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Development and Validation of HPLC Method For Quantitative and Qualitative Analysis of Asenapine Maleate from Polymeric Micelles

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Abstract

The precise and confirmed HPLC technique has been established to quantify Asenapine maleate (ASPM) concentration in polymeric micelles. The separation was performed using a C18 analytical column with a mobile phase comprised of acetonitrile: water with 0.2% TFA 50:50 v/v. Flow rate of mobile phase was 1 mL/min, with a UV detector set to a wavelength of 230 nm. Various analytical considerations such as linearity, sensitivity, precision and others, are essential for validating strategies to provide consistent, dependable, and precise findings. The analytical approach had been utilized to ascertain the ASPM level in polymeric micelles formulations. The assay was specific, exhibiting a retention time of 4.72± 0.08 minutes. Linearity was achieved throughout the concentration range of 10 **μ**g/mL to 150 **μ**g/mL. The established method is applicable for the assessment of ASPM polymeric micelles, including all of the method validation criteria, which are all within the permissible range.
Keywords: Asenapine Maleate, Polymeric Micelles, HPLC, Validation, Quantitative Analysis.

Introduction

Asenapine maleate (ASPM) is an antipsychotic medication utilized in the management of schizophrenia and bipolar I disorder. First-pass metabolism limits its oral bioavailability; hence its marketed dosage form was sublingual tablets. The drawbacks associated with sublingual pills encompass the discomfort experienced while retaining the dosage within the oral cavity, the occurrence of numbing sensation in tongue, and the possibility of medication loss should it be swallowed. [(1)].

ASPM is a tetracyclic medication composed by a dibenzo-oxepino pyrrole structure (Figure. 1). It is the ninth atypical antipsychotic drug that achieved authorization from the US Food and Drug Administration (FDA) in August 2009 for the treatment of schizophrenia and bipolar I disorder in adult [(2)].

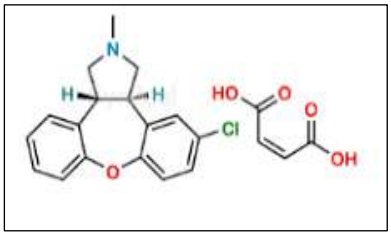


Figure 1. Chemical structure of Asenapine maleate

The assembly of polymeric micelles (PMs) in an aqueous environment occurs through the induction of core-shell architecture of amphiphilic block copolymers, ranging in dimensions from 10 to 100 nm. It has garnered significant attention for study and is among the most extensively researched systems. The core of a micelle can encapsulate pharmaceuticals with slightly aqueous solubility, thereby boosting drug solubility, improving pharmacokinetic profiles, and protecting degradable compounds from degradation. [(3)]. High-performance liquid chromatography (HPLC), a prevalent analytical method, is deemed optimal for the analysis of Asenapine owing to its superior separation efficiency, elevated sensitivity, accuracy, and rapidity [(4)].

At present, a variety of analytical techniques for the assessment of Asenapine maleate have been established. Such methods encompass ultraviolet-visible spectroscopy (UV) [(5)], thin layer-chromatography (TLC) [(6)], high performance thin layer-chromatography (HPTLC) [(7)]. These approaches provide unique characteristics. UV spectroscopy provides simplicity and rapidity; yet, it is particularly vulnerable to interference in intricate samples, potentially leading to erroneous outcomes. TLC and HPTLC offer adequate resolution; nevertheless, their sensitivity is

comparatively low, and repeatability is suboptimal [8]. In the past few years, NMR and UPLC-MS/MS have exhibited exceptional accuracy and sensitivity for qualitative and quantitative analysis. Nevertheless, the higher costs associated with these processes, coupled with their operational complexity, significantly limit their frequent utilization within laboratory settings. In contrast, High-Performance Liquid Chromatography (HPLC) is recognized as the preferred methodology for the analytical assessment of Asenapine maleate within laboratory environments, attributable to its sensitivity, efficiency in separation, and selectivity [9].

The preparation and optimization of liquid chromatography methodologies continue to be a prominent field of inquiry among researchers, who exhibit a considerable interest in this subject matter. The development of methodical techniques for complex mixtures or samples necessitates a thorough and systematic approach, which encompasses the precise modeling of the retention behavior of the analyte in question. [10].

The primary objective of this work is to create a technique for the measurement of Asenapine utilizing HPLC with UV detection. During the process, the optimal conditions for chromatography, such as mobile phase composition, flow rate, with a number of additional characteristics, were established. In accordance with the standards that were established by the "International Council for Harmonization" (ICH), the new method was validated as extensively as possible and developed.

