IJPSR (2018), Volume 9, Issue 9 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 08 December, 2017; received in revised form, 16 February, 2018; accepted, 19 February, 2018; published 01 September, 2018

DEVELOPMENT AND VALIDATION OF UV VISIBLE SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF ACECLOFENAC AND TRAMADOL IN BULK AND DOSAGE FORM

Suresh Mali *, Sufiyan Ahmad and V. M. Shastry

Department of Quality Assurance, Gangamai College of Pharmacy, Nagaon, Dhule - 424005, Maharashtra, India.

Keywords:

Acecloenac, Tramadol, Method development, Validation, UV spectrophotometric, Dosage forms

Correspondence to Author:
Mr. Suresh Mali

H. No. 29, Rajukmar Nagar, Near Songir Police Station, Songir, Dhule - 424309, Maharashtra, India.

E-mail: sufimpharm@rediffmail.com

ABSTRACT: Objective: Simple, rapid, sensitive, precise and reproducible specific UV spectrophotometric method for the determination of Aceclofenac (ACE) and Tramadol (TRM) in bulk drug and pharmaceutical dosage form were developed and validated. **Methods:** A simple double beam UV spectrophotometric method has been developed and validated with different parameters such as linearity, precision, repeatability, limit of detection (LOD), Limit of Quantification (LOQ), accuracy as per ICH guidelines. **Results:** UV-visible spectrophotometric method, measurement of absorption at maximum wavelength in 10 ml methanol and volume make with water solvent system as reference ACE and TRM were found to be at 203 nm and 241 nm respectively. The drug obeyed the Beer's law and showed good correlation. Beer's law was obeyed in concentration range 5 - 25 µg/ml for ACE and 2 - 10 µg/ml for TRM respectively with correlation coefficient was 0.999. The LOD and LOQ of ACE were found to be 4.7862 µg/ml and 14.50 µg/ml, TRM were found to be 2.0518 µg/ml and 6.2176 µg/ml, respectively. Percentage assay of ACE and TRM in tablets. **Conclusion:** The proposed method is simple, precise, accurate and reproducible can be used for routine analysis of ACE and TRM in bulk and tablet dosage form.

INTRODUCTION: Aceclofenac (ACE) is chemically [(2, 6-dichlorophenyl) amino] phenyl-acetoxyacetic acid **Fig. 1** is used as an effective non-steroidal anti-inflammatory drugs (NSAIDs) derived from the phenylacetic acid with pronounced anti-inflammatory, analgesic and antipyretic properties [1, 2]. Tramadol hydrochloride (TRM) is chemically (lRS, 2RS)- 2- [(dimethyl amino) methyl]- I- (3-methoxyphenyl) cyclo-hexanol hydrochloride [3] **Fig. 2**.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.9(9).3852-57

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.9(9).3852-57

Tramadol HCl is a synthetic, centrally acting analgesic with no anti-inflammatory activity and one of the most interesting and useful weak opioids for treatment of moderate to moderately severe pain with weak µ-receptor agonist properties and noradrenergic and serotonergic neurotransmission effects [4, 5, 6, 7, 8, 9, 10, 11].

The review of literature revealed that many analytical methods involving UV Spectro-photometric [12, 13] RP-HPLC, [14, 15, 16] HPTLC [17] and UPLC [18] have been reported for TRM individually and in combination with other drugs. UV spectrophotometric methods have been reported for determination of ACE in single or in combination with other drugs [19, 20]. Spectrophotometric methods for simultaneous estimation of ACE with other drugs also reported [21, 22].

There is no evidence of determination of the drug combination by UV spectrophotometry. Thus the present study is to develop simple, precise and accurate UV Spectrophotometric methods for the quantification of ACE and TRM in combined dosage form.

