Eurasian Journal of Analytical Chemistry
Volume 2, Number 3, 2007

Validated Absorption Factor Spectrophotometric and Reversed-Phase High-Performance Liquid Chromatographic Methods for the Determination of Ramipril and Olmesartan Medoxomil in Pharmaceutical Formulations

Chintan V. Patel[a], Amit P. Khandhar[b], Anandi D. Captain[a], Kalpesh T. Patel[c]

[a] A.R.College of Pharmacy and Institute of Science and Technology for Advance Studies and Research (I.S.T.A.R) Vallabh Vidya nagar- 388120; Gujarat, India.

[b] Analytical Development Laboratory, Cadila Healthcare Limited, Moraiya Ahmedabad, Gujarat,India.

[c]Torrent Research Center, Bhatt, Ahmedabad-382 210; Gujarat, India.

Abstract

Two simple and sensitive spectrophotometric and liquid chromatographic (LC) methods are described for the determination of ramipril and olmesartan medoxomil. The first method was based on the absorption factor. Ramipril and olmesartan medoxomil exhibit λ_{max} at 210 nm and 256 nm respectively. Olmesartan medoxomil has some interference at 210nm, while ramipril do not show any absorption at 256 nm.Quantitative estimation of ramipril was carried out by subtracting the absorption due to olmesartan medoxomil at 210 nm using experimentally calculated absorption factor. Beer's law was obeyed for ramipril and olmesartan medoxomil at 2-6 µg mL^{-1} and 8-24 µg mL^{-1} respectively. The second method, high-performance liquid chromatographic method was developed for the determination of ramipril and olmeasartan medoxomil using sodium perchlorate: acetonitrile (60:40, v/v) as the mobile phase and measuring the response at λ_{max} 210 nm. The analysis was performed on a Phenomenex C_8 (250 X 4.0 mm), 5 µm column. The calibration curve was obtained for ramipril and olmesartan medoxomil at 1-6 µg mL^{-1} and 4-24 µg mL^{-1} respectively. The mean recovery was 99.80 ± 0.20% and 99.86 ± 0.20% for ramipril and olmesartan medoxomil respectively. The methods were validated according to the ICH guidelines.

***Key words*:** Ramipril; Olmesartan medoxomil; Absorption factor method; Reversed-phase; HPLC;

1. Introduction

Ramipril and olmesartan medoxomil fixed dose combination tablet contains ramipril and olmesartan medoxomil as antihypertensive agent.

