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Der Pharmacia Lettre, 2011, 3(2): 194-202
(http://scholarsresearchlibrary.com/archive.html)

Der Pharmacia Lettre
Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Validated RP-HPLC Method for simultaneous determination of Atorvastatin and Ramipril and its application in drug formulation

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ABSTRACT:

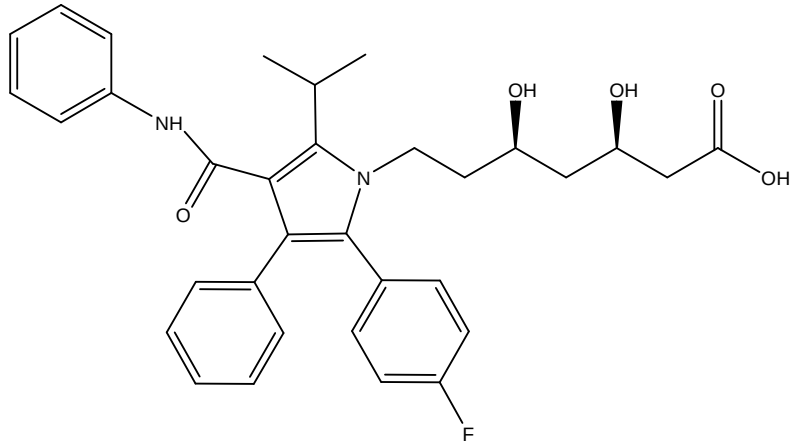
A rapid, sensitive and specific RP-HPLC method involving UV detection was developed and validated for determination and quantification of Atorvastatin and Ramipril. Chromatography was carried out on a Phenomenex – Luna, C18 (250 x 4.6 mm i.d.,5µ) column. using filtered and degassed mixture of acetonitrile ,water and methanol (55:40:5) as mobile phase at a flow rate of 1.0 ml/min and effluent was monitored at 237nm. The method was validated in terms of linearity, precision, accuracy and specificity. The assay was linear over the concentration range of 5.0-25.0 mcg/ml and 2.5 to 12.5 mcg/ml for Atorvastatin and Ramipril respectively. Accuracy of the method was determined through recovery studies by adding known quantities of standard drug to the pre analyzed test solution and was found to be 98.5-99.96% and 99.99%-100.25% within precision RSD of 1.04 and 1.24 for Atorvastatin and Ramipril respectively. The method requires less than 10 minutes as run time for analysis which prove the adoptability of the method for the routine quality control of the drug.

Key words: Atorvastatin, Ramipril, Method development, Validation.

INTRODUCTION

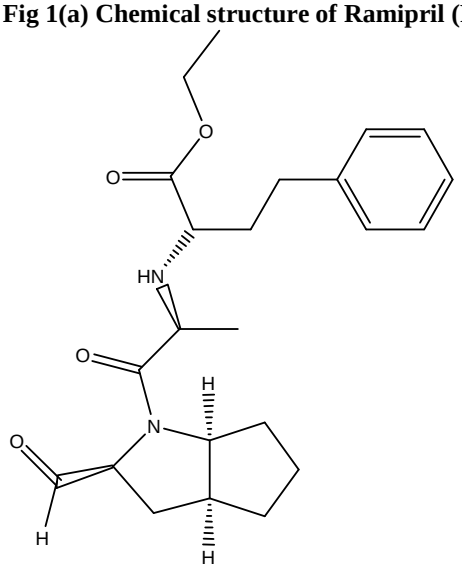
The determination of low concentration and poorly absorbing analytes in pharmaceutical associations constitutes a challenging problem in current pharmaceutical analysis. Capsules containing the pharmaceutical association between Atorvastatin and Ramipril (10 and 5 mg, respectively) are employed as antihypertensive [1,2]. In this combination, Atorvastatin [[R-(R*, R*)]-2-(4-fluoro- phenyl)- ß-δ- dihydroxy-5-(1-methylethyl) -3- phenyl 4-[(phenylamino) carbonyl]-1H- pyrrole-1- hepatonoic acid] is anti hyperlipoproteinemic drug and Ramipril[(2s,3as, 6as)- 1- [(s)-2- [(s)-1-(ethoxycarbonyl)-3-phenyl propyl] amino propanoyl]

octahydrocyclopenta[6] pyrrole-2- carboxylic acid] is ACE inhibitor which are used for relieving the symptoms of hypertension and pain relief in angina [3,4]. Both drugs are insoluble in water and their chemical structures are shown in Fig. 1 (a) and (b) [5,6].



