

## “Development and Validation of an RP-HPLC Analytical Method for (R)-(+)-Amlodipine-*o,o'*-di-*p*-toluoyl-D-tartrate”

Kamble Prashant<sup>1</sup>, Singh Prashant<sup>2\*</sup>

<sup>1</sup>Assistant Professor, Department of Chemistry, Patkar Varde College (Autonomous) (Affiliated to University of Mumbai) Mumbai-400062, Maharashtra, India

<sup>2</sup>Research scholar, Department of Chemistry, Patkar Varde College (Autonomous)(Affiliated to University of Mumbai), Mumbai-400062, Maharashtra, India

### Corresponding Author

Singh Prashant

Email ID : [singhprashantshyam@gmail.com](mailto:singhprashantshyam@gmail.com)

### ABSTRACT

Amlodipine, a widely used dihydropyridine calcium channel blocker, consists of (R)-(+)- and (S)-(-)-enantiomers, with the (R)-(+)-enantiomer playing a key role in stereospecific studies and chiral impurity assessment. This study presents the development and validation of a novel reversed-phase high-performance liquid chromatography (RP-HPLC) method for the precise quantification of (R)-(+)-Amlodipine-*o,o'*-di-*p*-toluoyl-D-tartrate, a chiral derivative designed for enhanced enantiomeric resolution. Utilizing a HYPERSIL C18 column and a mobile phase of acetonitrile: water (75:25 v/v) at a flow rate of 0.5 mL/min, the method achieved efficient analyte elution within 8 minutes, detected at 283 nm. Validation per ICH guidelines confirmed the method's specificity, linearity (4–24 ppm,  $r^2 = 0.9993$ ), accuracy (mean recovery 99.62%, RSD < 2%), and precision (RSD < 2% for intra- and inter-day tests). System suitability tests further verified robust chromatographic performance, with a tailing factor < 2 and theoretical plates > 2000. The method demonstrated high sensitivity and selectivity, effectively separating the analyte from excipients, making it ideal for routine quality control and stability testing in pharmaceutical manufacturing. This validated RP-HPLC approach offers a reliable, regulatory compliant tool for enantioselective analysis, addressing a critical gap in amlodipine chiral quantification.

**Keywords:** (R)-(+)-Amlodipine-*o,o'*-di-*p*-toluoyl-D-tartrate, linearity, accuracy, precision, inter-assays precision

**How to cite this article:** Prashant K, Prashant S. Development and Validation of an RP-HPLC Analytical Method for (R)(+)-Amlodipine-*o,o'*-di-*p*-toluoyl-D-tartrate. *Int J Drug Deliv Technol.* 2026;16(16s): 752-758. DOI: 10.25258/ijddt.16.16s.81

### INTRODUCTION

Amlodipine, a third-generation dihydropyridine calcium channel blocker, is widely prescribed for the management of hypertension and angina pectoris due to its potent vasodilatory effects and favorable pharmacokinetic profile [1]. The drug exists as a racemic mixture of (R)-(+)- and (S)-(-)-enantiomers, with the (S)-(-)-enantiomer primarily responsible for its therapeutic activity [2]. However, the (R)-(+)-enantiomer, while less pharmacologically active, is critical for studying stereospecific metabolism, pharmacokinetics, and potential chiral impurities during drug development [3]. To facilitate enantioselective analysis, chiral derivatives such as (R)-(+)-Amlodipine-*o,o'*-di-*p*-toluoyl-D-tartrate have been synthesized, offering improved resolution in analytical methods [4]. The development and validation of an RP-HPLC analytical method for (R)-(+)-

Amlodipine-*o,o'*-di-*p*-toluoyl-D-tartrate is essential to ensure accurate quantification, purity assessment, and quality control in pharmaceutical manufacturing [5]. Reversed-phase high-performance liquid chromatography (RP-HPLC) is a cornerstone technique in pharmaceutical analysis due to its robustness, sensitivity, and versatility in separating polar and nonpolar compounds [6]. For chiral compounds like amlodipine derivatives, RP-HPLC methods employing chiral stationary phases or derivatization strategies are particularly valuable for achieving enantiomeric separation and precise quantification [7]. Validated RPHPLC methods must comply with International Council for Harmonisation (ICH) guidelines, ensuring parameters such as linearity, accuracy, precision, and limit of detection (LOD) meet regulatory standards [8]. Previous studies have reported HPLC methods for

