International Standard Serial Number (ISSN): 2249-6807

International Journal of Institutional Pharmacy and Life Sciences 3(6): November-December 2013

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Pharmaceutical Sciences

Research Article……!!!

Received: 05-10-2013; Revised: 01-12-2013; Accepted: 12-12-2013

DIFFERENCE ABSORBANCE SPECTROPHOTOMETRIC DETERMINATION OF PARACEMATOL IN TABLET DOSAGE FORM CONTAINING PARACETAMOL AND DICLOFENAC

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Keywords:

Paracetamol, diclofenac, difference spectroscopy

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ABSTRACT

The selectivity and accuracy of spectrophotometric analysis of formulation containing absorbing interferents can be done by the technique of difference spectrophotometry. Estimation of paracetamol is simply difference absorbance between two equimolar solutions of the analyte in different chemical forms which exhibit different spectral characteristics. The projected method is based upon measuring the difference absorbance in pH 7.5 and pH 11.5 where absorbance of diclofenac remains unaffected. The Beer's range of paracetamol in both buffer solution i.e. pH 7.5 and pH 11.5 was found to be 5-10 µg/ml. The wavelength of analysis was chosen as256 nm, where ΔA has remained positive. The precision and accuracy of method was acceptable and the relative standard deviation did not exceed 1.2% (n=6) and recovery was between 98.02% - 101.3% for paracetamol. The proposed method has been validated and successfully could be applied for the determination of the drug in solid dosage form and in the form of granulation.

INTRODUCTION

Paracetamol is antipyretic and analgesic which is an ingredient of many proprietary preparations. Chemically it is N(4- Hydroxyphenyl) acetamide. It is official in IP, BP and USP. It is slightly soluble in cold water,more soluble in hot water, soluble in ethanol, methanol, ethylene chloride, acetone and chloroform, slightly soluble in ether, practically insoluble in petroleum ether, pentane and benzene. Dissociation constant of paracteamol (pKa) is 9.5. It shows UV absorption in aqueous acid at 245nm ($A_{1cm}^{1\%}$ =668) and more absorption in aqueous alkali at 257nm ($A_{1cm}^{1\%}$=715). Diclofenac is non-steroidal anti-inflammatory analgesic drug. Chemically it is 2-[(2, 6, - Dichlorophenyl) amino] benzene acetic acid. Solubility at 25^{o}C (mg/ml) in deionised water (pH 5.2)>9, methanol>24, acetone 6, acetonitrile<1, cyclohexane<1. Dissociation constant (pKa) of Diclofenac is 4.2. It shows UV absorption in aqueous acid at 273nm ($A_{1cm}^{1\%}$= 309) and also more UV absorption in aqueous alkali at 275nm $A_{1cm}^{1\%}$= 315).

Literature survey shows that estimation of paraceteamol and diclofenac is done by UV spectrophotometry and Chromatography. Proposed method is only for analysis of paracetamol in presence of diclofenac. The technique of difference absorbance spectrophotometric method is based upon the absorbance difference (ΔA) which can be induced by changing the pH of solvent medium of equimolar solution of paracetamol in 2 different buffer system where interfering substance (here diclofenac) has same absorbance in both the buffer system. The choice of wavelength is based upon the consideration of pKa of analyte (i.e. paracetamol) and interferant (i.e. diclofenac). Both the drugs are pH sensitive but since pKa difference between both the drug is greater than 4, difference spectroscopy method development is possible. The selection of wavelength is based upon the condition that ΔA should be positive. Practically ΔA is having positive value at 256nm. So, by determining Δa (standard absorptivity) we can determine the concentration, by formula ΔA= Δabc. The developed method is simple, rapid, precise and economic technique for determination of paracetamol in presence of diclofenac.

MATERIALS AND METHOD

INSTRUMENTS

A Shimadzu Double beam UV- Visible spectrophotometer 1700 with 1 cm matched quartz cells, bandwidth 1nm was used for recording of spectrum. Calibrated Systronic pH system 361 was used to record and maintain the record. Pure drug samples of paracetamol and diclofenac (Okasa

Pharma, Satara) were used having 99.2% and 99.83% purity respectively. The wavelength of analysis chosen is 256nm where ΔA remains positive. Since paracetamol and diclofenac are both pH sensitive chemicals, but the difference between pKa of paracetamol and diclofenac (interference) is more than 4, so we can apply difference spectroscopy to above formulation. Buffer used were phosphate buffer pH 7.5 and pH 11.5. All reagents used were of analytical grade. Double distilled deionised water was prepared by Millipore system (Sartorius, USA) using 0.2 micrometer membrane filter. Tablets were procured locally. Electronic balance of Shimadzu type AX120 was used for weighing purpose.