7-[2-(4-Fluoro-phenyl)-5-isopropyl-3-phenyl-4-phenylcarbamoyl-pyrrol-1-yl]-3,5-dihydroxy-heptanoic acid (Ramipril)

Fig 1(a) Chemical structure of Ramipril (RM)



2-[2-(2-Formyl-hexahydro-cyclopenta[*b*]pyrrol-1-yl)-1-methyl-2-oxo-ethylamino]-4-phenyl-butyric acid ethyl ester (Atorbastatein)

Fig.1 (b) Chemical structure of Atorvastatin(AB)

Many analytical methods like simultaneous estimation of Atorvastatin and Ramipril by first order derivative spectroscopy [7], stability indicating HPLC methods [8-11], Ultra-HPLC tendem mass spectroscopy [12], LC- tendem mass spectroscopy method for determination of Ramipril [13] and other methods were reported for determination of AB and Ramipril alone or in combination with other antihypertensive drugs [14-21]. Analytical method by RP-HPLC has been reported for the combination but due to the use of expensive chemicals the method was costly.[22]

A comprehensive literature search revealed the lack of a suitable and economic procedure for the simultaneous determination of these two drugs in pharmaceutical dosage forms. Therefore, the aim of the present work is the development and validation of a simple and reliable RP-HPLC method for the simultaneous determination of AB and RM in their combined capsule formulations, and its application to the determination of both analytes in commercial brand of their combined capsule formulation. Hence, an attempt was made in this study to develop a rapid, economical, precise and accurate method for simultaneous estimation of AB and RM in capsule formulation by RP-HPLC.

MATERIALS AND METHODS

2.1. Chemicals and reagents

All experiments were performed with pharmaceutical-grade AB and RM, and analytical-grade reagents. HPLC-grade solvents were employed for analysis. Solvents were filtered through 0.45 µm membrane filters. All dilutions were performed in standard volumetric flasks. The pharmaceutical preparations, declaring to contain 10 mg AB, 5mg RM and excipients, were obtained from a local drugstore.

2.2. Instrumentation and chromatographic conditions

The separations were performed with a Schimadzu ^{R} 1100 series liquid chromatograph consisting of quaternary pumps, a manual injector fitted with a 20 µl loop and a dual-wavelength UV–vis detector set at a working wavelength of 237 nm. Compounds were separated on a 250 mm×4.6 mm C18 column (Luna, Phenomenex, 5µm particle size). The mobile phase was a 55:40:05 (v/v/v) mixtures of acetonitrile, water and methanol pumped at a flow rate of 1.0 ml min−1. Chromatograms were recorded employing lab solutions software.

2.3. Preparation of stock and working standard solutions

The stock solution of AB (1.0 mg ml−1) was prepared in a 100.0 ml volumetric flask by dissolving an accurately weighed amount (100.0 mg) of AB in methanol. The stock solution of RM (1.0 mg ml−1) was prepared in a 100 ml volumetric flask by dissolving in methanol 100.0 mg of accurately weighed RM. The solutions, which proved to be stable for a period of 3 months, were conserved at 4◦C, in light-resistant containers and were left to attain room temperature before use.

Solutions containing mixtures of AB and RM were prepared by dilution of appropriate volumes of the working solutions in methanol. All the solutions were protected from light throughout the experiments.

2.4. Sample preparation

Pharmaceutical formulation of one brand (average weights of 176.9 mg/capsule) was evaluated. In this, 20 capsules were accurately weighed and their average weight was calculated. The capsule powder was taken and a quantity equivalent to one capsule was weighed and transferred to a 100.0 ml volumetric flask and the volume was made up to 100.0 ml using methanol. The flask was sonicated on a water bath for 10 min at 37 ^{0}C. A 10.0 ml portion of this solution was diluted up to 100.0 ml with methanol to get concentration of 10.0 µg/ml of AB and 5.0 µg/ml of

RM. The process was repeated with five aliquots of capsule powder. The solutions were filtered through a 0.45 µm nylon membrane filter before the analysis.