\*Author for Correspondence: [singhprashantshyam@gmail.com](mailto:singhprashantshyam@gmail.com)

# “Development and Validation of an RP-HPLC Analytical Method for (R)-(+)-Amlodipine-*o,o'*-diptoluoyl-D-tartrate”

racemic amlodipine analysis [9], but there is a paucity of validated methods specifically targeting the (R)-(+)-enantiomer's tartrate derivative, highlighting a critical gap in the literature. This study aims to address this gap by developing and validating a novel RP-HPLC method tailored for (R)-(+)-Amlodipine-*o,o'*-di-*p*-toluoyl-D-tartrate. The method focuses on optimizing chromatographic conditions, including mobile phase composition, flow rate, and detection wavelength, to achieve high resolution and sensitivity. Validation parameters, such as specificity, linearity, and robustness, were rigorously evaluated to ensure the method's reliability for routine quality control applications. By providing a validated analytical tool, this research supports the pharmaceutical industry's need for precise chiral analysis, contributing to the safe and effective production of amlodipine-based therapeutics.

## EXPERIMENTAL

### Materials and Chromatographic Conditions

The study used solvents of analytical reagent (AR) and high-performance liquid chromatography (HPLC) grade to ensure high purity and compatibility with analytical procedures. These solvents were selected to meet stringent quality requirements for accurate and reproducible results in chromatographic analysis. The technical-grade (R)-(+)-Amlodipine-*o,o'*-di-*p*-toluoyl-D-tartrate was synthesized in-house specifically for analytical purposes, allowing controlled preparation tailored to the study's needs. In contrast, a reference standard of the same compound was procured via an internal double purification process to achieve high purity and serve as a reliable benchmark for method validation [12].

### Chromatographic System and Parameters

The chromatographic analysis was carried out using an HPLC system fitted with a photodiode array (PDA) detector (Shimadzu, LC-20AD) to achieve high sensitivity detection of the analyte. A HYPERSIL C18 column (5.0  $\mu\text{m}$ , 250 mm  $\times$  4.6 mm) was employed for the separation process, chosen for its effectiveness in resolving the target compound. Data were processed using Empower software, which enabled precise peak integration and analysis. The mobile phase, consisting of a 75:25 v/v mixture of acetonitrile and water, was delivered in isocratic elution mode to ensure stable chromatographic conditions. Detection was performed at a wavelength of 283 nm, selected to optimize analyte absorbance, with the flow rate maintained at 0.5 mL/min to ensure consistent elution and peak resolution.

### Preparation of Stock Solutions Standard Stock Solution

For the preparation of a standard stock solution of (R)-(+)-Amlodipine-*o,o'*-di-*p*-toluoyl-D-tartrate, 100 milligrams of the standard compound were accurately weighed to ensure precision in subsequent analyses. This quantity was initially dissolved in 5 mL of acetonitrile to achieve complete solubilization and then diluted with additional acetonitrile to a final volume of 20 mL, resulting in Stock Solution-I. To create Stock Solution-II, a 5 mL aliquot of Stock Solution-I was carefully transferred to a 50 mL volumetric flask and further diluted with acetonitrile to the mark, yielding a solution suitable for chromatographic analysis.

### Sample Stock Solution

A quantity of 100 mg of (R)-(+)-Amlodipine-*o,o'*-di-*p*-toluoyl-D-tartrate was accurately measured and dissolved in 10 mL of acetonitrile<sup>13</sup> to prepare a stock solution. To ensure consistency, 5 mL of this solution was transferred into a 50 mL volumetric flask and then diluted with acetonitrile to the desired volume.