ISSN: 1306-3057

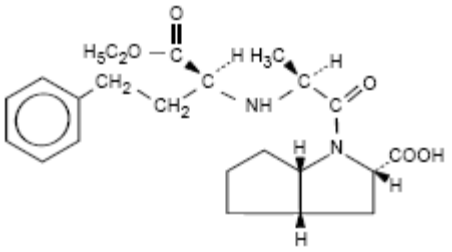

Fig.1. Structure of Ramipril

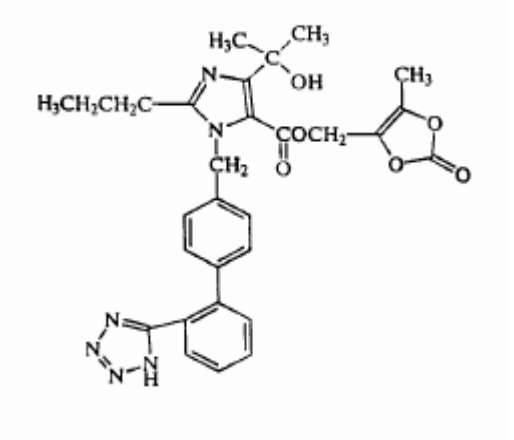

Fig.2. Structure of Olmesartan medoxomil

Ramipril's chemical name is (2S, 3aS, 6aS) -1[(S)-N-[(S) -1-Carboxy-3-phenylpropyl] alanyl] octahydrocyclopenta[b] pyrrole-2-carboxylic acid, 1-ethyl ester. Ramipril is an angiotensin-converting enzyme (ACE) inhibitor. An inactive prodrug, ramipril is converted to ramiprilat in the liver and is used to treat hypertension and heart failure, to reduce proteinuria and renal disease in patients with nephropathies, and to prevent stroke, myocardial infarction, and cardiac death in high-risk patients. Ramiprilat, the active metabolite, competes with angiotensin I for binding at the angiotensin-converting enzyme, blocking the conversion of angiotensin I to angiotensin II [1]. As angiotensin II is a vasoconstrictor and a negative-feedback mediator for renin activity, lower concentrations result in a decrease in blood pressure and an increase in plasma rennin. Ramiprilat may also act on kininase II, an enzyme identical to angiotensin-converting enzyme that degrades the vasodilator bradykinin [2]. Olmesartan medoxomil is described chemically as 2, 3-dihydroxy-2-butenyl 4-(1-hydroxy-1-methylethyl)-2-propyl-1-[p-(o-1H-tetrazol-5-ylphenyl) benzyl] imidazole-5-carboxylate, cyclic 2, 3-carbonate. Olmesartan medoxomil, a specific angiotensin II type 1 antagonist, is used alone or with other antihypertensive agents to treat hypertension. Unlike the angiotensin receptor antagonist losartan, Olmesartan does not have an active metabolite or possess uricosuric effects. Blockade of the angiotensin II receptor inhibits the negative regulatory feedback of angiotensin II on renin secretion, but the resulting increased plasma renin activity and circulating angiotensin II levels do not overcome the effect of olmesartan on blood pressure. Angiotensin II is formed from angiotensin I in a reaction catalyzed by angiotensin converting enzyme (ACE, kininase II) [3]. Angiotensin II is the principal presser agent of the renin-angiotensin system, with effects that include vasoconstriction, stimulation of synthesis and release of aldosterone, cardiac stimulation and renal reabsorption of sodium [4]. Olmesartan blocks the vasoconstrictor effects of angiotensin II by selectively blocking the binding of angiotensin II to the angiotensin 1

receptor in vascular smooth muscle [5, 6]. Fixed dose combination of both these component in one tablet is 5 milligrams of Ramipril/ 20 milligrams of Olmesartan medoxomil once daily.

Literature survey did not reveal any reported method for the analysis of olmesartan medoxomil neither in combination with any other drug nor alone. Even it is not official in Indian Pharmacopoeia (IP), British Pharmacopoeia (BP), and United State Pharmacopoeia (USP). But various analytical methods for quantitative determination of ramipril in pharmaceutical formulations have been reported in literature like LC-MS (Liquid chromatography-mass spectrophotometry) [7], Atomic-absorption spectrometry [8], Capillary electrophoresis [9], HPLC (High-performance liquid chromatography) [10, 11],. Spectrophotometry and atomic-absorption spectrometry [12], Spectrophotometry [13], RP-HPLC (Reverse phase-high performance liquid chromatography) [14].

The non-availability of UV-Spectrophotometry method and High-Performance LC method until now for the simultaneous analysis of these components made it worthwhile objective to pursue the present research work. Therefore, in the proposed work, a successful attempt has been made to develop analytical method with due consideration of accuracy, sensitivity, rapidity, economy.

2. Experimental

2.1. Instrument and Condition

Ultraviolet-visible (UV-Vis) spectrophotometer : Model UV-1700 (Shimadzu, Tokyo, Japan). HPLC system: Shimadzu LC 2010C integrated system equipped with quaternary gradient pump, 2010C UV-Vis detector, and 2010C-column oven and 2010C programmable auto sampler controlled by CLASS-VP software.

