INTERNATIONAL JOURNAL IN PHARMACEUTICAL SCIENCES

Journal Homepage: https://www.ijpsjournal.com

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Research Article

A Novel Validated Stability Indicating Qbd Based Assay Method For The Quantification Of Desmopressin Acetate By High Performance Liquid Chromatography

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ARTICLE INFO

Received: 02 Dec 2023
Accepted: 04 Dec 2023
Published: 09 Dec 2023

Keywords:
Desmopressin Tablet, RP-HPLC-DAD, Forced degradation, Validation, Method development.

DOI:
10.5281/zenodo.10316417

ABSTRACT

The purpose of present analytical research was to develop and validate a new, rapid, selective, specific, accurate and efficient stability indicating RP-HPLC-DAD assay test method for the estimation of Desmopressin in Desmopressin tablets. Several liquid chromatographic parameters viz change in mobile phase, buffer solution and solvent composition, analytical column (stationary phase) and column compartment temperature were studied. The elution pattern of Desmopressin are obtained with improved peak shape under a set of gradient chromatographic condition: a reverse phase Inertsil C18 (250mm x4.6mm, 5.0µm) column with a mobile phase A consisting of methanol and mobile phase B is phosphate buffer pH 4.5 (25:75), flow rate 1.2 mL/minute, UV detection wavelength 220 nm. The chromatographic run time was 12 minutes with Desmopressin peak eluting at 6.6 minutes. The developed assay test method was found to be very specific and linear in the range of 5- 15 µg /mL (r2 = 0.9998). The best precision was obtained because the maximum RSD was 1.25%. Mean recoveries was 100.3%. The percent RSD was less than 2.0%. The test method is robust w.r.t changes in flow rate and wavelength. Solution stability evaluation showed no evidence of degradation product. Standard solution was stable for 88 hours and test sample solution was for 48 hours at room temperature. Forced degradation study (Specificity) of Desmopressin shown that peak was pure and there was no coeluting peaks when samples were assayed against reference solution. The developed analytical test method was validated as per ICH guideline and found precise, robust, accurate, linear, specific and stability indicating ensuring suitability of the test method for estimation of Desmopressin in tablets. Assay test method was successfully used for routine analysis of Desmopressin formulations and Oral solid dosage forms.

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

INTRODUCTION

Desmopressin is a synthetic analogue of the natural pituitary hormone 8-arginine vasopressin, an antidiuretic hormone affecting renal water conservation and found in body. It prevents reabsorption of water and thereby decreases frequency of urination. Hence, it can be used to treat polyuria in diabetic patients [2, 3]. Now, Desmopressin is available as tablet, Nasal spray and parenteral formulations [4-6]. Analytical technique which has been reported for assay of Desmopressin in dosage forms is high performance liquid chromatography (HPLC), for example, development and validation of Reverse phase HPLC method for Desmopressin from polymeric nanoparticles by Dipti et al is reported [1]. This work shows peak shape of analyte is yet to improve, need stability indicating method. The solution stability need to establish. Based on literature search to the best of knowledge the stability indicating method for Desmopressin quantitation in tablet form is not yet reported.

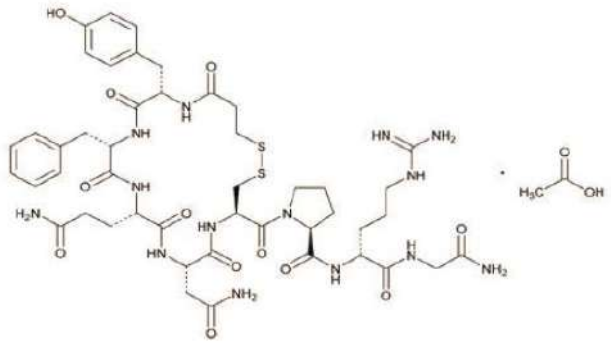
Chromatographic conditions has been also referred in the United States pharmacopoeia (USP) and European Pharmacopoeia as an officially validated analytical method for desmopressin. USP method state, use of Buffer solution and acetonitrile in proportion of 78:22 as mobile phase, run time at flow rate of 1ml/minute [8]. EP method discusses Phosphate buffer solution and solvent acetonitrile as a mobile phase in proportion of 60:40, at a flow rate of 2.0 ml/min [9]. Both methods suggest use of buffers solution with higher proportion. Assay of Desmopressin in nasal spray by HPLC – UV detector was reported by Hazim et al [11]. RP-HPLC determination of desmopressin tablet and desmopressin acetate Injection worked by Ren et al [12]. Development and Validation of a RP-HPLC method for determination of Desmopressin in Chitosan Nanoparticles is reported by Taghizadeh et al [13]. Development and Validation of RP-HPLC-DAD stability indicating assay method for the determination of Desmopressin in Desmopressin tablets was reported by Rashidul Islam et al [14] in this reported mobile phase, chromatographic method contains acetonitrile as organic phase which is costly compared to methanol, hence method needs to develop and validate using methanol as organic phase instead acetonitrile. Also Hypersil BDS C18 column was used but inertsil C18 column must be used which is easily available as an alternative stationary phase for quantification.

Reported analytical methods are not stability indicating and no solution stability of reference and test is specified. [1,13]. Hence it needs for more research work.

