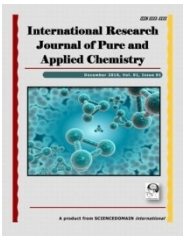

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Development of a New Eco-Friendly Validated *UV* Spectrophotometric Method for Quantitative Determination of Acetaminophen in Tablet Dosage Form

Swati Jain[1], Ashutosh Pareek[1*], Sarita singh[1], Pratima[1], Sangeeta Kumari[1], Seema Verma[1], Vivek Jain[1] and Yashumati Ratan[1]

[1]Department of Pharmacy, Banasthali University, Banasthali-304022, Rajasthan, India.

Authors' contributions

This work was carried out in collaboration between the authors. Author AP designed the study, wrote the protocol, performed statistical analysis and did the manuscript editing. Authors SJ, SS, Pr, SK, SV carried out the analysis, validation and literature search. Authors YR and VJ wrote the first draft managed the analysis of the study, and did review of the write-up. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: The present study was undertaken to develop a new *UV* spectroscopic method of acetaminophen using mix solution of methanol: phosphate buffer 7.4 (in 1:9 ratio) (further diluted with phosphate buffer 7.4 only) as a solvent system for analysis of acetaminophen.
Place and Duration of Study: Department of Pharmacy, Banasthali University, Rajasthan between July 2012 and August 2012.
Methodology: Method was developed and various validation parameter as per ICH Q2B guideline were tested like LOD, LOQ, precision, molar absoptivity, sandells' sensitivity and stability testing for the assessment of newly developed method. Furthermore method was also compared with already existed official method using pure methanol (Class II) as solvent. Finally marketed acetaminophen tablets were subjected for determination of percent purity and recovery test in three different levels (80%, 100%

**Corresponding author: Email: ashu83aadi@gmail.com;*

and 120%) using the developed method.
Results: The λ_{max} (absorption maxima) of the drug was found to be 247 nm. A linear response was observed in the range of 0-45µg/ml with a regression coefficient of 0.997. Beside this solution was significantly stable for 15 days. The percent purity (99.09%) and % recovery at 80, 100 and 120 % were found to be 99.89, 99.00, and 100.02 respectively.
Conclusion: Result suggested that the newly developed method having significant similar data irrespective of decrease in methanol used, which revealed that new developed method is a simple, accurate, precise, specific, sensitive, cost effective, reproducible and an eco-friendly alternative for UV spectrophotometric determination of acetaminophen.

Keywords: Acetaminophen; spectroscopic; molar absorptivity; sandell's sensitivity.

1. INTRODUCTION

Acetaminophen (Paracetamol) chemically is, N-acetyl-p-aminophenol, have been extensively used as antipyretic and analgesic drug. [1,2] It is also effective in treating neuralgia and pain of musculoskeletal origin. [3] Acetaminophen is official in Indian Pharmacopoeia and British Pharmacopoeia; both suggest titrimetric and UV spectrophotometric assay method for acetaminophen in bulk and tablet formulations. The various methods for determination of acetaminophen include chromatographic[4,5,6,7] electrochemical[8,9] and spectrophotometric techniques[10,11,12,13,14,15] are available in research papers. Acetaminophen is available in different dosage forms - tablets, capsules, suspensions, syrups, drops, elixirs and suppositories. [16] In many official compendia, pure methanol and other organic solvents (class 2) have been used for the analysis of paracetamol which is toxic and costly. [3] Beside this most of the developed methods are complex, non eco friendly, costly and time consuming; these suggested thrust area in the field analysis of acetaminophen. For this reason, in present study it was envisaged to develop a less toxic, cheap, eco-friendly and equally sensitive spectroscopic method for the determination of acetaminophen. In line to this we used mixed solution of methanol and phosphate buffer 7.4 (in 1:9 ratio) for initial solubilization of the drug, (further diluted with phosphate buffer 7.4) in place of pure methanol.

2. MATERIALS AND METHODS

2.1 Instruments

UV-visible double beam spectrophotometer, Labindia 3000 model with spectral bandwidth of 0.1 nm, and a pair of 10 mm matched quartz cells was used.

2.2 Materials

The Acetaminophen reference standard was purchased from S. D. Fine Chem. Ltd. (Mumbai, India). The commercial fixed dose formulation containing 500 mg acetaminophen (Paracip: Cipla) was procured from the local market. Methanol and phosphate buffer 7.4 was used as solvent for the preparation of stock and working standard solutions. All the chemicals and reagents were of analytical grade.

