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Pyrocatechol Violet in Pharmaceutical Analysis

Part II. A Spectrophotometric Method for the Determination of Paracetamol in Pure and in Pharmaceutical Dosage Forms

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Abstract:- A spectrophotometric method for the selective determination of paracetamol based on its reaction with pyrochatechol violet under basic conditions to form an ion-pair complex is described. The absorption maximum of the coloured ion-pair formed is observed at 652 nm and the molar absorptivity is 4.54×10^{3} l mol^{-1} cm^{-1} . Beer's law is obeyed over the concentration range 0.5-34.0 μg ml^{-1}, while that obtained using Ringbom method is in the range 3.5 -32.0 μg ml^{-1}. There is no interference from common additives, excipients and commercial drugs present in their formulations suggesting a highly selective procedure compared with others. Statistical analysis of the obtained results showed that there is, no significant difference and absence of any systematic error in the method compared with the official one. The method is simple, rapid and convenient and was applied successfully to the determination of paracetamol in pure and in its dosage forms compared with the official method.

Key word: Paracetamol analysis, pyrocatechol violet, spectrophotometry, dosage forms.

Introduction

Paracetamol (N-acetyl-4-aminophenol) occupies a prominent position amongst the extensively employed antipyretic analgesics. It is frequently formulated in drugs with acetyl salicylic acid, salicylamide, caffeine, codeine phosphate, phenyl propanolamine HCl, analgin and oxyphenbutazone. Paracetamol is widely used as an analgestic and antipyretic agent[1]. Analytical interest in paracetamol has been discussed in reviews[2,3]. Overdoses of paracetamol causes hepatic necrosis, probably owing to its metabolite, N-acetyl-p-benzoquinone. Diagnosis must be quick and rapid methods for the determination of paracetamol are therefore needed. Several types of

analytical procedures for determination have been proposed, e.g., titrimetric, gravimetric, electroanalytical[4], colorimetric[5,6], UV-Visible absorption[7-10] and chromatographic[11-13] methods. Various oxidative reagents have been proposed for spectrophotometric[14,15] or fluorimetric[16] determinations of paracetamol. Moreover, a flow-injection spectrofluorimetric determination has been reported based on its oxidation with hexacyanoferrate[17,18]. Most of these methods require lengthy treatment and lact the simplicity and selectivity needed for routine analysis.

A simple, rapid and selective spectrophotometric method for the determination of paracetamol in drug formulations is described. Reaction with pyrocatechol violet in aqueous basic media, almost instantaneously pink colour that reaches its maximum intensity within 2.0 min, the wave length of maximum absorption being 652 nm.

Experimental

Reagents and apparatus

All experiments were performed with analytical grade chemical. Doubly distilled water was used throughout.

5 x 10^{-3} M stock solution of pyrocatechol violet (Aldrich product) was prepared by dissolving 0.1932 g in bidistilled water and completed to the mark in a 100 ml calibrated flask with water.

Stock solutions of 500 μg ml^{-1} paracetamol, anhydrous caffeine, analgin, phenyl propanolamine HCl, chlorpheniramine maleate, acetyl salicylic acid, codeine phosphate or oxyphenbutazone were prepared by dissolving the respective standard compound in water. Standard compounds were obtained from ICN Biomedical products. Stock solutions were stored below 6.0 °C in the dark. Solutions of the desired concentration were obtained by diluting the stock solutions to volume with water.

A borate buffer solutions of pH 5.5 to 10.5 were prepared as recommended previously[19].

All spectral measurements were made using a Perkin-Elmer λ3B and the pH of the solutions was checked using an Orion Research Model 601A/Digital Ionlyzer.

Procedures

Sampling:

A known number of tablets are weighed and ground into a fine powder. A portion of powder containing about 50 mg of paracetamol is weighed accurately, mixed with 30 ml of water and stirred for 10 min. The insoluble mass is filtered off on a Whatman No. 41 filter-paper, washed with water and the filtrate plus washings are diluted to 500 ml with water in a calibrated flask.

Known volumes of syrup are directly diluted with water. Suppositories of paracetamol produce viscous slurry owing to the presence of gelatinous matter that is difficult to filter. Such samples, and those, which also contain other drugs, are extracted with three successive 20 ml portions of anhydrous acetone. The combined extracts are warmed in a boiling water-bath to evaporate acetone and the remaining residue is dissolved in water in a 100 ml calibrated flask.

