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Development and Validation of RP-HPLC Method for Simultaneous Estimation of Combination of Eperisone Hydrochloride and Naproxen Sodium

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ABSTRACT

A simple, rapid and precise Reverse Phase High Performance Liquid Chromatographic method was developed for simultaneous estimation of Eperisone hydrochloride and Naproxen sodium in newly developed tablet dosage form by reverse phase HiQSil C-18 column (250 mm, 4.6 mm, and 5 µm). The sample was analyzed using: Acetonitrile: 10mM n-hexane sulfonic acid buffer (70:30 v/v), as a mobile phase at a flow rate of 1.0 mL/min. and detection at 250 nm. The retention time for Eperisone hydrochloride and Naproxen sodium was found to be 2.77 min and 9.56 min, respectively. The linearity of developed method was achieved in the range of 1-10 µg/mL for Eperisone hydrochloride and 1-25 µg/mL for Naproxen sodium. The method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation, robustness and ruggedness as per ICH guidelines.

Keywords: RP-HPLC, Eperisone hydrochloride (EPE), Naproxen sodium (NAP), validation.

INTRODUCTION

Eperisone hydrochloride is chemically 1-(4-ethylphenyl)-2-methyl-3-piperidin-1-ylpropan-1-one (**Figure:1.1**) and is the well-known antispasmodic drug.[1,2,3,4] Mechanism of action of Eperisone includes inhibition of angiotensin II-induced relaxations, mediated possibly by endogenous PGI_2.[5]Eperisone also possesses the property of a Ca^{2+} antagonist on smooth muscle tissues, in addition to the action of antispastic agent, i.e., this agent blocks the voltage-dependent influx of Ca^{2+} at the smooth muscle membrane.[6,7] Eperisone hydrochloride is official in Japanese Pharmacopoeia only[8] and non-aqueous titrimetric method is reported for the estimation. Many methods like HPLC,[9]HPLC-MS [10,11,12] and GC-MS [13] methods were reported for estimation of Eperisone hydrochloride.
Naproxen sodium is an anti-inflammatory agent, analgesic, antipyretic and propanoic acid derivative compound (**Figure:1.2**) Mechanism of action of Naproxen sodium includes inhibition of arachidonic acid cyclo-oxygenase enzyme (COX) and thereby inhibits production of prostaglandins.

Naproxen sodium is official drug in Indian Pharmacopoeia,[14] British Pharmacopoeia [15] and United state Pharmacopoeia.[16] Many methods like UV-Visible spectroscopy, HPLC, HPTLC methods are reported for estimation Naproxen sodium in single as well as combined dosage form.[17,18,19,20]

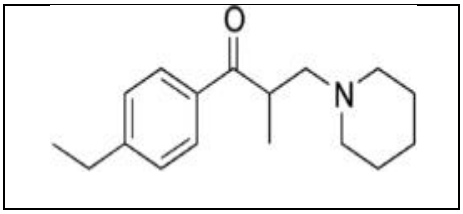

Figure 1.1: Chemical Structure of Eperisone HCl

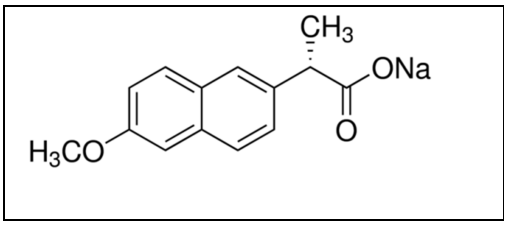

Figure 1.2: Chemical Structure of Naproxen sodium

MATERIALS AND METHODS

2.1Chemicals and solvents:
Methanol (HPLC grade), water (HPLC grade), n-hexane sulfonic acid (AR grade),NaOH(AR grade), hydrochloric acid (AR grade) and 30% Hydrogen Peroxide (AR grade) were used for experimental work. HPLC grade Acetonitrile was used. All reagents were purchased from Merck pharmaceuticals Pvt. Ltd. Pure sample of Eperisone hydrochloride & Naproxen Sodium was a gift sample from Akum drug & pharmaceutical Ltd.