The aim and objective of this research work was to develop stability indicating cost effective analytical method to quantify Desmopressin from Desmopressin tablets using RP-HPLC-DAD techniques. There are several marketed Desmopressin formulations which needs dedicated analytical methodology to quantify drug moiety present in dosage forms in required specification limits.

In present research work, the difficulty faced during study was, Desmopressin is large molecule and was freely water soluble. This was pH and moisture sensitive due to this the drug was unstable. The storage of drug was maintained at refrigerated condition throughout study. The recovery did not achieved due to water as mobile phase and diluent during development trials. The pH of mobile phase was selected after several trials and same used for diluent to maintain solution stability. It was found to be stable in Phosphate buffer pH 4.5 rather than water or pH 7.4. Hence, buffer could not replace with water but use of organic solvent have been minimized to obtain optimum retention time, good peak shape and greater peak resolution. It was possible by selecting phosphate buffer pH4.5 in mobile phase

and as diluent along with optimum proportion of methanol. Buffer is also kept optimum to increase retention of analyte. Forced degradation study for stability indicating method and solution stability study of reference and test solutions was established. cellular concentration of GSH, and decreases the activity of SOD and CAT. It has also known to decrease the detoxification system produced by GST. Increasing evidence indicates that oxidative stress causes liver injury, cirrhosis growth and carcinogens. In our studies, it exposes that OSME could restore the movement of both these antioxidant enzymes.



Structure of Desmopressin

MATERIAL AND METHODS

Chemicals

HPLC grade methanol was procured from Merck Inc. Purified HPLC grade water by Millipore Make machine was used. Rest chemicals utilized are AR grade. Reference standard Desmopressin and Desmopressin tablets were obtained as gift sample from Sanofi-Aventis Pharma, Goa, India.

Instrumentations:

HPLC System equipped with 2595 separation module PDA 2896 (Waters Co), Waters 2488 dual wavelength detector (Empower Software), Inertsil C18, 250 x 4.6mm, 5µm analytical column, Digital ultra sonicator, pH meter (Thermo scientific Make), electronic analytical balance (Mettler Toledo Make), Fisher scientific vacuum oven and humidity desiccator were used.

Analytical method development

The rationale of the present study is to create a simple, precise, specific, accurate, stability indicating HPLC analytical assay test method for determination of Desmopressin from desmopressin tablets.

Selection of chromatographic condition

In this study, the assay method development and validation of Desmopressin in Desmopressin tablet was performed by RP-HPLC instrument equipped with Photo Diode array detector (PDAD).

Selection and preparation of mobile phase

The standard solution of Desmopressin were injected in to the HPLC autosampler instrument and run at various solvent proportions. Different mobile phases like Acetonitrile and water, Acetonitrile and Phosphate buffer pH7.4 [1], Acetonitrile and Phosphate buffer pH 4.5, Methanol and water, methanol and phosphate buffer (pH4.5) were tried in order to find the best conditions for the elution. It was found that Potassium dihydrogen phosphate buffer (pH 4.5) and Methanol in the ratio of 75:25 gave satisfactory results as compared to other mobile phases, good resolution, peak shape and desired elution was obtained. The mobile phase with different proportions was applied and using various flow rates. Finally, the optimized composition of the mobile phase was discovered.

Preparation of Phosphate Buffer pH 4.5

Dissolve 3.4 grams of potassium dihydrogen phosphate in 1 litter of water in glass bottle. Adjust pH to 4.5 ± 0.05 with Ortho- phosphoric acid and filtered through 0.45 µm membrane filter and degassed for 10 minutes using sonicator before use.

Mobile phase A: Phosphate Buffer pH 4.5, Mobile phase B: Methanol, use Gradient system in HPLC, Phosphate Buffer pH 4.5: Methanol (75:25).

Preparation of diluent:

Use mobile phase as diluent pH 4.5 Phosphate Buffer: Methanol (75:25v/v)

Selection of analytical wavelength

Working standard solution was injected & run at 220nm in PDA detector to obtain analytical Chromatogram of Desmopressin component, wavelength selected was 220 nm as the drug indicates significant absorbance at this wavelength, [8,9].

Selection of analytical column (stationary phase)

Various chromatographic trials were taken by injecting and running standard solution of Desmopressin in various analytical column having different stationary phases which are of C18, 150mm and 250 mm length, including ODS column 150 x 3mm, 5µ. [1] Hypersil BDS C18, 250 x 4.6mm [14], Inertsil C18, 250 x 4.6mm 5µ column. The best elution pattern and peak shape achieved at Inertsil C18, 250 x 4.6mm, 5µ column in comparison to other columns.

Preparation of Standard Solution:

Weigh and transfer accurately 25 milligram of Desmopressin working standard into a 250 ml volumetric flask, transfer 70 ml of diluent, sonicate to dissolve and made up to the mark with diluent. Pipette out 5 ml of this solution and dilute to 50 ml with diluent.

Preparation of Test sample solution:

Weigh and transfer 10 tablets of Desmopressin of 0.2mg strength, into 200 ml volumetric flask, add approximate 140 ml of diluent and sonicate to dissolve for 15- 20 minutes with occasional shakings. Make up to volume with diluent. Mix & allow to settle for 5-10 minutes. Filter through 0.45µm membrane filter (Teflon).

