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Method development and validation for the simultaneous estimation of tolperisone hydrochloride and diclofenac sodium by RP-HPLC

Madasu Sravan Kumar[1*], P.Sunitha[2]

M.Pharmacy,Teegala, Krishna Reddy College of Pharmacy, Hyderabad, India.

***Corresponding author: Madasu Sravan Kumar**

E-mail id: sravanmadasu90@gmail.com

ABSTRACT

A validated RP-HPLC method has been developed for the determination of Tolperisone hydrochloride and Diclofenac Sodium in tablet dosage form. This method is developed by using C_{18} column (Inertsil ODS, 250 mm length, 4.6 mm internal diameter and 5 µm particle size) and a mixture Phosphate Buffer pH 3.0, Acetonitrile and Methanol (60:20:20) as a mobile phase. The drug was quantified by a UV detector at 242 nm. The method is linear for Tolperisone hydrochloride and Diclofenac Sodium in range of 90 to 210 µg/ ml and 30 to 70µg/ml, respectively. The percentage mean recovery of the method for Tolperisone hydrochloride and Diclofenac Sodium was found to be 99.75% and 99.38%, respectively. The proposed method was found to be linear, precise and accurate for the quantitative estimation of Tolperisone hydrochloride and Diclofenac Sodium in tablets and can be used for commercial purposes.

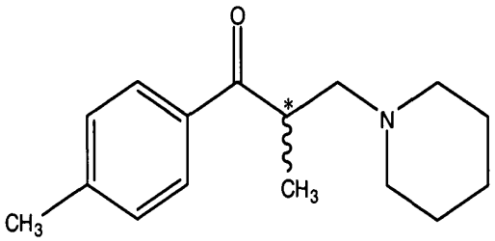
Keywords: Tolperisone hydrochloride, Diclofenac Sodium, Phosphate Buffer, RP-HPLC

TOLPERISONE HYDROCHLORIDE

Tolperisone hydrochloride is a centrally acting skeletal muscle relaxant used in the treatment of spasticity, muscle spasms and chronic pain. It

DESCRIPTION

exhibits membrane stabilizing potency, which is characteristic of anti-arrhythmic and local anesthetic agents.



.HCl

Structure of Tolperisone hydrochloride

IUPAC name: 2-methyl-1-(4-methylphenyl)-3-(1-piperidyl)-propan-1-one

Molecular formula: $C_{16}H_{23}NO.HCl$

Molecular weight: 245.36 g/mol

Mechanism of action:

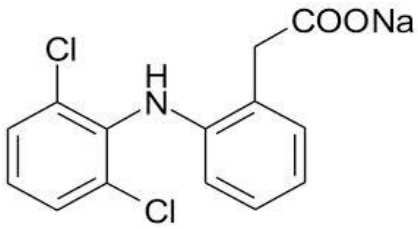
Tolperisone hydrochloride acts at the level of spinal cord and exerts its spinal reflex inhibitory action predominantly via a pre-synaptic inhibition of the transmitter release from the primary afferent endings via a combined action on voltage-gated sodium and calcium channels.

INDICATION

Tolperisone hydrochloride is indicated as centrally acting skeletal muscle relaxant used in the treatment of spasticity, muscle spasms and chronic pain.

DICLOFENAC SODIUM

Diclofenac Sodium is a non-steroidal anti-inflammatory drug taken or applied to reduce inflammation as an analgesic reducing pain in certain conditions.



Structure of Diclofenac Sodium

IUPAC name: Sodium {2-[(2,6-dichlorophenyl) amino] phenyl}acetate

Molecular formula: $C_{14}H_{10}Cl_2NNaO_2$

Molecular weight: 318.13g/mol

Category:

- Antispastic agent
- Analgesic
- Non-steroidal anti-inflammatory agent

MECHANISM OF ACTION

During injuries, arachidonic acid is converted to prostaglandins in the presence of enzyme cyclo-oxygenases (cox-1 and cox-2). These prostaglandins sensitize pain receptors. Diclofenac works by blocking the effect of chemicals called cyclo-oxygenase (COX) enzymes on arachidonic acid and responsible for the analgesic effects of diclofenac.

INDICATION

A non-steroidal anti-inflammatory drug taken to reduce inflammation and pain.