Chromatographic parameters:

- Column- Phenomenex C_8 (250 X 4.0 mm), 5 µm
- Detector- UV-Visible.
- Wavelength- 210nm,
- Flow rate- 1.5 mL min^{-1}.
- Mobile phase- Sodium perchlorate buffer: acetonitrile (60: 40)
- Sodium perchlorate buffer- 3 g Sodium perchlorate in to 1000 mL stoppered glass cylinder. Add 500 mL milli-Q water and dissolve it. Makes volume up to the mark with milli-Q water + add 3.0 mL triethylamine, adjust pH 3.0 with orthophosporic acid
- Ratio of sodium perchlorate buffer: triethylamine (100: 0.3)
- Diluent – acetonitrile: water (50:50)

Table 1. System suitability and system precision (For HPLC)

Compound	**Retention time (Mean ± SEM)**	**n**	**k'**	**R**	**T**	**α**
Ramipril	6.92 ± 0.0020	924.50	3.62	-	1.12	-
Olmesartanmedoxomil	9.00 ± 0.0010	7317.55	5.01	3.14	1.31	1.38

n= Theoretical plates
k'= Capacity Factor
R= Resolution
T= Asymmetry
α = Selectivity

2.2. Reagents

Ramipril reference standard. - Assigned purity, 99.8% (Dr Reddy's Laboratory, Hyderabad, India).

Olmesartan medoxomil reference standard - Assigned purity, 98.9% (Hetro Drugs Limited, India).

Acetonitrile. – AR grade (Spectrochem).

Sodium perchlorate, ortho-phosphoric acid and triethylamine. – AR grade, E-Merck Limited.

Commercially available ramipril and olmesartan medoxomil tablets. - Claimed to contain 5 mg and 20 mg of the drug. Procured from the Cadila Healthcare Limited, Moraiya, Ahmedabad, India.

2.3. Standard Stock Solution (for spectroscopy method)

Standard stock solution of 200 $\mu g.mL^{-1}$ and 800 $\mu g.mL^{-1}$ was prepared by dissolving 20 mg of ramipril and 80 mg of olmesartan medoxomil in to a 100 mL of volumetric flask add 50 mL of diluent and sonicate it to dissolve. Make up volume up to mark with diluent and prepare final concentration 4 $\mu g.mL^{-1}$ for ramipril and 16 $\mu g.mL^{-1}$ olmesartan medoxomil.

2.4. Sample preparation (for HPLC method)

An accurately weighed five intact tablets equivalent to 25 mg of ramipril and 100 mg of olmesartan medoxomil in to a 100 mL volumetric flask. Add 50 mL of diluent and sonicate it for 30 min. make up volume up to mark with diluent, mix and filter it through 0.45 µm HVLP filter. Make appropriate dilution to get final concentration of ramipril 5 $\mu g.mL^{-1}$ and olmesartan medoxomil 20 $\mu g.mL^{-1}$.

2.5. Spectrophotometric method

2.5.1. Absorption factor methods

2.5.1.1. Construction of calibration curve

λ_{max} of ramipril (4 µg.mL^{-1}) and olmesartan medoxomil (16 µg.mL^{-1}) was determined by scanning the drug solution in diluent and was found to be at 210 nm and 256 nm respectively. Olmesartan medoxomil also showed absorbance at 210 nm, while ramipril did not show any interference at 256 nm. To construct Beer's plot for ramipril and olmesartan medoxomil, dilutions were made in diluent using stock solution. Also Beer's plot was constructed for ramipril and olmesartan medoxomil in solution mixture at different concentration (2:8 3:12, 4:16, 5:20, and 6:24) µg.mL^{-1} levels. Both the drugs followed linearity individually and in mixture within the concentration range 2-6 µg.mL^{-1}and 8-24 µg.mL^{-1}for ramipril and olmesartan medoxomil respectively.

