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SPECTROPHOTOMETRIC DETERMINATION OF PARACETAMOL DRUG USING 8-HYDROXYQUINOLINE

R.B. Dixit* and J.A. Patel

Ashok and Rita Patel Institute of Integrated Study and Research in Biotechnology and Allied Sciences, affiliated to Sardar Patel University, New Vallabh Vidyanagar – 388121, Gujarat, India

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Correspondence to Author:

R.B. Dixit

Ashok and Rita Patel Institute of Integrated Study and Research in Biotechnology and Allied Sciences, affiliated to Sardar Patel University, New Vallabh Vidyanagar – 388121, Gujarat, India

E-mail: ritsdixit@yahoo.co.in

ABSTRACT: A rapid and simple spectrophotometric method is reported here for the determination of paracetamol in a commercially available tablet formulation. The method is based on the diazotization of hydrolyzed paracetamol with 8-hydroxyquinoline as a coupler to form stable azo dyes color solution. The concentration of drug paracetamol was investigated by spectrophotometrically. The azo dyes formed with 8-hydroxyquinoline as coupling agents follow the Lambert-Beer's law in the range of 2 to 10 μgmL^{-1} of paracetamol. Sandells sensitivity and molar absorptivity observed for azo dye coupled with 8-hydroxyquinoline was found 7.9 $\mu g\ mL^{-1}cm$ and 1.9 $\times 10^4$ $Lmol^{-1}cm^{-1}$ respectively. The percentage recovery of the drug was found in the range of 97.4 to 100.2 % the coupling agent 8-hydroxyquinoline. The data obtained using 8-hydroxyquinoline as a coupler were compared with the data obtained with 2-naphthol as a coupler suggests that dye formed with 8-hydroxyquinoline is more stable than the 2-naphthol. The method reported here may be used to determine the trace amount of paracetamol in any clinical samples with accuracy and precision.

INTRODUCTION: Paracetamol is a chemically *N*-(4-hydroxyphenyl)acetamide. It has antipyretic, analgesic and anti-inflametory actions. It has a highly targeted action in the brain, blocking of an enzyme involved in the transmission of pain. Its mode of action was known to be different compare to other pain relievers, but although it produces pain relief throughout the body. Looking to the importance of paracetomol drug several methods have been developed for evaluation, validation and assaying of paracetamol in a drug formulation [1-11].

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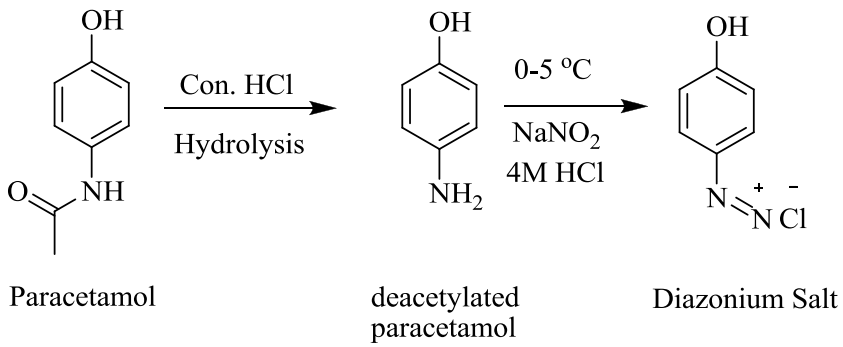
These methods are as diverse as a simple titrimetric method to HPCL and spectrophotometric methods. A very little work has been attempted to estimate paracetamol using Griess reaction [12, 13]. This method is based on hydrolysis of drug and then diazotization of paracetamol using aromatic phenol as couplers to produce deep orange or red color with a maximum absorption wavelength at 470 nm.

Therefore, in the present study, we had planned to develop a new method for assaying paracetomol drug by following Griess reaction using 8-hydroxyquinoline as a coupler.

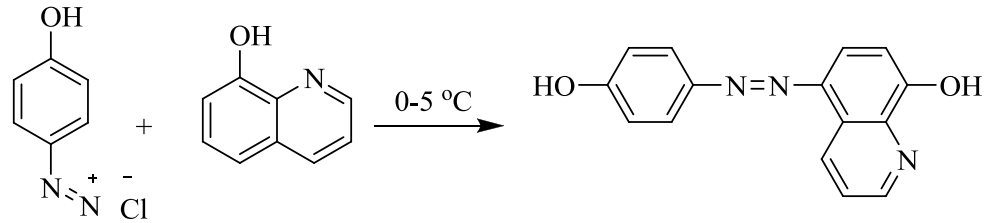
The same was compared using 2-napthol as a coupler. The reaction mechanism assumed to be followed during the present study is shown in **Scheme 1**.

