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"Development Of A Quantitative Method For The Assessment Of (S)-Amlodipine Di-P-Toluoyl-D-Tartaric Acid"

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ABSTRACT

A reverse-phase high-performance liquid chromatography (RP HPLC) method was developed and validated for the analysis of (S)-amlodipine di-p-toluoyl-D-tartaric acid. The analysis used a mobile phase of acetonitrile and water (80:20 v/v) at a flow rate of 0.8 ml/min, with UV detection at 249 nm, and was performed on a HYPERSIL column (250 x 4.6 mm, 5 µm). The retention time for (S)-amlodipine di-p-toluoyl-D-tartaric acid was 5.742 minutes. The method demonstrated a linear response for concentrations ranging from 4 to 24 ppm, with a correlation coefficient ('r' value) of 0.999. The method's linearity, accuracy, precision, selectivity, robustness, and forced degradation studies were thoroughly evaluated, meeting all the criteria set by ICH guidelines. Intra- and inter-day precision showed relative standard deviations below 2%. The percentage recoveries of (S)-amlodipine di-p-toluoyl-D-tartaric acid in pharmaceutical formulations were between 98.74% and 101.11%, with an overall mean recovery of 99.59%.

Keywords: Method validation, linearity, accuracy, precision, inter-assays precision.

INTRODUCTION

Amlodipine is a well-known dihydropyridine calcium channel blocker widely prescribed for the treatment of hypertension and angina pectoris. Its mechanism of action involves inhibiting the influx of calcium ions into vascular smooth muscle and cardiac muscle cells, leading to vasodilation and reduced cardiac workload. Amlodipine exists as a racemic mixture, containing equal parts of (R)- and (S)-enantiomers. Notably, the (S)-enantiomer is predominantly responsible for the therapeutic effects. Enantiomers are molecules that are mirror images of each other and cannot be superimposed. They often exhibit different pharmacological effects despite having identical chemical compositions. The (S)-enantiomer of amlodipine has been found to possess greater pharmacological activity compared to the (R)-enantiomer. This has led to an increased interest in developing formulations that exclusively contain the (S)-enantiomer to enhance efficacy and reduce potential side effects. The formulation of pure (S)-amlodipine poses several challenges, particularly concerning its solubility, stability, and bioavailability. To address these issues, researchers have explored various strategies, including the use of co-crystals, salts, and derivatives. One innovative approach involves combining (S)-amlodipine with di-p-tolyl-D-tartaric acid to form (S)-amlodipine di-p-tolyl-D-tartaric acid. Di-p-tolyl-D-tartaric acid is an organic acid derivative known for its ability to enhance the physicochemical properties of drugs. This compound acts as a chiral resolving agent, improving the solubility and stability of (S)-amlodipine. By forming a stable complex with (S)-amlodipine, di-p-tolyl-D-tartaric acid aids in maintaining the integrity and bioavailability of the active ingredient.

In analytical chemistry, the determination of composition is primarily reliant on modern technology and analytical techniques [1-5]. These complex approaches are essential for efficiently employing analytical instruments and obtaining dependable, high-quality analytical results during investigations. A vital aspect of analytical technique development is the careful selection of an accurate assay procedure, which is required to determine the composition of formulations. This stage is critical in building a reliable analytical procedure that allows for the exact identification and quantification of distinct components in a sample.

Furthermore, validation of analytical methods is critical since it includes establishing the method's capability for measuring concentrations in laboratory settings, particularly for future sample analyses. The validation approach thoroughly ensures that the method is effective and acceptable for producing accurate and meaningful outcomes [6-7].

Adherence to Good Laboratory Practices (GLP) and Good Manufacturing Practices (GMP) are essential in the context of instrumental reverse-phase high-performance liquid chromatography (RP-HPLC). The development of analytical procedures within these regulated environments [8-11] guarantees that analyses are not only precise and accurate, but also meet defined quality requirements. This emphasizes the importance of strictly adhering to protocols to ensure the dependability of analytical results and the integrity of the entire analytical process.

EXPERIMENTAL:

The experiment used HPLC/AR grade solvents. The standard sample of (S)-amlodipine di-p-toluoyl-D-tartaric acid was prepared in-house through a double purification process. Additionally, the technical grade of (S)-amlodipine di-p-toluoyl-D-tartaric acid was also produced in-house.

Chromatographic conditions:
conducted using a HYPERSIL column (C18, 5.0 µm, 250 x 4.6 mm). Data processing was carried out using Empower software on the HPLC system. Isocratic elution was performed with a mobile phase of acetonitrile and water in an 80:20 (v/v) ratio. The flow rate was maintained at 0.8 ml/min, and detection was done at a wavelength of 249 nm.

