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Original Research article

Simultaneous Determination of Ramipril and Amlodipine Besylate in Tablet Dosage form by First Order Derivative Spectrophotometric Method

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ABSTRACT

The aim of present study was to develop a simple, precise, accurate and reproducible spectrophotometric method for simultaneous determination of ramipril and amlodipine besylate by UV-visible spectrophotometer using the first order derivative method. According to our present knowledge, no first order derivative method was reported so far. Thus, in present study it was decided to carryout first order derivative method and it was validated in compliance with ICH (Q2 R1) guideline. Ramipril and amlodipine besylate showed absorbance at the working wavelength of 211.87 nm (zero crossing point of amlodipine besylate) and 254.34 nm (zero crossing point of ramipril) respectively using distilled water as a diluent. Linearity was established over the concentration range of 2-25 µg/mL and 2-50 µg/mL for ramipril and amlodipine besylate with correlation coefficient 0.999 and 0.998 respectively. Accuracy was obtained between 99.91-101.06% and 99.66-100.66% for ramipril and amlodipine besylate respectively. LOD were found to be 0.078 µg/mL and 0.059 µg/mL and LOQ were 0.239 µg/mL and 0.178 µg/mL for ramipril and amlodipine besylate respectively. The results revealed that the developed method is suitable for the routine analysis of determining of ramipril and amlodipine besylate in a tablet dosage form.

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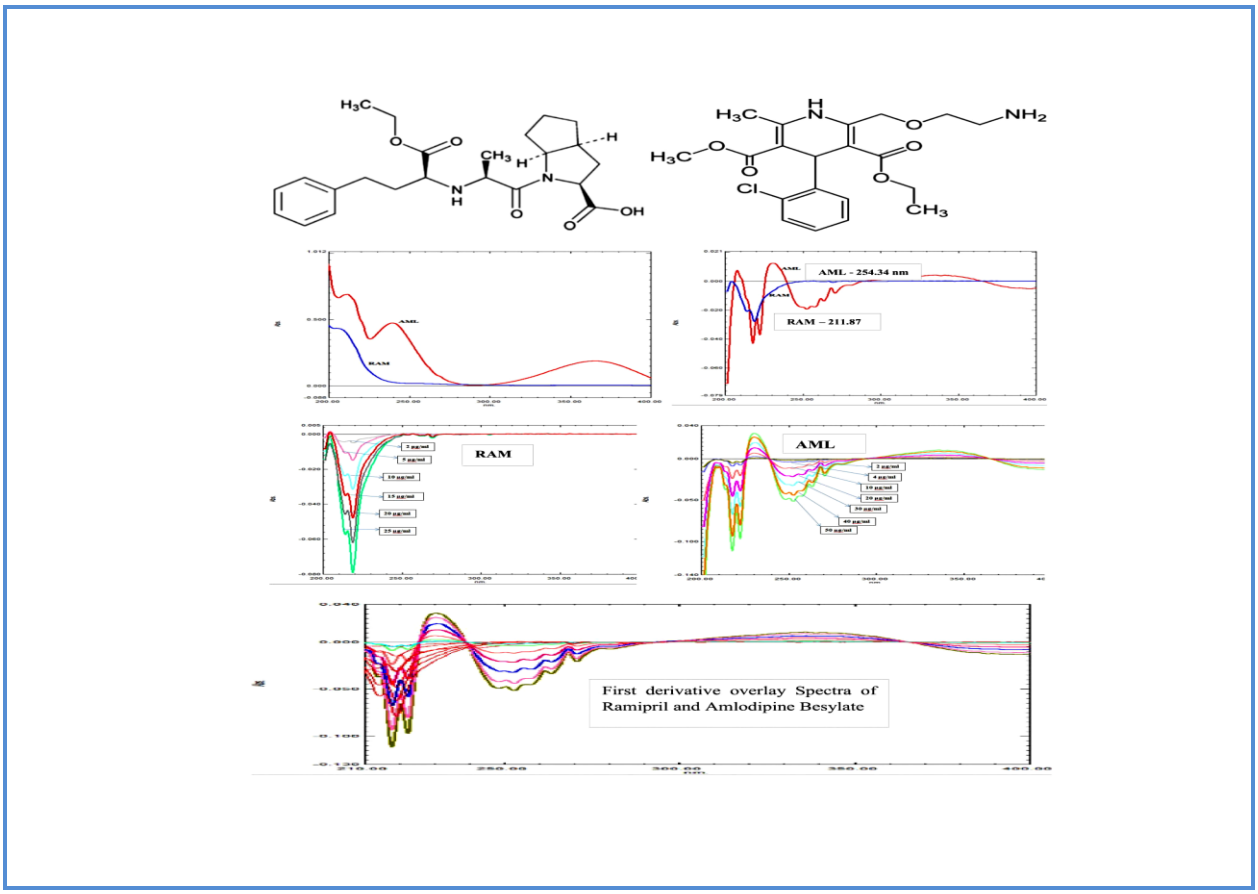
Graphical Abstract



