ASSAY OF PROMAZINE AND ITS SEPARATION FROM CHLORPROMAZINE AND PROMETHAZINE

By L. Cavatorta

From the Analytical Department, Lepetit S.p.A., Milan, Italy.

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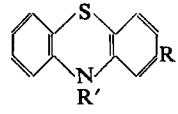
A colorimetric method is described to identify and quantitatively determine promazine, in pharmaceutical preparations. The method is applicable to promazine and promethazine mixtures, and promazine and chlorpromazine in mixtures. With a second colorimetric method promethazine or promethazine in the presence of the other two substances can be determined.

Among phenothiazine derivatives chlorpromazine (I) and promazine (II) have been much used as tranquillising drugs. Both may be associated in pharmaceutical preparations with the antihistamine drug promethazine (III), an isomer of promazine.



(I) R = Cl; R′ = $CH_2CH_2CH_2NMe_2HCl$
(II) R = H; R′ = $CH_2CH_2CH_2NMe_2HCl$
(III) R = H; R′ = $CH_2—\underset{\displaystyle CH_3}{\underset{|}{CH}}NMe_2HCl$

Promazine may be assayed by methods similar to those for chlorpromazine and promethazine. The more important of these are as follows.

(1) Colorimetric assay based on the colour developed by oxidizing the phenothiazine ring to thionyl-like derivatives[1–6]. Among the oxidizing agents, bromine water, nitric acid, sulphuric acid, potassium persulphate, iodic acid and ferrous salts are mentioned; but difficulty in stabilising the colour is encountered. Calò and colleagues[6] find sulphuric acid poorly specific, while the colour developed by potassium persulphate with chlorpromazine has a poor stability. Stable and reproducible colours for chlorpromazine were obtained with both iodic and phosphoric acids by these authors.

(2) Ultra-violet spectrophotometric assay after dissolving in dilute hydrochloric acid.

(3) Assay in non-aqueous medium: the dimethylamino group is titrated with perchloric acid[7] in dioxane.

(4) Gravimetric assay, by precipitating the bases in the form of picrates[8].

For methods 2, 3 and 4, high purity is required; the presence of only traces of basic compounds may alter the ultra-violet absorption curve and may also produce erroneous results with both the perchloric acid titration and the picrate determination.

The separation of promazine from chlorpromazine or promethazine has not been described, while the separation of chlorpromazine from promethazine has been described by Berti[9], who used chromatographic methods, and recently by Calò with an ionophoretic method[6].

Principle of the Proposed Method

The method described for the assay of promazine, chlorpromazine and promethazine mixtures is based on the method described by Overholser and Yoe[10] for the assay of phenothiazine by the formation of a complex with palladium chloride and also on the fact that promethazine differs from chlorpromazine in having a substituted *iso*propyl instead of a propyl group. Promethazine may be differentiated from the two other products using mercuric sulphate as reagent, which besides acting as oxidizing agent is also used to detect *iso*propanol in the presence of propanol[11].

Complex with Palladium Chloride

According to Overholser and Yoe[10] palladium chloride reacts with phenothiazine giving blue coloured solutions or a precipitate which is not an oxidation product but a complex having the formula

$$Pd(C_{12}H_9NS_2)Cl_2$$

For the reaction, 1 ml. of an aqueous solution of 0·03 mg./ml. of $PdCl_2$ and a solution of phenothiazine in an amount of acetone which must not exceed 20 per cent by volume of the total solution, are added to 5 ml. of a buffer at pH 2·9. The reading must be made and compared with a standard immediately as the colour is not stable. The optimum amount of phenothiazine is 40 μg.; with amounts greater than 100 μg. the colour is very unstable. The colour obtained for pure phenothiazine ranges between blue and purple. The coloured complex may be extracted with ethyl acetate or chloroform yielding a red coloured extract which is less stable than the blue colour of the buffered solution. The buffer is necessary, as small quantities of salts or acids may alter the intensity of colour.

