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Development And Validation Of An Analytical Method For Estimating Residual Solvents In Amlodipine Using Headspace Gas Chromatography

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Abstract
Organic volatile contaminants and Residual impurities present in pharmaceutical products are estimated by using Head Space Gas Chromatography. In this study a Head Space Gas Chromatography method was used to detect residual solvents and organic volatile contaminants present in Amlodipine tablet dosage form. Amlodipine is a well-established, extended-release calcium channel blocker (CCB) known for its efficacy in reducing blood pressure. In this study an attempt was made to analyze the residual organic solvents such as Methanol and Ethanol present in Amlodipine tablet dosage form by headspace gas chromatography (HS-GC). The carrier gas streamed was nitrogen, the method was developed and optimized by using HP-InnoWax Column (30 m × 320 µm × 0.5 µm) coated with Polyethylene Glycol as stationary phase and coupled with flame ionization detector. An injector temperature of 250°C was programmed to prevent degradation. A temperature of 40°C was set as the initial oven temperature and monitored at a final temperature of 125°C. N, N-dimethylacetamide was selected as the sample solvent. The validation studies were performed with regard to International Council for Harmonization (ICH) Q2 guidelines for validation of analytical experiments. All the validation parameters complied with the specification limit. Hence, the optimized method developed and validated can be utilized for the concurrent detection of residual solvents in tablet formulations.

Keywords: Amlodipine Tablets; GCFID; Methanol; Ethanol; Organic volatile Contaminants; Residual Solvents

Introduction

Elevated blood pressure typically precedes the onset of cardiovascular disease (CVD), which stands as the leading global cause of mortality [1,2]. Uncontrolled blood pressure is considered a critical risk factor for cardiovascular diseases. As per the World Health Organization (WHO), approximately 1.28 billion individuals worldwide, aged between 30 and 79, experience hypertension, with notable prevalence observed in nations categorized as low- and middle-income [3]. Hence, addressing hypertension effectively serves as a pivotal approach in curtailing the incidence of cardiovascular diseases [2]. Employing pharmaceutical interventions alongside modifying behavioral risk factors, such as quitting smoking, shedding excess weight, engaging in regular physical exercise, limiting alcohol consumption, and adopting a balanced diet, contributes to the reduction of hypertension and the risk of developing CVD [1].

The primary antihypertensive medications commonly utilized include calcium-channel blockers (CCBs), angiotensin-converting enzyme inhibitors (ACEIs), angiotensin-receptor blockers (ARBs), beta blockers (BBs), and diuretics (DIUs). CCBs stand out as one of the most frequently prescribed classes of antihypertensive drugs. Each type of approved CCB operates by blocking calcium influx through transmembrane channels, thereby diminishing the interaction between actin and myosin in myocytes. This action leads to the relaxation of vascular smooth muscle tone, ultimately resulting in decreased blood pressure. Numerous clinical trials have extensively explored and validated the substantial hemodynamic effects of CCBs on cardiovascular and hemodynamic parameters [4,5].

Amlodipine stands as a well-established, extended-release calcium channel blocker (CCB) known for its efficacy in reducing blood pressure [6]. It is chemically characterized as a fully substituted dialkyl 1,4-dihydropyridine-3,5-dicarboxylate derivative, employed in managing hypertension, chronic stable angina, and confirmed or suspected vasospastic angina. 2D and 3D conformer of amlodipine is in figure 1 [7]. This compound functions as an antihypertensive agent, calcium channel blocker, and vasodilator. Initially sanctioned by the FDA in 1987, amlodipine is a widely utilized antihypertensive medication categorized under dihydropyridine calcium channel blockers. Its preference for peripheral blood vessels endows it with a reduced likelihood of inducing myocardial depression and cardiac conduction abnormalities compared to other calcium

channel blockers. Amlodipine is commonly prescribed for hypertension and angina treatment. It possesses antioxidant properties and fosters the production of nitric oxide (NO), a crucial vasodilator that aids in blood pressure reduction. The convenience of once-daily dosing further enhances the appeal of amlodipine as a therapeutic option.

