

## Simultaneous estimation of dicyclomine hydrochloride and diclofenac sodium in bulk and in tablets by UV-spectrophotometry

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### ABSTRACT

A simple UV-spectrophotometric method has been developed for the simultaneous estimation of dicyclomine hydrochloride and diclofenac sodium in bulk and its formulation. The Vierodt's method implemented for the formation and solving simultaneous equations. The absorbance maxima of dicyclomine and diclofenac used were 267nm and 280 nm, respectively. The Calibration curves were linear with correlation coefficient of 0.997 and 0.999 over the concentration range of 2-10 µg/mL and 5-25µg/mL for dicyclomine and diclofenac respectively. The mean percent recovery was found to be 100.46±1.09 and 100.47±1.45 for dicyclomine and diclofenac respectively. The method is validated according to ICH guidelines. From the results, one can state that, the method was is precise, accurate and sensitive. The present developed method was successfully applied for routine laboratory analysis of drugs.

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### KEYWORDS

Dicyclomine hydrochloride;  
Diclofenac sodium;  
Vierodt's method;  
Validation.

### INTRODUCTION

The drugs and combinations of the drugs are needed to be analyzed qualitatively and quantitatively. For analysis of the drugs different analytical methods are routinely used. The analytical methods are classified as classical and instrumental. The classical methods are further classified into gravimetric, titrimetric etc. As these methods are simple but less precise and more time consuming so nowadays these methods are not suggested for routine analysis. But more precise and simple methods are needed for the estimation of the drugs. Diclofenac sodium (DICLO) is chemically sodium{2-(2-(2,6-

dichlorophenylamino)-phenyl)}acetate Figure 1. It is official in IP,<sup>[1]</sup> BP<sup>[2]</sup> and USP<sup>[4]</sup> is a potent analgesic, non-steroidal anti-inflammatory drug (NSAID). It is used in inflammatory and painful diseases of rheumatic and non-rheumatic origin. The exact mechanism of action<sup>[5]</sup> is unknown but is thought to be inhibiting prostaglandin synthesis by inhibiting cyclo-oxygenase. Diclofenac so-

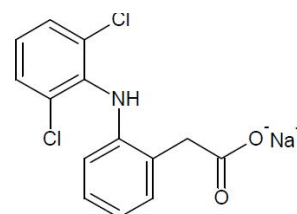


Figure 1 : Structure of diclofenac sodium

dium acts by cyclo-oxygenase inhibition, reduction of arachidonic acid release and enhancement of arachidonic acid uptake. It also exhibits bacteriostatic activity by inhibiting bacterial DNA synthesis<sup>[6]</sup>.

Diclofenac results in a dual inhibitory effect on both the cyclo-oxygenase and lipoxygenase pathways<sup>[7]</sup>. Diclofenac sodium is used for the relief of signs and symptoms of osteoarthritis, rheumatoid arthritis and ankylosing spondylitis. It is often used to treat chronic pain associated with cancer<sup>[8]</sup>.

Dicyclomine is chemically 2-(diethylamino)-ethyl-bi-(cyclohexane)-1-carboxylate. It is used as an antispasmodic and anticholinergic agent.<sup>[2]</sup> Figure 2, Dicyclomine relieves smooth muscle spasm of the gastrointestinal tract<sup>[8]</sup>. Dicyclomine action is achieved via a dual mechanism: a specific anti-cholinergic effect (antimuscarinic) at the acetylcholine-receptor sites and a direct effect upon smooth muscle (musculotropic).

It is used as an anti spasmotic and in urinary incontinence. This dual mode of action provides a specific anti-cholinergic effect at acetylcholine receptor and a direct effect upon smooth muscle, but with a rarely causes any side effect<sup>[9]</sup>.

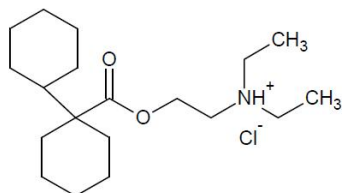


Figure 2 : Structure of dicyclomine hydrochloride

Dicyclomine is an anticholinergic drug. It exerts its action by inhibiting muscarinic receptors on smooth muscles and prevents the effect of acetylcholine. Inhibition of acetylcholine produces relaxation of smooth muscles of gastrointestinal tract and genitourinary tract and reduces the painful spasm and cramp. It inhibits gastrointestinal propulsive motility and reduces gastric acid secretion. It also has a direct relaxant effect on smooth muscle. It readily crosses the blood brain barrier and produces CNS effects<sup>[10]</sup>. Detailed literature survey for diclofenac sodium, dicyclomine hydrochloride revealed that few methods of estimation are available based on different techniques<sup>[11-20]</sup>.