2.2Selection of mobile phase and chromatographic conditions:
Chromatographic separation studies were carried out on the working standard solution of Eperisone HCl (10 µg/mL) and Naproxen Na (10 µg/mL). Initially, trials were carried out using methanol and acetonitrile in various proportions along with buffer of varying pH, to obtain the desired system suitability parameters. After several trials, mixing Acetonitrile and 10 mM n-hexane sulfonic acid buffer in the ratio of (70: 30 v/v) was chosen as the mobile phase, which gave good resolution and acceptable peak parameters.

2.3Preparation of mobile phase:
Initially phosphate buffer was prepared by dissolving 1.882 gm of n-hexane sulfonic acid in sufficient water to produce 1000 mL. Mobile phase was prepared by mixing Acetonitrile and 10 mM n-hexane sulfonic acid buffer in the ratio of 70: 30 v/v. It was then filtered through 0.45 µ membrane filter paper using filtration assembly and then sonicated on ultrasonic water bath for 10 min.

2.4 Preparation of Standard stock solution:
Standard stock solution of EperisoneHCl and Naproxen Na were prepared separately by dissolving 10 mg of drug in 10 mL of Acetonitrile to get concentration of 1000 µg/mL. From the standard stock solution, mixed working standard solution was prepared to contain 100 µg/mL of EperisoneHCl and 100 µg/mL of Naproxen Na in mobile phase. Also from stock solution of EperisonHCl and Naproxen Na (1000 µg/mL), final solution of EperisoneHCl (10 µg/mL) and Naproxen Na (10 µg/mL) was preparedseparately in mobile phase.

2.5 Selection of Detection Wavelength:
From the standard stock solution further dilutions were made using Acetonitrile and scanned over the range of 200 - 400 nm and the spectra were obtained. It was observed that both the drug showed.

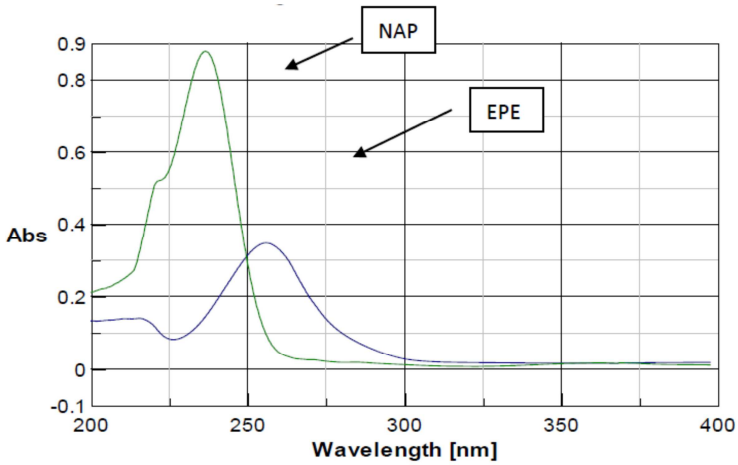

Figure:1.3 Overlain UV Spectra of EPE (10 µg/mL) and NAP (10 µg/mL)

3.0 Method Validation [21]

3.1 System suitability parameter of both drugs:

The column was saturated with the mobile phase (indicated by constant back pressure at desired flow rate). Working standard solution of each drug as individual (10 µg/mL) as well as in combination (10 µg/mL) was injected on system. The retention time for both the drug was found as,

EPE = 2.773 ± 0.04 min and
NAP = 9.560 ± 0.15 min

Chromatogram of Mobile phase blank, EPE and NAP are shown in figure 1.4,1.5,1.6,and1.7 respectively.
System suitability parameters of EPE and NAP are summarized in Table 1.1