Materials and Methods

Materials

Asenapine maleate (Purity >99.0 %) has been supplied from Suzhou Zehou Biochemical Technology Co., LTD, (China), TPGS was obtained from Shanghai Aladdin Biochemical Technology (China), Tetronic® 701 had purchased from MERYER Co (China). Acetonitrile (ACN) of HPLC grade was gathered from (Biosolve BV-Chemicals.com, Netherlands), methanol and trifluoracetic acid (TFA) were received from Sisco Research laboratories pvt. Ltd, India. This investigation employed only analytical-grade chemicals and reagents.

Methods

Instrumentation

The HPLC was performed with a CECIL apparatus (UK) equipped with a UV detection (CECIL CE 4200-Adept detector, UK), four channel low pressure LPG pump 4104, Harmony column (4.6×250 mm, pore size 5 µm, C18, Perkin Elmar) and temperature controller Varian Metathern®, USA. The Lab Solution software (Power Stream by Cecil instrument, Ltd, version 4.2) was utilized to oversee instrument both data and functionality collection.

Preparation of ASPM polymeric micelles by thin film-hydration method

PMs was produced using the thin film hydration approach, wherein drug and copolymers TPGS:Tetronic® 701 at concentrations 2% and 4% (w/v) as demonstrated in Table 1, were dissolved in methanol within a round-bottom flask. The solvent was gradually evaporated at 60 °C under decreased pressure with a rotary evaporator (Stuart rotary evaporator, RE300DB, North Yorkshire, UK), rotating at 80 rpm, resulting in a thin dry layer of ASPM-copolymer matrix on the interior surface of the flask. The desiccated film has been rehydrated using 10 ml of phosphate- buffered saline (PBS) solution (pH 7.4) while maintaining a temperature of approximately 40 ˚C, with agitation at 60 rpm for one hour in a rotary evaporator to get a polymeric micelle solution [11].

Table 1. Composition of polymeric micelles formulation

Formula	Drug (%) w/v	Copolymer/surfactant	Copolymer/surfactant (%) w/v	PBS pH 7.4 (ml)
F1	0.5 %	TPGS:Tetronic®701	2%	10
F2	0.5 %	TPGS:Tetronic®701	4%	10

Characterization of ASPM Polymeric Micelles

Particle size and polydispersity index (PDI) Measurement

Size of the particles and the polydispersity index (PDI) for the prepared micelles had been measured via dynamic light scattering technique, using zetasizer (Malvern Zetasizer UK). Throughout the measurement process, the samples were not diluted in any way. Every measurement was performed three times [12].

Determination of Encapsulation efficiency (EE)

The encapsulation efficiency (EE) of ASPM in micelles were quantified by centrifugation technique, 2 ml of each formula was centrifuged using Millipore filter membrane (Amicon®) ultra-4 tube with molecular weight cut-off of 10,000 Da (Sigma, Ireland). Micelles carrying the encapsulated drug stayed in the outer chamber following 30 minutes of centrifugation at 4000 rpm, while the free drug was filtered into the

sample recovery chamber. The unentrapped drug amount in micelles was diluted to a suitable volume and assessed by HPLC with UV detector at 230 nm in order to calculate the entrapment efficiency according to equation 1. The experiment was performed in triplicate [13].

$$\text{EE (\%)} = \frac{\text{Weight of the drug encapsulated in micelles}}{\text{weight of the feeding drugs}} \times 100$$

Equation 1

Chromatographic conditions

Prior to use, the drug and copolymers solutions were prepared by dissolving in methanol and filtered through a filter 0.45 μm nylon membrane to eliminate particulates. Mobile phase consists of acetonitrile and water with 0.2% TFA at ratio 50:50 (v/v), which degassed with negative pressure degassing (Degasser 4040) to eliminate dissolved gases [14]. A constant flow rate of 1mL/min was maintained, and the wavelength for detection was set at 230 nm [15], 10 μL as a volume of injection was used, and the duration of the run was 10 minutes. Prior to the measurement of wavelengths, the drug was subjected to UV–Visible spectrophotometry at a concentration of (30 ***μ***g/ml) in order to ascertain the suitable nanometer value for HPLC [16]. All experimental analyses were executed at room temperature to guarantee consistency and reproducibility of results.