## MATERIALS AND METHODS

Methanol, diclofenac sodium and dicyclomine HCl tablets were obtained from local market. Pure samples of diclofenac sodium and dicyclomine hydrochloride were obtained from manufacturer as gift samples. All the chemicals used throughout the experiment are of analytical grade and used as such supplied by the manufacturer.

### Preparation of stock standard solutions of DCY and DICLO

Accurately weighed 10 mg of DCY and 10 mg of DICLO were transferred into separate 100 mL volumetric flasks. Each drug was dissolved in 50 mL methanol and shaken for 10 min. Then, the volume was made up to the mark with distilled water to give final strength of 100  $\mu\text{g/mL}$  of DCY and 100  $\mu\text{g/mL}$  of DICLO.

### Selection of analytical wavelengths

Appropriate volume, 1.0 mL of DCY and 1.0 mL of DICLO standard solution was transferred to separate 10 mL volumetric flasks and the volume was adjusted up to the mark with distilled water to get concentration 10  $\mu\text{g/mL}$  of DCY and 10  $\mu\text{g/mL}$  of DICLO. Both the drug solutions were scanned separately between 200-400 nm Figure 3, 4. The overlain spectra of both drugs were recorded. From overlain spectra, wavelengths 267 nm ( $\lambda_{\text{max}}$  of DCY) and 280 nm ( $\lambda_{\text{max}}$  of DICLO) were selected for analysis of both drugs using simultaneous equation method (Cramers rule) Figure 5.

### Linearity study of DCY and DICLO

Aliquot portions of DCY were transferred into 10 mL volumetric flasks. The volume was adjusted up to the mark with distilled water to obtain concentrations 2, 4, 6, 8 and 10  $\mu\text{g/mL}$  of DCY. Absorbance of these solutions were measured at 267 nm; TABLE 1. Calibration curve was constructed by plotting absorbance *versus* concentration as shown in Figure 6.

Aliquot portions of DICLO were transferred into 10 mL volumetric flasks. The volume was adjusted to the mark with distilled water to obtain concentrations 5, 10, 15, 20 and 25  $\mu\text{g/mL}$  of DICLO. Absorbance of these solutions was measured at wavelength