Introduction

Ramipril (RAM) is (2S,3As,6As)-1-[(2S)-2-[[(2S)-1-ethoxy-1-oxo-4phenylbutan-2-yl]amino]propanoyl]-3,3a,4,5,6,6a-hexahydro-2H-cyclopenta[b] pyrrole-2-carboxylic acid [1]. It is angiotensin converting enzyme (ACE) inhibitor, indicated for the treatment of hypertension, heart failure and myocardial infarction. Structure of the ramipril is shown in Figure 1 [2].

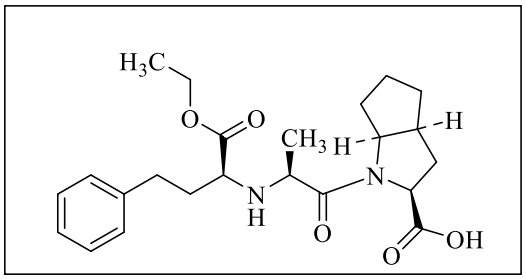


Figure 1. Structure of ramipril

Amlodipine besylate (AML) is benzenesulfonic acid; 3-O-ethyl 5-O-methyl 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6methyl-1, 4-dihydropyridine-3, 5-dicarboxylate, It is a Calcium channel blocker, indicated for the treatment of hypertension and angina. Structure of amlodipine besylate is illustrated in Figure 2 [3].

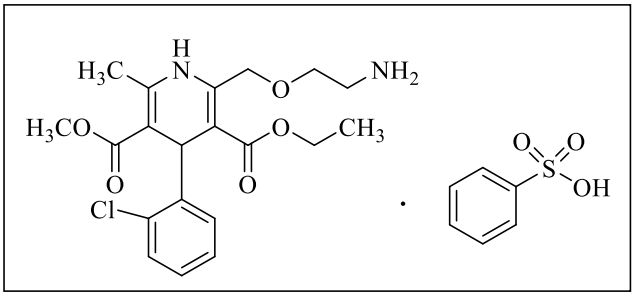


Figure 2. Structure of amlodipine besylate

The drug combination offers more beneficial effects over single drug therapy in the treatment of hypertension in patients [4]. Various analytical methods are available to determine the ramipril and amlodipine besylate individually and in combination. It includes simultaneous equation methods [5], RP-HPLC [6-8], HPTLC [9] for combination of both drugs and simultaneous equation methods [10, 11], RP-HPLC [12-16], stability indicating HPLC [17, 18], UPLC [19], LC-MS [20], Chemometry [21], absorption maxima method [22] and area under curve method for ramipril and amlodipine besylate alone and with combination with others are available.

According to our present knowledge, no first order derivative method was reported so far. Therefore, in this study it was decided to carry out the first order derivative method. This method was validated in compliance with the ICH guideline (Q2 R1) [23]. First order derivative spectroscopy was found to be more selective, accurate, precise and simple for the estimation.

