European Journal of Chemistry

View Journal Online
View Article Online

RP-HPLC method development and validation for simultaneous estimation of ramipril and felodipine

Elham Anwar Taha [1], Manal Mohammed Fouad [2,3,*], Ali Kamal Attia [1] and Zainab Mahmoud Yousef [1]

[1] *National Organization for Drug Control and Research (NODCAR), Giza, 12553, Egypt*
dr_elhamtaha@hotmail.com (E.A.T.), alikamal1978@hotmail.com (A.K.A.), zeze.badawy@gmail.com (Z.M.Y.)
[2] *Analytical Chemistry Department, Faculty of Pharmacy, Al-Azhar University, Cairo, 11651, Egypt*
manalfoad2000@yahoo.com (M.M.F)
[3] *Analytical Chemistry Department, Faculty of Pharmacy, October University for Modern Sciences and Arts, 6 October City, Giza, 12585, Egypt*

* *Corresponding author at: Analytical Chemistry Department, Faculty of Pharmacy, Al-Azhar University, Cairo, 11651, Egypt.*
Tel: +202.0122.3337669 Fax: +202.002.37603811 e-mail: manalfoad2000@yahoo.com (M.M. Fouad).

RESEARCH ARTICLE

10.5155/eurjchem.10.2.113-117.1832

Received: 09 February 2019
Received in revised form: 20 April 2019
Accepted: 04 May 2019
Published online: 30 June 2019
Printed: 30 June 2019

KEYWORDS

Isocratic
Ramipril
Felodipine
Routine analysis
Pharmaceuticals
Chromatography

ABSTRACT

A rapid and sensitive High Performance Liquid Chromatography (HPLC) method has been developed and validated as per ICH guideline for simultaneous determination of ramipril and felodipine binary mixture. Chromatographic separation was achieved on a Hyperchom C18 column (250 × 4.6 mm i.d., 5 µm) using an isocratic mobile phase of potassium dihydrogen phosphate (pH = 3.4): methanol: acetonitrile in the ratio 15:15:70 (*v*:*v*:*v*). The flow rate was 1.5 mL/min, temperature of the column was maintained at 30 °C and detection was made at 210 nm. Linearity studies indicated that the drugs obey Beer's law over the range of 10-80 µg/mL for ramipril and 5-80 µg/mL for felodipine. The proposed method is precise, accurate, linear and robust. The short retention time allows the analysis of a large number of samples in a short period of time and, therefore, considered to be cost-effective that can be used for routine analysis of both drugs in the pharmaceutical industry.

Cite this: *Eur. J. Chem.* **2019**, *10(2)*, 113-117 **Journal website**: www.eurjchem.com

1. Introduction

Today's pharmaceutical industries are looking for new ways to cut cost and shorten time for the development of drugs while simultaneously improving the quality of their products. The high-performance liquid chromatography is a well-established reliable technique used in controlling the quality and consistency of active pharmaceutical ingredients and dosage forms, it is one of the most promising developments in the area of fast chromatographic separations with its unique characteristics of high chromatographic resolution, speed, and sensitivity analysis [1]. In the present work, this technology has been applied to the method development and validation study of mixture of ramipril and felodipine.

Ramipril (RAM) is (2S,3aS,6aS)-1-[(2S)-2[[(1S)-1-(ethoxy carbonyl)-3-phenylpropyl]amino]propanoyl] octahydro cyclo penta[b]pyrrole-2-carboxylic acid, used as angiotensin converting enzyme inhibitor (ACE inhibitor) Figure 1 [2]. Angiotensin-converting enzyme (ACE) inhibitors lower blood pressure by reducing peripheral vascular resistance without reflexively increasing cardiac output, heart rate, or contractility [3]. These drugs block the enzyme ACE which cleaves angiotensin I to form the potent vasoconstrictor angiotensin II [4]. RAM is officially listed in British Pharmacopoeia, which describes a potentiometric titration with 0.1 M sodium hydroxide for its assay in bulk [2].