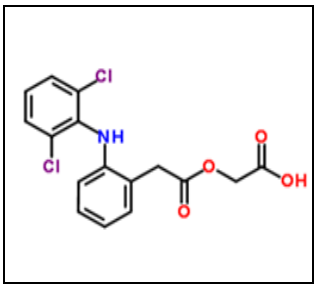

FIG. 1: STRUCTURE OF ACELOFENAC

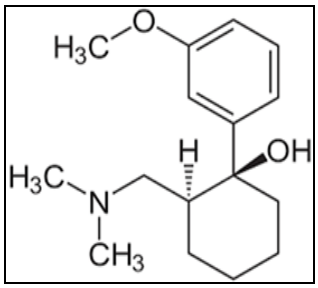

FIG. 2: STRUCTURE OF TRAMADOL

MATERIALS AND METHODS:

Materials and Reagents: A Shimadzu UV/Visible double beam spectrophotometer (Model 1700) with 1 cm matched quartz cells was used in present study for multi component analysis. Aceclofenac and Tramadol in the form of gift samples were kindly supplied by R. S. I. T. C, Jalgaon respectively. HPLC grade methanol used for UV method (Merck Specialities Pvt. Ltd., Shiv Sager Estate 'A' Worli, Mumbai). Methanol: Acidic water (0.05% OPA), prepared in solvent double distilled water was used as solvent throughout the study. A combination of aceclofenac (100 mg) and tramadol (37.5 mg) in tablet formulation was procured from Dewcare Concept brand name Taxidol.

Preparation of Standard Stock Solution:

Aceclofenac Standard Stock Solution: (Stock I): An accurately weighed quantity, 100 mg of Aceclofenac was dissolved in Methanol in a 10 ml volumetric flask and volume made up to 10.0 ml to produce a solution of 10,000 µg/ml **Fig. 3.**

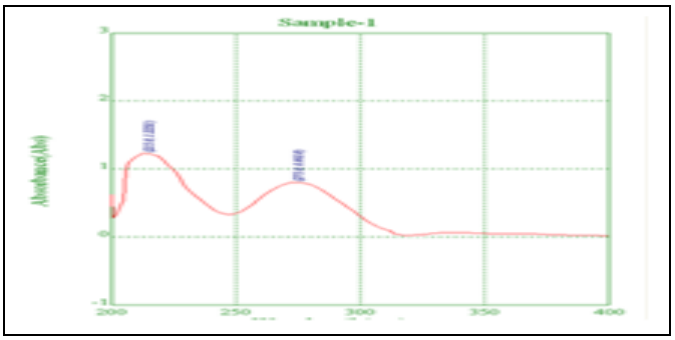

FIG. 3: UV SPECTRUM OF ACECLOFENAC

Tramadol Standard Stock Solution: (Stock II): An accurately weighed quantity, 40 mg of Tramadol was dissolved in Methanol in 10 ml volumetric flask and volume made up to 10.0 ml to produce a solution of 4000 µg/ml **Fig. 4.**

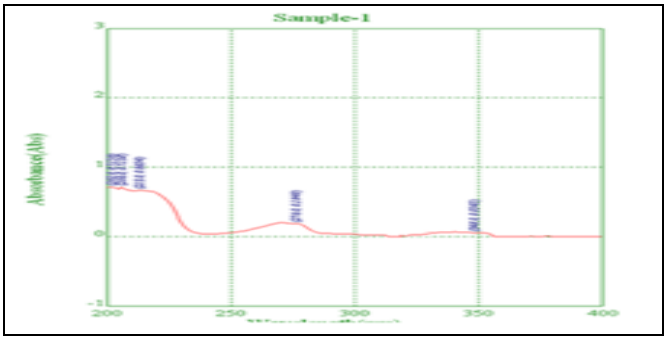

FIG. 4: UV SPECTRUM OF TRAMADOL

Preparation of Stock Standard Combination Solution: (Stock III) [ACE + TRM]: Accurately weight and transfer 100 mg Aceclofenac and Tramadol 40 mg working standard into 10 ml volumetric flask as about diluents methanol completely and make volume up to the mark with the same solvent to get 1000 µg/ml standard (stock solution) and 15 min sonicate to dissolve it and remove the unwanted gas, further an aliquots portion of Aceclofenac and Tramadol stock solution in ratio of 70:30 were mixed in volumetric flask in 10 ml and volume was adjusted up to mark with mobile phase from the resulting solution 0.1 ml was transferred to 10 ml volumetric flask and the volume was made up to the mark with Methanol: Acidic water, prepared in (7 ml Methanol: 3 ml Acidic water) solvent. Result as shown **Fig. 5.**

