DIRECT COLORIMETRIC DETERMINATION OF SMALL QUANTITIES OF *m*-AMINOPHENOL IN SODIUM AMINOSALICYLATE

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A colorimetric method has been developed for the determination of *m*-aminophenol in sodium *p*-aminosalicylate by formation of a blue colour with phosphomolybdic acid. Although both sodium aminosalicylate and *m*-aminophenol form blue complexes with this reagent, prior isolation of the *m*-aminophenol is unnecessary because the extinction coefficient for the *m*-aminophenol complex is 90 times that of sodium *p*-aminosalicylate complex.

SINCE sodium *p*-aminosalicylate was introduced in therapy the amount of *m*-aminophenol, its most common impurity, has been of major interest to many authors. Although its toxic effect is not as great as initially believed, control of commercial sodium aminosalicylate is undoubtedly necessary because of the high toxicity of its oxidation products (Haberland, 1951; Boymond and Adatto, 1955; Kasalicky and Nejedly, 1945). According to various pharmacopoeias the limits of *m*-aminophenol vary from 0·1 to 1·0 per cent.

Most of the reported methods for the determination of *m*-aminophenol present as impurity in sodium aminosalicylate use an extraction or precipitation procedure for the preliminary separation (see, for example, Ettinger and Ruchloff, 1951; Faschalek, 1952; Simmonite, 1949). Others determine *m*-aminophenol directly. Haberland (1951) reported its direct determination by precipitation with formalin at pH 5·3. The drawback of this method is that after a time sodium aminosalicylate gives a precipitate with formalin. Ota, Nakajima and Nakagami (1956) elaborated a spectrophotometric method for the simultaneous determination of both substances, but co-existing substances seriously interfere in this determination. Franc (1955) reported a colorimetric method with 4-aminoantipyrine, which permits the direct determination up to 0·5 per cent of *m*-aminophenol in sodium aminosalicylate. The direct colorimetric method by the diazo method proposed by Pesez (1949) is unreliable because of the poor stability of the azo dye formed. A method based upon the formation of an indamine dye from *m*-aminophenol in the presence of oxidants has been described by Hrdý and Petříkova (1957).

We sought a simple, rapid and reliable method allowing the direct determination of small quantities of *m*-aminophenol in sodium aminosalicylate. As both substances are structurely very similar it was impossible to find a reagent which would react with the phenol but not with the aminosalicylate. From preliminary investigations only phosphomolybdic acid in the presence of ammonia gave promising results. Thus 20 mg. of the phenol gave an intense blue colour, while the same amount

of the aminosalicylate gave a pale blue colour. The extinction coefficient of the *m*-aminophenol complex was 90 times that of the sodium aminosalicylate complex. This observation suggested a simple colorimetric method.

EXPERIMENTAL

Reagents and Apparatus

m-Aminophenol, Bayer, redistilled, m.p. 122–123° (Koffler block), sodium *p*-aminosalicylate, $2H_2O$, Bayer, content of *m*-aminophenol less than 0·01 per cent, acetate buffer solution of pH 6·0 prepared from 0·2M sodium acetate and 0·2M acetic acid, phosphomolybdic acid, Carlo Erba, Reagent Grade.

Measurements were made with the Zeiss, Elko II, photometer, using a filter of wavelength 720 mμ and 1 cm. cells and a Jobin Yvon spectrophotometer, using 1 cm. quartz and glass cells respectively.

Methods of Assay

Reagent concentrations and reaction conditions were selected after evaluation of each factor over a range. The optimum conditions for the determination of *m*-aminophenol in sodium aminosalicylate are as follows:

To 50 mg. sodium aminosalicylate dissolved in 1 ml. of distilled water add 10 ml. acetate buffer and 7 ml. of 5 per cent aqueous solution of phosphomolybdic acid. Mix well and after 10 min. add 3 ml. of 2 per cent ammonia solution. After 5 min. measure the extinction against water of the blue reaction products of both the sample and a blank of 50 mg. "standard sodium aminosalicylate"* similarly treated, at a wavelength of 720 mμ. Correct the extinction of the sample by subtracting the reagent blank reading. The content of phenol is obtained by reference to a calibration graph.

