Journal of Pharmaceutical and Scientific Innovation
www.jpsionline.com
Research Article

NOVEL UV-SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS ESTIMATION OF OMEPRAZOLE AND DICLOFENAC IN BULK AND PHARMACEUTICAL FORMULATIONS

K. Siva Chaitanya* and Aziz Unnisa
Department of Pharmaceutical Analysis, KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada, Andhra Pradesh, India
*Corresponding Author Email: khushiazeez@yahoo.co.in
DOI: 10.7897/2277-4572.035189

Received on: 05/09/14 Revised on: 08/10/14 Accepted on: 15/10/14

ABSTRACT
The aim of present experiment was to develop validated UV spectroscopic method for simultaneous estimation of Omeprazole and Diclofenac in bulk and pharmaceutical formulation. Area under curve (Method 1), simultaneous equation (Method 2) and absorbance ratio (Method 3) were developed for the determination of Omeprazole and Diclofenac in their combined capsule formulation without prior separation. The solutions of standard and sample were prepared in methanol for all the methods. Quantitative determination of the drugs was performed at the wavelength ranges of 291-311 nm and 271-291 nm (method 1), at 301 and 281 nm (method 2) and 301 nm and 295 nm (method 3) for Omeprazole and Diclofenac respectively. The Proposed methods were evaluated for the different validation parameters like precision, reproducibility, linearity and accuracy as per ICH guidelines. Linearity was observed in the range of 5-25 µg/mL for Diclofenac and 1-5 µg/mL for Omeprazole with correlation coefficient of 0.998 and 0.999 for Omeprazole and Diclofenac respectively. These methods are simple, precise, sensitive and applicable for the simultaneous determination of these drugs in pure powder and formulation.
Keywords: Absorbance ratio method, Area Under Curve Technique, Diclofenac, Omeprazole, simultaneous equation, UV Spectroscopy.

INTRODUCTION

Omeprazole (OME) is 5- methoxy -2-(4-methoxy-3 - 5-dimethyl – 2 – pyridinyl methyl sulfinyl)-3H-benzimidazole (Figure-1) is used as an anti ulcerative (proton pump inhibitor). Omeprazole[1,2] is racemate (both R and S forms), in acidic condition of the stomach both (R and S forms) are converted to achiral products which reacts with the cystine group in H^+/K^+ ATPase there by destroying the ability of the parietal cells to produce Gastric acid. Diclofenac Sodium[3] (DIC) which is chemically sodium 2-[(2, 6-dichlorophenyl)-amino]phenyl acetate (Figure-2) is used in the treatment of signs and symptoms of osteoarthritis and rheumatoid arthritis. It acts by inhibition of both leukocyte migration and the enzyme cyclo-oxygenase (COX-1 and COX-2), leading to the peripheral inhibition of prostaglandin synthesis. As prostaglandins sensitize pain receptors, inhibition of their synthesis is responsible for the analgesic effects of Diclofenac. The Antipyretic effects may be due to its action on the hypothalamus, resulting in peripheral dilation, increased blood flow and subsequent heat dissipation. Literature survey revealed several analytical methods for estimation of DIC[4-13] and/ or OME[14-23] in bulk, pharmaceutical formulation or in biological fluids, in isolation or in combination with other drugs like Nimesulide[7], Paracetamol[8], Thiocolchicoside[9], Rabeprazole[10], Misoprostol[12], Eperisone Hydrochloride[13], Ondansetron[15], Drotaverine Hydrochloride[16] and Domperidone[19]. The analytical techniques such as HPLC, Spectrophotometry and HPTLC were used for these determinations. However, to the best of our knowledge no method has been reported for the simultaneous estimation of OME and DIC using UV spectrophotometry. Hence the objective of the present paper was to develop the first UV spectrophotometric methods which are simple, rapid, precise, accurate and economical and can be used for the simultaneous estimation of the drugs OME and DIC in the combined dosage form without prior separation and to validate the developed method in accordance with ICH gudilence[20-24].

MATERIALS AND METHODS

Pure OME and DIC were obtained as gift sample from Darwin labs pvt Ltd, Vijayawada, Andhra Pradesh, India. Diopra (Diclofenac 50 mg + Omeprazole 10 mg) capsules were procured from the local pharmacy. Methanol (analytical grade) was used as the solvent. A Shimadzu UV-1800 spectrophotometer, with a pair of 1 cm matched quartz cells were used for the spectral measurements.

