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Development and validation of a RP- HPLC method for simultaneous estimation of enalapril maleate and ramipril in bulk and tablet dosage form

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ABSTRACT

A simple reversed-phase high-performance liquid chromatographic (RP-HPLC) method has been developed and validated for simultaneous determination of Enalapril maleate and Ramipril in bulk and tablet dosage form. Chromatographic analysis was performed on a Oyster BDS C18 column (250x 4.6 mm, 5µm) column temperature 65˚C with a mixture of buffer A and buffer B in the ratio 50:50 [Buffer A preparation: 2 gm of sodiumperchloride in 800ml water and add 0.5 ml tri ethyl amine, adjust the pH to 3.6±0.1 with phosphoric acid and add 200 ml of acetonitrile. Buffer B preparation: 2 gm of sodiumperchloride in 300 ml water and add 0.5 ml tri ethyl amine, adjust the pH to 2.6±0.1 with phosphoric acid and add 700 ml of acetonitrile] as mobile phase, at a flow rate of 1.0 mL min^{-1}. UV detection was performed at 208 nm. The method was validated for accuracy, precision, specificity, linearity and sensitivity. The retention times of Enalapril maleate and Ramipril were 4.197 and 5.819 min, respectively. Calibration plots were linear over the concentration ranges 5–30 µg mL^{-1} and 5–30 µg mL^{-1} for Enalapril maleate and Ramipril, respectively. The Limit of detection was 0.571 and 1.090 µg mL^{-1} and the quantification limit was 1.733 µg mL^{-1} and 3.303 µg mL^{-1} for Enalapril maleate and Ramipril, respectively. The accuracy of the proposed method was determined by recovery studies and found to be 98.06% to 100.47%. Commercial tablet formulation was successfully analyzed using the developed method and the proposed method is applicable to routine analysis of determination of Enalapril maleate and Ramipril in bulk and tablet dosage form.

Keywords: Enalapril maleate, Ramipril, RP-HPLC, validation

INTRODUCTION

Enalapril

Enalapril is a prodrug which is hydrolysed in the body to Enalaprilat, which is an inhibitor of angiotensin-converting enzyme (ACE). It is indicated for treatment of hypertension, treatment of symptomatic heart failure and prevention of symptomatic heart failure in patients with asymptomatic left ventricular dysfunction (ejection fraction <35%) [1]. chemically it is ((S)-1-{N-[1-(ethoxycarbonyl)-3-phenylpropyl]-L-alanyl}-L-proline, (Z)-2-butenedioate (1:1), a derivative of two amino-acids, L-alanine and L-proline (fig.1). It is a white to off-white crystalline, odourless powder which melts in the range of 143–144 ◦C [2]. ACE is a peptidyldipeptidase that catalyzes the conversion of angiotensin I to the vasoconstrictor substance, angiotensin II, which stimulates aldosterone secretion by the adrenal cortex. Blocking the conversion of the angiotensin I to the angiotensin II, leads to a reduction in vasopressin activity and a decrease in peripheral vascular resistance [3, 4]. Oral enalapril is rapidly absorbed, with peak serum

concentrations of enalapril occurring within one hour. The extent of absorption of enalapril from oral enalapril tablet is approximately 60%. The absorption of oral enalapril is not influenced by the presence of food in the gastro-intestinal tract. The determination of enalapril has been carried out by numerous analytical methods such as HPLC [5–12], UV spectroscopic method [13].

