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DEVELOPMENT AND VALIDATION OF THE HPTLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF RAMIPRIL AND HYDROCHLOROTHIAZIDE IN SOLID DOSAGE FORM

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Keywords:

Validation, HPTLC, Simultaneous estimation, Ramipril, Hydrochlorothiazide

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ABSTRACT: Ramipril is an angiotensin-converting enzyme inhibitor and is used in treating high blood pressure. Hydrochlorothiazide is a diuretic drug that increases urine output. High-Performance Thin-Layer Chromatography is a sophisticated form of thin-layer chromatography which is a separation technique and is used in various bioavailability studies to study marketed formulations as well as novel therapeutic agents. We aimed to develop and validate a simple, precise, accurate, and rapid HPTLC method for the simultaneous estimation of Ramipril and Hydrochlorothiazide in the combined solid dosage form. Precoated silica gel $G_{60}F_{254}$ was used as the stationary phase. The mobile phase used was a mixture of methanol: toluene: ethyl acetate: glacial acetic acid (1:6:3:0.5 %v/v). Spot detection was carried out at 210 nm. Validation of the method was done in terms of linearity, accuracy, precision, and specificity. A linear calibration curve was obtained between 2000 to 12000 ng/spot for Ramipril and 500 to 3000 ng/spot for Hydrochlorothiazide. The limit of detection and the limit of quantification for the Ramipril was found to be 434.1 and 1315.483 ng/spot, respectively, and for Hydrochlorothiazide 80.6 and 244.31 ng/spot, respectively. The drug content of the marketed formulation was successfully determined by the proposed validate method.

INTRODUCTION: Nowadays, an abundant combination of drugs in various dosage forms is available in the market. Multicomponent formulations have gained a lot of importance due to greater patient acceptability, multiple action, increased potency, lesser side effects, and quick relief. Simultaneous analysis procedures are often used in the current scenario for the estimation of drugs in multicomponent pharmaceutical formulations because of their inherent advantages, avoid time-consuming, extraction, and separation, are economical in the manner that the use of expensive reagents is minimized, and are equally accurate and precise.

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One such combination of drug formulation is Ramipril and Hydrochlorothiazide marketed as Cardace-H 5 tablets (SanofiIndia Ltd.), which is used in the treatment of hypertension.

Chemically Ramipril is 4-[2-(1-ethoxycarbonyl-3-phenyl-propyl) aminopropanoyl]-4-azabicyclo [3.3.0] actane-3-carboxylic acid [1] **Fig. 1** and Hydrochlorothiazide is 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide 1, 1-dioxide [2] **Fig. 2.**

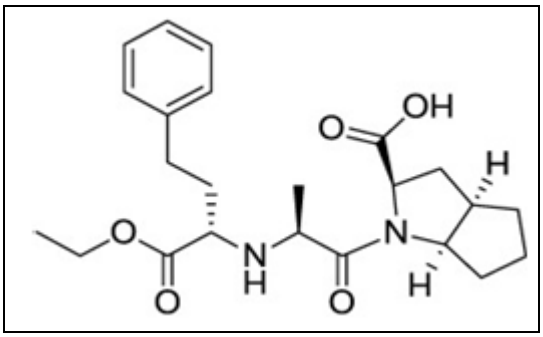

FIG. 1: STRUCTURE OF RAMIPRIL

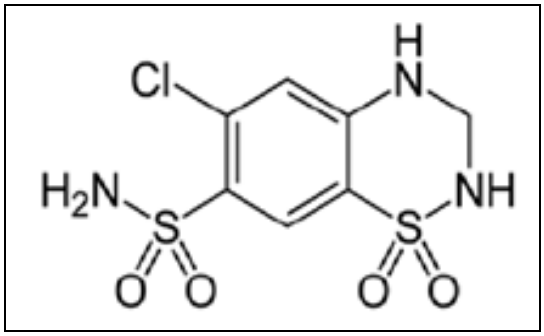

FIG. 2: STRUCTURE OF HYDROCHLOROTHIAZIDE

Ramipril is used for the treatment of essential hypertension and it is an ACE inhibitor and works by relaxing blood vessels which in turn helps the blood to flow more easily. This drug blocks the enzyme ACE which converts angiotensin I to form the potent vasoconstrictor angiotensin II [3]. The addition of Hydrochlorothiazide (A Thiazide diuretic class drug, also known as 'water pills') to Ramipril was found to be more effective than each reagent at lowering blood pressure in patients with hypertension. Hydrochlorothiazide is used to treat edema (fluid retention; excess fluid held in body tissues) and is prescribed alone or together with other medications to treat high blood pressure.