Preparation of Standard Stock Solutions

Accurately weighed 10 mg of OME and DIC were dissolved separately in small amount of methanol in 10 mL volumetric flasks and sonicated for 3 minutes. The final volume was adjusted up to the mark with methanol to get a solution of 1 mg/mL.

Preparation of Sample Solutions

Twenty capsules of Diopra (OME 10 mg and DIC 50 mg) were taken; the contents were removed from the shell as completely as possible and weighed. The amount of powder equivalent to 10 mg of DIC was transferred into a 10 mL volumetric flask, dissolved in methanol and sonicated for about 20 minutes. The solution was filtered through nylon disc filter (0.22 µ) and volume was made up to the mark using the same solvent. The filtrate was further diluted to get the concentration of both drugs in the linearity range.

Method 1

For the simultaneous determination using the area under curve (AUC) method, suitable dilutions of the standard stock solutions (1000 µg/mL) of OME and DIC were prepared separately in methanol. The solutions of drugs were scanned in the range of 200-400 nm, the wavelength of 301 nm and 281 nm were selected as λ_{max} of OME and DIC respectively. The areas of the two drugs were determined at the selected wavelengths ranges i.e., 291 to 311 nm and 271 to 291 nm (± 10 nm of λ_{max} of the both drugs). The 'X' values were determined as X= Area under curve of component (from 291 to 311 nm or 271 to 291 nm)/concentration of the component

in g/l. C_{OME} and C_{DIC} are the concentrations of OME and DIC respectively (g/l) in sample solution; $AUC_{291-311}$, $AUC_{271-291}$ are the area under curve of sample solution at the wavelength range, 291 – 311 nm and 271-291 nm, respectively. The 'X' values reported are the mean of six independent determinations. Applying equations (1) and (2), concentrations C_{OME} and C_{DIC} were obtained. Figure 3, linear response with increasing concentration was obtained for both of the drugs hence the same wavelength range was used for estimation of capsule formulations. Sample spectra were shown in Figure 4.

$$C_{OMP} = \frac{AUC(271-291)\times X_A(291-311) - AUC(291-311)\times X_A(271-291)}{X_D(271-291)\times X_A(291-311) - X_D(291-311)\times X_A(271-291)} \quad \text{..... (1)}$$

$$C_{DIC} = \frac{AUC(291-311)\times X_D(271-291) - AUC(271-291)\times X_D(291-311)}{X_D(271-291)\times X_A(291-311) - X_D(291-311)\times X_A(271-291)} \quad \text{..... (2)}$$

Where, C_{OME}, C_{DIC} are the concentrations of the OME and DIC in the sample solution, AUC (291 – 311), AUC (271-291) are the area of the mixture, X_A (291 – 311), X_A (271-291) are the absorptivities of OMP and X_D (291 – 311), X_D (271-291) are the absorptivities of DIC.

Method 2

For the determination of OME and DIC using the Simultaneous equation method, standard stock solutions of OME and DIC (1000 µg/mL) were diluted with methanol to get the concentration of 10 µg/mL and the solutions were scanned in the wavelength range of 400–200 nm. From the overlain spectrum of OME and DIC, two wavelengths i.e., 301 nm and 281 nm were selected for OME and DIC respectively. The calibration curves were constructed in the concentration range of 4-20 µg/mL at each of the wavelengths. The absorptivity coefficients were determined for both the drugs at the selected wavelengths and calculated by using the formula-3 and 4. OME and DIC overlay spectra was shown in Figure 5.

$$C_X = \frac{A_1 a y_2 - A_2 a y_1}{a x_1 a y_2 - a x_2 a y_1} \quad \text{..... (3)}$$

$$C_Y = \frac{A_1 a x_2 - A_2 a x_1}{a x_1 a y_2 - a x_2 a y_1} \quad \text{..... (4)}$$

Where, A_1 and A_2 are absorbance of sample at 301 nm and 281 nm, respectively, Cx and Cy are concentrations of OME and DIC respectively