RESULTS AND DISCUSSION

To select and optimize HPLC conditions for quantification of Desmopressin in test sample, some of the parameters have been varied like pH of buffer, ionization constant, proportion of aqueous to organic mobile phase composition and flow rate. On the basis of outcome from development trial, optimum chromatographic conditions considering total run time, retention time, solvent elution time and peak shape (symmetry and theoretical plate) were finalized. Based on the UV spectra of Desmopressin, the wavelength was adopted at 220 nm for better sensitivity and selectivity of Desmopressin in presence of formulation excipients (Placebo) [8,9]. Based on the high solubility of Desmopressin in aqueous solution, hydrophilic solvents were used to reduce the affinity of the drug for the stationary phase (column) and thus achieve low retention time. Firstly, feasibility was monitored as per the test method mentioned in the European Pharmacopoeia (EP) 7.0, written phosphate buffer pH 7.0 and acetonitrile (60:40) as mobile phase, with a flow rate of 2.0 mL/min [9]. However, for test samples, irregular peaks were observed using low resolution chromatography, possibly because of physicochemical properties, instrumental or analytical column differences. Chromatography were performed using acetonitrile and water in different ratio. Noticeable tailing and an irregular shape of the Desmopressin peak were obtained when the ratio of acetonitrile solvent was kept more than phosphate buffer solution in mobile phase. Increasing the phosphate buffer solution proportion in the mobile phase, the Desmopressin peak became more regular and appropriate, when the ratio of acetonitrile: Phosphate buffer pH4.5 of 25:75 was applied, an optimum and symmetric peak was achieved successfully. However acetonitrile is costlier than methanol and inertsil C18 column was an alternate of Hypersil BDS column, hence trial was taken using this column and replacing acetonitrile with methanol in same proportion in mobile phase and diluent, it was found exactly reproducible chromatographic pattern with appropriate system suitability parameter. The Desmopressin peak was detected

in approximately 6.7 minutes and run time was 12 minutes. Chromatogram of placebo showed no interference at the retention time of Desmopressin peak, when compared with freshly prepared standard solution and diluent, indicating the selectivity of developed method for Desmopressin in presence of Placebo (Figure 1, 2 and 4)

