



Quantitative Estimation of Four Sartans in Presence of Hydrochlorothiazide in Pharmaceutical Preparations by High Performance Liquid Chromatography

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Authors' contributions

This work was carried out in collaboration between all authors. Author PR designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors KM and VLS managed the literature searches, analyses of the study and author KR managed the experimental process and author PSB supervised the work. All authors read and approved the final manuscript.

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ABSTRACT

A simple, reliable, reproducible, economical and rapid isocratic RP-HPLC method was developed for the simultaneously separated and quantification of four angiotensin II-receptor antagonists namely Telmisartan (TELM), Losartan (LOSA), Olmesartan (OLME) and Valsartan (VALS) along with thiazide diuretic mostly Hydrochlorothiazide (HCTZ). All the above said drugs were separated by using Welchrom C₁₈ column having internal diameter of 4.6 X 250 mm with 5 µm particle size as stationary phase with mobile phase consisting a mixture of acetonitrile and phosphate buffer (pH-3.3, 50:50 v/v). The mobile phase was pumped at a flow rate of 1 ml/min. The wave length of the UV detection was done at 238 nm. All the sartans were separated within 6 minutes. The calibration curves were linear ($r^2 = 0.9998$) in all cases. The relative standard deviation was less than 2% and average recovery was above 99.95%. All four sartans were routinely assayed without interference.

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This method was statistically validated in different parameters like linearity, precision, specificity, accuracy, robustness and ruggedness. The optimized method proved to be accurate, specific and robust for the quality control of four angiotensin-II receptor blockers alone or in combination with HCTZ in bulk drug and tablet dosage forms.

Keywords: Hydrochlorothiazide; losartan; olmesartan; telmisartan; valsartan.

1. INTRODUCTION

Angiotensin II-receptor antagonists [1] exerts its antihypertensive effects by inhibiting the activation of angiotensin II type 1 (AT₁) receptors. This shows vasodilatation and reduces the secretions of vasopressin and aldosterone, thereby reducing BP. HCTZ is a thiazide diuretic. It exerts its antihypertensive effects by inhibiting Na⁺/Cl⁻ reabsorption from the distal convoluted tubules in the kidney. By reducing osmotic pressure in this way, HCTZ reduces the reabsorption of water in the distal convoluted tubules and thereby reduces plasma volume and cardiac output. Most hypertensive patients will not reach and maintain blood pressure goal on mono therapy. Therefore combinations containing ARBs along with HCTZ have been preferred because ARBs are associated with superior tolerability which may lead to improved adherence. ACE inhibitors are facing a drawback of angioedema and cough which are not seen with ARBs. Various combinations of ARB's are available with a fixed dose of Hydrochlorothiazide.

Several analytical methods for quantitative determination of ARBs individually or in combination with HCTZ in pharmaceutical formulations are described in scientific literature such as UV spectrophotometry [2-5], TLC [6], HP-TLC [7,8], Capillary electrophoresis [9-11], HPLC [12-36], Solid phase extraction and LC-MS [37], LC-MS/MS [38], UPLC-MS [39]. Some of these HPLC methods have been found to be longer retention time, cumbersome, uneconomical possessing poor precision and specificity. The objective of this research work was to develop and validate rapid, economical and sensitive method for quantitative determination of four sartans with HCTZ in tablets. Thus we have decided to develop a novel efficient method to determine all the mentioned drugs in binary combinations such as HCTZ with TELM, HCTZ with LOSA, HCTZ with OLME and HCTZ with VALS without changing the detection wave length and chromatographic conditions. The chemical structures of HCTZ, TELM, LOSA, OLME and VALS are shown in Fig. 1.

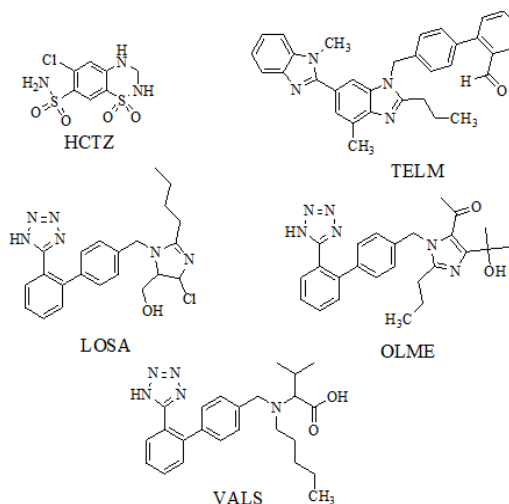


Fig. 1. Chemical structures of HCTZ, TELM, LOSA, OLME and VALS

2. MATERIALS AND METHODS

The pharmaceutical grade reference samples were obtained from Hetero Labs Ltd., Hyderabad, India. Triethylamine and acetonitrile of HPLC grade were purchased from Merck pharmaceuticals private Ltd., Mumbai, India. HPLC grade methanol and water were obtained from Merck specialties private Ltd., Mumbai, India. Commercial tablets of TELM, LOSA, OLME and VALS with HCTZ were obtained from local pharmacy.

2.1 Preparation of pH 3.3 Phosphate Buffer

6.056 g of potassium dihydrogen orthophosphate was dissolved in 445 ml of HPLC grade water to prepare 10 mM of phosphate buffer. To the above solution 55 ml of 0.1 M phosphoric acid was added and its pH was adjusted to 3.3.

2.2 Preparation of Mobile Phase

The above prepared phosphate buffer was mixed with acetonitrile in the ratio of 50: 50 v/v and the

solution was filtered through 0.45 µm nylon membrane filter and degassed by sonication.

2.3 Preparation of Stock Standard Solutions

Standard stock solutions containing (1.25, 0.4, 0.5, 0.4, 0.8 mg/ml) of HCTZ, TELM, LOSA, OLME and VALS were prepared by dissolving (12.5, 40, 50, 40, 80 mg) of each in methanol in 100 ml volumetric flask respectively. Then it was sonicated for 15 minutes and final volume was made up to 100 ml with methanol to get standard stock solutions.

2.4 Construction of Calibration Plot

Each solution was injected in triplicate and chromatographed under the above mentioned conditions. Linear relationships were obtained when average drug standard peak area were plotted against the corresponding concentrations for each drug. Regression analysis of the calibration data was then carried out.

