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Spectrophotometric method for the determination of paracetamol and phenacetin

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Abstract

A rapid, sensitive and simple spectrophotometric method is proposed for the determination of hydrolysis products of paracetamol (PRL) and phenacetin (PHN) with sodium 1,2-naphthoquinone-4-sulphonate and cetyltrimethyl ammonium bromide (CTA) in alkaline medium. The absorbances are measured at 570 and 500 nm and the molar absorptivities found to be 1.118×10^4 and 4.54×10^3 l mol^{-1} cm^{-1} for PRL and PHN, respectively. The coloured species conforms to Beer's law over the range 1–20 μg ml^{-1} for PRL and 2–24 μg ml^{-1} of PHN. The sensitivity is enhanced by the addition of CTA. The method is successfully employed for determination of PRL or PHN in various pharmaceutical preparations and laboratory made tablets and results have been statistically compared with those obtained by the official method.

Keywords: Cetyltrimethyl ammonium bromide; Paracetamol; Phenacetin; Sodium 1,2-naphthoquinone-4-sulphonate; Spectrophotometry

1. Introduction

Paracetamol (*N*-acetyl-*p*-aminophenol, PRL) and phenacetin (acetophenetidine, PHN) are widely used as analgesic and antipyretic drugs, together with caffeine, ibuprofen and diclofenac sodium. Several spectrophotometric methods have been reported for their determination. The majority of published methods for PRL and PHN depend on hydrolysis of the compounds, leading to the formation of a Schiff base with a substituted benzaldehyde [1,2], or reaction with *o*-cresol [3], sodium nitroprusside [4], cerium(lV) [5], and oxidative coupling with *m*-cresol [6] and sodium iodylbenzoate [7]. Other spectrophotometric methods are based on indophenol blue formation [8,9], nitrosation and subsequent chelation [10], ultraviolet absorption [11] and its change with pH [12]. Most of these methods require lengthy treatments and lack the simplicity and sensitivity needed for routine analysis.

Sanghavi et al. [13] reported a colorimetric method for the hydrolysed product of PRL with 1,2-naphthoquinone-4-sulphonate (NQS) in acid medium to form a Schiff base having an absorption maximum at 480 nm. However, in our labo-

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ratory, reaction of the hydolysis product, 4-aminophenol, of PRL with NQS in acid medium gives a yellow colour instead of a red colour, and a red colour in alkaline medium.

In the present communication we extend our reported method [14] for the determination of isoniazid to paracetamol and phenacetin, via their hydrolysis products and based on the formation of violet and red coloured products with NQS and CTA in alkaline medium. The method offers the advantages of sensitivity, simplicity and rapidity without the need for extraction.

2. Experimental

A JASCO Model UVIDEC-610 UV–Vis spectrophotometer with 1.0 cm matched cells was used for the electronic spectral measurements.

Paracetamol (BDH), phenacetin (BDH), sodium 1,2-naphthoquinone-4-sulphonate (BDH) and cetyltrimethyl ammonium bromide (Hopkins and Williams, UK) were used. All other chemicals used were analytical reagent grade. Deionized water was used to prepare all solutions and in all experiments.

2.1. Solutions

Accurately weighed (50 mg) paracetamol or phenacetin was transferred to a 100 ml round bottom flask containing 15 ml of 20% hydrochloric acid, and refluxed for 30 min. The solution was cooled and the condenser washed with water. The solution with the washings was transferred to a 100 ml calibrated flask and diluted to volume with water. This solution was then diluted to contain 50 μg ml^{-1} of PRL or PHN and standardized by a reported method [15]. A 0.02% aqueous solution of NQS was freshly prepared and protected from sunlight. A 1% aqueous solution of CTA, 2% sodium hydroxide solution (NaOH) and 2% sodium carbonate (Na_2CO_3) solution were used.

2.2. Procedure

Aliquots of standard solution of PRL (25–500 μg) or PHN (50–600 μg) were transferred into a 25 calibrated flasks; 6 ml of 0.02% NQS, 1 ml of 1% CTA and 2 ml of 2% NaOH (for PHN: 3 ml of 2% Na_2CO_3) were added and diluted to the mark. After mixing the solution thoroughly, the absorbance was measured at 570 nm (for PHN, 500 nm) against the corresponding reagent blank, and calibration graphs were constructed.

2.3. Procedure for assay of PRL and PHN in commercial and laboratory made samples

Twenty tablets (commercial tablets or laboratory made tablets containing talc, starch, glucose and magnesium stearate) were powdered and weighed. An amount equivalent to 50 mg (for syrup and injection forms an appropriate volume of the sample) of PRL or PHN was taken and subjected to hydrolysis using 15 ml of hydrochloric acid. The filtrate was made up to 100 ml and an aliquot of this solution was treated as described above for the determination of PRL or PHN.