The literature survey reveals that the determination of Ramipril was done by spectrophotometric methods, [4-7] HPLC, [8-11] HPTLC, and electrochemical methods [12-13]. On the other side, Hydrochlorothiazide was determined individually by UV spectroscopy [14-17] HPLC, [18] HPTLC, [19-21] and electrochemical methods. Meanwhile, some spectrophotometric, HPLC, and HPTLC [20-22] and many bioanalytical methods were reported for the simultaneous estimation of Ramipril and Hydrochlorothiazide in the biological fluids and formulations. The survey also gives information about a few newer approaches for the simultaneous determination of drugs in the combined dosage form. For instance, the study described the dissolution method development and validation for both drugs by HPLC method [23] and a stability indicating LC [24] method was used for the determination of Ramipril in the presence of its degradation product. Also, a modern approach of HPLC [25] utilizing a Photo Diode Array detector (HPLC-PDA) was utilized for the simultaneous estimation. The absorptivity factor method and ratio subtraction method are the two new smart spectrophotometric methods [26] used for the estimation of Hydrochlorothiazide. From the exhaustive literature survey, it was found that little work on the HPTLC method has been reported for the simultaneous estimation of Ramipril and Hydrochlorothiazide in the combined formulation. Therefore, it was felt necessary to develop an HPTLC method for the simultaneous estimation of these drugs. In the present investigation, an attempt has been made to develop a more accurate and precise HPTLC method for the simultaneous estimation of Ramipril and Hydrochlorothiazide in combined solid dosage forms.

MATERIALS AND METHODS: Ramipril and Hydrochlorothiazide standards were procured as a gift sample from Torrent Pharmaceuticals Ltd and Centaur Pharmaceuticals Ltd respectively. Tablets containing Ramipril (5 mg) and Hydrochlorothiazide (12.5 mg) were purchased from the local market (Cardace H 5, Sanofi India Ltd.).

A Camag HPTLC system comprising of Camag Linnomate V automatic sample applicator, Hamilton syringe (100 µl), Camag TLC Scanner 3, CamagWinCATS software, Camag Twin-trough chamber (10×10 cm), and ultrasonicator were used during the study. The stationary phase used for the study was prepared by Silica gel $G_{60}F_{254}$ TLC Plates (10 × 10 cm, layer thickness 0.2 mm, E. Merck, Mumbai) and all the analytical grade chemicals and reagents were used.

Preparation of Mobile Phase: A mixture of 1 ml methanol, 6ml toluene, 3ml ethyl acetate and 0.5% glacial acetic acid previously filtered through 0.45 µm filter paper in a flask was used as a mobile phase.

Preparation of Standard Stock Solution: Ramipril (30 mg) and Hydrochlorothiazide (15 mg) each were weighed accurately, dissolved, and diluted separately with methanol to obtain the final concentration of 3000 ng/µl and 1500 ng/µl of each drug. Four ml aliquot from standard Ramipril solution and 2 ml aliquot from standard Hydrochlorothiazide solution was taken and mixed in separate 10 ml volumetric flask to obtain a final concentration of 2000 ng/ml of Ramipril and 500 ng/ml of Hydrochlorothiazide.