2.5 Sample Preparation (Dosage form)

About twenty Telmisat-H, Zargo-H, Olmicep-H, Valent-H tablets were taken and make them to a fine powder with mortar and pestle separately. Average weight of tablet was calculated, and the amount of powder that is equivalent to HCTZ, TELM, LOSA, OLME and VALS (12.5, 40, 50, 40, 80 mg) respectively of each type of tablets were accurately weighed and separately transferred into 100 ml volumetric flasks and dissolved in methanol. Solutions were degassed then sonicated for 15 min and then filtered through 0.45 µm nylon membrane filters. Aliquots of appropriate volume (10 ml) were transferred to 100 ml calibrated flasks and diluted to volume with mobile phase to obtain the above mentioned concentrations.

3. RESULTS AND DISCUSSION

3.1 Optimization and Method Development

Numerous trials were carried out for the optimization of mobile phase in order to get proper optimized HPLC conditions. A number of trials with different mobile phases were

performed, such as commonly used blend of solvents acetonitrile: HPLC grade water, methanol: water, methanol: Acetonitrile: water in varying ratio and by adjusting pH to attain required separations. In the long run after reviewing the results, mobile phase of phosphate buffer mixture whose pH adjusted to 3.3, acetonitrile in the ratio of 50:50 v/v which meets all the conditions of system suitability and also a better separation which result good peak shape, better resolution, less run time, nominal peak tailing and reproducibility results were recognized. Hence this mobile phase was chosen for current study. The stationary phase made up of Welchrom C₁₈ column with dimensions of 4.6 X 250 mm, 5 µm is found to be utmost suitable for separation of Sartans together with HCTZ. The UV spectrum of the sartans were scanned separately in the region of 200 - 400 nm and the UV overlain spectra of four sartans demonstrated that they get absorbed appreciably at 238 nm. Hence this wave length was selected for the determination of all sartans. The UV overlain spectra of four sartans with HCTZ is shown in Fig. 2. A model chromatogram which depicts how the peaks are separated for the 4 sartans along with HCTZ is depicted in Fig. 3. The peaks were eluted at 3.300, 3.780, 4.467, 5.247, 5.570 minutes for HCTZ, TELM, LOSA, OLME and VALS respectively. System suitability and column performance parameters are shown in Table 1.

3.2 Method Validation

The present method was validated based on ICH Q2 (R1) [40] guidelines. The parameters like system suitability, specificity, linearity, precision, accuracy, limit of detection (LOD), limit of quantitation (LOQ) and robustness were validated.

3.2.1 System suitability

HPLC system is allowed to stabilize for 40 minutes. Then system suitability was conducted to verify the parameters such as theoretical plate count (NLT 3000), efficiency per meter, resolution (NLT 2.0), and tailing factor (NMT 1.5). If the system suitability parameters are satisfied then sample is injected twice and record the chromatograms. System suitability and column performance parameters are depicted in Table 1.