General procedure

In order to construct a calibration graph, a suitable aliquots of standard solution are transferred into 10 ml calibrated flasks to produce working solutions in the range of 5.0-340 μg of paracetamol solution. Then 1.0 ml of 5×10^{-3} M reagent solution and 6.0 ml of pH 8.5 buffer solution are added to each of the calibrated flasks. The reaction mixture is left to stand at room temperature (25 ± 2 °C) for 2.0 min to complete colour intensity. The absorbance is measured at 652 nm against a reagent blank solution prepared similarly. The calibration graph was prepared by plotting absorbance vs concentration of paracetamol.

Stoichiometric relationship

Job's method of continuous variation was employed; 5×10^{-3}M standard solution of paracetamol and 5×10^{-3} M solution of pyrocatechol violet was used. A series of solutions was prepared in which the total concentration of paracetamol and reagent was kept at 1×10^{-3}M in the final assay solution. The reagent was mixed in various proportions and diluted to volume in a 10 ml calibrated flask with 6.0 ml of pH 8.5 buffer solution, following the above mentioned procedure.

Results and discussion

This method for determining paracetamol is simpler, selective and more rapid compared with the existing procedures[2-18]. The indophenol method[20] is selective, sensitive and highly accurate but it requires prior hydrolysis of the drug and a number of sequential dilutions to obtain a suitable concentration. Also, phenacetin produces about 5.0 % higher results[18] and any free 4-aminophenol present causes a positive error.

In our previous work[21] , pyrocatechol violet has been used for the spectrophotometric determination of some β-lactom antibiotics in pure and in dosage forms. The reaction of pyrocatechol violet with paracetamol results in the formation of an intense pink product that exhibits an absorption maximum at 652 nm. Several parameters such as pH, reagent concentration, time, temperature and sequence of additions were optimized to achieve high sensitivity, low blank reading and high stability for the determination of paracetamol. To elucidate the best medium for quantitative determination, different volumes of borate buffer solution of varying pH values were used. The absorbance readings were maximal at pH 8.0-9.0 as shown in Fig. 1. Moreover 6.0 ml of pH 8.5 is the optimum volume of the best pH value for such reaction to form the ion pair product with high intense colour.

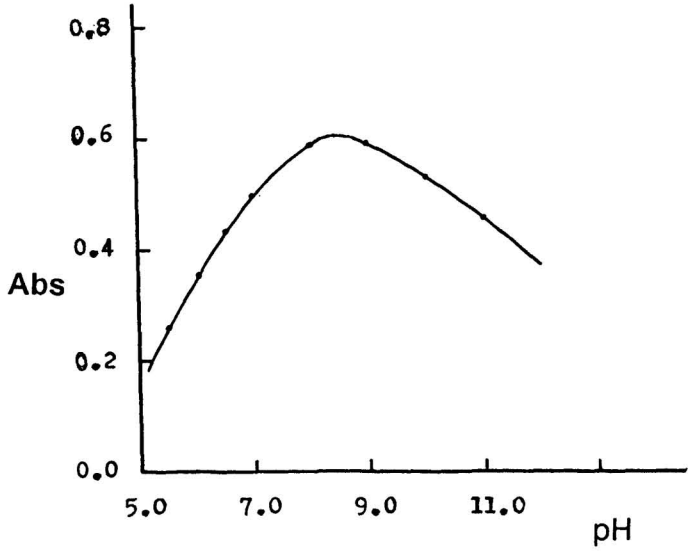


Fig.1: Effect of pH on the absorbance of 20μg ml^{-1} paracetmol using $5x10^{-4}$ M PCV measured at 652nm.

The effect of reagent concentration was investigated by using varying amounts of 5 x 10^{-3} M solution. 1.0 ml was found to be sufficient for the production of maximum and reproducible colour intensity. Higher concentrations of reagent did not affect the colour intensity (Fig. 2).