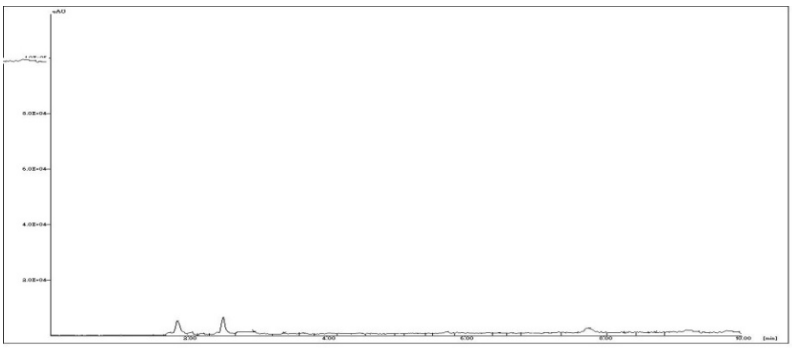

Figure:1.4 Mobile Phase blank

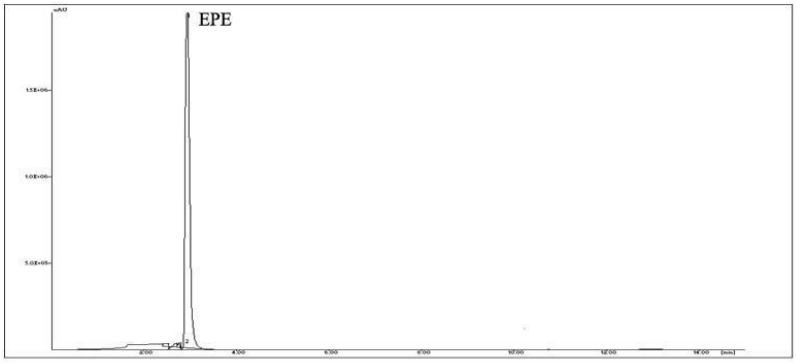

Figure:1.5 Chromatogram of EPE (10 µg/mL)

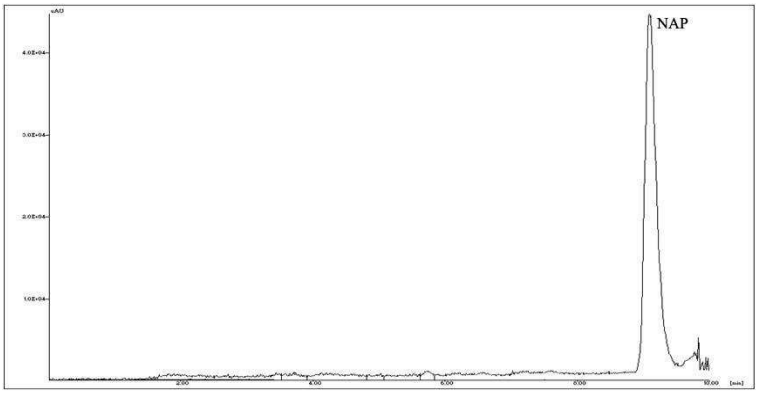

Figure:1.6 Chromatogram of NAP (10 µg/mL)

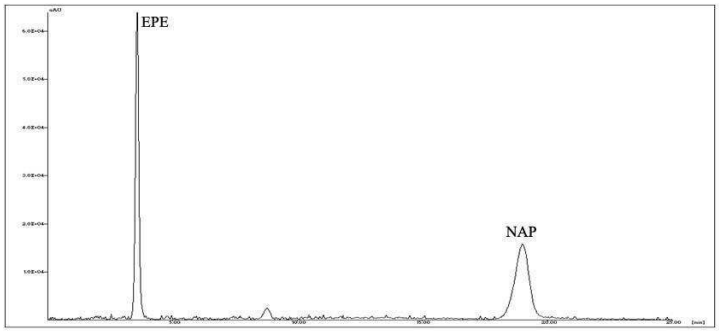

Figure:1.7 Chromatogram of EPE (10 µg/mL, RT = 2.773 ± 0.04 min) and NAP (10 µg/mL, RT = 9.560 ± 0.15 min)