Step I Deacetylation & diazotization of paracetamol



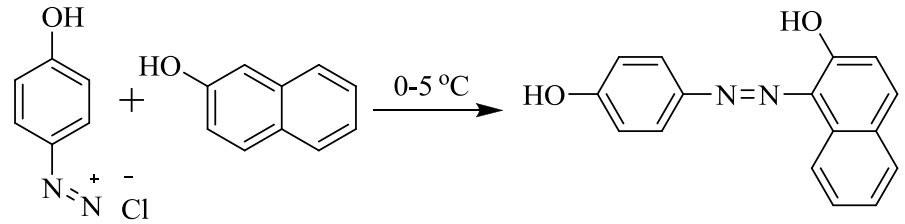
Paracetamol deacetylated paracetamol Diazonium Salt

Step II Coupling of diazonium salt with 8-hydroxyquinoline



Diazonium salt 8-HQ Azo dye-I

Step II Coupling of diazonium salt with 2-naphthol



Diazonium salt 2-Naphthol Azo dye-II

SCHEME 1: POSSIBLE REACTION PATH TO BE FOLLOWED DURING SPECTROPHOTOMETRIC DETERMINATION OF PARACETAMOL.

MATERIALS AND METHODS:

Instrument: OPTIZEN 3220 UV spectrophotometer, MECASYS Co. Ltd., with matched quartz cell corresponding to 1 cm path length and spectral bandwidth 1 nm was used.

Materials: A standard sample of paracetamol was obtained from Dwarkesh chemicals Pvt. Ltd., Ahmedabad, as gift sample. Paracetamol tablets of different brands were purchased from local market. All other chemicals used were of Analytical reagent grade.

Preparation of calibration curves of dye using two couplers: To prepare standard solution of pure authentic sample of paracetamol (PCM), 250 mg of drug was weighed accurately and refluxed with 25 ml of 4 M HCl for 30 minutes. 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 4 M 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 5 minutes. Then, 1.5 ml of 0.5% w/v solution of 8-hydroxyquinoline/2-napthol in 4 M NaOH was added as coupling agent. The absorbance of color produced due to azo dyes formation was measured using spectrophotometer at 470 nm and 490 nm respectively.

Similarly tablet of paracetamol was weighed out and powdered. The powdered sample equivalent to 250 mg of paracetamol (PCM) was accurately weighed out, same process for hydrolysis and color development was carried out. Absorbance was measured at appropriate wavelengths using Perkin Elmer Lambda 40 UV/VIS spectrophotometer and paracetamol was estimated from calibration curve. Calibration curve [14] for both dyes has been shown in **Figure 1 and 2** respectively. Molar extinction coefficient [15] (ε_{max}) and Sandell's sensitivity[16] (ss) were calculated from graph.