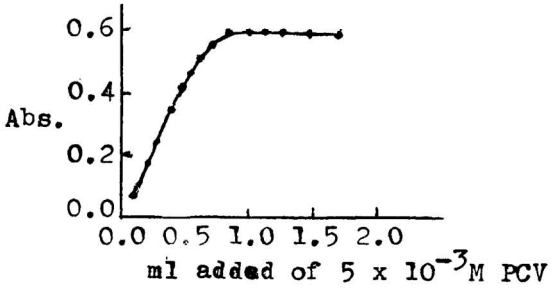
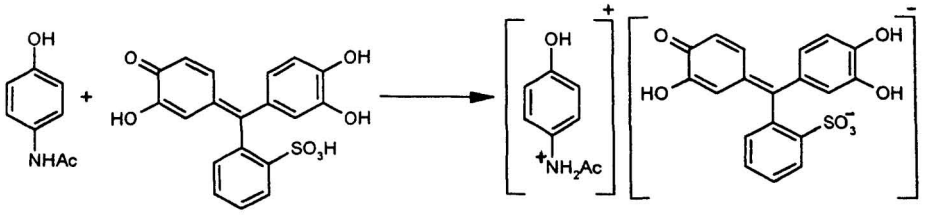


Fig.2: Effect of 5x10^{-3} M of PCV on the absorbance of 20µg ml^{-1} paracetmol at λ_{max} 652 nm.

The optimum reaction time was determined by following the colour development at ambient temperature (25 ± 2 °C). Complete colour intensity was attained after 2.0 min. The colour remained stable for 12 hrs at room temperature. Raising the temperature up to 50 °C, no change in the absorbance of the coloured product was observed, whereas raising above 50 °C the absorbance and λ_{max} of the coloured ion pair was decayed and retained to the origin of the reagent used.

The most favourable sequence is paracetamol-reagent-buffer for the highest colour intensity and the least time for developing maximum absorbance value. All other sequences needed longer times with lower value for absorbance.

Job's continuous variation graph for the reaction of paracetamol and pyrocatechol violet showed that the interaction between these two compounds occurs on an equimolar basis. The reaction occurs through the formation of ion pair complex. The coloured reaction product can be represented by the following structure.



Quantification

A linear correlation was found between absorbance and concentration in the range 0.5-34.0 μg ml^{-1}. The correlation coefficient, intercept and slope for the calibration data for paracetamol are calculated using the least-squares method and the regression equation was found to be A = 0.008 + 0.030C (where C is the concentration in μg ml^{-1}). To obtain more accurate range of determination, Ringbom method was applied in the range 3.5 -32.0 μg ml^{-1}.

The sensitivity of the proposed method can be determined by calculating the molar absorptivity and Sandell sensitivity which found to be 4.54 x 10^3 l mol^{-1} cm^{-1} and 0.033 μg cm^{-2}, respectively. In order to determine the accuracy and precision of the method, solutions containing eight different concentration of paracetamol were prepared and analysed in quintuplicate. The standard errors and relative standard deviations in Table 1 can be considered satisfactory, at least for the concentration levels examined. The reproducibility of the procedure was determined by running five replicate samples, each contains 20 μg ml^{-1} in the final assay solution. At this concentration, the relative standard deviation was 0.86%.

Table (1): Evaluation of accuracy and precision of the proposed method

Taken	Found * μg ml^{-1}				Standard	Confidence
μg ml^{-1}	P	O	S	Sr%	error	limits
4	3.97	3.93	0.03	0.48	0.012	3.97±0.035
8	8.05	7.90	0.05	0.74	0.020	8.05±0.060
12	12.10	12.20	0.08	1.05	0.033	12.10±0.050
16	15.85	16.25	0.04	0.63	0.016	15.85±0.050
20	19.88	20.30	0.06	0.86	0.024	19.88±0.070
24	24.20	23.70	0.10	1.31	0.041	24.20±0.120
28	27.75	28.40	0.09	1.18	0.37	27.75±0.110
32	32.30	32.50	0.11	1.39	0.042	32.30±0.135

* Average of five determinations

P- Proposed method O-Official method [23]

The performance of the proposed method was assessed by calculation of the t- and F-values compared with the official method. Mean values were obtained in a

Student's t- and F-test with 95% confidence limits for five degrees of freedom [22]. The results showed that the calculated t- and F-values did not exceed the theoretical values indicating the absence of any systematic error in the method. There is no significant difference between the proposed method and the official one[23] (based on titration with 0.1 M ammonium cerium (IV) sulphate using 0.1 ml of ferrion solution as indicator).

