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Novel Reverse Phase Liquid Chromatography Method for Accurate Determination of Vasopressin in Pharmaceutical Products

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ABSTRACT
A simple, gradient, reproducible, and selective RPHPLC technique was developed and validated in this work to quantify assay of drug substance and drug product.Separation was achieved with Agilent HPLC with C18 column, with mobile phase containing 0.1% Orthophosphoric acid in water and Acetonitrile. 50 µlof sample was injected in mobile phase at flow rate of 1 ml per minute at column oven of 25°C. Detection done at 220 nm by using UV detector. The developed method was validated for specificity, system suitability, Linearity, Precision, Accuracy and Robustness. The developed method was found to be acceptable in terms of specificity and system suitability.The excellentlinearity was found between 4 to 60 µg/mL with correlation coefficient(R) of 0.9994. The low %RSD values for both RT and peak area indicate excellent system precision. The average assay was found to be 101.4%, STDEV of 0.769, and %RSD of 0.76% demonstrating the method's precision. Across all spiking levels (80%, 100%, and 120%,) the results showed minimal variation, indicating the method'ssuitability for accurate determination. Parameters including column oven temperature, and flow rate did not hinder the RT, theoretical plates, asymmetry and area indicating robust method.The RP-HPLC method developed for vasopressin was validated and applied to the analysis of three different API lots and found to be acceptable. The measured vasopressin content for both formulations (19.76 IU/ml for BRAND-1 and 19.95 IU/ml for BRAND-2) closely matched their labelled amounts of 20.0 IU/ml. These results demonstrate that the developed method is robust and reliable for the quantitative analysis of drug substance and marketed vasopressin formulations, ensuring consistent and accurate drug content determination.

Keywords: Vasopressin, HPLC, UV detector, Validation, development

INTRODUCTION

Vasodilatory shock and diabetes insipidus are two cardiovascular diseases that are becoming more and more common worldwide. This has brought attention to the urgent need for effective diagnostic and treatment measures**[1]**. The peptide hormone vasopressin, which is produced by the hypothalamus and stored in the posterior pituitary, is essential for preserving hemodynamic stability because of its vasoconstrictive effects and its ability to encourage the kidneys' reabsorption of water**[2]**. Vasopressin has a crucial role in the clinical management of diseases such neurogenic diabetes insipidus, post-cardiotomy vasodilatory shock, and septic shock. According to recent global figures, sepsis, a significant indication for the use of vasopressin, is thought to impact over 49 million people yearly and cause around 11 million fatalities globally**[4]**.The global market for antidiuretic hormones is expected to rise dramatically over the next ten years, and sales of vasopressin and its analogues have been steadily rising. These patterns highlight how crucial it is to ensure the quality and accessibility of vasopressin formulations in order to satisfy patient demands**[5]**.

However, the exact dosing and effectiveness of vasopressin are crucial to its therapeutic use, therefore reliable analytical techniques are required to evaluate its quality. Vasopressin is a complicated peptide that poses special analytical chemistry issues because of its structural complexity, sensitivity to degradation, and requirement for strict regulatory compliance**[6]**. For the quantitative and qualitative estimation of peptides such as vasopressin, chromatographic methods in particular, RP-HPLC have become the gold standard. This approach is perfect for pharmaceutical applications since it has great sensitivity, resolution, and repeatability. The development of an ideal RP-HPLC technique for vasopressin is still being researched despite its broad use because of persistent issues such peptide aggregation, poor recovery rates, and the possibility of secondary interactions with chromatographic columns**[7]**.

Vasopressin is used extensively in pharmaceutical industries worldwide, which emphasizes the need for approved analytical techniques that comply with legal requirements. Variability in vasopressin's analytical data is now caused by irregularities in method development and validation procedures, which raise concerns for patient safety and product effectiveness**[8]**. For example, inaccurate dosing may result from inappropriate resolution of vasopressin's active and inactive forms during analysis, which might worsen side effects or impair therapeutic results. Furthermore, the growing use of biosimilar formulations has increased the need for accurate analytical techniques to ensure product integrity and bioequivalence.