Method 3

Absorbance ratio method uses the ratio of absorbances at two selected wavelengths, one which is an isoabsorptive point while the other being the λ-$_{max}$ of one of the two components. From the overlay spectra of two drugs, it is evident that OME and DIC show an isoabsorptive point at 295 nm. The second wavelength used is 301 nm, which is the λ-$_{max}$ of OME. Working standard solutions having concentration 1, 2, 3, 4 and 5 µg/ml for OME and 5, 10, 15, 20 and 25 µg/ml for DIC were prepared in methanol and the absorbances at 295 nm (isoabsorptive point) and 301 nm (λ-$_{max}$ of OME) were measured and absorptivity coefficients were calculated. The concentration of two drugs in the mixture can be calculated using following equations.

$$CX = [(QM - QY) / (QX - QY)] \times A1/ax1 \text{..... (1)}$$
$$CY = [(QM - QX) / (QY - QX)] \times A1/ay1 \text{ ..... (2)}$$

Where, A_1 and A_2 are absorbances of mixture at 295 nm and 301 nm; ax_1 and ay_1 are absorptivities of OME and DIC at 295 nm; ax_2 and ay_2 are absorptivities of OME and DIC respectively at 301 nm; $QM = A_2 / A_1$, $QX = ax_2 / ax_1$ and $QY = ay_2 / ay_1$

Validation

Validation of the proposed methods was carried out for its accuracy, precision, specificity and linearity according to ICH guidelines

Accuracy

Recovery studies were carried out at three different levels by adding the pure drug to previously analyzed tablet powder sample. Accurately weighed quantities of tablet powder equivalent to 80 %, 100 % and 120 % of label claim of OME were taken in a series of 10 mL volumetric flasks and appropriately diluted as described under the preparation of sample solution. From the amount of total drug present, percentage recovery was calculated by proposed two methods and results are shown in Table 2.

Precision

Inter-Day precision: - It was performed by analyzing the solution by same analyst on the alternate days till 5th day. Results indicate that the solution is stable up to 3 days. Thereafter degradation may have taken place leading lower percent label claim.

Intra-day precision: - It was done by analyzing the solution by same analyst within a day. Results indicated that the solution was stable for entire day.

Linearity

Method 1

Linearity was checked by diluting standards stock solution at five different concentrations. OME was estimated in the concentration range of 1-5 µg/mL at 291 nm - 301 nm while DIC was determined in the concentration range of 5-25 µg/mL at 271 nm - 291 nm. Calibration curves (n = 5) were plotted between concentration and area of drugs (Figure 7, 8) and optical parameters were calculated.

Method 2

Linearity of simultaneous equation method was checked by diluting standards stock solution at five different concentrations. Linearity for OME (1-5 µg/mL) and DIC (5-25 µg/mL) was recorded at 301 nm and 281 nm respectively. Calibration curve (n = 5) were plotted between concentration and absorbance are shown in Figure 8, 9.

Method 3

Linearity for absorbance ratio method was checked by diluting aliquots of standards stock solutions to obtain working standards in concentration range of 1-5 µg/mL (OME) and 5-25 µg/mL (DIC) was with the concentration range of at 295 nm. Isoabsorptivity point was linear in the concentration range of at 301 nm. Calibration curve (n = 5) were plotted between concentration and absorbance. Plot was shown in Figure 10, 11.

Limit of detection

The Limit of detection (LOD) is the smallest concentration of the analyte that gives the measurable response. LOD was calculated using the following formula and shown in Table 1.

$$LOD=3.3\ (\sigma /S)$$

Where, S= slope of calibration curve, σ =standard deviation of the response

Limit of quantification

The limit of quantification (LOQ) is the smallest concentration of the analyte, which gives a response that can be accurately quantified. LOQ was calculated using the following formula and shown in Table 1.

$$LOQ=10(\sigma /S)$$

Where, S= slope of calibration curve, σ=standard deviation of the response

RESULTS

Validation of the proposed methods was performed as per the ICH guidelines. The accuracy of the proposed method was determined by recovery studies. Pure OME and DIC were added to the pre analysed sample at three concentration levels viz 80, 100, 120 %. Three replicate analyses were carried out at each level. The mean percent recovery was found in the range of 99.4 to 100.3 % for all the methods shown in Table 2. For the two methods linearity was observed in the concentration range of 5-25 µg/mL for DIC and 1-5 µg/mL for OME both the drugs. Commercial formulations containing OME and DIC were analyzed by the proposed methods. Three replicate analysis of formulation were carried out and the mean assay values were found in the range of 98.4 to 99.5 % shown in Table 3. Precision is calculated as inter day and intraday variations for both the drugs. Percent relative standard deviations for estimation of OME and DIC under intraday and interday variations were found to be less than 1.