RESULTS AND DISCUSSION

3.1. Selection of the detection wavelength

The UV spectra of AB and RM in a 55:40:5 (v/v/v) mixture of acetonitrile, water and methanol, in the region between 220 and 240 nm, are shown in **Fig.2.** In their pharmaceutical association, RM is nominally two times less concentrated than AB, the latter having also better absorbing characteristics in the UV region.

As observed, AB exhibits fairly constant absorption throughout the spectrum with a maximum at 246 nm, while RM shows a maximum at 237 nm. This suggested the latter as the optimum detection wavelength in order to favor the quantification of RM, the less concentrated component of the mixture.

Fig . 2. Overlain spectra of Atorvastatin and Ramipril

3.2. Selection of the mobile phase composition

After a series of screening experiments, it was observed that mixtures of acetonitrile-water and methanol (55:40:5) produced satisfactory separations, the addition of methanol being useful for improving peak shapes. The retention times of AB and RM were 5.678 and 2.884 min, respectively, as shown in the typical chromatogram of **Fig. 3.**

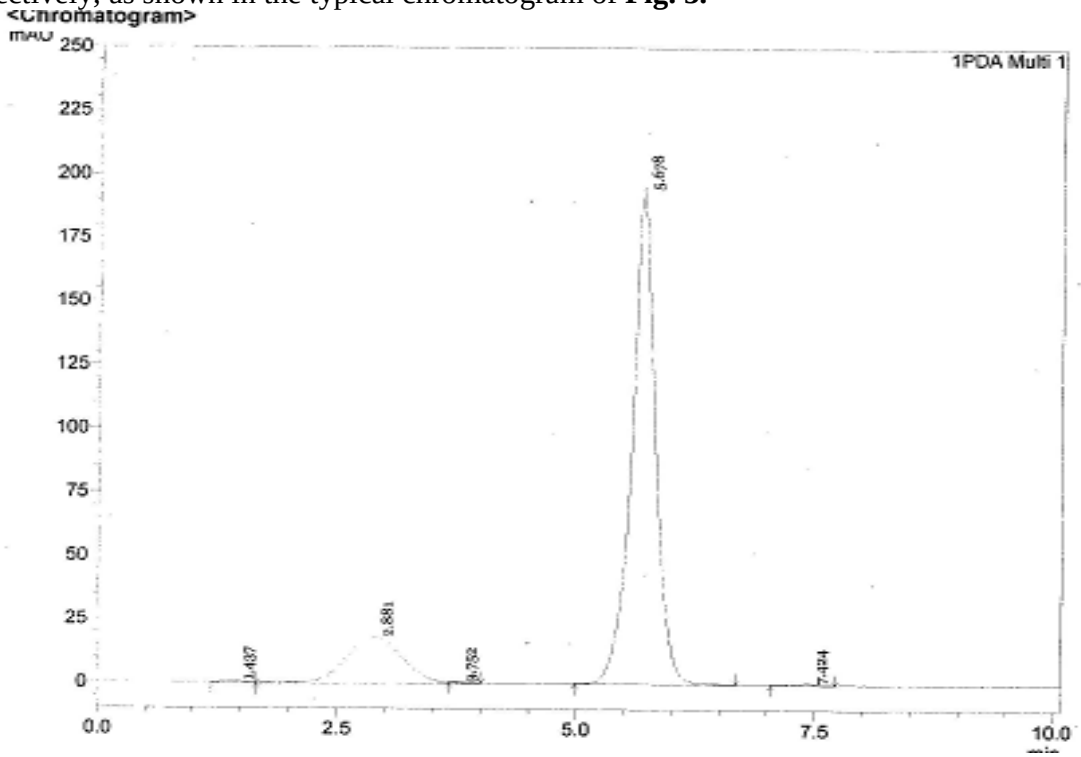


Fig 3 Chromatogram for combined spectra of AB and RM

3.3. Method validation

3.3.1. Linearity

Linearity of the proposed method was evaluated according to the ICH guidelines, by the analysis of working solutions of AB and RM at five different concentrations. Taking into account the purpose of the assay, the linear ranges were 5-25 µg ml−1 for AB and 2.5-12.5 µg ml−1 for RM. The linearity curve for Atorvastatin and Ramipril were shown in **Fig no.4** and **5** respectively. The results show excellent correlations within the tested concentrations ranges.

