THE ESTIMATION OF SODIUM GENTISATE IN TABLETS AND INJECTIONS

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Sodium salicylate is widely used in the treatment of acute rheumatic fever and usually controls the pain and swelling associated with the disease. It has been emphasised that adequate therapy with the drug demands a high plasma-salicylate level of between 30 and 40 mg. per 100 ml.[1,2]. Graham and Parker[3] demonstrated that plasma-salicylate levels greater than 35 mg. per 100 ml. are associated with the well-known toxic effects of salicylates on the gastro-intestinal tract and on the special senses. This must be considered one of the serious drawbacks of prolonged salicylate therapy.

Normal adults excrete 4 to 8 per cent. of ingested salicylate as gentisic acid[4] (2:5-dihydroxybenzoic acid) and it has been reported that in patients with acute rheumatic fever this fraction is appreciably increased[5]. The action of salicylates may be due to formation of gentisate, the latter substance acting as an inhibitor of the enzyme hyaluronidase[6]. This enzyme depolymerises hyaluronic acid which acts as an interfibrillar cement in the tissues and it has been suggested that there is increased hyaluronidase activity in rheumatic disease[7]. The sodium salt of gentisic acid has been used in the treatment of rheumatic fever[8] and seems to be at least as therapeutically active as sodium salicylate and has the advantage that it produces few, if any, toxic effects even in doses up to 18 g. per day[9].

Sodium gentisate may be administered orally as cachets or tablets or by intramuscular injection. Two independent methods of estimation of the substance in these preparations have been devised. One method is based on the photometric measurement of the blue colour produced when gentisate reacts with an aqueous solution of the Folin-Ciocalteu phenol reagent in alkaline solution. The colour production depends on the reduction of hexavalent molybdenum and tungsten in the reagent to coloured products of lower valency. The other method utilises the absorption given by gentisate in the ultraviolet region. The ultraviolet spectra of aqueous solutions of sodium gentisate show a maximum at 3200Å and the optical densities at this wavelength were found to be directly proportional to the concentration of gentisate ion.

Gentisic acid has been estimated in urine, after a preliminary extraction with ether, by means of the blue colour it gives with ferric chloride[9] but this colour is too transient for accurate work. It has also been assayed by the reduction of alkaline cupric solutions, carried out according to the Shaffer-Hartmann method for blood sugar, and by a bromine

consumption procedure, but this latter reaction has to be performed at 0°C. to make it quantitative[4].

Experimental

Method I.

A blue colour having an absorption maximum of 6600Å develops when sodium hydroxide is added to a mixture of a solution of sodium gentisate and the Folin-Ciocalteu phenol reagent[10]. The colour reaches a maximum after 1 minute but begins to fade after 20 minutes (Fig. 1); a

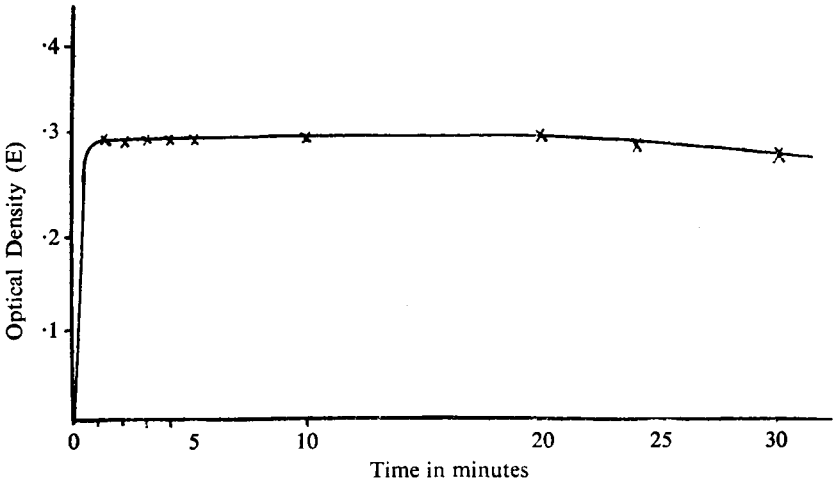

Fig. 1.—Rate of development and fading of Folin-Ciocalteu blue colour at room temperature.

similar behaviour has been reported for salicylates[11]. The absorption density of the coloured solution was found to be directly proportional to the concentration of gentisate ion up to 100 mg. per 100 ml.