Felodipine (FLD), chemically known as ethylmethyl(4RS)-4-(2, 3-dichlorophenyl)-2, 6-dimethyl-1,4dihydropyridine-3, dicarboxylate, used as calcium channel blocker, Figure 1 [2]. Felodipine was classified as dihydropyridines, this class of calcium channel blockers have the advantage of showing little interaction with other cardiovascular drugs, such as digoxin or warfarin, which are often used concomitantly with calcium channel blockers [4]. The drug is officially listed in British Pharmacopoeia, which describes a titration with 0.1 M cerium sulphate for its assay in bulk [2].

Ramipril in combination with felodipine are used in several antihypertension preparations. RMP was determined individually by spectrophotometric methods [5-7], HPLC [6,8-10], HPTLC [11,12] and electrochemical methods [13-16]. On

http://dx.doi.org/10.5155/eurjchem.10.2.113-117.1832

the other hand, FLD was determined individually by UV spectroscopy [17-20], HPLC [18,21-23], spectrofluorimetric [24], gas chromatography [25] and electrochemical methods [26,27]. Meanwhile, some spectrophotometric [28,29], HPLC [28-31] and HPTLC [32] methods were reported for the simultaneous determination of both drugs.

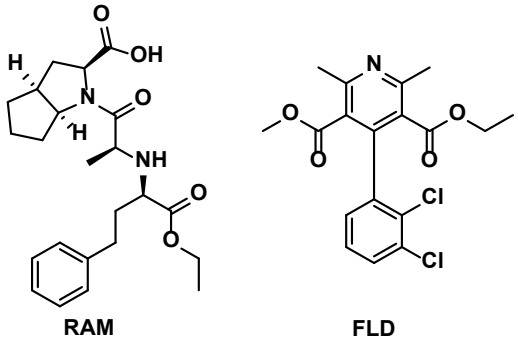

Figure 1. Chemical structures of ramipril and felodipine.

2. Experimental

2.1. Instrumentation

Chromatographic separation was performed on an Agilent 1100 HPLC (USA) with UV detector. Analysis was performed on a Hyperchom C 18 column (250 mm, 4.6 mm i.d., 5 µm) at 30 °C. Data acquisition and processing was performed using ChemStation software. An Elma S100 ultrasonic processor (Germany) was used for the degassing of the mobile phase.

2.2. Materials and reagents

2.2.1. Pure samples

Ramipril (99.96%) [2] was obtained from Kahira Pharmaceuticals & Chemical Industries Co. and felodipine (98.9%) [2] was kindly supplied from Egyptian Group for Pharmaceutical Industries (EGPi Co., Egypt).

2.2.2. Market samples

Triacor® (Aventis Pharma, Deutschland GmbH - Germany) labelled to contain 5 mg RMP and 5 mg FEL, purchased from local pharmacy.

2.2.3. Solvents

Ortho-phosphoric acid (Sigma-Aldrich, Germany), potassium dihydrogen phosphate (ADWIC Chemicals, Egypt), methanol and acetonitrile HPLC grade (Fisher, UK). Bi-distilled water was prepared and used throughout the procedure.

2.3. Solution preparation

2.3.1. Preparation of mobile phase

A mixture of phosphate buffer (pH = 3.4), methanol and acetonitrile in their appropriate ratio was prepared, degased in ultrasonic water bath for 5 minutes and filtered through 0.45 µm filter under vacuum filtration.

2.3.2. Stock solution preparation

25 mg of each of RAM and FLD were accurately transferred into separate 25 mL volumetric flasks to which about 10 mL of methanol was added, sonicated then diluted to the mark with the same solvent to get a standard solution of 1 mg/mL.

2.3.3. Tablet solution preparation

Twenty Triacor® tablets were weighed separately and powdered. Amount of powdered tablet equivalent to 25 mg RMP and 25 mg FLD were weighed, transferred into 25 mL volumetric flask and treated with 10 mL methanol. Solution was sonicated for 15 min, adjusted to volume with methanol and filtered through 0.45-micron membrane filter paper. The resulting solution was further diluted with methanol to get a final concentration of 1 mg/mL RMP and FLD.