3.3.2. Accuracy

The accuracy of the method was determined by measuring the drug recoveries by the standard addition method, in order to determine eventual positive or negative interferences produced by the excipients in the formulation [23].

Known amount of standard AB and RM were added in to pre-analyzed samples and subjected to proposed HPLC method. The results of recovery studies are shown in **Table-1.**

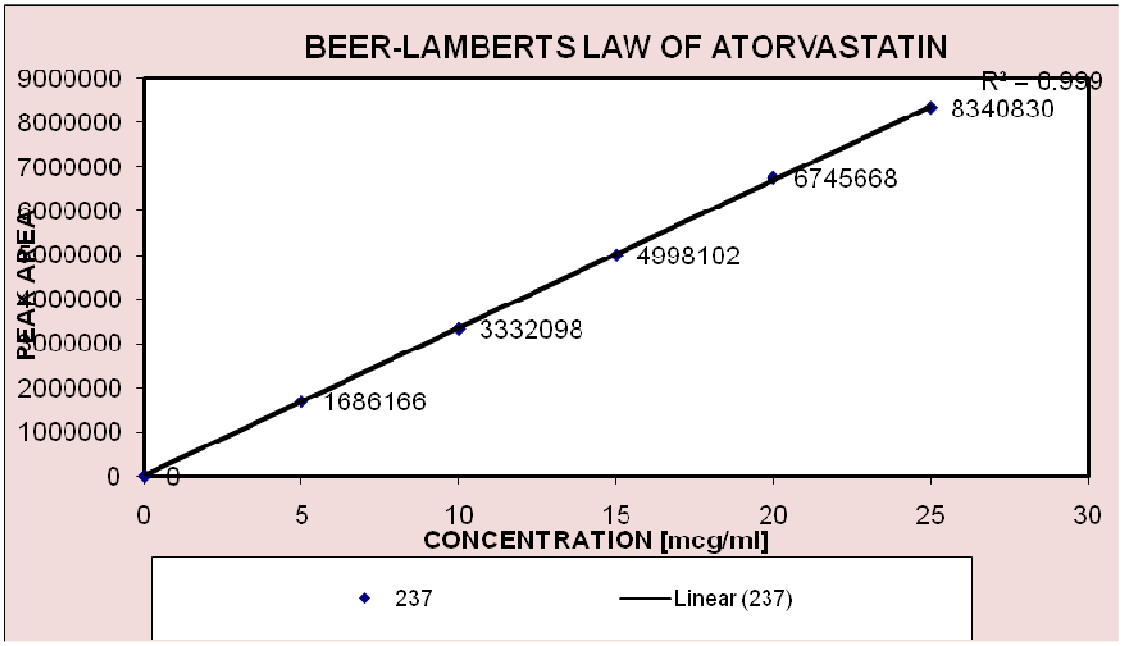


Fig .4 Linearity calibration curve of AB

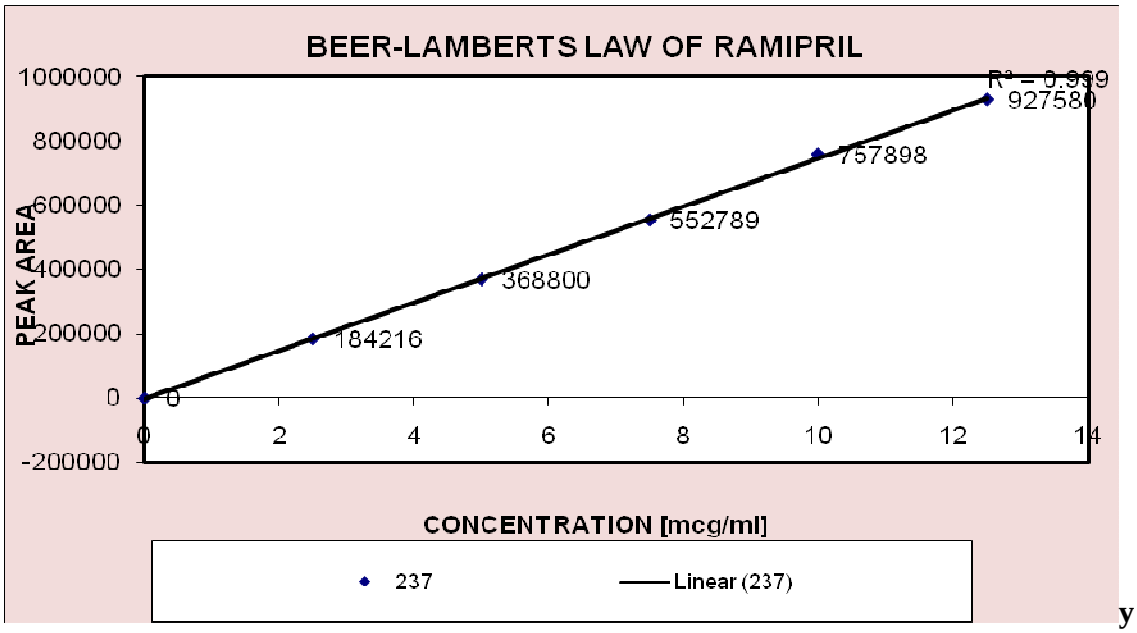


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Fig-5 Linearity calibration curve of RM

Table-1: Analysis of capsule containing Atorvastatin and Ramipril

FORMULATION	DRUG	INJECTED SAMPLE (in mg)	AMOUNT STD. ADDED (in mg)	AMOUNT RECOVERED (in mg)	% RECOVERY
CAPSULE	AB	17.59	1.5	1.6	98.5
	RM	17.59	0.75	1.2	99.0

3.3.3. Precision

Precision was evaluated at the repeatability and intermediate precision levels. Repeatability was studied by the determination of system precision for six replicate injections of the mixed standard solutions in groups of three, at three different levels .In inter-day precision same standard was injected on different system and the found ±SD were 0.60 and 2.15 for atorvastatin and Ramipril respectively. The results were depicted in **table no.2.**

Table 2 : Results for interday and intraday studies

	Interday		Intraday	
	AB	**RM**	**AB**	**RM**
Mean	**100.07**	**99.38**	**99.24**	**98.86**
±SD	**0.60**	**2.15**	**1.04**	**1.95**

3.3.4. System suitability