EXPERIMENTAL

EQUIPMENTS

The chromatographic technique performed on a Shimadzu LC20-AT Liquid chromatography with 2695 prominence UV-visible detector and Spinchrom software, reversed phase C18 column (Inertsil ODS, 250 mm × 4.6 mm, 5µ) as stationary phase. Thermo Electron Corporation double beam UV-visible spectrophotometer (vision pro-software), Ultrasonic cleaner, Shimadzu analytical balance AY-220, Vacuum micro filtration unit with 0.45µ membrane filter was used in the study.

MATERIALS

Pharmaceutically pure sample of Tolperisone HCl and Diclofenac Sodium were obtained as gift samples from Chandra Labs, Prashanthinagar, Kukatpally, Hyderabad, India. The purity of the drug was evaluated by obtaining its melting point and ultraviolet (UV) and infrared (IR) spectra. No impurities were found. The drug was used without further purification. HPLC-grade Acetonitrile was from Standard Reagents Pvt Ltd. KH_2PO_4 (AR grade) was from Merck. A tablet formulation of TOLPERISONE HCl and DICLOFENAC SODIUM (150 mg and 50 mg label claims) were procured from local market (Tolpidol-D, THEMIS MEDICARE Ltd., India).

CHROMATOGRAPHIC CONDITIONS

The sample separation was achieved on a C18 (5 µ, 25 cm X 4.6 mm id.) Inertsil ODS column, aided by mobile phase mixture of Phosphate Buffer (30mM) pH: 3.0: Acetonitrile : Methanol (60: 20:20), that was filtered and degassed prior to use, at a flow rate of 1ml/min. Injection volume is 20 µl and detected at 242 nm at ambient temperatures.

PREPARATION OF MOBILE PHASE

BUFFER PREPARATION

Weigh accurately about 6.8 gm of KH_2PO_4 and dissolve with 500ml of HPLC grade water than make up to 1000 ml with HPLC grade water then adjust the pH: 3.0 with Ortho-Phosphoric acid or Sodium hydroxide.

MOBILE PHASE

Then add 60 volumes of buffer, 20 volumes of Acetonitrile and 20 volumes of Methanol sonicated for 15 min and filtered through a 0.45µ membrane filter.

ANALYSIS OF FORMULATION

PREPARATION OF STANDARD SOLUTION

A 150mg of standard Tolperisone HCl and 50mg Diclofenac Sodium were weighed and transferred to 100 ml of volumetric flask and dissolved in mobile phase. The flask was shaken and volume was made up to mark with mobile phase to give a primary stock solution containing 150µg/ml Tolperisone HCl and 50µg/ml of Diclofenac Sodium. From the above solution 5ml of solution is pipette out into a 50 ml volumetric flask and volume was made up to mark with mobile phase to give a solution containing 150µg/ml Tolperisone HCl and 50µg/ml of Diclofenac Sodium.

PREPARATION OF SAMPLE SOLUTION (TABLET FORMULATION)

For the estimation of the drug in tablet formulation twenty tablets were weighed and their average weight was determined. The tablets were then finely powdered. Appropriate quantity equivalent to 150mg Tolperisone HCl and 50mg Diclofenac Sodium were accurately weighed and the powder was transferred to 100 ml volumetric flask and shaken vigorously with mobile phase and sonicated for 15 min and volume made up to the mark with mobile phase. The solution was shaken vigorously and filtered by using Whatmann filter No.41. from the above filtered clear solution 5ml of sample pipetted out into a 50 ml volumetric flask volume made up to the mark with mobile phase to give a solution containing 150µg/ml Tolperisone HCl and 50µg/ml of Diclofenac Sodium.