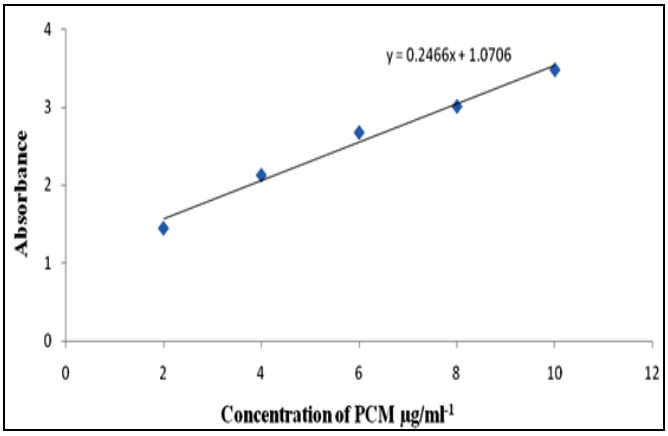


FIGURE 1: ADHERENCE TO LAMBERT-BEER'S LAW USING 8-HYDROXYQUINOLINE AS COUPLING AGENT

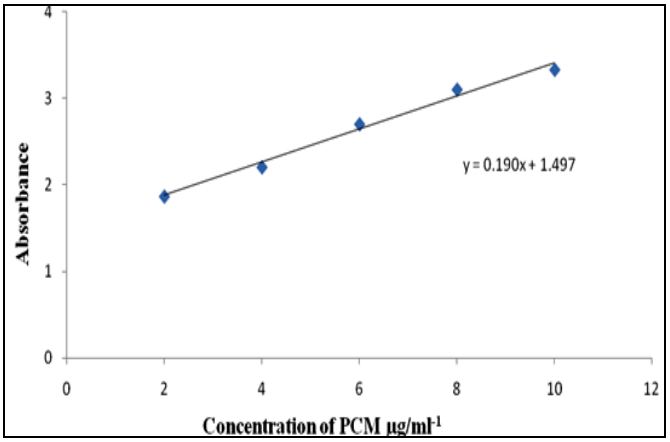


FIGURE 2: ADHERENCE TO LAMBERT-BEER'S LAW USING 2-NAPTHOL AS COUPLING AGENT

Stability test for dye prepared: Stability of prepared dyes were checked for 10 µg/ml solution of paracetamol by coupling it with 8-hydroxyquinoline/2-napthol. The successive readings have been taken at 470 nm and 490 nm respectively for 70 minutes using Spectrophotometer. Results [16] for the same have been incorporated in **Figure 3 and 4**.

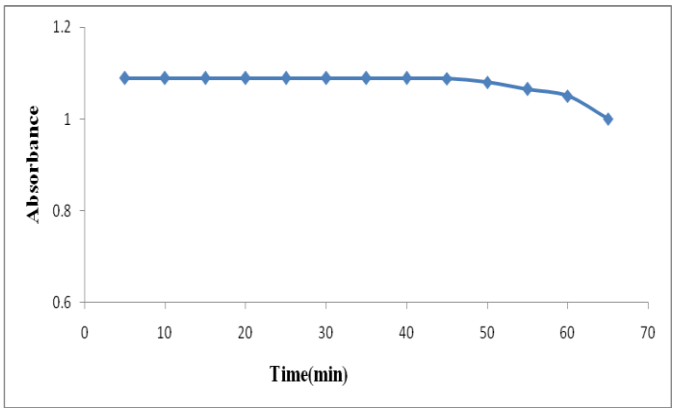


FIGURE 3: STABILITY OF THE DYE FORMED USING 8-HYDROXYQUINOLINE AS COUPLING AGENT.

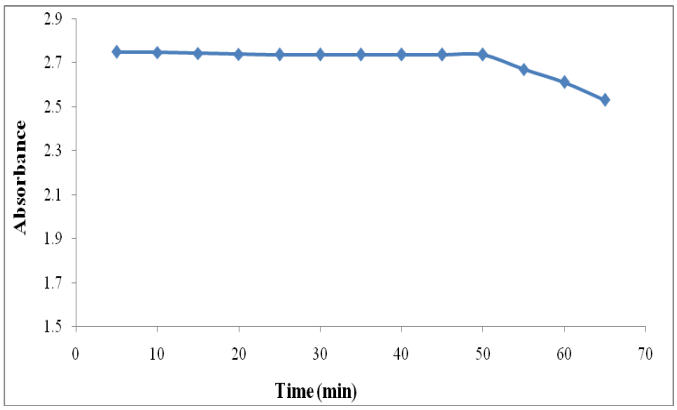


FIGURE 4: STABILITY OF THE DYE FORMED USING 2-NAPTHOL AS COUPLING AGENT

Reliability and suitability of the method: Reliability and suitability of the method have been checked via calculation recovery and % recovery using marketed available Paracetamol drug tablets as a standard. The authentic hydrolyzed paracetamol was added in the range of 76-116 % of the pre-analyzed paracetamol tablet. Recoveries of paracetamol at three different amounts (i.e. 75 mg, 100 mg and 125 mg) using 8-hydroxyquinoline and 2-napthol were determined. The results for the same are given in **Table 1 and 2**. The above discussed methods of recovery and % recovery were checked for marketed available formulations.