System suitability tests were performed in accordance with USP 30 to confirm that the equipment was adequate for the analysis to be performed. The test was carried out by injecting five replicates of a standard solution containing 10.0 µg ml−1 and 5.0 µg ml−1 of AB and RM, respectively. The corresponding observed R.S.D. values were 1.04% and 1.63% which were considered satisfactory, meeting the requirements of USP 30 (R.S.D. <2%). The results were shown in **table 3**

Table 3: Results for system suitability parameters.

	AB	RM
%RSD	1.04	1.63
Asymmetry	1.102	1.124

3.4. Application. Assay of pharmaceutical capsule

The validated HPLC method was used for the simultaneous determination of AB and RM in their combined dosage form. Five samples of each brand were weighed separately and analyzed. The results, expressed as percentage drug recovery related to label claim, are informed in Table 7. These indicate that the amounts of each drug in the capsule of both brands are within the USP requirements of 90–110% of the corresponding label claims. The results were shown in **table 4.**

Table 4: Results for Assay of pharmaceutical capsule brand

	% Label Claim	
	AB	RM
Mean	99.56	101.138
±SD	1.50	1.80

CONCLUSION

A simple and efficient HPLC method has been developed and validated for the isocratic separation and simultaneous determination of Atorvastatin and Ramipril in their combined dosage form. The method, suitable for routine quality control, has been successfully applied to

the determination of both analytes in their commercial brand of capsule containing this pharmacological association.From the results it was evident that method is more precise,accurate and inexpensive from the previously reported methods.