Table 1: Validation Data for Omeprazole and Diclofenac

Parameters	Method 1		Method 2		Method 3	
	OME	DIC	OME	DIC	OME	DIC
Area range (λ)	291-311	271-291	301	281	301	295
Beer's-Lambert's range (µg/mL)	1-5	5-25	1-5	5-25	1-5	5-25
Regression equation y =mx + c	y = 0.598x – 0.248	y = 0.078x + 0.040	y = 0.206x - 0.010	y = 0.041x - 0.001	y = 0.206x - 0.010	y = 0.031x - 0.004
Slope (m)	0.598	0.078	0.206	0.041	0.206	0.031
Intercept (c)	0.248	0.04	0.01	0.001	0.01	0.004
Correlation coefficient (r2)	0.999	0.998	0.998	0.998	0.998	0.998
Recovery + S. D. (n = 3)	99.5	99.4	99.31	99.63	99.21	99.45
Repeatability (% RSD, n = 6)	0.768	0.987	0.831	0.927	0.791	0.912
Intermediate precision (% RSD)						
Interday (n = 3) Intraday (n = 3)	0.514 0.615	0.426 0.715	0.431 0.632	0.520 0.691	0.431	0.692
LOD (µg/mL)	0.121	0.059	0.105	0.048	0.105	0.048
LOQ (µg/mL)	0.36	0.179	0.31	0.145	0.31	0.145

Table 2: Accuracy Data for Omeprazole and Diclofenac

Level of recovery	Drug	Amount of drug taken	Amount of std drug added	Recovery	%RSD
80	OME	2	3.8	99.5	0.51
	DIC	10	18	99.4	0.44
100	OME	2	4	99.6	0.643
	DIC	10	20	99.3	0.72
120	OME	2	4.2	100.1	0.518
	DIC	10	22	100.3	0.641

Table 3: Assay Data

S. No.	Sample solution concentration of OME (µg/mL)	Sample solution concentration of DIC (µg/mL)	Amount Found (%)	Mean Amount Found (%)	% RSD*
1	2	10	98.4	99	0.58
2	2	10	99.3		
3	2	10	99.5		