Table:1.1 System suitability parameters

Name	RT (Min)	Concentration (µg/mL)	Area (µV.Sec)	Plates	Asymmetry
EPE	2.773±0.04	10	1581773.62	8604	1.025
NAP	9.560±0.15	10	1396966.42	9235	1.036

3.2Linearity

From the standard stock solution (1000 µg/mL) further dilutions were made to prepare range of solution containing six different concentrations of EPE and NAP. Six replicates per concentration were injected. The linearity (relationship between peak area and concentration) was determined by analyzing six solutions over the concentration range of 1-10 µg/mL for EPE and 1-25 µg/mL for NAP.

The results obtained are shown in Table1.2 for EPE and in Table 1.3 for NAP. The peak areawere plotted against the corresponding concentrations to obtain the calibration curve as shown in Fig.1.8 for EPE and1.9for NAP.

Table: 1.2 Linearity study of EPE

Replicates	Concentration of EPE					
	1µg/mL	2µg/mL	4µg/mL	6µg/mL	8µg/mL	10µg/mL
	Peak Area					
1	171847	363780	670397	962148	1260364	1481773
2	171957	364758	680079	966897	1260790	1482691
3	172145	365263	677412	961177	1266631	1487857
4	171658	365090	673106	969060	1264420	1482124
5	172456	368037	673407	969938	1261330	1488260
6	171352	367030	678799	968407	1268673	1481874
Mean	171902.5	365659.7	675533.3	966271.2	1263701	1484097
SD	427.30	1424.58	3785.55	3718.27	3436.67	3088.05
%RSD	0.00249	0.0039	0.0055	0.00385	0.00272	0.00208

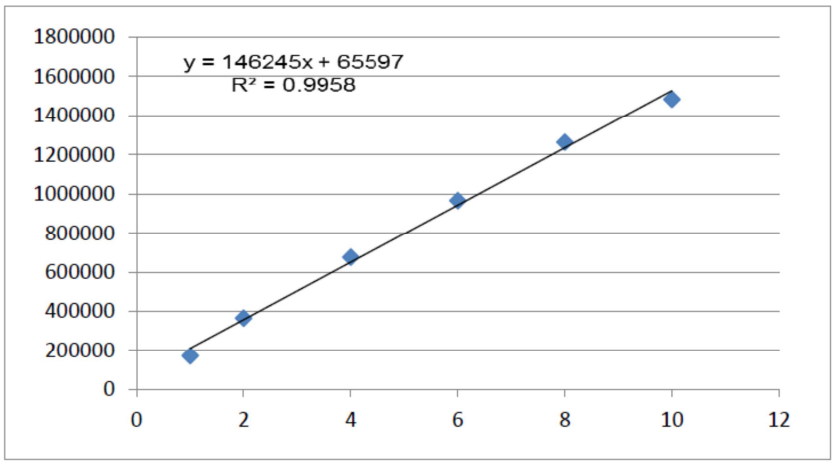

Figure:1.8 Calibration curve for EPE

Table:1.3 Linearity study of NAP

Replicates	Concentration of NAP					
	1µg/mL	5µg/mL	10µg/mL	15µg/mL	20µg/mL	25µg/mL
	Peak Area					
1	192885	736009	1356997	1875305	2654186	3168938
2	192270	738122	1344907	1874328	2665964	3164506
3	193720	740660	1336779	1888425	2655547	3171103
4	192824	740662	1357794	1879630	2655940	3158547
5	193848	746019	1345119	1884232	2661934	3164758
6	193690	739720	1350696	1879691	2657631	3155996
Mean	193206.1	740198.6	1348715.3	1880268.5	2658533.6	3163974.6
Std.Dev.	1818.53	3358.53	8060.32	5346.11	4519.65	5821.72
%RSD	0.00941	0.00454	0.00598	0.00284	0.0017	0.00184