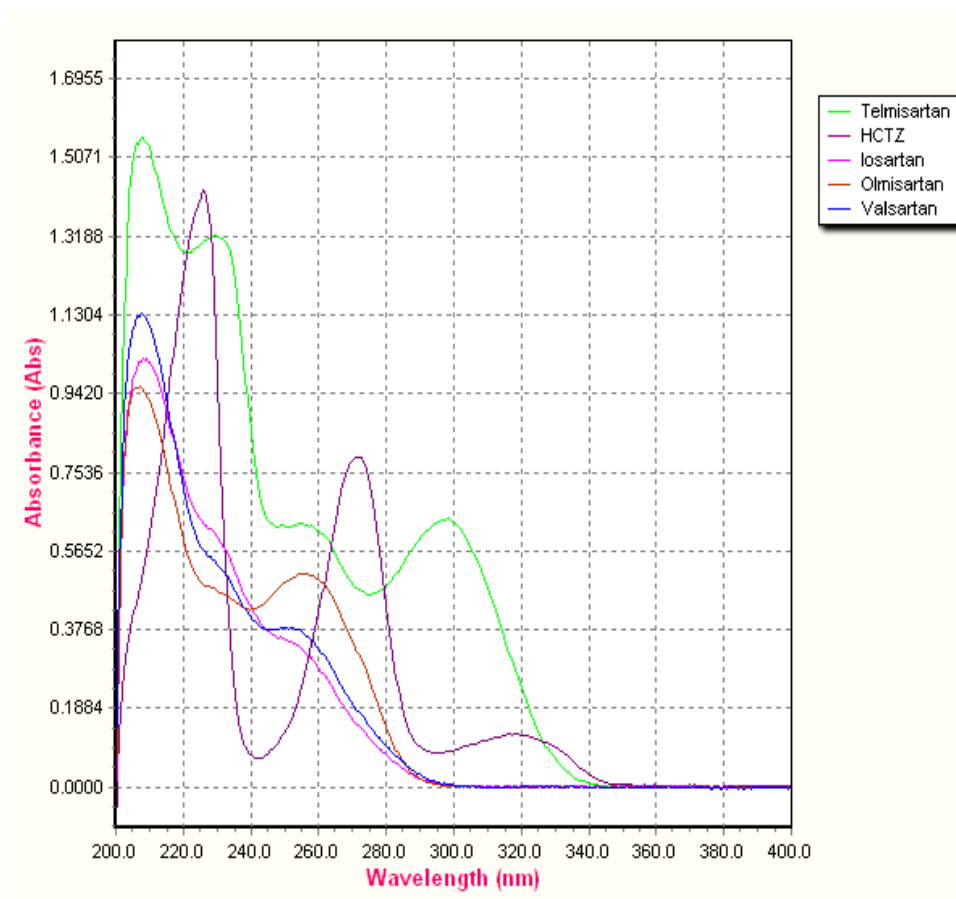


Fig. 2. UV overlay spectra of the four sartans & HCTZ

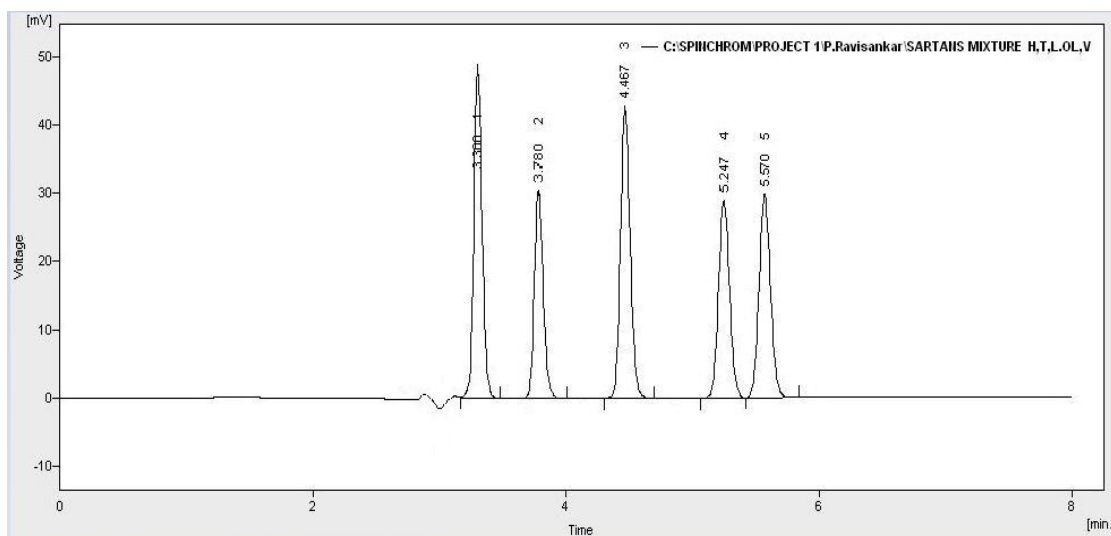


Fig. 3. A typical chromatogram showing the separation of HCTZ, TELM, LOSA, OLME, VALS in a synthetic mixture

Table 1. System suitability and column performance parameters

Parameter	Chromatographic conditions for 4 sartans & hydrochlorothiazide				
Instrument	Shimadzu LC-20AT Prominence liquid chromatograph				
Column	Welchrom C ₁₈ column (4.6 X 250 mm, 5 µm)				
Detector	Shimadzu LC-20AT Prominence UV-Visible detector				
Mobile phase	10 mM phosphate buffer (pH 3.3) : Acetonitrile 50:50, v/v				
Flow rate	1mL/min				
wave length	UV at 238 nm				
Run time	8 minutes				
Temperature	Ambient temperature (25 °C)				
Injection volume	20 µL				
Name of the drugs	HCTZ	TELM	LOSA	OLME	VALS
Retention time (minutes)	3.300	3.780	4.467	5.247	5.570
Th.PI (Efficiency)	12312	12468	14715	15250	17188
Theoretical plates per meter	246247	247368	2,94308	305005	343756
Resolution	-	3.776	4.862	4.931	2.008
Tailing factor	1.194	1.116	1.182	1.160	1.154

3.2.2 Specificity

The specificity of the method was evaluated with regard to interference due to presence of excipients. A placebo for each formulation was mixed by adding the particular excipients with mobile phase without the drug. Actually drug to excipients fraction employed were analogous to that in the commercial formulations and the solutions were prepared by following the sample preparation procedure that is mentioned above. Before injection the blend was filtered using 0.45 µm membrane filters. The results of specificity study are shown in Table 2.

3.2.3 Linearity

The linearity test was determined at five varying concentration levels of the drugs. ICH recommends minimum five concentrations for analysis. For that purpose drug concentration ranges of 2.5-12.5, 8-40, 5-50, 8-40, 16-80 µg/mL were prepared respectively. The calibration curve was constructed by plotting peak area versus concentration of drugs. The linear regression equations were $Y = 88.146 X + 0.0586$ ($r^2 = 0.9998$), $Y = 27.099 X - 6.5173$ ($r^2 = 0.9998$), $Y = 21.431 X - 8.5685$ ($r^2 = 0.9997$), $Y = 13.366 X - 25.101$ ($r^2 = 0.9981$), $Y = 14.576 X + 2.9327$ ($r^2 = 0.9999$) for HCTZ, TELM, LOSA, OLME and VALS respectively where Y is the peak area of analyte and X represents drug concentration.

3.2.4 Precision

The precisions of the method was performed to find out repeatability (Intra-day) and intermediate precision (Inter-day) for HCTZ with TELM, HCTZ with LOSA, HCTZ with OLME and HCTZ with VALS. The intra - day precision was done under the same experimental conditions by repeating the assay thrice for three levels in the same day where as inter - day precision was carried out by taking over the assay on 3 different days, three on every day for the 3 concentration levels respectively. The precision study outcomes were given in terms of % RSD. The % RSD was calculated which is within the satisfactory criteria of NMT 2.0. The precision study results are shown in Table 3.

3.2.5 Accuracy

Accuracy of the method was determined in terms of recovery by spiking the formula ion with the standards of each drug equivalent to 80 %, 100% and 120% of the amount initially present. Recovery tests were carried out by analyzing mixtures of HCTZ with TELM, HCTZ with LOSA, HCTZ with OLME and HCTZ with VALS with diverse compositions. Known amounts of standards drugs were added to a pre-analyzed sample at 3 different levels 80%, 100% and 120% and the mixed standard solutions were analyzed thrice at each level according to suggested method. The mean recovery data and % recovery results are shown in Table 3.

3.2.6 Robustness

The robustness of the present analytical method was verified by the analysis of HCTZ with TELM, HCTZ with LOSA, HCTZ with OLME and HCTZ with VALS under varied experimental conditions

like making intentional changes in chromatographic conditions like mobile phase composition ($\pm 5\%$), flow rate (± 0.2 ml/min), mobile phase p^H and detection wave length (± 5 nm). The results obtained were within tolerable limits. The results are shown in Table 3.

Table 2. Results of specificity

Name	HCTZ	TELM	HCTZ	LOSA	HCTZ	OLME	HCTZ	VALS
Mobile phase	No peaks	No peaks	No peaks	No peaks	No peaks	No peaks	No peaks	No peaks
Placebo	No peaks	No peaks	No peaks	No peaks	No peaks	No peaks	No peaks	No peaks
Individual Separate standard solutions.	Peak for HCTZ and TELM at 3.300 min. and 3.780 min. respectively.		Peak for HCTZ and LOSA at 3.300 min. and 4.467 min. respectively.		Peak for HCTZ and OLME at 3.300 min. and 5.247 min. respectively.		Peak for HCTZ and VALS at 3.300 min. and 5.570 min. respectively.	