The use of RP-HPLC is justified by its demonstrated effectiveness in peptide analysis. Peptides may be effectively separated according to their hydrophobicity using reverse-phase chromatography, which takes advantage of the hydrophobic interactions between the analyte and the nonpolar stationary phase**[9]**. Vasopressin is a short peptide of nine amino acids that is suited for RP-HPLC analysis due to its mild hydrophobicity**[10]**. However, meticulous method development is required due to the inherent difficulties of peptide chromatography, which include peak widening from secondary interactions and adsorption to the column surface. By concentrating on improving chromatographic parameters, the study seeks to lessen these problems and ensure precise quantification and reliable results from several studies. Apart from its therapeutic importance, vasopressin is a paradigm for developing peptide analysis methods. This study has greater implications for the analysis of other peptide-based treatments, a developing area of biopharmaceuticals with uses in infectious illnesses, cancer, and endocrinology. The development of a validated RP-HPLC method for vasopressin advances analytical techniques for complex biomolecules. Even though RP-HPLC is widely used, issues such peak tailing, peptide aggregation, and low repeatability make it difficult to standardize the procedure. Advanced techniques including gradient elution, ion-pairing agents, and temperature control are needed to address these problems. Robust validation is also necessary to take matrix effects in complicated formulations into consideration**[11]**. This study sets a standard for vasopressin analysis by taking a thorough approach to method development and validation. By improving quality control procedures, the optimised RP-HPLC method ensures the efficacy and safety of vasopressin formulations. In the end, it promotes worldwide healthcare initiatives for vital medical demands, enhances analytical standards, and develops peptide therapeutic analytics. The current study is designed with aim to develop and optimise a robust, reproducible RP-HPLC analytical method to determine vasopressin assay of drug substance & vasopressin levels in drug formulations. The study aims to close the existing gaps in analytical accuracy and precision by utilizing sophisticated chromatographic methods and methodical method optimization.

EXPERIMENTAL

Chemicals

Vasopressin API and other chemicals such as water, acetonitrile, acetic acid, and orthophosphoric acid of HPLC grade were used.Two differentinjection formulation brand of Vasopressinwere used in study

Instrumentation

A high-performance liquid chromatography system (Agilent) equipped with Inertsil C18 column (100 mm, 4.6 mm, 3µm) with G1311Apump and a UV-visible detector was employed in gradient mode.

Chromatographic conditions

A high-efficiency short and fine-pore C18column (Intertsil100 mm, 4.6 mm, 3.µm) was utilised as the stationary phase to accomplish chromatographic separation. 0.1% Orthophosphoric acid in water (Mobile phase A) and Acetonitrile (Mobile phase B) was sonicated, degassed and used as mobile phase with a gradient run timeof 15 minutes, 50µlof standard/samplewas injected at aflow rate of 1 ml per minute into the HPLC. Chromatographic separations were carried out by keeping column oven temperature at 25°C. Detection done at 220 nm by using UV detector.

Mobile phase selection gradient optimization

The mobile phase selection and optimization was performed in gradient as well as isocratic mode by varying composition of Mobile phase A and phase B. The initial trial was conducted with an isocratic method buffer:

ACN (98:2). The retention time of vasopressin was observed as about 4.2 minutes.To further reduce the run time isocratic method with 87:13 buffer : ACN was tried. However, Vasopressin and acetic acid eluted at about same retention time of about 2 minutes.
Further following gradient program was optimizedto fix this. The gradient program optimizations is presented in Table 1.

Table 1: Gradient Program

Time in min	MP A	MP B
0	95	5
6	20	80
6.1	95	5
15.0	95	5

Solution preparation
Preparation of standard solution
25 ml volumetric flask was used to dissolve 10 mg of vasopressin standard in order to prepare a stock solution with a 400 µg/ml concentration. Add the diluent as water and sonicate to dissolve it. Use diluent to dilute to the appropriate level. Further pipette out 1 ml of 400 µg/ml concentration standard stock solution into the 10 ml volumetric flask, add diluent shake well and dilute up to the mark with diluent. (40 µg/ml)

Active substanceSample Preparation
25 ml volumetric flask was used to dissolve 10 mg of vasopressin active substance sample in order to prepare a stock solution with a 400 µg/ml concentration. Add the diluent as water and sonicate to dissolve it. Use diluent to dilute to the appropriate level. Further pipette out 1 ml of 400 µg/ml concentration standard stock solution into the 10 ml volumetric flask, add diluent shake well and dilute up to the mark with diluent. (40 µg/ml)

Method Validation [12]
Specificity& system suitability
The method's specificity was studied by comparing a vasopressin-containing sample with a blank sample that contained diluent and acetic acid.The specificity of the procedure was assessed by looking for any interference in the vasopressin region.The system suitability was studied as per good chromatographic practice to ensure the chromatographic system is suitable for analysis.

