PHARMATUTOR
PHARMACY INFOPEDIA
ISSN: 2347-7881

Research Article

Analytical Method Development and Validation of First Order Derivative Spectrophotometric Method for Simultaneous Estimation of Paracetamol and Propyphenazone in their Combined Pharmaceutical Dosage Form

Mehul Kakdiya*, Avinash Nagapara, Darshan Madiya, Shital Faldu
Department of Quality Assurance,
Smt. R. D. Gardi B. Pharmacy College, Rajkot, Gujarat, India
*kakdiyamehul@gmail.com

ABSTRACT

A simple, precise, accurate and reproducible spectrophotometric method has been developed for simultaneous estimation of Paracetamol (PCM) and Propyphenazone (PP) by employing first order derivative zero crossing method in Methanol. The first order derivative absorption at 249 nm (zero cross point of Paracetamol) was used for quantification of Propyphenazone and 274 nm (zero cross point of Propyphenazone) for quantification of Paracetamol. The linearity was established over the concentration range of 1-12 µg/ml and 5-24 µg/ml for Paracetamol and Propyphenazone with correlation coefficient r2 0.999 and 0.996, respectively. The mean % recoveries were found to be in the range of 100.48-102.119% and 100.448-102.713 % for Paracetamol and Propyphenazone, respectively. The proposed method has been validated as per ICH guideline and successfully applied to the estimation of Paracetamol and Propyphenazone in their combined pharmaceutical dosage form.

Keywords: Paracetamol, Propyphenazone, Methanol, First order derivative method

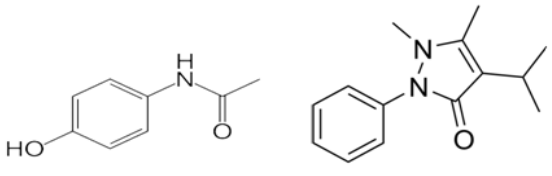
INTRODUCTION

Paracetamol (PCM) is chemically 4-hydroxyacetanilide (Figure 1-A) used as analgesic and antipyretic. [1]Paracetamol acts primarily in the CNS, increasing the pain threshold by inhibiting both isoforms of cyclooxygenase, COX-1, COX-2 and COX-3 enzymes involved in prostaglandin (PG) synthesis.[2]

Propyphenazone (PP) is chemically4-Isopropyl-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one.PP is a NSAID, analgesic, anti-pyretic drug having mechanism of action like by inhibiting COX-2 show the analgesic anti-pyretic effect.[3]

The therapeutic importance of these two compounds justifies establishing analytical methods for its determination in pharmaceutical dosage form.

The chemical structures of Paracetamol and Propyphenazone are shown in Figure 1 (A), (B).[1, 3]



Paracetamol (A) Propyphenazone (B)

Figure-1: Chemical structure of (A) Paracetamol and (B) Propyphenazone

How to cite this article: MG Kakdiya, A Nagapara, D Madiya, S Faldu; Analytical Method Development and Validation of First Order Derivative Spectrophotometric Method for Simultaneous Estimation of Paracetamol and Propyphenazone in their Combined Pharmaceutical Dosage Form; PharmaTutor; 2014; 2(5); 161-166

PHARMATUTOR
PHARMACY INFOPEDIA
ISSN: 2347-7881

Paracetamol is official in IP, BP and USP and is estimated by UV-Visible Spectrophotometric method as per IP, USP and BP. [3,4,5]In BP a redox titration for PCM is given for drug substance.[4]Propyphenazone is official in IP, BP Pharmacopoeia.[3,4]It is assayed by titrimetrically OR potentiometrically as per IP, BP. Literature review also reveals HPLC, UV spectrophotometric and HPTLC method for the estimation of PCM with other drugs.[6,7,8,]Literature survey does not reveal any simple spectrophotometric method of Paracetamol and Propyphenazone in Pharmaceutical dosage form. So the objective of this work was to develop simple, precise and rapid spectrophotometric methods for combined pharmaceutical dosage form containing Paracetamol, Propyphenazone and excipients.