Preparation of solutions of sodium gentisate for analysis. a. Powder. About 50 mg. of the powder is accurately weighed, dissolved in distilled water and made up to 100 ml.

b. Tablets. 2 tablets, each containing 0·25 g. of sodium gentisate, are dissolved in distilled water and made up to 1 l.

c. Injection. Solutions for intramuscular injection are prepared which contain 1 g. of sodium gentisate in 10 ml. of distilled water and are sterilised by autoclaving. 5 ml. of the injection is made up to 1 l. with distilled water.

The method of estimation is as follows: 1 ml. of sodium gentisate solution prepared from the powder, tablet or injection is added to 9 ml. of distilled water, 10 ml. of Folin-Ciocalteu reagent (diluted 1 to 3 with distilled water) are added and well mixed. 1 ml. of the mixture is removed and 5 m. of aqueous 0·5N sodium hydroxide added to it, the

solution is allowed to stand at room temperature for 5 minutes and the absorption density measured against distilled water in a photo-electric absorptiometer using a 1 cm. cell and a filter transmitting maximally above 6600Å. A Hilger Spekker photo-electric absorptiometer and an Ilford spectrum red filter No. 608 have been used in the present work. A calibration curve (Fig. 2) may be constructed from dilutions of anhydrous sodium gentisate in distilled water.

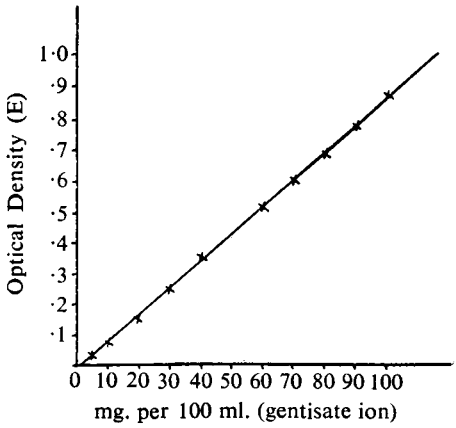

FIG. 2.—Calibration curve of gentisate in distilled water using the blue colour given by the Folin-Ciocalteu reagent in alkaline solution. The absorption densities were measured in a Hilger Spekker absorptiometer using an Ilford spectrum red filter, No. 608.

Method II.

The ultraviolet absorption spectrum of an aqueous solution of sodium gentisate (Fig. 3) shows a maximum at 3200Å, the *p*H of the solution being 5·6. The molecular extinction coefficients, $\epsilon_{(mol.)}$ at 3200Å of sodium gentisate and gentisic acid are 4312 and 3773 respectively, a value of 3750 ± 115 at 3225Å has been

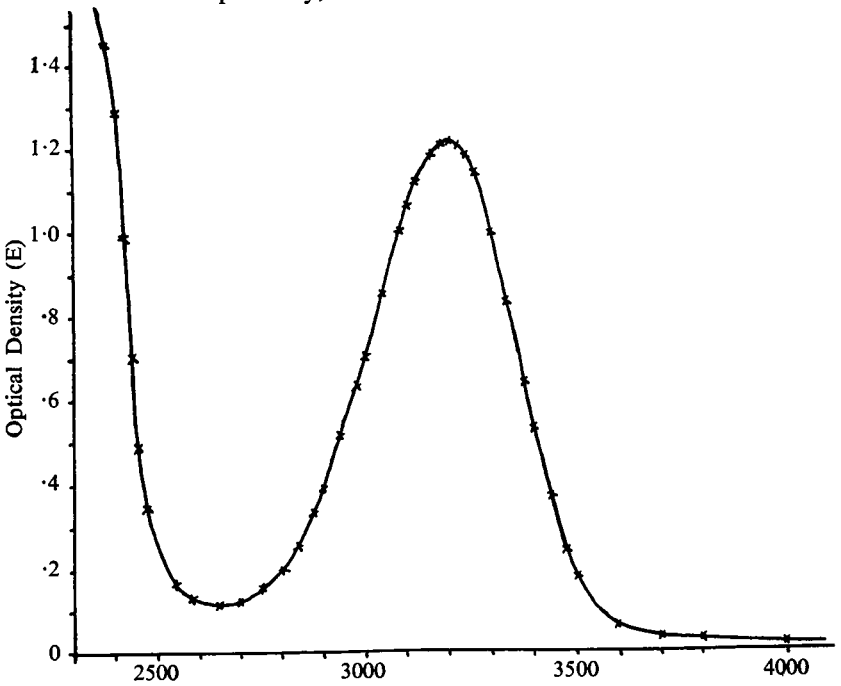

FIG. 3.—Ultra violet absorption spectrum of an aqueous solution of sodium gentisate at *p*H 5·6, the concentration of the solution was 5 mg./ml. per 100.

reported for gentisic acid[4]. It was found that the optical densities, measured at 3200Å, of solutions of sodium gentisate were directly proportional to the concentration up to 5 mg./100 ml.