Linearity
The concentrations of 4 µg/mL, 20 µg/mL, 40 µg/mL, 48 µg/mL, and 60 µg/mL were obtained by diluting (400 µg/mL) Stock solution. These values corresponded to approximately 10%, 50%, 100%, and 120% to 150% of the target concentration of Vasopressin, respectively. The chromatograms were then recorded after each concentration was injected using the autosampler equipment. The calibration curve was then obtained by plotting an area under the curve vs concentration. The coefficient of correlation (r), equation, slope, and intercept were obtained.

Precision
System Precision
Six replicate injections of a standard vasopressin solution were injected, and the percentage RSD was determined for retention time & area.

Method Precision
Examining six separate injections of sample solutions and vasopressin standards. By contrasting the sample solution response with the standard solution response, the sample's assay % was determined. The assay result's percentage RSD was computed.

Accuracy
Recovery experiments were used to assess the method's accuracy. Vasopressin was recovered from a spiked blank solution in a study. Vasopressin equivalent to 80%, 100%, and 120% of the target concentration of 32 µg/ml, 40 µg/ml, and 48 µg/ml, respectively, was used to create the samples. Three separate sample solutions were made for every spike level that was being assayed.

Robustness
The robustness of the method was evaluated by modifying specific parameters and observing the resulting changes in RT, Theoretical plates and asymmetry after three consecutive injections of the standard

concentration. To assess the impact on peak retention and peak area, the column temperature was varied by ±2°C from the established method temperature of 25°C. Additionally, the effect of alterations in flow rate was studied by adjusting the composition, providing insights into the method's stability under varying analytical conditions.

RESULTS AND DISCUSSION

Mobile phase selection and optimization

The first trial conducted with an isocratic method using buffer: ACN (98:2) showed the retention time of 4.2 minutes for vasopressin. Using an 87:13 buffer, the isocratic approach was used using ACN to further minimise the retentiontime. Nonetheless, acetic acid and vasopressin eluted at approximately the same retention time of about 2 minutes.To resolve this a gradient program of about 6 minutes was tried, herein the retention time of acetic acid was approximately 1.9 minutes, while vasopressin exhibited a retention time of approximately 3.3 minutes. however, subsequent analyses revealed inconsistencies in the retention times. To address this issue, a stabilization period of approximately nine minutes was introduced, extending the total runtime to approximately fifteen minutes. Thereafter, a short gradient elution method was developed, demonstrating improved precision, accuracy, and cost-effectiveness.

Specificity and system suitability

The specificity of the method was determined and it was found that There was no blank interference with the Vasopressin and acetic acid peak. Vasopressin and acetic acid peak were well separated from each other. The overlay of blank, acetic acid and standard solution are presented in Figure 1 showing the specificity of the developed method

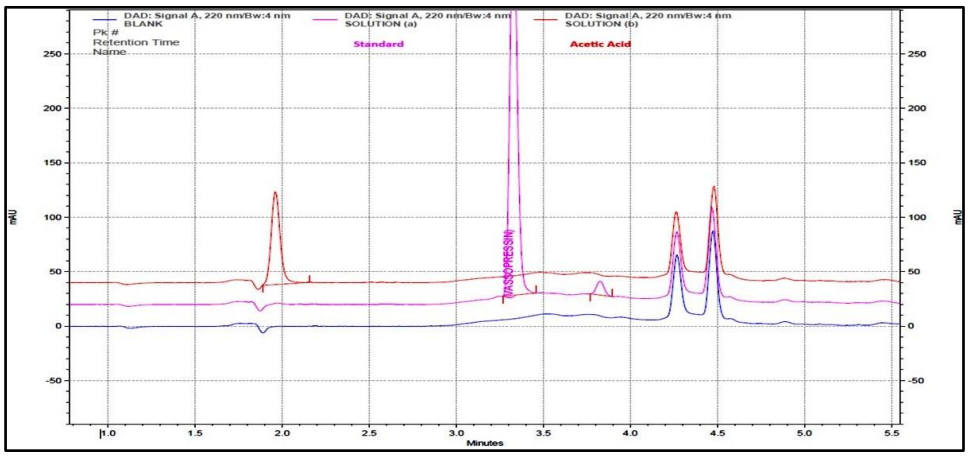

Figure 1: Overlay chromatogram of Vasopressin and acetic acid showing no blank interference