RESULTS AND DISCUSSIONS

DETERMINATION OF WORKING WAVE LENGTH (ΛMAX)

Dissolve 10 mg of working standard Tolperisone HCl and Diclofenac Sodium separately in 25 ml of Methanol. Take 0.5 ml of above solution in a 10 ml volumetric flask and dilute with methanol up to the mark. Take UV-visible spectra from 400-200 nm and determine the λmax of Tolperisone HCl and Diclofenac Sodium separately using Methanol as blank and then determine the isobestic point. The λmax of Tolperisone HCl (working standard) was found to be at 240 nm and λmax of Diclofenac Sodium (working standard) was found to be at 249 nm. The isobestic point of Tolperisone HCl and of Diclofenac Sodium was found to be at 242nm. The U.V Graph shown in Fig.No.1:

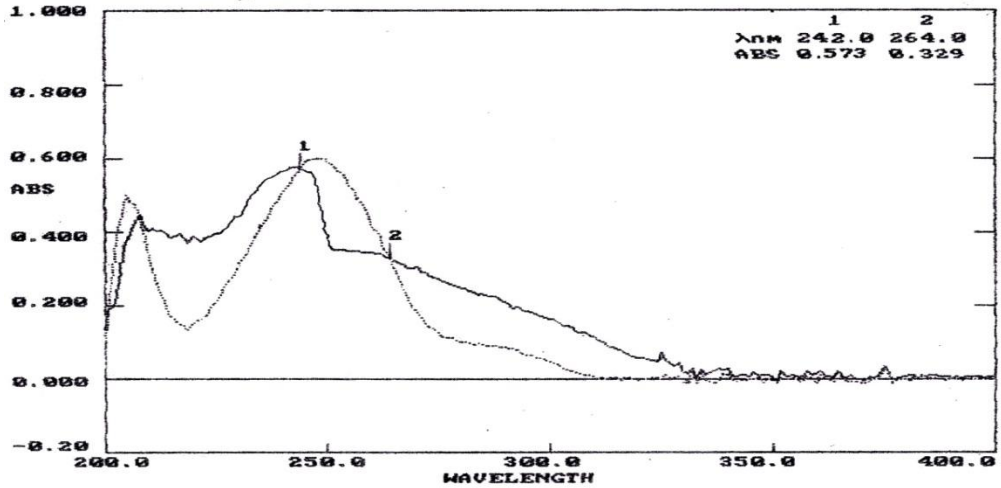


Fig.No.1: UV Tolperisone HCl and of Diclofenac Sodium

After several initial trails with mixtures of methanol, water, ACN and buffer in various combinations and proportions, a trail with a mobile phase mixture of Phosphate Buffer (30mM) pH: 3.0: Acetonitrile : Methanol (60:20:20) brought sharp and well resolved peaks. The chromatogram was shown in Fig.No.2:

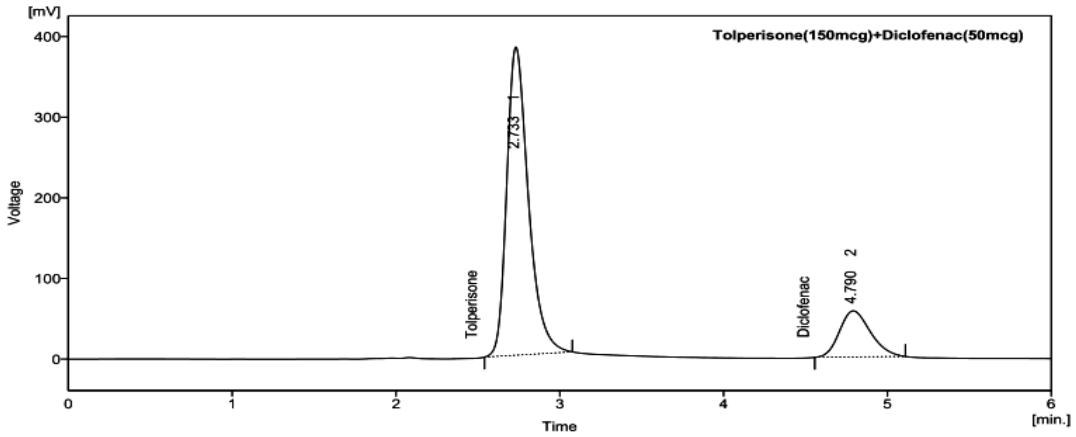


Fig.No.2: Chromatogram of Tolperisone HCl and Diclofenac Sodium

METHOD VALIDATION

SYSTEM SUITABILITY

Weigh accurately about 150mg of Tolperisone HCl and 50mg of Diclofenac Sodium into a 100ml of clean and dry volumetric flask, add 70ml of diluents, shake and sonicated to dissolve the content. Filter the solution through 0.45µm and make up the volume with diluents. Dilute 1ml of this solution in 10ml of diluents and prepare 6 replicate injections of standard.

