Simultaneous estimation of amlodipine besylate, Rosuvastatin calcium and Fimasartan potassium trihydrate combination used in the treatment of hypertension using LC method

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Abstract

The proposed sensitive, precise, and accurate reverse phase-liquid chromatography (RP-LC) method is used for simultaneous estimation Amlodipine besylate, Rosuvastatin calcium and Fimasartan potassium trihydrate in bulk and synthetic mixture which is under phase 3 trial. An accurate and cost effective isocratic approach is employed by using Phenomenex C-18 column (250 mm × 4.6 mm, 5 µm). A method was developed and validated by focusing on ICH Q2 (R1) guideline parameters, efficient separation of all three drugs were obtained by optimised solvent mixture of acetonitrile and 0.02 M potassium dihydrogen phosphate buffer (49:51, v/v) pH 3.0 adjusted with 1% O—phosphoric acid used as mobile phase, flow rate was maintained 1 ml/min and analysis was carried out by using PDA detector at common wavelength 242 nm. The linearity ranges were found to be 1–7 µg/mL for Amlodipine besylate with correlation coefficient (r^2) 0.9966, 2–12 µg/mL for Rosuvastatin calcium with correlation coefficient (r^2) 0.9998 and 6–36 µg/mL for Fimasartan potassium trihydrate with correlation coefficient (r^2) 0.9990, respectively. The proposed LC method has potential qualitative as well as quantitative applications for simultaneous estimation of Amlodipine besylate, Rosuvastatin calcium and Fimasartan potassium trihydrate in bulk and synthetic mixture.

Keywords Amlodipine besylate · Rosuvastatin calcium · Fimasartan potassium trihydrate · Validation · RP-LC

1 Introduction

Amlodipine besylate (AML) which is chemically 3-ethyl-5-methyl-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate which has empirical formula $C_{26}H_{31}ClN_2O_8$ and molecular weight of 567.05 gm/mole. The mechanism of action of amlodipine besylate is angio-selective calcium channel blocker which blocks the movement of calcium ions in the cardiac muscles which inhibits the contraction of cardiac muscles [1]. Rosuvastatin calcium (ROS) which is (3R, 5S, 6E)-7-(4-(4-fluorophenyl)-6-(1-methylethyl)-2-(ethyl(methylsulfonyl)

amino)-5-pyrimidinyl)-3,5-dihydroxy-6-heptenoic acid which has an empirical formula $C_{22}H_{28}FN_3O_6S$ and molecular weight is 1001.14 gm/mole. The mechanism of action of Rosuvastatin calcium is an HMG Co A reductase inhibitor, HMG Co A is an a rate-limiting enzyme that converts the 3-hydroxy-3-methylglutarate to mevalonate which is indeed precursor of cholesterol synthesis, Rosuvastatin Calcium with a good and balanced diet helps to reduce LDL (Low density Lipoprotein) which are harmful for our body and helps in conserving the HDL (High density lipoprotein) [2]. Fimasartan potassium trihydrate which is chemically 2-(2-butyl-4-methyl-6-oxo-1-[[2'-(1H-1,2,3,4-tetrazol-5-yl)-

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[1,1'-biphenyl]-4-yl)methyl]-1,6-dihydropyrimidin-5-yl)-*N,N*-dimethylethanethioamide which has an empirical formula $C_{27}H_{36}KN_7O_4S$ and molecular weight is 593.79 gm/mole (Fig. 1). The mechanism of action of Fimasartan is an angiotensin II receptor antagonist which is employed in treatment of hypertension as well as heart failure [3]. Currently the combination of Amlodipine besylate, Rosuvastatin Calcium and Fimasartan Potassium Trihydrate is under phase III clinical trial and the study has been completed in month of June 2019 under the observation of USFDA [4].

The extensive literature review revealed that various LC methods have been published for the estimation of Amlodipine besylate [5–13], Rosuvastatin calcium [14–16] and Fimasartan potassium trihydrate [17] in combination.

Literature review revealed that no method has been developed for simultaneous estimation of AML, ROS and FIM in combined dosage form. Therefore, the objective of present study involves the simultaneous estimation of AML, ROS and FIM in bulk and synthetic mixture by RP-LC method.