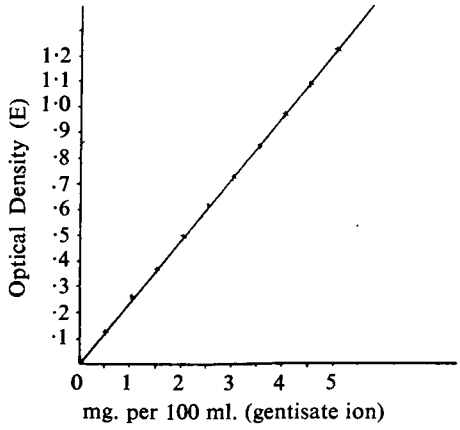

FIG. 4.—Calibration curve of gentisate in distilled water. The optical densities were measured at 3200Å in a Hilger Uvispek spectrophotometer.

Preparation of solutions. The solutions prepared for method I are accurately diluted 1 to 20. The method of estimation is as follows. The optical density of the solution is measured at 3200Å against distilled water using a 1 cm. cell in an ultraviolet spectrophotometer. The Hilger Uvispek ultraviolet spectrophotometer has been employed in the present work. A calibration curve may be constructed from dilutions of sodium gentisate in distilled water (Fig. 4). The results obtained in the estimation of sodium gentisate in various preparations are given in Table I; they are expressed as anhydrous sodium gentisate $C_7H_5O_4Na$.

TABLE I

Preparation	Method I	Method II
(1) Aqueous solution...	27·1	26·9
(2) Aqueous solution...	40·0	40·5
(3) Aqueous solution...	17·2	17·5
(4) Aqueous solution...	34·5	34·3
(5) Powder	95·2	95·4
(6) Powder	92·8	93·1
(7) Powder	93·4	93·2
(8) Powder	92·7	93·0
(9) Tablet	0·236	0·245
(10) Tablet	0·232	0·240
(11) Tablet	0·215	0·220
(12) Tablet	0·226	0·230
(13) Injection	9·42	9·50
(14) Injection	9·60	9·65
(15) Injection	9·24	9·40
(16) Injection	9·06	9·10

Nos. 1-4 are expressed as mg. per 100 ml. of sodium gentisate ($C_7H_5O_4Na$)
Nos. 5-8 are expressed as g. per cent. w/w of sodium gentisate
Nos. 9-12 are expressed as g. of sodium gentisate per tablet
Nos. 13-16 are expressed as g. per 100 ml. of sodium gentisate.

SUMMARY

1. Two independent methods of estimation of sodium gentisate are described, one method uses the blue colour which develops when gentisate reacts with the Folin-Ciocalteu phenol reagent in alkaline solution, the other method is based on the measurement of the optical densities of aqueous solution of gentisate at 3200Å.

2. The application of these methods to the assay of sodium gentisate in powder form, tablets and solutions for injection is described.

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REFERENCES

1. Reid, *Quart. J. Med.*, 1948, **66,** 139.
2. Coburn, *Bull. Johns Hopkins Hosp.*, 1943, **73,** 435.
3. Graham and Parker, *Quart. J. Med.*, 1948, **66,** 153.
4. Kapp and Coburn, *J. biol. Chem.*, 1942, **145,** 549.
5. Smith, *J. Pharmacol.*, 1949, **97,** 357.
6. Meyer, Ragan and Weinshelbaum, *Fed. Proc.*, 1948, **7,** 173.
7. *Lancet*, 1949, **257,** 473.
8. Meyer and Ragan, *Science*, 1948, **108,** 281.
9. Camelin, Accoyer, Pellerat, Lafuma and Coirault, *Bull. Soc. med. Hôp., Paris*, 1949, **21/22,** 826.
10. Peters and Van Slyke, *Quantitative Clinical Chemistry*, 1932, London, Balliere, Tindall and Cox, **2,** 655.
11. Smith and Talbot, *Biochem. J.*, 1950, **46,** v.