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Original Article

RP-HPLC Method Development and Validation for Simultaneous Estimation of Telmisartan, Rosuvastatin Calcium and Amlodipine Besylate in Combination

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Abstract

Introduction: Dyslipidemia-hypertension proves to be a major risk factor for cardiovascular diseases. In order to achieve better adherence and cost-effectiveness than free equivalent combination therapies, a fixed-dose combination therapy with telmisartan (TEL), rosuvastatin calcium (ROS) and amlodipine besylate (AML) is required in this type of patients.

Aim: A simple, selective and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method has been developed and validated for estimation of telmisartan, rosuvastatin calcium, and amlodipine besylate in synthetic mixture.

Materials and methods: Chromatographic separation was performed on a reversed-phase Luna C18 100Å column (250 mm × 4.6 mm i.d., particle size 5 μ) using an isocratic elution of mobile phase consisting of methanol and acetonitrile (pH 3.5 adjusted by orthophosphoric acid) (60:40 v/v) at a flow rate of 1.0 ml/min.

Results: Ultraviolet (UV) detection was performed at 242 nm and retention time of telmisartan, rosuvastatin calcium and amlodipine besylate was found to be 2.67, 4.70, and 7.44 min, respectively. The calibration curve was linear (correlation coefficient >0.999) in the selected range of analyte.

Conclusions: The method was validated for accuracy, precision, linearity, limit of detection, limit of quantitation and ruggedness. The system suitability parameter, such as theoretical plate, asymmetry, and resolution between standard five replicate were well within the limits.

Keywords

accuracy, ICH guideline, mobile phase, precision, retention time.

INTRODUCTION

Telmisartan is chemically described as 2-(4- {[4-methyl-6-(1-methyl-1H-1,3-benzodiazol-2-propyl-1H- 1,3benzodiazol-1-yl] methyl}phenyl) benzoic acid. It is used as an angiotensin II receptor antagonist (AT1) in the management of hypertension.[1] It selectively antagonizes angiotensin II binding to the AT1 subtype receptors. It is commonly administered through the oral route.

Rosuvastatin calcium is chemically calcium: (*E*,3*R*,5*S*)-7-[4-(4-fluorophenyl)-2- [methyl(methylsulfonyl)amino]-6-propan-2-ylpyrimidin-5-yl]-3,5-dihydroxyhept-6-enoate. It belongs to a class of drugs called statins, which are employed in lowering hypercholesterolemia, its related conditions and preventing cardiovascular diseases.[2]

Amlodipine besylate is chemically described as 3-ethyl-5-methyl (±)-2-[(2-aminoethox) methyl]-4-(2- chlorophenyl)-1, 4- dihydro-6-methyl-3, 5-pyridinedi carboxylate, mono benzene sulphonate. AML is a calcium channel blocker[2], which inhibits the influx of extracellular calcium across the myocardial and vascular smooth muscle cell membranes, thus decreasing the contractile process and hence dilating coronary and systemic arteries[3].

The literature survey revealed that methods available for the determination of telmisartan are such as [UV][4], [HPLC][5], [RP-HPLC][6], and [Tandem mass spectrometry][7]. The methods available for determination of rosuvastatin calcium are [UV][8], [RPHPLC][9], [HPTLC][10], etc. Similarly, for determination of ADB, the methods are [UV][11], [RP-HPLC][12], and [HPTLC][13]. Many methods have been described in the literature for determination of telmisartan, rosuvastatin calcium, and amlodipine besylate individually and in combination with other drugs. No single method was reported for the estimation in the combined form.

AIM

The present work described a validated reverse phase HPLC method for determination of TEL, ROS, and AML in synthetic mixture used in the management of hypertension with dyslipidemia.[14]

MATERIALS AND METHODS

Chemicals and materials

The raw materials for telmisartan and Rosuvastatin were received as gift sample from Dano Pharmacham Pvt.Ltd., Ankleswar. Amlodipine Besylate raw material was received as gift sample from Mccoy Drug Pvt. Ltd., Sachin. HPLC Grade Methanol was received from RANKEM and HPLC Grade Acetonitrile was received from E Merck Ltd. Membrane filter: 0.22 µm and Nylon membrane filters were received from RANKEM.

Instrumentation

Chromatographic analysis was carried out on liquid chromatography (UFLC Shimadzu Corporation, Japan) with LC-2010HT series binary pump system using a UV detector with Software CLASS –VP (version 2.31) software to acquire and process the data. HPLC condition is given in **Table 1**.