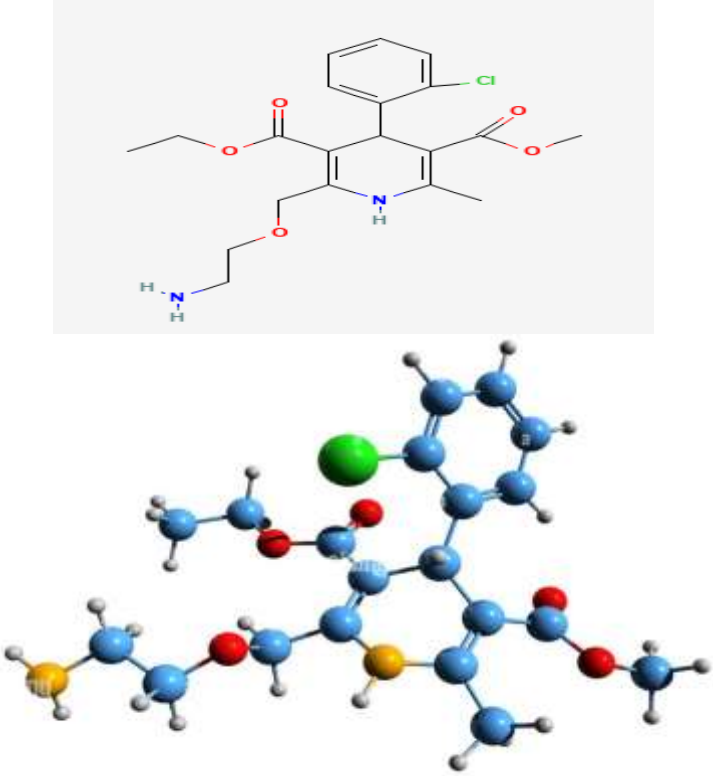

Figure 1 Structure of Amlodipine

Pharmaceutical residual contaminants and organic volatile solvents play significant roles in the manufacturing processes of finished products, drug excipients, and the preparation of drug substances and formulations. Despite efforts to mitigate their presence, practical manufacturing methods often fall short of completely eliminating residual solvents [8]. During bulk drug manufacturing or the coating of solid dosage forms, organic solvents become entrapped within the formulation. This occurs particularly when these solvents are used to dissolve film-coating materials for application onto tablet formulations. As a result, residual solvents persist in pharmaceutical products even after manufacturing processes, posing potential concerns for product quality and safety [9].

Headspace Gas Chromatography (HS-GC) proves highly effective for both qualitative and quantitative assessments of volatile contaminants and impurities in samples, which can be efficiently transferred from a matrix to the headspace gas chromatography. This technique is also renowned for its utility in trace element analysis [10]. Common applications of headspace analysis encompass the examination of volatile organic compounds (VOCs) from contaminated samples and wastewater treatment, residual solvents in pharmaceutical packaging, toxicology screening including blood alcohol levels, volatile constituents found in food and alcoholic beverages, and diagnostic gas analysis in oils [11].
Consequently, extensive literature review was conducted, focusing on relevant studies. Among them, one study introduced and assessed a dispersive liquid–liquid microextraction (DLLME) method coupled with gas chromatography–mass spectrometry (GC-MS) for monitoring and quantifying class 1 residual impurities in pharmaceutical formulations [12]. Another study detailed the development of a rigorously validated reversed-phase high-performance liquid chromatography (RP-HPLC) technique for quantifying amlodipine levels in tablet dosage form [13]. Furthermore, research investigated a cross-linked chitosan microspheres method utilizing spray drying for developing and validating a liquid chromatographic approach to estimate amlodipine in its pharmaceutical formulations [14]. Similarly, another study examined the analysis of amlodipine in plasma samples from healthy volunteers for method development and validation purposes [15]. An Ultra Performance Liquid Chromatography (UPLC) method was also evaluated for quantifying amlodipine in lipid-based formulations [16].

Additionally, studies explored the degradation pattern of amlodipine under various stress conditions to validate a stability-indicating HPLC method and develop an analytical method for estimating toxic impurities in amlodipine drug substances (17, 18). Novel methods were also developed and validated by liquid chromatography-tandem mass spectrometry (LC-MS/MS) to quantify amlodipine from plasma and dried plasma spots (DPS) [19]. Moreover, LC-MS/MS methods were established for assessing amlodipine using deuterated standards and measuring prodrug amlodipine and its metabolite in mouse and human plasma [20]. These studies aimed at providing precise, rapid, and robust methods for quantification of amlodipine in human

plasma, as well as evaluating bioequivalence of drug formulations in human plasma through simple, sensitive, and precise method development and validation.