2.4. Chromatographic conditions

The analysis was achieved on a Hyperchom C18 column (250 mm, 4.6 mm i.d., 5 µm). Isocratic elution was performed using a mobile phase of potassium dihydrogen phosphate (adjusted to pH = 3.4 using orthophosphoric acid): methanol: acetonitrile in the ratio 70:15:15 (*v*:*v*:*v*) at a flow rate of 1.5 mL/min. The detection was monitored at the wavelength of 210 nm. Analysis was performed at 30 °C column temperature and the injection volume was 10.0 µL.

2.5. Method validation

The method was validated in accordance with ICH guidelines [33].

2.5.1. Linearity

Linearity of the method was studied by injecting five concentrations of the drugs in triplicate having concentration ranges from 10-80 µg/mL for RMP and 5-80 µg/mL for FLD into the HPLC system. Linear graphs were plotted by using the peak areas against concentration in µg/mL from which the correlation coefficients, slopes and *y*-intercepts of the calibration curves were determined.

2.5.2. Accuracy

Five different concentrations (15, 30, 50, 70 and 75 µg/mL) covering the linearity range of both RAM and FLD were analysed for accuracy. The chromatograms were recorded and the %Recovery±S.D. was calculated from regression equations of the calibration curves.

2.5.3. Precision

Three different concentrations (20, 40 and 60 µg/mL) of both RAM and FLD were analysed in triplicates within the same day for intraday and for three successive days for interday precision. The chromatograms were recorded and the percentage relative standard deviation (RSD%) was calculated from regression equations of the calibration curves.

2.5.4. Limit of quantification

The limit of quantitation (LOQ) of the method was determined by standard deviation of response and slope method.

2.5.5. Specificity

In the present work, specificity was checked by analysing RMP with FLD in their laboratory prepared mixtures containing different ratios of the cited drugs within the linearity range. The concentration of each drug was calculated by substitution in the corresponding regression equation, from which mean % recovery can be calculated.

Table 1. System suitability parameters by the proposed HPLC method.

Parameter	Ramipril	Felodipine
Retention time (min)	2.522	3.87
Capacity factor (K')	3.2	4.3
Resolution	6.43	6.43
Theoretical plates	1269	13096

Table 2. Validation parameters of the proposed HPLC method for the determination of Ramipril and Felodipine.

Parameter	Ramipril	Felodipine
Linearity range (µg/mL)	10-80	5-80
LOQ (µg/mL)	2.15	1.21
Regression parameters		
Slope	30.67	55.30
Intercept	16.66	30.60
Correlation coefficient (r^2)	0.9998	0.9992
Accuracy		
(R% of added standard±SD)	100.88±0.84	100.76±1.19
Precision (RSD %)*		
Intraday	0.43-0.83	0.21-0.96
Interday	0.95-1.44	1.03-1.34

*Average of 9 determinations.

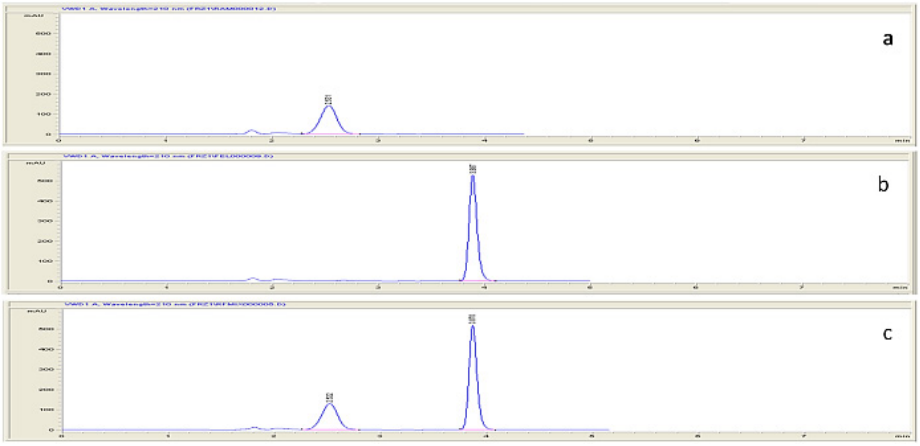

Figure 2. HPLC chromatograms of RAM and FLD using a mobile phase of phosphate buffer: methanol: acetonitrile (70:15:15, *v*:*v*:*v*). (a) RAM, (b) FLD and (c) RAM and FLD binary mixture.

