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Development and validation of a RP-HPLC-PDA method for simultaneous estimation of Hydrochlorothiazide and Irbesartan

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Abstract

A simple, precise, rapid and accurate RP-HPLC-PDA method has been developed for the simultaneous estimation of Hydrochlorothiazide and Irbesartan in tablet formulations. The chromatographic separation was achieved on Waters Symmetry C18 column (250 mm x 4.6 mm, 5.0 µ particle size) using methanol: THF: acetate buffer (60:10:30v/v) pH adjusted to 5.5 with acetic acid, flow rate was 0.75 ml/min and column was maintained at 50^{0}C. Quantification and linearity was achieved at 271 nm over the concentration range of 0.3 – 50 µg /ml for Hydrochlorothiazide and 3.6 - 600 µg/ml for Irbesartan. The method was validated for specificity, linearity, accuracy, precision, LOD, LOQ and robustness. The proposed method was optimized and validated as per the ICH guidelines.

Key words: Hydrochlorothiazide, Irbesartan, method validation, RP-HPLC-PDA.

INTRODUCTION

Hydrochlorothiazide (HTZ) is a diuretic. Chemically it is (6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide(1).Several analytical methods, including spectrophotometry [3–6] and HPLC [7 –12], have already been reported for its determination, either alone or in combination with other drugs. Irbesartan (IRBE) is an orally active specific angiotensin II receptor antagonist used, as a hypotensive agent does not required biotransformation into an active form. Chemically it is 2-butyl-3-[*p*-(*o*-1H-tIRBEazol-5-ylphenyl)benzyl]-1,3-diazaspiro[4.4]non-1-en-4-one(1). Literature survey reveals that irbesartan

can be estimated spectrophotometrically (5,16) and chromatographically (11-15) either alone or in combination with other drugs.

To our knowledge there is no HPLC method reported for the combination therefore aim of the present study was to develop a sensitive, precise, accurate and specific method for the determination of HTZ and IRBE simultaneously in formulation.

The present work describes a simple reverse phase LC method for the determination of HTZ and IRBE in tablets. The method was validated according to ICH guidelines and was found to be reproducible with good resolution between HTZ, internal standard (IS) and IRBE. The detector response was found to be linear in the concentration range of 0.3 – 50 µg/ml for HTZ and 3.6 – 600 µg/ml for IRBE.

MATERIALS AND METHOD

Reagents and chemicals:
Pure drug sample of IRBE (% purity 99.75) and HTZ (% purity 99.93) was kindly supplied as a gift sample by Cipla Pvt. Ltd. Mumbai. These samples were used without further purification. Tablets each containing 150 mg of IRBE and 12.5 mg HTZ used for analysis were IROVEL-H (Formulation I) and XARB-H (Formulation II) manufactured by Sun Pharma Sikkim, India and Piramal Health Care, India , Respectively.

HPLC grade Methanol and tetrahydrofuran (THF) were procured from Merck and Qualigens fine Chemicals, respectively (Mumbai, India). Analytical Reagent grade Ammonium Acetate and Acetic Acid was procured from Research Lab Fine Chem (Mumbai, India). Double distilled water was made at lab scale only.

Instrumentation and chromatographic conditions:
The HPLC system consisted of a binary pump (model Waters 515 HPLC pump), auto sampler (model 717 plus Auto sampler), column heater, and PDA detector (Waters 2998). Data collection and analysis were performed using Empower- version 2 software. Separation was achieved on Symmetry C18 (250 mm × 4.6 mm, 5.0 µ) and Kromasil C18 (250 mm × 4.6 mm, 5.0 µ) columns maintained at 55^0C using column oven. Isocratic elution with Methanol: THF: Acetate buffer (60:10:30 v/v) mobile phase at the flow rate of 0.75 ml/min was carried out. The column was supported with waters symmetry C18, (waters C18, 20 x 3.9 mm, 5µ) guard column. The detection was monitored at 271 nm (Fig.1) and injection volume was 20 µl. The peak purity was checked with the photodiode array detector. Shimadzu analytical weighing balance (Model AUW220D) and PH Meter (Equip-Tronics micro controller pH Meter Model EQ-621) was used for study.

Preparation of standard solutions and calibration curve:
Standard stock solution of HTZ and IRBE (1mg/ml) were separately prepared in methanol. To study the linearity range of each component, serial dilutions of HTZ and IRBE each were made from 0.3-50 µg/ml and 3.6-600 µg/ml respectively in mobile phase and injected on to column. Calibration curves were plotted as concentration of drugs versus peak area response. From the standard stock solutions, a mixed standard solution was prepared containing the analytes in the

given ratio and injected on to column. The system suitability test was performed from six replicate injections of mixed standard solution (Table 1).