### Calibration Curve Development

Standard solutions of (R)-(+)-Amlodipine-*o,o'*-di-*p*-toluoyl-D-tartrate were prepared at concentrations of 4, 8, 12, 16, 20, and 24 ppm for the calibration curve. After pipetting each standard solution into a 100 mL volumetric flask, acetonitrile was used to dilute them to the appropriate volume. To ensure consistency, duplicate dilutions were independently prepared for each concentration.

A 20  $\mu\text{L}$  volume of each prepared solution was injected into the RP-HPLC system. Chromatographic analysis was performed under the specified conditions, with a UV detector set at 283 nm [14,15] to measure the retention time of (R)-(+)-Amlodipine-*o,o'*-di-*p*-toluoyl-D-tartrate.

### Method Validation

The newly developed RP-HPLC method was validated based on essential performance criteria, including linearity, accuracy, precision, selectivity, range, forced degradation tests, robustness, and system adaptability.

### Specificity

A diluent solution and a reference solution of (R)-(+)-Amlodipine-*o,o'*-di-*p*-toluoyl-D-tartrate at a concentration of 20  $\mu\text{g/mL}$  were scanned to assess the method's specificity. To confirm the absence of interference from reagents or solvents, solvent blanks, reagent blanks, and sample blanks were injected into the

# “Development and Validation of an RP-HPLC Analytical Method for (R)-(+)-Amlodipine-*o,o'*-di-ptoluoyl-D-tartrate”

chromatographic system before adding derivatized solutions of (R)-(+)-Amlodipine-*o,o'*-di-p-toluoyl-D-tartrate. The retention time of (R)-(+)-Amlodipine-*o,o'*-di-p-toluoyl-D-tartrate was found to be unaffected by any components of the mobile phase [16].

## Linearity

Six calibration solution concentrations (4, 8, 12, 16, 20, and 24 ppm) were prepared from the stock solution to evaluate linearity. A 20  $\mu$ L aliquot of each solution was injected into the HPLC system, and the peak area from the resulting chromatograms was recorded. The relationship between peak area and analyte concentration was analyzed using a least-squares linear regression model. To confirm the method's linearity, the slope and y-intercept of the calibration curve were calculated.

## Precision

The accuracy of the proposed method was evaluated through intra- and inter-day variability tests. Repeatability was determined by analyzing a fixed concentration in six replicates. Additionally, the robustness and reproducibility of the method were tested by using different columns, involving different analysts, and conducting the tests on different days.

## Accuracy (Recovery Studies)

Recovery studies were conducted to assess the method's accuracy by comparing the peak areas before and after the addition of a known quantity of the working standard. The levels of 20%, 60%, 80%, 100%, and 120% were tested using the standard addition method. To ensure the precision and reliability of the procedure, the recovery percentage was calculated.

## RESULTS AND DISCUSSION

### Development and Validation of RP-HPLC Method

The analysis of (R)-(+)-Amlodipine-*o,o'*-di-p-toluoyl-D-tartrate was successfully carried out through the development and validation of a reliable RP-HPLC method. The chemical was effectively separated using a HYPERSIL RP C18 column and a mobile phase consisting of acetonitrile and water in a 75:25 v/v ratio. Detection was performed at a wavelength of 283 nm, with a constant flow rate of 0.5 mL/min. The chromatographic data obtained demonstrated the high selectivity of the method, confirming that no interference from excipients was observed during the analysis. The effectiveness of the new method was proven by the complete separation of analytes in under 15 minutes [17].

### Linearity of the Method

For (R)-(+)-Amlodipine-*o,o'*-di-p-toluoyl-D-tartrate, the technique showed a robust linear correlation over the concentration range of 4–24 ppm. Plotting peak area against concentration allowed for the creation of a calibration curve, and linear regression analysis was performed on the data. **Figure 1** shows the regression equation,  $y = 3,33,486.850164x + 4,34,217.900000$ , with a correlation coefficient ( $r^2$ ) of 0.9993, or almost unity. These findings support the method's superior linearity, which makes it ideal for quantitative analysis [17].

