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# Simultaneous estimation of lornoxicam and paracetamolin tablets by reverse phase HPLC method

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**ABSTRACT**

*A simple,fast, precise and accurate liquid chromatographic method was developed for simultaneous estimation of Lornoxicam and Paracetamol in combined tablet dosage form by Reverse phase HPLC method. Chromatography was carried out on a C-18 column (250 mm×4.6 mm,i.d. 5 µm) using Acetonitrile:0.05 M Ammonium Acetate buffer (pH adjusted to 6.5 with triethylamine) in the ratio of 98:2 (v/v) as amobile phase at a flow rate of 0.8 ml/min and eluents were monitored at 280 nm. The calibration curves were linear over the range of 2 – 20µg/ml for Lornoxicam and 10 – 20 µg/ml for Paracetamol. The average retention time ofLornoxicam and Paracetamol was found to be 2.086 min and 3.70 min respectively. The method was reproducible, with good resolution and sharp peak of Lornoxicam and Paracetamol. Theresults of the analysis have been validated statistically.*

**Key words:** Paracetamol, Lornoxicam, RP-HPLC, Validation, Acetonitrile.

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## INTRODUCTION

Chemically, Paracetamol (PAR) is N(4-hydroxyphenyl) acetamide. It has antipyretic and analgesic activity. Lornoxicam (LOR) is (3*E*)-6-chloro-3-[hydroxy(pyridine-2-ylamino) methylene]-2- methyl-2, 3-dihydro-4*H*-thieno[2,3-*e*][1,2]thiazin-4-one-1,1-dioxide. It has non steroidal anti-inflammatory activity. Paracetamol is official in IP[1], BP[2] and USP[3] while Lornoxicam is not official in any Pharmacopoeia, but listed in the Merck Index[4]. LOR belongs to the chemical class of oxicams, and is a cyclooxygenase inhibitor[5]. Literature survey reveals many analytical methods are available for determination of Paracetamol such as UV Spectrophotometry[6-8], HPLC[9-16], and Capillary electrophoresis[17] methods from pharmaceutical preparations. Few analytical methods for determination of Lornoxicam using UV spectroscopy[18-19],HPLC[20-22]and polarography[23] in plasma and pharmaceutical formulation have been reported. Extensive literature survey reveals, only one method is available that is based on estimation of Paracetamol and Lornoxicam by spectrophotometry[24].

This paper presents simple, rapid, reproducible and economical method for the simultaneous analysis estimation of both the drugs from pharmaceutical dosage form by using reverse phase HPLC. The proposed method was validated following ICH guidelines[25].

## MATERIALS AND METHODS

Standard gift samples of Paracetamol and Lornoxicam were procured from Glenmark Pharmaceuticals, Mumbai. The commercial fixed dose tablet formulation containing 500 mg of Paracetamol and 8 mg Lornoxicam were purchased from local market.All chemicals and reagents used were of HPLC grade, HPLC water was obtained by triple distillation. Chromatographic separation was performed at room temperature on aJasco PU-2080 plus HPLC solvent delivery system (pump),rheodyne injector with 20 µl loop volume, UV-2075 UV-VIS detector set at 280 nm, JASCO Borwin 1.50.8.0 version software were used for analysis. A Phenomenex LUNAC18 column (250 mm x 4.6 mm i.d., 5µm) was used for analysis. In addition, an electronic balance (Shimadzu AND HR200), a pH meter (Systronics model EQMKVI), a sonicator (PCi, Mumbai)were used in this study.

**Preparation of Mobile Phase:**

0.05 M ammonium acetate buffer was prepared by dissolving 3.85gm of ammonium acetate in 50ml HPLC water and volume made up to 1000 ml by HPLC water, 50ml of this solution diluted to 1000ml with 0.05M acetic acid and pH adjusted to 6.5 by triethylamine.The mobile phase containing Acetonitrile:0.05 M ammonium acetate buffer (98:2), pH adjusted to 6.5 with triethylamine was found to resolve LOR and PAR. Triethylaminewas used for pH adjustment of buffer. The mobile phase was filtered on a 0.45 micron membrane filter and then ultrasonicated for 15min. The flow rate was set to 0.8 ml/min. Both drugs showed good absorbance at 280 nm, which was selected as wavelength for further analysis. All determinations were performed at constant column temperature ($26 \pm 2^0$)

**Optimization of detection wavelength**

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. Drug solution of 10 µg/ml of LOR and 10µg/ml of PAR were prepared in methanol and acetonitrile. Each solution was scanned using double beam UV-Visible Spectrophotometer-1700 in the spectrum mode between the wavelength range of 400 nm to 200 nm and their spectra was overlaid. The wavelength selected was 280 nm as both the drugs showed significant absorbance at this wavelength.

