TECHNICAL METHODS

The Colorimetric Estimation of 3-Chlorpromazine in Biological Fluids

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With the increasing clinical use of chlorpromazine ("largactil") it was found desirable to obtain a satisfactory method for its determination in biological fluids. Procedures have been described for this substance or for closely related phenothiazine compounds using plant peroxidases (Solsona and Mora, 1951), ferric chloride (Fossoul, 1950), sodium persulphate (Fossoul, 1951), picric acid (Uyeo and Oishi, 1952), sodium nitrate in concentrated hydrochloric acid (Berti and Cima, 1954), and concentrated sulphuric acid (Dubost and Pascal, 1953). Many of these depend on the red colours produced by the controlled oxidation of phenothiazine derivatives.

Some of the methods examined proved insensitive to the small amounts of chlorpromazine likely to be found in biological material or they employed unstable reagents. The sulphuric acid method was both sensitive and employed a stable reagent, but difficulties have been encountered (Dubost and Pascal, 1955) with some batches of sulphuric acid due to fading of the colour. This can be overcome by the addition of traces of reducing agents to the sulphuric acid, but this

then produces a reagent stable for only a short time. Furthermore, both this method and that of Berti and Cima require high concentrations of strong acids in the final reaction mixture, and it was felt that the use of corrosive fluids of this strength should be avoided if possible.

Porter and Silber (1950) have described a modification of Brodie's method for basic organic substances which has been applied to the estimation of phenothiazine compounds, but this is not suitable for routine use.

Experimental

All optical measurements were made in a "unicam" S.P. 500 spectrophotometer using matched glass 1 cm. cells.

The reds produced by the phenothiazine derivatives in oxidizing conditions were considered to offer the best basis for colorimetric estimation, and the effect of various oxidizing agents and conditions was investigated.

In these experiments the reagents were added to 5 ml. of a solution of 50 μ g. in 0.1 N sulphuric acid.

Concentrated perchloric acid proved as sensitive as sulphuric acid (which can be used with as little as 1.25 mg. of chlorpromazine per litre), but since our intention was to avoid the use of concentrated acid solutions if possible this substitution offered no advantages.

The colour produced by traces of ferric iron in dilute mineral acids seemed to offer the best possibilities and was chosen for further study. In the case of ferric alum in dilute (4.4 N) perchloric acid the optimum conditions for the production of a sensitive

FIG. 1

and stable colour were not found despite many experiments in which the relative concentrations of the components were varied. It was found that ferric iron in sulphuric acid solutions became more sensitive with increasing concentrations of sulphuric acid, and this sensitivity was shown to be due to the presence of iron, since comparable concentrations of sulphuric acid without iron gave very pale or colourless solutions. The most satisfactory colour was produced in the presence of a final sulphuric acid concentration of approximately 16%, but the colour was very sensitive to the amount of ferric iron. By varying the concentration of iron it was found that the optimum concentration was between 0.03 and 0.06% of hydrated ferric nitrate. It is essential that the iron solution be added to the reaction mixture after the sulphuric acid, and under these conditions a colour stable for at least an hour is formed. Fig. 1 shows that the colour obeys Beer's law up to 100 μ g. with only slight deviations, of the order of 5%, up to 200 μ g. chlorpromazine in the final reaction mixture.

Methods

Reagents.—The following are required: 50% sulphuric acid, 0.1 N sulphuric acid, 1.0 N sodium hydroxide, 0.2 N sodium hydroxide, 50% potassium hydroxide, 2% ferric nitrate ($\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$) in 1.0 N H_2SO_4 , and ether. All reagents should be of A.R. quality where possible.

Extraction from Urine.—Urine, 20 ml., and 1 ml. of N NaOH is extracted four times with 20 ml. portions of ether. The pooled extracts are washed with 10 ml. 0.2 N NaOH followed by 10 ml. of water. After removing the wash water completely the extract is shaken vigorously with 10 ml. 0.1 N sulphuric acid and allowed to stand for five minutes. The acid extract is removed, warmed slightly, and aerated for a few seconds to remove traces of ether. Duplicate 4 ml. samples are taken for the final estimation. With quantities of chlorpromazine below 1 mg. per litre it is advisable to extract with only 6 ml. of the sulphuric acid solution. Occasionally intractable emulsions form, and if this happens the urine-ether emulsion should be transferred to a centrifuge tube and the extraction completed as for blood.

Extraction from Blood: Free Chlorpromazine.—Oxalated blood, 5 ml., is treated with 5 ml. of 50% KOH in a 50 ml. pyrex centrifuge tube and warmed on the boiling water-bath for two minutes with frequent and vigorous shaking. The mixture is cooled, 10 ml. of water added, and shaken well with 20 ml. of ether. The tube is centrifuged and the ether layer removed to a separating funnel. The ether extraction is repeated three times more. The transfer of the ether layer can easily be accomplished by the use of a two-hole stopper fitted with glass tubing similar to a wash bottle head. This is placed in the mouth of the separating funnel and by gentle suction the ether drawn over into the funnel with no risk of loss. If the end of the tube which draws up the

ether is bent back upon itself for the last 2 mm. after being drawn to a capillary it will be found that all but the last few drops of ether can be removed with no disturbance of the aqueous phase.

The pooled ethereal extracts are washed with NaOH and water as in the case of urine, extracted with 6 ml. of 0.1 N sulphuric acid and 4 ml. of the acid extract taken for colour development.

Extraction from Blood: Total Chlorpromazine.—Blood, 5 ml., and 5 ml. of concentrated hydrochloric acid are heated in a boiling water-bath for five minutes. The mixture is cooled, cautiously made alkaline, and extracted with ether as for free chlorpromazine.

Estimation.—The 4 ml. samples are treated with 2 ml. of 50% sulphuric acid and mixed well. Then 0.2 ml. of the ferric nitrate solution is added and the red colour read at 530 μ (Ilford spectrum green 604) against a water blank.

A calibration curve is prepared with amounts of chlorpromazine hydrochloride from 5 to 100 μ g. in 4 ml. 0.1 N sulphuric acid.

Recoveries.—It was found that at least 90% of chlorpromazine added to urine could be recovered down to 1.0 mg. per litre. With blood the recoveries were only slightly less down to 2.0 mg. per litre. Sample data are given in Table I.

TABLE I

Chlorpromazine Added (μ g.)	Found (μ g.)	%
<i>Recovery from Urine</i>		
10	8.7	87
20	18.7	93.5
35	32.5	93
50	47	94
<i>Recovery from Blood</i>		
10	8.7	87
20	18.4	92
50	43.9	87

Summary

A rapid and simple method for the determination of chlorpromazine ("largactil") in urine and blood using cheap and stable reagents has been described.

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