THE COLORIMETRIC DETERMINATION OF PHENACETIN IN TABLET MIXTURES

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Phenacetin may be quantitatively converted to nitrophenetidine by nitration and subsequent saponification with sodium hydroxide. The nitrophenetidine thus formed is soluble in alkaline solution and may be determined colorimetrically at 445 mμ. The method may be applied to samples containing 1 to 5 mg. of phenacetin. This method possesses several advantages over existing methods in that it is simple, sensitive and accurate and does not require the tedious separation of caffeine when applied to the determination of phenacetin in aspirin, phenacetin and caffeine mixtures. Recovery data of phenacetin in these mixtures are given. A simple and rapid procedure for the analysis of the components of aspirin, phenacetin and caffeine is also described.

THE rapid determination of the components of the time-honoured A.P.C. tablets has always been the goal of many analytical chemists. While satisfactory results can be obtained for the determination of aspirin in this mixture by the B.P.C.[1] or the A.O.A.C.[2] methods, the procedures recommended for the separation and determination of caffeine and phenacetin are time-consuming, tedious and lack specificity and reproducibility. Daoust[3] described a simple colorimetric method for the determination of caffeine as its phosphomolybdate derivative in A.P.C. tablets, and the main difficulty seems to be the lack of a reliable method for the estimation of phenacetin in this mixture.

Many methods for the determination of phenacetin have been reported. Wollish, Colarusso, Pifer and Schmall[4], described a procedure for the determination of phenacetin by non-aqueous titration. Casini[5] recommended the conversion of phenacetin to tetra-iodophenacetin by the addition of standard iodine solution, the excess of which can be determined with thiosulphate solution. Higuchi and Patel[6] have described a spectrophotometric method for the determination of phenacetin in A.P.C. mixture. Although satisfactory results can be obtained by these methods all require time-consuming extraction for the separation of phenacetin from caffeine before its determination. Horn[7] described a colorimetric method for the determination of phenacetin as 3-nitro-4-acetamidophenetole and he reported its applicability to A.P.C. mixtures. However, Casini[5] has studied this procedure in detail and reported the method to be neither sensitive nor reliable. Degner and Johnson[8] estimated phenacetin from the colour produced with concentrated hydrochloric acid and chromic acid. The reaction is dependent on temperature, time, and the concentrations of the chromic acid and ammonium citrate used. The colour goes through a maximum intensity and then fades, the time and intensity of maximum colour formation and rate of fading depends on the amount of chromic acid, hydrochloric acid and ammonium citrate used as well as

the temperature. Because of these limitations the need for a rapid and accurate method for the determination of phenacetin for control work is apparent.

EXPERIMENTAL

Reagents. Phenacetin standard solution—Recrystallised phenacetin, 20 mg., is dissolved in about 70 ml. of chloroform and diluted to 100 ml., 5 ml. contains 1 mg. of phenacetin. Nitric acid (50 per cent v/v). Sodium hydroxide solution (10 per cent) and chloroform, reagent grade.

Preparation of a standard curve. Aliquots of 5, 10, 15, 20, and 25 ml. of the standard solution of phenacetin are pipetted consecutively into each of five 50 ml. beakers on a steam bath. After evaporation of the chloroform 2 ml. of nitric acid is added and the solution allowed to stand for 30 minutes at room temperature. Sodium hydroxide solution, 10 ml., is then added to each beaker and the solutions heated on the bath for 30 minutes. Each solution is transferred to a 100 ml. volumetric flask and diluted to mark with distilled water. The optical density of the nitrophenetidine may be measured directly at 445 mμ on a Hilger spectrophotometer using distilled water as the blank. The calibration curve of phenacetin plotted on a linear scale at 1 mg. increments in the range for 1 to 5 mg. indicated that the method obeys Beer's Law with good agreement.