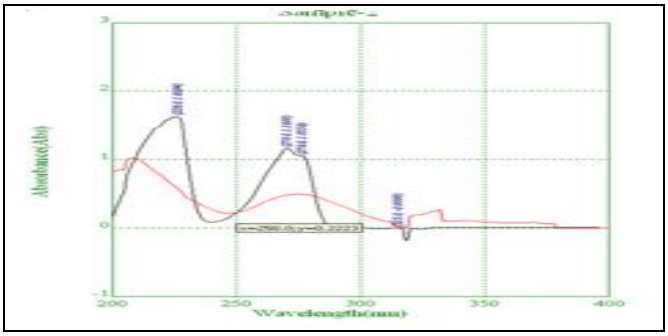

FIG. 5: ISO-ABSORPTIVE POINT OF ACECLOFENAC AND TRAMADOL

Procedure for Calibration Curve of Aceclofenac and Tramadol: The mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained. From the freshly prepared

standard stock solution, pipette out 10 mg Aceclofenac and 40 mg Tramadol in 10 ml of volumetric flask and diluted with mobile phase. From it 0.05, 0.1, 0.15, 0.2 and 0.25 ml of solution were pipette out in 10 ml volumetric flask and volume was made up to 10 ml with mobile phase to get final concentration 50, 100, 150, 200, 250 µg/ml of Aceclofenac and 20, 40, 60, 80, 100 µg/ml of Tramadol **Table 1** and **2**.

TABLE 1: LINEARITY DATA FOR ACECLOFENAC

Method	Conc. µg/ml	Peak area (µV.sec)		Average peak area (µV.sec)	S. D. of Peak Area	% RSD of Peak Area
		1	2			
UV Method	5	0.2498	0.2511	0.25	0.00	0.37
	10	0.4623	0.4716	0.47	0.01	1.41
	15	0.6516	0.6645	0.66	0.01	1.39
	20	0.8769	0.8851	0.99	0.01	0.59
	25	1.0836	1.0811	1.08	0.00	0.16
	Equation			y = 0.041x + 0.043		
	R^2			0.998		

TABLE 2: LINEARITY DATA FOR TRAMADOL

Method	Conc. µg/ml	Peak area (µV.sec)		Average peak area (µV.sec)	S. D. of Peak Area	% RSD of Peak Area
		1	2			
UV Method	2	0.1978	0.1956	0.20	0.00	0.79
	4	0.3578	0.3611	0.35	0.00	0.67
	6	0.5511	0.5624	0.56	0.01	1.44
	8	0.7111	0.7192	0.72	0.01	0.80
	10	0.9034	0.9123	0.91	0.01	0.69
	Equation			y = 0.089x + 0.007		
	R^2			0.998		

The respective linear equation for Aceclofenac was Y = 0.041+ 0.043 and Tramadol equation Y = 0.089X + 0.007 where x is the concentration and y is area of peak. The correlation coefficient was 0.998. The calibration curve of Aceclofenac and Tramadol is depicted in **Fig. 6** and **7.**