Preparation of standard solution

1 ml of the standard stock solutions (1000 µg/ml) of all three drugs (TEL, ROS, and AML) was taken in a common volumetric flask diluted up to 10 ml with mobile phase - acetonitrile : methanol, pH=3.5 adjusted using orthophosphoric acid (60:40) to make the final concentration of 100:100:100 µg/ml.

Preparation of a sample solution (assay procedure)

It was prepared as per the patent (telmisartan: 80 mg, rosuvastatin calcium: 20 mg, and amlodipine besylate: 10 mg) and talc quantity was sufficient. All the excipients were mixed in a 100-ml volumetric flask and sonicated for 15 min. The solution was filtered through Whattman filter paper no. 42. Finally, the solution concentrations were obtained as 800, 200, and 100 µg/ml for each of the drugs, respectively. From that pipette out 1 ml in a 10-ml volumetric flask and volume made up with mobile phase – methanol : acetonitrile (60:40 v/v) to make the final concentration of TEL (80 µg/ml), ROS (20 µg/ml), and AML (10 µg/ml) and recorded peak areas were noted for estimation of TEL, ROS and AML.[15]

RESULTS AND DISCUSSIONS

Method validation

System suitability studies

The system suitability was evaluated by five replicate analyses of TEL, ROS, and AML mixture at concentrations of 80, 20, and 10 µg/ml of each drug, respectively. The column efficiency, resolution, and peak asymmetry were calculated for the standard solution. The results of system suitability and system precision were presented **(Table 2)**.[16-18]

Linearity and range

The linearity response was determined by analysing five independent levels of concentration in the range of 40-200 µg/ml for TEL, 10-50 µg/ml for ROS, and 5-25 µg/ml for AML.[19-21] The results are presented in **Table 3**. A calibration curve was found to be linear with a regression coefficient (>0.99) **(Fig. 1)**.

Table 1. Chromatographic condition

Column	Luna C18 100Å column (250 mm * 4.6 mm i.d., particle size 5 µ)
Detector	242 nm
Injection volume	20 µl
Flow rate	0.1 ml/min
Mobile phase	Methanol : Acetonitrile : water (60:40 v/v) (PH3.5 Adjusting with orthophosphoric acid)

Table 2. System suitability studies

Parameters	Observed values			IP specification
	TEL*	ROS*	AML*	
Retention time (min)	2.07	4.65 7	6.99	-
Theoretical plates	7506.14	2190.34	11989	Not less than
Asymmetry (10%)	1.57	0.89	0.78	Not greater
Resolution	15.76	-	7.45	>2

Observed values for system suitability test *(n=5)

Table 3. Calibration curve data

Concentration (µg/ml)	TEL (Peak area)*	Concentration (µg/ml)	ROS (Peak area)*	Concentration (µg/ml)	AML (Peak area)*
40	10789	10	23450	5	54677
80	157889	20	142311	10	298777
120	246778	30	319008	15	356789
160	334556	40	497665	20	678990
200	410560	50	585261	25	846670

* n=5

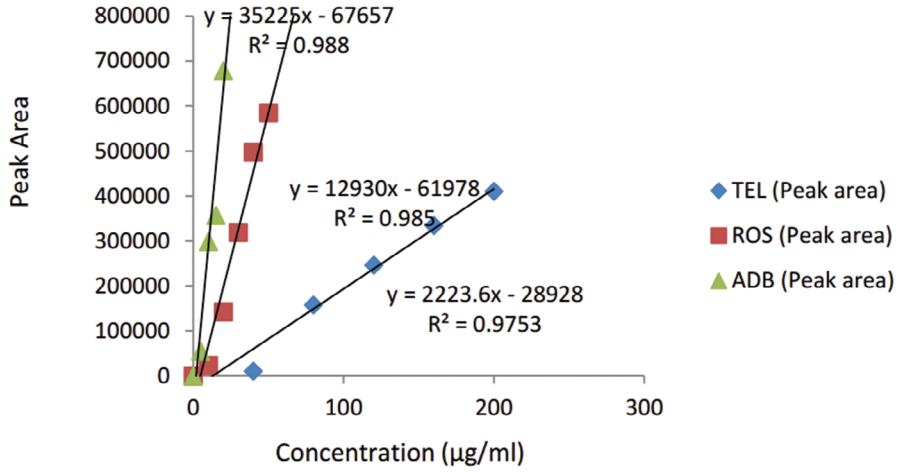

Figure 1. Calibration curve of telmisartan, rosuvastatin calcium, and amlodipine besylate.