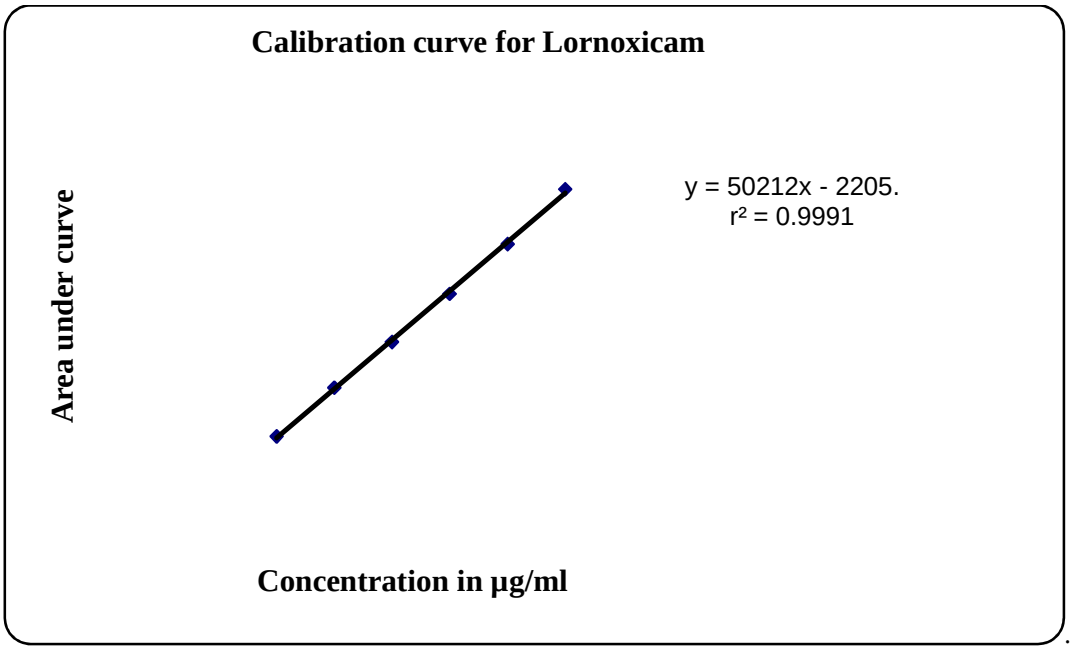


**fig. 1: Calibration curve for lornoxicam**

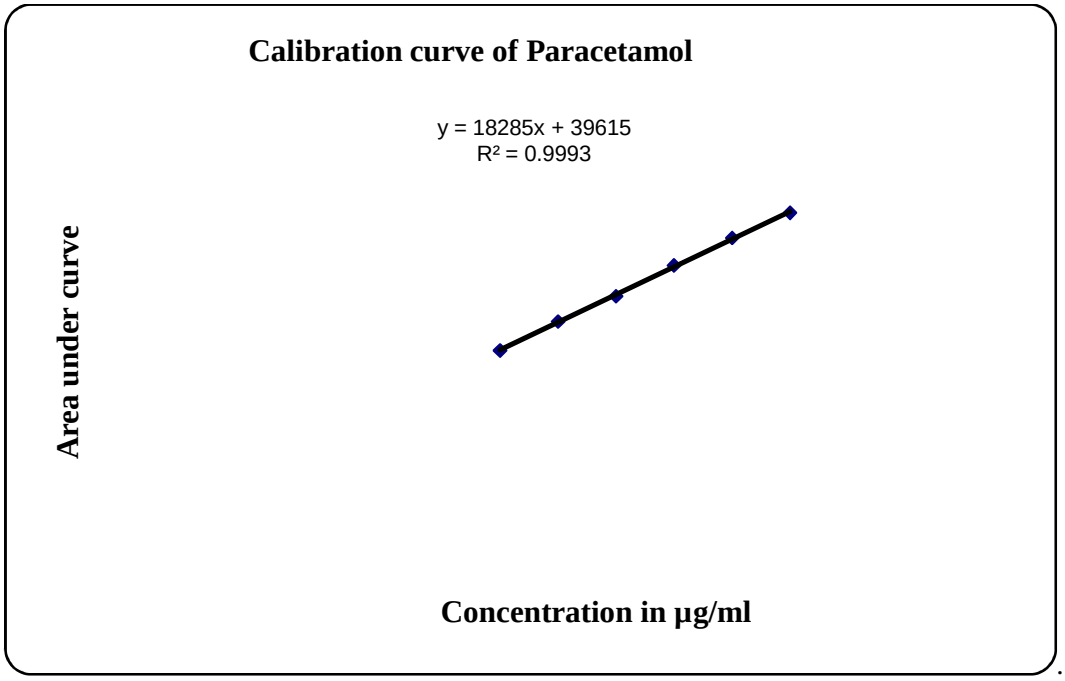


**fig. 2: Calibration curve forParacetamol**

**Preparation of standard stock solutions and standard calibration curves:**
Standard stock solution of LOR and PAR were prepared separately by transferring 10 mg of LOR and 10 mg of PARA in 100 ml volumetric flask. Sufficient amount of methanol and acetonitrile in1:1 V/V was added, sonicated and remaining volume was made up to the mark with same solvent. Aliquots of standard stock solution were appropriately diluted with acetonitrile to obtain concentration range of 2-20 µg/ml for LOR and 10-20 µg/ml for PAR. Accurately measured standard stock solution of LOR (0.2, 0.4, 0.6, 0.8, 1, 1.2 ml) & standard stock solution of Paracetamol (1, 1.2 1.4, 1.6, 1.8, 2 ml) were transferred to a separate series of 10 ml of volumetric flasks and dilutions were made by using mobile phase. The diluted standard solutions with varying concentration were injected (in triplicate) into the HPLC system separately and chromatographed under above mentioned chromatographic conditions. Chromatographic peaks of both drugs were recorded at 280 nm using UV detector. The calibration curves of mean peak area versus concentration of both drugs were plotted and show in fig No.1 and fig No.2