Experimental

Chemicals and reagents

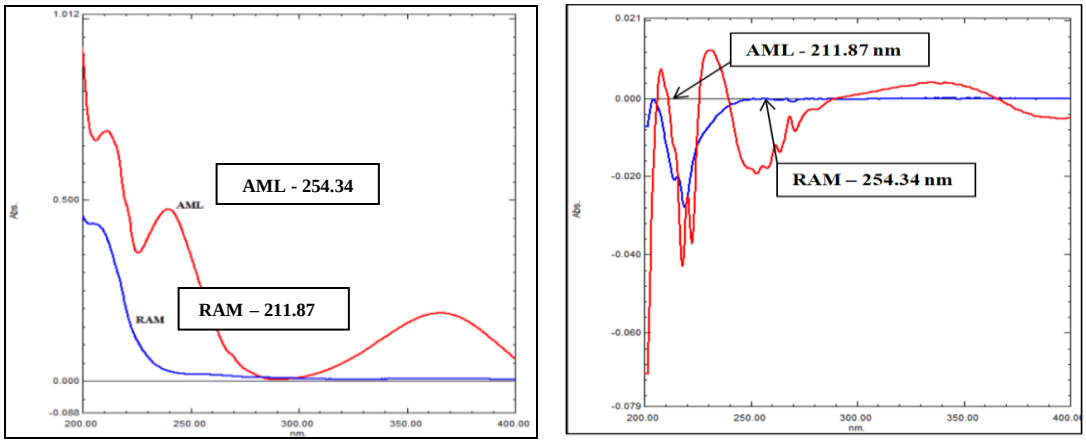
Ramipril, amlodipine besylate and distilled water were provided by the B.K. Mody government pharmacy college. UV-visible spectrophotometer (UV-1800 Shimadzu) used, data were processed using UV Prob (version 2.60) software.

Preparation of standard stock solution

Standard stock solution of 100 µg/mL for ramipril and 200 µg/mL for the amlodipine besylate was prepared in distilled water as diluent.

Selection of wavelength

By appropriate dilutions from the standard stock solution, 10 µg/mL and 20 µg/mL of the ramipril and amlodipine besylate respectively were separately prepared and scanned in the UV range 200–400 nm. The overlain zero-order absorption spectra of both drugs were obtained. These absorbance spectra were converted to the first order derivative spectra. After observing overlay first order derivative spectra with scaling factor 1 and Δλ 2 for ramipril and amlodipine besylate, zero crossing points of drugs were selected. The first wavelength selected was 211.87 nm (zero crossing of amlodipine besylate), where ramipril showed considerable absorbance. The second wavelength selected was 254.34 nm (zero crossing of ramipril), where amlodipine besylate showed considerable absorbance.



(a) Zero order specta (b) First order derivative spectra

Figure 3. Wavelength selection spectrum of RAM and AML

Method validation

Linearity

The standard stock solution was diluted appropriately to obtain the concentration of 2, 5, 10,15, 20, and 25 µg/mL of ramipril and 2, 4, 10, 20, 30, 40 and 50 µg/mL of amlodipine besylate in 10 mL volumetric flask, and diluted up to the mark with distilled water.

Specificity

Specificity was performed under 6 replicates at concentration 10 µg/mL of ramipril and 20 µg/mL of amlodipine besylate with and without addition of excipients to check the interference of excipients.

LOD and LOQ

The limit of detection (LOD) and limit of quantification (LOQ) were calculated by formula. The calibration curve was repeated five times and standard deviation (SD) of the intercepts was calculated.

LOD = 3.3 x Standard deviation/Slope

LOQ = 10 x Standard deviation/Slope

Accuracy

The accuracy of the method was performed in triplicate at three different concentration levels of 50, 100 and 150% (15, 20 and 25 µg/mL for ramipril and 30, 40 and 50 µg/mL for amlodipine besylate). Study was performed by spiking above concentration to placebo.

The accuracy of method was evaluated by calculating percentage recovery.