Accuracy

The difference between theoretical added amount and practically achieved amount is called accuracy of analytical method. Accuracy was determined at three different levels – at 50%, 100%, and 150% of the target concentration in triplicate. The results are presented in **Table 4**.

Precision

Intraday precision and interday precision

The precision of the developed method was assessed by analysing samples of the same batch with three combined solutions of TEL, ROS and AML in the concentration of 80,

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120, and 160 µg/ml of TEL, 20, 30, and 40 µg/ml of ROS, and 10, 15, and 20 µg/ml of AML, respectively in three replicates (n=3) each on same day.[22] The percentage of RSD value of the results corresponding to the peak area was expressed for intra-day precision. The precision of the developed method was assessed by analysing samples with three standard solutions of TEL, ROS, and AML similarly like above concentration respectively in three replicates (n=3) each on different day. The results are presented in **Table 5**. The results obtained were within 2% RSD.

Limit of detection (LOD) and limit of quantification (LOQ)[23,24]

The LOD & LOQ were found to be 1.46 and 4.21 µg/ml for TEL, 0.54 and 1.63 µg/ml for ROS, and 0.78 and 2.01 µg/ml for AML, respectively.

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Robustness

As defined by The International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH), the robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters. Robustness was performed by small variation in the chromatographic conditions and found to be unaffected by ±0.1 ml/min variation in flow rate of mobile phase, and ±0.1 variation in detection wavelength. These results are presented in **Table 6**.

Assay

The percentage assay of chromatogram analysis for bulk mixture of TEL, ROS, and AML were 99.98%, 100.21%, and 99.24%, respectively

The retention time of bulk mixture of TEL, ROS, and AML are 2.67 min, 4.70 min, and 7.44 min, respectively **(Fig. 2)**.

Table 4. Recovery data (accuracy)

Drug	Spiked level (%)	Amount taken (µg/ml)	Amount found (µg/ml)	Total amount Taken(µg/ml)	Amount found (µg/ml)	% Recovery
TEL	50%	80	40	120	118.14	98.45±0.56
	100%	80	80	160	159.13	99.46±0.21
	150%	80	120	200	199.48	99.74±0.88
ROS	50%	20	10	30	29.40	98.01±0.77
	100%	20	20	40	39.62	99.07±0.89
	150%	20	30	50	50.09	100.19±0.76
AML	50%	10	5	15	14.95	99.67±0.98
	100%	10	10	20	20.02	100.01±0.13
	150%	10	15	25	25.00	100.01±0.87

Table 5. Interday and intraday precision studies

INTRADAY PRECISION				INTERDAY PRECISION			
DRUG	Con. taken (µg/ml)	Mean Peak Area*	%RSD	DRUG	Con. taken (µg/ml)	Mean Peak Area*	%RSD
TEL	80	216788	0.67	TEL	80	206789	0.87
	120	288780	0.82		120	288685	0.80
	160	380998	0.56		160	374566	0.67
ROS	20	145677	0.78	ROS	20	155678	0.80
	30	312670	0.67		30	319080	0.69
	40	500134	0.21		40	526678	0.24
AML	10	298754	0.37	AML	10	289970	0.39
	15	354606	0.80		15	354567	0.81
	20	687888	0.88		20	698008	0.89

Table 6. Robustness studies

Conditions	Modification	TEL Peak Area	TEL %RSD	ROS Peak Area	ROS *%RSD*	AML Peak Area	AML %RSD
Flow rate (ml/min)	0.9	288678	0.56	309901	0.89	347891	0.82
	1.1	288689	0.59	309903	0.89	34893	0.83
Change of wavelength	241	276899	0.77	319960	0.70	346800	0.79
	243	276897	0.76	319950	0.71	346778	0.78