MATERIALS AND METHODS

Instrumentation

Double beam UV-visible spectrophotometer (hελios Alpha, Model - V 7.09) having two matched quartz cells with 1 cm light path. An Electronic analytical balance (Contech, CA34 Model) was used in the study.

Material and reagent

Paracetamol (PCM) was obtained from Meghmani pharma pvt ltd.,Ahmedabad and Propyphenazone (PP) bulk powder was kindly gifted by Vani pharma Pvt. Ltd, Hyderabad. Methanol reagent was from college supply.

Preparation of Standard Stock solution of PCM and PP:

Accurately weighed quantity 100 mg of PCM and PP were transferred into separate 100 ml volumetric flask, dissolved and diluted up to mark with methanol(100 ml). This will give a stock solution having strength of 1000 µg/ml of each.

Preparation of Working Standard Solution of PCM and PP:

100 µg/ml of PCM and PP solution were prepared by diluting 10 ml of stock solution to 100 ml with methanol in separate 100 ml volumetric flask.

Suitable aliquots of this solution were diluted up to the mark with methanol to get the concentration range of 1,3,5,7,9µg/ml for PCM and 7,11,15,19,23µg/ml for PP.

Selection of analytical wavelength:

1-12 µg/ml solutions of PCM and 5-24 µg/ml solutions of PP were prepared in methanol by appropriate dilution of working standard solution and spectrum was recorded between 200-400 nm and all zero order spectrums (D^0) were converted to first derivative spectrum (D^1) using delta lambda 1.0 and scaling factor 5.0. The overlain first derivative spectrums of PCM and PP at different concentration were recorded.The zero crossing point (ZCP) of PCM was found to be 249 nm (Figure 2) and ZCP of PP was found to be 274 nm (Figure 3).

Preparation of calibration curve:

Standard solutions of PCM in the concentration range of 1 to 12µg/ml obtained by transferring (0.1, 0.3, 0.5, 0.7, 0.9ml) of PCM working standard solution (100 µg/ml) to the series of 10 ml volumetric flasks and standard solutions of PP in the concentration range of 5 to 24 µg/ml were obtained by transferring (0.7, 1.1, 1.5, 1.9, 2.3ml) of PP working standard solution (100 µg/ml) to the series of 10 ml volumetric flasks. Then volume was adjusted up-to mark with methanol. All dilutions were scannedin wavelength range of 200 nm to 400 nm. All zero order spectrums (D^0) were converted to first derivative spectrum (D^1). Theabsorbance was plotted against the respective concentrations to obtain the calibration curves.

PHARMATUTOR
PHARMACY INFOPEDIA
ISSN: 2347-7881

VALIDATION PARAMETERS

Validation of developed method was carried out as per ICH guideline.[9] Parameters such as Linearity and range, Accuracy, Precision, LOD and LOQ were taken up as tests for analytical method validation.

Linearity andRange:

Appropriate volume of aliquot from PCM and PP working standard solution was transferred to volumetric flask of 10 ml capacity. The volume was adjusted to the mark with methanolto give a solutions containing 1-12 µg/ml PCM and 5-24 µg/ml PP. All D^1 Spectrum were recorded using above spectrophotometric condition. D^1 absorbance at 274 nm and 249nm were recorded for PCM and PP, respectively (n=5). Calibration curves were constructed by plotting average absorbance versus concentrations for both drugs. Straight line equations were obtained from these calibration curves.

Precision

Precision of the method was determined in the terms of Repeatability, Intraday and Interday precision. Repeatability (% RSD) was assessed by analyzing test drug solution within the calibration range, six times on the same day. Intraday variation (% RSD) was determined by analysis of this solution three times on the same day. Interday precision (%RSD) was determined by analysis of this solution on three different days.

Limit of detection (LOD) and limit of quantitation (LOQ)

They were calculated as 3.3 σ/S and 10 σ/S respectively. Where σ is the standard deviation of the response (y-intercept) and S, is the mean of the slope of calibration plot.

Recovery Studies:

Recovery studies were done so as to check the accuracy of the method. Known amounts of standard solutions of PCM and PP were added to pre-quantify dosage form solution of PCM and PP and D^1absorbance were determined at 274 nm and 249 nm respectively. Concentration of the drug in the mixture was calculated using the regression equations. The analysis was done in a set of 3 replicates.

