ISSN: 0975-766X
Available Online through
www.ijptonline.com
Research Article

# SPECTROPHOTOMETRIC DETERMINATION OF ATORVASTATIN AND EZETIMIBE USING 2,4-DNP IN BULK AND PHARMACEUTICAL DOSAGE FORMS

**A. Shravya, R.S. Chandan*, B. M. Gurupadayya, M. Sireesha**
Department of Pharmaceutical Analysis, JSS College of Pharmacy, JSS University, Mysore-570 015, India.
E-mail:chandan2211@rediffmail.com

*Received on 11-10-2010* *Accepted on 30-10-2010*

**ABSTRACT:**

Two simple sensitive and cost effective spectrophotometric methods are described for the determination of atorvastatin calcium and ezetimibe in bulk and pharmaceutical formulations. The method is based on the oxidation of 2, 4- dinitrophenylhydrazine and coupling of the oxidized product with drugs to give intensely colored chromogen. Under the proposed optimum condition, Beer's law was obeyed at the concentration range of 4-12µg $ml^{-1}$ and 4-10µg$ml^{-1}$ for Atorvastatin and Ezetimibe respectively. The results of tablet analysis were found to be 101.25% and100.3%. No interference was observed from common pharmaceutical adjuvants. The method was validated according to ICH guidelines by performing linearity, accuracy, and precision, limits of quantization, Detection and selectivity.

**Keywords:** Atorvastatin, Ezetimibe, 2,4-DNP, U.V.Spectrophotometry, Validation.

## INTRODUCTION:

Atorvastatin calcium (Figure 1), is [R-(R*, R*)]-2-(4-fluorophenyl)-β,δ-dihydroxy-5-(1-methyl ethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1Hpyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate. The empirical formula of atorvastatin calcium is $(C_{33}H_{34}FN_2O_5)_2Ca•3H_2O$ . Atorvastatin calcium is an inhibitor of 3-hydroxy-3 methyl glutarylcoenzyme A (HMG-CoA) reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, an early and rate-limiting step in cholesterol biosynthesis [1-2]. The typical dose of Atorvastatin calcium is 10-80 mg per day and it reduces 40-60% LDL[3].

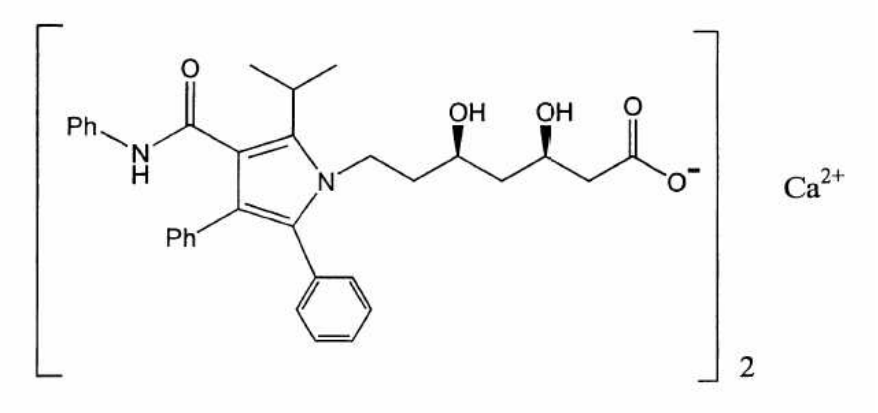



**Figure 1: Chemical Structure of Atorvastatin Calcium**

A few methods based on HPLC[4-5], GC-MS[6], LC-MS[7], HPLC – Electrospray tendem mass spectrometry[8] and HPTLC[9] were reported earlier for the determination of Atorvastatin calcium individually and in combination with other drugs.