Preparation of Sample Solution: To prepare the sample solution, twenty tablets were taken, weighed accurately, and ground to a fine powder. Equivalent to 5 mg of Ramipril and 12.5 mg Hydrochlorothiazide were weighed and transferred to a conical flask and mixed with methanol. The solution was sonicated for 15 min. The extracts were filtered with the help of Whatman filter paper No. 41 and the residue obtained was washed with methanol. The extracts and washings were pooled and transferred to a 25 ml volumetric flask and the volume was made up to 25 ml with methanol. Required dilutions were made to get 200 ng/µl of Ramipril and 500 ng/µl of Hydrochlorothiazide. Standard addition of 1800 µg/ml of Ramipril was done in the above solution.

HPTLC Method and Chromatographic Conditions: TLC plates were prewashed with methanol. Activation of plates was done in an oven at 50°C for 15 min. The chromatographic conditions maintained were precoated silica gel $G_{60}F_{254}$ aluminum sheets (10×10 cm) as stationary phase, the mixture of methanol: toluene: ethyl acetate: glacial acetic acid (1:6:3:0.5 %v/v) as mobile phase, chamber and plate saturation time was 30 min, migration distance allowed was 72 mm, wavelength scanning was done at 272 nm keeping the slit dimension at 5×0.45 mm. A deuterium lamp provided the source of radiation.

Four µl of standard solutions of Ramipril and Hydrochlorothiazide were spotted and developed at a constant temperature. Standard solutions of both drugs were run over 200-400 nm for selecting the working wavelength. Ramipril showed maximum absorbance at 205 nm and Hydrochlorothiazide at 266.74 nm. Both components show reasonably good response at 210 nm, therefore photometric measurements were performed at 210 nm with a Camag TLC scanner. The overlain UV spectra of Ramipril (2.0-10.0 µg/spot) and Hydrochlorothiazide (0.5-2.5 µg/spot) have appeared in **Fig. 3** and HPTLC chromatogram in **Fig. 4**.

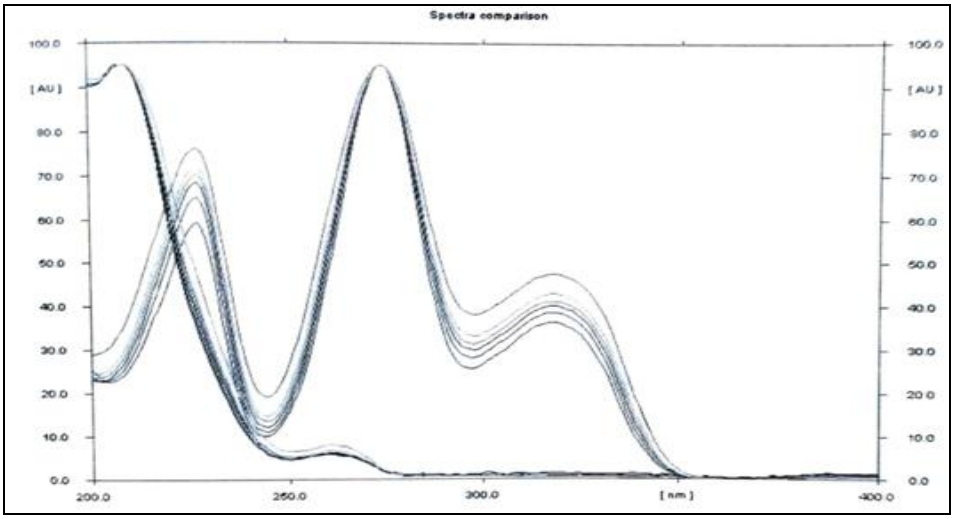

FIG. 3: OVERLAIN SPECTRA OF RAMIPRIL AND HYDROCHLOROTHIAZIDE

Method Validation: The method was validated following the ICH guidelines interms of linearity, accuracy, the limit of detection, the limit of quantification, intra-day and inter-day precision, and repeatability of measurement as well as repeatability of the sample application [27-28].

Linearity and Range (Calibration Curve): The linearity of the analytical method is its ability to elicit test results that are directly proportional to the concentration of analytes in a sample within a given range. The range of the analytical method is the interval between the upper and lower levels of analyte that have been demonstrated to be determined within a suitable level of precision, accuracy and linearity. Aliquots of 1, 2, 3, 4, 5, and 6 µl of the standard solution of both drugs were applied on the TLC plate. The TLC plate was dried, developed, and analyzed photometrically.