The RP-LC method has distinct advantage over conventional HPLC method, where in conventional HPLC method there is higher usage of organic solvents where as in RP-LC there is use of more aqueous solvent which is less harmful and hazardous to environment as well as the operator. RP-LC is more economic and much more sensitive than HPTLC. It is compatible with all major detectors of HPLC which are photodiode array (PDA) detector, UV-Visible detector as well as Refractive Index detector.

2 Material and chromatographic condition

2.1 Chromatographic condition

2.1.1 HPLC system

The HPLC system consisted of a binary pump (model Waters 515 HPLC pump), loop injector and PDA detector (Waters 2998). Data collection and analysis were performed using Empower—version 2 software. Separation was achieved on Phenomenex C-18 column (250 mm × 4.6 mm, 5.0 μ). Isocratic elution with Acetonitrile: 0.02 M potassium dihydrogen phosphate Buffer (49:51, v/v) pH 3.0 of mobile phase adjusted with 1% O—phosphoric acid at the flow rate of 1 mL/min was carried out. The detection was monitored at 242 nm and injection volume was 20 μL.

2.2 Reagent and material

2.2.1 Analytical balance

Mettler Toledo electronic balance (ME204, Mettler Toledo Group, Mumbai, India).

2.2.2 Pure sample

Analytically pure Amlodipine besylate, Rosuvastatin calcium and Fimasartan potassium trihydrate were obtained as gift samples from reputed pharmaceutical industry, Gujarat state, India.

Fig. 1 Chemical Structures of **a** amlodipine besylate, **b** Rosuvastatin calcium and **c** Fimasartan potassium trihydrate

2.2.3 Chemical reagent

Acetonitrile (HPLC Grade)—SRL Pvt Ltd., Mumbai, India, Acetonitrile(HPLC Grade)—E-Merck Pvt Ltd., Mumbai, India, Methanol(HPLC Grade)- SRL Pvt Ltd, Mumbai, India, HPLC grade water—Milli Q integral Water Purification System, Merck KGaA, Darmstadt, Germany, Potassium dihydrogen phosphate—S D Fine Chem Limited, Vadodara, Gujarat, India, Ortho-phosphoric acid(AR grade)- SRL Pvt Ltd., Mumbai, India. Microcrystalline Cellulose—Chiti-Chem Corporation Pvt. Ltd., Vadodara, Gujarat, India, Cross Povidone- Chemdyes Corporation Pvt. Ltd, Rajkot, Gujarat, India, Magnesium Stearate- Chemdyes Corporation Pvt. Ltd., Rajkot, Gujarat, India, Hydroxypropylmethylcellulose (HPMC) K100M- Chemdyes Corporation Pvt. Ltd., Rajkot, Gujarat, India, Calcium Phosphate- Chiti-Chem Corporation Pvt. Ltd., Vadodara, Gujarat, India, Cross Carmellose Sodium: Chemdyes Corporation Pvt. Ltd., Rajkot, Gujarat, India.

2.2.4 Preparation of synthetic mixture [18]

Synthetic mixture was prepared by taking active ingredients and excipients in following proportions.

1. Microcrystalline cellulose: 4080 mg
 2. Cross Povidone: 300 mg
 3. Calcium Phosphate: 300 mg
 4. Magnesium Stearate: 111 mg
 5. HPMC(Hydroxypropylmethylcellulose)K100M: 30 mg
 6. Cross Carmellose Sodium: 150 mg
- Mix all the above ingredients and to them add
7. Amlodipine besylate: 200 mg
 8. Rosuvastatin calcium: 400 mg
 9. Fimasartan potassium trihydrate: 1200 mg

Powder equivalent to 10 mg of Amlodipine besylate (20 mg of Rosuvastatin calcium and 60 mg of Fimasartan potassium trihydrate) was weighed and transferred to 100 mL volumetric flask containing few ml (50.0 mL) acetonitrile and mixture was sonicated for 15minutes. Make up volume upto the mark with acetonitrile. Filter the Solution with Whatman filter paper no. 42 in another 100 ml of volumetric flask which gave concentration of 100 µg/mL AML, 200 µg/mL ROS and 600 µg/mL of FIM, respectively.

2.2.5 Preparation of stock solution

Stock Solution were prepared by weighing 10 mg of each Amlodipine besylate, Rosuvastatin calcium and Fimasartan potassium trihydrate added to 3 different 10 mL volumetric flask containing few ml of acetonitrile, the volume was made upto the mark with acetonitrile to obtained the

concentration of stock solution 1000 µg/mL for each of these drugs.

