10·38	9·51
I	25	23·69	26·75	24·97
I	50	47·46	48·85	48·22
II	10	9·47	9·11	9·41
II	25	24·34	23·75	23·84
II	50	47·95	46·43	47·71

Precision of the method

Evaluation of the precision of the polarographic method, when applied to tablets, has been made by carrying out 10 determinations on the same batch of tablets (25 mg, Formulation I). The coefficient of variation obtained was 2·87%. The method therefore has adequate *precision* for stability evaluation.

Interference by precursors

Because of the method of synthesis likely impurities in the final product are II, the immediate precursor pyrimidoindolone, and III the penultimate precursor pyridoindolone ketal, below



III II I

Polarographic studies of these two compounds in TBACl showed that they have behaved very similarly to I. Both compounds exhibit one normal DC polarographic wave. The $E_{\frac{1}{2}}$ values were measured *vs* the saturated calomel electrode. A value of 1·5 V for III and −0·65 V for II compare with −1·4 V for the drug I.

Application of the method to solutions of I containing up to 15% II revealed no interference with recoveries of the drug (>98%). From four production batches recoveries of 99% were recorded. Since the penultimate precursor III has a reduction potential similar to that of I the separate determination of these materials is impossible by this assay procedure. Separation of III from I is readily accomplished by t.l.c.; however examination of batches of drug, manufactured to date, has revealed no trace of III.

Table 2. *Stability of drug at high temperatures and humidity* (75%RH)

		Polarography %	t.l.c. %
Initial		99·8	101·0
25° high humidity +	6 months	101·8	99·6
37° high humidity +	6 months	99·1	99·0
105°	6 months	99·9	98·2
Ultraviolet irradiation	10 months	97·3	96·9
170°	2 days	98·4	98·0
	4 days	80·1	81·5
	6 days	59·4	58·2

Application to stability trials

It was first necessary to establish that the polarographic method measured only intact drug. Samples of drug were therefore subjected to varying degrees of degradation and the amount of intact drug determined by t.l.c. on silica gel with a mixture of toluene–ethanol–ammonia (79:20:1) as developing solvent. The spots given by the degradation products were well defined and clearly separated from the drug spot. Parallel analyses were carried out by the polarographic method. The results in Table 2 indicate that the polarographic method of analysis is not subject to interference from the products of thermal degradation.

An examination of tablets stored at 25° with 75% RH, 37° and 37° with 75% RH all after 6 months and also samples stored in sunlight for 3 months showed the products to have good stability.

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