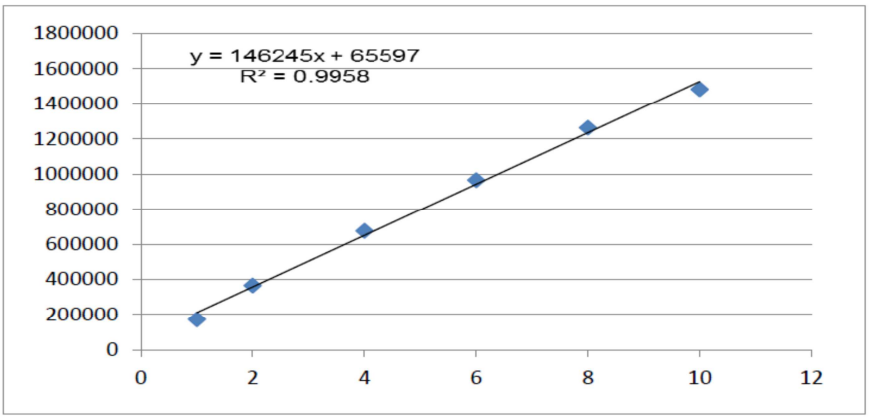

Figure1.9: Calibration curve for NAP

3.3 Range

EPE = 1-10 µg/mL
NAP = 1-25 µg/mL

3.4 Precision:

The precision of the method was demonstrated by Intra-day and Inter-day variation studies. In the Intraday studies, 3 replicates of 3 different concentrations of EPE (2, 4, 6 µg/mL) and of NAP (5, 10, 15 µg/mL) were analyzed in a day and percentage RSD was calculated. For the inter day variation studies, 3 replicates of different concentrations were analyzed on 3 consecutive days and percentage RSD were calculated. The results obtained for Intraday and Inter day variationsare shown in Table 1.4, 1.5, 1.6 and 1.7.

Table:1.4 Intra-day precision studies EPE

Concentration (µg/mL)	Area (µV.Sec)	% Recovery ± SD	Mean % Recovery*±SD	%RSD*
2	363780	101.51±0.47	101.5±0.29	0.28
2	361030			
2	362758			
4	660397	101.93±0.25		
4	661799			
4	663407			
6	951177	101.06±0.15		
6	953897			
6	952148			

Table:1.5 Inter-day precision of EPE

Concentration (µg/mL)	Area (µV.Sec)	% Recovery ± SD	Mean % Recovery*±SD	%RSD*
2	362037	101.45±0.43	101.4±0.21	0.20
2	363758			
2	361263			
4	660079	101.71±0.11		
4	661412			
4	660397			
6	953407	101.14±0.11		
6	953938			
6	952060			

**Average of three determinations*

Table:1.6 Intra-day precision studies NAP

Concentration(µg/mL)	Area (µV.Sec)	% Recovery ± SD	Mean % Recovery*±SD	%RSD*
5	719720	101.23±0.39	101.35±0.11	0.11
5	716019			
5	720662			
10	1356997	101.46±0.47		
10	1350696			
10	1345119			
15	1979691	101.36±0.23		
15	1975305			
15	1984232			

Table:1.7 Inter-day precision study NAP

Concentration(µg/mL)	Area (µV.Sec)	% Recovery ± SD	Mean % Recovery*±SD	%RSD*
5	720660	101.57±0.38	101.36±0.53	0.52
5	721122			
5	721009			
10	1344907	101.11±0.85		
10	1336779			
10	1357794			
15	1979630	101.42±0.38		
15	1974328			
15	1988425			