Materials and Methods

Instruments used

The study utilized a gas chromatograph fitted with a flame ionization detector. A headspace sampler, specifically the Agilent GC-HS 7890B Series, was employed in conjunction with an HP-InnoWax capillary column, coated with a polyethylene glycol (PEG) stationary phase, having an internal diameter of 0.5 μm, a length of 30 meters, and a film thickness of 320 μm. Additionally, an analytical balance (Radwag) and a micropipette (Eppendorf or equivalent) were used in the study.

Solvents and Chemicals

The chemicals used in the study were purchased from reputable suppliers. GC Grade chloroform and N, N-dimethylformamide, or their equivalents, were obtained from Sigma-Aldrich in Mumbai, India. Methanol and ethanol standards were procured from E. Merck. Additionally, a 10 mg amlodipine tablet dosage form was provided as a gift sample by Novartis Pharma (Pakistan) Ltd.

Instrumentation

The injection temperature was kept constant at 150 °C, with a split ratio of 5:1, utilizing the ultrapure nitrogen gas of 99.9995% purity (Air Products, Malaysia) was used as the carrier gas. The pressure was maintained consistently at 8 psi, and the column flow rate was set at 1.0 mL/min. The detector temperature was programmed to rise to 250 °C. The temperature program began with an initial hold at 40 °C for 1 minute, followed by an increase at a rate of 1 °C/min until reaching 45 °C, where it was held for another minute. The temperature was then ramped up at 5 °C/min until it reached 55 °C, followed by a 1-minute hold. Finally, the temperature was increased at a rate of 25 °C/min, reaching 125 °C. The flow rates were set as follows: zero-air at 250 mL/min, hydrogen at 25 mL/min, and makeup gas at 20 mL/min. The total analysis time was 15 minutes. Details regarding the headspace injector and GC parameters can be found in Table 1.

Table 1 Headspace Injector and GC conditions

Oven	
Equilibrium Time	1 min
Maximum Temperature	270 °C
Front SS Inlet	
Mode	Split
Heater	150 °C
Pressure	8 psi
Total flow	14.056 mL/min
Septum Purge Flow	3 mL/min
Split Ratio	5:1
Split Flow	9.2132 mL/min
Column	
Column Identification	HP-INNOWax coated with Polyethylene Glycol (PEG); dimensions 30 m x 320 μm x 0.5 μm
Temperature Range	40 °C – 260 °C
Pressure	8 psi
Flow	1.0 mL/min
Front Detector FID	
Makeup	N_2
Heater	250 °C
H_2 Flow	25 mL/min
Air Flow	250 mL/min
Makeup Flow	20 mL/min
Carrier Gas Flow Correction	Constant makeup and Fuel Flow
Total Run Time	15 min

Description of Analytical Method

Method validation

The study of validation parameters involved a comprehensive evaluation of several key metrics, as outlined in the ICH harmonized tripartite guidelines. These metrics included accuracy, linearity, limit of detection (LOD), limit of quantitation (LOQ), method precision, repeatability, ruggedness, specificity, and system suitability for residual solvents. Each parameter was carefully assessed to ensure the reliability and effectiveness of the analytical method used. By following these guidelines, the study aimed to establish a robust framework for analyzing residual solvents, ensuring that the methods applied are both precise and reliable across various conditions.

Preparation of standard stock solution:
The preparation of the standard stock solution was accomplished by weighing out 0.06 grams of methanol and 0.1 grams of ethanol. These amounts were then transferred into a 50.0 mL volumetric flask. After this, N, N-Dimethylacetamide was added to the flask to dilute the solution to the desired final volume. The solution was mixed thoroughly to ensure homogeneity. This prepared stock solution serves as a reference for subsequent analyses. The standard chromatogram for methanol is illustrated in Figure 2, providing a visual representation of the expected retention time and peak characteristics in the chromatographic analysis.