TABLE 1: RECOVERY TEST

Sr. No.	Amount of Drug (µg)	Recovery (µg) of		% Recovery of	
		2-naphthol	8-HQ	2-naphthol	8-HQ
1	75	65.5	67.4	98.2	100.2
2	100	97.8	94.2	97.8	98.1
3	125	117.8	118.6	98.8	97.4

TABLE 2: RESULTS OBTAINED FROM THE ANALYSIS OF PARACETAMOL TABLETS*

Sr. No.	Sample No.#	Mean % determined of		Relative standards deviation of	
		2-naphthol	8-HQ	2-naphthol	8-HQ
1	P_1	100.6	101.1	3.34	3.52
2	P_2	100.2	100.4	3.00	3.11
3	P_3	99.4	100.1	2.54	2.94

***** Paracetamol tablets containing 500 mg of paracetamol were analyzed. **#** $P_{1, 2, 3}$ = paracetamol tablet.

RESULTS AND DISCUSSION: The methods discussed in the present work provide a convenient and accurate way for the determination of paracetamol drug in the marketed table using spectroscopy in the visible range due to formation of deep orange or red colour of azo dyes.

The maximum absorbance of the azo dye formed in alkaline medium by the hydrolyzed product of 250 µg of paracetamol with 8-hydroxyquinoline and 2-napthol was observed at 470 nm and 490 nm respectively. The adherence to the Lambert Beers law[17] was tested by reacting aliquots of standard solution containing 2-10 µg mL^{-1} of paracetamol. The maximum absorbance of the azo dye formed in alkaline medium with 10 µg mL^{-1} of paracetamol using 8-hydroxyquinoline was observed at 470 nm.

The azo dye formed in alkaline medium with 10 µg mL^{-1} of paracetamol in 8-hydroxyquinoline have been found to be stable for 45 min. The plot of absorbance vs concentration [Figure 1] shows that the azo dye formed obeys Lambert-Beer's law from 2-10 µgmL^{-1} of paracetamol. In contrast to that, the maximum absorbance of the azo dye formed in alkaline medium with 10 µg mL^{-1} of paracetamol using 2-napthol was observed at 490 nm.

The plot of absorbance vs concentration [Figure 2] shows that the azo dyes formed obeys Lambert-Beer's law for 2-10 µgmL^{-1} of paracetamol. As well as the azo dye formed from 10 µg/ml paracetamol with 2-napthol as a coupling agent have been found to be stable for at least 50 minutes as shown in Figure 4.

Sandell's sensitivity [18] and molar absorptivity [19] when 8-hydroxyquinoline was used as a coupling component were 7.9 µg mL^{-1} cm and 1.9×10^4 $Lmol^{-1}cm^{-1}$ respectively. Similarly, Sandell's sensitivity[18] and molar absorptivity [19] when 2-napthol was used as a coupling component was found 5.9 µg mL^{-1}cm and 2.46×10^4 $Lmol^{-1}cm^{-1}$ respectively. According to Savvin S. B. later one is moderately sensitive then previous one [20].

Reliability and suitability of the method were determined by adding known quantities of authentic hydrolyzed paracetamol to the pre-analyzed paracetamol tablets. The marketed samples having different brands were taken for the purpose. The authentic hydrolyzed paracetamol was added in the range of 75 to 125 % of the pre-analyzed paracetamol tablet. Recoveries of paracetamol at three different amount using 8-hydroxyquinoline and 2-napthol are given in Table 1. It shows that the percentage recoveries were found in the range of 97.4 to 100.2 % for 8-hydroxyquinoline and 97.1 to 98.8 % for 2-napthol. This confirmed the validity of the method for the analysis of paracetamol in pharmaceutical formulations.

The quantitative determination of paracetamol in paracetamol tablets manufactured in India by three different manufacturers was carried out in this study. The results obtained are tabulated in Table 2. In general, the mean percentage determined in three replicate analyses is very close to the claimed amount by the manufacturers.

The relative standard deviations for all the three samples are in the range from 2.2-6.4 at 95% self-assurance. This indicates that the suitability of the presently used methods for the routine analysis of paracetamol tablets is highly appreciable.

CONCLUSIONS: 8-Hydroxyquinoline was investigated to estimate paracetamol in a pharmaceutical preparations using Spectrophoto-metric method. The amount of paracetamol determined from this method is found to be close with the amount claimed by the manufacturers. The percentage recovery was found to be in the range of 97.4-100.2 % for 8-hydroxyquinoline indicating the suitability of the method for the determination of paracetamol in pharmaceutical preparations.

The data obtained here shows that the dye formed with 8-hydroxyquinoline is more stable than the 2-naphthol. The present method of the azo dyes formation is quite stable for at least 45-50 minutes, is a simple and accurate method, and can be used for routine analysis of paracetamol in paracetamol tablets.

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