The system suitability study for Vasopressin was conducted to ensure the chromatographic system is suitable for analysis. System suitability is a critical part of method validation in chromatography, aiming to verify that the system will produce reliable and consistent results before sample analysis **(Figure 2)**.

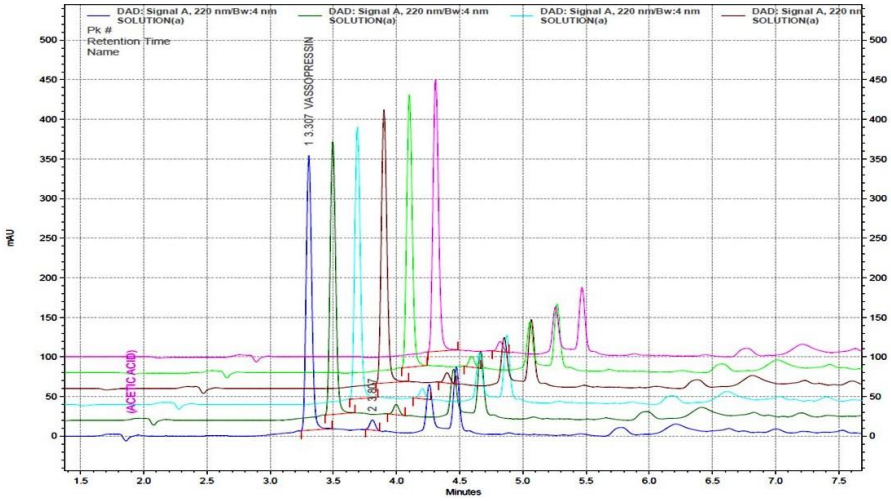

Figure 2: Overlay chromatograms of the system suitability study for Vasopressin

The retention time for Vasopressin was found to be 3.307 minutes. The RT provides a reliable measure of the analyte's elution, and its consistency across runs is validated by calculating the %RSD which was found to be less than 2%. Theoretical plates measurethe column efficiency. A higher number of theoretical plates indicates better separation efficiency**[13]**.Vasopressin, meet acceptance criteria of theoretical plate count (e.g., >2000 for acceptable performance).The peak shape is evaluated through the tailing factor, which ideally should be less than 2. Excessive tailing indicates issues like overloading, secondary interactions, or poor column packing. Vasopressin meet the accepatnce criteria of tailing factor (less than 2 is generally acceptable).The chromatogram demonstrates a well-defined and sharp peak for Vasopressin, with no excessive broadening, ensuring accurate quantification.**Table 2** describes the system suitability parameters.

Table 2: System suitability parameters of Vasopressin

Name	RT	Area	% Area	Asymmetry	Theoretical plates
Vasopressin	3.307 min	2223185	96.52	1.05	26481.6

(Moreover, for the assay test, similarity factor was found to be 1.0 (limit should be 0.98 to 1.02). Overall, this study confirms that the system adheres to the specified parameters for Vasopressin analysis, indicating its suitability for reliable and consistent analytical performance.**Figure 3** shows the system suitability chromatogram for Vasopressin standard