Determination of Phenacetin in the Presence of Caffeine

Known amounts of phenacetin and caffeine are weighed and dissolved in chloroform in a standard volumetric flask. Aliquots containing about 1 to 5 mg. of phenacetin are taken and evaporated in 50 ml. beakers on a water bath. Continue as described in the paragraph on the preparation of a standard curve, beginning with the words: "After evaporation of the chloroform . . ." to "measured directly at 445 mμ on a Hilger spectrophotometer." The quantity of phenacetin is calculated from the calibrated curve. Table I shows the results obtained using this procedure.

TABLE I

RECOVERY OF PHENACETIN IN MIXTURES WITH CAFFEINE

Phenacetin used, mg.	Phenacetin recovered, mg.	Recovery per cent
200	198	99·0
200	197	98·5
200	200	100·0
200	200	100·0
200	203	101·5
200	201	100·5

Determination of Phenacetin in the Presence of Aspirin and Caffeine

A mixture containing 0·230 g. of aspirin, 0·160 g. of phenacetin, and 0·030 g. of caffeine is dissolved in 500 ml. of chloroform. Portions of 10 ml. are taken and extracted once with 15 ml. of 6 per cent sodium bicarbonate solution. The sodium bicarbonate solution is then extracted thrice with 10 ml. portions of chloroform. The chloroform extracts are combined, washed once with 3 ml. of 6 per cent sodium bicarbonate

solution and evaporated to dryness in a 100 ml. beaker. After evaporation 2 ml. of nitric acid is added to the residue followed by 10 ml. of sodium hydroxide solution after 30 minutes. The phenacetin is determined as described previously. The recoveries of phenacetin using the above procedure are shown in Table II.

Determination of Aspirin, Phenacetin and Caffeine in Commercial A.P.C. Tablets and Mixtures

The average tablet weight is calculated from the weight of 20 tablets accurately weighed. A sample of about 0·5 g. of the pulverised tablet is transferred into a separating funnel. To this is added 10 ml. of water and 15 ml. of cold 6 per cent sodium bicarbonate solution together with 2

TABLE II

RESULTS OF ASSAYS FOR PHENACETIN ON KNOWN A.P.C. MIXTURES

Phenacetin present in 10 ml. $CHCl_3$ solution, mg.	Phenacetin recovered, mg.
3·20	3·15
3·20	3·17
3·20	3·22
3·20	3·16
3·20	3·17

TABLE III

RESULTS OF ASSAYS FOR ASPIRIN, PHENACETIN AND CAFFEINE IN COMMERCIAL A.P.C. TABLETS

	Aspirin		Phenacetin		Caffeine	
Samples	Amt. labelled per tablet (mg.)	Amt. found per tablet (mg.)	Amt. labelled per tablet (mg.)	Amt. found per tablet (mg.)	Amt. labelled per tablet (mg.)	Amt. found per tablet (mg.)
A	226·8	214	162·0	156	32·4	32·4
		217		157		31·6
B	226·8	216	162·0	156	32·4	32·4
		220		156		32·0
C	226·8	220	162·0	160	32·4	31·1
		224		162		30·6
D	226·8	223	162·0	158	32·4	31·3
		218		158		32·8

drops of dilute hydrochloric acid. The phenacetin and caffeine present are extracted with five 30 ml. portions of chloroform. Each chloroform portion is passed through a second separating funnel containing 3 ml. of cold 6 per cent sodium bicarbonate solution. The chloroform fractions are filtered through cotton wool and combined for later treatment. The sodium bicarbonate solutions are combined, acidified with 1 : 1 hydrochloric acid and the aspirin present is extracted and determined by the procedure described in A.O.A.C.[2]

The chloroform extracts are combined and transferred to a 200 ml. volumetric flask and made up to mark. Aliquots of 5 ml. are taken and evaporated in 50 ml. beakers and the phenacetin content is determined by the procedure already described. For the determination of caffeine 10 to 15 ml. aliquots of the chloroform solution are taken and evaporated

and the amount of caffeine is determined according to the procedure described by Daoust[3]. The results obtained for the analyses of four commercial samples of A.P.C. are shown in Table III.

