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Spectrophotometric Method for the Determination of Paracetamol

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Abstract

Paracetamol with 1-napthol or resorcinol gave azodye and the concentration of paracetamol was investigated spectrophotometrically. The azodyes formed with both 1-napthol and resorcinol as coupling agents follow Lambert Beer's law in the range of 0 to 10 μgmL^{-1} of paracetamol. The molar absorptivity and Sandell's sensitivity for azodye coupled with 1-napthol were found to be 1.68×10^4 $Lmol^{-1}cm^{-1}$ and 9.0 $ngmL^{-1}cm^{-2}$, respectively. The molar absorptivity and Sandell's sensitivity for azodye coupled with resorcinol were found to be 2.86×10^4 $Lmol^{-1}cm^{-1}$ and 5.3 $ngmL^{-1}cm^{-2}$, respectively. Both coupling agents had been applied successfully in the analysis of paracetamol in pharmaceutical preparation. The relative standard deviation for all five samples ranged from 2.2-6.4% at 95% confidence. The percentage recoveries were found to range from 97.8 to 103.4. Both methods used in the present study may be applied to the determination of trace amount of paracetamol in different clinical samples.

***Keyword**: Paracetamol, spectrophotometric, 1-napthol, resorcinol*

Introduction

Many methods are available in literature for assay of paracetamol in diverse types of samples including pharmaceutical preparations. These methods are as diverse as a simple titrimetric method to HPCL and spectrophotometric methods.[1] Owing to wide spread use of paracetamol in different kinds of pharmaceutical preparations, rapid and sensitive methods for the determination of paracetamol are being investigated. Many spectrophotometric methods of determination of paracetamol are available in literature.[1-9] These are based on hydrolysis of paracetamol to p-aminophenol and latter is reacted with specific reagents to produce colored substance which is monitored spectrophotometrically. Various spectrophotometric methods of analysis of paracetamol have been investigated. The method of assaying paracetamol was to react paracetamol with different reagents so that a colored species was formed, the absorbance of which was measured in visible region at appropriate

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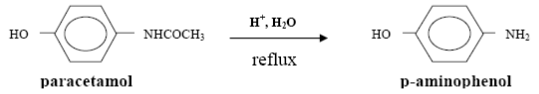
wavelength.[3-6] The methods of quantitative determination of paracetamol involved the hydrolysis of paracetamol to p-aminophenol. Hundreds of methods are available in converting the hydrolyzed product to colored species to estimate paracetamol.[1-3,6-8] Hydrolysis of paracetamol gives p-aminophenol and coupled with coupling agent to yield azodye. Diazotization of aromatic amine and coupling the product with phenols or aromatic amines is a famous Griess reaction which has been extensively used to estimate nitrate in water, soil, vegetables, meat products etc.[9,10] Surprisingly very little work has been done to estimate paracetamol using Griess reaction.[1] The following Griess reaction mechanism is assumed to be followed during the present study.

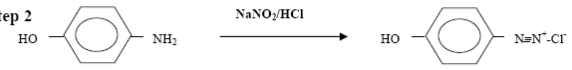
Scheme 1

Step 1



paracetamol **p-aminophenol**

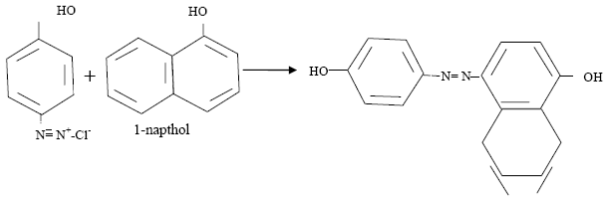
Step 2



p-aminophenol **diazonium salt**

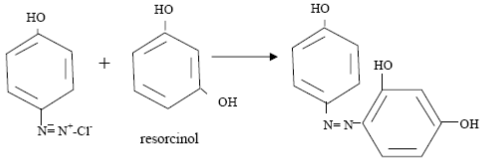
Step 3

a)1-Napthol as coupling agent



Azodye I

b)Resorcinol as coupling agent



Azodye II

The objective of the present work is to develop a simple, rapid and reliable method to assay paracetamol and to determine the paracetamol in medical paracetamol tablets.

Experimental Methods

Pure sample of paracetamol obtained from Nepal Drug Limited was used as standard and paracetamol tablets (500 mg) manufactured by different manufacturer were purchased from local market. Accurately 250 mg of pure authentic sample of paracetamol was weighed

out and refluxed with 20 mL of 4 M HCl with 30 mL of distilled water for about 30 minutes to prepare a standard solution. The content was appropriately diluted and required aliquots were taken for preparation of calibration curve.