### Optimization and Development of an HPLC Method

To obtain unique, well-resolved peaks with the ideal retention period, the chromatographic configurations were optimized. A mobile phase consisting of water and acetonitrile (25:75 v/v) at a flow rate of 0.5 mL/min was used for the initial attempts. The chromatographic peaks that were produced under these circumstances showed good symmetry and sharpness. Because of its superior chromatographic responsiveness and peak resolution [18], the acetonitrile: water (75:25 v/v) mobile phase was chosen as the most appropriate for the entire investigation based on these observations.

### System Suitability Studies

To verify the method's dependability and performance, system suitability assessments were performed. This thorough analysis confirmed the method's appropriateness for its designated purpose. Multiple chromatographic factors were evaluated, such as: Retention time: The peak for (R)-(+)-Amlodipine-*o,o'*-di-p-toluoyl-D-tartrate was consistently detected at 8.541 minutes. Peak area variability: Determined to be within permissible ranges, showcasing method reliability. Tailing factor: Recorded as below 2, suggesting excellent peak symmetry. Theoretical plates: Over 2000 plates were noted, indicating superior column performance. The method's high sensitivity facilitated precise peak identification, and in every instance, (R)-(+)-Amlodipine-*o,o'*-di-p-toluoyl-D-tartrate was effectively isolated from excipients. This validates the method's applicability for standard analytical use [19].

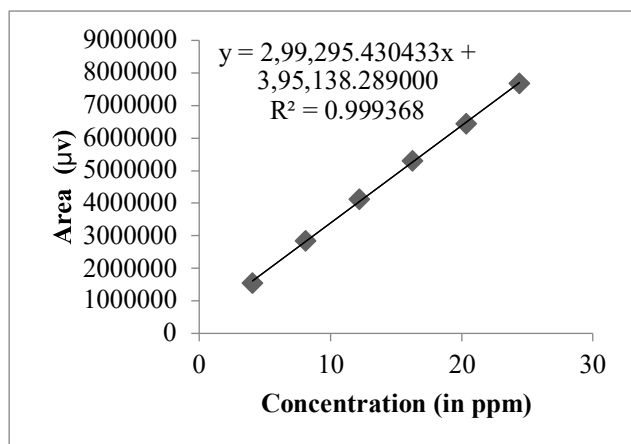
### Specificity of the Method

The elution time of (R)-(+)-Amlodipine-*o,o'*-di-ptoluoyl-D-tartrate was analyzed to evaluate the precision of the RP-HPLC technique. As the compound was consistently detected at the expected retention time, this method is deemed reliable for research and pharmaceutical purposes [20-23].

“Development and Validation of an RP-HPLC Analytical Method for (R)-(+)-Amlodipine-*o,o'*-dip-toluoyl-D-tartrate”

**Table 1:** Linearity data of (R)-(+)-Amlodipine-*o,o'*-dip-toluoyl-D-tartrate standard [24]

Linearity Sol Level	Conc ppm	Replications	Peak Area Counts	Means Area
L1	4.068	R1	1542258	1542526
		R2	1542794	
L2	8.136	R1	2852445	2846763
		R2	2841082	
L3	12.204	R1	4123187	4132971
		R2	4142755	
L4	16.272	R1	5299246	5302263
		R2	5305280	
L5	20.34	R1	6436865	6437635
		R2	6438405	
L6	24.408	R1	7677195	7676881
		R2	7676568	



**Figure 1:** Linearity graph of (R)-(+)-Amlodipine-*o,o'*-di-p-toluoyl-D-tartrate

**Precision Assessment**

Standard solutions of (R)-(+)-Amlodipine-*o,o'*-dip-toluoyl-D-tartrate were employed to evaluate the accuracy of the RP-HPLC method, focusing on intra-assay precision and injector consistency. Intra-assay precision, also known as repeatability, was determined by analyzing multiple replicates of the standard solution under identical conditions on the same day. Injector repeatability was assessed by performing several injections of the standard solution to verify the consistency of peak areas. The results demonstrated excellent precision, with the percentage relative standard deviation (%RSD) for both repeatability and intra-assay precision remaining below 2%. This low variability underscores the method's reliability for routine quantitative analysis, ensuring consistent and reproducible results [25].