Precision

Repeatability was performed under 6 replicates at concentration of 10 µg/mL of ramipril and 20 µg/mL of amlodipine besylate. Intra-day and inter-day variations of ramipril and amlodipine besylate were performed in triplicate at three different concentration levels 50, 100, 150% (5, 10, and 15 µg/mL and 10, 20, 30 µg/mL for ramipril and amlodipine besylate respectively). Results are expressed in the form of RSD.

Robustness

The robustness of method was established by introducing small change in experimental condition like wavelength. The changes made in wavelength ± 1 nm (211.87, 210.87, 212.87 nm for ramipril and 254.34, 253.34, 255.34 nm for amlodipine besylate).

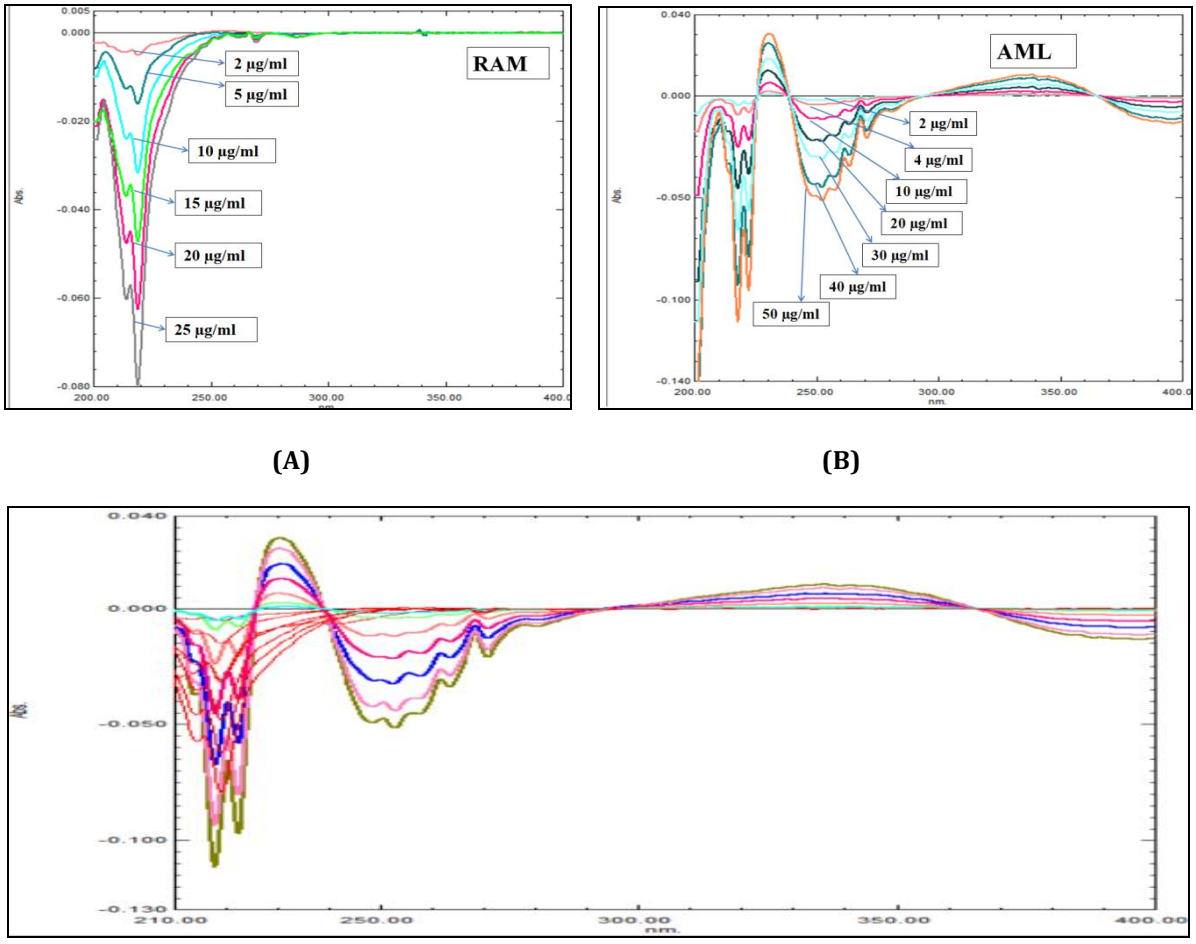
Assay of tablet dosage form

Twenty tablets was accurately weighed and finely powdered. A quantity of the powder equivalent to 10 mg ramipril and 20 mg amlodipine besylate was transferred into 100 mL volumetric flask. Then add 60 mL of distilled water and sonicate for 10 min, make up the volume with distilled water. Then passed it through whatman filter paper. From this solution made 10 µg/mL and 20 µg/mL solution for ramipril and amlodipine besylate respectively.

Results and discussion

Linearity: The calibration curve was obtained for ramipril and amlodipine besylate in the range of 2-25 µg/mL and 2-50 µg/mL and the correlation coefficient was found to be 0.999 and 0.998 respectively.