**Average of three determinations*

3.5 Accuracy

To check accuracy of the method, recovery studies were carried out by adding standard drug to sample at three different levels 50, 100 and 150 %. Basic concentration of sample chosen was 10 µg/mL of NAP from tablet solution. These solutions were injected under stabilized chromatographic conditions in triplicate to obtain the chromatograms. The drug concentrations of NAP were calculated by using linearity equation of NAP. The results obtained are shown in Table 1.8 Basic concentration of sample chosen was 2 µg/mL of EPE from tablet solution. These solutions were injected in stabilized chromatographic conditions in triplicate to obtain the chromatograms. The drug concentrations of EPE were calculated by using linearity equation of EPE. The results obtained are shown in Table 1.9

Table:1.8 Recovery study of EPE

Level	Conc.(µg/mL)		Area	Mean	Amount recovered(µg/mL)	% Recovery
	Sample	Std				
50%	2	1	499507 499828 854981	349740	2.96	98.82
100%	2	2	652257 658791 653357	654801.6	4.02	100.72
150%	2	3	812257 808791 803357	808135.00	5.07	101.55

Table :1.9 Recovery study of NAP

Level	Conc.(µg/mL)		Area	Mean	Amount recovered (µg/mL)	% Recovery
	Sample	Std				
50%	10	5	1951815 1944820 1934755	1943796.67	14.92	99.44
100%	10	10	2600576 2596339 2594364	2597093.00	20.17	100.86
150%	10	15	3161396 3187698 3199539	3182877.67	24.38	99.54

3.6.Limit of Detection (LOD)
LOD is calculated from the formula: -

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

Where,
σ = standard deviation of response for the lowest conc. in the range
S = slope of the calibration curve.
LOD of EPE = 0.028 µg/mL
LOD of NAP= 0.075µg/mL

3.7.Limit of Quantification (LOQ)
The quantitation limit is expressedas:

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

LOQ of EPE= 0.086 µg/mL
LOQ of NAP = 0.228µg/mL

3.8.Specificity
The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be more than 991, indicating the no interference of any other peak of degradation product, impurity or matrix.

3.9 . Robustness
Robustness of the method was determined by carrying out the analysis under conditions during which flow rate, wavelengths were altered and the effects on the area were noted. The results obtained are shown in Table 1.10

Table:1.10 Robustness study

Drug	%RSD found for Robustness study					
	Absorbance at Flow Rate (1mL/min)			Absorbance at Wavelength (nm)		
	0.9	1.0	1.1	240	239	241
EPE	0.2653	0.3115	0.0452	0.2309	0.3115	0.1755
NAP	0.2387	0.6947	0.1831	0.3684	0.6947	0.3097

3.10 Summary of validation study

The summary of validation parameters are summarized in Table 1.11

Table: 1.11 Summary of validation study

Sr.No	Validation Parameter	Results	
		EPE	NAP
1	**Linearity**	y=146245x+65597 R^2=0.9958	y=124307x+89599 R^2=0.9977
2	**Range**	1-10µg/mL	1-25 µg/mL
3	**Precision**	(%RSD)	(%RSD)
	A)Intraday Precision	0.28	0.11
	B)Interday Precision	0.20	0.52
4	**Accuracy**	%Recovery	%Recovery
	50%	98.82%	99.44%
	100%	100.72%	100.86%
	150%	101.55%	99.54%
5	**LOD**	0.028 µg/mL	0.075µg/mL
6	**LOQ**	0.086µg/mL	0.228µg/mL
7	**Specificity**	Specific	Specific
8	**Robustness**	Robust	Robust

CONCLUSION

A simple, economic, rapid and precise Reverse Phase High Performance Liquid Chromatographic method was successfully developed. Initially, trials were carried out using methanol and acetonitrile in various proportions along with buffer of varying pH, to obtain the desired system suitability parameters. After several trials, mixing acetonitrile and 10 mM n-hexane sulfonic acid buffer in the ratio of (70: 30, v/v) was chosen as the mobile phase, which gave good resolution and acceptable peak parameters. The retention time for EPE and NAP were found to be 2.773 ± 0.04 min and 9.560 ± 0.15 min respectively.