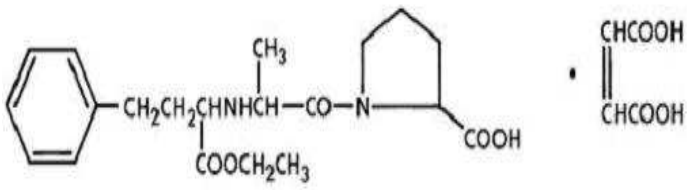

Fig. 1. Structure of Enalapril maleate

Ramipril

Ramipril is chemically (2*S*, 3*aS*, 6*aS*)-1-[(2*S*)-2-{[(2*S*)-1-ethoxy-1-oxo-4-phenylbutan-2-yl] amino} propanoyl]-octahydrocyclopenta[*b*]pyrrole-2-carboxylic acid [14], (fig.2). It is white or almost white crystalline powder. Freely soluble in Methanol, Slightly soluble in Water. Which melts in the range of 109 ◦C. It is an angiotensin-converting enzyme (ACE) inhibitor, used to treat hypertension and congestive heart failure. It is a prodrug and is converted to the active metabolite ramiprilat by liver esterase enzymes. It is an official drug in IP [15] and BP [16]. There are very few analytical methods reported for the estimation of Ramipril which includes RP-HPLC [17-24].

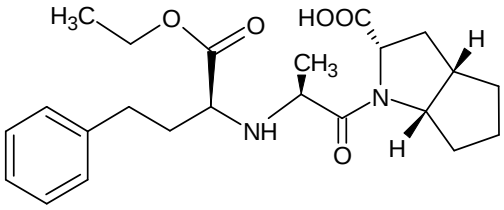

Fig. 2. Structure of Ramipril

But these methods are sophisticated, expensive and time consuming when compared to simple HPLC method. There is need for a interest to develop simple, accurate, specific, sensitive, precise and reproduciable simultaneous RP-HPLC method for the estimation of Enalapril maleate and Ramipril in bulk and its formulation.

MATERIALS AND METHODS

Pure standard drug of Enalapril maleate and Ramipril (Assigned purity 99.98%) was obtained as a gift sample from Hetero labs Pvt. Ltd, Baddi, India. The gift samples were used as standard without further purification. HPLC grade methanol (Qualigens), sodium per chloride, phosphoric acid and tri ethyl amine (S.D. fine chemicals, Mumbai, India), were used throughout the experiment. Commercial pharmaceutical preparation (ENVAS RB (Enalapril, Ramipril), VOLTA (CADILA)) which was claimed to contain 10mg of Enalapril maleate and 10 mg of Ramipril is used in analysis. The chemical structure and purity of the sample obtained was confirmed by TLC, IR, Melting point studies. HPLC grade Acetonitrile from Merck specialties Pvt Ltd, Mumbai. Water HPLC grade was obtained from Rankem laboratories.

2.2. Instrumentation and Chromatographic Conditions

High performance liquid chromatography, HPLC (WATERS 2695), UV-VIS detector was used. Isocratic elution of mobile phase comprising of Chromatographic analysis was performed on a Oyster BDS C18 column (250x 4.6 mm, 5µm) column temperature 65˚C, with a mixture of buffer A and buffer B in the ratio 50:50 [Buffer A preparation: 2 gm of sodiumperchloride in 800 mL water and add 0.5 mL tri ethyl amine, adjust the pH to 3.6±0.1 with phosphoric acid and add 200 mL of acetonitrile. Buffer B preparation: 2 gm of sodiumperchloride in 300 mL water and add 0.5 mL tri ethyl amine, adjust the pH to 2.6±0.1 with phosphoric acid and add 700 mL of acetonitrile] as mobile phase, at a flow rate of 1.0 mL min^{-1}. UV detection was performed at 208 nm. The retention times of enalapril maleate and ramipril were 4.197 and 5.819 min. The column temperature was maintained at ambient and the volume of injection was 20 µL. Prior to injection of analyte, the column was equilibrated for 30 min with mobile phase.

2.3. Preparation of mobile phase

The HPLC grade solvents were used for the preparation of mobile phase, isocratic elution of mobile phase comprising of with a mixture of buffer A and buffer B in the ratio 50:50 [Buffer A preparation: 2 gm of sodiumperchloride in 800mL water and add 0.5 mL tri ethyl amine, adjust the pH to 3.6±0.1 with phosphoric acid and add 200 mL of acetonitrile. Buffer B preparation: 2 gm of sodiumperchloride in 300 mL water and add 0.5 mL tri ethyl amine, adjust the pH to 2.6±0.1 with phosphoric acid and add 700 mL of acetonitrile] as mobile phase and filtered before use through a 0.45 µm membrane filter, sonicated and pumped from the solvent reservoir to the column at a flow rate of 1 mL min^{-1}.