Interferences

Owing to the fact that paracetamol is frequently commercialized together with other drugs such as acetyl salicylic acid, chlorpheniramine maleate, salicylamide, codeine phosphate, caffeine, analgin, oxyphenbutazone and phenylephrine HCl. The proposed method was applied to the determination of paracetamol in the presence of different ratios of those drugs to study their interference and tolerance limits. No interference was found up to the tolerance limits listed in Table 2. The results obtained indicated the selectivity of the proposed method for the determination of paracetamol in the presence of other drugs in pharmaceutical dosage forms. However, the present method has the advantage of ease of performance, less time consumption, convenience, and small sample and reagent consumption.

Table (2): Tolerance limits in determination of 20 μg ml^{-1} of paracetamol using pyrocatechol violet.

Drug added	Tolerance μg ml^{-1}	Drug added	Tolerance μg ml^{-1}
Fructose, lactose, maltose	8000	Salicylamide, acetyl salicylic acid	200
Glucose, galactose	5000	Analgin, phenyl propanolamine HC	160
Sucrose, reserpine, saccharin	3500	Codeine, caffeine	120
Acetate, phosphate, vaterate	2500	Oxyphenbutazone	85
Magnesium stearate, starch	1400	Chlorpheniramine maleate	48
Citric acid, benzoic acid	850	Phenylephrine HCl	30
Bicarbonate, barbiton, talc	400		

Analytical application

The proposed method was applied to commercially available pharmaceutical dosage forms containing paracetamol. The results obtained are summarized in Table 3

showed that the proposed procedure gives levels of accuracy and precision comparable to those obtained by the official method[23]. The accuracy with respect to the official method[23] was assessed by calculation of Student's t- values[22], whereas the precision was examined by calculation of F- values[22]. Mean values of t-and F- were 1.39 and 2.87, respectively, showing the absence of any systematic error in the method. The corresponding tabulated t- and F- values for five degrees of freedom and a 95% confidence level are 2.57 and 5.05, respectively. Therefore it can be concluded that there is no significant difference between the proposed method and the official one[23] (based on titration with 0.1 M ammonium cerium(IV) sulphate using 0.1 ml of ferroin solution as indicator).

Table (3): Assay of paracetamol in pharmaceutical dosage forms using PCV in aqueous basic media.

Dosage forms	Company	Combined drugs	Label to content mg	Found* (mg) Proposed	t**	Official [23]
Syrup						
Paracetamol	1		120	118.5	1.67	115
Pyremol™	2		150	153	1.91	144
Rhinemol	3	Phenyl propanolamine HCl	5			
		Chlorpheniramine maleate	1			
		Paracetamal	120	122	1.88	114
Tablets						
Vegaskine	4	Acetyl salicytic acid	300			
		Codeine phasphate	150			
		Paracetamol	200	203	1.83	210
Pyremol	2		500	506	1.54	510
Suppositorres						
Cerippo	3		120	122	1.49	116
			200	197	1.56	191
Pyremol™	2		300	305	1.38	290

1- Amriya for Pharmaceutical Industries Company, Alexandria, Egypt.

2- Glaxo Wellcome Egypt, Cairo, Egypt.

3- The Nile Company for Pharmaceuticals and Chemical Industries, Cairo, Egypt.

4- Alexandria Company for Pharmaceutical and Chemical Industries, Alexandria, Egypt.

Conclusion

The proposed method is simpler, selective, less time consuming and more sensitive than the official method (based on titration with 0.1 M ammonium cerium(IV) sulphate using 0.1 ml of ferroin solution as indicator). There is no interference from common additives, excipients and commercial drugs present in their formulations leading to a highly selective procedure of determination compared with others[14-18]. Suggesting application of the proposed method in routine analysis for the determination of paracetamol in the presence of acetyl salicylic acid, salicylamide, codeine phosphate, cafeine, analgin, phenyl propanolamine HCl, oxyphenbutazone, chlorpheniramine maleate, and phenylephrine HCl, in its dosage forms.

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