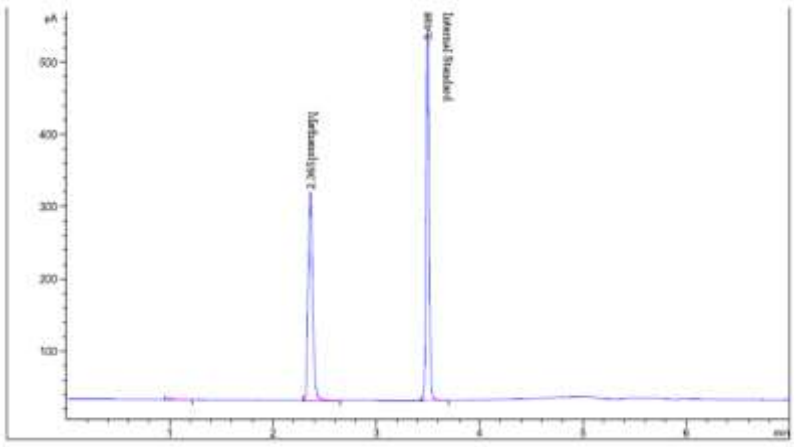

Figure 2 Standard Chromatogram of Methanol

Preparation of standard solution:
The procedure for preparing the standard solution began by transferring 1 mL of the stock solution into a 50.0 mL volumetric flask. This solution was then diluted to the desired volume using N, N-Dimethylacetamide, ensuring thorough mixing to achieve uniformity. Subsequently, 5 mL of this diluted solution was transferred into a 20 mL headspace vial, which was then securely sealed with a crimp cap to prevent any contamination or evaporation. The standard chromatogram illustrating the analysis of Ethanol is shown in Figure 3, highlighting its retention time and peak characteristics for reference in chromatographic evaluations.

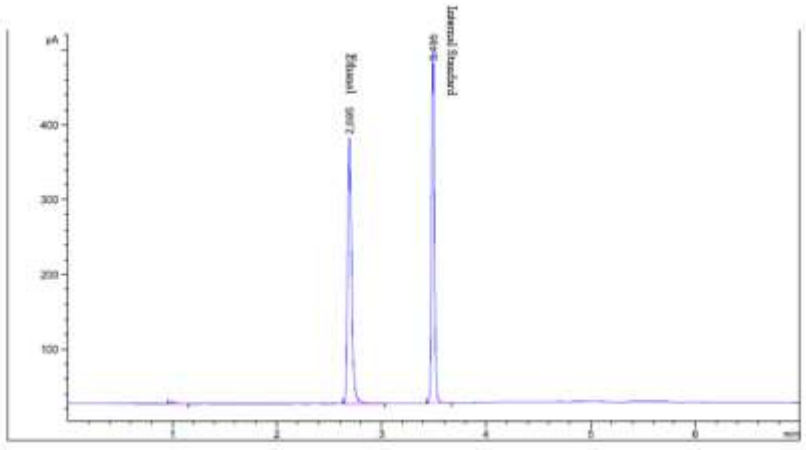

Figure 3 Standard Chromatogram of Ethanol

Preparation of sample solution:
The sample preparation involved weighing exactly 0.25 grams of the substance and placing it into a tared 20 mL headspace vial. N, N-Dimethylacetamide was used to dissolve the sample completely. After adding the solvent, the vial was sealed with a crimp cap and gently shaken for a few seconds to ensure that the sample was thoroughly dissolved and ready for analysis.

Results and Discussion
System suitability
A study was conducted to evaluate the system's suitability by preparing standard solutions according to the prescribed test method and injecting them into the GC/HS system. System suitability parameters, including USP resolution and the relative standard deviation for peak responses, were calculated using six replicate injections of the standard solution. The results

confirmed that all values were within the acceptable limits, demonstrating the system's accuracy and reliability. The outcomes of this analysis are summarized in Table 2.

Table – 2 System suitability data for Methanol and Ethanol

Injection No.	Peak area Methanol	Peak area Ethanol
01	204095	726701
02	206695	841853
03	207492	739557
04	206971	735806
05	205946	730027
06	207190	738755
Average	206398	35450
SD	2244.70	5914.09
%RSD	1.2	0.9

Method Precision

In order to assess the method precision, six unspiked samples were analyzed and subsequently injected into the GC/HS system. The study focused on calculating the concentrations of methanol and ethanol in each sample. The relative standard deviations (RSD) for the six different preparations of each substance were calculated in ppm and were found to comply with the acceptance criteria. This demonstrates the method's precision and reliability. The comprehensive results are summarized in Tables 3 and 4 and depicted in Figure 4.