2.5.1.2. Determination of absorption factor at selected wavelengths

Ramipril and olmesartan medoxomil solution in diluent of known concentrations were scanned against blank on spectrophotometer. The value of absorption factor was found to be 1.7456. Quantitative estimation of the ramipril and olmesartan medoxomil was carried out using following equation.

$$\text{Absorption of ramipril at 210 nm} = \text{abs}_{210}\ (\text{olme} + \text{rami}) - \left(\frac{\text{abs}_{210}\ (\text{olme})}{\text{abs}_{256}\ (\text{olme})}\right) \text{x}\ \text{abs}_{256}\,(\text{olme} + \text{rami})$$

Where; abs: Absorption value, rami: Ramipril and olme: Olmesartan medoxomil

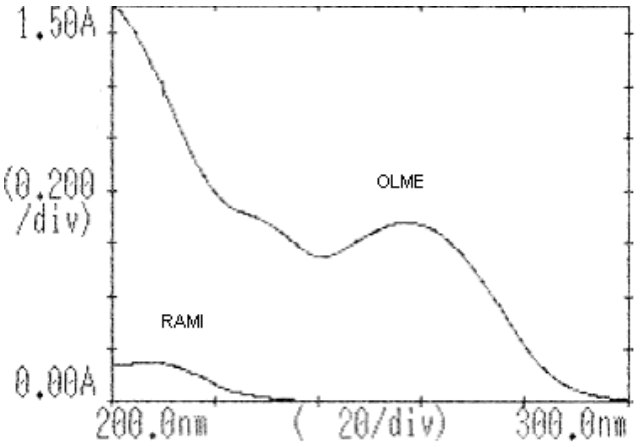

Figure 3. Overlay spectrum of ramipril and olmesartan medoxomil in water: acetonitrile (50:50). RAMI (4 µg.mL^{-1}) is ramipril, OLME is olmesartan medoxomil (16 µg.mL^{-1}) taken on UV-Vis spectrophotometer. (Shimadzu 1700).

2.5.1.3. Assay of tablet formulation

An accurately weighed five intact tablets equivalent to 25 mg of ramipril and 100 mg of olmesartan medoxomil in to a 100-mL volumetric flask. Add 50 mL of diluent and sonicate it for 30 min. filter it through 0.45 µm HVLP filter. Make appropriate dilution to get final concentration of ramipril 5 $\mu g.mL^{-1}$ and olmesartan medoxomil 20 $\mu g.mL^{-1}$. Appropriated aliquots were subjected to above methods and the concentrations of ramipril and olmesartan medoxomil were determined.

2.5.2. HPLC Method

2.5.2.1. Construction of calibration curve

To construct Beer's plot for ramipril and olmesartan medoxomil, dilutions were made in diluent using stock solution. Also Beer's plot was constructed for ramipril and olmesartan medoxomil in solution mixture at different concentration (1:4, 2:8 3:12, 4:16, 5:20, and 6:24) $\mu g.mL^{-1}$ levels. Both the drugs followed linearity individually and in mixture within the concentration range 1-6 $\mu g.mL^{-1}$and 4-24 $\mu g.mL^{-1}$ for ramipril and olmesartan medoxomil respectively.

2.5.2.1. Assay of tablet formulation

An accurately weighed five intact tablets equivalent to 25 mg of ramipril and 100 mg of olmesartan medoxomil in to a 100-mL volumetric flask. Add 50 mL of diluent and sonicate it for 30 min. filter it through 0.45 µm HVLP filter. Make appropriate dilution to get final concentration of ramipril 5 $\mu g.mL^{-1}$ and olmesartan medoxomil 20 $\mu g.mL^{-1}$. The concentrations of ramipril and olmesartan medoxomil were determined.

3. Result and discussion

3.1. Spectrophotometry method

The proposed analytical method is simple, accurate and reproducible. Ramipril and olmesartan medoxomil showed λ_{max} at 210 nm and 256 nm respectively. As their λmax differ more then 20nm, absorption factor method was tried for their simultaneous estimation in formulation. Olmesartan medoxomil also showed absorbance at 210 nm and give interference in determination of ramipril. Quantitative estimation of ramipril was carried out by subtracting interference of olmesartan medoxomil using experimentally calculated absorption factor.