The standard calibration curve was obtained using regression analysis with MS Excel. The mixed standard solution was also chromatographed. The graph of peak area versus concentration for both the drugs was plotted **Fig. 6** and **Fig. 7.**

Accuracy: Accuracy is the closeness of the test results obtained by the method to the true value. To study the accuracy, 20 tablets were weighed and powdered and the analysis of the same was carried out. Recovery studies were carried out by calculating recoveries of Ramipril and Hydrochlorothiazide by the standard addition method. For that known amount of standard solutions of Ramipril (3, 6, and 9 µg/spot) and Hydrochlorothiazide (1, 2, and 3 µg/spot) were added to quantified sample solution of tablet dosage form and their assay was done. The percentage recoveries are mentioned in **Table 1.**

TABLE 1: DETERMINATION OF ACCURACY

Amount of sample		Amount of drug added		Amount recovered		% Recovery	
RAM* (µg/spot)	HCZ# (µg/spot)	RAM (µg/spot)	HCZ (µg/spot)	RAM (µg/spot)	HCZ (µg/spot)	RAM	HCZ
6	2	0	0	6.05	1.95	-	-
6	2	3	1	9.12	2.97	101.33	99.00
6	2	6	2	12.10	4.027	100.83	100.67
6	2	9	3	14.87	5.05	99.13	101.00

*RAM – Ramipril, #HCZ – Hydrochlorothiazide

Precision: The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples.

It indicates random error results and was expressed as a coefficient of variation (CV). Three different concentrations of Ramipril (2, 4, 6 µg/spot) and Hydrochlorothiazide (0.5, 1.0, 1.5 µg/spot) were analyzed in triplicates within the same day for intra-day and three successive days for inter-day precision. CV was calculated **Table 2**.

Repeatability: Standard mixture solutions of Hydrochlorothiazide (0.5, 1.0, 1.5, 2.0, 2.5 µg/spot) and Ramipril (2.0, 4.0, 6.0, 8.0, 10.0 µg/spot) were prepared and recorded the chromatograms. The area measured of the same concentration solution five times and Relative Standard Deviation (RSD) was calculated **Table 2**.

Specificity and Selectivity: Specificity is a procedure to detect quantitatively the analyte in the presence of components that may be expected to be present in the sample matrix while selectivity is the procedure to detect qualitatively the analyte in the presence of components that may be expected to be present in the sample matrix.

Commonly used excipients in tablet preparation were spiked in a pre-weighed quantity of drugs and then absorbance was measured and the calculation was done to determine the quantity of drugs.

Analysis of the Marketed Formulation: Prepared sample solutions of the marketed formulation were spotted simultaneously onto the same plate followed by scanning development. The analysis was repeated in triplicate. The content of the drug was calculated from the peak areas recorded **Table 3**.

RESULTS: A solvent system that would give dense and compact spots with significant R_f values was desired for the quantification of Ramipril and Hydrochlorothiazide in pharmaceutical formulations.

The mobile phase consisting of methanol: toluene: ethyl acetate: glacial acetic acid (1:6:3:0.5 %v/v) gave R_f values of 0.27 and 0.56 for Ramipril and Hydrochlorothiazide, respectively **Fig. 4** and the image of developed HPTLC plate of Ramipril and Hydrochlorothiazide are depicted in **Fig. 5.**