3. Results and discussion

3.1. Spectral characteristics

The PRL (PHN) was hydrolysed in the presence of acid to form *p*-aminophenol (*p*-phenetidine). This reacts with NQS in the presence of NaOH (Na_2CO_3) in an aqueous medium to form a reddish-violet (red-yellow) coloured anionic product of λ_{max} 530 nm (465 nm). When CTA was added to the solution, an intense violet (red) coloured product was obtained with a strong bathochromic shift of 40 nm (35 nm) to 570 nm (500 nm). This displacement is due to the substitution of CTA with anionic product. Hence, these wavelengths were used for all subsequent measurements. The absorption spectra of these products and the reagent blank are shown in Figs. 1 and 2.

3.2. Optimum reagent concentration and order of addition

It was found that a 0.02% solution of NQS in

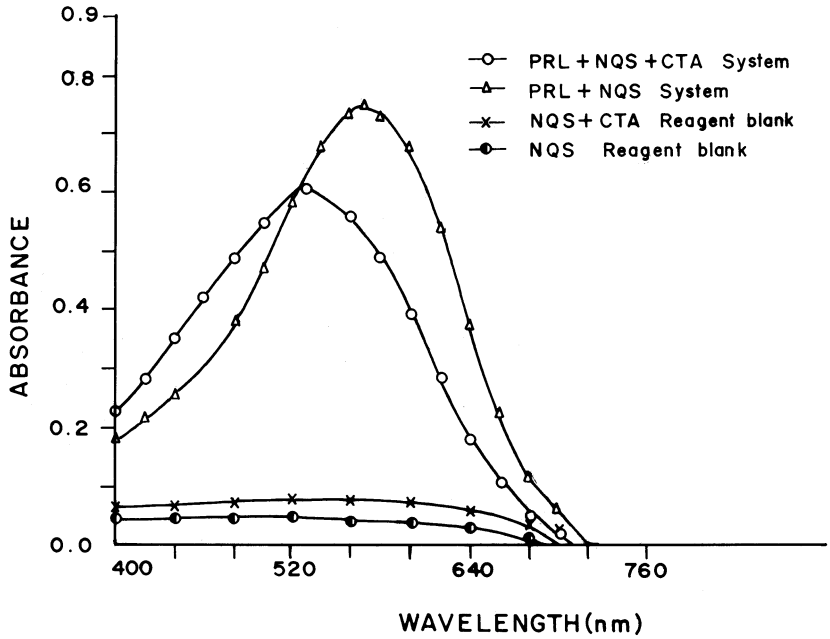


Fig. 1. Absorption spectra of PRL product and reagent blanks.

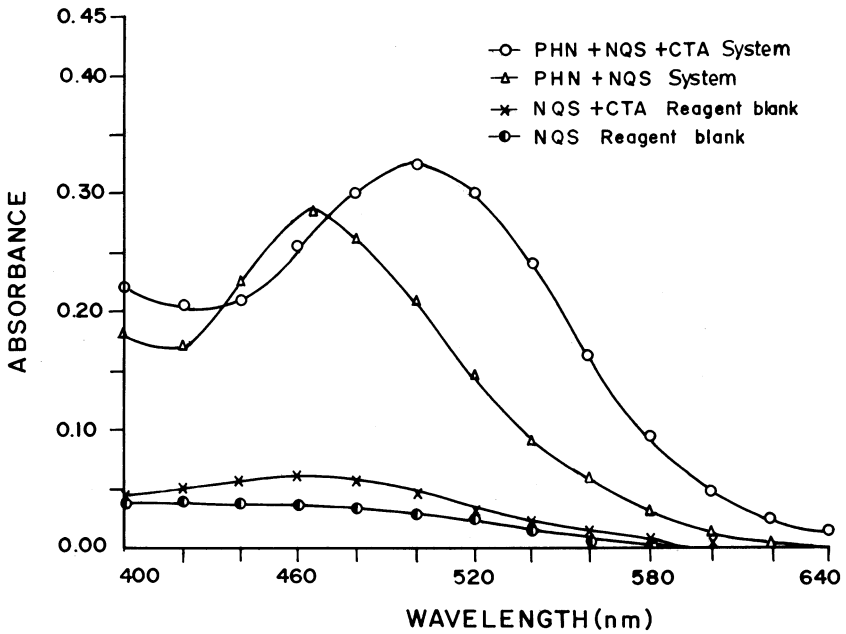


Fig. 2. Absorption spectra of PHN product and reagent blanks.