Establishment of chromatographic condition validation

Validation methods and their Importance

Process of validation is necessary for ensuring that the technique operates as intended, series of studies is required for validation to evaluate the method's efficacy. Upon implementation, the methodology should produce precise and reliable outcomes [17]. Laboratory testing is the exclusive way for assessing a technique's validity. Validation trials analyze several properties of the approach to evaluate its applicability [18].

Preparation of Calibration Standards Solutions

A stock solution of ASPM with a concentration of 1000 μg/ml was made by dissolving 0.05 g of the drug in methanol and then adding enough methanol to make 50 mL. A workable solution was made by taking 5.0 ml from the stock and adding methanol to make the total volume 50 ml. We made standard solutions with six different concentrations (10, 20, 40, 60, 80, and 150 μg/ml) by diluting the working solution with methanol in a series.

System Suitability

The aspects of the system's suitability with regard to the retention time, capacity factor (K), number of theoretical plates (N) and height equivalent to a theoretical plate (HEPT) were assessed by injecting a blank mobile phase followed by six replicates of Asenapine maleate at concentration 60 ***μ***g/ml [19].

Specificity and selectivity

The evaluation of the specificity and selectivity of the procedure was conducted through a comparative analysis of the mobile phase chromatogram obtained from a blank sample and that derived from a sample that had been simultaneously spiked with Asenapine. The analysis method specificity had been elaborated to ascertain that the co-polymers utilized during the composition process of polymeric micelles had no impact on the drug's determination [20]. The co-polymers caused spikes in the samples with the highest concentration utilized in the test formulations. To detect any peak that may appear during the retention time (tr) of Asenapine maleate, the chromatograms were checked.

Linearity

Standard solutions within the concentration range of 10 to 150 μg/ml were introduced into the HPLC system for analysis. Each solution underwent three separate measurements. The response signal was quantified at a wavelength of 230.0 nm. The calibration curve was developed by utilizing the respective concentrations associated with each response signal, followed by the calculation of the regression equation.

Accuracy

It was determined that the spiking approach was the most effective way to determine accuracy and recovery at three different concentration levels: 80%, 100%, and 120%. Three replicate results were performed for each level; the recovery rate and relative standard deviations (RSD) were calculated to evaluate the accuracy of the method. The recovery was determined using the following equation [21].

%Recovery= (Measured concentration) / (Theoretical concentration) × 100

Equation 2

While RSD was calculated from the mean values obtained in the studies as the following equation:

% RSD= (standard deviation of measured concentrations)/ (mean concentration) ×100

Equation 3

Precision

The improved chromatographic methods were used to observe at the standard solutions at 60, 80, and 150 μg/mL. Intraday accuracy was evaluated at disparate time intervals during the same day, whereas inter day precision was determined using identical concentrations over three successive days [22].

Sensitivity

Through the measurement of the limit of detection (LOD) and the limit of quantification (LOQ), which had been studied based on the signal-to-noise ratio at low concentrations of the standard solution, the method's sensitivity had been evaluated and graded with respect to its sensitivity [23]. Calculations for LOD and LOQ were carried out as per the guidelines provided by (ICH) and demonstrated in equations 4 and 5 respectively [24].

The calculation of the limit of quantification (LOQ) and the limit of detection (LOD) for Asenapine maleate was performed utilizing the standard deviation (SD) and slope (S) derived from the calibration curve.

The following formulas used to computation:

- LOD = (3.3×σ)/s ------- Equation 4
- LOQ = (10×σ)/s ------- Equation 5

Where, σ is the standard deviation (SD) of the response and s is the slope of the calibration curve [25].

Robustness

To evaluate the sturdiness of the HPLC technique, a variation in the temperature of the column was introduced. The actual temperature was the ambient and the variation was curried via escalating the temperature to 40 C˚, despite the flow rate being set at 1.0 mL/min. The impact of this variation on % recovery, % RSD and retention time was investigated in this study [26].

Results and Discussion

Characterization of ASPM Polymeric Micelles

The prepared polymeric micelles of ASPM were evaluated according to particles size, polydispersity index (PDI) and the entrapment efficiency and the results were summarized in Table 2, while size distribution graphs of each formula were shown in Figure 2 (A and B).