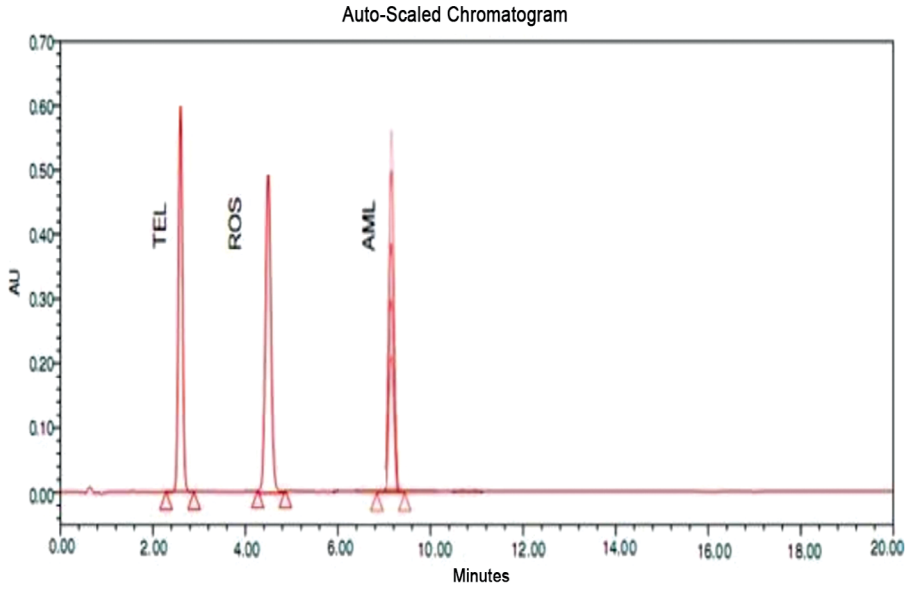

Figure 2. Typical chromatogram of TEL, ROS and AML. Chromatogram showing retention time 2.67, 4.70, and 7.44 min for telmisartan (TEL), rosuvastatin calcium (ROS), and amlodipine besylate (AML) in synthetic mixture, respectively.

CONCLUSIONS

A rational and valid attempt has been made for the development of telmisartan, rosuvastatin calcium, and amlodipine besylate in synthetic mixture. The accountability of the proposed method has been established by evaluating validation parameters as per ICH guidelines. The developed RP-HPLC methods are simple, economical, precise, and accurate for the simultaneous determination of telmisartan, rosuvastatin calcium, and amlodipine besylate in synthetic mixture.

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REFERENCES

1. Elseena J, Anjana CN, Merlin K, et al. Telmisartan and rosuvastatin: a review on the analytical methods for the individual and combined dosage forms. Int J Res Anal Rev 2020; 7(1):927–45.
2. Kaila H, Aambasana A, Thakkar S, et al. A new improved RP-HPLC method for assay of rosuvastatin calcium in tablets. Indian J Pharm Sci 2010; 72(5):592–8.
3. Kumar S, Ram B. Analytical method development and validation of amlodipine besylate in tablet dosage form. J Drug Deliv Ther 2019; 9(1):463–6.
4. Chavhan V, Lawande R, Salunke J, et al. UV spectrophotometric method development and validation for telmisartan in bulk and tablet dosage form. Asian J Pharm Clin Res 2013; 6(4):19–21.
5. Shen J, Jiao Z, Li ZD, et al. HPLC determination of telmisartan in human plasma and its application to a pharmacokinetic study. Int J Pharm Sci Res 2005; 60(6):418–20.
6. Surekha ML, Swamy GK, Ashwini GL. Development and Validation of RP-HPLC method for the estimation of telmisartan in bulk and tablet dosage form. Int J Drug Dev Res 2012; 4:200–5.
7. Penfei LI, Jinkai GU. Determination of telmisartan in human plasma by liquid chromatography - tandem mass spectrometry. J Chromatogr B 2005; 828(1):126–9.