Ezetimibe (Figure 2), (1-(4-flurophenyl)-3(R)-[3(S)-(4-flurophenyl) –3-hydroxy propyl] –4(S) (4 – hydroxyphenyl) azetidin- 2- one), prevents transport of cholesterol through the intestinal wall by selectively blocking the absorption of cholesterol from dietary and billiary sources. This reduces the overall delivery of cholesterol to the liver, thereby promoting the synthesis of LDL receptors and a subsequent reduction in serum LDL-C[10-11].

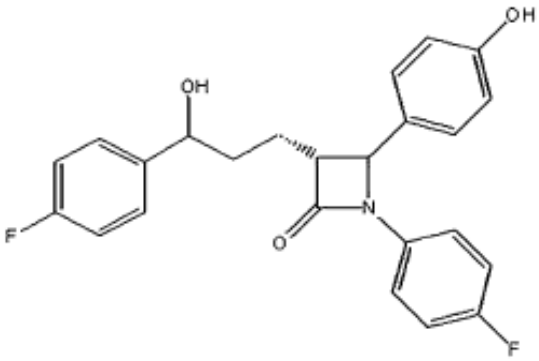



**Figure 2: Chemical Structure of Ezetimibe**

A few analytical procedures were also proposed for the determination of ezetimibe in dosage forms[12] in human serum, urine and feces[13]. A few analytical procedures were also proposed for the determination of atorvastatin

and ezetimibe both in combinations also in pure and pharmaceutical dosage forms in RP-HPLC[14], densitometric[15]. Some methods are developed using 2,4-DNP [16].

Although there are several methods developed on atorvastatin and ezetimibe in pure and pharmaceutical dosage forms no method was developed with 2,4-DNP.

## EXPERIMENTAL

### Apparatus

A Shimadzu UV-visible spectrophotometer model 1800 with 1 cm matched quartz cell was used for the absorbance measurements. Systonics electronic balance was used for weighing the samples.

### Chemicals and Reagents

Analytical reagent grade chemicals and double distilled water were used throughout the experiment. Standard atorvastatin and ezetimibe pure samples were obtained from Biocon pharmaceuticals, India and the formulation was purchased from the local market.

***2, 4-dinitrophenyl hydrazine (2, 4 DNP) 0.08 %( w/v)***

0.08 g of 2, 4 DNP was accurately weighed transferred into a 100 ml calibrated flask, dissolved in 10ml distilled water, and make up the volume up to the mark with distilled water to obtain a solution of 0.08% (w/v). The solution was freshly prepared and protected from light during the use.

***10 N Sodium hydroxide solution***

40 g of sodium hydroxide is accurately weighed and transferred into a 100.0ml volumetric flask and made up to the mark with distilled water.

***Potassium iodate 4% (w/v)***

4 g of potassium iodate is accurately weighed and transferred into a 100.0 ml volumetric flask and made up to the mark with distilled water.

***Standard solutions:***

The standard solutions of atorvastatin calcium and ezetimibe (1000 µg $ml^{-1}$) were prepared by dissolving 100mg of drug in 100ml of methanol. Working solutions of the drug were prepared by dilution of the stock solution. The marketed tablet form of atorvastatin calcium and ezetimibe used in the determination was Lipvas-

10 and Ezedoc10 with a labelled strength of 10 mg and 10mg which were manufactured by Cipla Pharmaceuticals Limited, Parwanoo, H.P, India and Lopin Ltd, Mumbai, India.

**Selection of Analytical Wavelengths for atorvastatin calcium and ezetimibe.**

A 1.5 ml quantity of 0.08% 2, 4 DNP solution, 1.5 ml of 4 % potassium iodate and 1ml of 10N sodium hydroxide were added into two test tubes and 0.7 ml of atorvastatin calcium and 0.8 ml ezetimibe stock solutions were added. The immediate coloured complex was formed. The solutions were made up to 10ml with water. The absorption spectrums of the complex were determined against blank solution and the wavelengths of maximum absorption ($\lambda_{max)}$ of the products of the reactions were noted.