Table 2. Characterization of ASPM Polymeric Micelles

Formula	Average value of particle size (nm) ± SD	PDI ± SD	Entrapment efficiency (EE%) ± SD
F1	10.103±0.899	0.047±0.01063	66.123±2.95
F2	10.32±0.603	0.0905±0.03271	78.913± 0.1815

The data was presented as mean ±SD, with n=3 and SD representing standard deviation

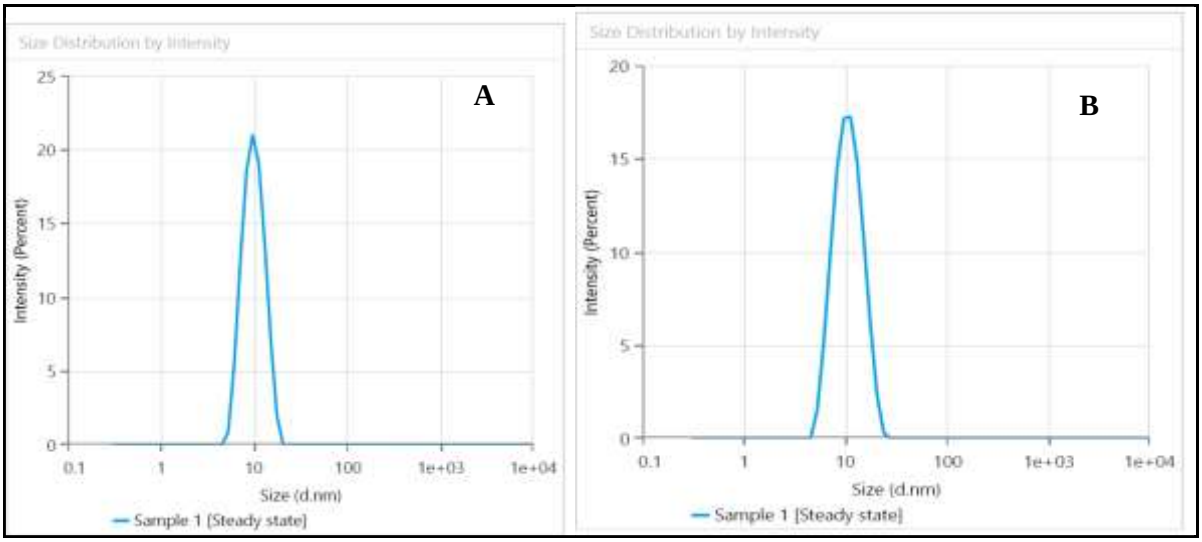


Figure 2. Size distribution for Polymeric Micellar Formulations: F1 (A) and F2 (B)

Particles size of the prepared formulations was within polymeric micelles size range as reported in the literatures.

The PDI values of each formula were below 0.3, indicating the monodispersity of the fabricated polymeric micelles.

EE% ranging from 66.123 ± 2.95 to 78.913 ± 0.1815 demonstrating variability depending on the % concentration of copolymers (TPGS: Tetronic®701) which are used in the formulation.

Selection of wavelength

UV spectrophotomer of ASPM at concentration 30μg/ml was shown in Figure 3, the UV detection wavelengths were ranged from 200 nm to 400 nm with low absorptivity and sensitivity of drug at wavelength of 270.5 nm. According to the Indian Pharmacopeia (I.P.), the wavelength maximum for compendial liquid

chromatographic procedures for determining related chemicals and estimating asenapine maleate is 230 nm. These methods are also used for determining other drugs that are relevant to the study [27,28].