3.2. HPLC Method

Considering the efficiency of HPLC, attempt has been made to develop simple, accurate, precise, rapid and economic method for simultaneous estimation of ramipril and olmesartan medoxomil in a tablet dosage form. Thus method described enables to the quantification of ramipril and olmesartan medoxomil. The advantages lie in the simplicity of sample preparation and the low costs of reagents used. It has been found that this method is also applicable for Inertsil ODS-2 and Phenomenex C-8 column (250X 4.6 mm), 5µm. The contribution of another important factor is its LOD. Experimental results were indicative of satisfactory precision and reproducibility. Hence, this HPLC method can be used for analysis of solid dosage form in quality control department.

3.3. Linearity

It can be seen that plot is linear over the concentration range of 2 to 6 µg.mL^{-1} and 8 to 24 µg.mL^{-1} for ramipril and olmesartan medoxomil with a correlation coefficient (r^2) 0.99982 and 0.99995 respectively in case of spectrophotometric method and 0.99958 and 0.99993 in case of HPLC method. The results obtained were presented in Table 2.

Table 2. Characteristics of the analytical method derived from the standard calibration curve.

Compound	LOD (µg mL^{-1})	LOQ (µg mL^{-1}) n=(5)	Linearity range (µg mL^{-1})	Correlation co-efficient r^2	Residual std. regression σ	Slope of regression S
Spectrophotometry						
Ramipril	0.5	1.5	2 to 6	0.99982	0.00167	0.03725
Olmesartan medoxomil	0.5	1.5	8 to 24	0.99995	0.00400	0.04138
HPLC						
Ramipril	0.08	0.024	2 to 6	0.99958	7815.145	34502.838
Olmesartan medoxomil	0.04	0.012	8 to 24	0.99993	10653.250	46087.139

LOD= Limit of detection
LOQ= Limit of quantification

3.4. Standard and sample solution stability

Standard and sample solution stability was evaluated at room temperature for 48 h. The relative standard deviation was found below 2.0%. It showed that both standard and sample solution was stable up to 48 h at room temperature.

3.5. Method precision

The relative standard deviation for six replicates of sample solution was less than 2.0%, which met the acceptance criteria established for spectrophotometric and HPLC methods. The results obtained were presented in Table 3.

Table 3. Method Precision

Spectrophotometric				
Compound	Concentration µg mL^{-1} (n=6)	Absorbance Mean ± SEM (n=6)	%Assay Mean±SEM (n=6)	% RSD (n=6)
Ramipril	4	0.199 ± 0.0013	101.0 ± 0.6591	1.6
Olmesartan medoxomil	16	0.879 ± 0.0037	101.8 ± 0.4070	1.0
HPLC				
Compound	Concentration µg mL^{-1} (n=6)	Retention time Mean ± SEM (n=6)	%Assay Mean±SEM (n=6)	% RSD (n=6)
Ramipril	4	7.49 ± 0.0000	100.5 ± 0.1902	1.2
Olmesartan medoxomil	16	9.70± 0.0000	100.5 ± 0.1197	0.7

3.6. Accuracy

Accuracy is performed at three levels 50%, 100% and 150% of target concentration. Percentage recovery and low relative standard deviation value show's accuracy of the spectrophotometric and HPLC methods. The data presented in Table 4.