PROCEDURE

1) Selection of buffer: pKa of paracetamol and diclofenac is 9.5 and 4.2 respectively, which indicates that both the drugs are pH sensitive. But since the difference between the pKa is more than 4, so we can apply difference spectroscopy to above formulation. So two buffer systems has been chosen one buffer having pH greater by 2 than pKa of analyte (i.e. paracetamol pKa 9.2) and another buffer having pH less by 2 than that of pKa of paracetamol. Since, pKa of paracetamol is 9.5 we have chosen buffer system with pH 7.5 and 11.5 where absorbance of diclofenac remains unaffected.

2) Preparation of drug solution: Stock solution of paracetamol and diclofenac in methanol was prepared followed by preparation of dilutions (10µg/ml) of both the drugs in buffer system having pH 7.5 and 11.5.

3) Selection of wavelength of analysis: Equimolar solution (10µg/ml) of the paracetamol in both buffers has been prepared followed by recording of spectra (**Figure 1**) in a spectrum mode. Both the spectra were overlain (**Figure 3**) and wavelength (256 nm) was selected where ΔA (difference in absorbance) is positive. Interference of diclofenac was same which is confirmed by recording absorbance of both equimolar solution of diclofenac at selected wavelength 256 nm.

4) Determination of Beer's range: Standard stock solution of paracetamol was prepared in methanol from which two sets of solutions in two different buffer systemswas prepared having concentrations 5, 6, 7, 8, 9 and 10µg/ml. The absorbance of above solutions at 256 nm was recorded which confirmed that individual absorbance of paracetamol in both buffer solutions obeyed Beer's law. So, automatically difference in absorbance of paracetamol i.e. ΔA obeys Beer's law.

5) Determination of difference Absorptivity of paracetamol (Δa of paracetamol): Five replicate dilutions of 10µg/ml were prepared separately in pH 7.5 and 11.5. The difference absorbance (ΔA) was directly determined by placing the drug solution of pH 11.5 in sample cell and a drug solution of pH 7.5 in reference cell. The procedure was repeated for all five solutions. ΔA was determined.Average of all absorbances was determined and multiplied by 1000 to get Δa. Value of Δa or $\Delta A_{1cm}^{1\%}$ or difference absorptivity was found to be 227nm.

6) Assay procedure for tablet: Twenty tablets were weighed and pulverized. From this sample powder equivalent to 100mg of paracetamol was dissolved in methanol so as to get standard stock solution of 1000µg/ml and filtered.The aliquots of the stock solution were pipetted out so as to get final dilutions of 10µg/ml in both buffer solutions i.e. in pH 11.5 and pH 7.5. The difference absorbance at 256nm was recorded. Practical concentration of paracetamol in µg/mlwas determined by formula ΔA = Δabc at 256nm.

Theoretical concentration of paracetamol was determined by weight and hence the assay of paracetamol in a given combination of paracetamol and diclofenac in solid dosage form was estimated.

METHOD VALIDATION

The developed method was validated according to **ICH Guidelines Validation of Analytical Procedures: Text and Methodology Q2 (R1)**. The method was validated with respect to following parameters accuracy, precision, linearity and specificity.

Accuracy: Accuracy parameter of developed method was determined by analyte recovery method for which nine measurements by using three concentrations of strengths 5 µg/ml, 10 µg/ml and 15µg/ml were done. All nine values were averaged and used for the final accuracy determination. The results found by this method were 99 to 101% at each level for a given reference substance.

Precision of method/ Repeatability (Intra-assay): The drug solutions were prepared in different concentration (5 µg/ml, 10 µg/ml and 15µg/ml) and the absorbance of solutions was recorded and the precision was calculated. Relative standard deviation was found to be less than 2%. Intermediate precision of the method was performed by repeating the same method by the analyst at different times of the day and following by determination of its relative standard deviation.

Linearity: Described method was found to be linear over the range of 5-15µg/ml for paracetamol. The linearity of calibration graphs and adherence to Beer's law were validated by the value of correlation coefficient.

Robustness: Robustness of the method was confirmed by altering the wavelength range and slit width but results show modest inference when change with slit width has not affected the absorbance of the drug indicating method is robust enough.