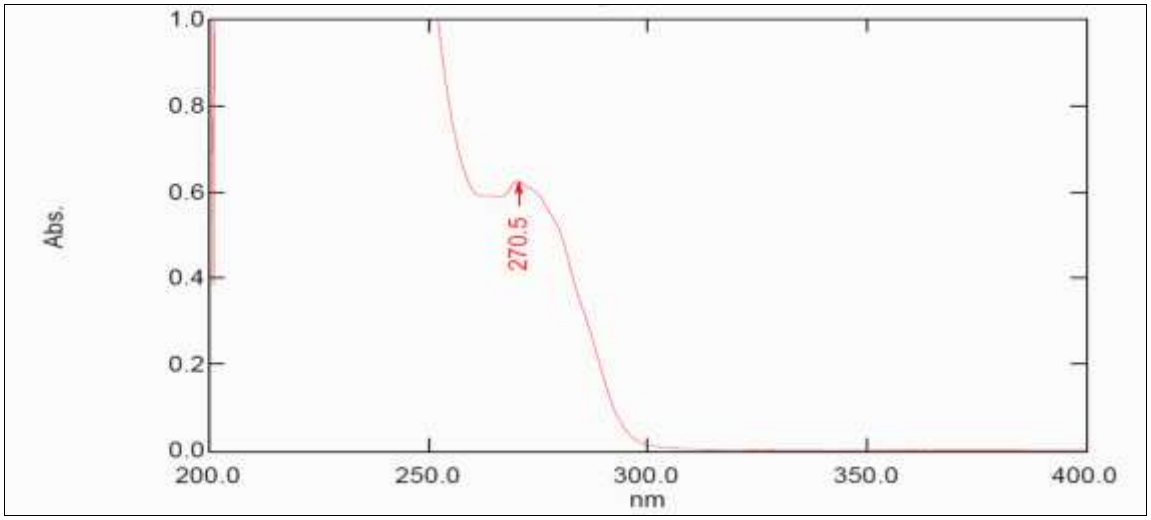


Figure 3. UV Spectrum of Asenapine maleate

System Suitability

The findings of six injections that were replicated at a concentration of 60 μg/ml are displayed in Table 3., showing the retention time, capacity factor, theoretical plates, and height equivalent to a theoretical plate, which were all within acceptable limits, thereby confirming the method's suitability for ASPM analysis.

Table 3. System suitability studies

Parameters	**Average values±SD**
Retention time ± SD	4.72± 0.08
Capacity factor (K) ± SD	1.35±0.0455
Number of Theoretical plates (N) ± SD	3292.492±1288.34
Height Equivalent to a Theoretical Plate (HEPT) ± SD	0.087±0.03344

The data are presented as mean (±SD), n = 6

Specificity and selectivity

Selectivity of the method was demonstrated after observing the chromatograms of drug solution, the copolymers solution, and the polymeric micelles, Figure 4 presents the chromatograms obtained from the specificity and selectivity test.

The retention time of drug was ranged from 4.6 -5 min when drug was analyzed from solution and in the determination of drug concentration in polymeric micelles (Figure 4, a and d). The chromatograms for TPGS and Tetronic® 701 (Figure 4, b and c) exhibited no absorption peaks corresponding to the retention time peak of ASPM in the chromatogram.

The specificity studies demonstrated that there was no interference observed; during the retention time, no peaks were identified that could potentially affect the analyte.