Comparison of results obtained by this method with those using the official method of Sanz (1952) which was proposed by the firm of Bayer gave the following results.

	m-Aminophenol per cent	
	Present method	Method of Sanz (1952)
1.	Not more	—
2.	than 0·01	—
3.	0·15	0·16
4.	0·30	0·28

Calibration Curve

1 ml. aliquots containing from 0·01 to 1 mg. *m*-aminophenol were added to 50 mg. amounts of standard sodium aminosalicylate. The solutions were treated with phosphomolybdic acid as above. After 10 min. extinction *E*, at 720 mμ of the blue reaction product was

* Sodium *p*-aminosalicylate Bayer containing less than 0·01 per cent *m*-aminophenol. Standards were used in all the experiments and gave constant extinction values of 0·091–0·093.

measured. A blank determination was also made using the standard sodium aminosalicylate with no added *m*-aminophenol and the extinction E_2 at 720 mμ also measured. $E_1 - E_2$ gave E due to the *m*-aminophenol complex above. The results of 86 determinations were plotted as E against mg. concentration of the *m*-aminophenol and a straight line obtained.

On statistical analysis of the results it was found that the limits of error for b = 0·2249, calculated according to Davies (1958), were between 95·1 and 104·9 per cent (P = 0·01).

Results and Discussion

The method described is applicable to the determination of *m*-aminophenol in the concentration range 0·1–2·0 per cent in sodium aminosalicylate, the range in which it occurs most frequently commercially. The use of 4 cm. cells may improve the sensitivity of the method.

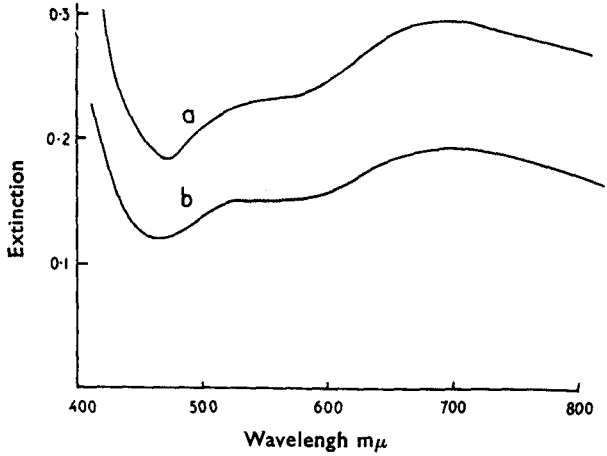

FIG. 1. Absorption curves for sodium aminosalicylate and *m*-aminophenol blue.
a. *m*-Aminophenol blue (prepared from *m*-aminophenol 1 mg./ml. solution).
b. Sodium aminosalicylate blue (prepared from 0·1 g./ml. solution).

The reaction of phenols with the phosphomolybdic acid reagent of Wavelet has been studied by Vignoli and Cristau (1952) and Cristau (1954). These authors have claimed that maximum absorption of the blue reaction product "molybden blue" occurs at 700 mμ, with maximum colour stability at pH 6·1.

Our preliminary investigations showed that a molybden-blue was formed from both *m*-aminophenol and sodium *p*-aminosalicylate. The absorption maximum for both was found to be between 680 and 700 mμ (Fig. 1) while maximum colour stability was attained at pH 6·2.

In the course of ultra-violet absorption studies of "molybden blues" obtained with various compounds Vaisberg and Ya Dain (1951) observed that the spectra of the "molybden blues" of the compounds examined between 200 and 450 mμ are similar to those of the original compounds.

From this they concluded that the original compound and the "Blue" produced therefrom had a similar structure.

The spectrum of *m*-aminophenol blue differs significantly from that of the *m*-aminophenol itself in showing no absorption in the region between 270 and 310 mμ. The ultra-violet spectrum of sodium aminosalicylate blue is identical to that of the original compound (Fig. 2).

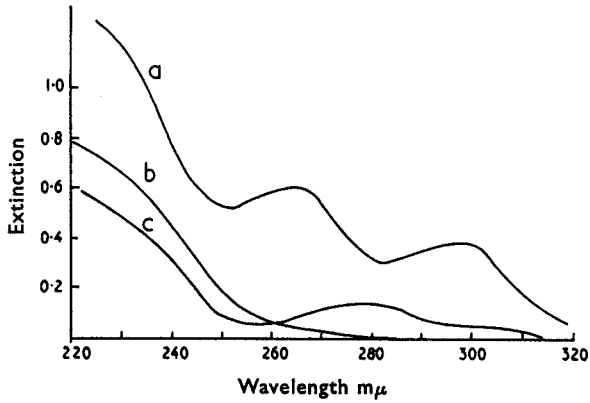

FIG. 2. Ultra-violet absorption curves of: a. Sodium aminosalicylate blue (0·02 mg./ml.) b. *m*-Aminophenol blue (0·002 mg./ml.) c. *m*-Aminophenol (0·08 mg./ml.) The curve for sodium aminosalicylate is identical with the curve for its blue complex.

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