2.5.6. Robustness

To prove the reliability of the analytical method during normal usage, some small but deliberate changes were made in the analytical method (e.g., flow rate, mobile phase composition and column temperature). Changes in the chromatographic parameters were evaluated for the studies.

3. Results and discussion

In the present work, HPLC method was applied to the method development and validation study of binary mixture of ramipril with felodipine.

3.1. Method development

Different chromatographic conditions were experimented to achieve better efficiency of the chromatographic system. Parameters such as mobile phase composition and pH, wavelength of detection, column and diluents were optimized. Choice of retention time, tailing, theoretical plates, and run time were the major tasks while developing the method. Hyperchem C18 column (250 mm, 4.6 mm i.d., 5 µm) was used for the elution. Several solvents (water, methanol and acetonitrile) were tried and different proportions of solvents were evaluated in order to obtain suitable composition of the mobile phase. Buffers like potassium di-hydrogen phosphate, di-potassium hydrogen phosphate, and di-sodium hydrogen phosphate were tried; potassium di-hydrogen phosphate (adjusted to pH = 3.4 using ortho-phosphoric acid): methanol: acetonitrile in the ratio 70:15:15 (*v*:*v*:*v*) gave perfectly eluted peaks. Trials were also done using different flow rates and detection was done at different wavelengths, in which 1.5 mL/min was optimum for separating mixture at a detection wavelength of 210 nm, simultaneously. Typical chromatograms obtained for the cited drug mixture under final optimized HPLC conditions showed retention time of 2.522, and 3.87 min for RMP and FLD, respectively (Figure 2).

3.2. Method validation

The proposed method was subjected to validation process to satisfy the requirements of ICH guidelines [33]. Freshly prepared stock solutions were used to establish system suitability tests. The variation in selectivity, retention time, resolution, and theoretical plates were well within the acceptable ranges for all analytes (Table 1).

The drugs concentrations and peak areas were plotted to construct the calibration curves. Good linearity was established with excellent correlations (>0.999) within the concentration range of 10-80 and 5-80 µg/mL for RMP and FLD, respectively. Regression parameters were computed and presented in Table 2.

The LOQ was determined for all the analytes (Table 2). The low quantification concentrations reflected the good sensitivity of the reported procedure. The mean percentage recovery was calculated to assess the accuracy of the newly developed method.

Table 3. Determination of ramipril/felodipine in their laboratory prepared mixtures by the proposed HPLC method.

Lab-prepared mixture (Ratio of Ramipril:Felodipine)	Recovery%	
	Ramipril	Felodipine
10:01	98.67	101.06
03:01	99.20	101.80
02:01	101.77	99.32
01:01 *	101.49	101.87
01:02	99.75	100.67
01:03	98.61	98.70
01:08	98.77	101.52
Mean±S.D	99.75±1.24	100.7±1.15

* Ratio in pharmaceutical formulation.

Table 4. Robustness study for the proposed HPLC method.

Parameters	Changed condition	%RSD	
		Ramipril	Felodipine
Flow rate, 1.5 mL/ min	±10%	1.18	0.86
Mobile phase ratio, 0.01 M KH_2PO_4 (pH = 3.4), methanol: acetonitrile (15:15:70, *v:v:v*)	±2%	1.56	1.00
Column temperature, 30 °C	±5 °C	1.29	1.85

Table 5. Statistical analysis of results obtained by the proposed and reported method [32] for the determination of ramipril/felodipine in their pharmaceutical formulation.

Parameters	HPLC method		Reported method	
	Ramipril	Felodipine	Ramipril	Felodipine
Mean%	100.57	100.70	99.68	100.13
N	5	5	5	5
SD	0.84	0.66	1.04	1.22
Variance	0.70	0.44	1.08	1.50
t-test	1.49	0.92	-	-
F-test	1.55	3.41	-	-

The theoretical t- and F-values at p = 0.05 (1.860) and (6.39), respectively [34].