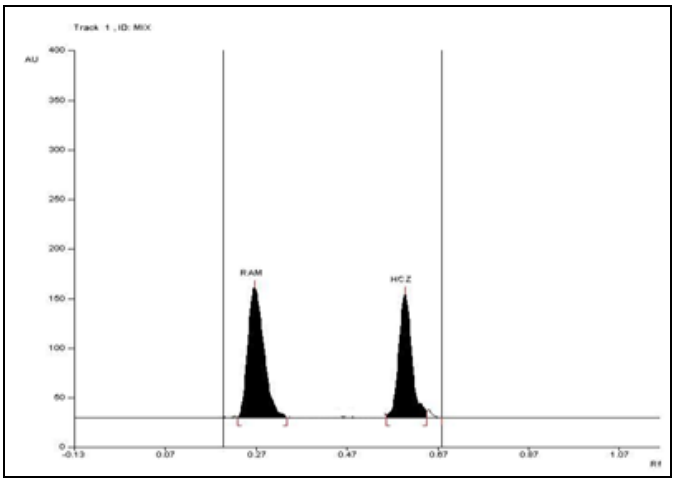

FIG. 4: HPTLC CHROMATOGRAM OF RAMIPRIL AND HYDROCHLOROTHIAZIDE

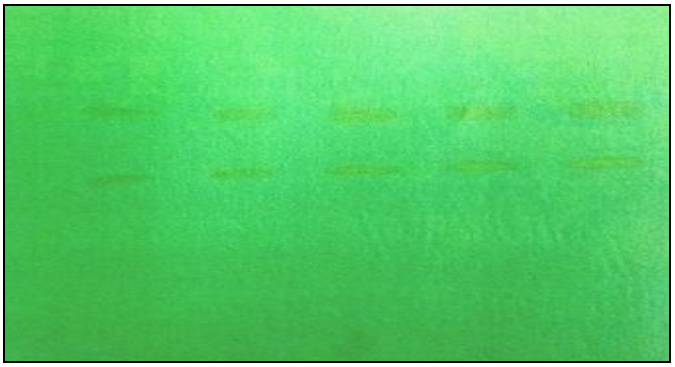

FIG. 5: IMAGE OF DEVELOPED HPTLC PLATE OF RAMIPRIL (A) AND HYDROCHLOROTHIAZIDE (B)

Linearity: The linear regression data *n=5* showed a good linear relationship over a concentration range of 2-10µg/spot for Ramipril **Fig. 6** and 0.5-2.5µg/spot Hydrochlorothiazide **Fig. 7.**

Calibration curves of both the drugs were plotted between the peak area and the concentrations and the linear regression coefficients of Ramipril and Hydrochlorothiazide were found to be 0.998 and 0.992 respectively **Table 2.**