Application of Proposed Method to dosage form:

First Order Derivative Spectrophotometry[10]

Weighed and finely powdered 20 Tablets. Accurately weighed and transferred equivalent to 100mg of Paracetamoland 100mg of Propyphenazone into a 100 ml volumetric flask. This solution was filtered through the Whatmann filter paper No. 41 and residues were washed with Methanol. The filtrate and washings were combined and volume was made-up to 100 ml with Methanol. 10 ml from above stock solution is transferred to 100ml volumetric flask and dilute to 100 ml with Methanol. Take 1 ml from this solution and dilute up to 10 ml with Methanol to get final concentration as 10µg/mL of Parcetamol and 10µg/mL of Propyphenazone.

The absorbance of the solution was measured using first order derivative Spectrophotometry at selected wavelengths for determination of PCM and PP.

The concentration of each drug was calculated using equation of straight line.

RESULTS AND DISCUSSION

Zero cross point (ZCP):

ZCP of PCM and PP were detected at 249 nm and 274 nm, respectively and overlain D^1spectra were recorded (figure 4).Regression characteristics forPCM and PP are shown in Table 4.

Method Validation:

The linearity range for PCM and PP were 1-12 µg/mL and 5-24 µg/mL respectively. Recovery studies was carried out by addition of standard drug solution to pre-analyzed dosage formsolution at three different

PHARMATUTOR
PHARMACY INFOPEDIA
ISSN: 2347-7881

concentrationlevels (80%, 100% and 120%) taking into consideration percentage purity of added bulk drug sample. The results of the recovery studies are found to besatisfactory for PCM and PP and shown in Table 1 and 2 respectively.The result of assay procedure obtained was showed in Table 3. Summary of Other validation parameters including Repeatability, Intraday, Interday, LOD and LOQ were found to be satisfactory and are shown in Table 5.

CONCLUSION

The results obtained by applying the suggested procedures, it is proved that the proposed method is accurate, precise, simple, sensitive, selective and rapid and can be applied successfully in routine analysis for the estimation of PCM and PP in their combined pharmaceutical dosage form. The developed method was validated as par ICH guidelines.

ACKNOWLEDGEMENT

The authors are thankful toSmt. R. D. Gardi B. Pharmacy College, Rajkot, Gujarat,India for providing necessary facilities to carry out this work. We are also thankful to Vani pharma Pvt. Ltd., Hyderabad for providing the free gift samples of propyphenazone which was required for our research work.

FIGURE AND TABLES

Figure-2: Overlain D^1Spectrum of Paracetamol (1-12 µg/ml) in methanol.