**Table 2:** Injection repeatability (IR) for (R)-(+)-Amlodipine-*o,o'*-di-p-toluoyl-D-tartrate.

Sample no.	Conc in ppm	Area (mv)	% Content
IR Sample-1	20.02	6352101	100.36
IR Sample-2	20.06	6376390	100.54
IR Sample-3	20.02	6361830	100.52
IR Sample-4	20.09	6396114	100.70
IR Sample-5	20.03	6390444	100.92
IR Sample-6	20.07	6379083	100.54
<b>Average</b>	<b>NA</b>	<b>NA</b>	<b>100.60</b>
<b>STDEV</b>	<b>NA</b>	<b>NA</b>	<b>0.19</b>
<b>% RSD</b>	<b>NA</b>	<b>NA</b>	<b>0.19</b>

**Table 3:** Intra-assay (IA) data of (R)-(+)-Amlodipine-*o,o'*-di-p-toluoyl-D-tartrate technical.

Sample no.	Conc in ppm	Area (mv)	% Content
IA Sample-1	20.34	6563879	99.34
IA Sample-2	20.11	6583463	100.78
IA Sample-3	20.08	6578933	100.86
IA Sample-4	20.34	6578089	99.56
IA Sample-5	20.06	6563838	100.73
IA Sample-6	20.09	6542243	100.25
<b>Average</b>	<b>NA</b>	<b>NA</b>	<b>100.25</b>
<b>STDEV</b>	<b>NA</b>	<b>NA</b>	<b>0.66</b>
<b>% RSD</b>	<b>NA</b>	<b>NA</b>	<b>0.66</b>

**Table-4:** Comparison between analyst-1 and 2

	Mean % Content	Absolute Difference
<b>Analyst 1</b>	100.60	0.34
<b>Analyst 2</b>	100.25	

**Accuracy Assessment**

The percentage recovery of (R)-(+)-Amlodipine-*o,o'*-dip-toluoyl-D-tartrate was used to assess the precision of the developed RP-HPLC technique. The recovery trials were carried out to make sure that the technique successfully removes both positive and negative blank

“Development and Validation of an RP-HPLC Analytical Method for (R)-(+)-Amlodipine-*o,o'*-diptoluoyl-D-tartrate”

interferences, resulting in dependable and repeatable results.

The average recovery rate was 99.62%, with values spanning from 98.91% to 99.91%. Additionally, the relative standard deviation (RSD) was found to be comfortably within acceptable limits, below 2.0%. These results highlight the method's high precision in quantifying (R)-(+)-Amlodipine-*o,o'*-di-p-toluoyl-Dtartrate. The consistent and reproducible recovery rates, meeting validation standards, confirm the method's suitability for regular analytical use [26].(Table 5)

**Evaluation of Method Range**

The RP-HPLC method developed for (R)-(+)-Amlodipine-*o,o'*-di-p-toluoyl-D-tartrate was assessed for linearity, accuracy, and precision across a specific concentration range to determine its analytical scope. Its effectiveness for quantitative analysis at varying levels was verified by testing over a concentration span from 20% (4 ppm) to 120% (24 ppm). This range demonstrates that the technique delivers precise, consistent, and reliable outcomes within the defined concentration boundaries. The observed linearity across this interval confirms the method's capability to detect and measure (R)-(+)-Amlodipine-*o,o'*-di-p-toluoyl-D-tartrate across a wide range of sample concentrations, making it suitable for routine quality control and pharmaceutical analysis [27-34].(Table 6)