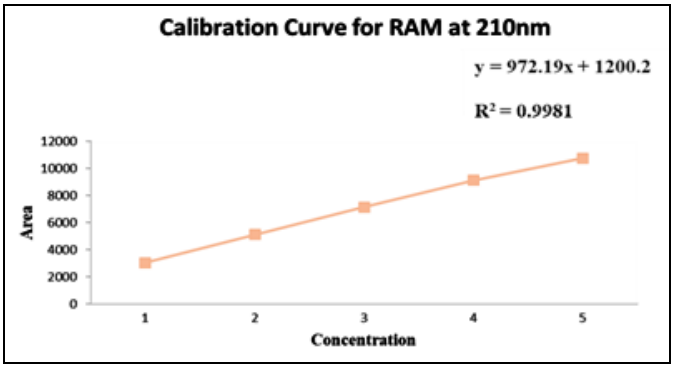

FIG. 6: CALIBRATION CURVE OF RAMIPRIL AT 210 NM

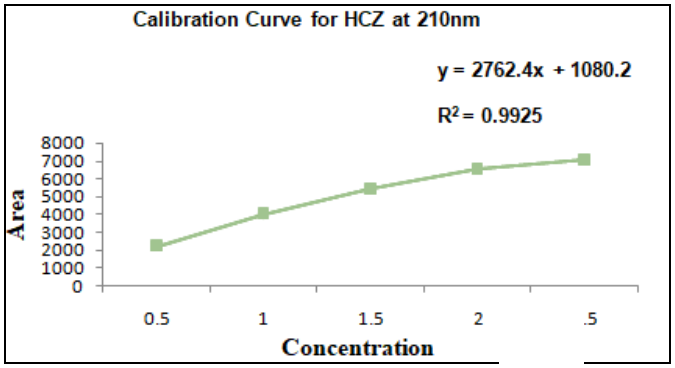

FIG. 7: CALIBRATION CURVE OF HYDROCHLOROTHIAZIDE AT 210 NM

TABLE 2: LINEARITY MEASUREMENTS OF RAMIPRIL AND HYDROCHLOROTHIAZIDE

S. no.	Ramipril (µg/spot)	Area (AUC)	Hydrochlorothiazide (µg/spot)	Area (AUC)
1.	2	3035.3	0.5	2231.76
2.	4	5115.57	1.0	4031.15
3.	6	7140.53	1.5	5435.7
4.	8	9120.46	2.0	6553.66
5.	10	10754.77	2.5	7076.43
6.	Correlation Coefficient (R^2)	0.9981	Correlation Coefficient (R^2)	0.9925

Limit of Detection and Limit of Quantification: The limit of detection and the limit of quantification for the Ramipril were found to be 434.1 and 1315.483 ng/spot, respectively, and for Hydrochlorothiazide 80.6 and 244.31 ng/spot, respectively **Table 3**.

TABLE 3: SUMMARY OF RAMIPRIL AND HYDROCHLOROTHIAZIDE VALIDATION PARAMETERS BY HPTLC METHOD

Parameters	Ramipril (210 nm)	Hydrochlorothiazide (210 nm)
Linearity range (µg/spot)	2 – 10	0.5 – 2.5
Slope (m)	972.19	2762.4
Intercept (c)	1200.2	1080.2
The standard deviation of the slope	26.16908	80.134
The standard deviation of the intercept	173.591	132.887
Limit of Detection (µg/spot)	0.4341	0.0806
Limit of Quantification (µg/spot)	1.3155	0.24431
RSD* %	2.36	1.504
Recovery %	99.16	100.67
Repeatability (RSD, n=5)	0.02361	0.01504
Precision (RSD, n=3)		
Intra-Day	0.8115	1.7315
Inter-Day	2.4950	1.2376
Specificity	Specific	Specific

*RSD - Relative Standard Deviation

Precision: The determination of intra-day precision was carried out 3 times on the same day by analyzing standard solutions in the concentration range of 2000 ng/spot to 6000 ng/spot for Ramipril and 500 ng/spot to 1500 ng/spot for Hydrochlorothiazide while inter-day precision was determined by analyzing corresponding standards daily for 3 days over a period of one week. The intra-day and inter-day coefficients of variation are given in **Table 3**. The repeatability of measurement of peak area was determined by spotting 4 µl of Ramipril and Hydrochlorothiazide solution on a TLC plate and developing the plate. The separated spot was scanned five times without changing the position of the plate and the %RSD for measurement of the peak area of Ramipril and Hydrochlorothiazide was found to be 2.36 and 1.50, respectively **Table 3**.

Specificity: The solution of the formulation was spotted on the TLC plate, developed, and scanned for confirming the specificity of the proposed method. It was observed that the excipients present in the formulation did not interfere with the peaks of Ramipril and Hydrochlorothiazide.

Accuracy: To check the accuracy parameter, recovery studies of the drugs were carried out. These studies were performed at three levels *i.e.*, multiple-level recovery studies. Sample stock solutions from tablet formulation were prepared. Dilutions were made and recovery studies were carried out. Percent recovery was following the limits listed in **Table 1**.

The assay value for the marketed formulation was found to be within the limits listed in **Table 4**. The low Relative Standard Deviation (RSD) value indicated the suitability of the method for routine analysis of Ramipril and Hydrochlorothiazide in solid dosage forms.

TABLE 4: ASSAY RESULTS OF MARKETED FORMULATION

Formulations	Actual concentrations (µg/spot)		Amount recovered (µg/spot)		% RAM*	% HCZ#
	RAM	HCZ	RAM	HCZ		
Cardace H 5 Tablet	2.0	0.5	1.9738	0.5052	98.69	101.05

*RAM – Ramipril, #HCZ – Hydrochlorothiazide

DISCUSSION: The developed HPTLC technique is simple, precise, specific, and accurate and the statistical analysis proved that the method is reproducible and selective for the analysis of Ramipril and Hydrochlorothiazide in bulk drug and tablet formulations. The method can be used for the routine analysis of Ramipril and Hydrochlorothiazide alone or in combination products and for the purity assessment of the two drugs in marketed formulations.