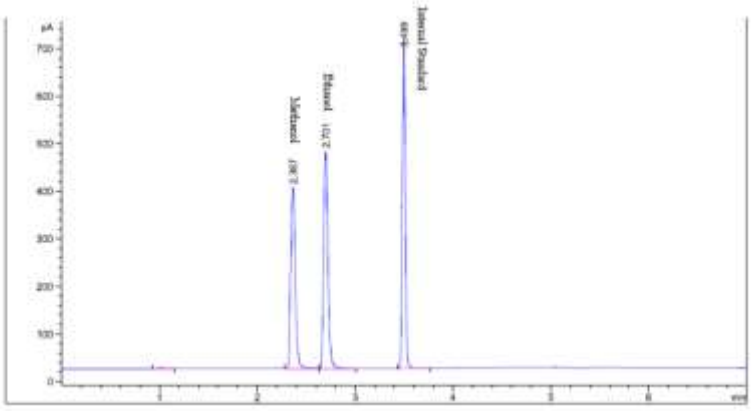

Figure 4 Chromatogram of Methanol and Ethanol

Table 3 Precision data for Methanol and Ethanol

Injection No.	Methanol Peak area	Ethanol Peak area
Std-1	204095	726701
Std-2	206695	741854
Std-3	207492	739557
Std-4	206971	735806
Std-5	205946	730027
Std-6	207190	738755
Average	206398	735450
SD	1244.71	5914.09
% RSD	1.2	0.9

Table 4 – Precision data for Methanol and Ethanol

Area of Methanol	Content of Methanol (ppm)	Content of Ethanol (ppm)
33028	65	ND
30983	59	ND
31458	60	ND
30311	58	ND
31122	59	ND
32286	62	ND
Average	60	NA
SD	2.86	NA
% RSD	4.7	0.0

Specificity

To assess the specificity of the method, a study was conducted where blank, standard solution, test solution, and individual standard solutions were prepared according to the analysis method and injected into the GC/HS system. The chromatograms were examined to detect any interference from blank peaks at the retention times of the known peaks in each solution. This step was crucial to confirm that the blank peaks did not overlap with the peaks of interest. The specificity results from this analysis are summarized in Table 5.

Table 5 – Specificity data for Methanol and Ethanol

Name of the peak	Retention time in Minutes
Methanol	3.366
Ethanol	4.700
Internal Standard (Acetonitrile)	4.498

Determination of LOD

The limit of detection (LOD) was determined through a series of precise steps involving methanol and Ethanol. Initially, 0.06 grams of methanol and 0.1 grams of ethanol were accurately weighed and placed into a tared 50.0 mL volumetric flask. The contents were then diluted to the final volume using N, N-Dimethylacetamide and mixed thoroughly to ensure uniformity. Following this, 1.0 mL of the standard stock solution was transferred into another 50.0 mL volumetric flask and diluted to volume with N, N-Dimethylacetamide. Subsequently, 2.5 mL of this prepared stock solution was transferred to a third 50.0 mL volumetric flask and further diluted with N, N-Dimethylacetamide to the final volume. This methodical dilution process was essential for accurately determining the LOD for both methanol and ethanol. The results of these analyses are comprehensively summarized in Tables 6 and 7, with additional visual representation in Figure 5.