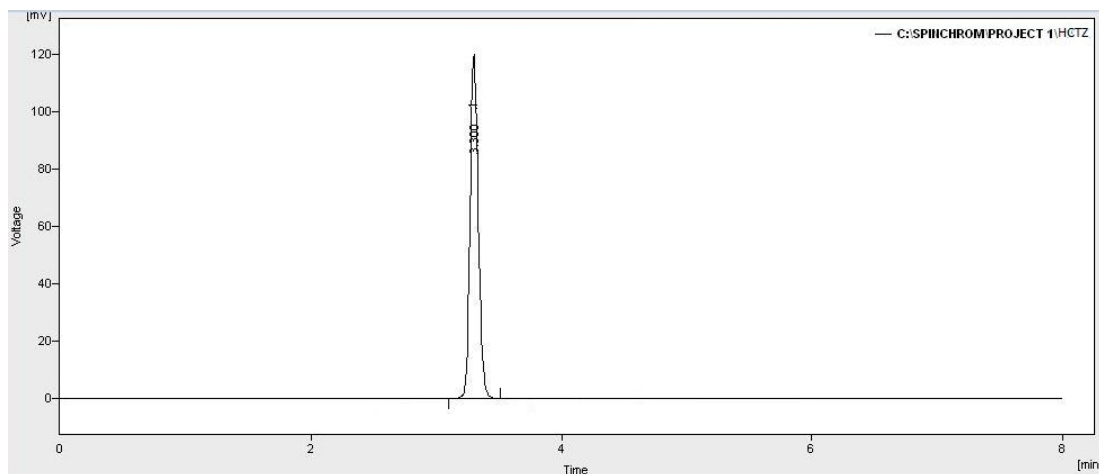


Fig. 4. Standard chromatogram relating to HCTZ

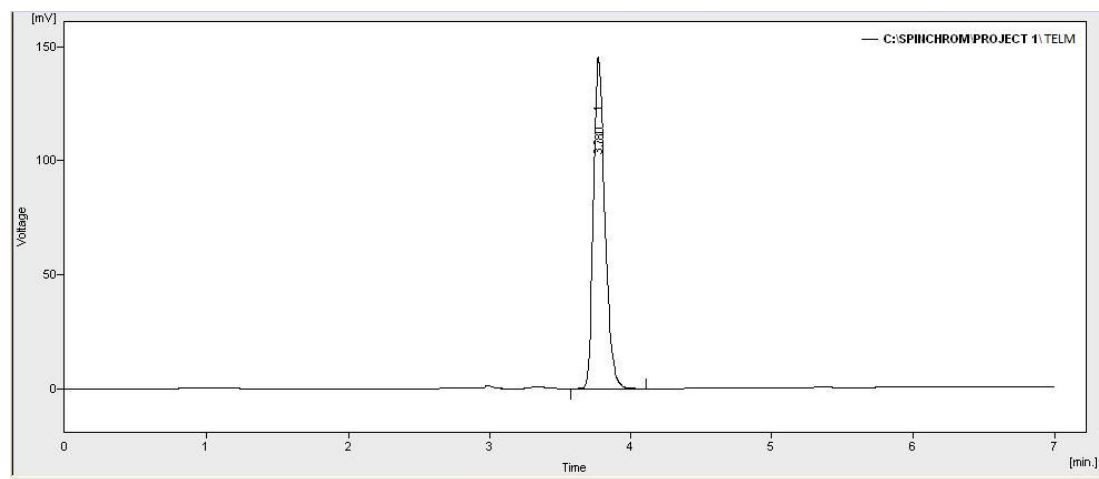


Fig. 5. Standard chromatogram relating to TELM

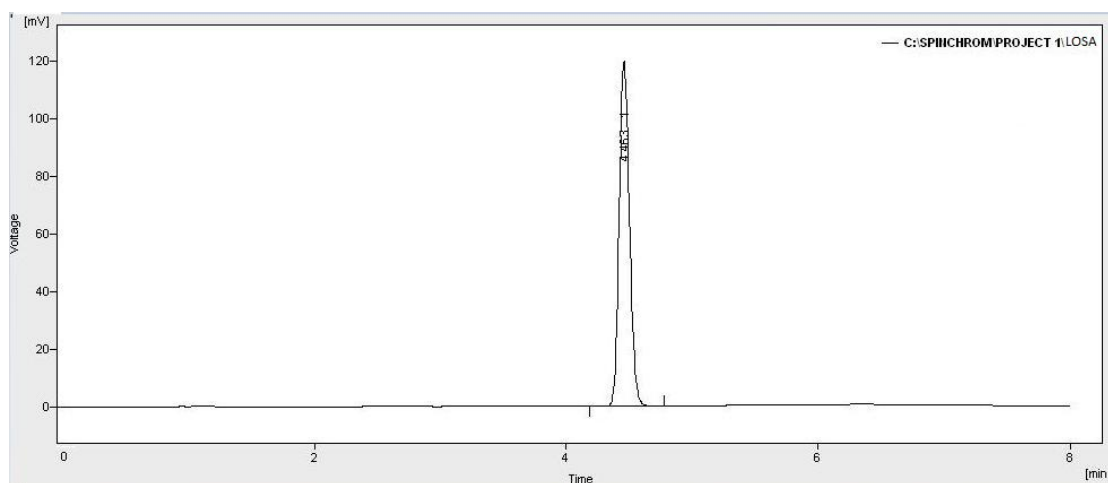


Fig. 6. Standard chromatogram relating to LOSA

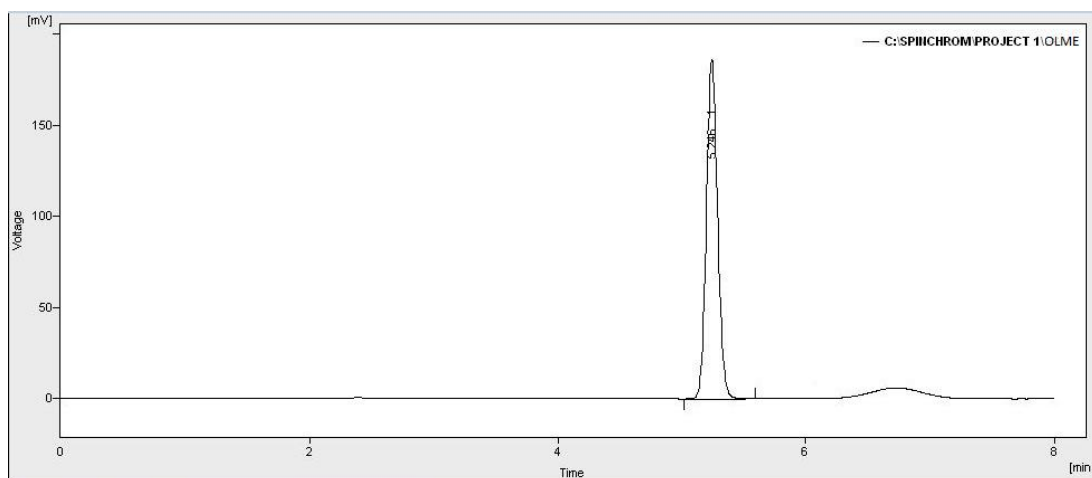


Fig. 7. Standard chromatogram relating to OLME

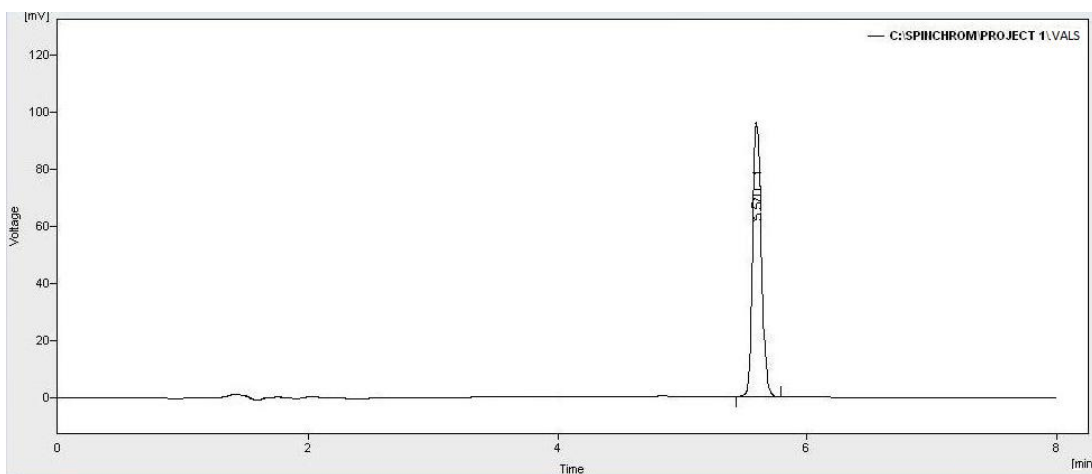