Analysis of tablet formulations:

Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 60 mg of IRB (5 mg of HTZ) was weighed and dissolved in the 40 ml of solvent with the aid of ultrasonication for 15 min and solution was filtered through Whatman paper No. 41 into a 50 ml volumetric flask. Filter paper was washed with the solvent, adding washings to the volumetric flask and volume was made up to mark with methanol.The solution was further diluted to get sample chromatogram. A typical chromatogram obtained from a sample solution is shown in Figure 1.

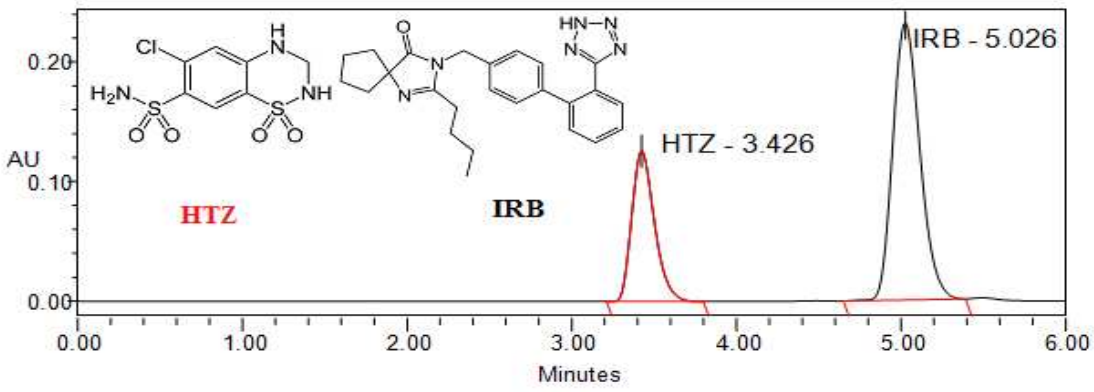

Figure 1 : A typical chromatogram of a tablet sample solution containing of 10 µg/ml of HTZ and 120 µg/ml IRBE with Structure of Hydrochlorothiazide and Irbesartan

RESULTS AND DISCUSSION

Method development:

The HPLC method was validated in terms of precision, accuracy and linearity according to ICH guidelines. Assay method precision was determined using nine independent test solutions. The intermediate precision of the assay method was also evaluated using different analyst on three different days. The accuracy of the assay method was evaluated with the recovery of the standards from excipients. Three different quantities (low, medium and high) of the authentic standards were added to the Placebo. The mixtures were extracted and analyzed using the developed HPLC method. Linearity test solutions were prepared as described in Formulation analysis. The LOD and LOQ for analytes were estimated by injecting a series of dilute solutions with known concentration. The Limit of Detection (LOD) and Limit of Quantification (LOQ) for analytes were estimated by injecting a series of dilute solutions with known concentration. Values of LOD and LOQ were calculated by using σ (standard Deviation of response) and b (Slope of the calibration curve) and by using equations, LOD=(3.3 x σ)/ b and LOQ=(10xσ)/b. To determine the robustness of the method, the final experimental conditions were purposely altered and the results were examined. The drug solution was determined using the samples for short-term stability by keeping at room temperature for 12 hrs and then analyzing. The long-term stability was determined by storing at 4^0C for 30 days. Auto-sampler stability was determined by

storing the samples for 24 hrs in the auto-sampler. For method development and optimization, retention factor (k) was calculated by using parameters t_R (retention time) and t_M (elution time of the solvent front) and by using the equation $k = (t_R - t_M)/ t_M$.

Choice of Internal Standard :
Different drugs like Nebivolol, Losartan, telmisartan, Amlodipine were investigated as internal standard. Most suitable internal standard producing a well resolved peak from the drug was Losartan (Figure 2).