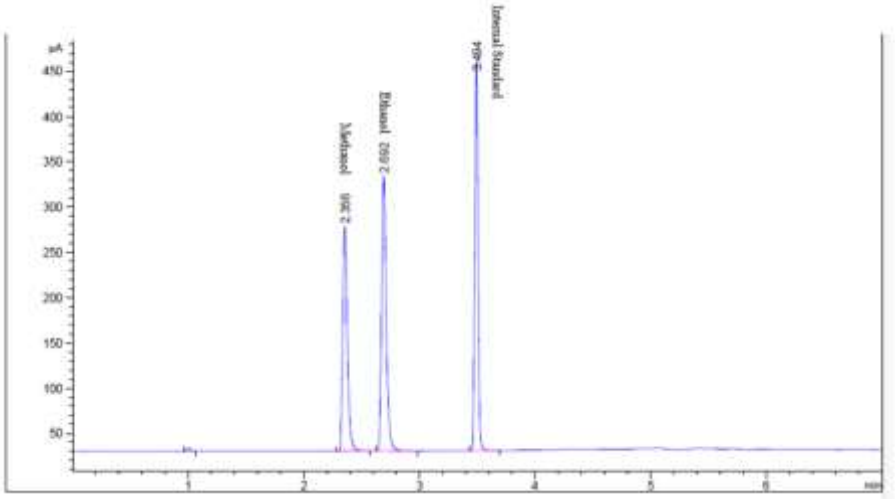

Figure 5 Chromatogram of Methanol and Ethanol

Table – 6 Establishment of LOD

Injection No	Methanol	Ethanol
Std-1	88714	704880
Std-2	88635	708956
Std-3	87214	797054
Std-4	87591	797074
Std-5	86455	790663
Std-6	89583	703955
Average	98033	600474
SD	1147.59	6652.99
%RSD	1.2	1.1

Table – 7 Establishment of LOD

Injection No	Methanol	Ethanol
Std-1	11281	31281
Std-2	10627	50823
Std-3	10935	51662
Std-4	11461	52164

Std-5	10281	51094
Std-6	11166	50921
Average	10959.5	41323.83
SD	440.2316	507.734
%RSD	2.1	1.2

To establish the linearity of the test method, standard solutions were prepared at concentrations ranging from 50% to 150% of the target concentration. These solutions were then analyzed according to the prescribed method. The correlation coefficient and Y-intercept were calculated from the data and were found to meet the acceptance criteria. A summary of these results can be found in Table 8.

Table 8 Linearity data for Methanol and Ethanol

Methanol			**Ethanol**		
Conc. ppm	**Area**	**Avg. Area**	**Conc. ppm**	**Area**	**Avg. Area**
7.27	52135	62150	20.14	214207	214205
	42163			214203	
10.91	68754	88731	30.20	280521	480167
	78706			279812	
14.54	103325	113126	40.27	524002	529669
	102935			535336	
18.17	123240	132918	50.34	668808	870930
	142594			773063	
21.81	138557	152696	60.41	923252	930280
	118842			943307	
	Slope	6528		Slope	25185
	Intercept	16207		Intercept	14096.28
	CC	0.9990		CC	0.9996
	Regression	1985.22		Regression	7405.51

The range of the test method was determined to be from 50% to 150% of the target concentration, as supported by the linearity, precision, and accuracy data. Methanol concentrations were evaluated from 7.27 ppm to 21.81 ppm, and ethanol concentrations ranged from 20.14 ppm to 60.41 ppm. The linearity plots for methanol and ethanol are shown in Figures 6 and 7, respectively.