**Effect of Reagent Concentration.**

The effect of varying the concentration of 2, 4 DNP was carried out using reagent concentrations of 0.05, 0.01, 0.02, 0.03, 0.04 ...0.08% in 10N NaOH and 4 % potassium iodate. After mixing 1.5 ml of each reagent concentration with the drug solutions of atorvstatin calcium and ezetimibe and made up to 10 ml with water, the absorbance readings of the complex formed were made at 479 nm and 457 nm on the UV-visible spectrophotometer.

**Optimization Studies.**

*Effect of 2, 4 DNP Concentration*

The studying of 2, 4-DNP concentrations revealed that the reaction was dependent on 2, 4 DNP reagent. The absorbance of the reaction solution increased as the 2, 4-DNP concentration increased, and the highest absorption intensity was attained at 2, 4-DNP concentration of 0.08 % (w/v). Higher 2, 4-DNP concentrations up to 1.5 % had no effect on the absorption values. Further experiments were carried out using 0.08 %.

**Preparation of calibration curve.**

Standard solutions of atorvastatin calcium and ezetimibe in methanol, having final concentrations in the range of 4-12 µg $ml^{-1}$ and 4-10 µg $ml^{-1}$, were transferred into a series of 10 ml volumetric flasks, to these solutions 1.5 ml of 0.08% 2, 4 DNP, 1.5 ml of 4% potassium iodate and 1 ml of 10N sodium hydroxide was added. The mixture was then gently shaken until the appearance of colour chromogen. The contents were diluted up to 10 ml with distilled water. The absorbance of each solution was measured at 479 nm and 457 nm respectively

against the reagent blank prepared in the same manner, without the analyte and the calibration curves (figure1,2) and absorption spectra(figure3,4) are represented respectively.