To avoid the use of acetone as solvent the phenothiazines are assayed as hydrochlorides, but colours obtained with a concentration of 0·03 mg./ml. of palladium chloride are too faint, and 1 mg./ml. is necessary. We also found that buffer at pH $2 \pm 0{\cdot}1$ gave a more stable colour. With this modification a bright red colour is obtained for promazine, chlorpromazine and promethazine; the calibration curve is the same for the three products. The Lambert-Beer law is closely observed; the colour rises to a maximum intensity after 10 minutes and is stable for about 2 hours having a maximum absorption at about 500 mμ. The optimum amount of compound for the assay is between 50 and 250 μg. The colour can be extracted by stirring the solutions with ethyl acetate, which extracts the colour due to chlorpromazine and promethazine but does not extract that due to promazine. The gold-yellow colour of the organic phase is stable for an hour and has the absorption maximum at 440 mμ. The optimum concentration ranges between 500 and 1,000 μg./ml.

Reaction with Mercuric Sulphate Reagent

Promethazine heated with this reagent gives either a red colouration or a red precipitate. Under the conditions described the red colour is stable for one hour and has an absorption maximum at 500 mμ, while promazine and chlorpromazine develop no colour.

Experimental

Determination of Promazine, Chlorpromazine and Promethazine with Palladium Chloride

Reagents: (1) Buffered solution at pH = 2 $\pm$ 0·1 : (10 g. of sodium acetate trihydrate are dissolved in 50 ml. of water: to the solution 80 ml. of N hydrochloric acid and water are added to a final volume of 200 ml.); (2) palladium chloride solution: (to 50 mg. of $PdCl_2$, hydrochloric acid is added to 50 ml.); (3) standard solutions of promazine hydrochloride in water, chlorpromazine hydrochloride in water, promethazine hydrochloride in water (99 per cent pure potentiometrically assayed).

Procedure: 0·5 ml. of $PdCl_2$ reagent is added to a mixture of 5 ml. of buffer solution and 1 ml. of aqueous solution containing from 50 to 150 μg. of the product to be assayed. Water is added to make 7 ml. The mixture is stirred, and after 15 minutes the red colour developed read a 500 mμ with a Beckman D.U. spectrophotometer using a 1 cm. cell, against a blank prepared in the same way without addition of the active substance.

TABLE I

Colour stability as obtained in the colorimetric assay of promazine, promethazine and chlorpromazine (200 μg./ml.) with $PdCl_2$

	E after minutes					
	5	10	20	30	60	120
Promazine	0·295	0·305	0·305	0·305	0·305	0·305
Chlorpromazine ..	0·295	0·295	0·300	0·300	0·300	0·300
Promethazine ..	0·290	0·290	0·295	0·295	0·295	0·295

TABLE II

Absorption values (*E*) at different wavelengths for promazine, chlorpromazine and promethazine (200 μg./ml.)

mμ	450	470	480	490	500	510	530	550
Promazine	0·16	0·25	0·295	0·300	0·305	0·298	0·265	0·210
Chlorpromazine	—	0·26	0·290	0·295	0·300	0·290	0·270	0·200
Promethazine	0·15	0·25	0·280	0·290	0·295	0·290	0·270	0·200

From the calibration curve, the concentration in μg./ml. of promazine, chlorpromazine and promethazine is obtained. At the same concentration two or three phenothiazine derivatives yield practically the same extinction as a single member of the mixture. Table I showing the values obtained with 200 μg./ml. after different times, confirms the stability of the colour. Table II gives the values of the absorption obtained from different wavelengths with 200 μg./ml. of each of the three substances.

Assay of Chlorpromazine and Promethazine by Extraction with Ethyl Acetate of Colour Developed with Palladium Chloride

The first reaction is repeated with solutions containing about 500 μg./ml. of either chlorpromazine or promethazine or a total of 500 μg./ml. of both. More compound is required in this reaction because the extinction of the gold-yellow solution obtained by extraction with ethyl acetate is lower than the red colour obtained in buffered solutions working at the same concentrations.