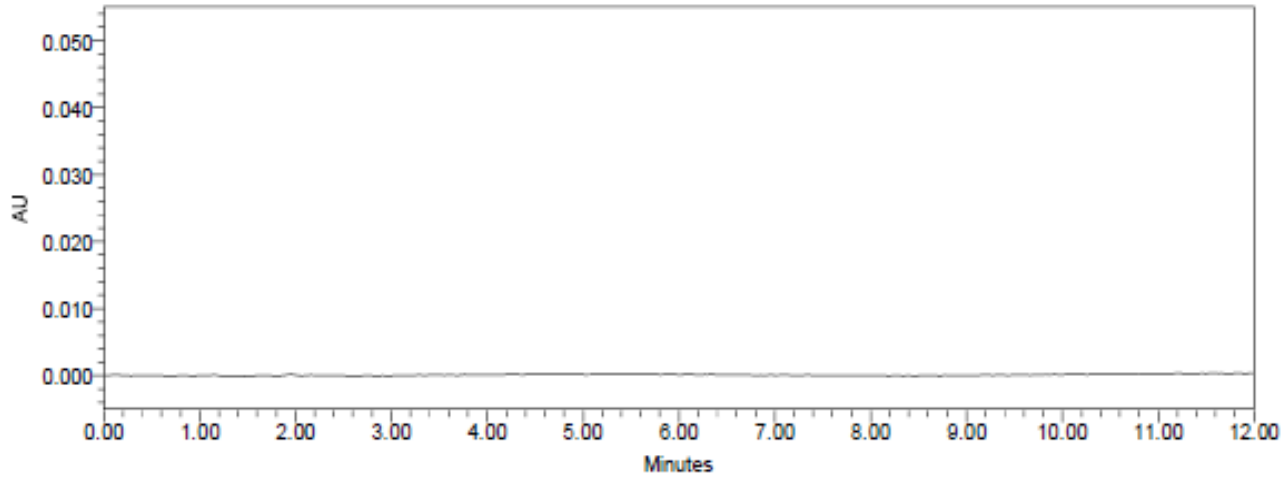


Figure 1: Diluent chromatogram, no peak observed

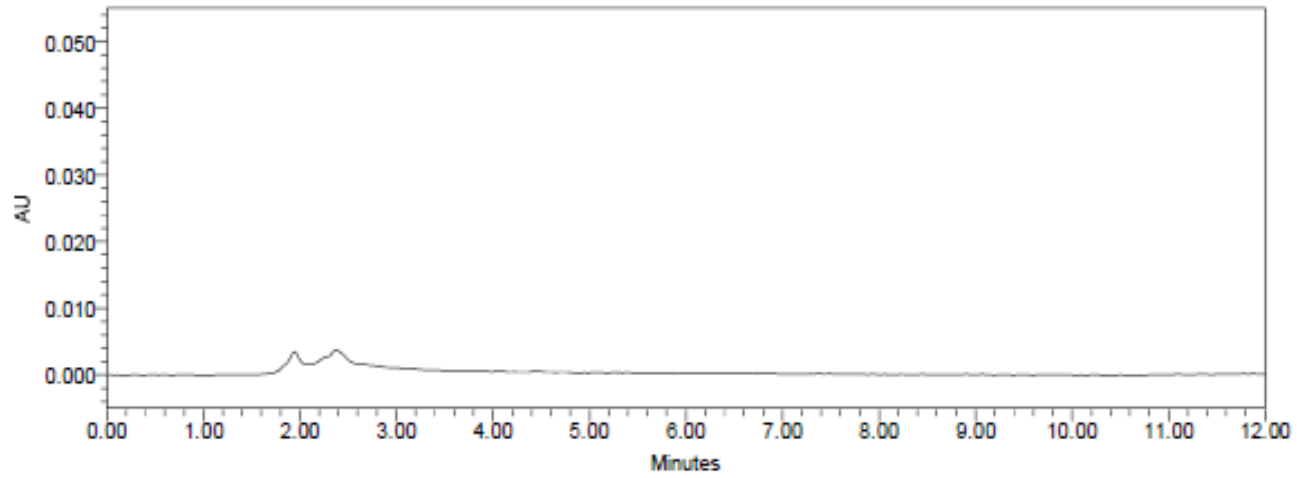


Figure 2: Placebo chromatogram, no interference of placebo peak

Optimized chromatographic conditions

The mobile phase was optimized at ratio of 75:25, Phosphate buffer pH 4.5: Methanol in a gradient mode over a wavelength of 220 nm. Other chromatographic parameters adjusted were injection volume 50 µl, flow rate at 1.2 ml/minute for 12 minute (run time). Column temperature is 30°C. Retention time is about 6.7 minutes, Column selected is Inertsil C18, 250 x 4.6mm, 5µ.

Calibration Curve

The calibration curve obtained by least square analysis showed linear relationship with regression coefficient (R^2) of 0.999 (Figure 3). At proposed concentration levels (Table 1), the standard deviation was optimum %RSD did not exceed 2%. The predicted concentrations were in close agreement with the theoretical concentrations

Sr no.	Concentration (ppm)	Response
1.	4.80	186787
2.	7.68	298426
3.	8.64	335316
4.	9.60	376926
5.	10.56	408157
6.	11.52	449254
7.	15.36	597486
Correlation Coefficient		0.99989

Table 1: Concentration and response in Linearity

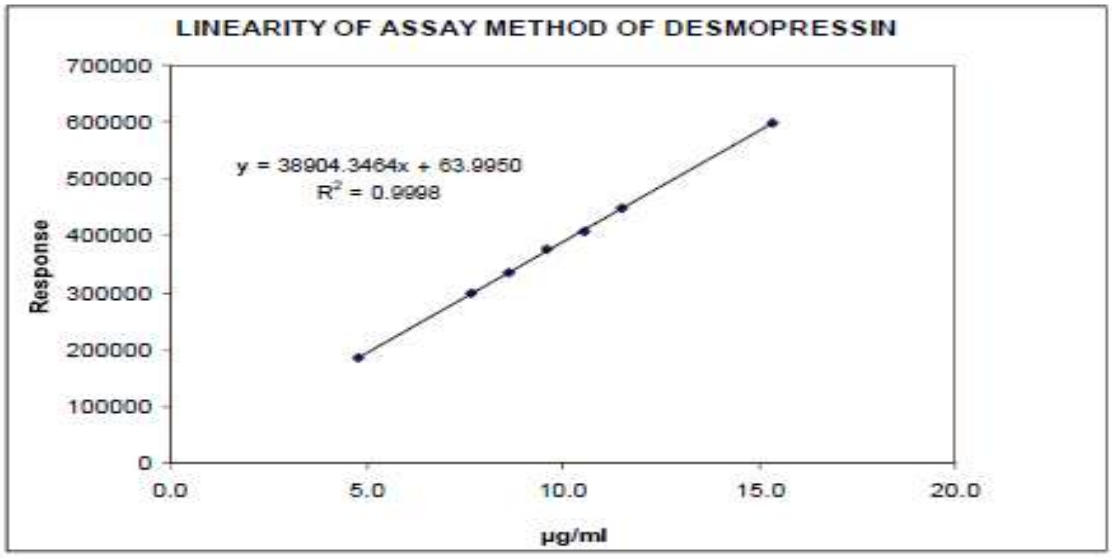


Figure 3: Calibration curve of Desmopressin

Analytical method validation

Method was developed to quantify Desmopressin from tablet dosage form and validated for various parameters as per ICH guidelines [10].

Specificity

The specificity of the method was determined by observing interference of any encountered ingredients present in the formulations. It was determined by comparing tablet samples with and without desmopressin (placebo) [10].

Selectivity

The selectivity of the developed analytical test method was demonstrated by a best separation of Desmopressin from other interfering peaks (Placebo) present in sample with better resolution. This is compared with standard chromatogram of fresh solution. Figure 4 and 5 indicates a representative chromatogram of the standard and sample solution analyzed