**Figure-1: Calibration graph of Atorvastatin.**

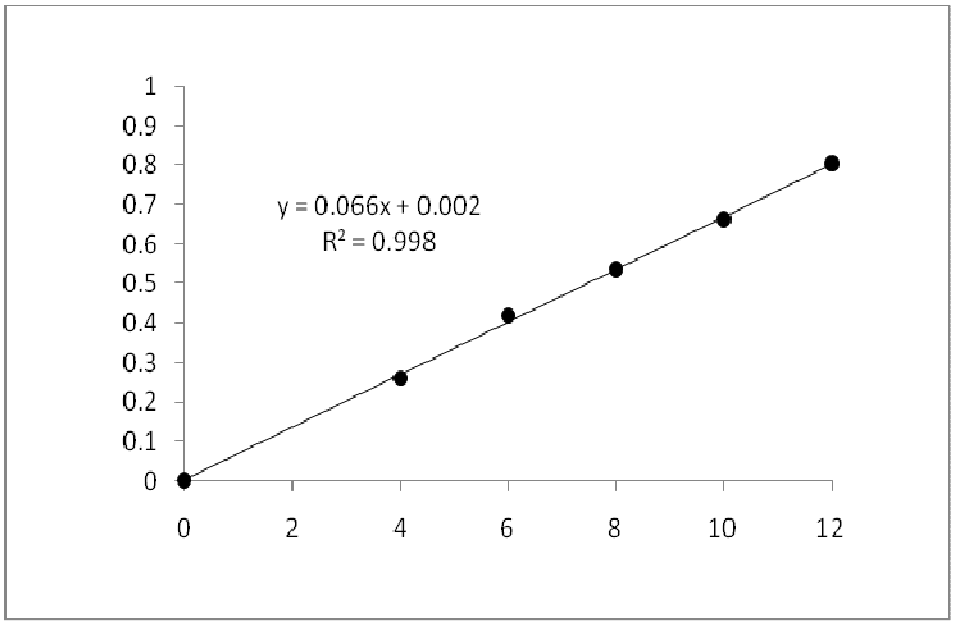



**Figure-2: Calibration graph of Ezetimibe.**

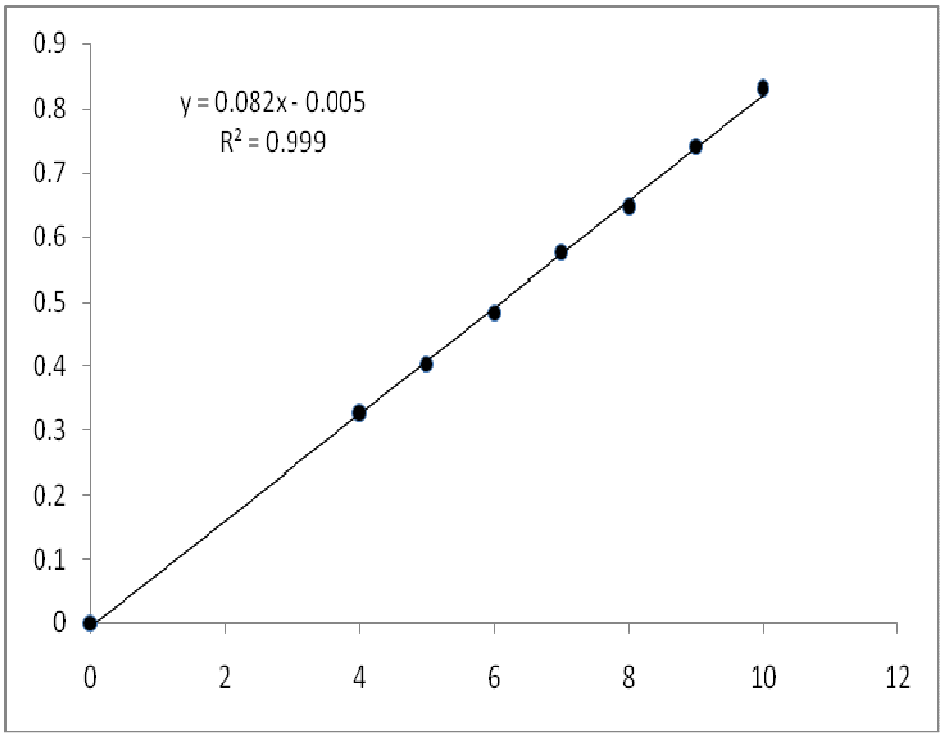


**Figure-3: Absorption Spectra of Atorvastatin.**

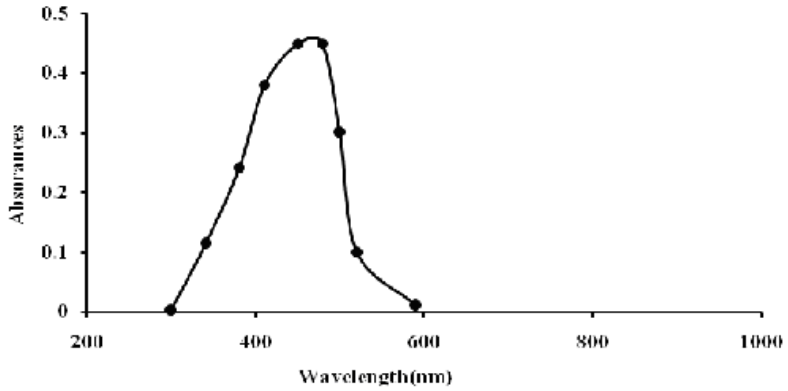
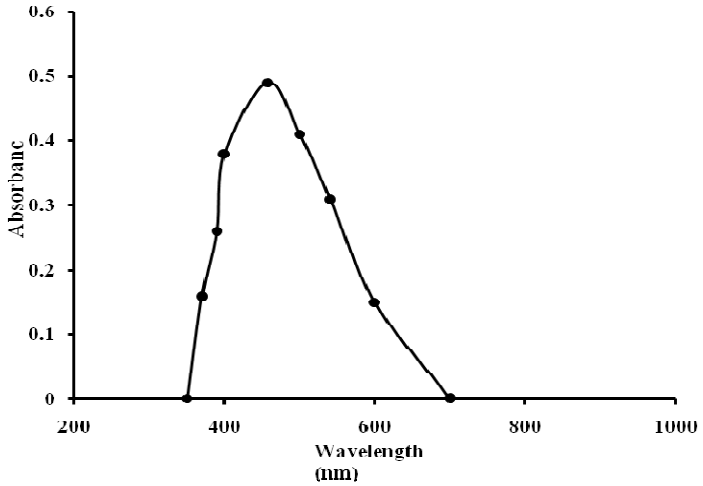