**Preparation of sample solution:**
Twenty tablets, each containing 500mg of paracetamol and 8mg of Lornoxicam were weighed and finely powdered. A quantity of powder equivalent to 100mg of paracetamol was taken and transferred to a glass mortar. Lornoxicam present in this tablet powder is 1.6mg, which could not be found out accurately, hence to increase accuracy,98.4mg pure Lornoxicam were weighed and transferred to same glass mortar to make 1:1 ratio and mixed properly. A quantity of powder equivalent to 10 mg of both the drugs were weighed and transferred to 100ml volumetric flask. To this 25 ml of methanol and 25ml of acetonitrilewere added, sonicated for 15 min and then the volume was made upto 100 ml with remaining quantity of methanol and acetonitrile and filtered through 0.22µ filter. Further dilutions were made to get concentration of 10µg/ml of paracetamol and 10µg/mlof lornoxicam. This solution was used foranalysis. With the optimized chromatographic conditions, a steady baseline was recorded.

From sample solution,20µl solution wasinjected into the column and chromatographed under above mentioned chromatographicconditions. Each sample solution was injected and chromatographed in triplicate. Six suchsamples were prepared and analyzed. The chromatogram recorded for sample solution is given in figure no. 3.The retention times of Lornoxicam and Paracetamol were found to be 2.086 and 3.70 min, respectively.

Content of LOR and PARA in tablet was calculated bycomparing mean peak area of sample with that of the standard. Concentrations of both drugs were calculated. Results of analysis of tabletformulation are shown in Table No. 1.

