Bulletin of Advanced Scientific Research 01 [03] (2015) e-ISSN: 2454-3691
www.asdpub.com/index.php/basr

Original Article

Zero order and area under curve spectrophotometric methods for determination of Paracetamol in pharmaceutical formulation

Mali Audumbar Digambar*, Hake Gorakhnath and Patil Manojkumar

Department of Pharmaceutics, Sahyadri College of Pharmacy, Methwade, Sangola-413307, Solapur, Maharashtra, India

***Corresponding Author**
Mali Audumbar Digambar
Department of Pharmaceutics,
Sahyadri College of Pharmacy,
Methwade, Sangola-413307, Solapur,
Maharashtra, India
E-mail: maliaudu442@gmail.com

Keywords:
Paracetamol,
UV visible spectrophotometry,
AUC,
Method Validation,
Antipyretic,
Accuracy.

Abstract

Objective: A simple, accurate, precise and specific zero order and area under curve spectrophotometric methods has been developed for determination of Paracetamol in its tablet dosage form by using methanol as a solvent.

Methods: (1) Derivative Spectrophotometric Methods: The amplitudes in the zero order derivative of the resultant spectra at 248 nm was selected to find out Paracetamol in its tablet dosage form by using methanol as a solvent.

(2) Area under curve (Area calculation): The proposed area under curve method involves measurement of area at selected wavelength ranges. Two wavelength ranges were selected 244-251 nm for estimation of Paracetamol.

Result & Discussion: The linearity was found to be 5-25 µg/ml for Paracetamol. The mean % recoveries were found to be 100.8% andzero order derivative and area under curve method of Paracetamol. For Repeatability, Intraday pr 100.66% of ecision, Interday precision, % RSD were found to be 0.7325, 0.0183 and 0.9768, 0.8742 for zero order and 0.8429, 0.8263 and 0.8570, 1.8017 for area under curve method respectively. Limit of Detection and Quantitation were found to be 0.4238µg/ml and 1.6987µg/ml for zero order and 0.8968µg/ml and 2.4278µg/ml for area under curve method respectively. Assay results of market formulation were found to be 100.25% for zero order and 100.88% area under curve method respectively. The proposed method has been validated as per ICH guidelines and successfully applied to the estimation of Paracetamol in its Tablet dosage form.

Conclusion: The developed methods can be concluded as accurate, sensitive and precise and can be easily applied to the pharmaceutical formulation.

1. Introduction

Chemically, Paracetamol is [N-(4-hydroxyphenyl) acetamide]. It is an analgesic antipyretic agent. Paracetamol is part of the class of drugs known as "aniline analgesics"; it is the only such drug still in use today[1]. It is not considered an NSAID because it does not exhibit significant anti-inflammatory activity (it is a weak COX inhibitor)[2,3]. This is despite the evidence that Paracetamol and NSAIDs have some similar pharmacological activity. It is effective in treating mild to moderate pain such as headache; neuralgia and pain of musculoskeletal origin[4,5]. Literature survey revealed several analytical methods UV spectrophotometry[6] and HPLC[7] have been reported in bulk, pharmaceutical dosage form for determination of Paracetamol. To our notice, so far no UV- spectrophotometric method using Zero order and Area under Curve (AUC) has been reported for the determination of Paracetamol in bulk and tablets. Hence an attempt has been made to develop new Zero order and Area under curve Spectrophotometry methods method for estimation of Paracetamol in bulk and pharmaceutical formulations with good accuracy simplicity, precision and economy.

Molecular formula: $C_8H_9NO_2$
Molecular weight: 151.163 g/mol

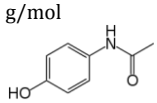

Figure 1: The structural formula of Paracetamol.

2. Materials and Methods

2.1 Apparatus and instrumentation

A shimadzu 1800 UV/VIS double beam spectrophotometer with 1cm matched quartz cells was used for all spectral measurements. Single Pan Electronic balance (CONTECH, CA 223, India) was used for weighing purpose. Sonication of the solutions was carried out using an Ultrasonic Cleaning Bath (Spectra lab UCB 40, India). Calibrated volumetric glassware (Borosil®) was used for the validation study.

2.2 Materials: Reference standard of Paracetamol API was supplied as gift sample Cipala Health Care (Pune, India). Tablet sample with label claim 500 mg per tablet were purchased from local market Solapur.