8. Mishra P, Shah K. Simple UV spectrophotometric determination of rosuvastatin calcium in pure form and in pharmaceutical formulations. J Chem 2009; 6(1):89–92.
9. Karunakaran A, Subhash V, Chinthala R, et al. Simultaneous estimation of rosuvastatin calcium and fenofibrate in bulk and in tablet dosage form by UV-spectrophotometry and RP-HPLC. Stamford J Pharm Sci 2011; 4(1):58–63.
10. Chaudhari BG, Patel NM, Shah PB. Determination of simvastatin, pravastatin sodium and rosuvastatin calcium in tablet dosage forms by HPTLC. Indian J Pharm Sci 2007; 69(1):130–2.
11. Kasture AV, Madhuri R. Simulataneous UV-spectrophotometric method for the estimation of atenolol and amlodipine besylate in combined dosage form. Indian J Pharm Sci 2006; 68(3):394–6.
12. Shah DA, Bhatt KK, Shankar MB, et al. RP-HPLC determination of atorvastatin calcium and amlodipine besylate combination in tablets. Indian J Pharm Sci 2006; 68(6):796–9.
13. Jain PS, Patel MK, Bari SB, et al. Development and validation of HPTLC method for simultaneous determination of amlodipine besylate and metoprolol succinate in bulk and tablet dosage form. Indian J Pharm Sci 2012; 74(2):152–6.
14. Jin X, Kim MH, Han KH, et al. Efficacy and safety of co-administered telmisartan/amlodipine and rosuvastatin in subjects with hypertension and dyslipidemia. J Clin Hypertens 2020; 22(10):1835–45.
15. Son M, Guk J, Kim Y, et al. Pharmacokinetic interaction between rosuvastatin, telmisartan, and amlodipine in healthy male Korean subjects: a randomized, open-label, multiple-dose, 2-period crossover study. Clin Ther 2016; 38(8):1845–57.
16. Huber JFK, Van der Linden R, Ecker E, et al. Column switching in high pressure liquid chromatography. J Chromatogr 1973; 2:267–71.
17. Yun KS, Zhu C, Parcher JF. Theoretical relationships between the void volume, mobile phase volume, retention volume, adsorption, and Gibbs free energy in chromatographic processes. Anal Chem 1995; 4:613–9.
18. Heinisch S, Rocca JL. Effect of mobile phase composition, pH and buffer type on the retention of ionizable compounds in reversed-phase liquid chromatography: application to method development. J Chromatogr A 2004; 1048:183–93.
19. International Conference on Harmonization of Technical Requirement for Registration of Pharmaceutical for Human use. Stability Testing of New Drug Substance and Products ICH Q2 (R1). 2003.
20. Chandran S, Singh RSP. Comparison of various international guidelines for analytical method validation. Pharmazie 2007; 62:4–14.
21. Quality Assurance of Pharmaceuticals. A compendium of guidelines and related materials. WHO Geneva 1997; 1(1):119–24.
22. Kirtan P, Patel KP, Chhalotiya UK, et al. A new RP-HPLC method for simultaneous quantification of perindopril erbumine, indapamide, and amlodipine besylate in bulk and pharmaceutical dosage form. Future J Pharm Sci 2020; 6:80.
23. Kansara D, Chhalotiya UK, Kachhiya HM, et al. Development of TLC method for simultaneous estimation of novel combination of amlodipine besylate, rosuvastatin calcium, and fimasartan potassium in synthetic mixture. J Chem Metrol 2020; 14:142–52.
24. Murtaza G, Akhtar Y, Mahmood T, et al. A novel UV-spectrophotometric method for simultaneous estimation of amlodipine and captopril. Pharm Chem J 2019; 52(11):952–8.

Разработка и валидация метода RP-HPLC для одновременной оценки телмисартана, розувастатина кальция и амлодипина безилата в комбинации

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Резюме

Введение: Дислипидемия-гипертензия является основным фактором риска развития сердечно-сосудистых заболеваний. Для достижения лучшей приверженности и экономической эффективности по сравнению с бесплатной эквивалентной комбинированной терапией у этого типа пациентов требуется комбинированная терапия с фиксированными дозами телмисартана (TEL), розувастатина кальция (ROS) и амлодипина безилата (ADB).

Цель: Разработан и утверждён простой метод селективной высокоэффективной жидкостной хроматографии с обращённой фазой (RP-HPLC) для оценки телмисартана, розувастатина кальция и безилата амлодипина в синтетических смесях.

Материалы и методы: Хроматографическое разделение на колонке с обращённой фазой Luna C18 100Å (250 мм × 4.6 мм i.d, размер частиц 5 µ) проводили изократическим элюированием подвижной фазы, состоящей из метанола и ацетонитрила (pH 3.5 доводили ортофосфорной кислотой) (60:40 v/v), со скоростью потока 1.0 мл/мин.

Результаты: Ультрафиолетовую (UV) детекцию проводили при 242 нм, а время удерживания телмисартана, розувастатина кальция и амлодипина безилата составляло 2.67, 4.70 и 7.44 мин соответственно. Калибровочная кривая была линейной (коэффициент корреляции > 0.999).

Заключение: Метод был валидирован на правильность, прецизионность, линейность, предел обнаружения, предел количественного определения и стабильность. Параметры пригодности системы, такие как теоретические плиты, асимметрия и разложение в стандартной пятикратной повторности, находились в пределах нормы.

Ключевые слова

точность, рекомендации ICH, подвижная фаза, прецизионность, время удерживания