n = 3, % RSD = % Relative Standard Deviation

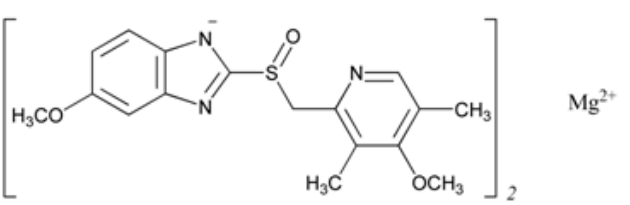

Figure 1: Structure of Omeprazole

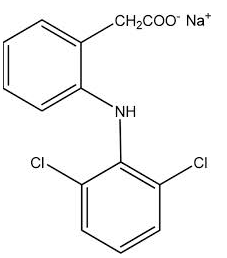

Figure 2: Structure of Diclofenac sodium

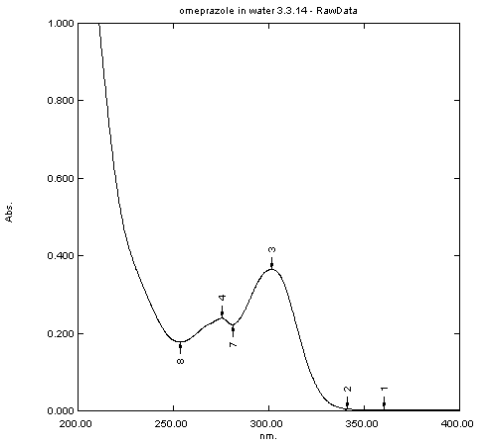

Figure 3: UV spectra of OME

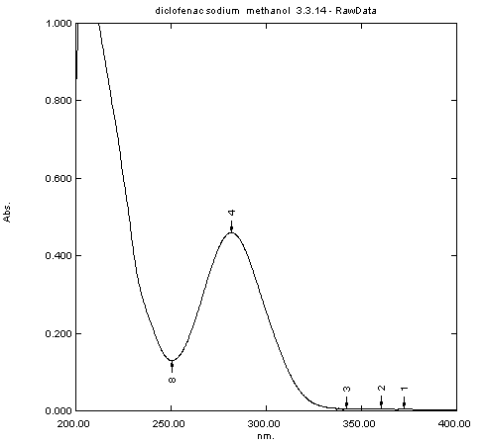

Figure 4: UV spectra of DIC

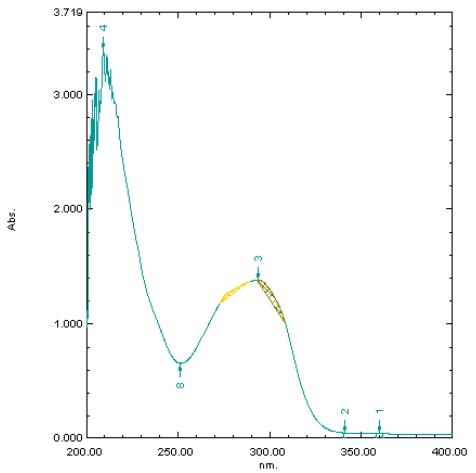

Figure 5: UV spectra of sample

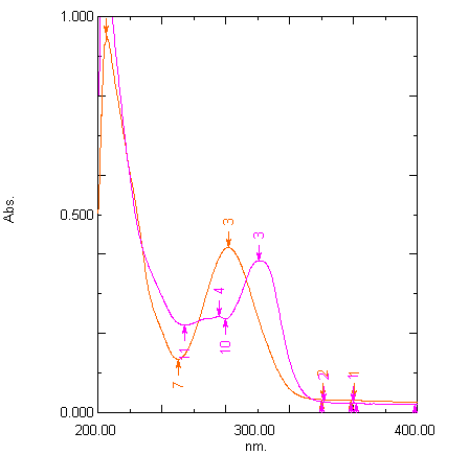

Figure 6: overlay spectra of OME and DIC

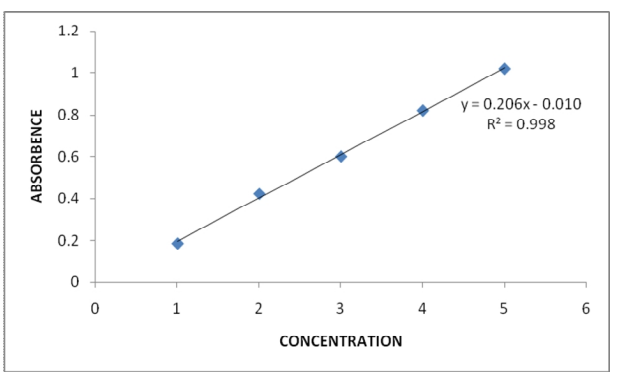

Figure 7: Linearity plot for OME

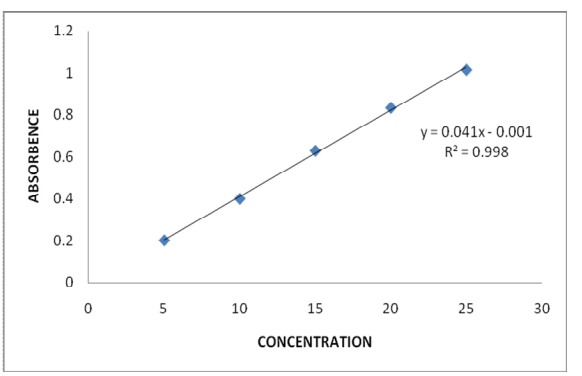

Figure 8: Linearity plot for DIC

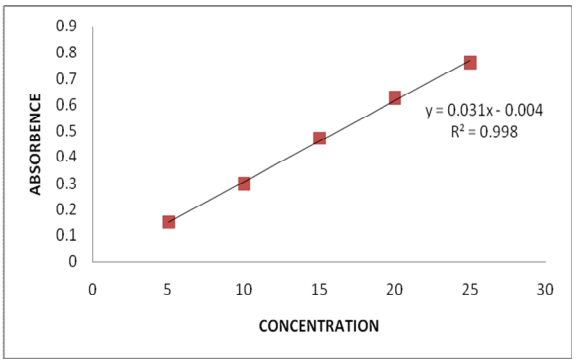

Figure 9: Linearity plot for DIC at isoabsorbtivity point

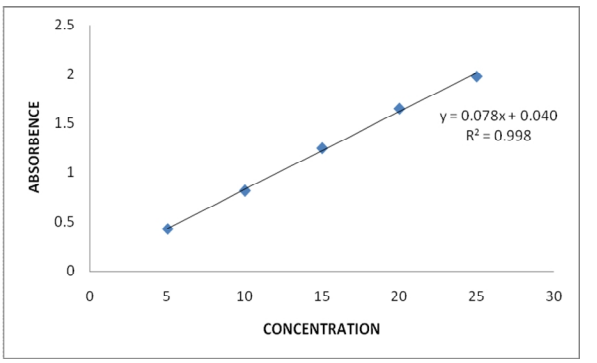