Fig. 8. Standard chromatogram relating to VALS

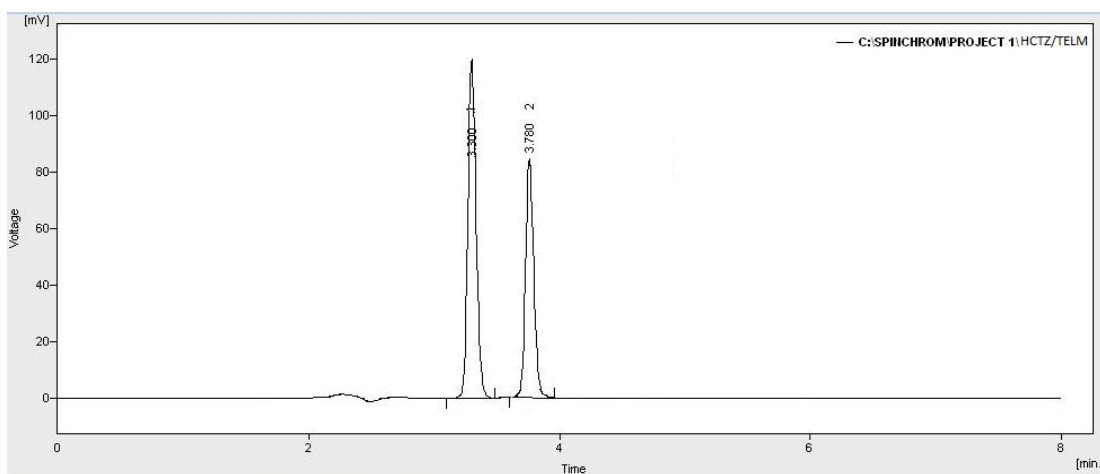


Fig. 9. Standard chromatogram relating to HCTZ (2.5 µg/ml) and TELM (8 µg/ml)

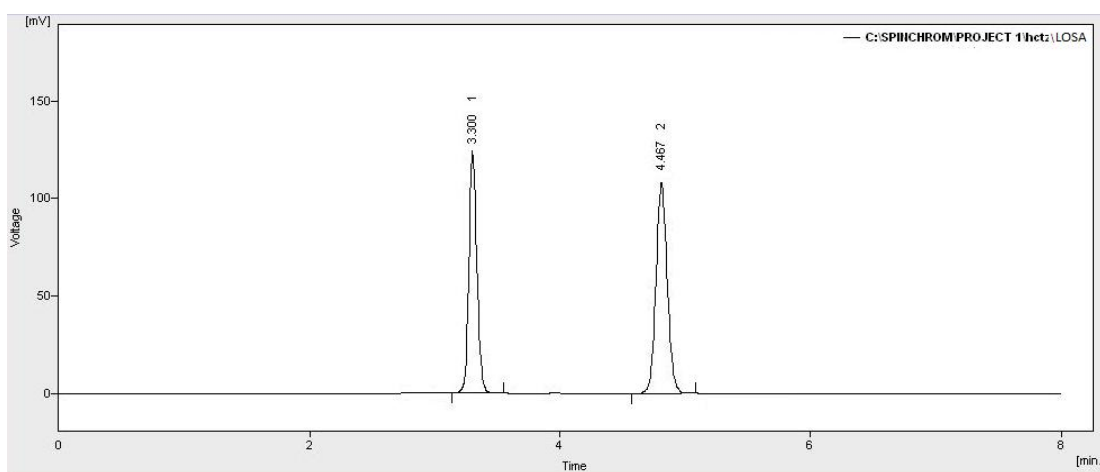


Fig. 10. Standard chromatogram relating to HCTZ (2.5 µg/ml) and LOSA (5 µg/ml)

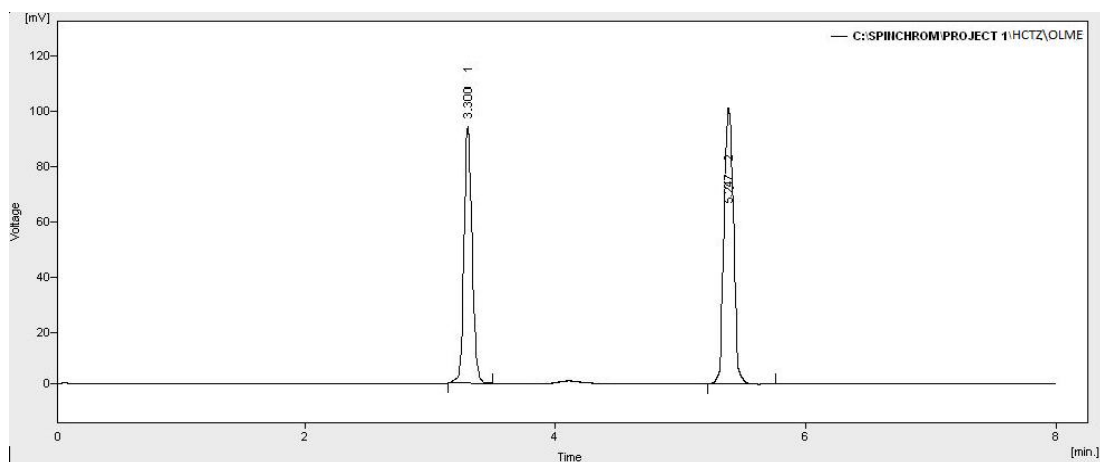


Fig. 11. Standard chromatogram relating to HCTZ (2.5 µg/ml) and OLME (8 µg/ml)