A stock solution of the standard:

A stock solution of (S)-amlodipine di-p-toluoyl-D-tartaric acid standard was prepared by accurately weighing 100 mg of the standard compound. It was then dissolved in 5 ml of acetonitrile and further diluted to a final volume of 20 ml with acetonitrile to prepare a stock solution I. From this solution, 5 ml was transferred to a 50 ml volumetric flask and diluted to the mark with acetonitrile, resulting in stock solution II.

Stock solution of sample

The (S)-amlodipine di-p-toluoyl-D-tartaric acid sample was weighed at 100 mg and diluted to a total volume of 10 mL with acetonitrile. From this solution, 5 mL was transferred into a 50 mL volumetric flask and further diluted to the mark with acetonitrile.

Calibration curve

The (S)-amlodipine di-p-toluoyl-D-tartaric acid was measured by pipetting a reference solution into a 100 mL volumetric flask. Concentrations of 4, 8, 12, 16, 20, and 24 parts per million (ppm) were prepared by diluting the (S)-amlodipine di-p-toluoyl-D-tartaric acid with acetonitrile to the mark. Each concentration was independently prepared for each dilution. These prepared concentrations were individually injected into the RP-HPLC system using 20 µL injections from duplicate solutions. Chromatography was performed under the specified conditions. The evaluation of (S)-amlodipine di-p-toluoyl-D-tartaric acid was conducted using a UV detector at a wavelength of 340 nm, as described [14-15].

Method Validation:

The method validation encompasses various aspects, including robustness, forced degradation, suitability of the system, precision, selectivity, accuracy, range, and linearity.

Specificity

Specificity was assessed by scanning both the diluent solution and the standard solution of (S)-amlodipine di-p-toluoyl-D-tartaric acid, each having concentrations of 20 µg/mL. Derivatized solutions containing (S)-amlodipine di-p-toluoyl-D-tartaric acid were then introduced into the chromatographic system, alongside a solvent blank, reagent blank, and sample blank. This step aimed to confirm the absence of interference from any reagent or solvent blank at the retention time of (S)-amlodipine di-p-toluoyl-D-tartaric acid.

Linearity:

The linearity test solutions were created by diluting a stock solution to various concentrations: 4, 8, 12, 16, 20, and 24 ppm. These solutions' concentrations were determined using the assay method. A 20 µL volume from each solution was injected into the HPLC system, and the resulting chromatogram was used to record the peak area. The data on peak area and concentration were then subjected to analysis using the method of least squares linear regression. The calibration curve yielded values for both y-intercept and slope.

Precision

The precision of the proposed method, encompassing intra-day precision and injector repeatability, was assessed by analyzing six replicates of a constant concentration of the (S)-amlodipine di-p-toluoyl-D-tartaric acid mixture. The linearity range of the mixture was evaluated on various days and under diverse conditions, including different analysts and columns.

Accuracy (Recovery studies)

The percentage recovery was calculated by comparing the area before and after introducing the operational standard. Both drugs underwent the same recovery procedure. The standard addition method was performed at levels of 20%, 60%, 80%, 100%, and 120%, and the percentage recovery was assessed.

RESULTS AND DISCUSSION

The RP-HPLC method was employed to develop and validate the separation of the compound (S)-amlodipine di-p-toluoyl-D-tartaric acid. Separation was accomplished using a HYPERSIL RP C18 column and a mobile phase composed of acetonitrile and water in an 80:20 (v/v) ratio. The flow rate was maintained at 0.8 ml/min, and detection occurred at a wavelength of 249 nm. In summary, the presence of excipients did not interfere with the detection of peaks for 4(S)-amlodipine di-p-toluoyl-D-tartaric acid, confirming the method's selectivity. The analytes were completely separated within a timeframe of less than 10 minutes.

HPLC method optimization and development:
The initial investigation included evaluating the mobile phase, which comprised a blend of water and acetonitrile in a ratio of 20:80 (v/v), with a flow rate of 0.5 mL/min. In this setup, peaks were distinctly separated with outstanding clarity and symmetry. Consequently, a mobile phase composed of acetonitrile and water in a volumetric ratio of 80:20 was chosen for the entire study owing to its superior chromatographic performance.

System suitability studies of method validation:
System suitability tests were conducted to verify the system's appropriateness for the intended purpose. Upon measurement, it was noted that the peak at 5.742 minutes exhibited an average retention time and a peak area variation of less than 2. Additionally, the tailing factor was below 2, and over 2000 theoretical plates were observed for the (S)-amlodipine di-p-toluoyl-D-tartaric acid peak. The proposed method demonstrates high sensitivity, enabling reliable peak detection. In all instances, 4(S)-amlodipine di-p-toluoyl-D-tartaric acid was successfully separated from the peak, along with the excipients.