2.2.6 Preparation of calibration standards

From the above prepared stock solution, six different concentrations for AML prepared with ranges from 1 to 6 µg/mL, for ROS with ranges from 2 to 12 µg/mL and for FIM with ranges from 6 to 36 µg/mL were prepared from their individual respective stock solutions.

2.2.7 Synthetic mixture sample solution

From the above prepared sample solution of Synthetic mixture, pipette out appropriate (0.3 mL) volume of solution was transferred into 10 mL of volumetric flask and make up the volume upto the mark with acetonitrile to obtained final concentration 3 µg/mL for AML, 6 µg/mL for ROS and 18 µg/mL for FIM, respectively.

2.3 Validation

Validation of the proposed HPLC method was carried out according to International Conference on Harmonization (ICH) guidelines Q2 (R1) for linearity, accuracy, precision, repeatability, specificity, sensitivity, and robustness [19].

2.3.1 Linearity of calibration curve

The linearity of method was analysed by establishing calibration curve at six concentration levels over the range of 1–6 µg/mL for AML, 2–12 µg/mL for ROS and 6–36 µg/mL for FIM, respectively. The calibration range was made in such a way that the ratio of combination was maintained throughout analysis. The calibration curve was established by plotting Peak area versus Concentration ($n=6$) and straight line equation was find out. The statistical data of regression analysis of the calibration curves for AML, ROS and FIM by proposed RP-LC method are shown in Table 1.

Table 1 Statistical data for AML, ROS and FIM by proposed RP-LC method

Parameters	AML	ROS	FIM
Range(µg/mL)	1–6	2–12	6–36
Regression coefficient (r^2)	0.9966	0.9998	0.999
Slope of regression equation	66,326	42,791	33,637
Standard deviation of slope	698.64	661.72	198.28
Intercept of regression	57,324	68,514	33,676
Standard deviation of Intercept	1998.90	2699.75	2462.80

2.3.2 Accuracy

The accuracy of method was determined by calculating recoveries of drug by standard addition method at three different level 80%, 100% and 120% of standards to pre-quantified sample solution of synthetic mixture. For AML 1.6 µg/mL, 2 µg/mL and 2.4 µg/mL were spiked to pre-quantified sample solution of synthetic mixture for AML 2 µg/mL, for ROS 3.2 µg/mL, 4 µg/mL and 4.8 µg/mL were spiked to pre-quantified sample solution of synthetic mixture for ROS 4 µg/mL and For FIM 9.6 µg/mL, 12 µg/mL and 14.4 µg/mL were spiked in pre-quantified sample solution of synthetic mixture for FIM 12 µg/mL, respectively. The data of accuracy studies by proposed RP-LC method are shown in Table 3.

2.3.3 Precision

Precision was estimated in terms of intraday and inter-day precisions. Intraday precision was determined by analyzing sample solutions of AML, ROS and FIM by three levels AML (1 µg/mL, 3 µg/mL, 6 µg/mL) ROS (2 µg/mL, 6 µg/mL, 12 µg/mL) and FIM (6 µg/mL, 18 µg/mL, 36 µg/mL) which covers low, medium, and high concentrations of the calibration curve three times on the same day. Interday precision was determined by analyzing sample solutions of AML, ROS and FIM at three levels covering low, medium, and high concentrations over the 3 different successive days. The peak areas obtained were used to calculate mean and %RSD values.

In RP-LC method, repeatability has been carried out by injection repeatability. Injection repeatability was carried out by analysing the samples of AML, ROS and FIM samples in which AML (3 µg/mL), ROS (6 µg/mL) and FIM (18 µg/mL) six times and peak area was measured.

2.3.4 System suitability

The system-suitability tests are integral part of gas and liquid chromatography. They are used to verify that the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The tests are based on concept that the equipment, electronics, analytical operations, and sample to be analysed constitute an integral system that can be evaluated as such. The system suitability parameters like resolution, theoretical plate and asymmetric factor were calculated and compared with standard values. The system suitability test was carried out on freshly prepared working standard stock solution of AML (3 µg/mL), ROS (6 µg/mL) and FIM (18 µg/mL), respectively. The system suitability data

of proposed RP-LC method for AML, ROS and FIM are shown in Table 5.