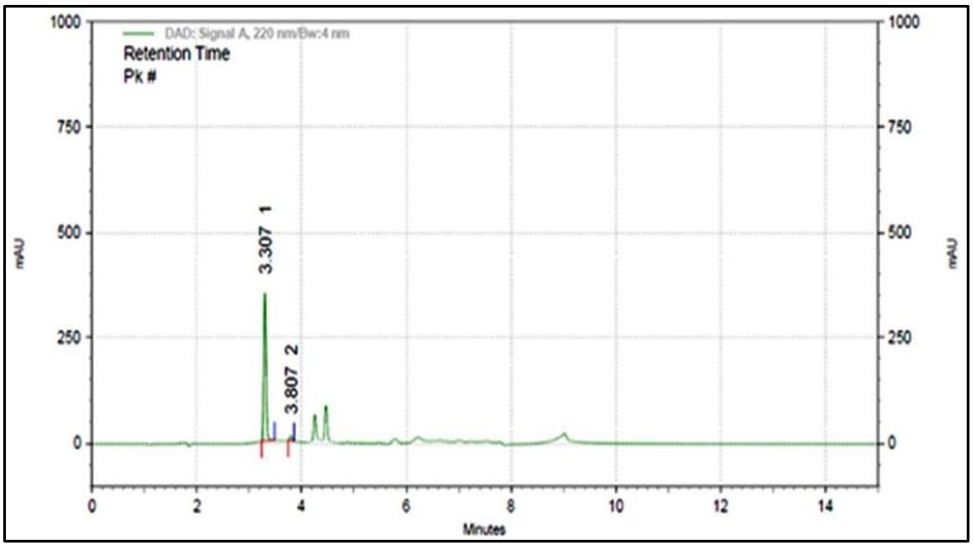

Figure 3: System suitability chromatogram for Vasopressin standard

Linearity

The linearity of vasopressin was established using an HPLC method by analyzing different concentrations (4, 20, 40, 48, and 60 µg/mL) and recording the corresponding peak areas **(Table 3)**. Excellent linear co relation was observed between concentration of Vasopressin and area between the range of 4 µg/mL to 60 µg/mL. The correlation coefficient (R) was found to be 0.9994 with % y intercept of 1.4305. This linearity confirms the reliability of the method for quantifying vasopressin, demonstrating its suitability for accurate and reproducible analysis in pharmaceutical applications.The linearity plot is presented in **Figure 4.**

Table 3: Linearity of vasopressin between 4-20 µg/ml and at 10 to 150% level

Level %	**Conc. in µg/ml**	**Area**	**Average Area**
10	4	306508	306494.50
		306481	
50	20	1306724	1306362.50
		1306001	
100	40	2583713	2589153.00
		2594593	
120	48	3192919	3186238.00
		3179557	
150	60	3886258	3883434.00
		3880610	

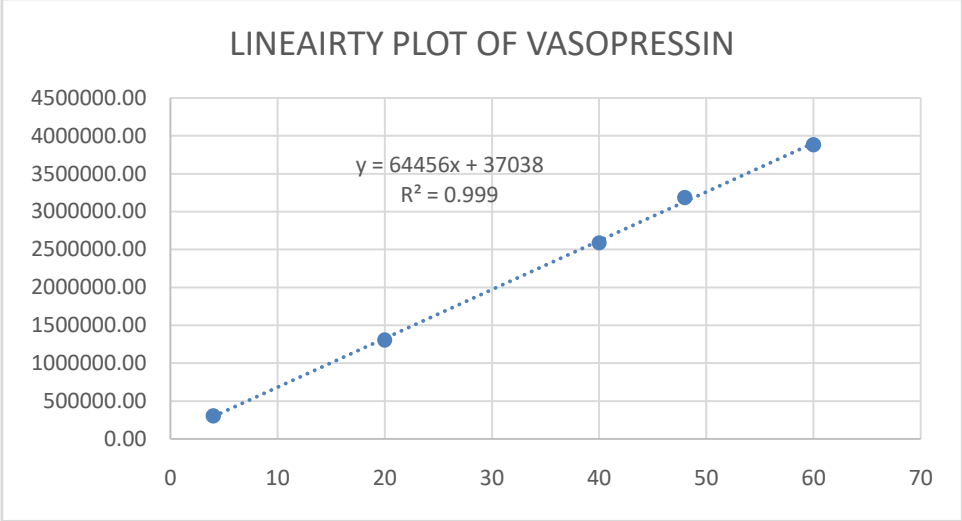

Figure 4: The linearity plot of Vasopressin

Precision

System Precision

The system precision of vasopressin was assessed using a standard solution analyzed through six consecutive injections in the HPLC system. The retention time (RT) for vasopressin across injections showed consistency, with an average RT of 3.30 minutes and a standard deviation (STDEV) of 0.006, resulting in a % RSD of 0.18% (Accepatnce limit NMT1.0%). Similarly, the peak areas were consistent, with an average value of 2,221,667.67, a STDEV of 5464.44, and a %RSD of 0.25% (Acceptance limit NMT 2.0%). The low %RSD values for both RT and peak area indicate excellent system precision, confirming the HPLC method's reproducibility and reliability for the quantification of vasopressin. The system precision results are presented in **Table 4 and Figure 5**.