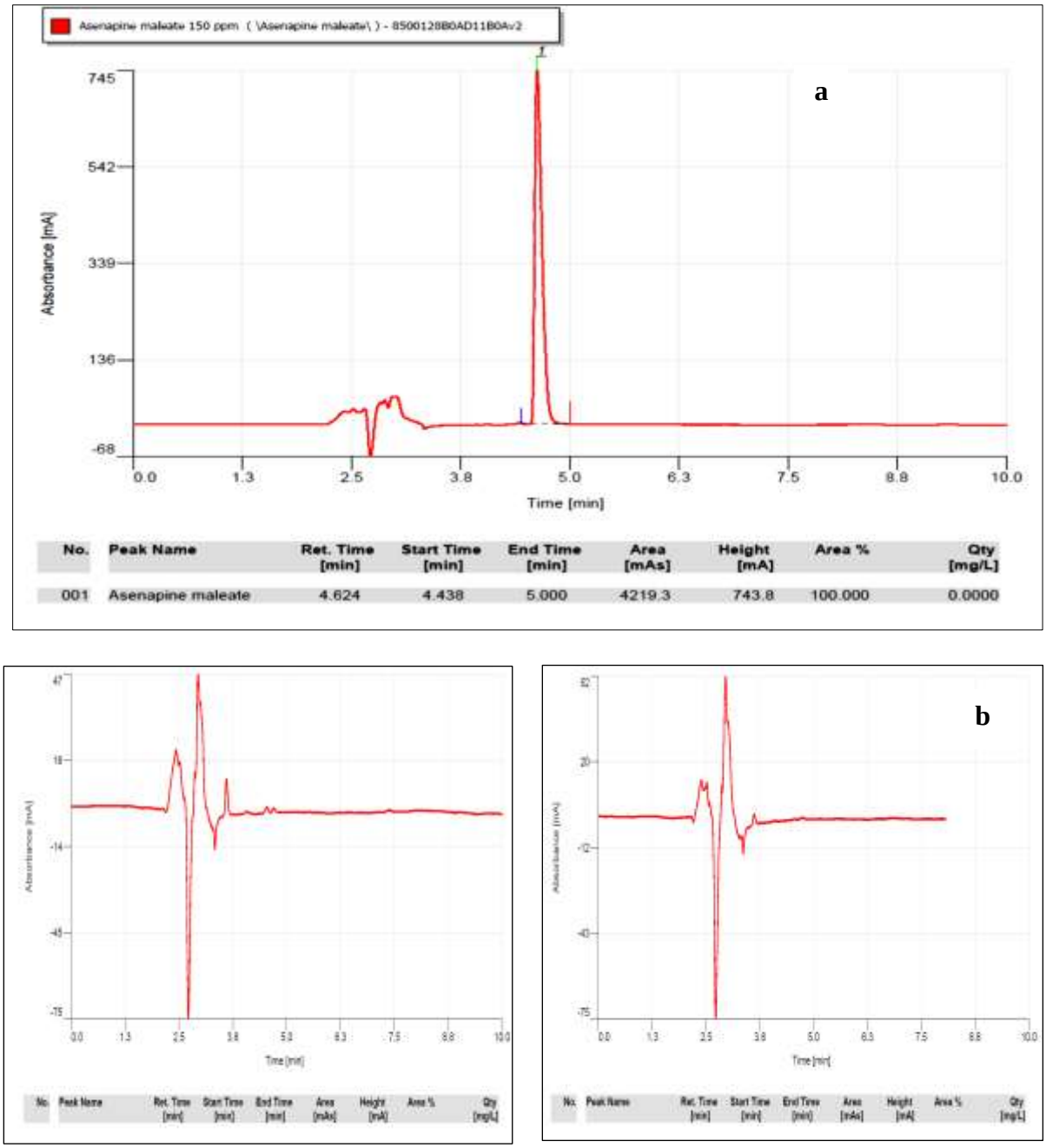
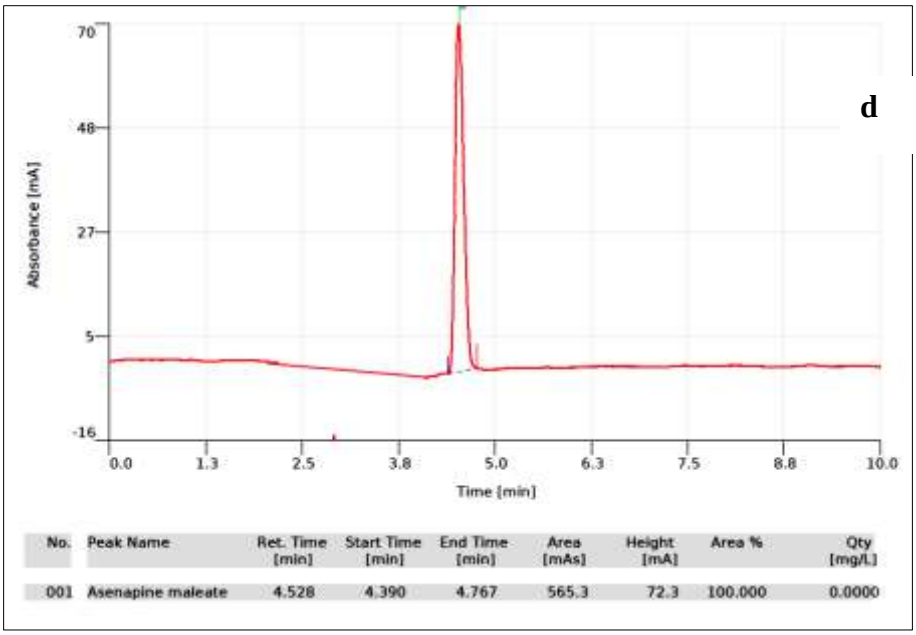


Figure 4. Chromatographs of a) Asenapine maleate at concentration 150 µg/ml, b) TPGS, c) Tetronic® 701 and d) unentrapped Asenapine maleate in polymeric micelle in entrapment efficiency study Conditions: mobile phase (acetonitrile/water with 0.2% TFA 50:50 v/v), flow rate at 1 mL/min; PDA detection wavelength at 230 nm; column temperature at 25 °C; sample temperature at 25 °C and injections volume was 10 µL.



Continued figure 4.

Linearity

Linearity means that the method may give findings that are directly proportional with the quantity of analyte in the sample. An evaluation of the linearity of the analytical technique was conducted using six concentrations ranging from 10 to 150 µg/mL, and a calibration curve was established This evaluation was carried out in triplicate. Then regression equation of the line was obtained (y = 34.091x - 45.851), resulting in the correlation coefficient R^2 value 0.9992, indicating the quality of curve.