Specificity:
The (S)-amlodipine di-p-toluoyl-D-tartaric acid compound exhibited a retention time of 5.742 minutes. No interfering peaks were detected from the blank at the corresponding retention period, affirming the specificity of the method for determining (S)-amlodipine di-p-toluoyl-D-tartaric acid. This specificity has been validated in prior research [20-22].

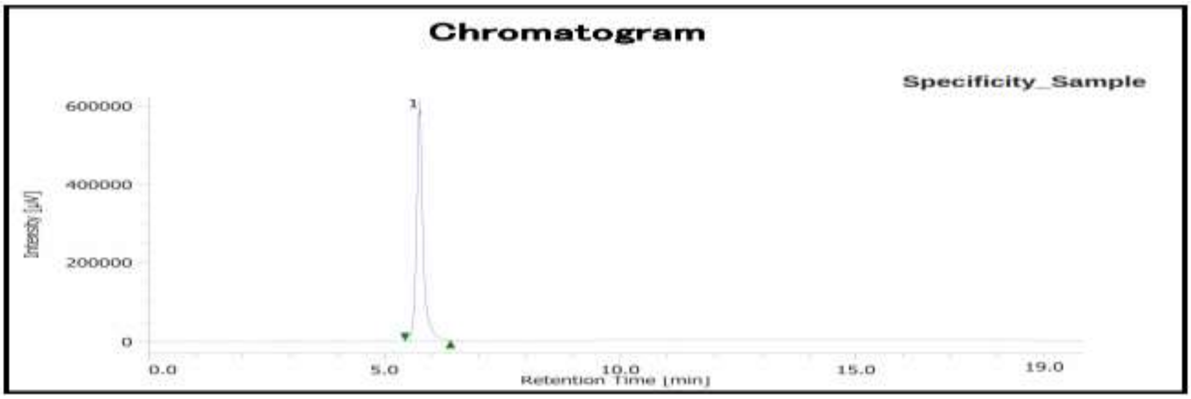

Figure-1: Specificity peak purity chromatogram of (S)-amlodipine di-p-toluoyl-D-tartaric acid

Linearity
The linear calibration curve for (S)-amlodipine di-p-toluoyl-D-tartaric acid was observed over a concentration range of 4.024–24.114 ppm. Peak area data for (S)-amlodipine di-p-toluoyl-D-tartaric acid in the treated samples underwent analysis using linear regression (**Table 1**) and calibration curves (**Figure 2**). The regression equation derived from the calibration curve was determined to be Y = 2,92,132.490770x - 52,790.166667 (as shown in Figure 2), with a correlation coefficient of 0.9999, indicating a robust positive correlation.

Table 1: Linearity data of (S)-amlodipine di-p-toluoyl-D-tartaric acid standard [24]

Linearity Sol Level	Conc ppm	Replications	Peak Area Counts	Means Area
L1	4.036	R1	1154876	1152441.5
		R2	1150007	
L2	8.072	R1	2284211	2283585
		R2	2282960	
L3	12.108	R1	3465205	3464652.5
		R2	3464100	
L4	16.144	R1	4618858	4618183.5
		R2	4617509	
L5	20.18	R1	5833185	5825213.5
		R2	5817242	
L6	24.216	R1	7030736	7025546.5
		R2	7020357	