Figure 10: Linearity plot for DIC AUC method

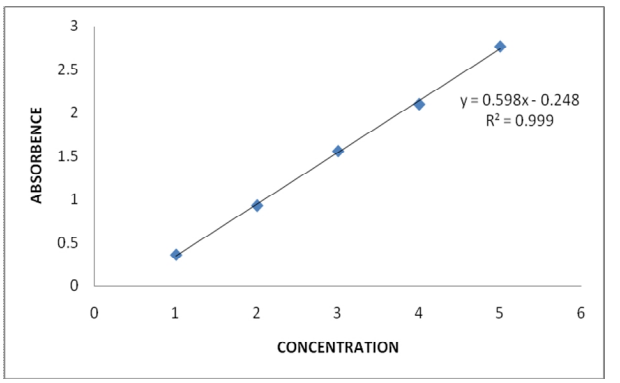

Figure 11: Linearity plot of OME for AUC method

DISCUSSION

The new UV spectrophotometric method developed and validated for simultaneous determination of OME and DIC in combined pharmaceutical dosage form was satisfactory with good precision and accuracy. The method was found to be simple, accurate, economical and rapid and can be applied for routine quality control of OME and DIC in bulk and their combined formulations.

ACKNOWLEDGEMENT

The authors are thankful to Darwin labs pvt Ltd, A.P, India for providing gift sample of OME and DIC, Management of Siddhartha Academy General and Technical Education and Principal of our college for providing required facilities to carry out this research work.