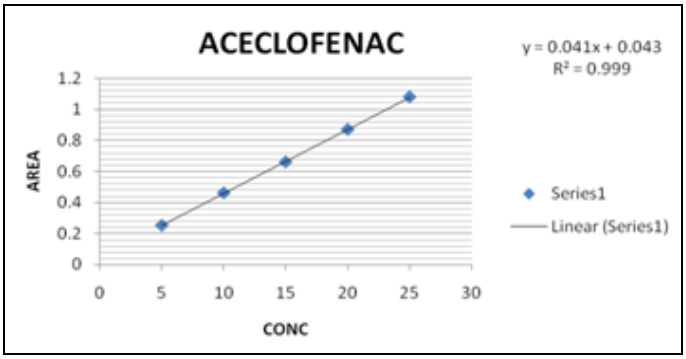

FIG. 6: CALIBRATION CURVE OF ACECLOFENAC

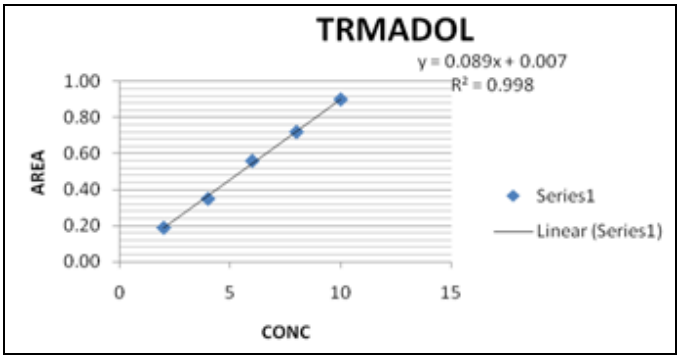

FIG. 7: CALIBRATION CURVE OF TRAMADOL

Selection of Detection Wavelength: Standard solutions were scanned in the range of 200 - 400 nm, against 10 ml Methanol and volume make with Methanol solvent system as reference Aceclofenac **Fig. 3** and Tramadol **Fig. 4** were showed absorbance maxima (λ_{max}) at 203 nm and 241 nm respectively **Fig. 5**.

If two Aceclofenac and Tramadol sample Interact with this point is called isobestic point Then detection of wavelength in isobestic point in 236 nm.

Procedure for Analysis of Tablet Formulation: Weigh 20 Aceclofenac and Tramadol combination tablets and calculated the average weight, accurately weigh and transfer the sample equivalent to 100 mg and 40 mg Aceclofenac and Tramadol into 10 ml volumetric flask. Add 10 ml Methanol of diluents and sonicate to dissolve it completely and make volume up to the mark with diluents. Mix well and filter through 0.45 µm filter. Further pipette 0.2 ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with

diluents 40 µg/ml. The simple chromatogram of test Aceclofenac and Tramadol Shown in **Fig. 8.**

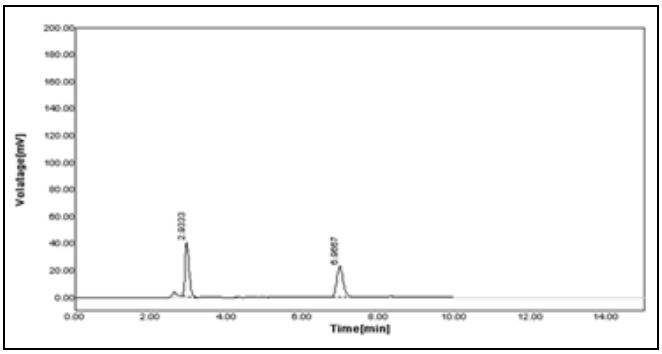

FIG. 8: CHROMATOGRAM FOR MARKETED FORMULATION

The amounts of Aceclofenac and Tramadol per tablet were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated five times with tablet formulation. Analysis of marketed formulation were also % lable claim was found to be 97 - 102% satisfactory are concluded **Table 3**.

TABLE 3: ANALYSIS OF MARKETED FORMULATION

Assay	Drug	label claimed	Amt. found	% label claim	S.D.	% RSD
UV Method	ACE	100	14.83	98.87	0.00	0.37
	TRM	37.5	6.13	99.88	0.01	0.19
	ACE	100	14.89	99.27	0.04	0.20
	TRM	37.5	6.11	102.92	0.01	0.06

Method Validation: The proposed methods were validated accordance to ICH Q2 (R1) guidelines for linearity, precision, accuracy, limit of detection, limit of quantification.