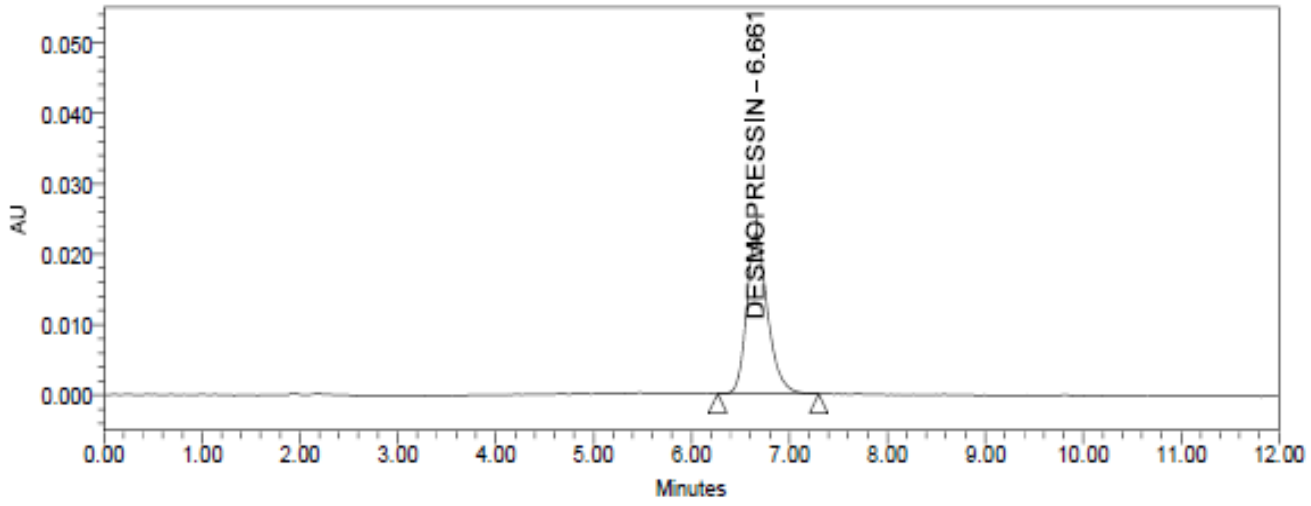


Figure 4: Desmopressin standard solution chromatogram

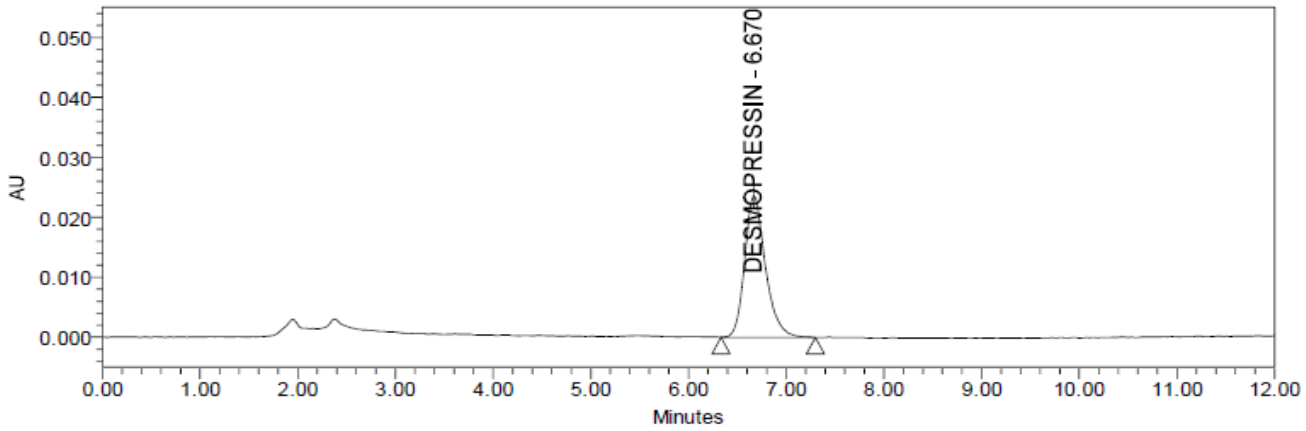


Figure 5: Desmopressin sample solution chromatogram

Identification

The retention time of Desmopressin peak in the chromatogram of the test sample solution preparation corresponds to that of the Desmopressin peak in the chromatogram of the standard preparation. Retention time of Desmopressin peak in reference solution is 6.661 minute and in sample solution is 6.670 minutes (Fig 4 and 5).

Placebo interference study: Prepared representative placebo solutions, standard solution and test sample solutions of Desmopressin Tablet. Placebo solution was prepared by weighing equivalent amount of placebo (excipients) present in sample to be taken for assay preparation diluted it as per the developed test method. Injected each of the diluent, placebo solution, sample solution and standard solution in duplicate into the HPLC using the developed chromatographic condition utilizing a HPLC-DAD. The Desmopressin peak observed is pure in standard solution and sample solution. No interference peak was observed from diluent and placebo at the retention time of Desmopressin peak. This is confirmed by purity angle is less than purity threshold. (Table 2 and Figure: 6 and 7).