2.3 Method development

2.3.1 Preparation of Standard and Sample Solutions

Stock solution of 10µg/ml of Paracetamol was prepared in Methanol for zero order and area under the curve spectrophotometric analysis. The standard solutions were prepared by dilution of the stock solution with Methanol in a concentration range of 5,10,15,20 and 25 µg/ml with Methanol or zero order and area under the curve spectrophotometric methods. Methanol was used as a blank solution. [10,11]

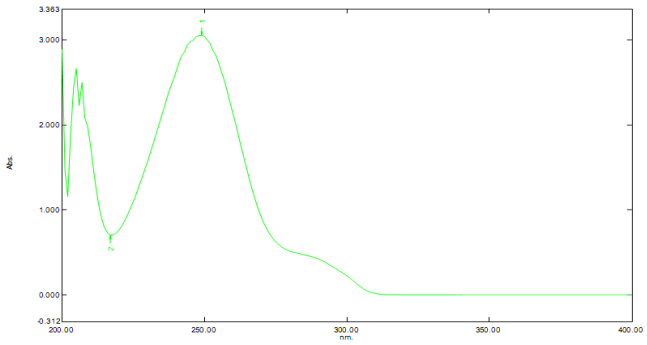

Figure 2: Zero order derivative spectrum of Paracetamol in Methanol (20µg/ml).

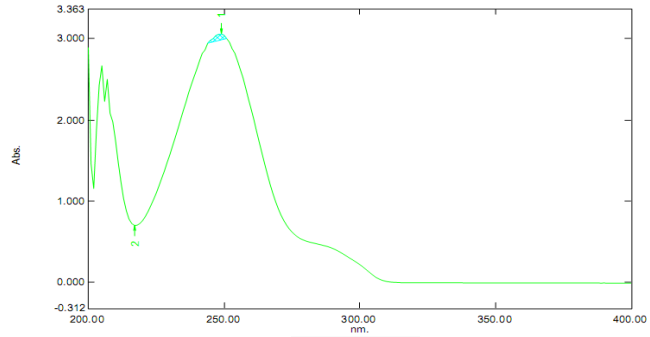

Figure 3: UV AUC spectrum of Paracetamol in Methanol (20µg/ml).

2.3.2 Area under curve (Area calculation)

Area under curve method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths such as λ1 and λ2 representing start and end point of curve region. The area under curve between λ1 and λ2 was calculated using UV probe software. In this study area was integrated between wavelength ranges from 244-251 nm.

Area calculation: $(\alpha+\beta) = \int_{\lambda 2}^{\lambda 1} A d\lambda$

Where, α is area of portion bounded by curve data and a straight line connecting the start and end point, β is the area of portion bounded by a straight line connecting the start and end point on curve data and horizontal axis λ1 and λ2 are wavelength range start and end point of curve region[8,9].

2.3.3 Assay Procedure

Twenty tablets each containing 500 mg of Paracetamol were weighed crushed to powder and average weight was calculated. Powder equivalent to 500 mg of Paracetamol was transferred in 100 ml of volumetric flask. A 50 ml of Methanol was added and sonicated for 15 minutes. Then solution was further diluted up to the mark with Methanol. The solution was filtered using Whatmann filter paper no. 41; first 5 ml of filtrate was discarded. This solution was further diluted to obtain 15µg/ml solution with water subjected for UV analysis using Methanol as blank. Appropriate dilutions were made with Methanol from stock solution for both zero order and area under the curve spectrophotometric methods.

Table 1: Assay of tablet dosage form

Sr. No.	Sample Solution Concentration (µg/ml)	Amount found (%)* Zero order derivative	Amount found (%)* Auc	Mean % Found zero order derivative	Mean % Found Auc	%RSD zero order derivative	%RSD Auc
1	15	98.53	101.19				
2	15	102.51	99.27	100.25	100.88	0.8927	0.9754
3	15	99.72	102.19				

*n=3, % RSD = % Relative Standard Deviation.

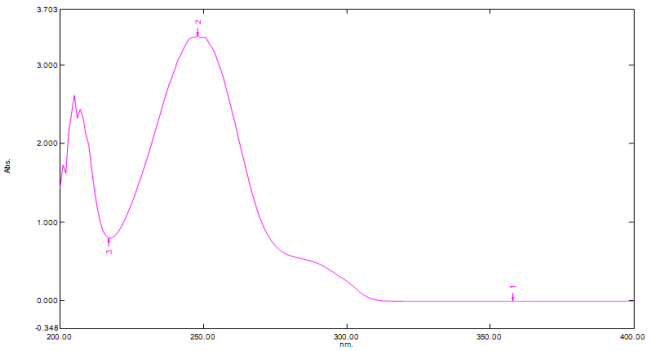

Figure 4: Zero order derivative spectrum of Paracetamol in Methanol (25µg/ml).