Table 4: System precision results of Vasopressin std solution

Standard solution		
Solution (a)		
Inj No.	**RT**	**Peak Area**
Inj- 1	3.307	2223185
Inj- 2	3.293	2223002
Inj- 3	3.293	2214524
Inj- 4	3.300	2224446
Inj- 5	3.300	2215893
Inj- 6	3.307	2228956
Average	**3.30**	**2221667.67**
STDEV	**0.006**	**5464.438**
%RSD	**0.18**	**0.25**

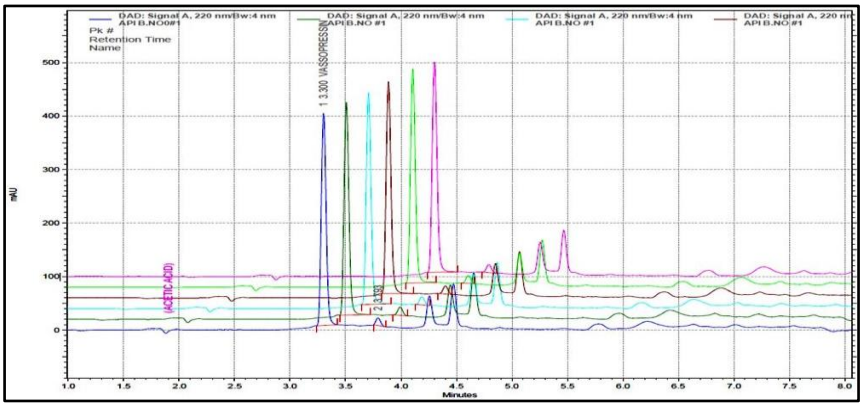

Figure 5: The overlay spectra of system precision results of Vasopressin sample

Method Precision

The method precision of Vasopressin was analyzed by injecting six sample injections of the same batch (API B.NO.1) using the HPLC method. The RT was found to be consistent across samples, with an average RT of 3.301 minutes, a STDEV of 0.008, and a %RSD of 0.24%, indicating stable chromatographic conditions. The assay results ranged from 99.9% to 102.1%, with an average of 101.4% (Acceptance limit 95% and 105%), a %RSD for assay values was 0.76% (Acceptance limit not more than 2.0%), demonstrating the method's precision and reliability for quantitative analysis of vasopressin in pharmaceutical products. The method precision results are presented in **Table 5**.

Table 5: Method precision results of Vasopressin sample

API B.NO.1			
Sample	**RT**	**Peak Area**	**ASSAY**
Sample- 1	3.300	2566958	**101.4**
Sample- 2	3.307	2570891	**101.6**
Sample- 3	3.307	2549884	**101.7**
Sample- 4	3.287	2552610	**99.9**
Sample- 5	3.307	2583827	**102.1**
Sample- 6	3.300	2550656	**101.7**
Average	**3.301**		**101.4**
STDEV	**0.008**		**0.769**
%RSD	**0.24**		**0.76**

Accuracy

The accuracy of the developed RPHPLC method for vasopressin was determined by spiking the sample at three different levels: 80%, 100%, and 120%, with three replicates at each level**(table 6)**. The % recovery at lower spiking ranged from 100.6% to 101.3%, with consistent results indicating accurate quantification at lower concentrations. At middle spiking, the % recovery values ranged from 101.1% to 101.8%, demonstrating the method's accuracy at the target concentration. For higher spiking, the recovery ranged from 100.6% to 101.8%, confirming reliablequantification at higher concentrations. Across all spiking levels, the results showed minimal variation, indicating the method's robustness and suitability for accurate determination of vasopressin. Moreover, % RSD for recovery values at three different levels 80%, 100%, and 120% are 0.33%, 0.36% and 0.58%respectively.