2.3.5 Specificity

The specificity of method was ascertained by analysing AML, ROS and FIM in presence of excipients (Microcrystalline cellulose, Cross Povidone, Calcium Phosphate, Magnesium Stearate, HPMC (Hydroxypropylmethylcellulose) K100M, Cross Carmellose Sodium) which are used to prepare synthetic mixture. The bands of AML, ROS and FIM were confirmed by comparing Rt value and comparing respective spectra of sample with those of standards. The summary of validation parameters are shown in Table 2.

2.3.6 Robustness

Robustness of the method was studied by observing the stability of the sample solution at $25 \pm 5^\circ\text{C}$ for 24 h, change in flow rate at 1 ± 0.1 ml, change in mobile phase ratio and change in wavelength of analysis. The data of robustness studies by proposed RP-LC method are shown in Table 4.

2.3.7 Solution stability

From the standard stock solution of AML, ROS and FIM, the concentration of 3 µg/mL for AML, 6 µg/mL for ROS and 18 µg/mL for FIM was prepared and stored at room temperature analyzed by proposed RP-LC at regular intervals of 0, 4, 8 and 24 h.

2.4 Analysis of synthetic mixture

The synthetic mixture of AML, ROS and FIM was prepared in ratio of 10: 20: 60 mg, respectively for 20 tablets. Common excipients like Microcrystalline cellulose (4080 mg), Cross Povidone (300 mg), Calcium Phosphate (300 mg), Magnesium Stearate (111 mg), HPMC

Table 2 Summary of validation parameters

Parameter	AML	ROS	FIM
Linearity (µg/ml)	1–6	2–12	2–36
Retention time (min)	3.15	6.32	9.22
Accuracy (%)	98.61–100.80	99.34–101.02	99.40–101.84
Precision (%RSD)			
Intra-day (n=3)	0.60–1.78	0.47–0.48	0.59–.88
Inter-day (n=3)	1.13–1.41	0.45–0.98	0.45–0.75
Specificity	Specific	Specific	Specific
Robustness	Robust	Robust	Robust
Solution stability	Stable for 24 h	Stable for 24 h	Stable for 24 h

(Hydroxypropylmethylcellulose) K100 (30 mg), Cross Carmellose Sodium (150 mg) were weighed accurately with respect to 20 tablets and transfer into motor pestle along with accurately weight quantity of active pharmaceutical ingredients of 200 mg of AML, 400 mg of ROS and 1200 mg of FIM which is equivalent to 20 tablets.

From the above mixture, weight accurate quantity which is equivalent to 10 mg of Amlodipine besylate, 20 mg of Rosuvastatin calcium and 60 mg of Fimasartan potassium trihydrate and transfer it in 100 mL volumetric flask containing few mL of acetonitrile and sonicated for 15 min. The solution was filtered using Whatman filter paper No.42 and collects the filtrate in another 100 mL volumetric flask and the residue was wash with few amount of acetonitrile, the filtrate and residue was combined and volume was diluted to the mark with the acetonitrile to obtained concentration 100 µg/mL for AML, 200 µg/mL for ROS and 600 µg/mL for FIM, respectively. Pipette out 0.3 mL aliquot from the above solution is transferred into 10 mL volumetric flask and volume was made upto the mark with acetonitrile to obtain final concentration of 3 µg/mL for AML, 6 µg/mL for ROS and 18 µg/mL for FIM, respectively. The possibility of interference from other components of the synthetic mixture in the analysis was studied. It was analysed under proposed chromatographic conditions and chromatogram recorded. The amount of AML, ROS and FIM were computed using regression equation.