Acknowledgement
The authors are thankful to the management of Technocrats Institute of Technology-Pharmacy,Bhopal.

REFERENCES

[1]Indian Pharmacopoeia, Govt. of India, Ministry of Health and Family Welfare, Indian Pharmacopoeia Comission ,Ghaziyabad, vol 2,**2007;**pp 749-752 and vol 3, pp. 1648-1650.
[2]Goodman & Gilman's; The Pharmacological Basic of thearapeutices, 10th Edition, Tata McGraw Hill Company,**2001**;pp. 984-989 , 820-828.
[3]Indian Pharmacopoeia, vol. II Govt. of India, Ministry of Health and Family Welfare, Published by controller of publication, New Delhi,**1996**,pp.371.
[4]Adele M. and Neil J.O., Eds, "The Merck Index"; 13th Edn., Published by Merck research lab, division of Merck & co, White house station, NJ, USA, **2001**,pp. 6909.
[5]Indian Pharmacopoeia vol. II**,** Govt. of India, Ministry of Health and Family Welfare, Published by controller of publication New Delhi,**1996,** pp.371.
[6]British Pharmacopoeia, vol. I, The stationary office under license from the Controller of Her Majesty Stationary Office for the Department of Health on behalf of the Health minister, **2004**, pp.979.
[7]Thamake SL, Jadhav SD, Pishawikar SA, *Asian Journal Research chemistry* 2 (1) **2009**, pp.52-54.
[8]Shah DA, Bhatt KK, Mehta RS and Gandhi TR.,*Indian Journal of Pharmaceutical Science*, **Nov-Dec 2008**, pp.754-760.
[9]Chaudhari BG, Patel NM, Shah PB, Patel LJ, Patel VP , *J AOAC Int*,90(6), **2007**,pp.1539-1546.
[10]Khedr Alaa, *J AOAC Int*;90(6): , Nov-Dec **2007**,pp. 1547-53
[11]Mohammdi A., Razanour N, *Journal of chromatography B*, **2007.** Pp. 215-221.
[12]Lucie Hnaa V. *The Journal of chromatography B*, **2009**, pp. 2093-2103.
[13]Veeren K, Vzclavokova, Dohanl J, Klimes J. *Journal of pharmaceutical and Bio-Medical analysis* **(**35**)**;,**2004** pp. 1179-1183.
[14]Stanisz Beata, Kania Lukasz.,*Acta Poloniae Pharmaceutica - Drug Research*, Vol. 63 No. 6 ,**2006** pp. 471-476
[15]Das SN, Mahapatra SK ,*Pharmabit* ,vol.18, no. 2, **2008**, pp.119-123.
[16]Brry L., Mark Williams, Anna Idiculla, Tarlk Veysogla., *Journal of Pharmaceutical &Bio-Medical Analysis* (23**)**,**2007**,pp.637-651.
[17]Hiral J**.** Panchal**,** Bhanubhai N. Suhagia, Natvarlal J. Patel, Ishwarsinh S**.** Rathod**,** Bhavesh H. Patel. *Chromatographia.* 69(1-2): **2009,** pp.91-95
[18]Meyyanathan S. N., Birajdar Arunadevi S. and Suresh Bhojraj, *Indian journal of pharmaceutical education and research* 43**(**2**) 2009**, pp 156-159
[19]Sonawane SS, Gide PS, Rishipathak DD, Nunnewar GS.,*Indian drugs*, 46(5) **2009,** pp 426-430.
[20]Chitlange SS, Bagri Kiran and Sakarkar DM. *Asian j. research chem.1* (1**),.2008,.** pp 15-18

[21]Thomas AB, Balap AR, Nanda, RK Dumbre NG, Kothapalli LP and Deshpande AD., *Indian journal of pharmaceutical research* 42(1), **2008**, pp 65-69.
[22]Joseph L, George M, Rao B VR. *Pak J Pharm Sci.* Jul;21(3),**2008,**pp.282-4.
[23]Baeyens W.R.G., Van der Weken G., D'haeninck E., A.M. Garcıa-Campana, Vankeirsbilck T., Vercauteren A., Deprez P., J. *Pharm. Biomed. Anal*. 32 **2003,**pp. 839–846.