SPECIFICITY (DIRECT COMPARISON METHOD)

Ability of the method to accurately measure the analyte response in the presence of all potential sample components. For specificity determination, all related substances of Tolperisone HCl and Diclofenac Sodium solutions were prepared individually. After that solution of Tolperisone and Diclofenac Sodium was prepared (control) and injected into HPLC to confirm the retention times. Then Tolperisone HCl and Diclofenac Sodium were spiked with all related substances prepared (spiked samples) as per methodology and injected into HPLC to confirm any co-elution with Tolperisone HCl and Diclofenac Sodium peak from any of related substance peak and diluents.

LINEARITY AND RANGE

Weigh accurately about 150mg of Tolperisone hydrochloride and 50mg of Diclofenac Sodium into a 100ml of clean and dry volumetric flask, add 70ml of diluents, shake and sonicated to dissolve the content, make up the volume with diluents. Filter the solution through 0.45µm. Dilute 0.6, 0.8. 1.2, 1.4 and 1.6 ml of this solution with 10ml of diluents and prepare a series of 5 dilutions.

ACCURACY

To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to sample solution at three different levels. To 80%, 100% and 120% of formulation the reference standards of the 20% of Tolperisone and 80% of Diclofenac Sodium were added. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated.

PRECISION

The closeness of agreement (degree of scatter) between a series of measurements obtained from multiple samplings of the same homogeneous sample. Six sample solutions were prepared individually using single batch of Tolperisone HCl and Diclofenac sodium as per test method and injected each solution into HPLC.

ROBUSTNESS

To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like different flow rates and wavelengths. It provides an indication about variability of the method during normal conditions of laboratory.

RUGGEDNESS

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the assay by two different analysts, different column, on different day.

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ)

LOD and LOQ of Diclofenac sodium and Tolperisone were determined by calibration curve method. Solutions of both Diclofenac sodium and Tolperisone were prepared in the range of 90-120 µg/ml and 30-70 µg/ml, respectively and injected in triplicate. Average peak area of three analyses was plotted against concentration.

$$\mathrm{LOD} = \frac{3.3\sigma}{S}$$

$$\mathrm{LOQ} = \frac{10\sigma}{S}$$

Where, σ = the standard deviation of the response

S = the slope of the calibration curve

DISCUSSION

In RP HPLC method, the primary requirement for developing a method for analysis is that the using different solvents and buffers and columns to get better retention time and theoretical plates, and better cost effective and time saving method than the previously developed methods. The isobestic Point of Tolperisone HCl and Diclofenac Sodium were found to be 242nm (Fig. No: 1) by scanning in UV region. The chromatographic method was optimized with mobile phase consisting of KH_2PO_4 Buffer (30mM): Acetonitrile: Methanol (60:20:20) and C18 INERTSIL ODS column. All the validation parameters were studied at the wavelength 242nm. Linearity (Fig.No.3; Table No.3) was observed in the range 90-210µg/ml for Tolperisone HCl (R^2 =0.9975) and 30-70µg/ml for Diclofenac sodium (R^2 =0.9966). The percentage recoveries were 99.75 and 99.38% for Tolperisone HCl and Diclofenac sodium which shows the accuracy of the method (Table No.4). The method developed was robust (Table No.6) against changes in flow rate, wavelength and rugged against changes in analyst, column and days (Table No.7).

Table No.1: System suitability of Tolperisone HCl and Diclofenac sodium

Injection	Area for Tolperisone HCl	Area for Diclofenac Sodium
1	3536.484	744.643
2	3527.142	740.028
3	3518.764	740.845
4	3527.142	751.016
5	3534.540	740.845
6	3534.540	751.651
Mean	3527.178	746.320 744.87647.464744.8764744.8764744.8764
SD	1.297	5.722
%RSD	0.367	0.766
USP Plate count	2380	2238