Effects of Time and Heat on the Stability of the Colour of Nitrophenetidine Solutions

A known amount of phenacetin is treated with 2 ml. of nitric acid and the solution is allowed to stand for 30 minutes. Ten ml. of sodium hydroxide solution is added and after 30 minutes the solution is transferred to a 100 ml. volumetric flask and the optical density of this solution

TABLE IV

EFFECTS OF HEAT AND TIME ON COLOUR OF THE SOLUTIONS

Time in hours	Optical density		
	Solution 1	Solution 2	Solution 3
0	0·40	0·425	0·886
1	0·430	0·480	0·880
2	0·505	0·555	0·880
3½	0·580	0·625	0·884
4	0·595	0·645	0·882
5	0·640	0·695	0·878
6	0·662	0·715	0·880
18	0·874	0·878	0·882
19	0·880	0·878	0·880
24	0·878	0·876	0·882

No heat was applied to solutions 1 and 2. Solution 3 was heated for 30 min. after addition of sodium hydroxide solution before dilution.

is measured at 445 mμ at hourly intervals. No heat is applied in this procedure and the results obtained are shown in Table IV, Columns II and III.

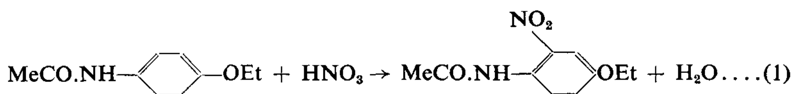
A similar quantity of phenacetin is treated with 2 ml. of nitric acid. After standing at room temperature for 30 minutes 10 ml. of sodium hydroxide solution is added and the alkaline solution is heated on a water bath for 30 minutes. The solution is transferred to a 100 ml. volumetric flask and diluted to mark with distilled water. The optical density of the solution was measured at 445 mμ at one hour intervals. There was no difference between the various readings as shown in Table IV, Column IV. It was observed that when no heat is applied the complete hydrolysis of nitrophenacetin to nitrophenetidine requires about 18 hours.

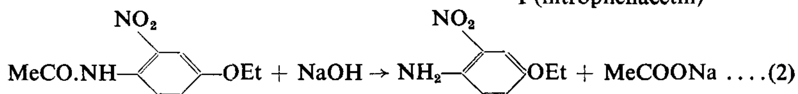
Effects of Amount of Alkali Used

Five 15 ml. aliquots of the standard solution of phenacetin are pipetted into each of five 50 ml. beakers on a water bath. After evaporation of the chloroform 2 ml. of nitric acid is added and the solution allowed to stand for 30 minutes after which 10, 15, 20, 25 and 30 ml. of sodium hydroxide solution is added to each beaker respectively. The resulting solutions are warmed on the water bath for a further 30 minutes and transferred to a 100 ml. volumetric flask and diluted to mark. The optical densities of these solutions were measured at 445 mμ and no differences between the readings were observed.

Results and Discussion

When phenacetin is reacted with nitric acid followed by warming with sodium hydroxide, nitration and hydrolysis take place in accordance with the following equations:





These two reactions form the basis of the proposed method.

Nitrophenacetin (I) when recrystallised from 20 per cent ethanol precipitates in the form of yellowish needles with a melting point of 97°. Nitrophenetidine (II), under suitable conditions is obtained as orange