Solution containing 2-10 µgmL^{-1} of paracetamol equivalent was taken in 25 mL volumetric flask. To this aliquot 0.6 mL of 4M HCl and 1 mL of 0.1% w/v solution of sodium nitrite were added for diazotization. One mL of 0.5% w/v solution of ammonium sulfamate was added after 3 minutes to destroy excess nitrous acid and left for 2 minutes. Then, 1.5 mL of 0.5% w/v solution of 1-Napthol in 4 M NaOH was added as coupling agent. The absorbance of this azodye was measured at 505 nm.

Tablet of paracetamol was weighed out and powdered. The powdered sample equivalent to 250 mg of paracetamol was accurately weighed out and exactly same process for hydrolysis and color development was carried out as was carried out for paracetamol. Absorbance was measured at appropriate wavelengths using Perkin Elmer Lambda 40 UV/VIS spectrophotometer and paracetamol was estimated from calibration curve. The detail procedure is described elsewhere.[1]

Results and discussion

The maximum absorbance of the azodye formed in alkaline medium with 250 µg of paracetamol in 1-napthol was observed at 505 nm. Such azodye is found to be stable for at least 45 minutes as shown in figure 1. The adherence to the Lambert Beers law was tested by reacting aliquots of standard solution containing 2-10 µg mL^{-1} of paracetamol.

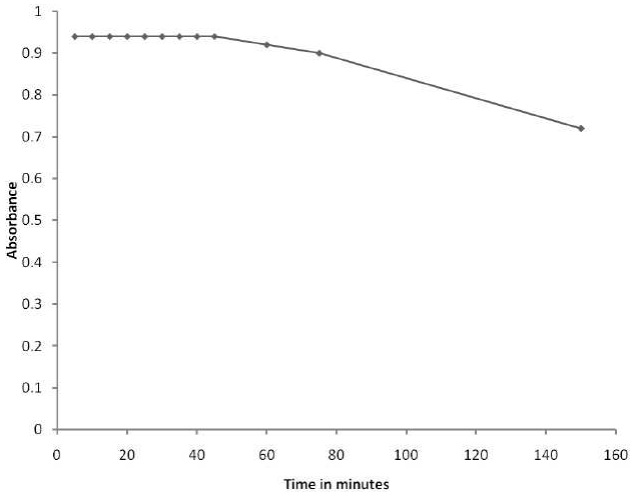


Figure 1: Stability of the formed dye using 1-napthol as coupling agent.

Figure 2 shows the plot of absorbance against concentration of paracetamol. The plot shows that the formed dye obeys Lambert Beer's law from 2-10 µg mL^{-1} of paracetamol.

The molar absorptivity and Sandell's sensitivity were found to be 1.68×10^4 $Lmol^{-1}cm^{-1}$ and 9 ng $mL^{-1}cm^{-2}$, respectively.

On the other hand, the maximum absorbance of the azodye formed in alkaline medium with 10 µg mL^{-1} of paracetamol in resorcinol was observed at 485 nm. Figure 3 shows the plot of absorbance against concentration of paracetamol. The plot shows that the formed dye obeys Lambert Beer's law from 2-10 µgmL^{-1} of paracetamol. The molar absorptivity and Sandell's sensitivity were found to be 2.86×10^4 $Lmol^{-1}cm^{-1}$ and 5.3 ng$mL^{-1}cm^{-2}$, respectively. These results revealed that resorcinol is found to be a more sensitive than 1-napthol as coupling agent.

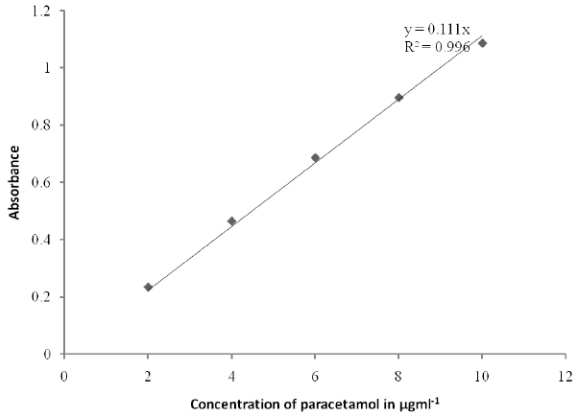


Figure 2: Adherence to Lambert Beer's law using 1-napthol as coupling agent.

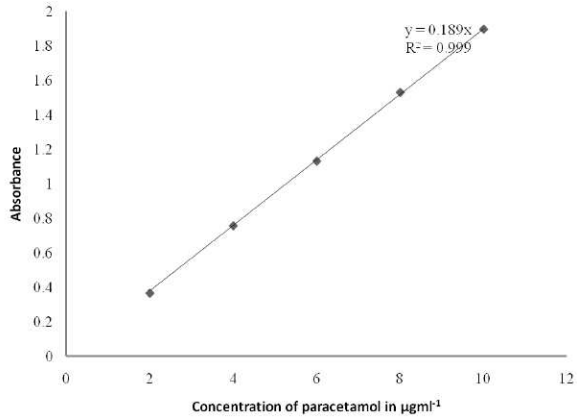


Figure 3: Adherence to Lambert Beer's law using resorcinol as coupling agent.