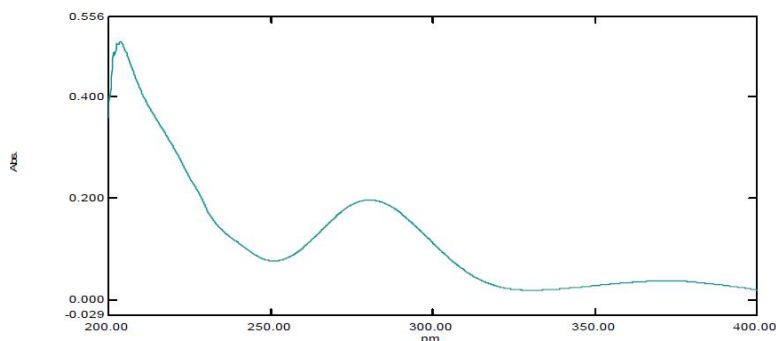


Figure 3 : UV spectra of diclofenac sodium

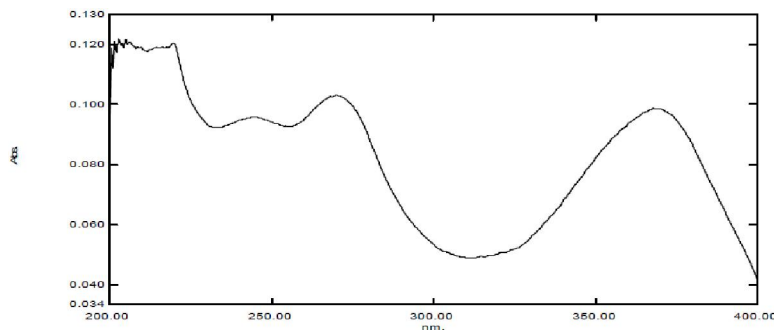


Figure 4 : UV spectra of dicyclomine hydrochloride

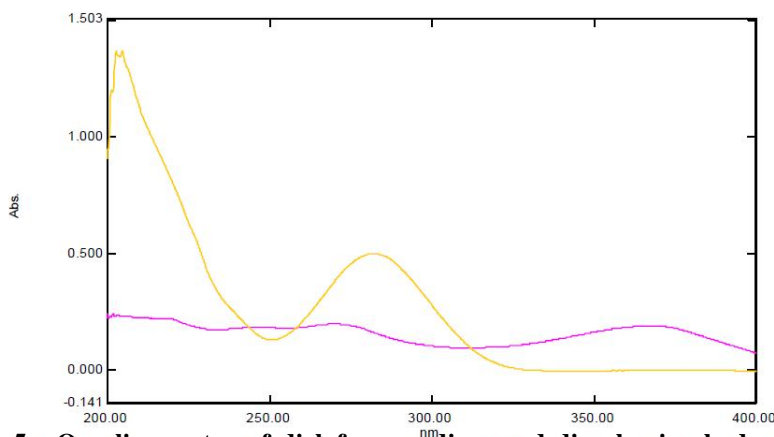


Figure 5 : Overlaid spectra of diclofenac sodium and dicyclomine hydrochloride

TABLE 1 : Linearity study for DCY at 267 nm

Conc. of DCY ( $\mu\text{g/mL}$ )	Absorbance $\pm$ SD (n = 5)	% RSD
2	0.081 $\pm$ 0.0013	0.80
4	0.143 $\pm$ 0.0043	1.26
6	0.196 $\pm$ 0.0035	0.70
8	0.270 $\pm$ 0.0063	0.94
10	0.337 $\pm$ 0.0063	0.74

280 nm TABLE 2. Calibration curve was constructed by plotting absorbance *versus* concentration as shown in Figure 7.

**Estimation of Dicyclomine and diclofenac in sample**

### Methodology

Simultaneous equation method uses two selected wavelengths, one is  $\lambda_{\text{max}}$  of dicyclomine and other is  $\lambda_{\text{max}}$  of diclofenac. The stock solutions of both the drugs were further diluted separately with methanol

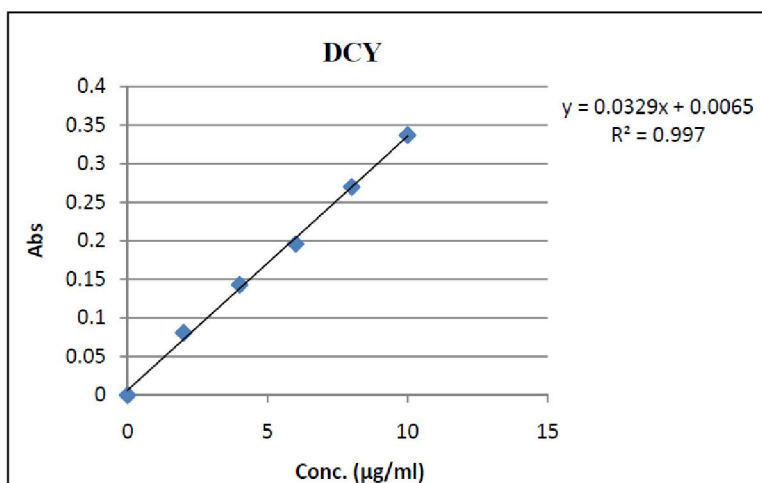


Figure 6 : Calibration curve of DCY

TABLE 2 : Linearity study for DICLO at 280 nm

Conc. of DICLO (µg/mL)	Absorbance ± SD(n = 5)	% RSD
5	0.207 ± 0.0052	1.24
10	0.374 ± 0.0063	1.15
15	0.606 ± 0.0071	1.38
20	0.818 ± 0.0066	0.97
25	1.033 ± 0.0087	1.42

to get a series of standard solutions of 2-10 µg/ml for dicyclomine hydrochloride and 5-25 µg/ml for diclofenac sodium for both drugs calculated at both wavelengths. Concentrations in the sample were obtained by using these equations.

$$C_X = \frac{A_2 a_{Y1} - A_1 a_{Y2}}{a_{X2} a_{Y1} - a_{X1} a_{Y2}} \quad (1)$$

$$C_Y = \frac{A_1 a_{X2} - A_2 a_{X1}}{a_{X2} a_{Y1} - a_{X1} a_{Y2}} \quad (2)$$

Where, A1 and A2 are absorbance of mixture at 267 nm and 280 nm respectively.