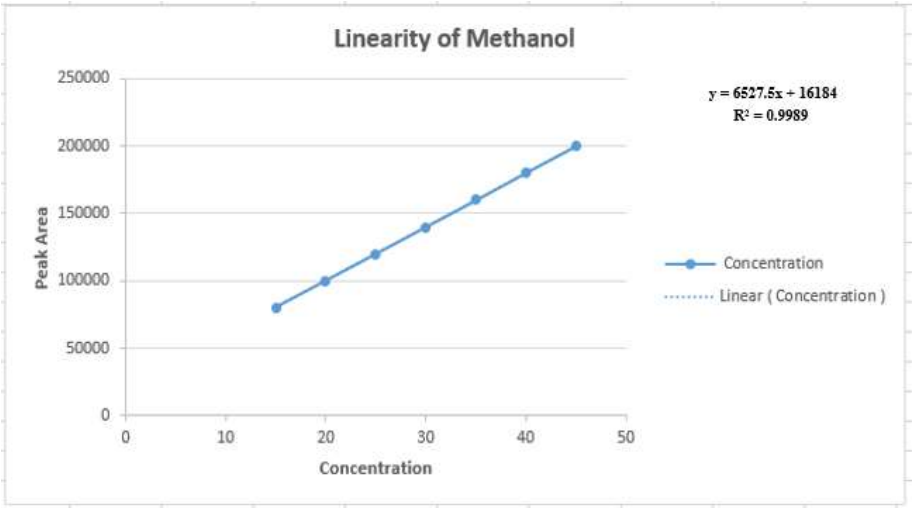

Figure 6 Calibration curve for Methanol

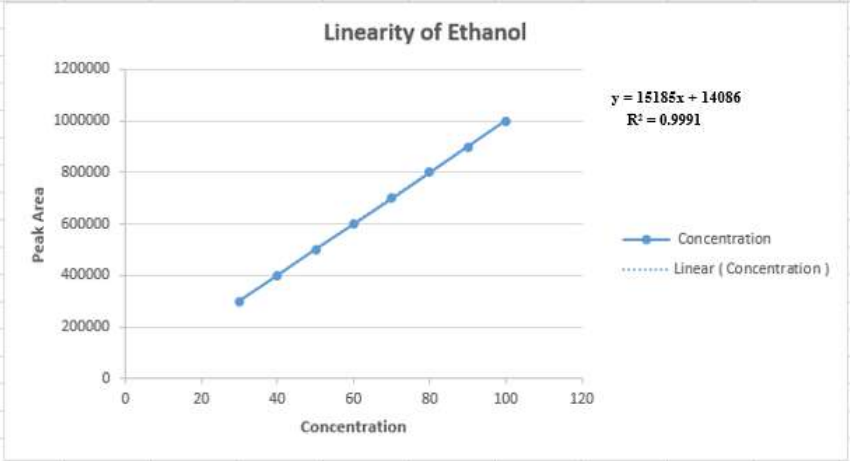

Figure 7 Calibration curve for Ethanol

Ruggedness

To evaluate the intermediate precision of the test method, six unspiked samples were analyzed and injected into the GC/HS system. This study was conducted over different days and by different analysts. The methanol and ethanol contents were calculated for each sample. The relative standard deviations (RSD) for each of the six preparations, measured in ppm, were found to meet the acceptance criteria. The results of this analysis are summarized in Tables 9 and 10, with additional visual data depicted in Figure 8.

Table –9 Intermediate precision data

Injection No.	Methanol	Ethanol
Std-1	101417	517704
Std-2	120120	511090
Std-3	109817	714144
Std-4	140700	508200
Std-5	129526	708809
Std-6	142035	721865
AVG	140603	513635
SD	873	5363.87
%RSD	0.9	0.9

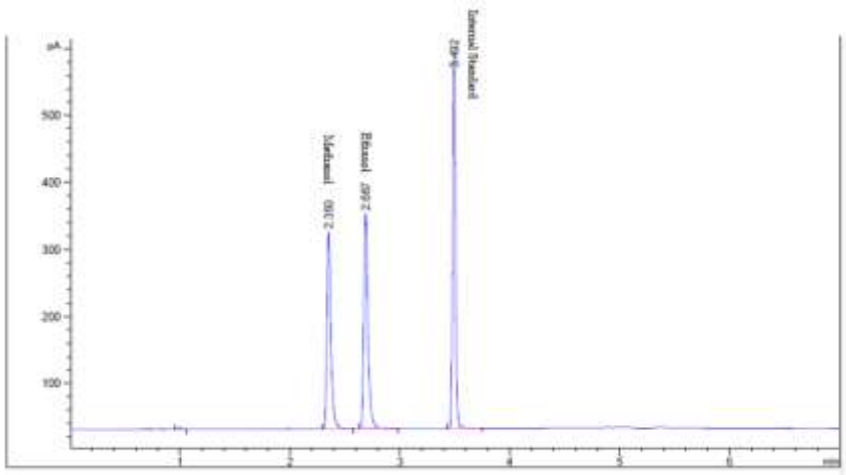

Figure 8 Precision Chromatogram

Accuracy/ Recovery

The accuracy of the test method was evaluated by assessing the recovery of methanol and ethanol from spiked samples. Samples were prepared by adding methanol and ethanol to the matrix at 50%, 100%, and 150% of the target concentration of known standards. These samples were prepared in triplicate at each spike level, and the content of the unspiked sample was subtracted to calculate recovery. The accuracy results are presented in Tables 11 and 12, with further data illustrated in Figure 9

Table – 11 Accuracy data (Methanol)

Recovery level	Sample No	'ppm' added	'ppm' recovered	% Recovery
50%	1	13.07	12.97	98.14
	2	13.07	12.92	97.73
	3	13.07	12.65	95.49
100%	1	23.15	25.33	120.75
	2	23.15	25.71	112.33
	3	25.15	24.20	105.36
150%	1	37.22	37.33	105.30
	2	37.22	32.40	95.21
	3	37.22	32.74	90.39
			Average	98.30
			% RSD	4.65