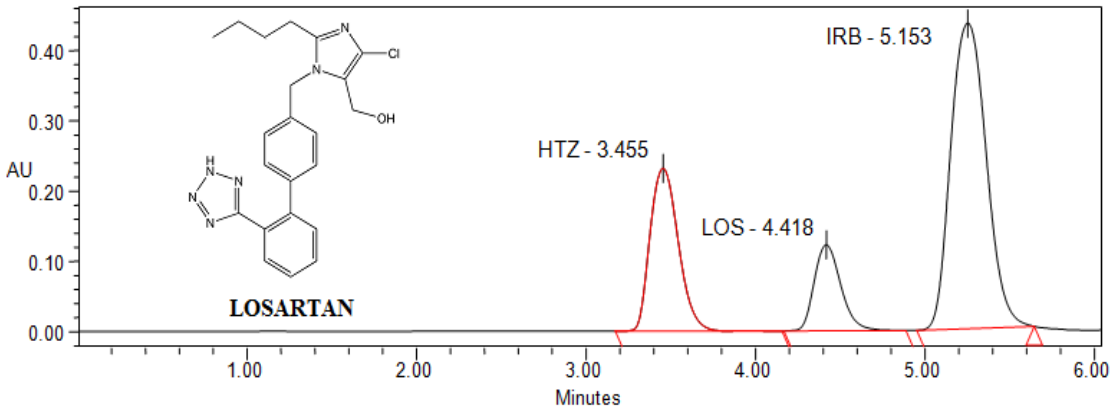

Figure 2: Chromatogram showing peaks of standard solutions of HTZ (20 µg/ml) , IRBE (240 µg/ml) and Losartan as an Internal Standard

Method optimization:
A well-defined symmetrical peak was obtained upon measuring the response of eluent under the optimized conditions after thorough experimental trials that can be summarized. Two columns were used for performance investigations, including Symmetry C18 (250 mm × 4.6 mm, 5.0 µ) and Kromasil C18 (250 mm × 4.6 mm, 5.0 µ), the First column was the most suitable one since it produced symmetrical peaks with high resolution. The UV detector response of HTZ and IRB was studied and the best wavelength was found to be 271 nm showing highest sensitivity.

Mobile phase composition
Several modifications in the mobile phase composition were performed in order to study the possibilities of changing the selectivity of the chromatographic system. These modifications included the change of the type and ratio of the organic modifier, the pH, the concentration of acetate buffer, the flow rate, the temperature and the stability of HTZ and IRB was also studied in methanol and mobile phase. The results obtained are shown in Table 1.

Type of organic modifier
Initially acetonitrile and water in different ratios were tried. But in that, both drugs showed peak broadening and the resolution was very less. So acetonitrile was replaced by methanol, THF and water was replaced with various buffers with different pH and concentration. Hence methanol: THF: acetate buffer pH 5 (60:10:30v/v) was suitable to get resolved and sharp peak. Methanol was the organic modifier of choice giving symmetrical narrow peaks and good Resolution reported in Table 1.

Ratio of organic modifier

The effect of changing the ratio of organic modifier on the selectivity and retention times of the test solutes was investigated using mobile phases containing concentrations of 60-40% methanol. Table 1 shows that 60% methanol was the best one giving well resolved peaks and higher no. of theoretical plates. Ratios less than 60% resulted in peaks with very long unacceptable retention times, where as ratios higher than 60% with decreased Resolution.

Effect of pH

The effect of changing the pH of the mobile phase on the selectivity and retention times of the test solutes was investigated using mobile phases of pH ranging from 4.0-7.0. Table 1 shows that a pH of 5.5 was the most appropriate one giving well-resolved peaks and highest no. of theoretical plates. At pH values > 5.0 produced peak broadening in the mobile phase.

Concentration of Acetate Buffer

The effect of changing the concentration of acetate buffer on the selectivity and retention times of the test solutes was investigated using mobile phases containing concentration of 10,15, 20,25,30,40 and 50 millimoles of acetate buffer. Figure 3 shows that 25 millimoles acetate buffer was found to be the most suitable giving best resolution and highest no. of theoretical plates because lower buffer concentrations showed Resolution problem.