**Figure-4: Absorption spectra of Ezetimibe.**




**Analysis of commercial pharmaceutical preparations.**

***Tablets***

An appropriate amount of atorvastatin calcium and ezetimibe were dissolved in methanol so as to prepare 1000 µg $ml^{-1}$ solution. An aliquot of this solution was diluted with methanol to obtain concentrations of 7 µg $ml^{-1}$ and 8 µg $ml^{-1}$ respectively. To that solution 1.5 ml of 0.08% 2, 4 DNP, 1.5 ml of 4 % potassium iodate and 1 ml of 10N sodium hydroxide is added. The mixture was then gently shaken until the appearance of colour chromogen. The contents were diluted up to 10 ml with distilled water.

**General procedure:**

Several standard solutions of atorvastatin calcium and ezetimibe were taken in individual standard flasks. To each standard flask, 1.5 ml of 0.08% 2, 4 DNP, 1.5 ml of 4 % potassium iodate and 1 ml of 10N sodium

hydroxide was added. The mixtures were then shaken until the appearance of colour chromogen. The absorbance was measured at λ max at 479 nm and 457 nm for atorvastatin calcium and ezetimibe respectively against a blank similarly prepared by omitting the drug solution with water. The concentration of atorvastatin calcium and ezetimibe in each standard flask was obtained by interpolating the corresponding absorbance value from Beer's plot of standard atorvastatin calcium and ezetimibe solutions.

**Quantification**

The limits of the Beer's law, the molar absorptivity and the Sandell's sensitivity values were evaluated. Regression analyses of the Beer's law plots at their respective $\lambda$max values revealed a good correlation. Graphs of absorbance versus concentration showed zero intercept, and are described by the regression equation, $Y = bX + c$ (where $Y$ is the absorbance of a 1 cm layer, $b$ is the slope, $c$ is the intercept and $X$ is the concentration of the drug in $\mu gml^{-1}$) obtained by the least-squares method. The results are summarized in Table 1.

**Table 1. Optical characteristics.**

| S.No | Parameter | Values | |
|---|---|---|---|
| | | **Atorvastatin** | **Ezetimibe** |
| 1. | $\lambda_{max}$/ nm | 479nm | 457nm |
| 2. | Beers law limits (µg/ml) | 4.0-12.0 | 4.0-10.0 |
| 3. | Molar absorptivity (1 /mol/cm) | $558.65 \times 10^3$ | $409.43 \times 10^3$ |
| 4. | Correlation coefficient (R) | 0.998 | 0.999 |
| 5. | Sandell's sensitivity(ng $cm^{-2}$) | 0.0152 | 0.0232 |
| 6. | Regression equation (y) | y = 0.066x + 0.002 | y = 0.082x - 0.005 |
| 7. | Slope, *b* | 0.066 | 0.082 |
| 8. | Intercept, *c* | 0.002 | 0.005 |
| 9. | Relative standard deviation% | 0.265 | 0.388 |
| 10. | Limit of detection (µg/ml) | 0.14 | 0.06 |
| 11. | Limit of quantification(µg/ml) | 0.44 | 0.23 |

**Validation of the method**

The validity of the method for the assay of atorvastatin calcium and ezetimibe were examined by determining the precision and accuracy. This was determined by analyzing six replicates of the drug within the Beer's law limits. The low values of the relative standard deviation (R.S.D.) indicate good precision of the methods. To study the accuracy of the methods, recovery studies were carried out by the standard calibration curve method. For this, known quantities of pure atorvastatin calcium and ezetimibe were mixed with definite amounts of pre-analyzed formulations and the mixtures were analyzed as before. The total amount of the drug was then determined and the amount of the added drug was calculated by difference. The results are given in Table 2, 3. The average percent recoveries obtained were quantitative indicating good accuracy of the methods.