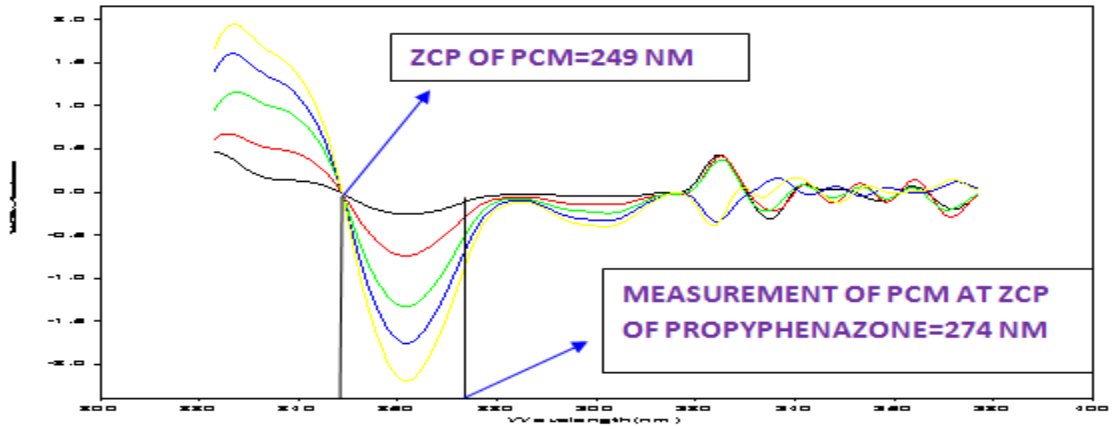
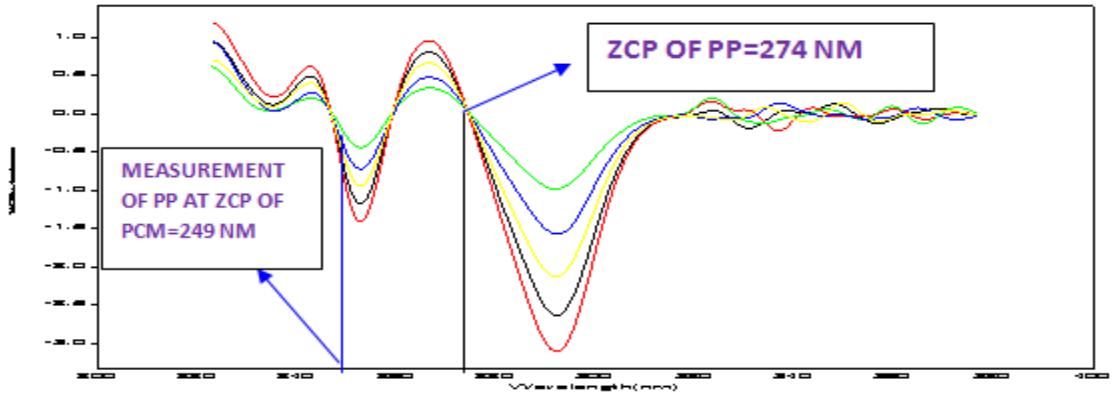


Figure-3: Overlain D^1 Spectrum of Propyphenazone (5-24 µg/ml) in methanol



PHARMATUTOR
PHARMACY INFOPEDIA
ISSN: 2347-7881

Figure-4: Overlain D1 Spectrum of PCM and PP in methanol.

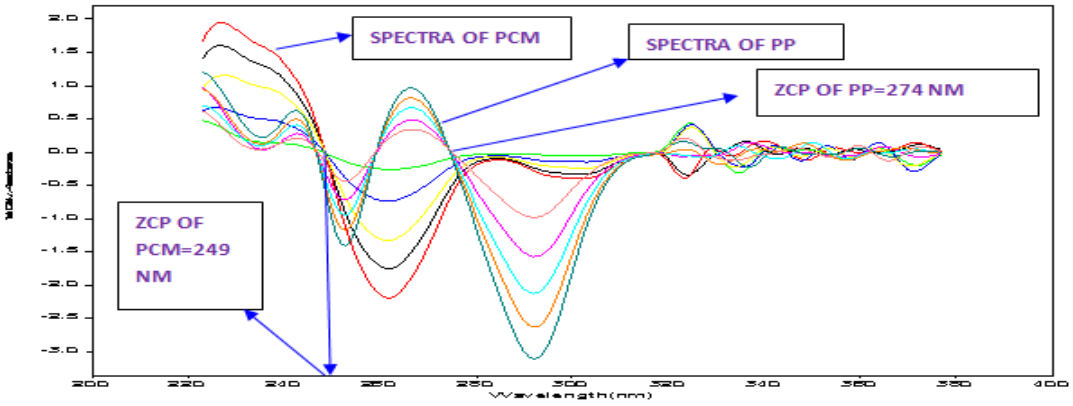


Table-1 Result of Recovery Studies for PCM in dosage form:

Amount of PCMin mixture (µg/ml)	Amount ofStd PCMadded (µg/ml)	Total amountof PCM(µg/ml)	Total amountof PCM found(µg/ml)Mean* ± SD	%Recovery
3	0	3	3.014 ± 0.0273	100.48
3	2.4	5.4	5.514 ± 0.0885	102.119
3	3	6	6.057 ± 0.0557	100.965
3	3.6	6.6	6.688 ± 0.0985	101.339

[*=mean value of 3 determination]