Table 6: Accuracy results of the vasopressin at 80%, 100%, and 120% spiking levels

Sr. no.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
(%) Spiked	80%	80%	80%	100%	100%	100%	120%	120%	120%
% recovery	100.6	101.3	101.1	101.6	101.1	101.8	101	101.8	100.6
% RSD for recovery	**0.33%**			**0.36%**			**0.58%**		

Robustness

Robustness is a critical parameter in the development of an analytical technique using High-Performance Liquid Chromatography (HPLC)**[14]**. The robustness study evaluates the method's ability to remain unaffected by minor variations in the experimental circumstances, for example changes in column oven temperature, and flow rate. The result of the robustness study is presented in **(Table7)**. Parameters including column oven temperature, and flow rate did not hinder the RT, theoretical plates, asymmetry and area. These observations showed that the RP-HPLC procedure was robust.

Table 7: Robustness study results

Sr. No	**Column temperature (°C)**	**RT**	**Theoretical plates**	**Asymmetry**	**Area**
1	23	3.309	26590.3	1.01	2227100
2	25	3.307	26481.6	1.05	2223185
3	27	3.306	26467.8	1.02	2221212
Sr. No	**Flow rate (ml/min)**	**RT**	**Theoretical plates**	**Asymmetry**	**Area**
4	0.9	3.299	26587.8	1.02	2224157
5	1	3.307	26481.6	1.05	2223185
6	1.1	3.309	26472.3	1.01	2225272

Analysis of API of different lots

The RP-HPLC method developed for vasopressin was validated and applied to the analysis of three API lots with different batch numbers. The retention times (RT) and peak area values for all samples were consistent, indicating the method's reliability and reproducibility. For API Batch No. 1, the average RTand assay values were 3.304and 101.5%, respectively, with low %RSD values (0.15 for RT and 0.14 for assay), confirming excellent precision**(Table 8)**. Similarly, API Batch No. 2 and Batch No. 3 showed consistent results with low variability (%RSD for assay: 0.91 and 0.21, respectively) and assay of 101.1% and 101.2 % respectively. These findings demonstrate that the developed RP-HPLC method provides accurate and reproducible results across different API batches, establishing its robustness for routine analysis.

Table 8: Three lot API analysis showing consistency in results

API B.NO.1				API B.NO.2			API B.NO.3		
Sample	**RT**	**Peak Area**	**Assay**	**RT**	**Peak Area**	**Assay**	**RT**	**Peak Area**	**Assay**
Sample- 1	3.300	2566958	101.4	3.280	2401998	100.4	3.327	2517431	101.3
Sample- 2	3.307	2570891	101.6	3.28	2432106	101.7	3.327	2508319	101.0
Average	3.304	2568924.50	101.5	3.280	2417052.00	101.1	3.327	2512875.0	101.2
STDEV	0.005		0.141	0.000	21289.571	0.919	0.000		0.212
%RSD	0.15		0.14	0.00	0.88	0.91	0.00		0.21
Assay	101.5			101.1			101.2		

Analysis of marketed formulation:

The developed RP-HPLC method was applied to analyze two marketed formulations of vasopressin, BRAND-1and BRAND-2. Being lower labelled claim of both brand samples, they were injected as such. The retention times (RT) for both formulations were consistent, with averages of 3.324 minutes for BRAND-1 and 3.320 minutes for BRAND-2, and low %RSD values (0.15 and 0.30, respectively), confirming the method's precision **(Table 9)**. The peak area %RSD values were also low (0.55 for BRAND-1 and 0.22 for BRAND-2), indicating excellent reproducibility. The measured vasopressin content for both formulations (19.76 IU/ml for BRAND-1 and 19.95 IU/ml for BRAND-2) closely matched their labelled amounts of 20.0 IU/ml. These results demonstrate that the developed method is robust and reliable for the quantitative analysis of marketed vasopressin formulations, ensuring consistent and accurate drug content determination.