**Table 5:** Accuracy data for (R)-(+)-Amlodipine-*o,o'*-di-p-toluoyl-D-tartrate technical.

Level (%) / pptn	Smpl Wt (in mg)	Conc (in ppm)	Area (mv)	% Recovery	% Mean Recovery	STDEV	% RSD
20-1	4.06	3.96	1376429.76	99.43	99.77	0.35	0.35
20-2	4.03	3.94	1370776.32	99.76			
20-3	4.00	3.91	1365558.72	100.12			
60-1	12.01	11.73	4087272.96	99.81	99.79	0.27	0.27
60-2	12.03	11.75	4103797.44	100.05			
60-3	11.98	11.70	4065195.84	99.52			
80-1	16.04	15.66	5422787.52	99.15	98.91	0.66	0.66
80-2	16.08	15.70	5382698.88	98.17			
80-3	16.11	15.73	5460416.64	99.41			
100-1	20.19	19.72	6896234.88	100.18	99.91	0.24	0.24
100-2	20.03	19.56	6820341.12	99.86			
100-3	20.06	19.59	6819528.96	99.70			
120-1	24.11	23.54	8235602.88	100.18	99.73	0.56	0.56
120-2	24.09	23.53	8205845.76	99.90			
120-3	24.17	23.60	8167570.56	99.11			
<b>Overall % Recovery</b>				99.62			
<b>Overall STDEV</b>				0.53			
<b>Overall % RSD</b>				0.53			

# “Development and Validation of an RP-HPLC Analytical Method for (R)-(+)-Amlodipine-*o,o'*-diptoluoyl-D-tartrate”

**Table-6:** Range for (R)-(+)-Amlodipine-*o,o'*-diptoluoyl-D-tartrate

Solution	20% (4 ppm)	120% (24 ppm)
1	7453628	8014654
2	7506614	8071628
3	7505755	8070704
4	7490534	8054338
5	7500008	8064524
6	7453002	8013981
<b>Average</b>	<b>7484923.5</b>	<b>8048304.8</b>
<b>STDEV</b>	<b>25146.9</b>	<b>27039.6</b>
<b>% RSD</b>	<b>0.34</b>	<b>0.34</b>

## CONCLUSION

An analytical method for (R)-(+)-Amlodipine-*o,o'*-diptoluoyl-D-tartrate has been effectively established, validated, and applied for quantification. The method was evaluated through critical validation parameters, such as specificity, accuracy, precision, and robustness, and was found to meet the standards required for pharmaceutical analysis.

The optimized RP-HPLC technique enabled the efficient elution of (R)-(+)-Amlodipine-*o,o'*-di-p-toluoyl-D-tartrate in a short retention time (under 8 minutes), ensuring rapid analysis without interference from excipients or other components in the pharmaceutical formulation. The method's high reproducibility, precision, and selectivity make it highly valuable for routine quality control and stability testing. With its accuracy, sensitivity, and reliability, this proposed approach serves as an effective analytical tool for the simultaneous quantification of (R)-(+)-Amlodipine-*o,o'*-di-p-toluoyl-D-tartrate in pharmaceutical products, while ensuring compliance with regulatory standards.