2.3 Preparation of Standard Stock Solutions

The standard stock solutions of pure acetaminophen were prepared by dissolving 100 mg of drug in 100 ml of mixture (10% methanol and 90% phosphate buffer (PB) 7.4). It was then sonicated for 10 minutes. From this solution 10 ml was taken and diluted to 100 ml with phosphate buffer 7.4 to get a stock solution containing 100 µg/ml of drug. The above process is repeated by using methanol to prepare same concentration of acetaminophen in methanol also.

2.4 Determination of Absorption Maxima

The stock solution was diluted to 10 µg/ml using phosphate buffer 7.4. Further a *UV* absorption maxima was determined by scanning this solution of acetaminophen in phosphate buffer 7.4 between 200- 400 nm by using Labindia 3000 UV spectrophotometer. Phosphate buffer 7.4 was used as a blank in the study. All the measurements were triplicate. Furthermore a representative overlain spectrum of acetaminophen in phosphate buffer 7.4 has drawn (Figure 1) for confirmation if any distortion in peak occurs. Similar procedure was repeated using methanol.

2.5 Preparation of Calibration Curve

Ten working standard solutions for the drug having concentration 2, 4, 6, 8, 10, to 20µg/ml were prepared with phosphate buffer 7.4 from the stock solution. The absorbance of resulting solutions of pure acetaminophen drug were measured at 247 λ max and a calibration curve was plotted to get the linearity and regression equation. All the measurements were triplicate to meet minimum statistical requirements.

2.6 Assay of Acetaminophen in Marketed Tablet by New Developed Method

The homogenized powder from twenty tablets with average weight equivalent to amount of 100 mg acetaminophen was transferred to a 100 ml volumetric flask. Approximately 10 ml of methanol were added for extraction of drug, solution was further stirred for 10 minutes. Then phosphate buffer 7.4 was added to make up the volume up to 100ml and continuously shaking in vortex mixture has been done until uniform mixture was not formed. Resulting mixture was filtered through Whatman filter paper No.41 and the filtrate was suitably diluted to produce the desired concentration using phosphate buffer 7.4. Appropriate aliquots of acetaminophen within the Beer's range were taken and samples were tested in triplicate manner for percentage purity and recovery test at three different levels (80 %, 100% and 120%).

2.7 Statistics

All the data were tested using two-way ANOVA followed by Tukey post hoc multiple comparison test using *statistical software graph pad prism 5*. The level of significance was considered $p<0.05$.

2.8 Validation

2.8.1 Accuracy

The accuracy of the developed methods was determined by calculating % recovery at three different levels (80%, 100% and 120%) in pre analyzed samples using standard addition method. [17]

2.8.2 Precision

Precision is the degree of aggregate among individual test results when a method is applied repeatedly to multiple sampling of a homogenous sample is known as precision of the analytical method.[7] Variation in (Intraday) and between days (inter-day) were analyzed and statistically tested. The Intraday and Inter-day precision was determined by analyzing same concentrations of acetaminophen (10µg/ml).

2.8.3 Linearity and range

Standard stock solution was prepared by dissolving 100 mg of pure acetaminophen drug in methanol and PB 7.4 (1:9) in 100 ml volumetric flask and the volume was made up with phosphate buffer 7.4. Ten working standard solutions for the drug having concentration 2, 4, 6, 8, 10, to 44µg/ml were prepared with phosphate buffer 7.4 from the stock solution. The absorbances of resulting solutions of pure acetaminophen were measured at 247λ max using phosphate buffer 7.4 as blank. All the measures were triplicate. Absorbance Vs concentration were plotted to obtain the calibration graph.

2.8.4 Limit of detection and limit of quantification

The limits of quantification (LOQ) and limit of detection (LOD) were evaluated based on the standard deviation of the response and the Slope by serial dilution of acetaminophen. LOD and LOQ of acetaminophen were determined using calibration standards. LOD and LOQ were calculated as 3.3σ/S and 10σ/S respectively, where S is the slope of the calibration curve and σ is the standard deviation of response. The limits of quantification (LOQ) and limit of detection (LOD) were evaluated based on the standard deviation of the response and the slope by serial dilution of acetaminophen. LOD and LOQ of acetaminophen were determined using calibration standards. LOD and LOQ were calculated as 3.3σ/S and 10σ/S respectively, where S is the slope of the calibration curve and σ is the standard deviation of response. [18]

2.8.5 Stability testing

The sample was subjected for stability studies under room temperature. Stabilities were studied by performing experiment to check the changes the absorbance with the freshly prepared standard solution. [18] the calibration curve was plotted on 1, 3, 7, and 15^{th} day using the stock solution. All the measurements were triplicate to meet minimum statistical requirements. Data were subjected for two-way anova testing using $p<0.05$ as significance level.