Table 4 Method accuracy

Spectrophotometric				
Level	Drug added (mg)	Drug recovered (mg)	% Assay (Mean ± SEM) (n=3)	% RSD of assay (n=3)
Ramipril				
50%	10.20	10.18	100.0 ± 0.0000	0.6
100%	20.00	19.96	100.0 ± 0.0003	0.4
150%	29.80	29.87	100.4 ± 0.0003	0.4
Olmesartan medoxomil				
50%	40.00	39.57	100.0 ± 0.0006	0.2
100%	80.20	79.14	99.8 ± 0.0005	0.2
150%	120.20	118.70	99.8 ± 0.0000	0.2
HPLC				
Level	Drug added (mg)	Drug recovered (mg)	% Assay (Mean ± SEM) (n=3)	% RSD of assay (n=3)
Ramipril				
50%	10.20	10.18	99.9 ± 0.2268	0.7
100%	20.10	20.06	99.6 ± 0.2081	0.6
150%	30.10	30.04	99.9 ± 0.2775	0.8
Olmesartan medoxomil				
50%	40.20	39.77	100.0 ± 0.2589	0.8
100%	80.30	79.43	99.9 ± 0.0986	0.3
150%	120.10	118.50	99.7 ± 0.2673	0.8

3.7. Method ruggedness

Ruggedness test was determined between two different days, analysts and instruments. The value of RSD was to be found below 2.0% showed ruggedness of developed spectrophotometric and HPLC method. The results of ruggedness were presented in Table 5.

Table 5. Method ruggedness

Spectrophotometric		
Compound	% Assay Mean ± SEM (n=6)	% RSD of assay (n=6)
Day 1, Analyst-1, Instrument-1		
Ramipril	101.0 ± 0.6591	1.6
Olmesartan medoxomil	101.8 ± 0.4070	1.0
Day 2, Analyst-2, Instrument-2		
Ramipril	101.9 ± 0.3071	0.7
Olmesartan medoxomil	101.7 ± 0.2587	0.6
HPLC		
Compound	% Assay Mean ± SEM (n=6)	% RSD of Assay (n=6)
Day 1, Analyst-1, Instrument-1		
Ramipril	100.5 ± 0.1902	1.2
Olmesartan medoxomil	100.5 ± 0.1197	0.7
Day 2, Analyst-2, Instrument-2		
Ramipril	100.1 ± 0.2038	1.3
Olmesartan medoxomil	100.4 ± 0.1934	1.2

3.8. Method robustness

The method was found to be robust, as small but deliberate changes in the method parameters have no detrimental effect on the method performance as shown in Table 6. Low value of relative standard deviation indicating the method was robust.

Table 6. Method robustness

Compound	% RSD in normal	Changed condition (n=5)	
Temperature		(-5°C)	(+5°C)
Ramipril	0.31	0.64	0.40
Olmesartan medoxomil	0.47	0.41	0.21
pH		(-0.2 unit)	(+0.2 unit)
Ramipril	0.31	0.94	0.44
Olmesartan medoxomil	0.47	0.66	0.32
Compound	% RSD in normal	Changed condition (n=5)	
Flow rate		(-10%)	(+10%)
Ramipril	0.31	0.70	0.94
Olmesartan medoxomil	0.47	0.29	0.63
Mobile phase ratio		(-2%)	(+2%)
Ramipril	0.31	0.85	0.25
Olmesartan medoxomil	0.47	0.39	0.22

3.9. Specificity

There was no interference from placebo and peak purity of ramipril and olmesartan medoxomil were 0.99994 and 0.99992 respectively. It shows that developed analytical method was specific for its intended purpose.

4. Conclusions

The methods described in this paper for the determination of ramipril and olmesartan medoxomil in pharmaceutical formulation are simple, accurate, sensitive, and reproducible. The proposed methods use inexpensive solvents and instruments that are available in developing countries. The proposed methods could be applied for routine analysis in quality control laboratories.

Acknowledgements

The authors are thankful to Zydus Cadila Limited, Ahmedabad, India for providing reference standards and all facilities to complete this research work.