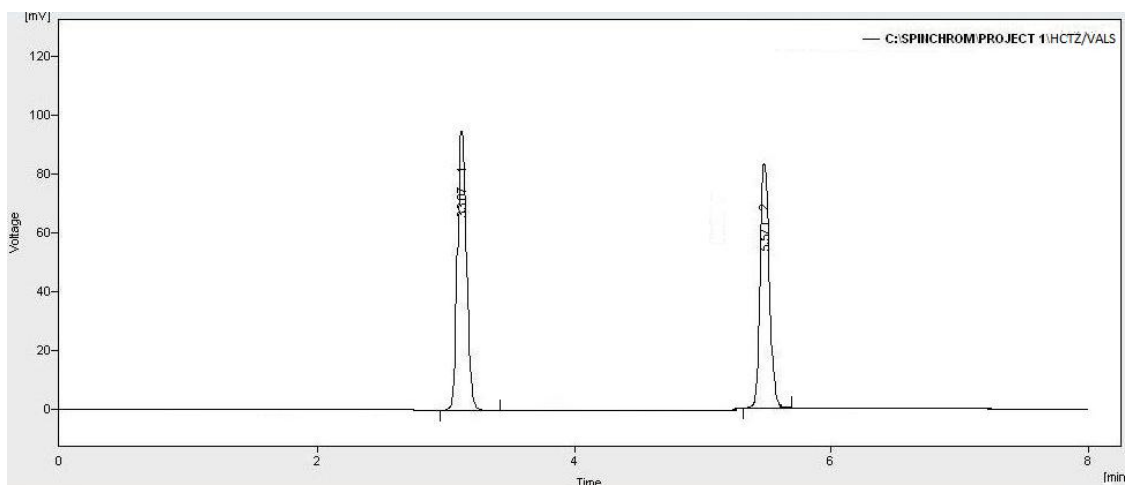


Fig. 12. Standard chromatogram relating to HCTZ (2.5 µg/ml) and VALS (16 µg/ml)

3.2.7 LOD and LOQ

LOD is the lowest concentration of analyte in a sample that can be detected, but not essentially quantified under the stated experimental conditions. LOQ is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy. LOD and LOQ were calculated by means of following formula $LOD = 3.3\sigma/S$ and $LOQ = 10\sigma/S$, where σ means standard deviation of response (peak area) and S stands for slope of the calibration curve. The results of LOD and LOQ are shown in Table 3.

3.2.8 Stability of analytical solution

In general stability of analytical solution was calculated by observing the peak area response. Stock standard solutions were analyzed after one, two and three days at 5°C and for a day at room temperature. These solutions were found to be stable for 3 days at 5°C and for a day at room temperature also.

3.3 Application of Pharmaceutical Preparation

The method developed was effectively useful for quantitative determination of commercial formulations such as Telmisat-H 40 mg, Zargo-H 50 mg, Olmecip-H 40 mg and Valent-H 80 mg. 6 replicate determinations were conducted. Excellent outcomes were obtained for all the compounds. The mean assay values obtained were in good conformity with that of label claim which are shown in Table 3.

3.4 Discussion

The most important objective of the current study is to develop a novel, quick, simple, accurate and precise RP-HPLC method for simultaneous separation and quantitative determination of HCTZ with TELM, HCTZ with LOSA, HCTZ with OLME and HCTZ with VALS in combined dosage forms. The important goal of method development is to achieve constant reproducible separation. On the other hand many trial and error methods were done in identifying the best operational and environmental conditions for optimizing appropriate method for separations.

RP-HPLC method was preferred for the separation process as all the mentioned drugs are relatively polar. C_{18} column of 4.6 mm internal diameter, 250 mm length and 5 µm particle size was preferred. Numerical trails with varying compositions of mobile phases, variable flow rate and temperature were done. At last an optimum separation condition was achieved with a blend of phosphate buffer of pH-3.3 and acetonitrile in a proportion of 50:50 v/v. The flow rate of mobile phase was set at 1mL/min, a common detection wave length of 238 nm was fixed and the temperature of the column was maintained at ambient conditions. After adjusting all the operational parameters at their subsequent optimum values, improved chromatographic peaks were acquired with characteristics of good resolution, symmetry and minimal tailing factors.

Table 3. Summary of validation parameters

Formulation	Telmisat - H (40 mg)		Zargo-H (50 mg)		Olmeceip-H (40 mg)		Valent-H (80 mg)		
Composition	TELM	HCTZ	LOSA	HCTZ	OLME	HCTZ	VALS	HCTZ	
Linearity ($\mu\text{g/mL}$)	8-40	2.5-12.5	5-50	2.5-12.5	8-40	2.5-12.5	16-80	2.5-12.5	
LOD & LOQ ($\mu\text{g/mL}$)	0.570 & 1.889	0.295 & 0.982	0.736 & 2.43	0.299 & 0.986	1.195 & 3.958	0.292 & 0.983	0.116 & 0.352	0.296 & 0.980	
Assay \pm SD (n=6)	99.95 \pm 1.13	98.16 \pm 0.38	99.85 \pm 1.16	98.21 \pm 0.45	99.90 \pm 1.10	99.19 \pm 0.16	100.12 \pm 0.15	99.59 \pm 0.12	
Mean % recovery	100.02 \pm 0.68	100.01 \pm 0.15	100.06 \pm 0.20	99.95 \pm 0.09	100.41 \pm 0.35	99.93 \pm 0.11	100.05 \pm 0.14	99.96 \pm 0.15	
Precision	Intra day (n= 6) (% RSD)	0.125	0.131	0.346	0.129	0.435	0.171	0.312	0.213
	Inter day (n=3) (%RSD)	0.165	0.321	0.326	0.434	0.657	0.322	0.319	0.435
Robustness, % Assay\pm% RSD									
Flow rate (\pm 2 mL/min)	0.8 mL/min	99.81 \pm 1.05	97.94 \pm 0.46	99.98 \pm 1.04	98.93 \pm 0.49	98.70 \pm 1.02	98.96 \pm 0.25	99.91 \pm 0.29	100.96 \pm 0.23
	1.2 mL/min	100.12 \pm 0.97	99.54 \pm 0.23	98.76 \pm 1.32	98.04 \pm 0.36	100.03 \pm 1.38	99.97 \pm 0.35	100.54 \pm 0.77	98.74 \pm 0.71
Detection wavelength (\pm 5 nm)	233 nm	99.97 \pm 1.46	99.43 \pm 0.29	98.42 \pm 1.46	97.95 \pm 0.98	100.21 \pm 1.37	99.96 \pm 0.84	100.45 \pm 0.97	99.98 \pm 0.54
	243 nm	97.98 \pm 1.09	98.04 \pm 0.76	100.05 \pm 1.03	98.94 \pm 1.08	98.79 \pm 0.73	98.76 \pm 1.09	99.94 \pm 1.43	98.47 \pm 1.11
Mobile phase composition (\pm 5%)	45:55 v/v	98.74 \pm 0.79	99.42 \pm 0.99	99.93 \pm 1.42	97.92 \pm 0.68	98.91 \pm 0.94	100.07 \pm 0.93	99.01 \pm 0.61	98.76 \pm 0.37
	55:45 v/v	99.96 \pm 1.2	97.81 \pm 0.27	98.54 \pm 0.86	98.86 \pm 0.30	98.12 \pm 1.57	98.72 \pm 1.09	99.49 \pm 0.74	100.03 \pm 1.18