REFERENCES

1. Indian Pharmacopoeia. 6th ed. New Delhi: The Controller of Publication, Government of India; Vol. II; 2010. p. 1710.
2. United States Pharmacopoeia and National Formulary. 34th ed. Rockville (MD): The United States Pharmacopoeia Convention Inc; Vol. II; 2011. p. 2546- 2547.
3. Japanese Pharmacopoeia. 15th ed. Tokyo: Japanese Society of Pharmacopoeia; 2007. p. 618.
4. Khaskheli AR, Abro K., Sherazi ST, Afridi HI, Mahesar SA, Saeed M. Simpler and faster spectrophotometric determination of Diclofenac Sodium in Tablets, Serum and Urine samples, Pak J Anal Environ Chem 2009; 10: 53-58.
5. Ahemad NR. High Performance Liquid Chromatographic Method for the determination of Diclofenac sodium in pharmaceutical preparations and in environmental samples. Iraqi Nat J Chem 2011; 44: 467-473.
6. Atto RA. New method for determination of Diclofenac sodium by High Performance Liquid Chromatography. Tikrit J Pharma Sci 2012; 8(1): 60-67.
7. Shafiee A, Amini M, Hajmahmodi M. Improved chromatographic method for determination of Diclofenac sodium in injectable solution and prediction of chemical stability, Journal of Sciences 2003; 14(1): 21-25.
8. Rawat S, Gupta A. Spectrophotometric method for simultaneous estimation of Nimesulide and Diclofenac Sodium in Pharmaceutical Dosage Forms, Asian J Pharm Ana 2011; 1(4): 85-87.
9. Sharma MC, Sharma S. Determination and Validation of UV Spectrophotometric method for Estimation of Paracetamol and Diclofenac Sodium in Tablet dosage forms using hydrotropic solubilizing agents, Int J PharmTech Res 2011; 3(1): 244-247.
10. Joshi RR, Gupta KR. Simultaneous UV-Spectrophotometric determination of Thiocolchicoside and Diclofenac in pharmaceutical formulation, Der Pharmacia Sinica 2010; 1(2): 44-51.
11. Lohe RW, Suruse PB, Kale MK, Barethiya PR, Kasture AV, Lohe SW. Spectrophotometric methods for simultaneous estimation of Rabeprazole and Diclofenac from combined Tablet dosage form, Asian J Res Chem 2008; 1(1): 26-28.
12. Patel RK, Patel HR, Patel VA, Ganure AL, Patel LJ. Development and validation of RP-HPLC method for simultaneous determination of Omeprazole and Diclofenac Sodium in capsule dosage form, J Pharma Res 2012; 5(3): 1640-1642.
13. Dhaneshwar SR, Bhusari VK. Validated HPLC Method for Simultaneous quantitation of Diclofenac Sodium and Misoprostol in bulk drug and formulation, Der Chemica Sinica 2010; 1(2): 110-118.
14. Nayak D, Vankar K, Patnaik A. Simultaneous Estimation of Rabeprazole Sodium and Diclofenac Sodium by RP-HPLC method in combined tablet dosage form, Int J PharmTech Res 2010; 2(2): 1488-1492.
15. Jhanwar B, Banerjee J, Kumar A, Nagori BP. Development and Validation of UV spectrophotometric method for estimation of Diclofenac Sodium and Eperisone Hydrochloride as API and in formulated sustained release granules, Indo American J Pharma Res 2013; 3(3): 2672-2685.
16. Woolf EJ, Matuszewski BK. Simultaneous determination of Omeprazole and 5'- hydroxyomeprazole in human plasma by liquid chromatography–tandem mass spectrometry, Journal of Chromatography A 1998; 828(1-2): 229-238.
17. Dedania Z, Dedania R, Karkhanis V, Vidya Sagar G, Baldania M, Sheth NR. RPHPLC Method for simultaneous estimation of Omeprazole and Ondansetron in combined dosage form. Asian J. Research Chem 2009; 2(2): 108-111.
18. Topagi KS, Jeswani RS, Sinha PK, Damle MC. A Validated Normal phase HPLC method for simultaneous determination of Drotaverine Hydrochloride and Omeprazole in Pharmaceutical formulation. Asian J. of Pharmaceutical and Clinical Research 2010; 3(1): 20-24.

19. Shim SH, Bok SJ, Kwon KI. Determination of Omeprazole in rat plasma by HPLC with column switching. Arch Pharm Res 1994; 7(6): 458-61.
20. Flor S, Tripodi V, Scioscia S, Revello L, Lucangioli S. Fast and sensitive new HPLC-UV method for determination of omeprazole and major related substances in pharmaceutical formulation. Journal of Liquid Chromatography and Related Technologies 2010; 33(18): 1666 – 78.
21. Rajesh KP, Hiren RP, Vinas AP, Ashok LG, Laxmanbhai JP. Development and validation of RP-HPLC method for simultaneous determination of omeprazole and diclofenac sodium in capsule dosage form, Journal of Pharmacy Research 2012; 5(3): 1640-1642.
22. Patel B, Patel M, Patel J, Suhagia B. Simultaneous Determination of Omeprazole and Domperidone in Capsules by RP-HPLC and Densitometric HPTLC, Int. J. Pharma. Bio. Res 2010; 1(1): 1-5.
23. Ray S, Kumar P. HPTLC and TLC method for rapid quantification and identification of Omeprazole, Indian Drugs 1994; 31(11): 543-547.
24. The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human use, Validation of Analytical Procedure: Text and Methodology, ICH Q2 (R1); 2005.

Source of support: Nil, Conflict of interest: None Declared

QUICK RESPONSE CODE	ISSN (Online) : 2277 –4572
	Website http://www.jpsionline.com

How to cite this article:
K. Siva Chaitanya and Aziz Unnisa. Novel UV-spectrophotometric methods for simultaneous estimation of omeprazole and diclofenac in bulk and pharmaceutical formulations. J Pharm Sci Innov. 2014;3(5):428-433 http://dx.doi.org/10.7897/2277-4572.035189