RESULTS:

Linearity and Range: From Aceclofenac and Tramadol standard stock solution, different working standard solutions (50 - 250 µg/ml) were prepared in the mobile phase. Likewise from Aceclofenac and Tramadol standard stock solution different working standard solutions (20 - 100 µg/ml) were prepared in the mobile phase. 20 µl of sample solution was injected into the column using fixed volume loop injector. Chromatograms were recorded. The area for each concentration were recorded **Table 4** and **5** shows linearity study. The calibration curve of Aceclofenac and Tramadol were shown in **Fig. 7** and **8.**

TABLE 4: LINEARITY OF ACECLOFENAC

Concentration µg/ml	Area Aceclofenac
50	144.969
100	291.402
150	471.014
200	617.934
250	767.878

TABLE 5: LINEARITY OF TRAMADOL

Concentration µg/ml	Area Tramadol
20	73.87
40	134.312
60	201.4
80	254.654
100	323.50

Accuracy: Recovery studies were performed to validate the accuracy of developed method. To pre analyzed tablet solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed **Table 6**.

TABLE 6: RESULT OF RECOVERY DATA FOR ACECLOFENAC AND TRAMADOL

Method	Drug	Level (%)	Amt. taken (µg/ml)	Amt. added (µg/ml)	Absorbance Mean* ± S.D.	Amt. recovered Mean*± S.D.	% recovery Mean*± S.D.
UV Method	ACE	80%	10	8	18.11 ± 0.08	8.06 ± 0.08	100.70 ± 0.99
		100%	10	10	20.04 ± 0.03	10.03 ± 0.03	100.31 ± 0.29
		120%	10	12	22.14 ± 0.03	12.14 ± 0.03	101.03 ± 0.22
	TRM	80%	4	3.2	7.23 ± 0.01	3.23 ± 0.01	100.91 ± 0.40
		100%	4	4	8.09 ± 0.01	4.09 ± 0.01	102.33 ± 0.24
		120%	4	4.8	8.81 ± 0.01	4.81 ± 0.01	100.23 ± 0.33

*mean of each 3 reading for UV method

Accuracy of UV spectroscopic method were ascertained by recovery studies performed at different levels of concentrations (80%, 100% and 120%). The % recovery was found to be within 98 - 102%. Statistical validation of recovery studies shown in **Table 7**.

Precision: Precision was studied to find out intra and inter-day variations in the test method of ACE and TRM. Intra-day precision was determined by analyzing three concentrations in three replicate measurements of within linearity range of drugs on three different times in the same day. Interday

precision was conducted during routine operation of the system over a period of 3 consecutive days. Intraday and interday Precision studies on UV method for ACE and TRM which shows the high precision % amount in between 98% to 102% indicates to analytical method that concluded **Table 8**.

TABLE 7: STATISTICAL VALIDATION OF RECOVERY STUDIES ACE AND TRM

Method	Level of recovery (%)	Drug	Mean % recovery	S. D.*	% RSD
UV Method	80%	ACE	101.40	0.99	0.98
		TRM	100.91	0.40	0.40
	100%	ACE	100.31	0.29	0.29
		TRM	102.33	0.24	0.23
	120%	ACE	101.34	0.22	0.22
		TRM	100.23	0.33	0.32

*Denotes average of three determinations for UV method

TABLE 8: INTRA AND INTER DAY PRECISION STUDIES ON UV METHOD FOR ACE AND TRM

Method	Drug	Conc. (µg/ml)	Intraday Precision		Interday Precision	
			Mean ± SD*	% Amt found	Mean ± SD*	% Amt found
UV Method	ACE	1	0.25 ± 0.00	100.97	0.24 ± 0.00	100.90
		1.5	0.66 ± 0.01	100.32	0.67 ± 0.00	100.30
		2	1.09 ± 0.00	102.12	1.09 ± 0.00	102.15
	TRM	2	0.19 ± 0.00	102.80	0.19 ± 0.00	102.78
		6	0.55 ± 0.00	100.84	0.52 ± 0.00	100.03
		10	0.92 ± 0.00	101.96	0.92 ± 0.00	101.63

*Mean of each 3 reading for UV method

System Suitability Parameters: Repeatability studies on UV method for Aceclofenac and Tramadol was found to be, the % RSD was less than 2%, which shows high percentage amount found in between 98% to 102% indicates the analytical method that concluded **Table 9**.