2.4. Standard solution

Standard stock solutions 1 mg mL^{-1} of enalapril maleate and ramipril were prepared in mobile phase and further diluted in mobile phase. The working standard solutions were prepared in mobile phase to contain mixture of Enalapril maleate and Ramipril in over the linearity range from 5-30 µg mL^{-1} and 5-30 µg mL^{-1}.

2.5. Assay in formulation

Twenty tablets each containing and their average weight was calculated. The tablet were crushed to furnish a homogeneous powder and a quantity equivalent to one tablet were weighed in to a 100 mL volumetric flask, dissolve in mobile phase, sonicated for about 15 min and then made up to volume with mobile phase. The solution was stirred for 10 min using a magnetic stirrer and filtered into a 100 mL volumetric flask through 0.45 µm membrane filter. The residue was washed 3 times with 10 mL of mobile phase, and then the volume was completed to 100 mL with the same solvent. Further add mobile phase to obtain an expected concentration of 10 µg mL^{-1} enalapril maleate and 10 µg mL^{-1} ramipril. All determinations were conducted in triplicate.

RESULTS AND DISCUSSION

The proposed HPLC method required fewer reagents and materials and it is simple and less time consuming. This method could be used in quality control test in pharmaceutical industries. The chromatogram of enalapril maleate and ramipril were shown in (Fig.3). There was clear resolution between enalapril maleate and ramipril with retention time of 4.197 and 5.819 minutes, respectively.

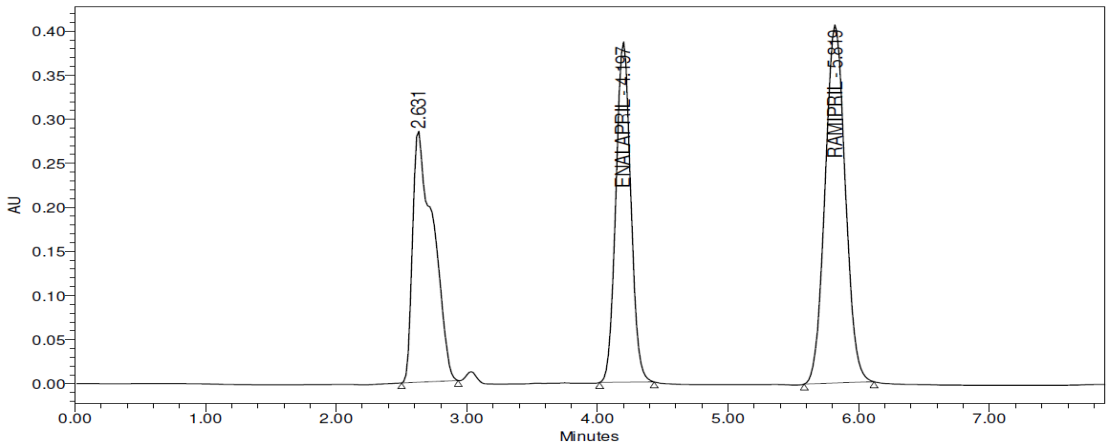

Fig. 3. Typical chromatogram of Enalapril maleate and Ramipri.

3.1. Linearity

The response was determined to be linear over the range of 5µg mL-1 to 30µg mL^{-1} (5, 10, 15, 20, 25, 30) for Enalapril maleate and 5- 30 µg mL^{-1} (5, 10, 15, 20, 25, 30) for Ramipril. The solutions were injected into HPLC system. Each of the concentration was injected to get reproduciable response. The run time was 15 min and the peak areas were measured (Table 1 & 2). The calibration curve was plotted as concentration of the respective drug versus the response at each level. The purposed method was evaluated by its correlation coefficient and intercept value calculated by statistical study. They were represented by the linear regression equation (Fig 4 and 5 calibration curve).