This method is fond linear. Linearity spectra and graph is revelead in Figure 5 and data is shown in Table 1.



(A) **(B)**

(C)

Figure 4. Linearity spectra for (A) RAM, (B) AML, (C) Overlay spectra

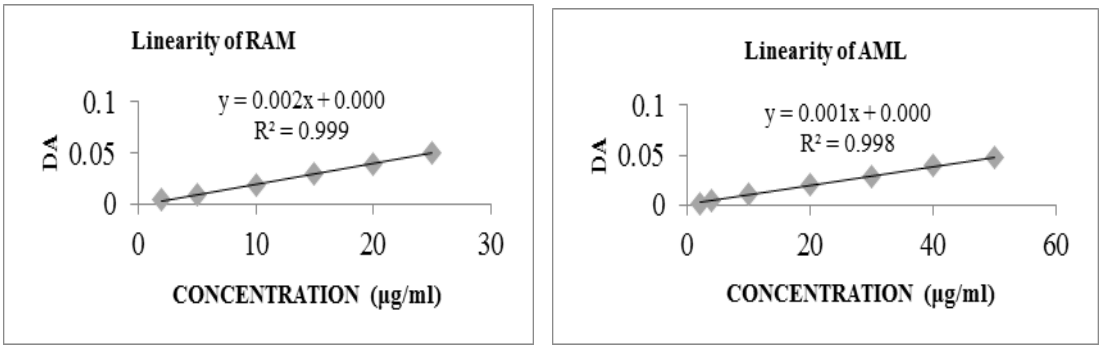


Figure 5. Linearity graphs for ramipril and amlodipine besylate

Table 1. Linearity for ramipril and amlodipine besylate

Ramipril		Amlodipine besylaye	
Conc (µg/mL)	**Absorbance**	**Conc (µg/mL)**	**Absorbance**
2	0.0040	2	0.0022
5	0.0102	4	0.0041
10	0.0190	10	0.0110
15	0.0300	20	0.0203
20	0.0392	30	0.0281
25	0.0500	40	0.0400
-	-	50	0.0473

Specificity: Excipients interference is not observed at the working wavelength 211.87 nm for ramipril and 254.34 nm for amlodipine besylate, and % interference was found less than 0.5%. Thus method is specific. The data of specificity is given in Table 2.

Table 2. Specificity study for ramipril and amlodipine besylate

	Conc (µg/mL) (n=6)	Absorbance		Concentration (µg/mL)		Difference	% Interference
		With excipients	Without excipients	With excipients	Without excipients		
		Mean ± SD		Mean ± SD			
RAM	10	0.019 ± 0.0		9.5 ± 0.0		0	0
AML	20	0.020 ± 0.0		20 ± 0.0		0	0

LOD and LOQ: LOD and LOQ were found to be 0.078 µg/mL and 0.239 µg/mL for ramipril and 0.059 and 0.178 µg/mL for amlodipine besylate respectively.

Accuracy: This method was accurate and % recovery was found in the range of 99.66-101.06%. Therefore, the recovery of drugs are acceptable as shown in Table 3.