3 Result and discussion

3.1 Optimization of mobile phase

The objective of the method development was to resolve chromatographic peaks for active drug ingredients (AML, ROS and FIM). Various solvents containing aqueous buffer, water and acetonitrile were tried as mobile phase. Acetonitrile: Water (70:30v/v) was used but it was found that peak shape of AML was not symmetrical. Acetonitrile: Water (80:20v/v) was tried where AML showed tailing, ROS and FIM had eluted on same retention time. Acetonitrile: Water (40:60 v/v) pH 8.0 with 1% Triethylamine was tried in which FIM and ROS had eluted on same retention time and AML peak shape was also not symmetrical. Acetonitrile: Water (40:60v/v) pH 3.0 adjusted with 1% Ortho-phosphoric acid was tried and it was found that peak separation was very good but tailing was found in FIM peak. Acetonitrile: Water (55:45v/v) pH 3.0 adjusted with Ortho-phosphoric acid was tried where peak separation was very good but AML peak shape was disturbed when it was tried in combination. Acetonitrile: Water (55:45v/v) pH 3.0 adjusted with Ortho-phosphoric acid where working standard solution were diluted with Acetonitrile and it was found that peak shape of all of the drug was excellent but peak of FIM and ROS were overlapping. The mobile phase consisting of Acetonitrile: 0.02 M KH_2PO_4 (49:51v/v) pH 3.0 adjusted with O-phosphoric acid was selected as it gave sharp, symmetric well resolved peak for AML, ROS and FIM. The flow rate was maintained at 1.0 mL/min. The retention time for AML, ROS and FIM were 3.1 min, 6.2 min and 9.2 min at common λ_{max} 242 nm, respectively (Figs. 2, 3).

Fig. 2 Chromatogram for amlodipine besylate (3 µg/mL), Rosuvastatin calcium (6 µg/mL) and Fimasartan potassium trihydrate (18 µg/mL) using mixture of acetonitrile and 0.02 M potassium dihydrogen phosphate buffer (49:51, v/v) pH 3.0 adjusted with 1% O—phosphoric acid as a mobile phase

Fig. 3 Overlay UV spectra of AML (1–6 $\mu\text{g/mL}$), ROS (2–12 $\mu\text{g/mL}$) and FIM (6–36 $\mu\text{g/mL}$) for selection of wavelength

Fig. 4 Overlay chromatogram of AML (1–6 $\mu\text{g/mL}$), ROS (2–12 $\mu\text{g/mL}$) and FIM (6–36 $\mu\text{g/mL}$)

3.2 Method validation

The calibration curves were obtained by plotting the peak area versus concentration over the range of 1–6 $\mu\text{g/mL}$ for AML, 2–12 $\mu\text{g/mL}$ for ROS and 6–36 $\mu\text{g/mL}$ for FIM, respectively. The data of regression analysis of the calibration curves are shown in Table 1. Overlay chromatogram of calibration curve for AML, ROS and FIM was shown in Fig. 4.

Instrumental precision was determined by performing injection repeatability test. The % RSD values were found to be 1.46 for AML, 1.30 for ROS and 1.69 for FIM, respectively.

The intra-day and inter-day precision studies were carried out. For the intra-day precision study, the % RSD values were found to be 1.79 for AML, 0.60 for ROS and 0.89 for FIM, respectively. For the inter-day precision study the % RSD values were found to be 1.42 for AML, 0.13 for ROS and 1.19 for FIM, respectively. The low % RSD values indicate that the method is precise. Summary of validation parameters are shown in Table 2.

The accuracy of the method was determined by calculating recoveries of AML, ROS and FIM by method of standard addition. The recoveries were found to be 98.60–100.80% for AML, 101.01–99.34% for ROS and 99.40–101.83% for

Table 3 Accuracy data for AML, ROS and FIM by proposed RP-LC method

Drug	% Level of spiking	Amount of drug taken from formulation (µg/mL)	Amount of standard drug spiked (µg/mL)	Mean area $n = 3 \pm SD$	Amount of drug found (µg/mL)	%Recovery
Amlodipine besylate	0	2	0	74,011 ± 715.54	1.9721	98.61
	80	2	1.6	182,081 ± 727.116	3.609	100.48
	100	2	2	209,044.33 ± 599.36	4.0160	100.80
	120	2	2.4	235,251.33 ± 1005.64	4.4111	100.56
Rosuvastatin calcium	0	4	0	104,059.3 ± 602.954	4.0407	101.02
	80	4	3.2	241,301.3 ± 955.93	7.240	101.01
	100	4	4	272,693 ± 1272.868	7.9738	99.34
	120	4	4.8	309,720 ± 863.6957	8.8391	100.98
Fimasartan potassium trihydrate	0	12	0	437,201 ± 657.721	11.928	99.40
	80	12	9.6	765,707 ± 1010.11	21.689	100.75
	100	12	12	839,455 ± 738.486	24.024	100.20
	120	12	14.4	930,920 ± 575.5	26.6236	101.84

FIM, respectively. The values indicate that the method is accurate. The detection limits for AML, ROS and FIM were 0.104 µg/mL, 0.165 µg/mL and 0.41 µg/mL, respectively, while quantitation limits were 0.315 µg/mL, 0.500 µg/mL and 1.245 µg/mL, respectively. The data of accuracy studies by proposed RP-LC method are shown in Table 3.