$Y_{Enalapril}$ = 53087x - 98013 Coefficient of correlation (r^2) value = 0.999
$Y_{Ramipril}$ = 65905x - 16789 Coefficient of correlation (r^2) value = 0.999

Table 1. For Peak area of Enalapril maleate

Conc in µg mL^{-1}	Area
0	0
5	2431068
10	5161578
15	7885747
20	10589529
25	13206310
30	15781198

Table 2. For Peak area of Ramipril

Conc in µg mL^{-1}	Area
0	0
5	2967718
10	6325745
15	9721440
20	13133747
25	16235347
30	19641463

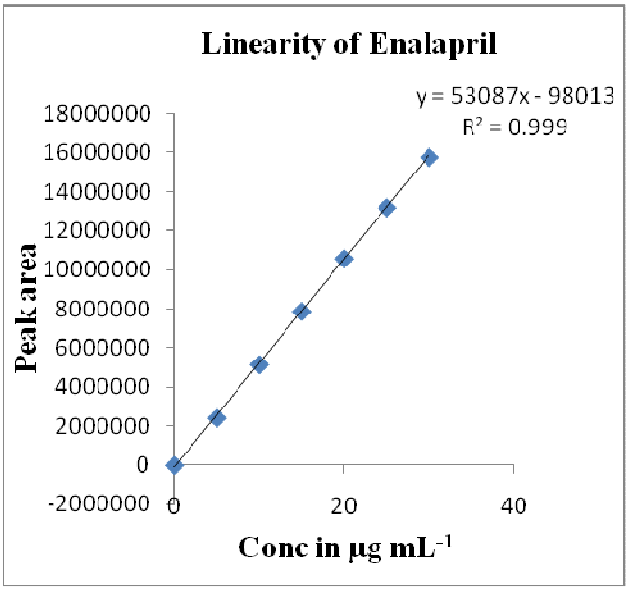

Fig. 4. Calibration curve for Enalapril

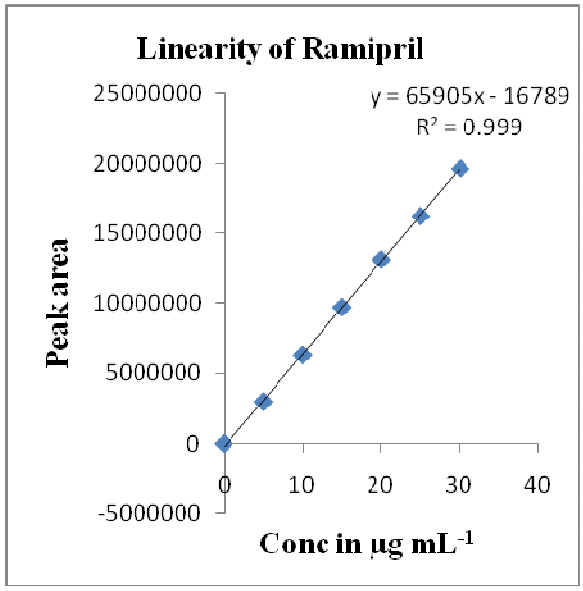

Fig. 5. Calibration curve for Ramipril

3.2. Accuracy:

The accuracy is the closeness of the measured value to the true value for the sample. Accuracy was found out by recovery study from prepared solution (three replicates) with standard solution, of the label claim. Aliquots of 50%, 100%, 150% weight of sample drug solution were pipetted into each of three volumetric flasks and prepare the serial dilution to get 20 µg mL^{-1}. To prepare for two each standard drug solution were pipetted into each of six volumetric flasks. To this 10 mL of enalapril standard drug solution of 100 µg mL^{-1} was added to each of three volumetric flask respectively. To this 10 mL of ramipril standard drug solution of 100 µg mL^{-1} was added to each of three volumetric flask respectively. The volume was made up to 100 mL with mobile phase. 20 µL of each solution was injected and chromatograms were recorded. The range was found between 98.06 % to 100.47 % respectively. The values of recovery justify the accuracy of the method. The % recovery values were obtained within the standard limit which confirms that the method is accurate and free from any positive or negative interference of the excipients (Table 3).