Table 3. Accuracy study for ramipril and amlodipine besylate

% Recovery level	Target Conc. (µg/mL)		Spiked Conc. (µg/mL)		% Mean recovery (n=3)	
	RAM	AML	RAM	AML	RAM	AML
50	10	20	5	10	100.44%	100.21%
100	10	20	10	20	99.9 %	99.66%
150	10	20	15	30	101.06%	100.66%

Precision: Repeatability and intermediate precision express in term of RSD. The result is summarized in Table 4 and 5 respectively.

Table 4. Repeatability study for ramipril and amlodipine besylate

Drug	Concentration (µg/mL) (n=6)	Concentration found (µg/mL) Mean ± SD	RSD
RAM	10	9.65 ± 0.00025	1.294
AML	20	20.2 ± 0.00013	0.680

Table 5. Intermediate study for ramipril and amlodipine besylate

Precision		Intra-day (n=3)		Inter-day (n=3)	
Drug	**Level (%)**	**Absorbance (Mean ± SD)**	**RSD**	**Absorbance (Mean ± SD)**	**RSD**
RAM	50	0.0101 ± 0.00017	1.7406	0.0098 ± 0.00018	1.8690
	100	0.0197 ± 0.00027	1.3726	0.0201 ± 0.00028	1.4065
	150	0.0305 ± 0.00035	1.1566	0.0300 ± 0.00040	1.3360
AML	50	0.0097 ± 0.00005	0.5237	0.0101 ± 0.00005	0.5697
	100	0.0205 ± 0.00014	0.7087	0.0200 ± 0.00015	0.7624
	150	0.0309 ± 0.00031	1.0192	0.0312 ± 0.00028	1.0900

Robustness: Making a deliberate change in wavelength was take place and RSD of absorbance found to be less than 2, specify that the method was robust. The results are shown in Table 6.

Table 6. Robustness study for ramipril and amlodipine besylate

Conc(µg/mL)	Absorbance at different wavelength (ramipril)		
10	**211.37 nm**	**211.87 nm**	**212.37 nm**
	0.0195	0.0196	0.0192
	0.0191	0.0192	0.0193
	0.0195	0.0195	0.0199
Mean ± SD	0.0193 ± 0.00023	0.0194 ± 0.00020	0.0196 ± 0.00020
RSD	1.1924	1.0711	1.0748
Conc(µg/mL)	**Absorbance at different wavelength (amlodipine besylate)**		
20	253.84 nm	254.34 nm	254.84 nm
	0.0203	0.0206	0.0203
	0.0200	0.0203	0.0206
	0.0201	0.0204	0.0205
Mean ± SD	0.0201 ± 0.00015	0.0204 ± 0.00015	0.0204 ± 0.00015
RSD	0.7587	0.7475	0.7463

Assay of tablet dosage form

% Drug content of tablet dosage form of ramipril and amlodipine besylate was found between 99.33-101.00%. The data is given in Table 7.

Table 7. Assay

Conc (µg/mL) RAM:AML	Absorbance (n=5) Mean ± SD		Conc. Found Mean ± SD		% Drug content	
	RAM	**AML**	**RAM**	**AML**	**RAM**	**AML**
(10:20)	0.0198 ± 0.00025	0.0202 ± 0.00026	9.93 ± 0.1258	20.20 ± 0.2645	99.33	101.00

Conclusions

The developed method for the spectrophotometric determination of the ramipril and amlodipine besylate was found to be simple, rapid, sensitive, reproducible specific, robust, with good accuracy and precision. As there was no interference of excipients at the working wavelength, it was very fast, with great reproducibility and good response. It allows reliably the analysis of the Ramipril and

AmLodipine Besylate in binary mixture. Linearity were established over the concentration range of 2-25 µg/mL and 2-50 µg/mL for ramipril and amlodipine besylate with correlation coefficient 0.999 and 0.998 respectively. Accuracy was obtained between 99.91-101.06% and 99.66-100.66% for ramipril and amlodipine besylate respectively. LOD were found to be 0.078 µg/mL and 0.059 µg/mL and LOQ were 0.239 µg/mL and 0.178 µg/mL for Ramipril and AmLodipine Besylate respectively.