Table No. 2: Specificity of Tolperisone and Diclofenac sodium

Tolperisone HCl	% Purity	Diclofenac sodium	% Purity
Control-1	98.3	Control-1	98.3
Control-2	98.8	Control-2	98.8
Spiked sample-1	98.5	Spiked sample-1	98.5
Spiked sample-2	98.3	Spiked sample-2	98.3
Mean (control)	**98.7**	Mean (control)	**98.6**
SD	0.32	SD	0.31
%RSD	0.4	%RSD	0.31
Mean (spiked)	**98.3**	Mean (spiked)	**98.3**
SD	0.25	SD	0.24
%RSD	0.3	%RSD	0.24
% Difference	0.4	% Difference	0.3

Table No. 3: Linearity of Tolperisone HCl & Diclofenac sodium

Tolperisone HCl			Diclofenac sodium	
S. No	Con.(mcg)	Area	Con.(mcg)	Area
1	90	527.728	30	2389.857
2	120	693.781	40	2958.940
3	150	849.276	50	3678.640
4	180	1064.668	60	4324.481
5	210	1204.743	70	5108.230

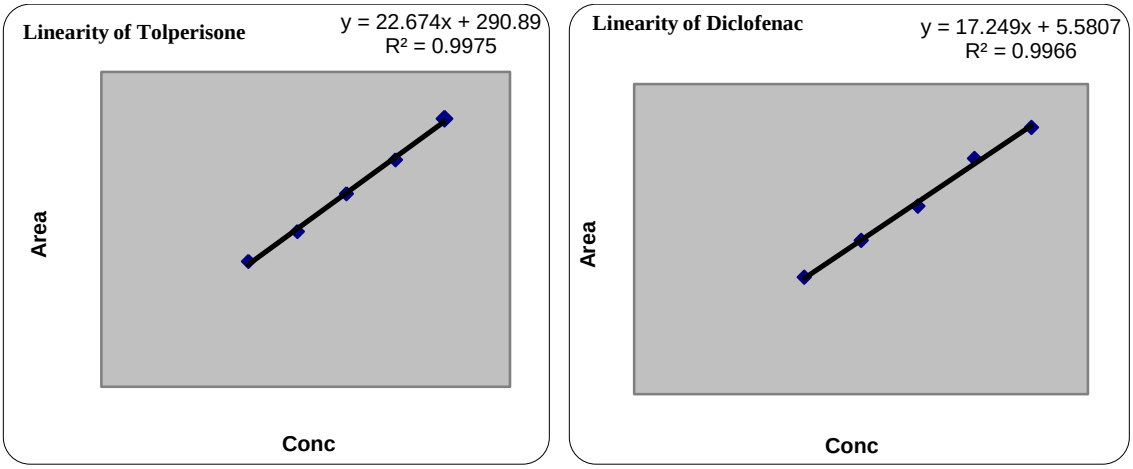


Fig.No.3: Linearity graphs of Tolperisone HCl and Diclofenac sodium

Table No.4: **Recovery of Tolperisone HCl and Diclofenac**

	Tolperisone		**Diclofenac**		
Standard Area	120mcg	2958.940	**Standard Area**	40mcg	693.781
	150mcg	3678.640		50mcg	849.276
	180mcg	4324.481		60mcg	1064.668

Table No.5: Method Precision of Tolperisone HCl and Diclofenac sodium

TOLP.	**120+30**	**150mcg**	**DICLO.**	**40+10**	**50 mcg**
	1	3678.640		1	879.276
	2	3646.139		2	860.942
	3	3777.092		3	852.977
	Avg	3700.624		Avg	864.398
	Result	150.08 mcg		Result	49.84mcg
	% Rec	100.05		% Rec	99.67

TOLP.	**150+30**	**180 mcg**	**DICLO.**	**50+10**	**60 mcg**
	1	4305.590		1	1007.187
	2	4366.372		2	1013.884
	3	4365.612		3	1034.896
	Avg	4345.8mcg		Avg	1018.656
	Result	177.21mcg		Result	59.97mcg
	% Rec	98.45mcg		% Rec	99.95mcg

TOLP.	**180+30**	**210 mcg**	**DICLO.**	**60+10**	**70 mcg**
	1	5117.878		1	1242.136
	2	5159.905		2	1226.559
	3	5126.088		3	1202.624
	Avg	5134.624		Avg	1223.77mcg
	result	213.72mcg		result	68.97mcg
	% Rec	101.77		% Rec	98.52