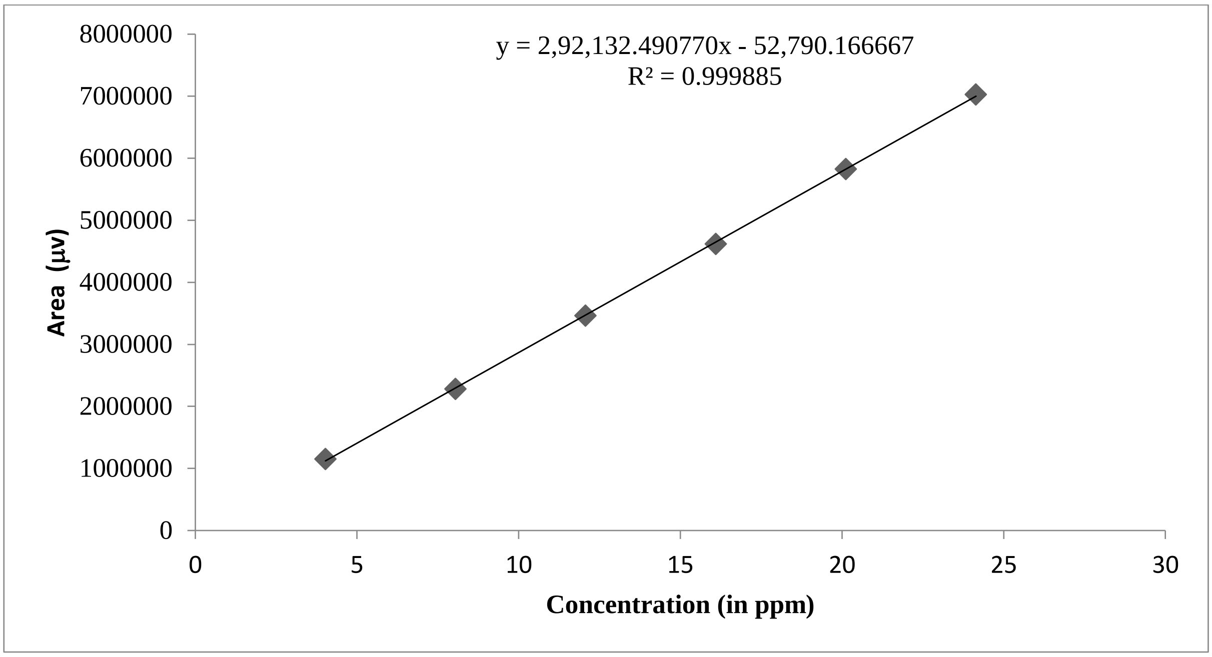

Figure-2: Linearity graph of (S)-amlodipine di-p-toluoyl-D-tartaric acid standard

Precision

The precision of the method was evaluated by examining the intra-assay and injector repeatability of the standard solutions of (S)-amlodipine di-p-toluoyl-D-tartaric acid. The % RSD for both repeatability and intra-assay precision was below 2%, demonstrating a high level of precision.

Table 2: Injection repeatability (precision) for (S)-amlodipine di-p-toluoyl-D-tartaric acid.

Sample no.	Conc in ppm	Area (mv)	% Content
Sample-1	20.32	5791853	99.93
Sample-2	20.36	5796396	99.81
Sample-3	20.42	5817114	99.88
Sample-4	20.40	5830713	100.21
Sample-5	20.30	5780994	99.84
Sample-6	20.40	5805699	99.78
Average	NA	NA	99.91
STDEV	NA	NA	0.16
% RSD	NA	NA	0.16

Table 3: Intra-assay (precision) data of (S)-amlodipine di-p-toluoyl-D-tartaric acid technical.

Sample no.	Conc in ppm	Area (mv)	% Content
Sample-1	20.02	5854048	99.84
Sample-2	20.10	5878601	99.86
Sample-3	20.20	5869923	99.22
Sample-4	20.23	5894371	99.49
Sample-5	20.11	5832468	99.03
Sample-6	20.05	5867483	99.92
Average	NA	NA	99.56
STDEV	NA	NA	0.37
% RSD	NA	NA	0.38

Table-4: Comparison between analyst-1 and 2

	Mean % Content	Absolute Difference
Analyst 1	99.91	0.35
Analyst 2	99.56	

Accuracy

The resultant recovery % RSD for (S)-amlodipine di-p-toluoyl-D-tartaric acid ranged from 98.74 -101.11%, with an overall mean recovery of 99.59 %. This indicates that the method is not influenced by any positive or negative interferences from the blank. Given these findings, it was concluded that the analyte's recovery data falls within the acceptable range, affirming the accuracy of the proposed method [26].

Table 5: Accuracy data for (S)-amlodipine di-p-toluoyl-D-tartaric acid technical.

% Recovery					
Level (%) / pptn	**Smpl Wt (in mg)**	**Conc. (in ppm)**	**Area (mv)**	**% Recovery**	**% Mean Recovery**
20_1	4.02	4.02	1153876	98.74	99.26
20_2	3.96	3.96	1151152	100.00	
20_3	4.01	4.01	1154450	99.04	
60_1	12.04	12.04	3464265	98.98	99.31
60_2	12.01	12.01	3463840	99.22	
60_3	11.95	11.95	3464384	99.73	
80_1	16.02	16.02	4615098	99.10	98.92
80_2	16.15	16.15	4650942	99.07	
80_3	16.11	16.11	4617318	98.60	
100_1	20.05	20.05	5827585	99.99	99.92
100_2	20.14	20.14	5836985	99.70	
100_3	19.98	19.98	5812639	100.08	
120_1	24.31	24.31	7029200	99.47	100.43
120_2	24.47	24.47	7192099	101.11	
120_3	24.56	24.56	7190309	100.72	

Range:

The assessed concentration range for (S)-amlodipine di-p-toluoyl-D-tartaric acid spans from 4 ppm (at 20% concentration) to 24 ppm (at 120% concentration).