Table 9: Marketed formulation analysis

BRAND-1			**BRAND-2**		
Inj No.	**RT**	**Peak Area**	**Inj No.**	**RT**	**Peak Area**
Inj- 1	3.320	2821911.47	Inj- 1	3.313	2841891.97
Inj- 2	3.327	2799911.88	Inj- 2	3.327	2832879.95
Average	3.324	2810911.68	Average	3.320	2837385.96
STDEV	0.005	15556.065	STDEV	0.010	6372.460
%RSD	0.15	**0.55**	%RSD	0.30	0.22
CONTENT OF VASOPRESSIN (IU/ml)		**19.76**	CONTENT OF VASOPRESSIN (IU/ml)	19.95	
Vasopressin labelled amount (IU/ml)		**20.0**	Vasopressin labelled amount (IU/ml)	20.0	

CONCLUSION

The study successfully developed and validated a robust, precise, and selective RP-HPLC method for the quantitative analysis of vasopressin in both bulk drug substances and finished pharmaceutical formulations. The method demonstrated excellent specificity, linearity (R= 0.9994), precision, and accuracy, with minimal variation across all spiking levels. The robustness of the method was confirmed through stability under varying analytical conditions, including changes in column temperature and flow rate, without affecting critical parameters such as retention time, theoretical plates, asymmetry, and peak area. The assay results for three API

lots and two marketed formulations closely aligned with their labelled claims, further confirming the method's reliability. Overall, this validated RP-HPLC method is suitable for routine quality control of vasopressin in pharmaceutical products.

REFERENCES

1. Marino BS, Tabbutt S, MacLaren G, Hazinski MF, Adatia I, Atkins DL, Checchia PA, DeCaen A, Fink EL, Hoffman GM, Jefferies JL. Cardiopulmonary resuscitation in infants and children with cardiac disease: a scientific statement from the American Heart Association. Circulation. 2018 May 29;137(22):e691-782.
2. Sparapani S, Millet-Boureima C, Oliver J, Mu K, Hadavi P, Kalostian T, Ali N, Avelar CM, Bardies M, Barrow B, Benedikt M. The biology of vasopressin. Biomedicines. 2021 Jan 18;9(1):89.
3. García-Álvarez R, Arboleda-Salazar R. Vasopressin in Sepsis and other Shock States: state of the art. Journal of Personalized Medicine. 2023 Oct 29;13(11):1548.
4. Wayland J, Teixeira JP, Nielsen ND. Sepsis in 2024: A review. Anaesthesia& Intensive Care Medicine. 2024 Aug 19.
5. Muttenthaler M, King GF, Adams DJ, Alewood PF. Trends in peptide drug discovery. Nature reviews Drug discovery. 2021 Apr;20(4):309-25.
6. Glavaš M, Gitlin-Domagalska A, Dębowski D, Ptaszyńska N, Łęgowska A, Rolka K. Vasopressin and its analogues: from natural hormones to multitasking peptides. International journal of molecular sciences. 2022 Mar 12;23(6):3068.
7. Ferrazzano L, Catani M, Cavazzini A, Martelli G, Corbisiero D, Cantelmi P, Fantoni T, Mattellone A, De Luca C, Felletti S, Cabri W. Sustainability in peptide chemistry: current synthesis and purification technologies and future challenges. Green Chemistry. 2022;24(3):975-1020.
8. Kumar S, Sanap SN, Vasoya M, Handa M, Pandey P, Khopade A, Sawant KK. USFDA-approved parenteral peptide formulations and excipients: Industrial perspective. Journal of Drug Delivery Science and Technology. 2024 Mar 15:105589.
9. Barbosa I, Domingues C, Ramos F, Barbosa RM. Analytical methods for amatoxins: A comprehensive review. Journal of Pharmaceutical and Biomedical Analysis. 2023 Aug 5;232:115421.
10. Randall CS, Malefyt TR, Sternson LA. Approaches to the analysis of peptides. InPeptide and protein drug delivery 2024 Nov 1 (pp. 203-246). CRC Press.
11. Williams ML, Olomukoro AA, Emmons RV, Godage NH, Gionfriddo E. Matrix effects demystified: Strategies for resolving challenges in analytical separations of complex samples. Journal of Separation Science. 2023 Dec;46(23):2300571.
12. Validation Of Analytical Procedures: Text And Methodology Q2(R1): Ich guideline
13. Bidlingmeyer BA, Warren Jr FV. Column efficiency measurement. Analytical Chemistry. 1984 Dec 1;56(14):1583A-96A.
14. Siddique I. Unveiling the Power of High-Performance Liquid Chromatography: Techniques, Applications, and Innovations. European Journal of Advances in Engineering and Technology. 2021 Sep 21;8(9):79-84.