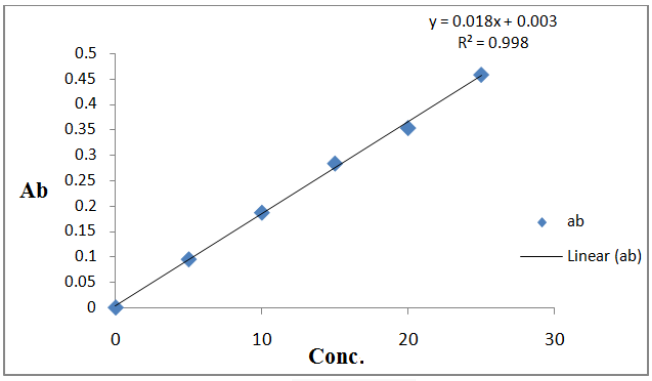

Figure 5: Linearity of Paracetamol by Absorbance

3. Results and Discussion

The zero order and area under the curve spectra for Paracetamol were recorded at the wavelength of 248 nm and 244-251 nm respectively.

3.1 Linearity and Range

Under the experimental conditions described, the graph obtained for zero order and area under the curve spectra showed linear relationship. Regression analysis was made for the slope, intercept and correlation coefficient values. The regression equations of calibration curves were y=0.021x-0.017 (r^2=0.995) at 224nm for zero order derivative spectrophotometry and y=0.023x-0.028 (r^2=0.988) at 218-227 nm for area under the curve spectrophotometry. The range was found to be 5-25 µg/ml for both zero order and area under the curve spectrophotometric methods.

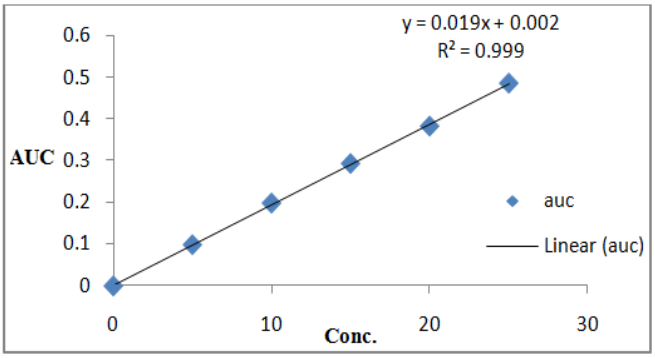

Figure 6: Linearity of Paracetamol by AUC.

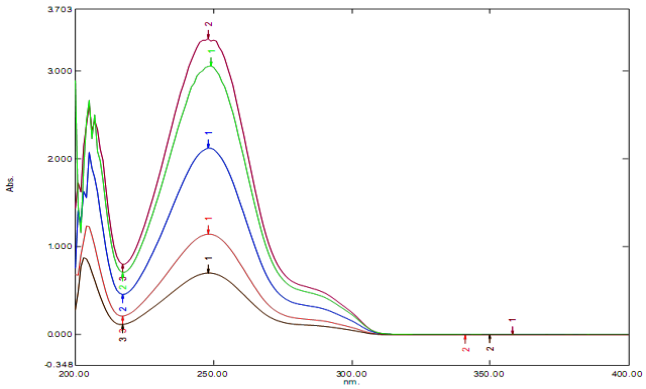

Figure 7: Zero order derivative overlay of Paracetamol at diff. Concentration.

Table 2: Stastical data for the calibration graphs for determination of Paracetamol by Proposed methods.

Parameters	Zero order derivative	Area Under the Curve
Linearity range (µg/ml)*	5-25	5-25
r^2	0.998	0.999

3.2 Accuracy

To study the accuracy of the proposed methods and to check the interference from excipients used in the dosage forms, recovery experiments were carried out by the standard addition method. The accuracy for the analytical method was evaluated at 80%, 100% and 120% levels of 15µg/ml standard solution. For Area under curve (AUC) was measured in wavelength range 244-251 nm and for zero order derivative at 248 nm and results were obtained in terms of percent recovery. Three determinations at each level were performed and % RSD was calculated for each level.

Table 3: Accuracy results for Paracetamol

Accuracy level	Sample conc (µg/)	Std. conc	Total amnt. Added (µg/m)	%Recovery zero derivatie	% Recovery Auc*	Mean of Zero derivative*	Mean of Auc derivative*	% RSD Zero derivative	% RSD Auc
80	15	12	27	100.41	102.07				
100	15	15	30	102.02	98.91	100.8	100.66	0.758	1.148
120	15	18	33	99.97	101.01				

*n=3, % RSD = % Relative Standard Deviation.

3.3 Precision

To determine the precision of the method, Paracetamol solutions at a concentration of 20 µg/ml were analysed each three times for both zero order and area under the curve spectrophotometric methods. Solutions for the standard curves were prepared fresh every day.