There was no interfering peak at the RT value of AML, ROS and FIM from excipients added in the synthetic formulation thereby confirming the specificity of the method.

The low values of RSD obtained after introducing small, deliberate changes in parameters of the developed RP-LC method confirmed its robustness. Robustness data of

Table 4 Robustness study of AML, ROS and FIM by proposed RP-LC method

Parameters	Normal condition	Change in condition drug	Mean area ± SD (n = 3)			% RSD		
			AML (3 µg/mL)	ROS (6 µg/mL)	FIM (18 µg/mL)	AML (3 µg/mL)	ROS (6 µg/mL)	FIM (18 µg/mL)
Change in Mobile Phase Ratio, ACN:0.02 M KH_2PO_4 , pH 3.0 adjusted with 1% O – phosphoric acid	ACN:0.025 M KH_2PO_4 49:51 v/v (pH 3.0 adjusted with 1% OPA)	50:50	138,593 ± 981.437	187,645 ± 1823.569	635,224 ± 4204.94	1.708	0.971	1.661
Change in Wavelength	242 nm	48:52	138,055 ± 721.388	185,547 ± 2180.48	634,836 ± 3127.71	1.522	1.174	1.492
		240	138,203 ± 1027.9	187,186.7 ± 1309.346	635,261 ± 1906	1.743	1.699	1.300
Change in Temperature	25°C	244	137,943 ± 1666.6	184,637.3 ± 1651.289	637,363 ± 1733.09	1.209	1.894	1.489
		30	138,619 ± 1099.73	186,410 ± 680.976	637,091 ± 2428.26	0.793	1.365	1.381
Change in Flow rate	1 ml/min	20	138,523 ± 1045.12	186,296 ± 1129.961	635,819 ± 1623.93	1.754	1.606	1.255
		0.9 ml/min	138,565.05 ± 1523.12	186,016 ± 2110.365	636,128.02 ± 2100.369	0.985	1.032	0.874
		1.1 ml/min	137,520 ± 1084.36	185,237.3 ± 1420.32	635,819.03 ± 1623.93	1.563	1.321	1.002

Table 5 The system suitability data of proposed RP-LC method for AML, ROS and FIM

Parameter	AML	ROS	FIM
Retention time (Min)	3.15	6.21	9.2
Peak resolution	6.1	2	–
Theoretical plate	2695.12	5323.94	5201.06
Asymmetric factor	0.95	0.94	1.05
Capacity factor	1.05	4.8	7.1

proposed method is shown in Table 4. The solution stability study revealed that AML, ROS and FIM solutions were stable for 24 h without detectable degradation. The percentage amount of both the drugs was found to be more than 98%.

System suitability test was carried out on freshly prepared standard stock solutions of AML, ROS and FIM. The system suitability test parameters are shown in Table 5.

4 Analysis of synthetic mixture

The proposed method was applied for the determination of AML, ROS and FIM in their synthetic mixture. The results for AML, ROS and FIM were comparable with the

corresponding labelled amounts. The % amount of drug found for the analysis of synthetic mixture is more than 98%. The assay overlay chromatogram was shown in Fig. 5.

5 Conclusion

RP-LC method has been developed for the simultaneous estimation of AML, ROS and FIM in bulk and its synthetic mixture. The low % RSD value was found for validation parameter which indicates that the suitability of this method for routine analysis and quantitative determination of Amlodipine besylate, Rosuvastatin calcium and Fimasartan potassium trihydrate in bulk and synthetic mixture by reverse phase liquid chromatographic method. The statistical analysis of data found proves that the method is reproducible, selective, accurate, robust and precise.

Fig. 5 Overlay chromatogram comparison of API and synthetic mixture

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Compliance with ethical standards

Conflict of interest The authors have no conflict of interest.

Code availability Software Empower version 2 was used on HPLC system.

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