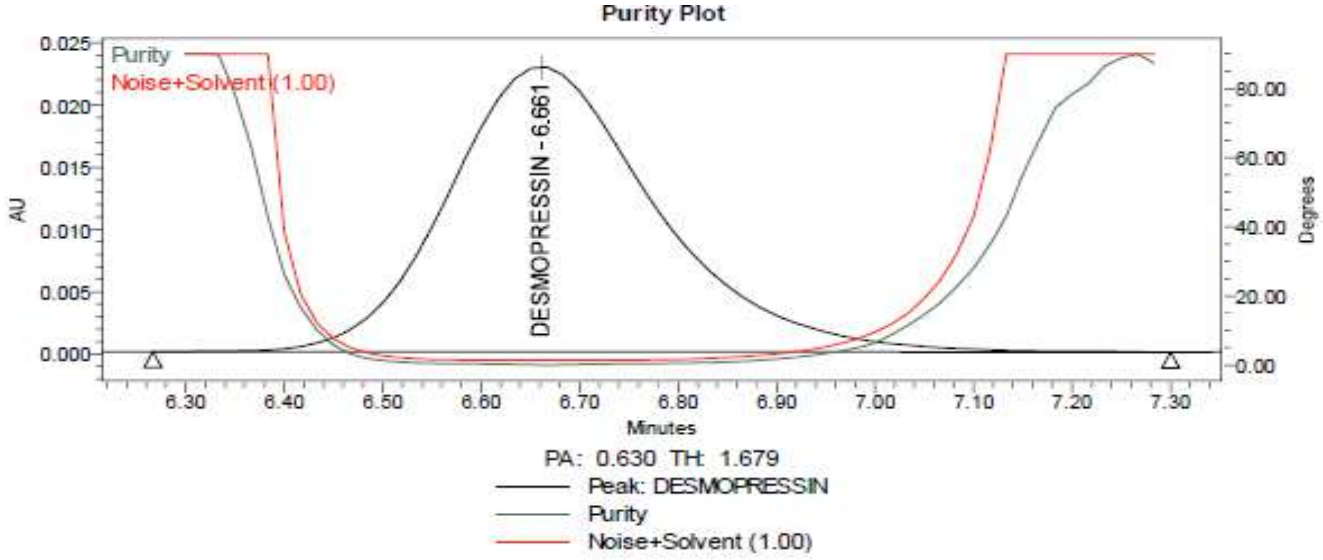


Figure 6: Desmopressin standard solution Spectral purity at 220nm on RP-HPLC-DAD. Purity threshold is more than Purity angle.

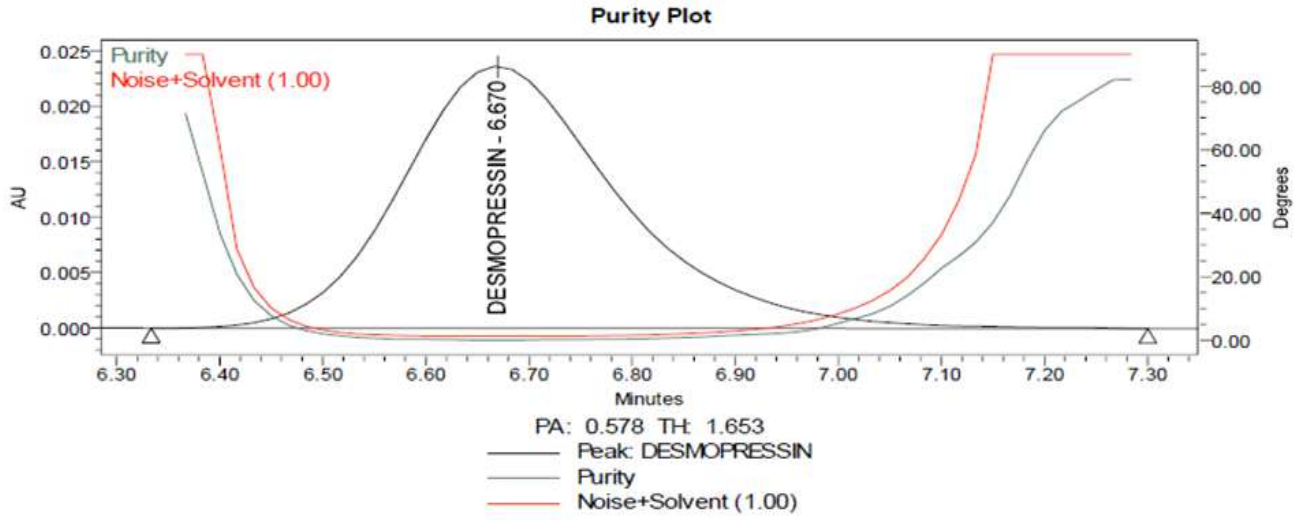


Figure 7: Desmopressin sample solution Spectral purity at 220nm on RP-HPLC-DAD. Purity threshold is more than Purity angle

Name	Purity Angle	Purity Threshold
Standard Solution	0.630	1.679
Sample Solution	0.578	1.653

Table 2: specificity data for desmopressin

Forced Degradation Studies

The sample solution was subjected to forced degradation using 5N Hydrochloric acid, 2N Sodium hydroxide, 50% Hydrogen peroxide at different time intervals at room temperature, data shown in Table 3. Further samples were exposed to Thermal Degradation at105°C for 72 hours Humidity Degradation at 25°C/92%RH for 72 hours and Photolytic Degradation at 1.2 million lux hours then analyzed using developed method (Table 3).The peak purity data of Desmopressin peak in every degradation sample indicate that the Desmopressin peak is homogeneous and there are no co eluting peaks indicating that the method is stability indicating and specific. The table 3 shows that purity angle is less than purity threshold which confirms purity of desmopressin peak.