CONCLUSION: A newly developed and validated HPTLC method for simultaneous analysis of Ramipril and Hydrochlorothiazide in pharmaceutical preparations was very simple, rapid, accurate, and precise. The method was successfully applied for the determination of Ramipril and Hydrochlorothiazide in their pharmaceutical tablet formulations.

Hence, for routine control analysis of Ramipril and Hydrochlorothiazide in their pharmaceutical formulation, this method can be conveniently used. The validation studies as per ICH guidelines following linearity, accuracy, precision, repeatability, specificity, and selectivity proved the suitability of the method for the intended use. Also, the non-interference of additives and excipients makes it suitable for the determination of the studied drugs in bulk and their combined solid dosage forms. The proposed HPTLC method can be used for Stability studies by performing forced degradation of drugs and also Chemometric and DoE-based analytical quality risk management to the HPTLC method can be considered as a great future concerned area.

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CONFLICTS OF INTEREST: The authors declare no conflict of interest.

REFERENCES:

1. http://en.wikipedia.org/wiki/Ramipril
2. http://en.wikipedia.org/wiki/Hydrochlorothiazide
3. Tripathi KD: Essential of Medical Pharmacology.8th ed. New Delhi: Jaypee Brothers Medical Publishers; 2022.
4. Sahu R and Vandana PB: Simultaneous spectrophotometric determination of Ramipril and Hydrochlorothiazide from their binary mixture by simultaneous equation method. Indian Drugs 2006; 43(3): 226.
5. Kumar M, Jindal M, Bhatt S, Panduranagan A, Malik A, Kaushik V, Upadhaya PK and Arunachalam G: Simultaneous estimation of Amlodipine Besylate and Ramipril in tablets dosage form by UV spectrophotometric method. Journal of Pharmaceutical Scienceand Research 2019; 11(2): 667-670.
6. Attala K and Elsonbaty A: Smart UV Spectrophotometric methods based on simple mathematical filtration for the simultaneous determination of Celecoxib and Ramipril in their pharmaceutical mixtures with amlodipine. A Comparative Statistical study. Spectrochimica Acta Part A Molecular and Biomolecular Spectroscopy 2021; 244:118533, https://doi.org/10.1016/j.saa.2020.118853
7. Nagar A, Deore S, Bendale A, Kakade R and Sonawane C: Analytical method development and validation of Ramipril and Candesartan Cilexetil in synthetic mixture. Innovations in Pharmaceuticals and Pharmacotherapy 2020; 8(2): 14-20.
8. Gandhimathi M, Ravi TK, Minan A and Varghese A: RP HPLC determination of Losartan Potassium and Ramipril in tablets. Indian Drugs 2004; 41(1): 36.
9. Valiyare GR, Chandra A, Apte SK and Mahadik AA: HPLC determination of Amlodipine, Losartan, and Ramipril in pharmaceutical preparation. Indian Drugs 2005; 42(5): 309.
10. Ahmed SI and Zaheer Z: Determination of Losartan potassium andRamipril hydrochloride in pharmaceutical preparation by RP-LC technique. International Journal of Research and Analytical Reviews 2019; 6(2): 929-934.
11. Taha EA, Fouad MM, Attia AK and Yousef ZM: RP-HPLC method development and validation for simultaneous estimation of Ramipril and Felodipine. European Journal of Chemistry 2019; 10(2): 113-117.
12. Taha EA, Attia AK, Fouad MM and Yousef ZM: Simultaneous determination of Ramipril and Felodipine using carbon paste electrode in Micellar medium. Analytical and Bioanalytical Electrochemistry 2019; 11(2): 150-164.