3. RESULTS AND DISCUSSION

The absorption maxima for acetaminophen in both solvents were 247 as shown in Fig. 1. Furthermore beers range was found to be 2- 44µg/ml for the new method as well as existed method.

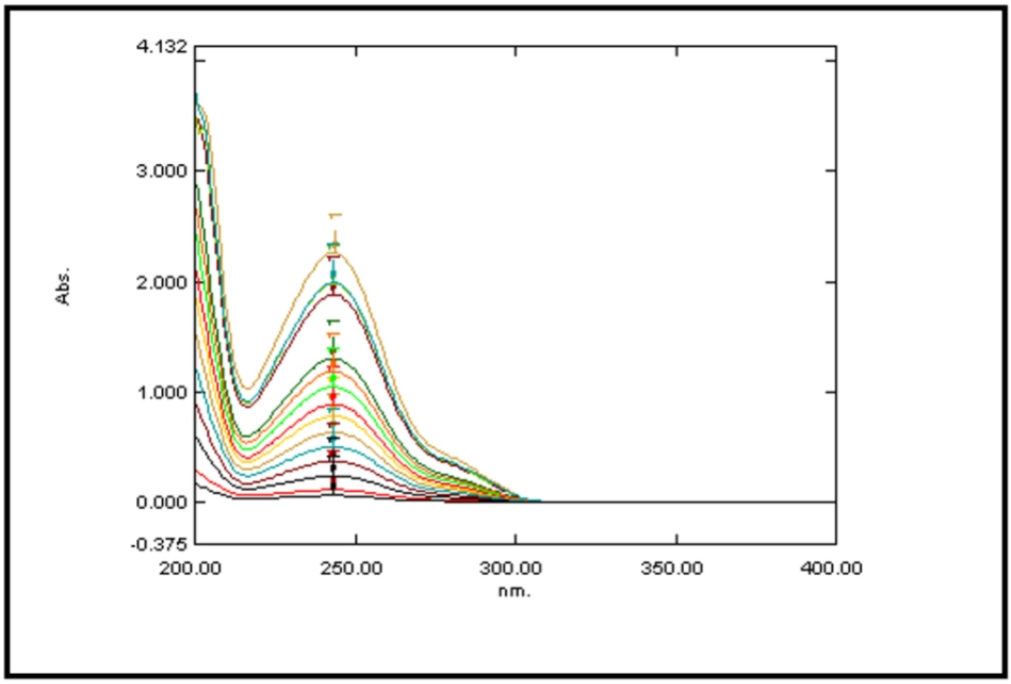

Fig. 1. Overlay spectrum of acetaminophen showing various spectra at different concentration of drug

The proposed method was validated as per the ICH Q2B guidelines. LOD and LOQ were found to be 0.502, 1.673 for acetaminophen in ratio of methanol and PB 7.4 (1:9) and 0.432, 1.440 for acetaminophen in methanol respectively. Inter-day and intraday precision studies showed % RSD values < 1 % that signifies the precision of the method. Stability studies also subjected to the new mixed solvent solution under room temperature and were significant stable up to 15 days. The graph has been drawn in between absorbance and concentration as shown in Fig. 2 at different days.