Table-6: Range for (S)-amlodipine di-p-toluoyl-D-tartaric acid

Solution	**20% (4 ppm)**	**120% (24 ppm)**
1	1154736	7030736
2	1150337	7030736
3	1154876	7030725
4	1150216	7020278
5	1154876	7020099
6	1150007	7020309
Average	**1152508**	**7025480.5**
STDEV	**2545.59769**	**5753.543282**
% RSD	**0.22**	**0.08**

Force Degradation Studies:

Under various conditions, (S)-amlodipine di-p-toluoyl-D-tartaric acid exhibited stability as a drug substance. It maintained stability under metallic conditions (0.05M $FeCl_3$) at room temperature, basic conditions (1N NaOH) at room temperature, acidic conditions (1N HCl) at room temperature, oxidation conditions (3% H_2O_2) with exposure to light at room temperature, reduction conditions (1% Na_2S), photolytic conditions (exposure to 1.2 million lux/hour), and thermal degradation conditions at 105°C.

In each degradation scenario mentioned above, every degradant peak could be distinguished from both the blank and the main peak. The observed correlation between the increase in degradant impurities and the decrease in assay result for (S)-amlodipine di-p-toluoyl-D-tartaric acid is considered satisfactory. Based on the provided validation data, it can be affirmed that the HPLC technique for (S)-amlodipine di-p-toluoyl-D-tartaric acid is specific and serves as a stability-indicating method. [27].

Table 7: Forced degradation calculation of (S)-amlodipine di-p-toluoyl-D-tartaric acid technical

Condition	**Smpl Wt (in mg)**	**Conc (in ppm)**	**Area (mv)**	**% Assay**	**% Total Imp.**	**Mass Balance**
As such	20.07	20.07	5780994	99.09	20.07	20.07
0.05M_$FeCl_3$_24 Hrs	20.03	20.03	5703930	97.96	20.03	20.03
1N_NaOH_24 Hrs	20.17	20.17	5790221	98.76	20.17	20.17
1N_HCl_24 Hrs	20.09	20.09	5739130	98.27	20.09	20.09
3% H_2O_2_24 Hrs	20.11	20.11	5715517	97.77	20.11	20.11
1% Na_2S_24 Hrs	19.95	19.95	5715517	98.56	19.95	19.95
Photo @ 1.2 million lux/Hr	19.96	19.96	5713584	98.47	19.96	19.96
Thermal @ 105°C_24 Hrs	20.03	20.03	5709788	98.06	20.03	20.03

Table 8: Force degradation of (S)-amlodipine di-p-toluoyl-D-tartaric acid impurity profile

% impurity (by Area normalization)								
RT about -->	**Unk @ 2.90**	**Unk @ 3.15**	**Unk @ 3.26**	**Unk @ 3.45**	**Unk @ 3.99**	**Unk @ 6.82**	**Unk @ 8.32**	**Total Imp**
As such	ND	ND	ND	ND	ND	ND	ND	0.000
0.05M_$FeCl_3$_24 Hrs	ND	ND	ND	ND	ND	0.138	ND	0.138
1N_NaOH_24 Hrs	0.09	ND	0.274	0.254	ND	ND	ND	0.618
1N_HCl_24 Hrs	ND	0.064	ND	ND	0.048	ND	ND	0.112
3% H_2O_2_24 Hrs	ND	ND	0.826	ND	ND	ND	ND	0.826
1% Na2S_24 Hrs	ND	ND	0.826	ND	ND	ND	ND	0.826
Photo @ 1.2 million lux/Hr	ND	ND	ND	ND	ND	ND	0.657	0.657
Thermal @ 105°C_24 Hrs	ND	ND	ND	ND	ND	ND	ND	0.000

CONCLUSION:

The (S)-amlodipine di-p-toluoyl-D-tartaric acid compound was verified, developed, and utilized for determining (S)-amlodipine di-p-toluoyl-D-tartaric acid. The method was identified as specific, accurate, precise, and robust. (S)-Amlodipine di-p-toluoyl-D-tartaric acid elutes rapidly, within a timeframe of fewer than 10 minutes, and does not exhibit any interference with the components of the pharmaceutical dosage form. The proposed approach is deemed suitable due to the high reproducibility, accuracy, excellent selectivity, and sensitivity of (S)-amlodipine di-p-toluoyl-D-tartaric acid for simultaneous determination.

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