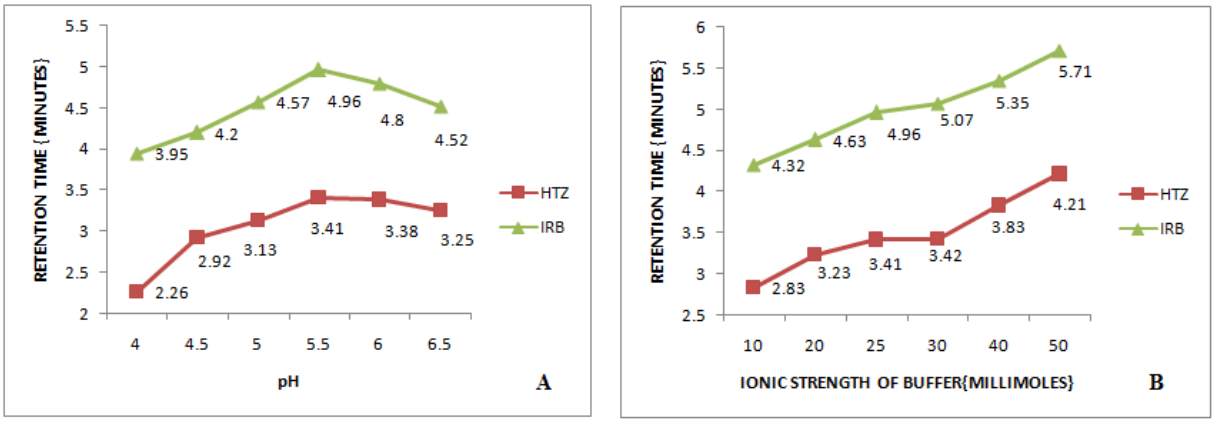

Figure 3

A. Effect of pH of Acetate Buffer in mobile phase on Retention times of HTZ and IRBE

B. Effect of Ionic strength of Acetate Buffer in mobile phase on Retention times of HTZ and IRBE

Effect of Flow rate

The effect of flow rate on the formation and separation of peaks was studied by varying the flow rate from 0.5-1.0; a flow rate of 0.75 ml/min was optional for good separation and resolution of peaks in a reasonable time.

Effect of Temperature

The effect of Temperature on the formation, separation and resolution was studied by varying the Temperature from 35 - 55^{o}C; we found that at lower Temperatures the peaks are not well resolved, whereas at 50 ^{o}C the peaks shows good symmetry and resolution.

Validation of method:

Linearity and range:

For the construction of calibration curves, six calibration standard solutions were prepared over the concentration range. Linearity was determined for HTZ in the range of 0.3-50 µg/ml and for IRBE 3.6-600 µg/ml. The correlation coefficient ('r') values were >0.999(n = 6). Typically, the regression equations for the calibration curve was found to be y = 1.2087x−6.6637 for HTZ, y=2.2752x−3.1905 for IRBE.

Precision and Accuracy:

The precision of repeatability of the method was determined by performing six replicate analyses of the same working solution. The relative standard deviation (R.S.D.) obtained for HTZ and IRBE were 0.60 and 0.81%, respectively. Intra-day precision of the developed LC method was determined by preparing the tablet samples of the same batch in nine determinations with three concentrations and three replicate each. The inter-day precision was also determined by assaying the tablets in triplicate per day for consecutive 3 days. The results indicated the good precision of the developed method (Table 1). Accuracy of the method was calculated by recovery studies at three levels by standard addition method. The mean percentage recoveries obtained for HTZ and IRBE were 99.50 and 99.35%, respectively (Table 2).

Table 1.System suitability parameters and results of precision at optimized chromatographic conditions

Compound	System Suitability		Precision of the Method b (n=5)		
	Parameter	Value	Actual Conc. (µg mL^{-1})	Measured conc. (µg/ml),% R.S.D	
				Intra-day	Inter-day
HTZ	Therotical plates	2912	5	4.97,0.65	5.09,0.75
	Peak Tailing	1.27	10	9.74,0.29	10.02,0.60
	% R.S.D.	0.69	15	15.07,0.87	15.06,0.98
IRBE	Therotical plates	4355	60	59.85,0.58	59.71,0.83
	USP resolution	5.65	120	119.69,0.73	120.05,0.55
	Peak Tailing	1.11	180	180.03,1.14	180.08,1.23
	% R.S.D.	0.77			

Table 2: Results of formulation analysis and accuracy studies

Compound (Label Claim)	Formulation Study (n=6)		Recovery (accuracy) Study	
	Tablet Batch	% Assay Found, % RSD	Recovery Level	% Recovery, % RSD(n=3)
HTZ	Batch I	98.82, 1.05	50	99.24, 0.39
	Batch II	99.13, 1.26	100	99.80, 0.83
			150	99.46, 0.31
IRBE	Batch I	99.80, 0.97	50	99.08, 0.59
	Batch II	99.48, 1.32	100	99.04,0.33
			150	99.94,0.42

Limit of detection (LOD) and Limit of quantitation (LOQ):

The LOD and LOQ values were found to be 0.3 and 0.99 µg/ml for both HTZ and IRBE, respectively.