SD: Standard deviation, RSD: Relative standard deviation: n= Number of determinations.

The present developed method was validated according to the ICH guidelines in different parameters like specificity, accuracy, linearity, precision, robustness, LOD and LOQ. System suitability was performed to make sure the suitability of complete testing system for the deliberate purpose. Accordingly system suitability parameters like retention time, resolution, number of theoretical plates, efficiency/meter, tailing factor of the peaks were computed for the optimized chromatographic conditions. As a result retention times of 3.300, 3.780, 4.467, 5.247 and 5.570 minutes, resolution of 3.776, 4.862, 4.931 and 2.008 for TELM, LOSA, OLME and VALS were obtained. And the plate number of 12312, 12468, 14715, 15250 and 17188, efficiency per meter of 246247, 247368, 294308, 305005 and 343756 and tailing factors of 1.194, 1.116, 1.182, 1.160 and 1.154 were obtained for HCTZ, TELM, LOSA, OLME and VALS respectively. As the results obtained were within the satisfactory limits, this method is appropriate for the separation and determination of the above drugs.

Linearity of developed method was determined by taking 5 different concentrations. The calibration curves for five different drugs showed linearity over a concentration ranges of 2.5-12.5, 8-40, 5-50, 8-40 and 16-80 µg/mL for HCTZ, TELM, LOSA, OLME and VALS respectively. In Telmisat-H 40 mg tablet 8-40 µg/mL for TELM and 2.5-12.5 µg/mL for HCTZ; for Zargo-H 50 mg tablet 5-50 µg/mL for LOSA and 2.5-12.5 µg/mL for HCTZ; for Olmecip-H 40 mg tablet 8-40 µg/mL for OLME and 2.5-12.5 µg/mL for HCTZ; for Valent-H 80 mg tablet 16-80 µg/mL for VALS and 2.5-12.5 µg/mL for HCTZ. The correlation coefficients were calculated from the linear regression analysis and it was found to be above 0.9998 in all cases. The obtained results were satisfactory as there exist a significant correlation between concentrations of each drug and their peak areas.

Precision of the analytical method was determined by using intra-day and inter-day studies. Triplicate samples of standard quality were taken in varying concentration levels and estimated for the intra-day and inter-day precision of the developed method. For repeatability and intermediate precision the % RSD values for all the drugs were calculated and % RSD of all the specific drugs were found less than 2% which explains that the current method is precise.

As per the ICH guidelines, to test the specificity of the developed method a combination of pure drug samples with proper excipients were injected to the system for quantifying each drug individually and also in combination of two drugs namely HCTZ with TELM, HCTZ with LOSA, HCTZ with OLME and HCTZ with VALS. In the same way blank solution with only commonly utilized excipients and synthetic mixture solutions were also injected separately. Peak responses for analyte and the blank were compared with each appropriate drug. The results revealed that there is no interference because of the commonly used excipients. Thus the method clearly proves to be specific for determining the above said drugs.

Accuracy of the present method was evaluated by combining the known quantity of pure standard drugs to pre-analyzed samples at 3 levels mainly 80%, 100% and 120%. Then the recovery levels were observed clearly. The above mentioned solutions were again prepared and analyzed in triplicate carefully. The same procedure was followed for all the individual drugs and also for drug combinations like HCTZ with TELM, HCTZ with LOSA, HCTZ with OLME and HCTZ with VALS and % RSD calculated was also known to be less than 2% for each of the drug.

Robustness of the present method was determined accordingly with minor modifications in the chromatographic conditions such as flow rate, λ_{max} and mobile phase composition. It was observed that in chromatograms there were no distinct changes. The % RSD values obtained in all the cases are less than 2%, hence this method is robust.

The limit of detection (LOD) and limit of quantitation (LOQ) results for combined drugs like TELM with HCTZ, LOSA with HCTZ, OLME with HCTZ and VALS with HCTZ were found to be 0.570 µg/mL and 1.889 µg/mL, 0.295 µg/mL and 0.982 µg/mL; 0.736 µg/mL and 2.434 µg/mL, 0.299 µg/mL and 0.986 µg/mL; 1.195 µg/mL and 3.958 µg/mL, 0.292 µg/mL and 0.983 µg/mL; 0.116 µg/mL and 0.352 µg/mL, 0.296 µg/mL and 0.980 µg/mL respectively. The lowest LOD and LOQ values indicated that the method is more sensitive. The mean assay values for HCTZ with TELM, HCTZ with LOSA, HCTZ with OLME and HCTZ with VALS combination of drugs were found to be 98.16±0.38 and 99.95±1.13; 98.21±0.45 and 99.85±1.16; 99.19±0.16 and 99.90±1.10; 99.59±0.12 and 100.12±0.15

respectively. Thus by the developed method all the specified drugs were recovered perfectly from pharmaceutical dosage forms. Thus the

method developed was found to be appropriately suitable for determination of the marketed formulations.