The intercept, slope, and R^2 were obtained by linear regression, and the collected evidence are displayed in (Table 4). The linear fits of the points (n=3) and of the average response are shown in Figure 5.

Table 4. The calibration curve parameters (n=3) show the linearity of HPLC method for determining Asenapine maleate.

Concentration (µg/ml)	AUC 1	AUC 2	AUC 3	Average AUC
10	337.7	308.1	299.1	314.9667
20	698.8	629.4	663.6	663.9333
40	1310.4	1325.5	1282.3	1306.067
60	2209.7	1937.9	1901.8	2016.467
80	2578.6	2728	2453	2586.533
150	5354.9	5112.1	4862.5	5109.833
Slope	35.574	34.428	32.272	34.091
Intercept	-52.784	-58.819	-25.955	-45.851
R^2	0.9955	0.9996	0.9989	0.9992

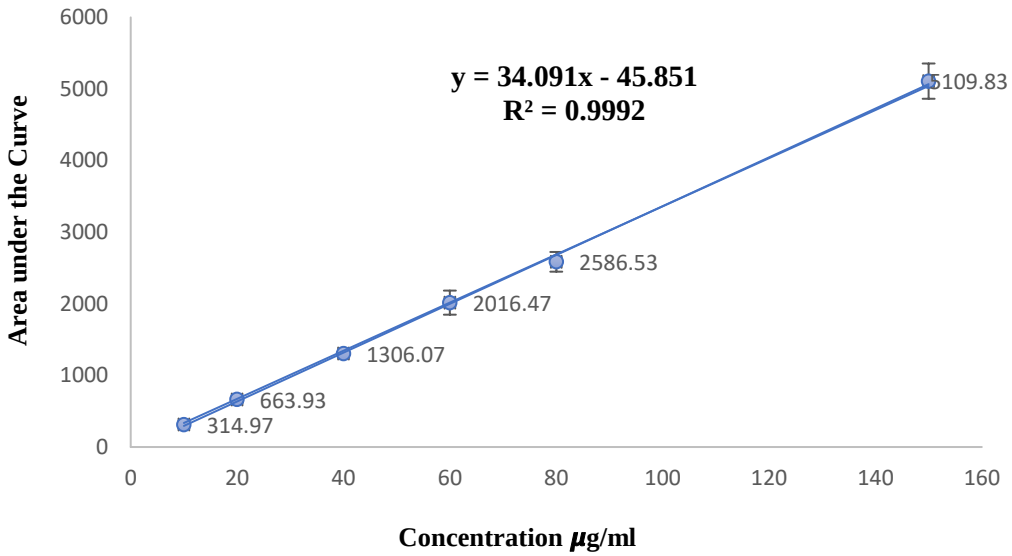


Figure 5. Linear fit of the calibration curve for average AUC ±SD (n=3)

Accuracy

Presented in Table 5 are the accuracy findings that were acquired through the employing of the HPLC method. The accuracy assessment involved a comparative analysis of the theoretical concentrations against the actual concentrations obtained from spiked samples. The percentage of recovery for all of the samples ranged from 98.5 to 102.32%, demonstrating that our approach exhibits an exceptional level of accuracy.

Table 5. Evaluated accuracy, expressed as percentage recovery, along with the percentage relative standard deviation (RSD) of the developed HPLC method, (n= 3)

Sample Level (%)	Theoretical (claimed) concentration in µg/ml	The concentration found in µg /ml	Recovery (%)	Recovery mean (%)	RSD%
80	20	20.05	100.25	98.5	1.6
		19.58	97.9		
		19.45	97.25		
100	30	29.89	99.63	98.9	1.59
		30.04	100.133		
		29.16	97.2		
120	40	40.8	102	102.32	0.28
		40.97	102.425		
		41.015	102.54		

Precision

Under consistent experimental conditions, we determined the percentage relative standard deviation (RSD) for both intraday and inter day assessments.

This allowed for an evaluation of the rigor of the established technique, the percentage relative variance had determined to be less than 2 %, indicating the accuracy of the developed procedure. The values obtained were summarized in Table 6. All values of precision were within recommended limits. Intraday RSD ranged from 0.27% to 1.4% whereas the inter day RSD was from 0.34% to 0.8%.