Tolperisone hydrochloride	Peak area	Diclofenac sodium	Peak area
1	3536.484	1	744.643
2	3527.142	2	740.028
3	3518.764	3	740.845
4	3527.142	4	751.016
5	3534.540	5	740.845
6	3534.540	6	751.651
Mean	3527.178	Mean	746.320
SD	1.297	SD	5.722
%RSD	0.367	%RSD	0.766

Table No.6: Robustness study

Parameter	TOLPERISONE HYDROCHLORIDE		DICLOFENAC SODIUM	
	Retention time(min)	Tailing factor	Retention time(min)	Tailing factor
Flow Rate				
0.8 ml/min	3.133	1.613	4.017	1.513
1.4 ml/min	2.373	1.373	4.160	1.483
Wavelength				
240nm	2.683	1.602	4.713	1.391
244nm	2.707	1.513	4.740	1.783

Table No.7: Ruggedness

TOLPERISONE HCl	% Purity	DICLOFENAC SODIUM	% Purity
Analyst 01	99.73	Analyst 01	99.90
Analyst 02	99.80	Analyst 02	99.80
%RSD	0.05%	%RSD	0.071%
Column ID	VLC-508	VLC-509	
Day	2-08-2014	3-08-2014	

Table No.8: LOD and LOQ of Tolperisone HCl and Diclofenac sodium

Tolperisone hydrochloride			Diclofenac sodium	
S. No	Con.mcg	Area	Con.mcg	Area
1	30	527.728	90	2389.857
2	40	693.781	120	2958.940
3	50	849.276	150	3678.640
4	60	1064.668	180	4324.481
5	70	1204.743	210	5108.230
SD	47.4	1077	15.811	273

CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation of Tolperisone HCL and Diclofenac Sodium was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories.

REFERENCES

1. Indian Pharmacopeia 2007, Indian Pharmacopoeia Commission, Ghaziabad, Vol.2, Pg.No.1199
2. British Pharmacopeia, Medicines Commission Pursuant to the Medicines Act 1968, Vol.1, Pg.No.680
3. United states Pharmacopeia, United States Pharmacopoeial Convention, Vol.2, Pg.No.2545
4. Lioyd. R. Snyder. Joseph J. Kirkland Practical method development by HPLC 2nd edition, Pg.No.123-126
5. B.K. Sharma Instrumental method of chemical analysis 28th edition. Goel publication house, Meerut, Pg.No.489-49
6. Douglas.A.Skoog, D.M.West, F.J Holler Fundamentals of analytical chemistry, Saunders college, 1996 Pg.No.100-105
7. Beckett, A.H and Stenlake J B, Practical Pharmaceutical Chemistry 4th edition part-2 CBS publishers and distributors, New Delhi, 1996, Pg.No.112-116
8. Arunadevi S. Birajdar, Subramania Meyyanathan and Bhojraj Suresh, a RP-HPLC Method for Determination of Diclofenac with Rabeprazole in Solid Dosage form, Pharma Science Monitor An international Journal of Pharmaceutical Sciences, 2(2), 2011, Pg.No.171-178
9. Bhavin P Marolia and Divyesh J Vanparia, Application of RP-HPLC Method for Simultaneous Estimation of Thiocolchicoside and Diclofenac in Commercially Available Capsules, American Journal of Pharmtech Research, 2, 2012, Pg.No.807-818
10. Bindiya Jadeja and Prakash Davadra, Stability Indicating Method Development and Validation for Estimation of Lornoxicam and Tolperisone Hydrochloride in Combined Dosage form, An International Journal of Pharmaceutical Sciences, 4(1), Jan 2013, Pg.No.3429-3445
11. Carolin Nimila. I, N. Chiranjeevi and Vinnakota.V.V.M. Kumar, P.Balan, Development and Validation of a RP-HPLC Method for Simultaneous Estimation of Tolperisone Hydrochloride and Paracetamol in Tablet Dosage Form, International Journal of Pharmacy and Pharmaceutical Sciences, 4(5), 2012, Pg.No. 84-88
12. Choudary B and Goyal A, Simultaneous Estimation of Diclofenac Sodium and Rabeprazole by HPLC Method in Combined Dosage Forms, International Journal of Pharmaceutical Sciences and Drug Research 1(1) 2009, Pg.No.43-45.