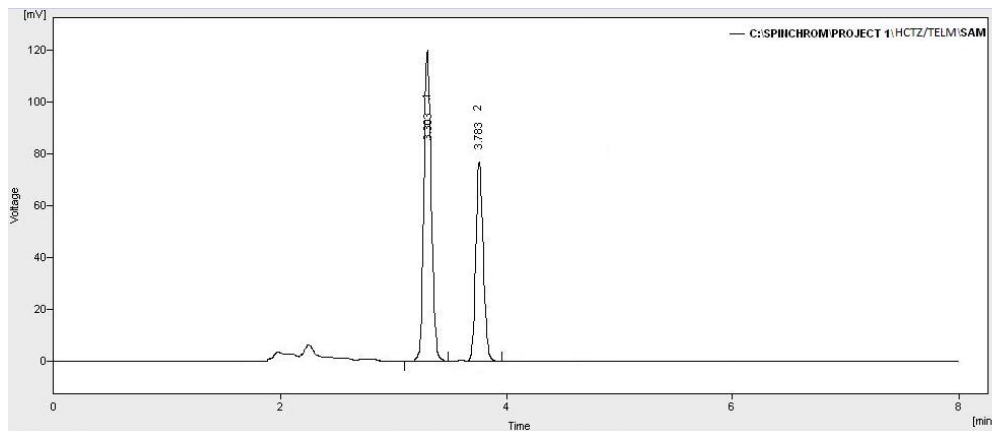


Fig. 13. Sample chromatogram relating to HCTZ and TELM

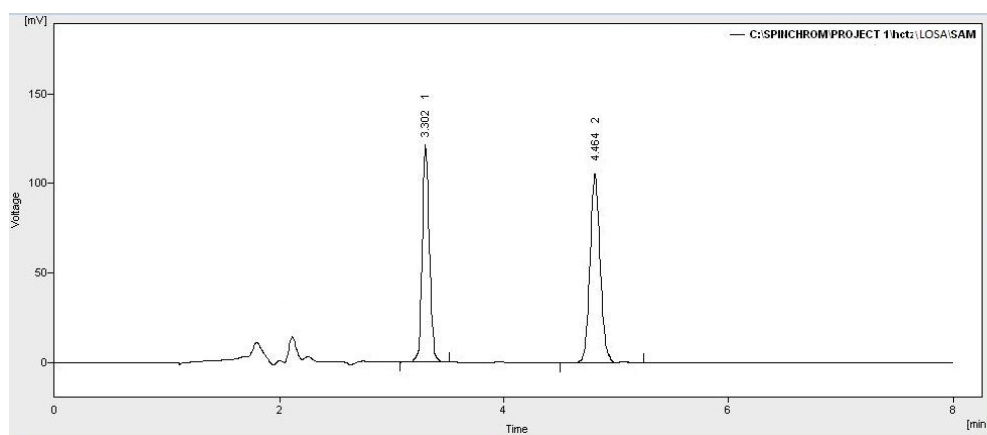


Fig. 14. Sample chromatogram relating to HCTZ and LOSA

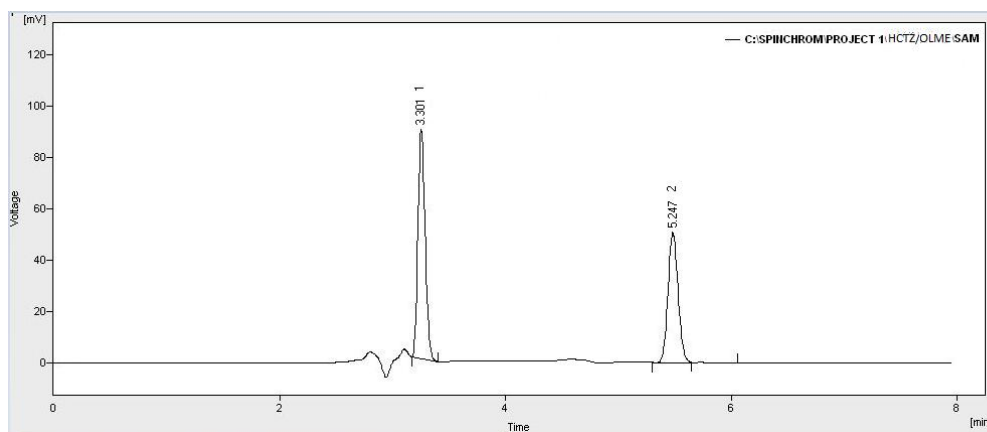


Fig. 15. Sample chromatogram relating to HCTZ and OLME

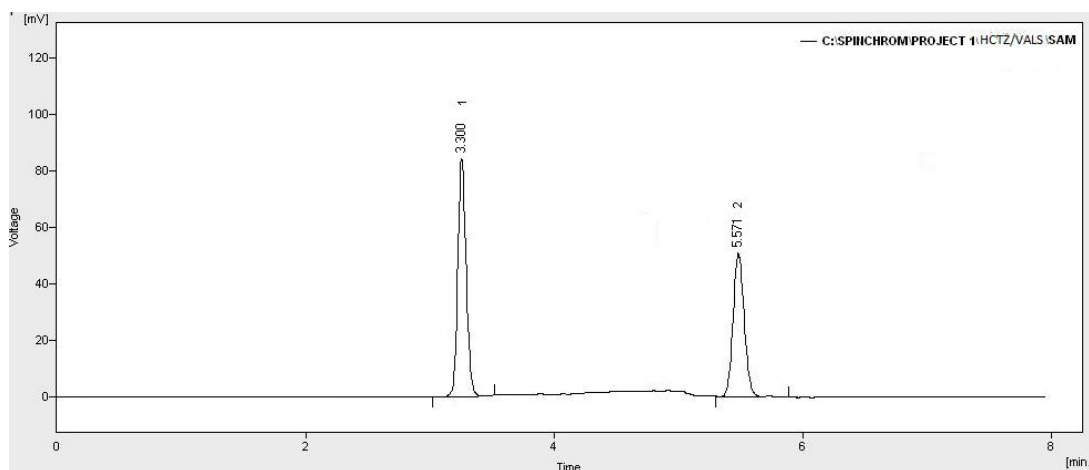


Fig. 16. Sample chromatogram relating to HCTZ and VALS

4. CONCLUSION

The novel analytical method can be used for separation and also proves that it is possible for analysis of Angiotensin receptor blockers like TELM, LOSA, OLME and VALS with HCTZ in their formulations without altering the flow rate, mobile phase composition and other chromatographic conditions. This novel RP-HPLC method of separation possess less retention time, better separation and improved reproducible results. The developed analytical method is low cost, rapid, precise, highly efficient, accurate, robust and agreeable than other existing methods reported hitherto. After validation it was also found that the method is free from interventions of additives and excipients employed in the preparation of above pharmaceutical dosage forms. Therefore, this method is aptly feasible in routine analysis of individual drugs and also in combinations like HCTZ with TELM, HCTZ with LOSA, HCTZ with OLME and HCTZ with VALS in quality control laboratories.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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