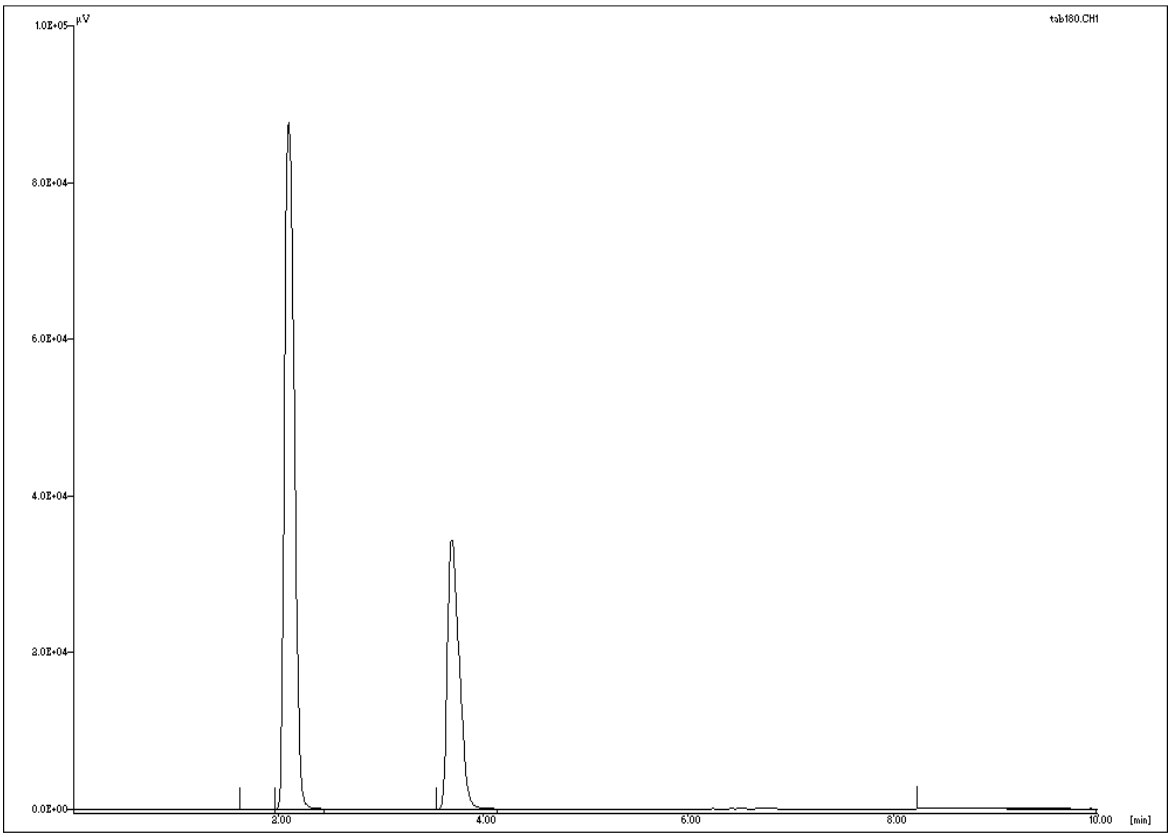


**fig 3:typical chromatogram of lornoxicam (rt= 2.086min) and paracetamol (rt= 3.70min)simultaneously in tablet formulation.**

**Table 1: Analysis of marketed tablet formulation**

| Conc. | Area Under Curve | | Retention Time (min) | | % Estimated (Mean ± SD) | |
|---|---|---|---|---|---|---|
| | LOR | PARA | LOR | PARA | LOR | PARA |
| 10 | 498320.2 | 179997.8 | 2.10 | 3.692 | 99.2 | 98.44 |
| 10 | 499016.12 | 181012.568 | 2.075 | 3.708 | 99.38 | 98.9 |
| 10 | 501032.9 | 181416.2 | 2.083 | 3.700 | 99.7 | 99.21 |
| Mean | 499456.4067 | 180808.856 | 2.086 | 3.700 | 99.4±0.264 | 98.8±0.404 |

## METHOD VALIDATION

### Linearity and Range:

The linearity of the method was determined at six concentration levels ranging from 10 to 20 µg/ml for PAR and 2-20µg/ml for LOR. The calibration curve wasconstructed by plotting response factor againstconcentration of drugs.

**Table 2: % Recovery Study Data**

| % Standard Addition | % Estimated (Mean ± SD) | | % R.S.D. | |
|---|---|---|---|---|
| | LOR | PARA | LOR | PARA |
| **80** | 98.37±0.202 | 98.29±0.311 | 1.14 | 1.76 |
| **100** | 98.77±0.135 | 98.92±0.2558 | 0.68496 | 1.29 |
| **120** | 99.73±0.776 | 99.80±0.09073 | 3.541 | 0.4 |

### Accuracy:

To ascertain the accuracy of the proposed method recovery studies were carried out by standardaddition method, adding known amount of each drug to the preanalysed tablet powder, at threelevels 80 %, 100 % and 120 % of the

label claim. Recovery studies were carried out in triplicateand the percentage recovery andstandard deviation of the percentage recovery were calculated and given in following table no.2.