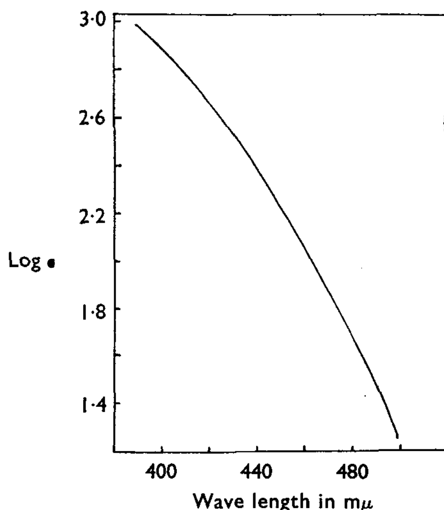


FIG. 1. Light absorption curves of nitrophenacetin in water.

red crystals having a melting point of 78° after recrystallisation from light petroleum. Nitrophenacetin has very low absorption at 465 mμ and its absorption spectra possesses no maximum between 400 mμ and 500 mμ. A typical absorption spectrum of nitrophenacetin is shown in Figure 1. It is therefore not unexpected that the determination of phenacetin as its nitro derivative at 465 mμ as proposed by Horn[7] lacks both sensitivity and reliability as reported by Casini[5]. Nitrophenetidine on the other hand exhibits very strong absorption between 400 mμ and 500 mμ and its absorption spectra has a maximum at 445 mμ as shown in Figure 2. This compound is very stable in alkaline solution and its absorption

spectra is not affected by the amount of alkali present. However, when nitrophenetidine is dissolved in an acid media a light yellow solution results. The spectra of the yellow solution is shown in Figure 3.

Heat is essential for the rapid and complete hydrolysis of nitrophenacetin to nitrophenetidine. Hydrolysis in the cold requires about 18 hours to

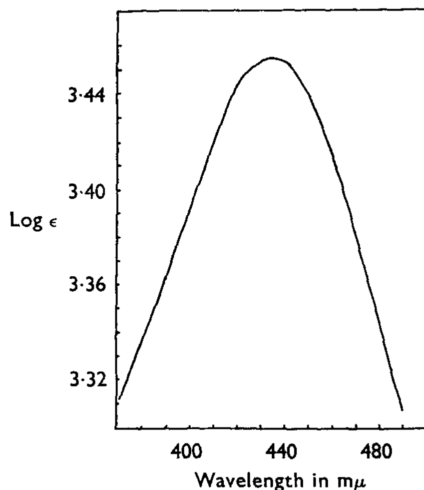


FIG. 2. Light absorption curves of nitrophenetidine in 10 per cent sodium hydroxide solution.

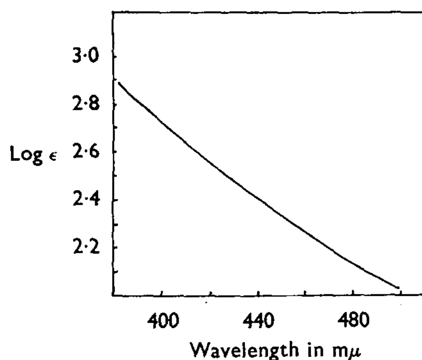


FIG. 3. Light absorption curves of nitrophenetidine in 10 per cent nitric acid.

reach completion. The necessity for heating the alkaline solution for 30 minutes before dilution cannot therefore be over-emphasised for reproducible results.

Recovery results by the proposed method are presented in Tables I to III. The values obtained showed that caffeine does not interfere in the procedure and the recoveries of phenacetin were good indicating satisfactory precision.

The proposed method fulfils the requirements for a routine procedure for the determination of phenacetin in tablet mixtures. The procedure

for the determination is simple and of general applicability and the colour developed is independent of time and the amount of alkali used. The tedious separation of caffeine from phenacetin is not required. The method is sensitive and reliable and may be advantageously used for the determination of phenacetin in A.P.C. mixture. Together with the A.O.A.C.[2] procedure for aspirin and the Daoust[3] method for caffeine, it makes feasible the rapid determination of the components in A.P.C. This proposed method for the analysis of the components of A.P.C. gave satisfactory results as shown in Table III. One analyst can make 6 to 8 complete assays of A.P.C. mixtures in an 8-hour day.

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