The mean recoveries were from 100.76 to 100.88 %±0.84-1.19 for the added drugs (Table 2), representing good accuracy of the method. Intraday and interday precision were undertaken to determine the reproducibility of the process. The % RSD values for the inter-day and intra-day measurements were ranging from 0.21-1.44%. The results listed in Table 2 showed that the proposed procedure is precise.

Specificity was determined by applying the proposed method to laboratory prepared mixtures containing different ratios of each of the two drugs in the two mixtures. Good recoveries for the studied drugs in both mixtures were obtained and results were presented in Table 3.

The robustness of the suggested method was confirmed by performing the analysis with modifications to the flow rate of the mobile phase (±10%), mobile phase composition (±2%) and column temperature (±5 °C). The results showed in Table 4 declares that slight modifications did not affect the resolution and tailing factor, indicating good robustness of the HPLC method.

Table 5 showed statistical comparisons of the results obtained by the proposed method and reported method [32]. The calculated t- and F-values were less than the theoretical ones indicating that there was no significant difference between the proposed and the reported methods with respect to accuracy and precision.

4. Conclusion

A newly developed and validated HPLC method for simultaneous analysis of RAM and FLD in pharmaceutical preparations was very simple, rapid, accurate, and precise. The method was successfully applied for determination of RAM and FLD in their pharmaceutical tablet formulation. Hence, this method can be conveniently used for routine quality control analysis of RAM and FLD in their pharmaceutical formulation. The validation studies as per ICH guideline in accordance to linearity, accuracy, precision, LOD, LOQ and robustness proved suitability of the method for the intended use. Also, the non-interference of additives and excipients makes it suitable for determination of the studied drugs in bulk and in their combined dosage forms.

Acknowledgements

The authors would like to express their gratitude to National Organization for Drug Control and Research (NODCAR, Egypt) for providing instruments and chemicals.

Disclosure statement

Conflict of interests: The authors declare that they have no conflict of interest.
Author contributions: All authors contributed equally to this work.
Ethical approval: All ethical guidelines have been adhered.
Sample availability: Samples of the compounds are available from the author.

ORCID

Elham Anwar Taha
http://orcid.org/0000-0002-8588-8242
Manal Mohammed Fouad
http://orcid.org/0000-0002-7991-0706
Ali Kamal Attia
http://orcid.org/0000-0002-9833-3414
Zainab Mahmoud Yousef
http://orcid.org/0000-0002-9717-7798

References

[1]. Raja, A. P.; Venkateshwar, R. J. *Asian J. Pharm. Clin. Res.* **2013**, *6(3)*, 178-181.
[2]. British Pharmacopoeia. 9th edition, The Council of Europe: 2018; Vol. I 768-771& II 1003-1005.
[3]. Brunton, L.L.; Goodman and Gilman's., 2011. *The Pharmacological Basis of Therapeutics*, The McGraw-Hill Co., Inc., China.
[4]. Whalen, K., Finkel, R., Panavelil, T.A., 2015. *Lippincott's Illustrated Reviews: Pharmacology*, Lippincott Williams and Wilkins, Philadelphia, Baltimore, New York, London.
[5]. Salama, F. M.; El-Sattar, O. I. A.; El-Aba, S. N. M.; Fuad, M. M. *Al-Azhar J. Pharmaceu. Sci.* **2001**, *27*, 121-132.