Sr no.	Experiment	Degradation Condition	% Assay	% Degradation	Purity Angle	Purity Threshold
1.	Control	--	99.7	--	0.578	1.653
2.	Acid degradation	5N HCL/0 hour	91.9	7.8	0.668	1.707
		5N HCL/1hour	88.0	11.7	0.956	1.886
		5N HCL/24hour	26.6	73.3	3.655	5.129
3.	Base degradation	2N NaOH/0 hour	89.0	10.7	0.787	1.842
		2N NaOH/24 hour	3.3	96.7	13.122	18.158
4.	Peroxide degradation	50% H_2O_2/0 hours	91.1	8.6	1.327	2.687
		50% H_2O_2/ 5 hours	78.1	21.7	0.805	1.948
		50% H_2O_2/24 hours	--	100.0	--	--
5.	Thermal degradation	105° C/72 hours	82.9	16.9	0.735	1.803
6.	Humidity degradation	25° C/92%RH-72 hours	100.2	--	0.603	1.666
7.	Photolytic degradation	1.2 million lux hours of light	97.7	--	0.544	1.685

Table 3: Forced degradation study data of Desmopressin

Linearity and Range

Linearity is the ability of a test analytical method to elicit test results that are directly pro89portional to the analyte concentration within a given range. 5 concentration levels prepared along with certain minimum specified ranges are required. The acceptance criterion for linearity is correlation coefficient (r2) should not be less than 0.990 for the least squares method of the analysis of the line [10]. To evaluate the linearity of the method, several concentrations of Desmopressin reference solutions were analyzed by HPLC-DAD, and area responses were recorded. [10,13].The linearity of method was assessed by analyzing a series of calibration standards solution, Calibration curve was obtained by plotting peak area vs concentration of drug and correlation coefficient was found to confirm the linearity.[10]

A series of standard preparations of Desmopressin was prepared over a range of 50% to 150% of working concentration of Desmopressin in Desmopressin tablets. (Minimum Five points should be in the range 80-120% of Standard / sample concentration for Assay). Since the working concentration is 10 µg per ml of Desmopressin, the linearity range was found 5 µg per ml to 15 µg per ml of Desmopressin. Correlation coefficient is 0.99989, hence developed method is considered linear (Table 1 and 4) and (Figure 3).

% Concentration	Concentration (µg per mL)	Response (Area)	Statistical Analysis	
50%	4.80	186787	**Slope**	38904.3
80%	7.68	298426	**Intercept**	64.0
90%	8.64	335316	**Correlation Coefficient**	0.9989
100%	9.60	376926		
110%	10.56	408154		
120%	11.52	449254		
150%	15.36	597486		

Table 4: Linearity of detector response for Desmopressin

Accuracy (Recovery)

Accuracy study was performed by recovery study of Desmopressin. Known amount of standard was added to the placebo solution and subjected to the proposed HPLC method. The study was performed at triplicate levels [10]. Placebo of Desmopressin Tablets was spiked with Desmopressin drug substance at three different levels, 80%, 100% and 120% of the label claim in triplicate (total nine determinations) and then proceeded as per developed sample solution preparation method. As described in table 5. Mean recovery is 100.3 % & RSD is 0.87 %. The relative standard deviation (RSD) of the replicates provides the analysis variation and gives an indication of the precision of the test method; while the mean of replicates indicates the accuracy of the test method [10].Therefore, the HPLC method is accurate

Sample No.	Amount added (mg)	Amount recovered (mg)	% Recovery
Acc.80%-1	1.62	1.63	100.9
Acc.80%-1	1.62	1.63	100.9
Acc.80%-1	1.62	1.64	101.5
Acc.100%-1	2.01	2.02	100.3
Acc.100%-1	2.01	2.04	101.3
Acc.100%-1	2.01	2.01	99.8
Acc.120%-1	2.41	2.40	99.5
Acc.120%-1	2.41	2.40	99.5
Acc.120%-1	2.41	2.39	99.5

Mean	**100.3**
SD	**0.875**
%RSD	**0.87**

Table 5: Accuracy results for Desmopressin Precision

Precision

The precision of the analytical method was performed and determined as inter and intra-day variability expressed as relative standard deviation (%RSD). The precision of the developed test method was assessed by calculating the %RSD of the peak response areas of five replicate injections of standard solutions (Table 6). The system precision RSD is 0.18 %, hence the method is precise.

Injection	Area
1	376849
2	376941
3	376738
4	375688
5	375513
Mean	**376346**
SD	**686.941**
%RSD	**0.18**

Table 6: system precision for Desmopressin

Six sample solutions of Desmopressin Tablets, 0.2 mg were prepared and run into the RP-HPLC-DAD using the developed method as described in table 7. The RSD of method precision is 1.30 %, hence the method is found reproducible.[10]

Sample	%Assay
1	101.9
2	100.4
3	98.3
4	100.4
5	101.7
6	101.1
Mean	**100.6**
SD	**1.305**
%RSD	**1.30**

Table 7: Method precision for Desmopressin

Ruggedness (Intermediate precision)

Ruggedness of method was verified by preparing Six sample preparations of the same lot of Desmopressin Tablets, 0.2 mg by different analyst, by using another instrument (HPLC), different column, and injected in duplicate into a another HPLC instrument on a different day. Assay of Desmopressin in Desmopressin tablet was calculated. The Overall RSD for twelve results of intermediate precision is 1.25%. Developed analytical method meets the acceptance criteria for Intermediate Precision (Ruggedness). Hence, developed method is precise and rugged. (Table 8).