Table – 12 Accuracy data (Ethanol)

Recovery level	Sample No	'ppm' added	'ppm' recovered	% Recovery
50%	1	19.95	20.18	101.15
	2		20.48	102.65
	3		20.72	103.86
100%	1	39.90	39.85	99.87
	2		40.03	100.32
	3		39.9	100.00
150%	1	59.85	60.03	100.30
	2		59.18	98.88
	3		59.54	99.48
			Average	100.72
			% RSD	1.58

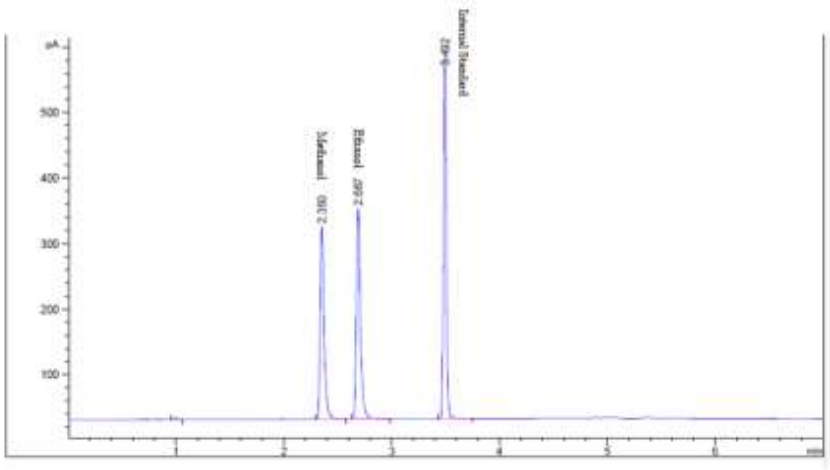

Figure 9 Accuracy Chromatogram for methanol and Ethanol

Discussion

Residual solvents are volatile organic compounds that can either remain in or be produced during the manufacturing process of drug excipients and active pharmaceutical ingredients (APIs). These solvents are often not entirely removed by standard production techniques. Therefore, rigorous quality control is essential for detecting impurities in APIs and drug formulations. This research focuses on developing a GC-HS method with a flame ionization detector to estimate methanol and ethanol in amlodipine tablets. The method employs a widely used HP-InnoWax column, measuring 30 meters in length, 0.5 µm in internal diameter, and 320 µm in film thickness.

The goal of this study was to create a fast, efficient, and reliable GC-HS method for the simultaneous estimation of methanol and ethanol in amlodipine tablets. The validation parameters adhered to ICH guidelines, with system suitability requiring a % RSD of no more than 15.0% for six replicate standard injections. Methanol and Ethanol achieved values of 1.2 and 0.9, respectively. Method precision dictated that the % RSD for methanol and Ethanol content in six samples should not exceed 15.0%, and the observed values were 1.2 and 0.9. Specificity testing ensured no interference at the retention time of the test solution from blank or standard solutions, a condition that was met in this study.

The LOD was determined to be 1.2 ppm for methanol and 1.1 ppm for Ethanol. The linearity requirement, with a correlation coefficient of at least 0.99, was met with values of 0.9989 for methanol and 0.9991 for Ethanol. Ruggedness criteria stipulate that the % RSD for methanol and Ethanol content in six samples should not exceed 15.0%, and the cumulative % RSD for method precision and intermediate precision should be under 20.0%. Both methanol and Ethanol achieved % RSDs of around 0.9. In terms of accuracy, the % recovery should be between 85% and 115%, with methanol and Ethanol showing recoveries between 90.39 - 105.36% and 98.88 – 103.86% respectively. These results validate the method's applicability for estimating methanol and Ethanol.

Conclusion

The GC-HS method that was developed and validated has been found to be both efficient and precise for the quantification of methanol and Ethanol residual solvents in amlodipine. The sample and standard preparation process is straightforward, fast, and highly sensitive. This method is adequate for the simultaneous detection of residual impurities and organic volatile solvents in pharmaceutical dosage forms. Additionally, it is well-suited for use in analyzing marketed formulations.

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