$a_{X1}$  and  $a_{X2}$  are absorptivity of diclofenac at  $\lambda_1$  and  $\lambda_2$  respectively.

$a_{Y1}$  and  $a_{Y2}$  are absorptivity of dicyclomine at  $\lambda_1$  and  $\lambda_2$  respectively.

$C_X$  and  $C_Y$  are concentrations of diclofenac and dicyclomine respectively.

#### Application of proposed method for physical laboratory mixture

Physical laboratory mixture of DCY and DICLO was prepared by dissolving 20 mg of DCY and 50 mg of DICLO in 100 mL of volumetric flask with methanol: distilled water (50:50). Appropriate volume 1.0 mL was transferred to 10 mL of volumetric flask and diluted up to mark with the same solvent to obtain concentration 20 mg/mL and 50mg/mL of DCY and DICLO, respectively. The solutions were

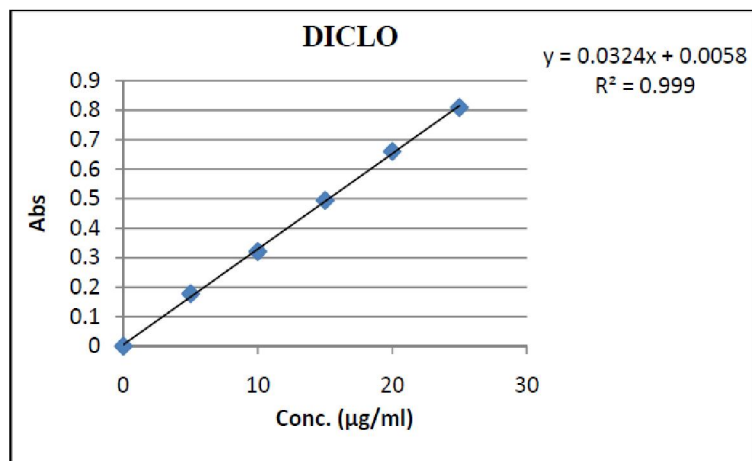


Figure 7 : Calibration curve of DICLO

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TABLE 3 : Application of proposed method for physical laboratory mixture

Drugs	Amount Taken ( $\mu\text{g/mL}$ )	Amount Found (n=6)	% Amount Found (n=6)	% RSD
DCY	20	19.98	99.9	1.31
DICLO	50	50.21	100.42	1.24

TABLE 4 : Results of analysis of marketed formulation

Tablet sample	Label claim (mg/tab)	Amount found (n=6)	% Amount found (n=6)	% RSD
DCY	20	20	98.9	1.43
DICLO	50	50	100.37	1.38

scanned in the range 200–400 nm, absorbance of the sample solutions were recorded at 267 nm and 280 nm i.e.  $A_1$  and  $A_2$ , respectively. The concentrations of the two drugs in sample solution ( $C_{\text{DCY}}$  and  $C_{\text{DICLO}}$ ) were determined, by using equation (1) and (2). Results are as shown in TABLE 3.

### Analysis of marketed formulation

Twenty tablets (Dipospas<sup>®</sup>) were weighed; average weight determined and ground into fine powder. A quantity of powder sample equivalent to 20 mg of DCY and 50 mg of DICLO was transferred into 100 mL volumetric flask containing 50 mL methanol and after that volume adjusted up to the mark with methanol. The resulting solution was further diluted to get concentration 20 mg/mL of DCY and 50  $\mu\text{g/mL}$  of DICLO. Prepared solution was scanned in the range of 200–400 nm and absorbance of sample solutions at selected wavelengths were recorded against blank. The concentrations of the two drugs in sample solutions ( $C_{\text{DCY}}$  and  $C_{\text{DICLO}}$ ) were determined, using equation (1) and (2). The results of the same are shown in the TABLE 4.