Straight-line calibration graphs were obtained in the concentration range 1-10 µg/mL for EPE and 1-25 µg/mL for NAP with high correlation coefficient. The proposed method was also evaluated by the assay of prepared formulation. The % drug content (Mean±S.D.) was found to be 98.86±0.41 for EPE and 99.64±0.07 for NAP respectively. Robustness of the method checked after deliberate alterations of the analytical parameters showed that areas of peaks of interest remained unaffected by small changes of the operational parameters (% R.S.D. < 2).

For EPE, the recovery study results ranged from 98.82 to 101.55 %. For NAP, the recovery results ranged from 99.44 to 100.86 %. The method was found to be accurate and precise, as indicated by recovery studies as recoveries were close to 100 % and % R.S.D. not more than 2. The lower values of % R.S.D. (< 2) obtained for both the drugs indicated that method was found to be precise.

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REFERENCES

[1] Craig CR, Stitzel RE. Modern pharmacology with clinical applications. Lippincott Williams & Wilkins. 6th edition. New York. **2003**; 339.
[2] Tariq M, Akhtar N, Ali M, Rao S, Badshah M, Irshad M. *J Pak Med Assoc* **2005**; 55: 202-4.
[3] Bresolina N, Zuccab C, Pecori A. *European Review for Medical and Pharmacological Sciences* **2009**; 13:365-70.

[4] Biering SF, Nielsen Jb, Klinge K. *Spinal Cord* **2006**; 44: 708-22.
[5] Inoue S, Bian K, Okamura T, Okunishi H, Toda N. *Jpn J Pharmacol*.**1989** ; 50(3):271-82.
[6] Fujioka M, Kuriyama H. *JPET* **1985**; 235(3):757-63.
[7] Bose K. *ExpClinPharmacol* **1999**; 21: 209-13.
[8] The ministry of Health, Labour and Welfare. Japanese Pharmacopeia. Prefectural office in Japan. 15th edition **2006**: 618.
[9] Fu CM, Li B, Guo RC. *Chin. JMAP* **2001**, 18: 57–9.
[10] Ding L, Wei X, Zhang S, Sheng J, Zhang Y. *J ChromatogrSci* **2004**; 42: 254-8.
[11] Shi A, Li K. *Chin. J. Pharm. Anal*. **2002**; 22:45–7.
[12] Wei X, Ding L, Gao J. Rapid and sensitive chromatography- electrospray ionization mass spectrometry method for the determination of Eperisone in human plasma: method and clinical applications. Yao XueXueBao. **2004**; 39: 309–11.
[13] Takamatsu T, Yamazaki K, Kayano M. *J. Chromatogram* **1992**; 584: 261–6.
[14] Government of India. Ministry of health and family welfare. Indian pharmacopoeia, Vol.–II. The Controller of Publications. New Delhi.**2010**: 770, 1479
[15] Great Britain: The department of health. Medicinal and healthcare products regulatory agency. British Pharmacopoeia. Vol. – II. The stationary office.**2009**: 1429.
[16] The United States Pharmacopoeia Convention. Food and drug administration. The United States pharmacopoeia 32. National Formulary 27. Vol. – II. Asian Edition, Rockville.**2009**: 2124-25.
[17] Solanki S, Dr. Patel P. *International Journal of Pharmacy and Pharmaceutical Science*, Vol 4, Suppl 1, **2012**.
[18] Sojitra C. *International Journal of Pharmacy and Pharmaceutical Sciences* Vol 4, Suppl 3, **2012**.
[19] HaqueA**,**Amrohi S, Nasare M, Diwan P. *IOSR Journal of Pharmacy*, Volume 2 Issue 4, July-August **2012**, PP.19-24.
[20] *Y.*Swapnaa *IJPBS*, Volume 3 Issue 1 JAN-MAR **2013**, 179-185.
[21] International Conference on Harmonization. Guidance for industry. Q2R1.Validation on Analytical Procedures: Methodology. Switcherland: IFPMA **1996**; 1-8.