Table 3. Result of recovery studies

Recovery studies	**Enalapril**	**Ramipril**
Accuracy std	10588754	13154765
	10587895	13161276
	10578943	13170432
Avg	10585197.33	13162157.67
Accuracy sample	10586587	13157863
50%spike	15720959	19763154
	15821783	19778034
	15830256	19768790
Avg	15790999.33	19769992.67
Amt recovered	**49.17**	**50.24**
%Recovery	**98.33**	**100.47 %**
100%spike	20825663	26358495
	21031548	26364587
	21041432	26370032
Avg	20966214.33	26364371.33
Amt recovered	**98.06**	**100.34**
%Recovery	**98.06**	**100.34 %**
150%spike	26760044	32893798
	26762154	32893798
	25770843	32891084
Avg	26431013.67	32892893.33
Amt recovered	**149.68**	**149.94**
%Recovered	**99.79**	**99.96 %**

3.3. Limit of Detection and Quantification

Limit of detection is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected. The detection limit (LOD) and quantitation limit (LOQ) may be expressed as:

$$L.O.D. = 3.3(SD/S)$$
$$L.O.Q. = 10(SD/S)$$

Where, SD = Standard deviation of the response, S = Slope of the calibration curve. The slope S may be estimated from the calibration curve of the analyte.

The LOD was found to be 0.571 µg mL^{-1} and 1.090 µg mL^{-1} and LOQ was found to be 1.733 µg mL^{-1} and 3.303 µg mL^{-1} for enalapril and ramipril respectively which represents that sensitivity of the method is high.

3.4. Precision

Repeatability involves analysis of replicates by the analyst using the same equipment and method and conducting the precision study over short period of time while reproducibility involves precision study at different occasions, different laboratories, and different batch of reagent, different analysts, and different equipments. The repeatability study which was conducted on the solution having the concentration of about 10 µg mL^{-1} for enalapril and 10 µg mL^{-1} for ramipril showed a RSD of 0.09 % for enalapril and 0.17 % for ramipril. It was concluded that the analytical technique showed good repeatability (Table4).

Table 4. Results of reproducibility analysis (Method precision)

Enalapril

S.No.	RT	Area
1	3.602	10591539
2	3.601	10553534
3	3.601	10569019
4	3.602	10592134
5	3.602	10565347
6	3.602	10570126
Avg	3.601667	10573617
SD	0.000516	**15291.34**
%RSD	**0.01**	**0.14 %**

Ramipril

S.No.	RT	Area
1	6.897	13176780
2	6.893	13138144
3	6.887	13172805
4	6.897	13169845
5	6.895	13141542
6	6.895	13153265
Avg	6.894	13158730
SD	0.003742	**16712.44**
%RSD	**0.05**	**0.13 %**

Table 5.Results of Repeatability analysis (System precision)

Enalapril

S.No.	RT	Area
1	3.608	10616395
2	3.603	10597338
3	3.604	10603294
4	3.606	10621453
5	3.602	10605438
6	3.602	10602543
Avg	3.604167	10607744
SD	0.002401	**9200.346**
%RSD	**0.07**	**0.09 %**

Ramipril

S.No.	RT	Area
1	6.933	13170043
2	6.915	13175060
3	6.905	13204350
4	6.935	13165763
5	6.902	13211543
6	6.902	13212154
Avg	6.915333	13189819
SD	0.01524	**21769.45**
%RSD	**0.22**	**0.17 %**

3.5. Reproducibility and Ruggedness

The ruggedness of an analytical method is determined by analysis of aliquots from homogenous lots by different analysts using operational and environmental conditions that may differ but are still within the specified parameters of the assay. The assay was performed in different condition, different analyst, and different dates **(**Table 6).