Table no.1: Analytical data for estimation of paracetamol using the proposed method

Parameter	Paracetamol
Beer's range	5-15 µg/ml
Wavelength of analysis	256 nm
$\Delta A_{1cm}^{1\%}$ = Δa (Difference in Absorptivity)	227 dl gm^{-1} cm^{-1}
pKa of Paracetamol	9.5
pKa of Diclofenac	4.2
pH of buffer system	7.5 and 11.5

Table no.2: Evaluation of the accuracy of the data for the proposed method

Theoretical concentration of paracetamol [n=9] (µg/ml)	Practical concentration of paracetamol (µg/ml)	Amount found (%)	Relative standard deviation
5 µg/ml (triplicate)	5.1	102%	±0.04
10 µg/ml (triplicate)	10.008	100.08%	±0.07
15 µg/ml (triplicate)	15.05	100.34%	±0.03

Table no.3: Evaluation of the repeatability of data for the proposed method

Theoretical concentration of paracetamol [n=9] (µg/ml)	Practical concentration of paracetamol (µg/ml)	Amount found (%)	Relative standard deviation
5 µg/ml (triplicate)	5.1	102	±0.04
10 µg/ml (triplicate)	10.008	100.08	±0.07
15 µg/ml (triplicate)	15.05	100.34	±0.03

Table no.4: Evaluation of the intermediate precision of data for the proposed method (Inter-day analysis)

Theoretical concentration of paracetamol (µg/ml)	Practical concentration of paracetamol (µg/ml)	Amount found (%)	Relative standard deviation
10.12 µg/ml	10.10	99.8	±0.03
10.08 µg/ml	10.02	99.4	±0.05
10.09 µg/ml	10.13	100.4	±0.08

Table no.5: Evaluation of the intermediate precision of the data for the proposed method by changing the analyst

Theoretical concentration of paracetamol (µg/ml)	Practical concentration of paracetamol (µg/ml)	Amount found (%)	Relative standard deviation
10.01 µg/ml	9.99	99.8	±0.04
10.07 µg/ml	10.12	100.5	±0.08
10.11 µg/ml	10.02	99.11	±0.07

Table no.6: Evaluation of percent purity of paracetamol for three different commercially marketed brands of Paracetamaol

Brand name	Label claim of (paracetamol + diclofenac) in mg	Amount of paracetamol found (mg)	% Purity
Diclomol, (Win-Medicare ltd..)	500+50	498.5	99.7
Argesic Plus (Centaur Drug House)	500+50	502.3	100.46
Diclomove-P (Emcure Pharmaceuticals ltd.)	500+50	499.7	99.98

Table no.7: Evaluation of the linearity parameter data for the proposed method

Parameter	Observation
Linearity concentration	5-15 µg/ml
Slope	0.012
Intercept	0.00043
Correlation coefficient	0.999

RESULT AND DISCUSSION

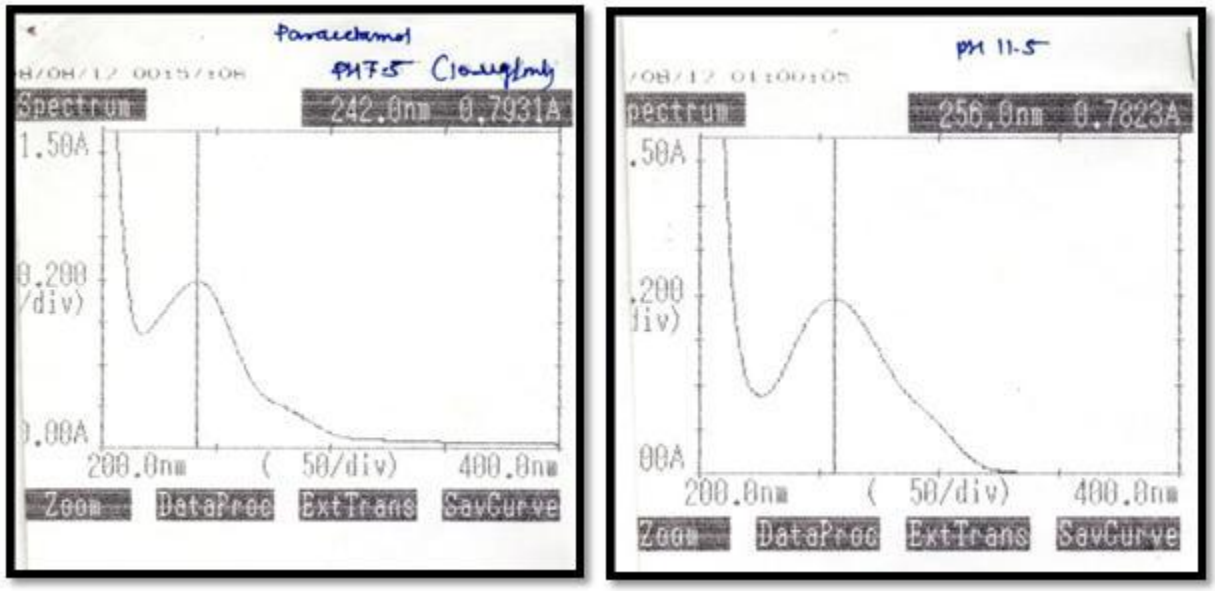

Figure 1: λ_{max} values of Paracetamol at two different pH i.e. pH 7.5 and pH 11.5.