## RESULTS AND DISCUSSION

The present method was validated in terms of

linearity, accuracy, precision and ruggedness.

### a) Accuracy

Accuracy of the method was assessed by % recovery experiments performed at three different levels i.e. 80, 100 and 120 %. To the pre-analyzed sample solution a known amount of drug standard of DCY and DICLO were added. The solutions were re-analyzed by proposed method. Results of recovery studies are shown in TABLE 5.

### b) Precision study:

Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions.

### Intra-day and inter-day precision

Intra-day and inter-day variations were determined by analyzing three different solutions of DCY and DICLO within the same day and three different days over a period of week. Intra-day precision was estimated by analyzing 6 mg/mL, 8 mg/mL and 10 mg/mL of DCY and 15 mg/mL, 20 mg/mL and 25 mg/mL of DICLO for three times within the same day. Inter-day precision was estimated by analyzing above mentioned concentrations of both the drugs for three different days over a period of week. The

TABLE 5 : Results of recovery studies

Level of recovery	Initial amount ( $\mu\text{g/mL}$ )		Concentration of excess drug added ( $\mu\text{g/mL}$ )		% Recovery (n=3)		% RSD	
	DCY	DICLO	DCY	DICLO	DCY	DICLO	DCY	DICLO
80	6	15	4.8	12	100.47	100.59	0.71	1.27
100	6	15	6	15	100.66	100.23	1.55	1.87
120	6	15	7.2	18	100.25	100.6	1.01	1.21

TABLE 6 : Results of intra-day and inter-day precision study

Drug	Amount taken ( $\mu\text{g/mL}$ )	Intra-day (n = 3)		Inter-day (n = 3)	
		Amount found ( $\mu\text{g/mL}$ )	% RSD	Amount found ( $\mu\text{g/mL}$ )	% RSD
DCY	6	6.13	1.07	6.34	1.40
	8	8.16	1.45	8.11	1.35
	10	10.14	0.97	10.21	1.27
DICLO	15	15.67	1.19	15.62	0.89
	20	20.48	1.41	20.15	1.55
	25	25.26	1.31	25.61	0.83

TABLE 7 : Results of ruggedness study

Drug	Analyst I (n = 6)		Analyst II (n = 6)	
	% amount found	% RSD	% amount found	% RSD
DCY	100.69	1.37	101.24	1.67
DICLO	98.86	1.05	99.27	1.51

TABLE 8 : Limit of detection and limit of quantification

Parameter	Measured Value ( $\mu\text{g mL}^{-1}$ )	
	DCY	DICLO
Limit of Detection	0.037	0.141
Limit of Quantification	0.421	0.345

results are shown in TABLE 6.

### c) Ruggedness

Ruggedness of the method was proved by analyzing the standard solutions 6  $\mu\text{g/mL}$  of DCY and 15  $\mu\text{g/mL}$  of DICLO by two different analysts using the same experimental and environmental conditions. The results are shown in TABLE 7.

### d) Limit of Detection and Limit of Quantification:

The LOD and LOQ for DCY and DICLO were studied by a series of dilute solutions with known concentrations. Limit of Detection (LOD) = 3.3 (SD/S), where SD is the residual standard deviation and S is the slope of the line. The calculation method is again based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula LOQ = 10(SD/S). The LOD and LOQ for DCY and DICLO were found suitable as represented in TABLE 9.

## CONCLUSIONS

The proposed method is simple for simultaneous

estimation of dicyclomine hydrochloride and diclofenac sodium in bulk and tablet formulation. The analysis of the mixture was done without any interference of excipients and additives. The simultaneous determination of the drugs in pure and tablet forms were done without any preliminary separation step, so the present method is more economical and less time consuming compared to other chromatographic methods.