Specificity:

The specificity of the HPLC method is illustrated in (Figure 2 & 4)**,** where complete separation of HTZ and IRBE was noticed in presence of tablet placebo. In addition there was no any interference at the retention time of HTZ and IRBE in the chromatogram of tablet solution. In peak purity analysis with photo diode array detector, purity angle was always less than purity threshold for all the analytes. This shows that the peak of analytes was pure and excipients in the formulation did not interfere the analytes.

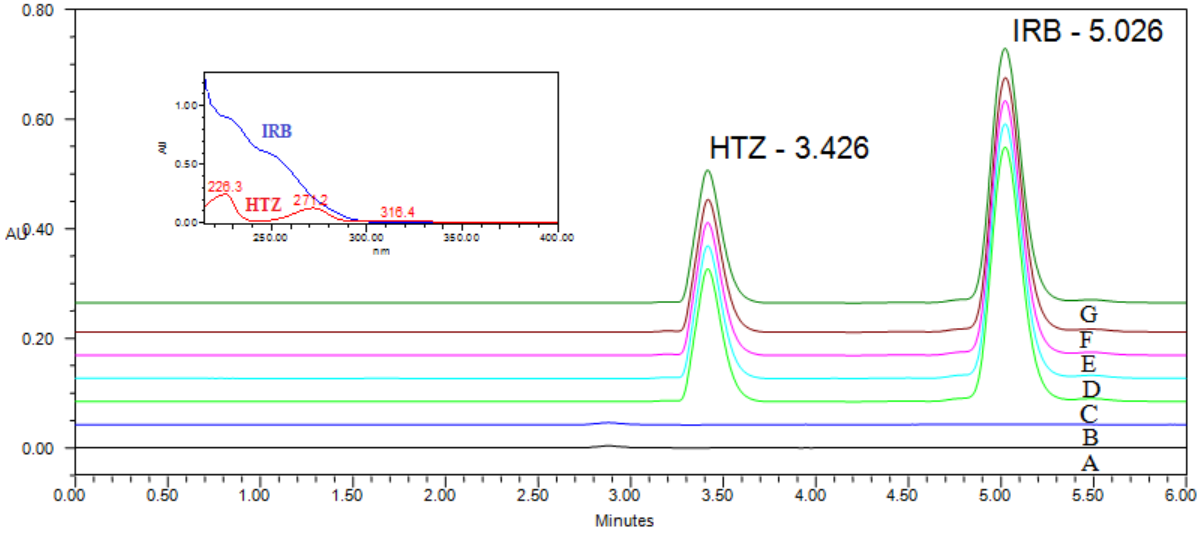

Figure 4: Specificity Chromatogram consists of A) Mobile Phase, B) Placebo, C) formulation, D-G) system suitability standards of HTZ (20 µg/ml) and IRBE (240 µg/ml) with online overlain PDA spectra

Robustness:

Robustness of the method was investigated under a variety of conditions including changes of flow rate, column temperature, column from different suppliers and wavelength of measurement. The mixed standard solution is injected in three replicates and sample solution of 100% concentration is prepared and injected in triplicate for every condition and % R.S.D. of assay was calculated for each condition. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters has proven that the method is robust (Table 3).

Table 3. Result of robustness study

Chromatographic parameters	**Retention time**		**Capacity factor**		**Tailing Factor**	
1: Flow Rate: -	**IRBE**	**HTZ**	**IRBE**	**HTZ**	**IRBE**	**HTZ**
0.71 (-5%)	5.368	3.615	4.36	2.61	1.16	1.29
0.75 (normal)	5.10	3.45	4.05	2.42	1.21	1.30
0.79 (+5%)	4.77	3.24	3.77	2.24	1.22	1.32
Mean ± SD (n=3)	5.07±0.23	3.43±0.18	4.06±0.29	2.42±0.36	1.19±0.072	1.31±0.03
2: Percentage of methanol in mobile phase:						
84	4.936	3.420	3.93	2.42	1.24	1.30
85(normal)	5.097	3.436	4.12	2.45	1.23	1.27