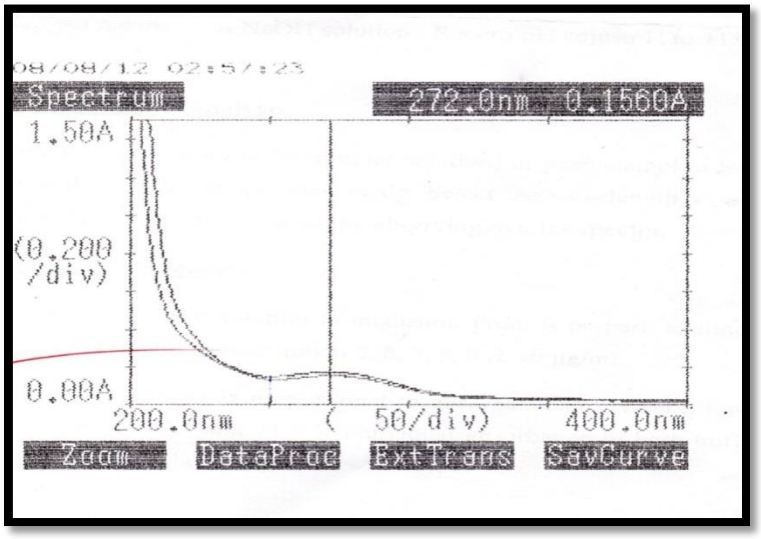

Figure 2: Overlay spectra of Diclofenac (10 µg/ml) at pH 7.5 and pH 11.5 showing no significant change in absorbance.

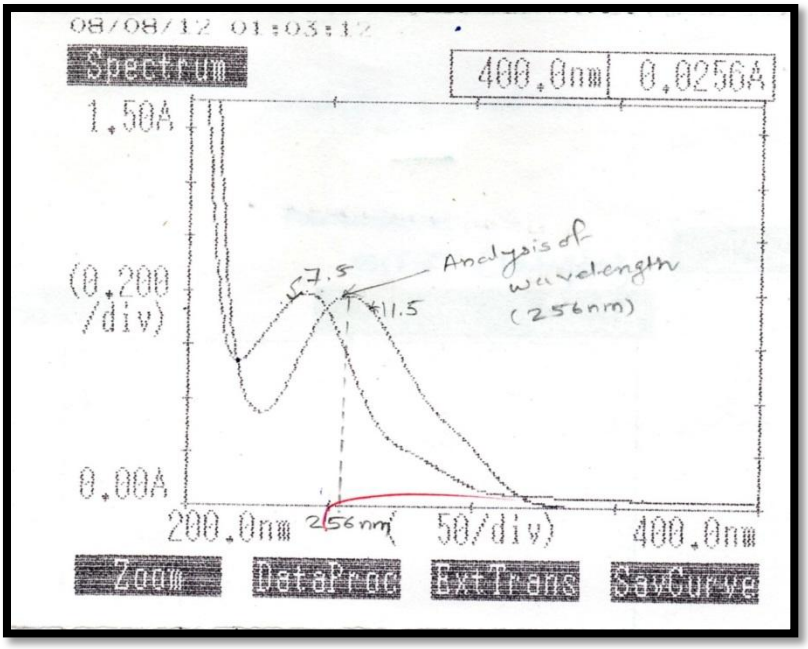

Figure 3: Overlay spectra of Paracetamolat two different pH i.e pH 7.5 and pH 11.5.

The proposed validated method for determination of paracetamol in solid dosage form in presence of diclofenac was very accurate and simple. Diclofenac though pH sensitive, but selection of appropriate buffer system helped in successful determination of paracetamol in presence of diclofenac. Buffer system of pH 11.5 and 7.5 was chosen by considering the pKa of both the drugs. In this method difference absorbance (ΔA) of paracetamol was obtained at 256 nm, where absorbance of diclofenac remains constant in both buffer systems.

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