## REFERENCES

- [1] Indian Pharmacopoeia, The Controller of Publications, Ministry of Health and Family Welfare, Govt. of India, New Delhi, 6<sup>th</sup> Edition, **2**, 1710 (2010).
- [2] Indian Pharmacopoeia, The Controller of Publications, Ministry of Health and Family Welfare, Govt. of India, New Delhi, 5<sup>th</sup> Edition, **2(404)**, 726-27 (2007).
- [3] British Pharmacopoeia, Medicinal and Healthcare Products Regulatory Agency, London, **2**, 2606 (2009).
- [4] United States Pharmacopoeia Convention, Food and Drug Administration. United States Pharmacopoeia National Formulary, Rockville, 34<sup>th</sup> Edition, **2**, 2546



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- (2011).
- [5] A.Merry, I.Power, Perioperative NSAIDs: Towards greater safety, *Pain Rev.*, **2**, 268–91 (1995).
- [6] W.S.Dietrich; C.K.Edmond; Pharmacology of diclofenac sodium, *The American Journal of Medicine*, **80**, **4**(2), 34–38 (1986).
- [7] L.B.Laurence, J.S.Loza, Analgesics; Antipyretic and anti-inflammatory agents, *Goodman & Gilman's The Pharmacological basis of Therapeutics*, 11<sup>th</sup> Edition, Medical Publishing Division, New York, 671-675 (1986).
- [8] ICH, Q2A, harmonized tripartite guideline, text on validation of analytical procedures, IFPMA, in proceeding of international conference on harmonization, Geneva, March (1994).
- [9] Drug bank, Drug profile, Dicyclomine HCl, <http://drugbank.ca/drugs/DB00863> (APRD00113), As accessed on: February 14, (2012).
- [10] Ashutosh kar; *Pharmaceutical Drug Analysis*, Minerva press, **369-390**, 565-591 (2001).
- [11] D.Prajapati, H.Raj; Simultaneous estimation of mefenamic acid and dicyclomine hydrochloride by RP-HPLC method, *Int.J.Biol.Sci.*, **3**(3), 611-625 (2012).
- [12] M.Bachani, D.Acharya, K.Shah; Development and validation of HPLC method for simultaneous estimation of dicyclomine hydrochloride, acetaminophen and clidinium bromide in solid dosage form, *Int.J.Pharm and Pharm Sci.*, **5**(2), 462-466 (2013).
- [13] R.Hedpara, V.Vibhuti Chhatarala, A.Agola; A validated RP- HPLC method for the simultaneous estimation of paracetamol and dicyclomine hydrochloride in pharmaceutical formulations. *Int J.Pharm. Sci.Res.*, **4**(6), 2400-2405 (2013).
- [14] S.R.Lokhande, S.M.Mhetre, S.S.Pekamwar, T.M. Kalyankar; Development and validation of reverse phase HPLC method for simultaneous estimation of dicyclomine hydrochloride, Mefenamic acid and paracetamol in tablet dosage form, *World J.Pharm. Sci.* **1**(3), 968-980 (2012).
- [15] A.Birajdar, S.Meyyanathan, S.Bhojraj; A RP-HPLC method for determination of diclofenac with rabeprazole in solid dosage form, *Int.J.Pharm.Sci.*, **2**(2), 171-178 (2011).
- [16] N.Kamble, S.Mahajan, N.Pradhan, S.Prabhune, S.Reddy; Development and validation of RP-HPLC method for simultaneous estimation of eperisone hydrochloride and diclofenac sodium in bulk and pharmaceutical dosage form, *Int J.Pharm.Chem.and Biol.Sci.*, **3**(4), 1286-1292 (2013).
- [17] M.Azeez, K.Kumar, G.Venkteshwarlu, G. Shreesailam, S.Kumarpatro; Spectrophotometric determination of paracetamol and diclofenac in combined dosage form by simultaneous equation method using UV-Vis spectrophotometry, *World J.Pharm Res.*, **2**(4), 917-923 (2013).
- [18] J.Kallol, A.Lopamudra, S.Moitra, A.Behera; Analysis of multicomponent drug formulations (diclofenac and paracetamol). *Asian J.Pharm.Clin.Res.*, **4**(2), 41-43 (2011).
- [19] V.Vemula, P.Sharma; RP-HPLC method development and validation for simultaneous estimation of diclofenac and tolperisone in tablet dosage form, *Asian J.Pharm.Clin.Res.*, **6**(3), 186-189 (2013).
- [20] B.Choudhary, A.Goyal, S.Khokra, D.Kaushik; Simultaneous estimation of diclofenac sodium and rabeprazole by high performance liquid chromatographic method in combined dosage forms, *Int J.Pharm.Sci. and Drug.Res.*, **1**(1), 43-45 (2009).