13. Moraes JT, Salamanca-Neto CAR, Eisele APP, Coldibeli B, Ceravolo GS and Sartori ER: Fast and sensitive simultaneous determination of antihypertensive drugs Amlodipine Besylate and Ramipril using an electrochemical method: application to pharmaceuticals and blood serum samples. Analytical Methods 2019; 11: 4006-4013 https://doi.org/10.1039/C9AY01232J
14. Lande NR, Shetkar BM, Kadam SS and Dhaneshwar SR: Simultaneous spectrophotometric estimation of Losartan Potassium and Hydrochlorothiazide from the combined dosage form. Indian Journal of Pharmaceutical Science 2001; 63(1): 66.
15. Jain SK, Jain D, Tiwari M and Chaturvedi SC: Simultaneous spectrophotometric determination of Propranolol hydrochloride and Hydrochlorothiazide in pharmaceutical preparation. Indian Journal of Pharmaceutical Science 2002; 64(3): 267.
16. Gandhimathi M, Vikram K, Baskaran A and Ravi TK: Simultaneous spectrophotometric determination of Losartan Potassium and Hydrochlorothiazide in combination. Indian Journal Pharmaceutical Science 2001; 63(2): 165.
17. Veerasekaran V, Katakdhond Shrinivas J, Kadam Shivajirao S and Janhavi Rao R: Simultaneous spectrophotometric estimation of Hydrochlorothiazide and Metoprolol Tartrate from the combined dosage form. Indian Drugs 2001; 38(4): 187.
18. Pawar S, Phadke H and Jadhav Y: Simultaneous determination of Lisinopril and Hydrochlorothiazide in Lisinopriland Hydrochlorothiazide tablets using stability indicating RP HPLC method. Indian Drugs 2006; 43(5): 429.
19. Walode SG, Charde MS, Tajne MR and Kasture AV: Development of HPTLC method for simultaneous estimation of Captopril and Hydrochlorothiazide. Indian Drugs 2005; 42(6): 340.
20. Baing MM, Vaidya VV, Singh G, Mhaske H and Dhotre D: Simultaneous determination of Losartan potassium, Ramipril, and Hydrochlorothiazide by HPTLC method. Indian Drugs 2006; 43(4): 333.
21. Patel LJ, Suhagia BN and Shah PB: HPTLC method for the simultaneous estimation of Bisoprololfumarate and Hydrochlorothiazide in tablets. Indian Drugs 2006; 43(8): 630.
22. Desai DA, Chauhan RS and Kushwaha RD: Development and validation of HPTLC method for simultaneous estimation of Hydrochlorothiazide and Ramipril in their combined tablet dosage form and stability indicating HPTLC method for estimation of Hydrochlorothiazide. International Journal of Research Analytical Review 2018; 5(3): 293-301.
23. Rout PV, Padwal SL, Bachute MT and Polshettiwar SA: Development and validation of RP-HPLC chromatographic dissolution method for the simultaneous estimation of Ramipril and Hydrochlorothiazide from solid dosage formulation. Journal of Pharmaceutical Research and Innovation 2021; 33(42B): 203-217
24. Belal F, Al-Zaagi IA, Gadkariem EA and Abounassif MA: A stability-indicating LC method for the simultaneous determination of ramipril and hydrochlorothiazide in dosage forms. Journal of Pharmaceutical and Biomedical Analysis 2021; 24(3): 335-342. https://doi.org/10.1016/S0731-7085(00)00474-X
25. Dawud ER and Shakya AK: HPLC-PDA analysis of ACE inhibitors, hydrochlorothiazide, and indapamide utilizing the design of experiments. Arabian Journal of Chemistry 2019; 12: 718–728.
26. Mohammed FF, Badr El-Din KM and Derayea SM: Two smart spectrophotometric methods for simultaneous determination of Lisinopril and Hydrochlorothiazide in binary mixtures. Journal of Advanced Biomedical and Pharmaceutical Sciences 2019; 2: 47-253.
27. ICH Harmonised Tripartite Guidelines. Validation of analytical procedures: text and methodology, Q2 (R): Nov 2005.
28. ICH, Validation of analytical procedures: methodology, Q2 (R1), International Conference on Harmonization, IFPMA, Geneva 1996.

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