**Precision**

The precision of the method was demonstrated byinter-day and intra-day variation studies. In the intraday studies, six repeated injections of standard andsample solutions were made and the response factor of drug peaks and % RSD were calculated. In theinter-day variation studies, six repeated injections ofstandard and sample solutions were made for threeconsecutive days and response factor of drugs peaksand percentage RSD were calculated.

**Limit of Detection and Limit of Quantification:**

The limit of detection (LOD) and limit ofquantification (LOQ) of the developed method were determined by injecting progressively lowconcentrations of the standard solutions using the developed RP-HPLC method. LOD and LOQ for both the drugs were calculated by using the values of slopes and intercepts of the calibration curves.

**Robustness**

Robustness of the proposed method was ascertained by deliberately changing the chromatographic conditions such as change in flow rate of the mobile phase (± 0.1 ml/min), change in composition of the mobile phase (± 1 ml) and change in pH of the buffer solution used in mobile phase. Effect of change in chromatographic parameters on resolution and tailing factor of peak was studied.

**System suitability studies:**

The column efficiency, resolution and peak asymmetry were calculated for the standard solutions and given in Table no 4.The values obtained demonstrated the suitability of the system for the analysis of this drug combinations, system suitability parameters may fall within ± 3 %standard deviation range during routine performance of the method.

**Table 3: System Suitability Parameters**

| Parameters | LOR | PARA |
|---|---|---|
| **Linearity range** | 2-12 | 10-20 |
| **Regression equation** | y=50212x-2205.7 | y=18285x+39615 |
| **Coefficient of Correlation** | 0.9991 | 0.9993 |
| **Theoretical plate / meter** | 2567.833 | 3713.66 |
| **Resolution Factor** | 1.33 | 4.5 |
| **Assymetric Factor** | 1 | 1 |
| **Tailing Factor** | 1.00 | 1.00 |
| **LOD (µg/mL)** | 0.09259 | 0.13189 |
| **LOQ(µg/mL)** | 0.280059 | 0.39967 |
| **Retention Time** | 2.07 | 3.714 |

**Solution stability:**

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 5 hours at room temperature. The results show that for both solutions, the retention time and peak area of PARA and LOR remained almost unchanged (% RSD less than 2.0 and no significant degradation within the indicated period, this indicated that both solutions were stable for at least 5 hours, which was sufficient to complete the whole analytical process.

## RESULTS AND DISCUSSION

In this wok an analytical HPLC method for assay and determination of content uniformity of lornoxicam and paracetamol in tablet formulation was developed and validated. The HPLC procedure was optimized with a view todevelop precise and stable assay method. Phenomenex C18 column (250 mm x 4.6mm i.d., 5µ), with a mobile phase Acetonitrile: 0.05M Ammonium Acetate buffer (pH adjusted to 6.5 with triethylamine) in the ratio of 98:2 (v/v) at a flow rate of 0.8 ml/min were used and eluents were monitored at 280 nm to give sharp and symmetrical peaks with retention time 2.086 min and 3.70 min forlornoxicam and paracetamolrespectively. The typicalchromatogram of sample solution is shown in Fig.3.The peak area ratio of standard and sample solutionswas calculated. The assay procedures were repeatedfor six times and mean peak area and mean weight ofstandard drugs was calculated. The percentage ofindividual drugs found in formulation was 99.4 % for LOR and 99.8 % for PAR.

The results of analysis shows that the amount of drugs was in good agreement with thelabel claim of the formulations.The standard deviation and % RSD calculated for the proposed method is low, indicating high degree of precision of the method. The accuracy of the method was determined byrecovery experiments.The results of the recovery studies performed shows high degree of accuracy of the proposed method. The LOD for lornoxicam and paracetamol were0.092 µg/ml and 0.13µg/ml respectively; LOQ were 0.28µg/ml and 0.39µg/ml respectively. Hence the present RP-HPLC method is suitable for the quality control of the raw materialsand formulations.