Stability of Analytical solutions

The test sample and standard solutions were stored at room temperature and evaluated against freshly prepared standard solution preparations for 88 hours. Standard solution is stable for 88 hours and test sample solution is stable for 48 hours at room temperature as per this study. (Table 9)

Sr.No	Name	% Content	% Correlation
1	Standard Solution-Initial	100	--
2	Standard Solution-24 hours	99.5	99.5
3	Standard Solution-47 hours	100.7	100.7
4	Standard Solution-87 hours	99.7	99.7
5	Standard Solution-Initial	101.9	--
6	Standard Solution-24 hours	101.3	99.4
7	Standard Solution-47 hours	101.8	99.9
8	Standard Solution-87 hours	101.9	106.9

Table 9: Stability of Desmopressin analytical solution at Room Temperature

Robustness

Influence of small changes in chromatographic conditions such as change in flow rate and wavelength of detection was studied to determine the robustness of the method and its %RSD was determined. Three Sample preparations of the same lot of Desmopressin Tablets were prepared as per developed method. The sample along with standard solution were injected in duplicate under different chromatographic condition. The results indicate that the test method is Robust for all variable conditions outlined in the tables 10 and 11

Control	(+0.1 mL/min.)	(-0.1 mL/min.)
98.3	98.1	98.0
98.7	99.5	100.8
98.6	98.9	99.7
Cumulative Mean	**98.7**	**99.0**
Cumulative SD	**0.492**	**1.046**
Cumulative %RSD	**0.50**	**1.06**

Table 10: Robustness: Change in Flow rate (±10%)

Control	(+5 mn.)	(-5 mn.)
98.7	98.5	98.4
98.8	98.2	98.4
98.2	99.1	98.8
Cumulative Mean	**98.8**	**98.7**
Cumulative SD	**0.373**	**0.299**
Cumulative %RSD	**0.38**	**0.30**

Table 11: Robustness: Change in wavelength (± 5nm)

Filter equivalency

Suitability of filters were checked against centrifuged samples. Sample solutions were subjected to centrifuge and filter in triplicate by Nylon and Teflon filters. Results as shown in table 12. The Mean Filtration Recovery is within limits for Nylon 0.45 µ and Teflon 0.45µ filter. The correlation lies between 98.0% and 102.0%. Therefore, Teflon 0.45µ and Nylon 0.45µ filter are suitable for filtration

Sample No.	%Assay		
	Centrifuged	Nylon 0.45 µ	Teflon 0.45 µ
1	100.2	98.0	101.8
2	101.9	92.3	101.9
3	98.2	98.0	102.9
Mean	100.1	98.1	101.9
RSD	1.85	0.18	0.10
%Correlation With Centrifuged	--	98.0	101.8

Table 12: Filter equivalency stud

System Suitability

Standard solution was injected and run five times. The RSD of five replicate injections should not be greater than 2.0%. The Tailing factor for Desmopressin peak should not be greater than 2.0 and the theoretical plate should not be lesser than 2000. System suitability criteria was meeting the requirements. The reported methods in literature shown that peak shape of Desmopressin is not good [1], solution stability study and forced degradation study is not established [11-13]. Present work reveal that the Desmopressin is pH and moisture sensitive in solution and bulk form respectively. In Present research work, the solutions were made stable by selection of appropriate pH of mobile phase and diluent along with optimum column stationary phase. It was observed that the retention and resolution of Desmopressin peak in this chromatographic condition is enhanced compared to other chromatographic conditions during development.

CONCLUSION

The simple, specific, selective, linear, precise, accurate, and robust stability indicating RP-HPLC-DAD method for assay of Desmopressin in Desmopressin tablets was developed and validated as per ICH guidelines. Hence this method can be introduced for routine analysis for quantification of Desmopressin content in pharmaceutical dosage forms. The developed analytical method has several advantages like method is Cost effective, precise chromatographic condition, low proportion of acetonitrile in mobile phase and diluent which allows analysis of number of samples in less volume of organic solvent hence reduced contamination to eco system. Further this method is found to be suitable for estimation of Desmopressin in Desmopressin Tablets. The developed Desmopressin assay method shown optimum stability of solution in mixture of pH 4.5 phosphate buffer and methanol. Recommended future research- LC method can be developed and validated for solid oral dosage forms to quantify related substances and degradation products by RP-HPLC-DAD.

Conflict of interest

Authors declare that there is no conflict of interest.

List of Abbreviation

RP-HPLC-DAD- Reverse Phase-High Performance Liquid Chromatography-Diod Array Detector
UV- Ultra Violet
PDA- Photo Diode Array
LC- Liquid Chromatography
HCL- Hydrochloric acid
NaOH- Sodium hydroxide
H2O2- Hydrogen Peroxide
Acc- Accuracy
Nm- nanometer
RSD- Relative standard Deviation
SD- Standard Deviation

ACKNOWLEDGEMENT

The authors are grateful to Sanofi- Aventis Pharmaceuticals, Goa, for gifting samples of Desmopressin tablet-0.2mg and active pharmaceutical ingredient. The author is highly oblige to the principal and management of K.B.H.S.S. trust's Institute of Pharmacy and Research center, Malegaon (Nashik) for providing laboratory space for this research work.

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HOW TO CITE: Rashid Azeez*, Vinod A. Bairagi, Ziyaurrahman Azeez, A Novel Validated Stability Indicating Qbd Based Assay Method For The Quantification Of Desmopressin Acetate By High Performance Liquid Chromatography, Int. J. in Pharm. Sci., 2023, Vol 1, Issue 12, 166-178. https://doi.org/10.5281/zenodo.10316417