Table 6. Precision study of the developed method of Asenapine maleate

Con. (μg/ml)	Intraday				Inter day			
	Average values of AUC	SD	%RSD	% Recovery	Average values of AUC	SD	%RSD	% Recovery
60	1984.8	5.46900	0.27	99.3	2145.81	17.0869	0.8	106
80	2686.533	38.1242	1.4	103.9	2700.3	9.189048	0.34	104.4
150	5080.167	27.7073	0.55	99.4	5316.89	19.83411	0.373	104

The data is presented as a mean ± SD, with n=3

Sensitivity

Under the parameters of the experiment that have been created, the LOD is the smallest sample concentration that can be detected, but it cannot be quantified with any degree of accuracy. The limit of quantification, on the other hand, is defined as the smallest sample concentration that can be determined with parameters that are acceptable in terms of accuracy and precision [29].

In the current study, to determine the lowest concentration at which an analyte could be detected (LOD) or measured (LOQ) with a level of precision and accuracy that was considered acceptable, the standard deviation of the response and the slope from linear regression of the calibration curve were utilized. The LOD and LOQ values obtained in the method analysis were 8.78 μg/mL and 26.61 μg/mL, respectively, built on the standard curve equation.

Robustness

When a technique does not change even when the analytical parameters undergo minute shifts, it could be said that the approach is as robust as possible [30]. In order to evaluate robustness, the percentage of recovery and the relative standard deviation of the mean concentration of the analyte were used when the temperature of column rise to 40 ˚C. The results were shown in Table 7; the approach was shown to be reliable when these variations in chromatographic parameters were taken into consideration.

Table 7. Robustness results for Asenapine maleate at column temperature 40 ˚C

Concentration μg/ml	Area under the curve at column temperature 40 ˚C	Calculated concentration	Rt (min)
150	5205.6	154	4.429
	5080.9	150	4.65
	5012.1	148	4.66
Average	5132.866667	150.6666667	4.579666667
SD	98.08650944	3.055050463	0.130576925
Recovery (%)	100.45	100.44	95
RSD (%)	1.923	2.03	2.85

Conclusion

The current HPLC method was executed for the quantification of Asenapine maleate in polymeric micelle formulations. This method exhibits remarkable system suitability, reproducibility, and precision, facilitating swift qualitative and quantitative analysis of ASPM under standard laboratory conditions. The HPLC approach exhibited enhanced sensitivity and a wider range, rendering it more appropriates for intricate analytical scenarios and determine the accurate content of drug without any interference with the analyte. By using these sustainable practices, researchers aid in cultivating a more ecologically aware and sustainable scientific community.

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Conflicts of Interest

The authors claimed that they had no conflicts of interest.

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Ethics Statements

We confirmed that no animals were employed in this investigation.

Author Contribution

Zainab Eassa Jassim conceptualized and developed the study, conducted the experiments, evaluated the data, and composed the report. Nawal A. Rajab contributed to data interpretation, assisted in experimental design, and critically revised the manuscript. Hamid Akbari Javar provided supervision, and final approval of the version to be published. All authors have read, reviewed the results and approved the final manuscript.

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تطوير وتحقق من صحة طريقة HPLC للتحليل الكمي والنوعي لأسينابين ماليات من المذيلات البوليمرية

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الخلاصة

تقنية HPLC الدقيقة والمؤكدة أسست لتحديد تركيز أسينابين ماليات (ASPM) في المذيلات البوليمرية. تمت عملية الفصل باستخدام عمود تحليلي C18 مع طور متحرك يتكون من أسيتونيتريل : ماء مع ٠,٢TFA % بنسبة ٥٠:٥٠ حجماً. معدل تدفق الطور المتحرك كان ١ مل/دقيقة، مع كاشف UV محدد على طول موجي ٢٣٠ نانومتر. تعد اعتبارات تحليلية مختلفة مثل الخطية والحساسية والدقة وغيرها، ضرورية للتحقق من استراتيجيات توفير نتائج متسقة وموثوقة ودقيقة. تم استخدام النهج التحليلي لتحديد مستوى ASPM في تركيبات المذيلات البوليمرية. كان التحليل محددًا، حيث أظهر زمن احتباس قدره ٤,٧٢ ± ٠,٠٨ دقيقة. تم تحقيق الخطية في نطاق التركيز من ١٠ µg/mL إلى ١٥٠ µg/mL. الطريقة المعتمدة قابلة للتطبيق لتقييم المذيلات البوليمرية ل ASPM ، بما في ذلك جميع معايير التحقق من الطريقة، والتي تقع جميعها ضمن النطاق المسموح به.

الكلمات المفتاحية: أسينابين ماليات، مذيلات بوليمرية، HPLC، التحقق، التحليل الكمي.