**Table 2: Results of recovery study by standard addition method for Atorvastatin.**

| S.no | Standard Atorvastatin (µg) | Sample Atorvastati (µg) | Absorbance at 479nm | Amount of Atorvastatin from std.graph | Recovery of std (mg) | % Recovery |
|---|---|---|---|---|---|---|
| 1 | 4 | 4 | 0.523 | 7.9 | 3.9 | 97.5% |
| 2 | 6 | 4 | 0.646 | 9.9 | 5.9 | 98.3% |
| 3 | 8 | 4 | 0.803 | 12.1 | 8.1 | 101.25% |

**Table 3: Results of recovery study by standard addition method for Ezetimibe.**

| S.no | Standard Ezetimibe (µg) | Sample Ezetimibe (µg) | Absorbance at 457nm | Amount of Ezetimibe from std.graph | Recovery of std (mg) | % Recovery |
|---|---|---|---|---|---|---|
| 1 | 2 | 2 | 0.407 | 3.98 | 1.98 | 99.0% |
| 2 | 3 | 2 | 0.624 | 6.01 | 3.01 | 100.3% |
| 3 | 4 | 2 | 0.826 | 7.90 | 3.9 | 97.5% |

**Precision**

The precision of the proposed methods was ascertained by actual determination of six replicates of fixed concentration of the drug within the Beer's range and finding out the absorbance by the proposed method in all the three drugs. The results are given in Table 4.

**Table 4: Evaluation of accuracy and precision**

| Drug | S.no | Label Claim (mg) | Amount found* (mg) | % Purity* | Average (%) | S.D | %R.S.D |
|---|---|---|---|---|---|---|---|
| Atorvastatin | 1 | 10 | 9.85 | 98.5 | 98.65 | 0.6 | 0.61 |
| | 2 | | 9.90 | 99.0 | | | |
| | 3 | | 9.82 | 98.2 | | | |
| | 4 | | 9.96 | 99.6 | | | |
| | 5 | | 9.79 | 97.9 | | | |
| | 6 | | 9.87 | 98.7 | | | |
| Ezetimibe | 1 | 10 | 9.90 | 99.0 | 98.9 | 0.6431 | 0.65 |
| | 2 | | 9.94 | 99.4 | | | |
| | 3 | | 9.96 | 99.6 | | | |
| | 4 | | 9.80 | 98.0 | | | |
| | 5 | | 9.83 | 98.3 | | | |
| | 6 | | 9.93 | 99.3 | | | |

SD. Standard deviation; RSD.relative standard deviation.

**Accuracy**

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts of bulk samples of atorvastatin calcium and ezetimibe within the linearity range were taken and added to the pre-analyzed formulation.

**Ruggedness**

To ascertain the ruggedness of the methods, six replicate determinations at different concentration levels of the drugs were carried out. The intra-day RSD values were less than 1%. The values of between-day RSD for different concentrations of drugs obtained from the determinations and indicate that the proposed method has

reasonable ruggedness. The within-day RSD values were less than 1%. The values of inter-day RSD for different concentrations of drugs obtained from the determinations and indicate that the proposed method has reasonable ruggedness.

**Results and discussion:**

***Spectral characteristic***

The absorption spectra of the reaction product of oxidized 2, 4 DNP with drugs show maximum absorption (λmax) at 479 nm and 457 nm for of atorvastatin calcium and ezetimibe respectively. The blank solution had negligible absorbance at the λmax in which the drugs were analysed. The thus formed color was stable for more than two hours.