[6]. Ustun, F. G.; Ustun, O.; Atay, O. *Turk. J. Pharm. Sci.* **2004**, *2(1)*, 65-76.
[7]. Rahman, N.; Ahmad, Y.; Azmi, S. N. H. *AAPS Pharm. Sci. Tech.* **2005**, *6(3)*, E543-E551.
[8]. Patil, K. R.; Rane, V. P.; Sangshetti, J. N.; Shinde, D. B. *Chromatographia* **2008**, *67(7-8)*, 575-582.
[9]. Rajput, P. S.; Kaur, A.; Gill, N. K.; Mittal, K.; Sarma, G. S. *J. Appl. Pharm. Sci.* **2012**, *2(7)*, 160-165.
[10]. Szpot, P.; Buszewicz, G. *Acta Pharmaceut.* **2015**, *65(2)*, 159-169.
[11]. Patel, A. V.; Patel, P.; Chaudhary, B.; Rajgor, N.; Rathi, S. *Int. J. Biol. Pharm. Res.* **2010**, *1(1)*, 18-24.
[12]. Parmar, D. A.; V. Thakkar, D.; Patel, R. B.; Patel, M. R. *Thai J. Pharm. Sci.* **2015**, *39(3)*, 83-88.
[13]. Belal, F.; Al-Zaagi, I. A.; Abounassif, M. A. *J. AOAC Int.* **2001**, *84(1)*, 1-8.
[14]. Prieto, J. A.; Jimenez, R. M.; Alonso, R. M. *Il Farmaco* **2003**, *58(5)*, 343-350.
[15]. Mattos, G. J.; Scremin, J.; Salamanca-Neto, C. A. R.; Sartori, E. R. *Electroanalysis* **2017**, *29(4)*, 1180-1187.
[16]. Silva, T. A.; Fatibello-Filho, O. *Anal. Methods-UK* **2017**, *9(32)*, 4680-4687.
[17]. Basavaiah, K.; Chandrashekar, U.; Prameela, H. C. *Il Farmaco* **2003**, *58(2)*, 141-148.
[18]. Salem, H.; Abdallah, O. M. *Am. J. Appl. Sci.* **2007**, *4(9)*, 709-717.
[19]. Nimje, H.; Oswal, R.; Kshirsagar, S. S.; Chavan, M. *Res. J. Pharm. Tech.* **2011**, *4(12)*, 1805-1806.
[20]. Jadhav, N. R.; Kambar, R. S.; Nadaf, S. *Adv. Chem.* **2014**, Article ID 131974, 1-6.
[21]. Karlsson, A.; Pettersson, K.; Hernqvist, K. *Chirality* **1995**, *7(3)*, 147-153.
[22]. Cardoza, R.; Amin, P. *J. Pharmaceut. Biomed.* **2002**, *27(5)*, 711-718.
[23]. Baranda, A.B.; Jimenez, R. M.; Alonso, R. M. *J. Chromatogr. A* **2004**, *1031(1-2)*, 275-280.
[24]. Walash, M. I.; Belal, F. F.; El-Enany, N. M.; El-Maghrabey, M. H. *Chem. Cent. J.* **2011**, *5*, 61, 1-11.
[25]. Dru, J. D. Y.; Hsieh, J. Y. K.; Matuszewski, B. K.; Dobrinska, M. R. *J. Chromatogr. B* **1995**, *666(2)*, 259-267.
[26]. Jammal, A. E.; Vire, J. C.; Patriarche, G. J.; Palmeiro, O. N. *Electroanalysis* **1992**, *4(1)*, 57-64.
[27]. Sikkander, A. R. M.; Vedhi, C.; Manisankar, P. *Int. J. Indust. Chem.* **2012**, *3(1)*, 29-37.
[28]. Rontogianni, M. A.; Markopoulou, C. K.; Koundourellis, J. E. *J. Liq. Chromatogr.* **2006**, *29(18)*, 2701-2719.
[29]. El-Yazbi, F. A.; Mahrous, M. E.; Hammud, H. H.; Sonji, G. M.; Sonji, N. M. *Anal. Lett.* **2008**, *41(5)*, 853-870.
[30]. B. Raja; Rao, A. L. *Asian J. Res. Chem.* **2013**, *6(11)*, 1018-1022.
[31]. Gawai, A. A.; Shaikh, T.; Kolhe, S.; Shaikh, F.; Deokar, N. *Int. J. Chem. Tech. Res.* **2018**, *11(2)*, 228-239.
[32]. Mohamed, A. M.; Omar, M. A.; Hammad, M. A.; Mohamed, A. A. *Biomed. Chromatogr.* **2016**, *30(2)*, 200-207.
[33]. ICH Harmonised Tripartite Guideline. USA, 2005.
[34]. Miller, J. N.; Miller, J. C. Statistics and Chemometrics for Analytical Chemistry. 6th edition, Pearson Education Limited: Harlow, England, 2010.