86	5.267	3.451	4.26	2.49	1.21	1.25
Mean ± SD (n=3)	5.1±0.16	3.435±0.19	4.10±0.12	2.46±0.06	1.23±0.053	1.28±0.08
3: Temperature:						
48 (-2%)	5.078	3.438	4.07	2.43	1.20	1.29
50 (normal)	5.054	3.427	4.01	2.42	1.20	1.30
52 (+2%)	4.921	3.420	3.92	2.42	1.25	1.32
Mean ±SD (n=3)	5.01±0.13	3.428±0.28	4.00±0.05	2.42±0.02	1.23±0.03	1.31±0.02
4: Columns form different manufacturers:						
Symmetry	5.058	3.421	4.03	2.42	1.21	1.32
Kromasil	5.12	3.518	4.25	2.51	1.25	1.35
Mean ±SD (n=3)	5.089±0.25	3.469±0.38	4.14±0.27	2.46±0.11	1.23±0.08	1.33±0.07
5: pH:						
5.4 (-0.1)	5.059	3.430	3.98	2.39	1.20	1.29
5.5 (normal)	5.054	3.427	4.06	2.43	1.21	1.30
5.6 (+0.1)	5.078	3.429	4.27	2.51	1.21	1.32
Mean ± SD (n=3)	5.063±0.068	3.429±0.021	4.10±0.24	2.46±0.11	1.21±0.032	1.31±0.042
6: Wavelength: % RSD of calculated contents (n= 6)						
269 (-1)	5.054	3.427	4.063	2.43	1.21	1.31
271 (normal)	5.053	3.427	4.062	2.43	1.21	1.30
272 (+ 1)	5.054	3.427	4.062	2.42	1.22	1.30
Mean ± SD (n=3)	5.054±0.024	3.427±0.10	4.062±0.022	2.42±0.021	1.30±0.021	1.30±0.021

Solution stability studies:
Stability as described in method validation under experimental section was studied. Result of short-term, long-term and the auto sampler stability of the HTZ and IRBE solutions were calculated form nominal concentrations and found concentration. Results of the stability studies were within the acceptable limit (97–103%).

CONCLUSION

Linear, precise, and accurate RP-HPLC-PDA method has been developed and validated for quantitative determination of HTZ and IRBE from two tablet formulations. The manuscript describes, for the first time simultaneous estimation of the combination. All the parameters for the two titled drugs met the criteria of ICH guidelines for method validation. The method is very simple, specific, reliable, rapid and economic nature as all peaks are well separated and there is no interference by excipients peaks with total runtime of 6 min, which makes it especially suitable for routine quality control analysis work and Dissolution studies. This method is also applicable for analysis of Plasma samples.

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REFERENCES

[1] S. C. Sweetman, Martindale; The Complete Drug Reference. Pharmaceutical Press, London, **1999**, 32, 316.
[2] ICH-Q2B Validation of Analytical Procedures: Methodology International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human

Use, Geneva, Switzerland, 1996.
[3] E. Satana, S. Altına, N.G.Gogera, S.A. Ozkan, Z. Senturk, *J. Pharm. Biomed.Ana.*,**2001**, 5, 1009
[4] A.R. Rote, P.D. Bari, *AAPS PharmSciTech.*, **2009**, 4,1200.
[5] C. Vetuschi, A. Giannandrea, G. Carlucci, P. Mazzeo, *Il Farmaco.*,**2005**,8,665.
[6] P.S. Tarte, S. P. Wate, P.B.Khedikar, G. Pawnikar, *Asian J. Research Chem.*, **2008,** 2, 74.
[7] Al-momani I F, *Turk J Chem.*,**2001**, 25, 49.
[8] S. J. Joshi, P. A. Karbhari, S. I. Bhoir, K. S. Bindu, C. Das, *J. Pharm.Biomed. Ana.*, **2010**, **52**, 362.
[9] S. S. Qutab, S. N. Razzaq, M. Ashfaq, Z. A. Shuja, I. U. Khan, *Acta Chromatographica.*, **2007**, 19,119.
[10] Z. Vujic, M. Crevar, V. Obradovic, V. Kuntic, S.U. Markovic. *Chromatographia.*, **2009**, 70, 1221.
[11] N. Erk, *J. Chromatogrphy B., 2003*,1, 195.
[12] S. Najma, A.M. Saeed, A.S. Shahid, S. Shahnawaz, *Chinese J. chromatography.*, **2008**,5, 544.
[13] A.K. Shakya, Y.M. Al-Hiari, M. O. Alhamami Omran, *J.chromatography B.*, **2007**, 2, 245.
[14] E. Caudron, S. Laurent, E. M. Billaud, P. Prognon, *J. chromatography B.*,**2004**,2, 339.
[15] S.Y. Chang, D.B. Whigan, N.M. Vachharajani, R. Patel, *J.chromatography B.*,**1997**,1-2, 149.
[16] H.E. Abdellatef, 2006 : *Spectrochimica Acta Part A.*, **2007**,4-5,1248.