TABLE III

EXTINCTION VALUES AT 440 mμ OF ETHYL ACETATE SOLUTIONS OF PROMAZINE, CHLORPROMAZINE AND PROMETHAZINE (500 μG./ML.)
(3 assays)

Promazine ..	0	0	0
Chlorpromazine ..	0·135	0·135	0·140
Promethazine ..	0·07	0·07	0·07

To a 100 ml. separating funnel is added 5 ml. of buffer solution, 0·5 ml. of palladium chloride reagent, 1 ml. of a solution of the compounds at the above mentioned concentration and 1 ml. of water. The mixture is stirred ten times and 10 ml. of ethyl acetate added. The mixture is again stirred ten times, the aqueous layer is removed and the organic layer is washed with 5 ml. of N HCl. After 5 minutes the colour extracted into the organic phase is read at 440 mμ against a blank and against standard solutions prepared in the same way using 500 μg. of promethazine or chlorpromazine or a mixture of both, the proportions varying according to the composition of the mixture to be assayed. The results are reported in Table III.

TABLE IV

COLOUR STABILITY AS OBTAINED IN THE COLORIMETRIC ASSAY OF CHLORPROMAZINE AND PROMETHAZINE WITH $PdCl_2$ AFTER EXTRACTION WITH ETHYL ACETATE (500 μG./ML.)

	E after minutes					
	5	10	20	30	60	90
Chlorpromazine ..	0·130	0·135	0·135	0·135	0·135	0·120
Promethazine ..	0·07	0·07	0·07	0·07	0·07	0·065

TABLE V

ABSORPTION VALUES (*E*) AT DIFFERENT WAVELENGTHS FOR ETHYL ACETATE SOLUTION OF CHLORPROMAZINE AND PROMETHAZINE (500 μG./ML.)

mμ	400	420	430	440	450	460	480
Chlorpromazine	0·11	0·120	0·125	0·135	0·130	0·120	—
Promethazine	0·06	0·065	0·065	0·07	0·06	0·05	—

Table IV reports the values of reading with 500 μg./ml. of chlorpromazine obtained after different times showing the stability of the colour in ethyl acetate. Table V presents the absorption values at different wavelengths obtained with 500 μg./ml. of these substances.

Promethazine Assay with Mercury Sulphate

Reagent: 5 g. of yellow mercury oxide is dissolved in a mixture of 80 ml. of concentrated sulphuric acid and 20 ml. of water.

Procedure: to 1 ml. of reagent is added 1 ml. of an aqueous solution containing from 50 to 100 μg./ml. of promethazine. The mixture is heated on a boiling water bath for ten minutes, cooled, and water added to 5 ml. The colour is read after ten minutes at 500 mμ in a 1 cm. cell with a Beckman D.U. against a blank prepared from 1 ml. of reagent and 4 ml. of water. The concentration of promethazine is read from the calibration curve.

Table VI gives the values of readings with 100 μg./ml. of promethazine obtained after different times, showing the stability of colour.

TABLE VI

COLOUR STABILITY AS OBTAINED IN THE COLORIMETRIC ASSAY OF PROMETHAZINE WITH $HgSO_4$ (100 μG./ML.)

E after minutes					
3	5	10	20	30	60
0·270	0·280	0·280	0·280	0·280	0·278

TABLE VII

ABSORPTION VALUES (*E*) AT DIFFERENT WAVELENGTHS OBTAINED WITH 100 μg./ml. OF PROMETHAZINE

mμ	450	475	500	510	520	530
	0·235	0·250	0·275	0·270	0·220	0·185

Table VII gives the values of absorption with the different wavelengths obtained with 100 μg. of promethazine. No colour developed with the conditions described, on proportional quantities of promazine and chlorpromazine. With mixtures of promethazine and promazine or chlorpromazine, the calibration curves so obtained were equal to that already obtained with pure promethazine.

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