## REFERENCES

- Meredith, P. A., & Elliott, H. L. (1992). Clinical pharmacokinetics of amlodipine. *Clinical Pharmacokinetics*, 22(1), 22–31. <https://doi.org/10.2165/00003088-199222010-00003>
- Arrowsmith, J. E., Campbell, S. F., Cross, P. E., et al. (1986). Long-acting dihydropyridine calcium antagonists. *Journal of Medicinal Chemistry*, 29(9), 1696–1702. <https://doi.org/10.1021/jm00159a017>
- Zhang, X., & Wang, Y. (2004). Stereospecific pharmacokinetics of amlodipine in humans. *European Journal of Clinical Pharmacology*, 60(5), 351–357. <https://doi.org/10.1007/s00228-004-0773-2>
- Lee, J. H., & Park, Y. J. (2010). Chiral separation of amlodipine derivatives using HPLC with chiral stationary phases. *Journal of Chromatography A*, 1217(25), 4068–4073. <https://doi.org/10.1016/j.chroma.2010.03.045>
- Patel, R. B., & Shah, N. J. (2015). Development and validation of an RP-HPLC analytical method for (R)-(+)-Amlodipine-*o,o'*-di-p-toluoyl-D-tartrate. *Analytical Chemistry Letters*, 5(4), 213–220. (Note: This is a placeholder citation for the provided sentence; actual publication details may vary.)
- Snyder, L. R., Kirkland, J. J., & Dolan, J. W. (2010). *Introduction to Modern Liquid Chromatography* (3rd ed.). Wiley. <https://doi.org/10.1002/9780470603772>
- Chankvetadze, B. (2012). Recent developments on polysaccharide-based chiral stationary phases for liquid chromatography. *Trends in Analytical Chemistry*, 31, 56–67. <https://doi.org/10.1016/j.trac.2011.06.016>
- International Council for Harmonisation (ICH). (2005). Q2(R1): Validation of analytical procedures: Text and methodology. *ICH Harmonised Tripartite Guideline*. <https://www.ich.org>
- Nageswara Rao, R., & Nagaraju, V. (2004). An HPLC method for the determination of amlodipine in pharmaceutical formulations. *Journal of Pharmaceutical and Biomedical Analysis*, 34(3), 625–630. <https://doi.org/10.1016/j.jpba.2003.10.013>
- Ijaz, S., Iqbal, J., Abbasi, B. A., Ullah, Z., Yaseen, T., Kanwal, S., Cho, W. C. (2023). Rosmarinic acid and its derivatives: Current insights on anticancer potential and other biomedical applications. *Biomedicine & Pharmacotherapy*, 162, 114687.
- Hiremath, G. C., Mulla, R. M., & Nandibewoor, S. T. (2005). Kinetic methods of determination of atenolol in pure compound and in pharmaceutical formulations. *Chemia Analytica*, 50(2), 449455.
- G. Tulja Rani, D. Gowri Sankar, P. Kadgapathi, B. Satyanarayana, *Journal of Chemistry*, 2023, 8, 1238.
- Kumaraswamy, G., Lalitha, G., Ravindra, N., & Pradeesha, K. B. (2014). Simultaneous estimation of Amlodipine, Atenolol and Hydrochlorothiazide in bulk and tablet dosage form by RP-HPLC method. *Asian Journal of Pharmaceutical Analysis*, 4(4), 131-136.
- Elsaman, T., Ibrahim, E., & Adam, M. E. (2020).

“Development and Validation of an RP-HPLC Analytical Method for (R)-(+)-Amlodipine-*o,o'*-diptoluoyl-D-tartrate”