***Reaction sequence and stoichiometric relationship***

The 2, 4 DNP is oxidized by potassium iodate to give diazonium cation that reacts with drugs by electrophilic substitution at the phenolic ring to give deep colored chromogens. The proposed reaction sequence for atorvastatin calcium and ezetimibe .

**CONCLUSION**

The reagents utilized in the proposed methods are cheap, readily available and the procedures do not involve any critical reaction conditions or tedious sample preparation. Moreover, the methods are free from interference by common additives and excipients. The wide applicability of the new procedures for routine quality control was well established by the assay of atorvastatin calcium and ezetimibe in pure form and in pharmaceutical preparations.

**ACKNOWLEDGEMENTS**

The authors express their sincere thanks to Biocon pharmaceuticals, Bangalore, India for supplying the gift samples of atorvastatin calcium and ezetimibe. Thanks to the Principal, JSS College of Pharmacy, Mysore, for providing the necessary facilities.

**REFERENCES:**


1. M.J.O Neil. The Merck Index. 14th edn. Merck Research Laboratories Station, NJ.**2006** P.864
2. P. Beringer, Remingtons – The Science and Practice of Pharmacy, 21st edn.VOL-II, Mack publishing, Easton, PA, **2005**, P.1368
3. Page, Curtis, Sutter, Walkar and Hoffmann (**2002**) Integrated Pharmacology 2nd, Mosby International Ltd., p 303
4. Altuntas. T. G. and Erk, N., (2004 ), *J.Liq. Chro.* , 27 (1) : 83
5. Gowari, Sankar D. ,Raju, M.S.M Sumanth, Kalyan S. and Latha, P.V.M., (**2005**), *Asian J. Chem*, 17 ( 4) : 2571
6. McKenny. J.M., Mccormik, L.S., Weis.S., Korean, M., Kotonek.S.. and Black. D.M., A (**1998**), *Amer. J. Med*., 104: 137.
7. Black, A. E. Sinz, M.W., hayees, R.N. and Woolf, T.F., (**1998**) *Drug Metab. Dispor*.,26:755
8. Bullen, W. W., Miller, R.A., Hayes, R.N., (**1999**), *Amer. Soc. Mass Sprectrum*., 10:55
9. Yadav, S.S., Mhaske, D.V., Kakad, A.B., Patil, S.R., A (**2005**) *Indian Journal. Pharm. Sci*.,67 (2): 182.
10. E. Leitersdorf. *Int. J. Clin.Pract.* 56(2), 116 (**2002**).
11. M.Von Heek, C. Farley, D.S. Compton. *Br. J. Phamacol.* 134, 409 (**2001**).
12. Sistla R, Tata V S S K and Diwan P V, *J Pharm Biomed Anal.*, **2005**, 39, 517.
13. Oswald S, Scheuch E, Cascorbid I. and Siegmund W, *J Chromatogr B* , **2006**, 830**,** 143-150.
14. Jayapal Reddy Sama, Rama Rao Kalakuntla, V. Surya Narayana Rao, P. Reddanna, Scholars Research Library, Der Pharmacia Lettre, 2010: 2 (1) 427-436.
15. S. S. Dhaneshwar, S. R. Dhaneshwar, P. Deshpande, and M. Patil, Acta Chromatographica, 2007, No. 19.
16. Padmarajaiah Nagaraja and Ashwinee Kumar Shrestha , E-Journal of Chemistry, 2010, 7(2), 395-402.
17. ICH Draft Guidelines on Validation of Analytical Procedures: Definitions and Terminology, Federal Register. Vol. 60, IFPMA, Switzerland, 1995, p.11-260, 1995.



**Corresponding author:**

**R.S.Chandan***

JSS College of Pharmacy,

JSS University,

Mysore-570015,

**E-mail**:*chandan2211@rediffmail.com*