Table-2 Result of Recovery Studies for PP in dosage form:

Amount ofPPin Mixture(µg/ml)	Amount ofStd PP added (µg/ml)	Total amount of PP(µg/ml)	Total amountofPP found(µg/ml)Mean* ± SD	%Recovery
11	0	11	11.12 ± 0.1502	101.136
11	8.8	19.8	19.88 ± 0.2511	100.448
11	11	22	22.59 ± 0.2094	102.713
11	13.2	24.2	24.43 ± 0.3780	100.952

[*=mean value of 3 determination]

Table-3: Analysis of PCM and PP in dosage form:

Tablet dosage form	Label claim(mg)		%Recovery ± SD (% of label claim*)	
	PCM	PP	PCM	PP
	100mg	100mg	100.73% ± 1.10	101.83% ± 1.94

[*=mean value of 5 determination]

Table-4: Regression Characteristics:

Characteristics	PCM at 274 nm	PP at 249 nm
Linearity (µg/ml)	1-12	5-24
Regression Equation	y = 0.092x+0.047	y = 0.024x-0.044
Slope	0.092	0.024
r2	0.999	0.996
Intercept	0.047	0.044
S.D. of Intercept	0.004	0.001155

PHARMATUTOR
PHARMACY INFOPEDIA
ISSN: 2347-7881

Table-5: validation parameters:

Parameters	PCM at 274 nm	PP at 249 nm
Repeatability(%RSD)(n=6)	0.84262	1.4361
Precision (%RSD)		
Intra-day (n=3)	0.6026-1.6889	0.7957-1.5742
Inter-day (n=3)	0.7908-1.2067	1.5811-1.9776
LOD (µg/ml)	0.145055	0.158813
LOQ (µg/ml)	0.43956	0.48125
% Recovery (n=3)	100.48%-101..339%	100.95%-101.13%
Assay (mean ± S.D.) (n=5)	100.73% ± 1.107	101.83% ± 1.94

LOD: Limit of Detection, LOQ: Limit of Quantitation, R.S.D.: Relative standard deviation, S.D.: Standard deviation

↓ REFERENCES

1. Sweet man SC., The Martindale: The Complete Drug Reference; 36th Edn; Pharmaceutical Press, London, UK, 2009, pp 978.
2. Rang HP, Dale MM, Ritter JM and Moore PK, Pharmacology, 5th Edn; Elsevier, New Delhi 2006, pp 251,442.
3. Indian Pharmacopoeia, Volume 3, Ghaziabad: Govt. of Indian Ministry of Health and Family Welfare, the Controller of Publication, 2007, The Indian Pharmacopoeial Commission, pp 900-03.
4. British Pharmacopoeia, Volume I-II, London: The Department of Health,: HMSO Publication; 2009, pp 2789
5. The United States Pharmacopoeia-27, NF-22, Asian edition, Rockville, MD: The US Pharmacopoeial Convention, Inc., 2004, pp 1397-98.
6. Vichare V, Mujgond P, Tambe V and Dhole SN. Simultaneous Spectrophotometric determination of Paracetamol and Caffeine in Tablet formulation. International Journal of PharmTech Research, 2010, 2(4), 2512-2516.
7. Pawar PY, Joshi RS, Jangale KN and Wagh SK. Development and Validation of a Reversed Phase HPLC Method for Simultaneous Estimation of Enalapril maleate, Hydrochlorothiazide and Paracetamol in Pure and its Pharmaceutical Dosage Form. Pelagia Research Library, 2011, 2(5), 121-127.
8. Vyas AJ, Patel JK, Bhandari A, Chavda JR and Sheth NR. SimultaneousEstimation of Lornoxicam and Paracetmaol by Vierodt's Method in APIand in Synthetic mixture. International Journal of ChemTech Research,2011, 3(3), 1269-1273.
9. ICH guideline Q2 (R1). Validation of analytical procedures: text and methodology. Geneva: 1996.
10. Beckett AH. And Stenlake JB. Practical Pharmaceutical Chemistry, part-II; 4th Edn; CBS Publisher and Distributors, New Delhi, 2007, pp 275-336