Table 4: Results of Intra and Inter Day Precision

Parameters	Intra Day Precision		Inter Day Precision	
	S.D*	% RSD*	S.D*	% RSD*
Zero derivative	0.7325	0.9768	0.0183	0.8742
Area under the curve	0.8429	0.8570	0.8263	1.8017

3.4 Sensitivity

The limit of detection (LOD) and limit of quantification (LOQ) were calculated by using the equations LOD = 3xσ/ S and LOQ = 10xσ/S, where σ is the standard deviation of intercept, S is the slope. The LOD and LOQ were found to be 0.4238µg/ml and 1.6987µg/ml respectively for zero order derivative and The LOD and LOQ were found to be 0.8968µg/ml &2.4278 µg/ml for area under the curve methods respectively.

3.5 Analysis of the Marketed Formulation:

There was no interference from the excipients commonly present in the tablets. The drug content was found to be 100.25% and 101.88% zero order and area under the curve spectrophotometric methods respectively. It may therefore be inferred that degradation of Pracetamol had not occurred in the marketed formulations that were analysed by this method. The low % R.S.D. value indicated the suitability of this method for routine analysis of Paracetamol in pharmaceutical dosage form.

Table 5: Summary of validation parameters

Parameter	Zero derivative	AUC
λ range	200-400 nm	200-400 nm
Regression Equation (y=mx+c)	Y=0.018x+0.003	Y=0.019x+0.002
Measured wavelength	248nm	244-251nm
Linearity range	5-25µg/ml	5-25µg/ml
Slope	0.018	0.019
Intercept	0.003	0.002
Correlation coefficient (R^2)	0.998	0.999
Limit of Detection (LOD) µg/ml	0.4238	0.8968
Limit of Quantitation (LOQ) µg/ml	1.6987	2.4278
Accuracy (Mean % Recovery)	100.8	100.66
Precission (%RSD)	0.758	1.148

4. Conclusion

No UV or Area under Curve spectrophotometric methods have been described for the determination of Paracetamol. Therefore simple, fast and reliable derivative spectrophotometric methods were developed for the routine determination of Paracetamol. The developed methods can be concluded as accurate, sensitive and precise and can be easily applied to the pharmaceutical formulation.

Acknowledgement

Authors were thankful to by Cipla Health Care (Pune, India) for providing gift sample of Paracetamol. We would also like to thanks Principal and Management of Sahyadri College of Pharmacy, Methwade, Sangola, Solapur, Maharashtra, India for providing all the facilities to complete this work successfully.

References

[1] Lakshmi S., Lakshmi K.S, Tintu.T. Simultaneous Spectrophotometric Estimation of Paracetamol and Lornoxicam in tablet dosage form. *International Journal of Pharmacy and Pharmaceutical Science.* 2(4): 166-168.

[2] Bhavsar K. C., Gaikwad P. D., Bankar V. H., Pawar S. P., Development And Validation of UV Spectrophotometric Method For Simultaneous Estimation of Paracetamol And Lornoxicam In Bulk And Tablet Dosage Form. *International Journal of Pharmacy & Technology*. 2010; 2(2): 429 – 439.

[3] Gandhi S V., Kulkarni V. G., Deshpande P. B., Chaube P. H., High Performance Thin Layer Chromatographic Analysis of Paracetamol and Lornoxicam in Combined Tablet Dosage Form. *Pelagia Research Library Der Chemica Sinica*. 2011; 2(1): 182-188.

[4] Patil P., Chivate N. D., Shinde S. S., More H. N., Pishwikar S. A., Simultaneous determination of Olmesartan medoxomil and Amlodipine besylate from Tablet Formulation by Multiwavelength Method. *Int.J. ChemTech Research*.2011; 3(2): 267-273.

[5] Siladitya Behera, Subhajit Ghanty, Fahad Ahmad, Saayak Santra, and Sritoma Banerjee. UV-Visible Spectrophotometric Method Development and Validation of Assay of Paracetamol Tablet Formulation. *J Anal Bioanal Techniques*. 2012; (3)6: 1-6.

[6] Rupali S. Joshi*, Nilima S. Pawar, Sameer S. Katiyar, Devendra B. Zope and Amol T. Shinde. Development and validation of UV spectrophotometric methods for simultaneous estimation of Paracetamol and Ibuprofen in pure and tablet dosage form. *Der Pharmacia Sinica*. 2011; 2(3): 164-171.

[7] Prasanna Reddy Battu and MS Reddy. RP-HPLC Method for Simultaneous Estimation of Paracetamol and Ibuprofen in Tablets. *Asian J. Research Chem.*2009; 2(1): 2009.

[8] ICH, 3QB Validation of Analytical Procedures: Methodology, *International Conference on Harmonization.* November 1996.

[9] ICH, Q2A Text on Validation of Analytical Procedures, *International Conference on Harmonization*. October 1994.