TABLE 9: REPEATABILITY STUDIES ON UV METHOD FOR ACE AND TRM

Method	Conc. of ACE and TRM (mg/ml)	Peak area	Amount found (mg)	% Amount found
UV method for ACE	10	0.4587	10.11	101.96
	10	0.4611	10.17	101.70
	10	0.4605	10.16	101.70
	10	0.4613	10.17	101.70
	10	0.4645	10.25	102.56
		Mean	10.17	101.92
		SD	0.05	0.37
		%RSD	0.49	0.37
UV method for TRM	4	0.3598	3.96	99.10
	4	0.3591	3.95	98.90
	4	0.3601	8.96	99.18
	4	0.3605	3.97	99.29
	4	0.3613	3.98	99.52
		Mean	3.96	99.20
		SD	0.01	0.23
		%RSD	0.29	0.23

Limit of Detection (LOD) and Limit of Quantification (LOQ): LOD is the lowest amount of analyte in a sample that can be detected but not necessarily quantify under the stated experimental conditions. LOQ is the lowest concentration of analyte in a sample that can be determined with the acceptable precision and accuracy under stated experimental conditions. The LOD and LOQ of aceclofenac were found to be 4.7862 µg/ml and 14.50 µg/ml, Tramadol were found to be 2.0518 µg/ml and 6.2176 µg/ml, respectively.

DISCUSSION: The proposed methods for simultaneous estimation of Aceclofenac and Tramadol in tablet dosage forms were found to be simple, accurate, economical and rapid. The method was validated as per the ICH Q2 (R1) guidelines. Standard calibration yielded correlation coefficient (r^2) 0.999 for both Aceclofenac and Tramadol at all the selected wavelengths. The values of % RSD are within the prescribed limit of 2%, showing high precision of methods and recovery was close to 100% for both drugs. Results of the analysis of pharmaceutical formulations reveal that the proposed method is suitable for their simultaneous determination with virtually no interference of any additive present in pharmaceutical formulations. Hence, the above methods can be applied successfully for simultaneous estimation of ACE and TRM in formulations.

CONCLUSION: The developed UV spectrophotometric method in that linearity, precision, range and robustness were found to be more accurate, precise and reproducible. The methods were found to be simple and time saving. All proposed methods could be applied for routine analysis in quality control laboratories.

ACKNOWLEDGEMENT: The authors are thankful to the Principal, Gangamai College of Pharmacy, Nagaon, Dhule for providing necessary facilities for research work. They are also grateful to Dewcare concept for giving gift samples of pure drugs.

CONFLICT OF INTEREST: Authors have no conflicts of interest to declare.

REFERENCES:

1. Government of India Ministry of Health and Family Welfare: Indian Pharmacopoeia. 5th edition Ghaziabad (India). The Indian Pharmacopoeia Commission 2007; 2: 63-64.
2. Moffat AC, Osselton MD, Clarke EGC, Widdop B and Galichet LY: Clarke's analysis of drugs and poisons, 3rd edition, Pharmaceutical Press 2004; 2: 570-571.
3. Indian pharmacopoeia. Delhi: Government of India. Ministry of health and family welfare, the controller and publication 2010; 3: 2245-7.
4. López - Muñoz FJ, Díaz-Reval MI, Terrón JA and Déciga-Campos M: Analysis of the analgesic interactions between ketorolac and tramadol during arthritic nociception in rat. European Journal Pharmacology 2004; 484(2-3): 157-65.
5. Lehmann KA: Tramadol in acute pain. Drugs 1997; 53(9): 25-33.
6. Klotz U: Tramadol - the impact of its pharmacokinetic and pharmacodynamic properties on the clinical management of pain. Arzneimittelforschung 2003; 53(10): 681-7.
7. Abanmy NO, Zaghloul Y and Radwan MA: Compatibility of tramadol hydrochloride injection with selected drugs and solutions. American journal of health system Pharm 2005; 62(12): 1299-302.
8. Barcia E, Martín A, Azuara ML, Sánchez Y and Negro S: Tramadol and hyoscine N-butyl bromide combined in infusion solutions: Compatibility and stability. Support Care Cancer 2007; 15(1): 57-62.
9. Scott LJ and Perry CM: Tramadol: A review of its use in peri-operative pain. Drugs 2000; 60(1): 139-76.
10. Lin TF, Lin FS, Chou WH, Yeh YC, Lin CP and Fan SZ: Compatibility and stability of binary mixtures of ketorolac tromethamine and tramadol hydrochloride injection concentrate and diluted infusion solution. Acta Anaesthesiol Taiwan 2010; 48(3): 117-21.
11. Cabrera J, Mancuso M, Cabrera-Fránquiz F, Limiñana J and Díez A: Stability and compatibility of the mixture of tramadol, ketorolac, metoclopramide and ranitidine in a solution for intravenous perfusion. Farm Hosp 2011; 35: 80-3.
12. Prajapati JM and Prajapati HR: Development and validation of derivative spectrophotometric method for the quantitative estimation of tramadol hydrochloride and aceclofenac in tablet dosage forms. Journal of Pharmacy research 2011; 4(9): 2950-3.
13. Puranik M, Hirudkar A, Wadher SJ and Yeole PG: Development and validation of spectrophotometric methods for simultaneous estimation of Tramadol hydrochloride and Chlorzoxazone in tablet dosage form. Indian Journal of Pharmaceutical Sciences 2006; 68(6): 737- 739.
14. El - sayed AY, Mohamed KM, Hilal MA, Mohamed SA, Aboul - Hagag KE and Nasser AY: Development and validation of high performance liquid chromatography - diode Array detector method for the determination of Tramadol in human Saliva. Journal of chromatography separate technique 2011; 2(4): 1-6.
15. Ahir KB, Patelia EM and Mehta FA: Simultaneous Estimation of Tramadol HCl, Paracetamol and Domperidone in Pharmaceutical formulation by RP-HPLC method. Journal of chromatography separate technique 2012; 3(8): 1-5.
16. Chandra P, Rathore A, Lohidasan S and Mahadik KR: Application of HPLC for the simultaneous determination of Aceclofenac, Paracetamol and Tramadol hydrochloride in Pharmaceutical dosage. Journal of Form Sci Pharm 2012; 80: 337-51.
17. Desai P, Captain A and Kamdar S: Development and validation of HPTLC method for estimation of Tramadol HCl in bulk and in capsule dosage form. International Journal of Pharm Tech Res. 2012; 4(3): 1261-5.
18. Kanakapura BV, Hosakere DR, Xavier CM, Pavagada JR and Madihalli SR: A stability indicating UPLC method for the determination of Tramadol hydrochloride: Application to Pharmaceutical Analysis. Journal of chromatography research international 2012; 1-9.
19. El-Saharty YS, Refaat M and El-Khateeb SZ: Stability-indicating spectrophotometric and densitometric methods for determination of aceclofenac. Drug development and industrial Pharmacy 2002; 28: 571-582.
20. Gandhi SV, Barhate NS, Patel BR, Panchal DD, Bothara KG: A validated densitometric method for analysis of aceclofenac and paracetamol as the bulk drugs and in combined tablet dosage forms. Acta Chromatographica 2008; 202: 175-182.
21. Singhvi I and Goyal A: Visible spectrophotometric determination of aceclofenac and indepamide from tablets using folin-ciocalteu reagent. International Journal of Pharmaceutical Science 2007; 69: 164-165.
22. Srinivasan K, Alex J, Shirwaikar A, Jacob S, Kumar S and Prabu S: Simultaneous derivative spectrophotometric estimation aceclofenac and tramadol with paracetamol in combination solid dosage forms. International Journal of Pharmaceutical Science 2007; 69: 540-545.

How to cite this article:
Mali S, Ahmad S and Shastry VM: Development and validation of UV visible spectrophotometric method for estimation of aceclofenac and tramadol in bulk and dosage form. Int J Pharm Sci & Res 2018; 9(9): 3852-57. doi: 10.13040/IJPSR.0975-8232.9(9).3852-57.