Table 6. Results of reproducibility

Parameters	Enalapril	Ramipril
Average Percentage Recovery	98.71%,	100.25%
SD between set of analysis on same date	15291.34	16712.44
SD between set of analysis on different date	9200.34	21769.45
RSD between set of analysis on same date	0.14%	0.13%
RSD between set of analysis on different date	0.09%	0.17%

3.6. Robustness

The robustness of the method was determined by delibrate changes in the method like alteration in pH of the mobile phase, percentage organic content, changes in the wavelength. The robustness of the method shows that there were no marked changes in the chromatographic parameters, which demonstrates that the method developed is robust.

3.7. Specificity

The selectivity of an analytical method is its ability to measure accurately and specifically the analyte of interest in the presence of components that may be expected to be present in the sample matrix. If an analytical procedure is able to separate and resolve the various components of a mixture and detect the analyte qualitatively the method is called selective. It has been observed that there are no peaks of diluents and placebo at main peak's. Hence, the chromatographic system used for the estimation of enalapril and ramipril is very selective and specifi. Specificity studies indicating that the excipients did not interfere with the analysis. For demonstrating the specificity of the method for drug formulation the drug was spiked and the representative chromatogram (Fig.6).

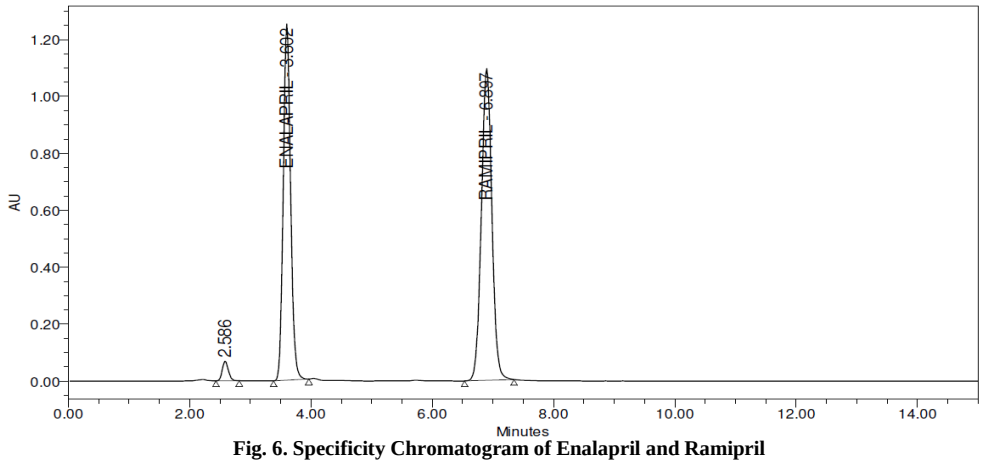

Fig. 6. Specificity Chromatogram of Enalapril and Ramipril

3.8. System Suitability

A binary solution of 10 µg $mL^{-1}$of enalapril and 10 µg mL^{-1} of ramipril (in triplicate) was prepared and same was injected, then the system suitability parameters like resolution factor (*Rs*), tailing factor (*Tf*) and theortical plates (*N*) were calculated and recorded in Table 6. The values for system suitability parameters showed feasibility of this method for routine pharmaceutical application.

Table 7. Results of system suitability parameters

S.No.	Parameters	Enalapril values	Ramipril values
1.	Theoritical Plates (N)	4155.00	7641.00
2.	Resolution (*Rs*)	5.03	12.36
3.	Tailing factor (*Tf*)	1.11	1.02

CONCLUSION

The proposed RP-HPLC method is found to be simple, accurate, precise, linear, and specific for quantitative estimation of enalapril and ramipril in bulk and its formulation. The proposed RP-HPLC method is cost effective and less time consuming. The values for system suitability parameters showed feasibility of this method for routine pharmaceutical application. Hence the present HPLC method is suitable for routine assay of enalapril and ramipril in raw materials and in pharmaceutical formulations in the quality control laboratories.