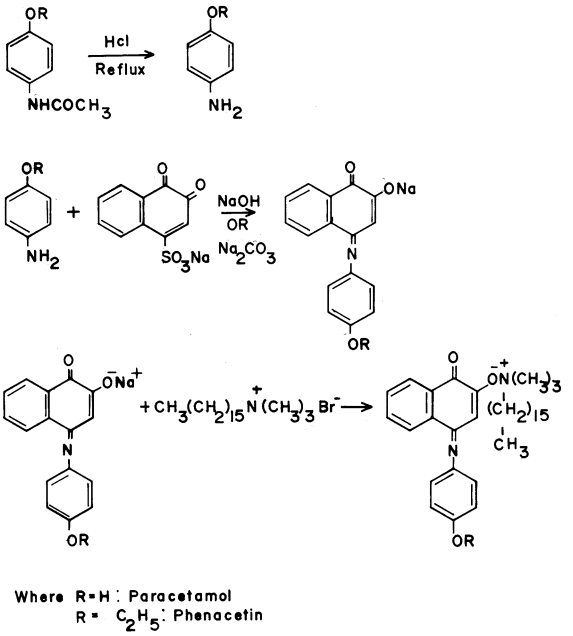
the range 4–8 ml and a 1% solution of CTA in the range 0.5–2 ml was necessary to achieve maximum colour intensity. Hence, 6 ml of NQS and 1 ml of CTA solution were selected. There was no appreciable change in the absorbance or colour of the product if the order of addition of reactants was varied.

3.3. Quantification

Beer's law holds good over the ranges 1–20 μg ml^{-1} for PRL + NQS + CTA and 2–24 μg ml^{-1} for PHN + NQS + CTA products. Molar absorptivity, specific absorptivity, Sandel sensitivity, percentage relative standad deviation, correlation coeffficient, intercepts and slopes for the calibration data of PRL and PHN by the suggested method are given in Table 1.

3.4. Choice and effect of alkali

Choice of alkali is important for the drugs investigated. For PRL, various volumes of 2% NaOH were tried and good results were obtained between 1–4 ml for the stable violet product. The coloured product of PHN was unstable in NaOH, but in 2% Na_2CO_3 it gives a stable red coloured product and the best results were obtained with between 1 and 6 ml of Na_2CO_3. Below 1 ml of base, colour intensity decreased. Therefore, 2 ml of 2% NaOH and 3 ml of 2% Na_2CO_3 were chosen for the investigation.



Scheme 1.

Table 1
Optical characteristics of paracetamol and phenacetin

Optical character	Values of PRL	Values of PHN
Beer's law range (μg ml^{-1})	1.0–20.0	2.0–24.0
Molar absorptivity (l mol^{-1} cm^{-1})	1.118×10^{4}	4.534×10^{3}
Specific absorptivity (ml g^{-1} cm^{-1})	7.4×10^{-2}	2.53×10^{-2}
Sandel's sensitivity (μg cm^{-2})	1.4×10^{-2}	3.953×10^{-2}
Regression equation (y)[a]		
Slope (a)	7.18×10^{-2}	2.57×10^{-2}
Intercept (b)	3.1×10^{-3}	9.6×10^{-3}
Correlation coefficient	1.004	0.9989
R.S.D. (%)[b]	1.44	2.7

[a] $y = ax + b$, where x is the concentration (μg ml^{-1}) of PRL or PHN.
[b] Relative standard deviation ($n = 5$).

3.5. Reaction sequence

A characteristic reddish violet (reddish yellow) coloured product of λ_{max} 535 nm (465 nm) is formed when the hydrolysis product of PRL (PHN) is allowed to react with NQS in the presence of NaOH (Na_2CO_3) in aqueous medium. Under the experimental conditions, the light yellow alkaline solution of the *o*-quinoidal NQS reacts with compounds containing one removable hydrogen atom [16] attached to one nitrogen atom, to yield an anionic reddish violet (reddish yellow) coloured paraquinoid imide condensation product. When CTA is added to the reddish violet (reddish yellow) product, an intense violet (red) coloured product of λ_{max} 570 nm (500 nm) is formed [14]. A reaction mechanism based on the above reaction is shown in Scheme 1.