References

1) Kukushkin S K, Lebedev A V, Manoshkina E M, Shamarin V M (1998) Ramipril effects on 24-hour profile of blood pressure in patients with mild and moderate hypertension. J Pharm Biomed Anal 70(9): 69-71.
2) Reinhard H A., Becker M D, Bernard M D, Schölkens M D (1987) Ramipril: Review of pharmacology. J Clin Pharmacol 59(10): 3-11.
3) Strawn W B, Chappell M C, Dean R H (2000) Inhibition of early atherogenesis by losartan in monkeys with diet-induced hypercholesterolemia, J.Pharm.Biomed Anal., 101(7):1586.
4) Püchler K, Laeis P, Stumpe K O (2001) A comparison of the efficacy and safety of the oral angiotensin II antagonist Olmesartan medoxomil with those of atenolol inpatients with moderate to severe hypertension under continuous treatment with hydrochlorothiazide. J Pharm Biomed Anal 19 (2): 153.
5) http://www.fda.gov/medwatch/SAFETY/2004/nov_PI/Benicar_Tab_PI.pdf
6) Ball K, A (2001) Multicentre, double-blind, efficacy, tolerability and safety study of the oral angiotensin II antagonist Olmesartan medoxomil versus losartan in patients with mild to moderate essential hypertension. J Pharm Biomed Anal 19 (2): 153.
7) Zhimeng Z, Andre V, Len N.(2002) Liquid chromatography–mass spectrometry method for determination of Ramipril and its active metabolite ramiprilat in human plasma. J Chromatogr B 779(2): 297-306.
8) Magda M, Ayad A, Shalaby E, Abdellate F (2002) Spectrophotometric and AAS determination of Ramipril and Enalapril through ternary complex formation J Pharm Biomed Anal 28 (2): 311.

9) Hillaert S, Grauwe K, Bossche W (2001) Simultaneous determination of hydrochlorothiazide and several inhibitors of angiotensin-converting enzyme by capillary electrophoresis. J Chromatogr A 924 (1):439.
10) Belal F, Al-Zaagi A, Gadkariem E, Abounassif M (2001) A stability-indicating LC method for the simultaneous determination of ramipril and hydrochlorothiazide in dosage forms J Pharm Biomed Anal 24(3):335.
11) Barry L, Mark Williams, H, Anna I, Veysoglu T, Parente E (2000) Development and validation of a liquid chromatographic method for the determination of the related substances of ramipril in Altace capsules. J Pharm Biomed Anal 23 (4):637-651.
12) Hisham E, Magda M, Elham A (1999) Spectrophotometric and atomic absorption spectrometric determination of ramipril and perindopril through ternary complex formation with eosin and Cu (II). J Pharm Biomed Anal 18 (6):1027.
13) Bonazzi D, Gotti R, Andrisano V, Cavrini V (1997) Analysis of ACE inhibitors in pharmaceutical dosage forms by derivative UV spectroscopy and liquid chromatography (HPLC). J Pharm Biomed Anal 16(3):431-438.
14) Zarapkar S S, Rane S H (2000) RP- HPLC determination of ramipril and hydrochlorothiazide in tablet Indian. J Pharma Sci 37(12):589-593.
15) Daharwal S J, Garg G, Saudagar R B, Saraf S (2006) Methods of estimation of multi-component formulations. Indian J Pharma Sci 19(8):102.
16) Stenlake J B, Backett A H (1997) Practical pharmaceutical Chemistry, 4th Edition, Part-2, C. B. S. Publishers, p281.
17) Tipre D N, Kasture A V (2000) Simultaneous estimation of Ciprofloxacin and Tinidazole in tablet dosage form using spectrophotometric and HPLC methods. Indian Drugs 37(3): 148-154.

Received: April 10, 2007; Accepted: August 22, 2007

Corresponding author:
A.R.College of Pharmacy and Institute of Science and Technology for Advance Studies and Research (I.S.T.A.R) Vallabh Vidya nagar- 388120; Gujarat India.

Phone: +91-9909418342

Fax: +91-2717-250319

E-mail: chintanpatel84@gmail.com