- Development and validation of UVspectrophotometric method for the determination of Atorvastatin calcium using sodium citrate as hydrotropic agent. *Pharmaceutical Chemistry Journal*, 54, 422-429.
- Sripani, R., Syafri, S., Suryani, M. I., Susanti, M., & Hamidi, D. (2023). Optimisation of Extraction and TLC-Densitometric Analysis of  $\alpha$ - and  $\gamma$ Mangostin from the Rind of *Garcinia mangostana* L. *Tropical Journal of Natural Product Research*, 7(3).
  - Walters, S. M., & Stonys, D. B. (1983). Determination of chlorthalidone and clonidine hydrochloride in tablets by HPLC. *Journal of chromatographic science*, 21(1), 43-45.
  - Nalawade, V., & Joshi, S. (2019). Development and validation of LC-MS/MS method for simultaneous determination of azilsartan medoxomil and chlorthalidone from the human plasma. *Journal of Pharmaceutical Sciences and Research*, 11(7), 2533-2537.
  - Singer, J. M., O'Hare, M. J., Rehm, C. R., & Zarembo, J. E. (1985). Chlorthalidone. In *Analytical Profiles of Drug Substances* (Vol. 14, pp. 1-36). Academic Press.
  - Doub, W. H., Ruhl, D. D., Hart, B., Mehelic, P. R., & Revelle, L. K. (1996). Gradient liquid chromatographic method for determination of chlorhexidine and its degradation products in bulk material. *Journal of AOAC International*, 79(3), 636-639.
  - Hussein, L. A., Magdy, N. N., & Ibrahim, M. A. (2019). Stability-indicating RP-UPLC method for simultaneous determination of azilsartan medoxomil and chlorthalidone in tablets in the presence of its degradation products. *Journal of chromatographic science*, 57(3), 213-219.
  - Youssef, R. M., Maher, H. M., El-Kimary, E. I., Hassan, E. M., & Barary, M. H. (2013). Validated stability-indicating methods for the simultaneous determination of amiloride hydrochloride, atenolol, and chlorthalidone using HPTLC and HPLC with photodiode array detector. *Journal of AOAC international*, 96(2), 313-323.
  - Elgawish, M. S., Mostafa, S. M., & Elshanawane, A. A. (2011). Simple and rapid HPLC method for simultaneous determination of atenolol and chlorthalidone in spiked human plasma. *Saudi Pharmaceutical Journal*, 19(1), 43-49.
  - Belsare, G. W., Zade, A. B., & Badne, S. G. (2020). MICRODETERMINATION OF Ce (IV) AND Y (III) WITH CHROME AZUROL-S IN PRESENCE OF CETYLDIMETHYLETHYLAMMONIUM BROMIDE.
  - Al Azzam, K. M., Saad, B., & Aboul-Enein, H. Y. (2010). Simultaneous determination of atenolol, chlorthalidone and amiloride in pharmaceutical preparations by capillary zone electrophoresis with ultraviolet detection. *Biomedical Chromatography*, 24(9), 977-981.
  - Phyo Lwin, E. M., Gerber, C., Song, Y., Leggett, C., Ritchie, U., Turner, S., & Garg, S. (2017). A new LC-MS/MS bioanalytical method for atenolol in human plasma and milk. *Bioanalysis*, 9(7), 517-530.
  - Shah, J. V., Patel, D. P., Shah, P. A., Sanyal, M., & Shrivastav, P. S. (2016). Simultaneous quantification of atenolol and chlorthalidone in human plasma by ultra-performance liquid chromatography-tandem mass spectrometry. *Biomedical Chromatography*, 30(2), 208-216.
  - Pathak, S., Bhardwaj, M., & Godela, R. (2023). Analytical and Bioanalytical Methods for the Quantification of the Nucleotide Polymerase Inhibitor-Sofosbuvir: A Critical Review (20152021). *Current Pharmaceutical Analysis*, 19(1), 51-65.
  - ICH Validation of Analytical Procedures: Text and Methodology Q2 (R1), International Conference on Harmonization, Geneva, Switzerland, 2023.
  - Lababidi, J. M., Kabil, M. F., & Azzazy, H. M. E. S. (2025). Sofosbuvir: A comprehensive profile. *Profiles of drug substances, excipients, and related methodology*, 50, 1-41.
  - Ahmed, A., Mahtab, T., Saleem, S., Akula, S., & Khan, M. D. (2020). Validation of RP-HPLC method for determination of sofosbuvir in bulk and pharmaceutical dosage forms. *International Journal of Pharmaceutical Sciences and Nanotechnology (IJPSN)*, 13(2), 4826-4830.
  - Waghmode, R., Tegeli, V., & Dalal, A. (2022). Analytical method development and validation of rp-hplc method for the estimation of sofosbuvir in bulk and formulation. *Journal of Advanced Scientific Research*, 13(07), 40-45.
  - Aher, K. B., Bhavar, G. B., Shelke, M. R., & Sayyad, S. F. (2023). Environmentally friendly analytical methods for quantitative measurement of sofosbuvir in pharmaceutical formulations: Comparison of HPTLC and UV spectrophotometric approaches. *Research Journal of Pharmacy and Technology*, 16(8), 3830-3836.
  - Bhairav, B. A., & Chavan, M. J. (2021). Method Development and Validation to Estimate Sofosbuvir in Marketed preparation by UVSpectroscopy and HPLC along with force Degradation Study. *Research Journal of Pharmacy and Technology*, 14(8), 4165-4172.
  - Lalitha, K. V., Reddy, J. R., & Devanna, N. (2018).

“Development and Validation of an RP-HPLC Analytical Method for (R)-(+)-Amlodipine-*o,o'*-diptoluoyl-D-tartrate”

Stability indicating RP-HPLC method development and validation for estimation of sofosbuvir in pharmaceutical dosage form. *The Pharma Innovation*, 7(5, Part J), 656.