Table 2
Determination of paracetamol and phenacetin by the proposed method

Product	Composition (mg)	Recovery (%, mean ± R.S.D.)[b]	
		Proposed method	Official method
Dolopar[a]			
Paracetamol	250	99.7 ± 1.06	99.4 ± 1.1
Analgin	250		
Caffeine	250		
Calpol[a]			
Paracetamol	500	101.3 ± 0.68	100.1 ± 0.65
Pacimol[a]			
Paracetamol	500	102.1 ± 0.98	99.9 ± 0.81
Pyrigesic[a]			
Paracetamol	500	100.4 ± 0.39	99.9 ± 0.53
Ibugesic Plus[a]			
Ibuprofen	200		
Paracetamol	325	102.6 ± 0.46	101.5 ± 0.73
Diclogesic[a]			
Diclofenac sodium	50		
Paracetamol	500	101.3 ± 0.45	100.4 ± 0.49
Flamar-P[a]			
Chlorzoxaone	250		
Paracetamol	300	99.9 ± 0.82	99.8 ± 0.81
Dolo (syrup)[a]			
Paracetamol	500	101.0 ± 1.02	100.3 ± 0.99
Crocin Drops[a]			
Paracetamol	150	100.8 ± 0.55	100.2 ± 0.54
H-Mol-75[a]			
Paracetamol	75	99.9 ± 1.14	99.8 ± 1.21
Benzyl alcohol			
Phenacetin (laboratory made)	200	99.8 ± 0.78	–

[a] Trade name.
[b] Average of five determinations, assayed as a percentage of label claim. R.S.D., relative standard deviation ($n = 5$).

3.6. Stability

The products resulting from the suggested method were studied at different temperatures. It was found that the absorbance values remain constant in the temperature range 5–80°C. Above 80°C, the absorbance decreases, indicating dissociation. Hence, room temperature was recommended for the proposed method. The violet and red coloured products were stable for more than 4 days and the results were reproducible.

3.7. Interference

The effects of various substances that often accompany PRL in pharmaceutical preparations were studied. Of the drugs commonly contained in formulations of PRL, indomethacin, promethazine, chlorpheniramine maleate, phenylephrine hydrochloride and ascorbic acid interfered, while analgin, caffeine, ibuprofen, diclofenac sodium, chlorzoxazone, benzyl alcohol did not interfere, in the determination of PRL by the proposed method. Anothor attractive feature of the proposed method is that it is free from interference by diluents and excipients such as magnesium stearate, glucose, talc, starch, lactose and dextrose.

3.8. Application

The applicability of the method to assay pharmaceutical preparations was examined. The assay of PRL preparations, singly and in various combinations is shown in Table 2. The formulations of PHN are not available in the Indian market. Hence we prepared tablets of PHN in the laboratory and used these for the assay. The results obtained compare favourably with the results obtained by the official method [17].

4. Conclusions

The method is found to be simple, economical, rapid and sensitive. The statistical parameters and the recovery study data clearly indicate the reproducibility and accuracy of the method. Thus the method can be adopted for routine analysis in quality control laboratories.

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References

[1] A.A. D'Souza, K.G. Shenoy, Can. J. Pharm. Sci. 3 (1968) 90–92.
[2] E. Kalatzis, I.J. Zarbi, Pharm. Sci. 65 (1976) 71–74.
[3] L. Davey, D. Naidoo, Clin. Chem. 39 (1993) 2348–2349.
[4] C.S.P. Sastry, K.V.S.S. Murthy, Indian Drugs 19 (1982) 158–161.
[5] S.M. Sultan, Z.I. Abdullah, M.A. Alrahaman, S.A. Altamrah, Y. Asha, Analyst 111 (1986) 919–921.
[6] S.Z. Qureshi, A. Saeed, N. Rahman, Chem. Anal. 37 (1992) 227–229.
[7] K.K. Verma, A. Jain, K.K. Stewart, Anal. Chim. Acta 261 (1992) 261–267.
[8] C.T.H. Ellcock, A.G. Fogg, Analyst 100 (1975) 16–18.
[9] Z. Bouhsain, S. Garrigues, A.M. Rubio, M.D. Guardia, Anal. Chim. Acta 330 (1996) 59–69.
[10] S.F. Belal, M.A.H. Elsayed, A. Elwalily, H. Abdine, Analyst 104 (1979) 919–927.
[11] British Pharmacopoeia 1973, HM Stationary Office, London, 1973, p. 340.
[12] M.A.H. Elsayed, S.F. Belal, A.F.M. Elwalily, H. Addine, Analyst 104 (1979) 620–625.
[13] N.M. Sanghavi, M.G. Pai, East. Pharm. 17 (1974) 53–54.
[14] P. Nagaraja, K.C. Srinivasa Murthy, H.S. Yathirajan, Talanta 43, (1996) 1075–1080 and references cited therein.
[15] I.P. Koka, Farmatsiya 33 (1984) 37–42.
[16] J. Saurina, S. Hemandez-Cassou, R. Tauler, Anal. Chem. 67 (1995) 3722–3726.
[17] British Pharmacopoeia 1993, Office of the British Pharmacopoeia Commission, London, 1993, p. 483.