Reliability and suitability of the method were determined by adding known quantities of authentic hydrolyzed paracetamol to the pre-analyzed paracetamol tablets. The authentic hydrolyzed paracetamol was added in the range of 70 to 116 % of the pre-analyzed paracetamol tablet. Recoveries of paracetamol at three different amounts using 1-Napthol

and Resorcinol are given in Table 1. The percentage recoveries were found to range from 97.8 to 103.4 % which confirmed the validity of the method for the analysis of paracetamol in pharmaceutical formulations.

Table 1: Recovery test

S.N.	Amount Added (μg)	Recovery		% Recovery	
		1-napthol	resorcinol	1-napthol	resorcinol
1	75	74.0	77.6	98.6	103.4
2	100	103.3	102.0	103.3	102.0
3	125	122.2	127.6	97.8	102.0

The quantitative determination of paracetamol in paracetamol tablets manufactured in Nepal by five different manufacturers was done in this study. The results obtained are tabulated in Table 2. The manufacturer specifications are shown in Table 3. In general, the mean percentage determined in five replicate analyses is very close to the claimed amount by the manufacturers. The relative standard deviation for all five samples ranges from 2.2-6.4% at 95% confidence. This indicates that the suitability of the presently used methods for the routine analysis of paracetamol tablets is highly appreciable. Needless to say that if any ingredients that are added to paracetamol formulation contain aromatic amine it is likely to interfere in the determination of paracetamol. The extent of interference and technique to eliminate such interfering ingredient is left for future study due to the time limitation of the study.

Table 2: Results obtained from the analysis of paracetamol tablets (all paracetamol tablets containing 500 mg of paracetamol were analyzed).

S.N.	Sample No.	Mean % determined		Relative standard deviation (95% confidence)	
		1-napthol	resorcinol	1-napthol	resorcinol
1	PT-1	101.8	102.0	5.8	2.4
2	PT-2	100.2	100.8	6.0	4.6
3	PT-3	105.4	104.8	6.4	6.0
4	PT-4	100.6	101.6	4.2	2.8
5	PT-5	100.2	99.0	2.2	2.2

Conclusions

Two coupling agents, 1-napthol and resorcinol, were investigated to estimate paracetamol in raw material and pharmaceutical preparations using a simple and sensitive spectrophotometric method. The amount of paracetamol determined from this method is found to be in close agreement with amount claimed by the manufacturers. The percentage recovery was found to range form 97.8-103.4% indicating the suitability of the method for the determination of paracetamol in pharmaceutical preparations. The present method of the azodye formed is quite stable for at least 45 minutes. Therefore, the present method is

simple, accurate can be used for routine analysis of paracetamol in raw material and paracetamol tablets.

Table 3: Manufacturer specifications.

S.N.	Sample No.	Manufacturer specification	Amount in mg
1	PT-1	Paracetamol	500
2	PT-2	Paracetamol B.P. Phenyl ephedrine HCl I.P. Chlorpheniramine maleate I.P.	500 7.5 4
3	PT-3	Paracetamol B.P. Phenyl ephedrine HCl I.P. Pheniramine maleate I.P.	500 10 7.5
4	PT-4	Paracetamol B.P. Pseudoepherine HCl B.P. Chlorpheniramine B.P.	500 30 2
5	PT-5	Paracetamol I.P. Chlorzoazone USP	500 250

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References

1. P. D. Sethi, "*Quantitative Analysis of Drugs in Pharmaceutical Formulations*", CBS Publishers, 1993.
2. B. Morelli, *J. Pharm. Biomed. Anal.*,1989, **7**, 577.
3. P. B. Issopoulos, *Acta. Pharm. Hung.*,1992, **6**, 3138.
4. M. Knochen, J. Giglio and B.F. Reis, *J. Pharm. Biomed. Anal.*, 2003, **33**, 191.
5. C.S. Frings and J.M. Saloom, *Clin. Toxicol*, 1979, **15**, 67.
6. C. Xu and B. Li, *Spectrochim. Acta. A. Mol. Biomol. Spectrosc.*, 2004, **60**, 1861.
7. C. O. Usifoh, S. A. Adelusi and R. F. Adebamco, *Pak. J. Sci. Lnd. Res*., 2002, **45**, 7.
8. S. M. Hassan, M. I. Walash and S. M. EL-Sayed, *J. Assoc. off. Anal. Chem.*, 1981, **64**, 1442.
9. J. B. Fox, *Crit. Rev. Anal. Chem.*, 1985, **15**, 283.
10. L. S. Clesceri, A. E. Greenberge and A. D Eton, *Standard Methods for Examination of Water and Waste water*, 1998**,** APHA.
11. B. R. Shrestha, *Spectrophotometric Determination of Paracetamol*. M. Sc. Dissertation, Central Department of Chemistry, Tribhuvan University, Kathmandu, Nepal.