## CONCLUSION

The rapid and reliable RP-HPLC method has been developed for the analysis of lornoxicam and paracetamol in the combined dosage form. The developed method was found to be precise, simple and reproducible. The method is new and can be employed for routine analysis lornoxicam and paracetamolin pure and tablet dosage form.

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## REFERENCES

[1] The Indian Pharmacopoeia, Govt. of India, Ministry of Health and Family Welfare Controller of Publication, **2007**, Vol.III, 1514
[2] The British Pharmacopoeia, HMSO publication: London; **2007** edition, Vol. II, 1575.
[3] United States pharmacopoeia 24 NF19 United States Pharmacopeial Convention. Inc. 12601 Asian ed.**1999**, 17
[4] The Merck Index.14th edition.Merck and Co., Inc White House Station, NJ, USA,**2006**, 5612.
[5] The Merck Index.14th edition.Merck and Co., Inc. Whitehouse Station, NJ, USA, **2006**,5582
[6] S. J. Wadher, P. R. Pathankar, M.Puranik, R.O.Ganjiwale, P. G.Yeole *Indian J Pharm Sci*,**2008**; 70 (3):393-395.
[7] R. Sawant, R. Joshi, D. Kawade , V.Sarode, *Der Pharmacia Lettre*, **2010**, 2 (2): 471-478
[8] L. P. Kothapalli, A. K. Karape, A. B. Thomas, R. K. Nanda,P. Gaidhani, M. E. Choudhari, *Der Pharma Chemica*, **2011**, 3(1):365-371
[9] M.Ghada, M.Hadad, E. Samy, M. M.Mahmand *Talanta* **2009**; 79: 1360-1367.
[10] N.Udupa, A.Karthik, G. Subramanian, A. R. Kumar, *Indian J Pharm Sci* **2007**; 69 (1): 140-144.
[11] G Subramanian, M Vasudevan, S Ravisankar, B Suresh, *Indian J Pharm Sci* **2005**; 67 (2): 260-263.
[12] Z. Fijalek, E. Wyszecka-Kaszuba, MWarowna-Grzeskiewicz, *JPharm Boimed Anal* **2003**; 32: 1081-1086.
[13] M. Lotfi, D. Frida, *J Pharm Biomed Anal*.**2002**; 27: 851-860.
[14] M. Vasudevan, S.Ravisankar, T.Ravibabu, Nagarajan. *Indian J Pharm Sci* **2000**; 62(2): 122-125.
[15] A. B. Devkhile, K. A. Shaikh, *Der Pharmacia Lettre*, **2010**, 2(5): 237-244
[16] D. V. Pokharkar, R.Korhale, S. Jadhav, N. Birdar, D. C. Puri, N. P.Wani *Der Pharmacia Lettre*, **2011**, 3 (6):49-57
[17] S. Zahao, D. Xiao, W. Bai, H. Yuan, *Anal Chim Acta* **2006**; 559: 195-199.
[18] B. Singh, G. Saini, D. N. N. Sharma, S. D. Roy, N. Gautam *Int. Jr. Pharm Sci Research* **2011**; 2(1): 102-106
[19] N. Jain, R. Jain, V. Sahu, H. Sharma, S. Jain, D.K. Jain, *Der Pharma Chemica*, **2010**, 2(6):165-17
[20] Y. H. Kim, H. Y. Ji, E. S Park, S. Chae, H. S. Lee, *Archieves of Pharmacal Research* **2007**; 30 (7): 905-910.
[21] K. R.Patil, D. B.Shinde,V. P.Rane,J.N.SangshettiJr. *Chromatographia* **2009**; 69: 1001-1005
[22] Savita S Yadav, Anuradha S Jagtap, Janhavi R Rao *Der Pharma Chemica*, **2012**, 4(5):1798-1802.
[23] I. Cetin, N.Kocak,S.Aycan, C.B.U. *J. Sci*. **2009**; 5.1: 11-18
[24] L. Sivasubramanian, Lakshmi KS and Tintu T.*Int J Pharm PharmSci* **2010**; 2(4): 166-168
[25] ICH, Q2 (R1), Hormonised Tripartite Guideline, Validation of analytical procedures. Text and methodology, International Conference on Harmonization (ICH),Geneva, Nov **2005**.