References

[1] Indian pharmacopoeia, government of India, ministry of health & family welfare; 8th Edition; Indian pharmacopoeia commission, Ghaziabad, 2018, Volume-II, Page No. 3090

[2] Rajput P.S., Kaur A., Gill Kaur N., Mittal K., Sarma G.S. *J. Appl. Pharm. Sci*, 2012, **2**:160

[3] Indian pharmacopoeia, government of India, ministry of health & family welfare; 8th Edition; Indian pharmacopoeia commission, Ghaziabad, 2018, Volume-II, Page No.1219

[4] Bhatt J.U., Shah D.A., Chhalotiya U.K., Bhatt K.K. *Int. J. Institut. Pharm. Life Sci.*, 2013, **3**:100

[5] Kumar M., Jindal M., Bhatt A., Pandurangan A., Malik A., Kaushik V., Upadhaya P.K., Arunachalam G. *J. Pharm. Sci. Res.*, 2019, **11**:667

[6] Dai S.Y., Qiu S.T., Fu C.M. J. *Pharm. Ana.l*, 2013, **3**:440

[7] Kumar A.P.V., Kumar A., Nasare M., Rao V., Prasad V.V.I.N., Diwan P.V. *J. Adv. Pharm. Educat. Res.,* 2012, **2**:137

[8] Patel J., Patel M. *J. Chem. Pharm. Res.,* 2014, **6**:725

[9] Gupta K.R., Wankhede S.B., Tajne M.R., Wadodkar S.G. *Asian J. Chem.,* 2007, **19**:4177

[10] Kalra N., Choudhary S. *Adv. J. Pharm. Life Sci. Res.,* 2013, **1**:16

[11] Macêdo I.Y.L., Gil E.S., *J. Anal. Pharm. Res*., 2017, **4**:1

[12] Yilmaz B. *Int. J. Pharm. Sci. Rev. Res*., 2010, **1**:39

[13] Verma A.R., Sanmukha J.V., Reddy S.M. *J. Chem. Pharm. Res*., 2015, **7**:1060

[14] Mayank J., Sukriti T., Vinay Kumar M., Sugat S., Saima S., *Int. J. Pharm. Life Sci.,* 2010, **1**:428

[15] Khazali E.A.A., Hasan N.S., Abd alwahab H.S., Ali I.K., *World J. Pharm. Sci.,* 2017, **5**:45

[16] Bhaisare M., Sahu K., Karthikeyan C., Moorthy N.S.H.N., Trivedi P. *Latin Am. J. Pharm.,* 2011, **30**:342

[17] Gandhi B.M., Kapuganti A.N.J., Vatchavai B.R., Sumanth K.S., Kogitapurapu V.K., Kolli S., Parimi H. *Asian J. Biomed. Pharm. Sci.*, 2016, **6**:14

[18] Kiarie-Makara M.W., Lee D-K., *IOSR J. Pharm. Biol. Sci.,* 2016, **11**:24

[19] Waghmare A.N., Muddukrishna B.S., , Vasantharaju S.G., *Mintage J. Pharm. Med. sci.*, 2014, **3**:22

[20] Ponneri V., Vasu B.R., Inamadugu J.K., Rao N.P., Vudagandala S. *J. Pharm. Sci.,* 2012, **2**:319

[21] Nagawalli D., Vaidhyalingam V., Santha A., Sankar A.S.K., Divya O. *Acta Pharm.*, 2010, **60**:141

[22] Iftequar S., Swaroop L., Zaheer Z., Shahid M., Imran S., Dehghan M.H., *Int. J. Drug Dev. Res.,* 2012, **4**:286

[23] Guideline I.H.T., *Validation of analytical procedures: text and methodology Q2 (R1).* International Conference on Harmonization, IFPMA, Geneva, Swizerland, 2005

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