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REFERENCES

[1] http://www.medicines.org.uk/EMC/medicine/22427/SPC/Enalapril/
[2] Ip DP, Brenner GS, in: K. Florey (Ed.) Analytical Profiles of Drug Substances, Academic Press Orlando FL: **1987**, 207–243.
[3] Franz DN, Gennaro AR. Remington, The Science and Practice of Pharmacy, 19th Edition, Mack Publishing Company: Pennsylvania. 2: p 951.
[4] Warner GT, Perry CM, *Drugs*. **2002**, 62: 1381-1405
[5] Al-Momani IF , *Turk J Chem*. **2001**, 25: 49-54.
[6] Thongnopnua P, Poeaknapo C , *J Pharm Biomed Anal*. **2005**,37: 763-769.
[7] Tajerzadeh H, Hamidi M , *J Pharma Biomed Anal*. **2001**,24: 675-680.
[8] Qin XZ, Demarco J, Dominic PI , *J Chromatogr* A. **1995**, 707: 245–254.
[9] Safila Naveed, Najma Sultana and M.Saeed Arayne, *Analytical & Bioanalytical Techniques*. **2012**, 3: 1-4.

[10] Mehrdad Hamidi, Hosnieh Tajerzadeh, *Journal of Pharmaceutical and Biomedical Analysis*. **2001**, 24 (4): 675–680.
[11] Tamboli A.M,Chetan Chavan, Mohite S.K , *Journal of Pharmacy Research*. **2010**, 3(11): 2564-2567.
[12] Marta de Diego, Gloria Godoy, Sigrid Mennickent and Ricardo Godoy, *International year of chemistry*. **2011**, 34(3): 450-454.
[13] Sunil Kumar Dubey, Sandeep Kumar, Rajeev J Mudakavi, Swapnil Deshpande, Akash Kumar Jain, *International Journal of Advances in Pharmaceutical Sciences*. **2010**, 1: 375-380.
[14] Laurence L Brunton , John S Lazo, Keith Parker. Goodman & Gilman's,The pharmacological basis of therapeutics, 11th Edition, MC Graw Hill Medical publishing division: New Delhi, **2005**, p 803.
[15] Indian Pharmacopoeia Indian Pharmacopoeia Commission: Ghaziabad. **2010**, 3: p 2038.
[16] British Pharmacopoeia Ph Euro monograph 1590. Medicines and Health care products Regulatory Agency (MHRA): London. **2003**, 2: p 1609.
[17] Kalyana Ramu B and Raghubabu K , *International Journal of Chemistry Research*. **2011**, 2(2): 15- 19.
[18] Priyanka R Patil, Sachin U Rakesh, Pandurang N Dhabale, and Kishor B Burade , *Research J Pharm Tech*. **2009**, 2(2): 304-307.
[19] Srinivasa Rao and Srinivas K, *Indian Journal of Pharmaceutical Sciences*. **2010**, 72 (1): 108-111.
[20] Chintan V. Patel, Amit P. Khandhar, Anandi D. Captain, Kalpesh T. Patel , *Eurasian Journal of Analytical Chemistry*. **2007**, 2(3), 159-171.
[21] Yogesh gupta, shanti nagar, Gopal pura, Tonk road, *Asian journal of pharmaceutical and clinical research*. **2009**,2(4): 104-111.
[22] Manju Latha YB, Gowri Sankar D , *Inventi journals Pharma Ana & Qual Assur*. **2012**: 270.
[23] Sunil Jawla, Jeyalakshmi K, Krishnamurthy T, Kumar Y, *International Journal of PharmTech Research*. **2010**, 2(2): 1625-1633.
[24] Lakshmi K. S and Lakshmi Sivasubramanian , *Journal of the chilean chemical society*. **2010**, 55(2): 223-226.