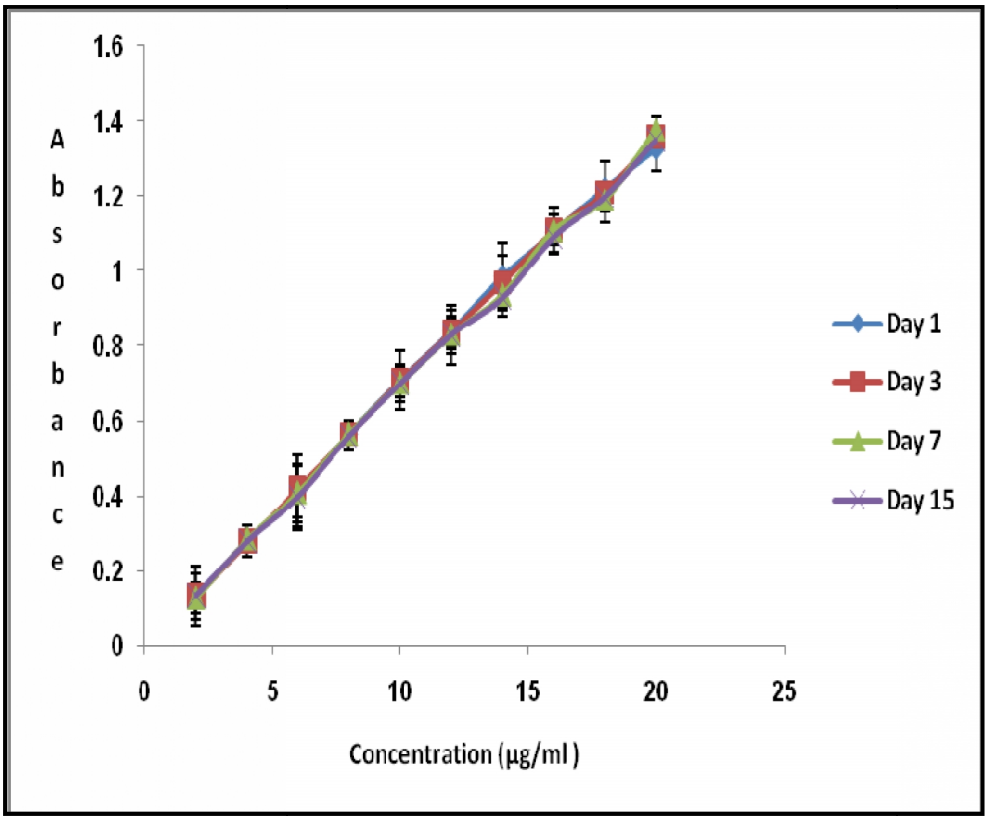

Fig. 2. Stability of acetaminophen solution at various days

Values are expressed as mean ± SEM (n=3) and analyzed by Two-way ANOVA; P<0.05 considered as significant level

The % recovery for acetaminophen in ratio of methanol and PB 7.4 (1:9) at level 80%, 100%, 120% were found to be 99.89±2.027, 99.00±4.913, 100.02±4.819 and for acetaminophen in methanol was 99.90±3.170,99.97±2.270, 100.11±1.680 respectively. Recovery study revealed that the method is accurate for quantitative estimation of acetaminophen, in tablet dosage form as the statistical parameters are within the acceptance range.

Percent purity for acetaminophen in marketed tablets were found to be 99.09% in mixed solvent methanol and phosphate buffer 7.4 (10:90 ratio) which is close to acetaminophen in methanol. All the comparative results have shown in Table 1.

Molar absorptivity and sandell's sensitivity results suggested that developed method has shown moderate sensitivity which favored its suitability toward analysis. Besides this comparison results with pure methanol were also found statistically significant (P< 0.05) similar which revealed its superiority and suitability over already existing official methods using methanol.

In addition to this, developed method reduced the use of class II organic solvents without compromising its sensitivity and accuracy; which proved its utility as a good alternate for quantitative *UV* spectroscopic analysis of acetaminophen in bulk and dosage form.

Table 1. Validation parameters for acetaminophen in different solvents

S. No.	Validation Parameter	Values Methanol: PB 7.4 (1:9)	Pure methanol
1.	Regression coefficient(R^2)	0.997	0.998
2.	Equation of straight line (x represent concentration in µg/ml, y represent absorbance)	y=0.065x+0.004	y=0.112x+0.007
3.	Linearity Range (µg/ml)	2-44	2-44
4.	LOD (µg/ml)	0.502	0.432
5.	LOQ (µg/ml)	1.673	1.440
6.	Precision (% COV)	1.837	0.9213
7.	Inter Day (% RSD)		
a.	1st Day	0.043	0.033
b.	2nd Day	0.045	0.037
c.	3rd Day	0.044	0.041
8.	Intra Day (% RSD)		
a.	1st hours	0.032	0.031
b.	3rd hours	0.035	0.025
c.	5th hours	0.043	0.025
9.	Molar Absorptivity (L mol^{-1} cm^{-1})	1.008×10^4	0.906×10^4
10.	Sandell's Sensitivity (µg/cm^2/0.001absorbance unit)	0.01497	0.0164
11.	Accuracy		
	80%	99.89±2.027	99.90±3.170
	100%	99.00±4.913	99.97±2.270
	120%	100.02±4.819	100.11±1.680

4. CONCLUSION

From the above results and data, it may be concluded that the proposed new method is simple, sensitive**,** eco-friendly, precise, and cost-effective. Besides this developed method has suitability for quality control testing.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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