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ANALYTICAL METHOD OF RELATED SUBSTANCES FOR DESMOPRESSINACETATE INJECTION DILUENT COMPATABILITY STUDY 0.2 mcg/Ml BY HPLC

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ABSTRACT

Desmopressin acetate is a synthetic analogue of vasopressin widely used in the management of diabetes insipidus, nocturnal enuresis, and certain bleeding disorders. Due to its high potency and low therapeutic concentration, the accurate determination of related substances and evaluation of diluent compatibility are essential to ensure the quality, safety, and stability of the injectable formulation. The present study describes the development and validation of a stability-indicating High-Performance Liquid Chromatography (HPLC) method for the determination of related substances in Desmopressin Acetate Injection Diluent Compatibility Study (0.2 0.2 mcg/Ml).

Keywords: Desmopressin Acetate; High-Performance Liquid Chromatography (HPLC); Diluent Compatibility; Injectable formulation.

INTRODUCTION

Desmopressin acetate is a synthetic analogue of the natural antidiuretic hormone arginine vasopressin, developed to provide prolonged antidiuretic activity with reduced vasopressor effects. It is widely prescribed for the treatment of central diabetes insipidus, primary nocturnal enuresis, nocturia associated with nocturnal polyuria, and certain bleeding disorders such as mild hemophilia A and von Willebrand disease. Owing to its high pharmacological potency, desmopressin acetate is administered in very low doses, making the accurate determination of impurities and degradation products a critical aspect of pharmaceutical quality control.

The quality, efficacy, and safety of injectable drug products are highly dependent on the presence of related substances, which may arise during synthesis, manufacturing, storage, or degradation. Even trace levels of impurities can influence the therapeutic performance and safety profile of peptide-based drugs such as desmopressin acetate. Therefore, regulatory agencies require comprehensive impurity profiling using validated analytical methods to ensure that impurity levels remain within acceptable limits throughout the product's shelf life.

High-Performance Liquid Chromatography (HPLC) has become the analytical technique of choice for the determination of related substances because of its high sensitivity, selectivity, reproducibility, and ability to separate closely related impurities and degradation products. A stability-indicating HPLC method is particularly valuable because it can distinguish the active pharmaceutical ingredient from its degradation products generated under various stress conditions, thereby supporting stability studies and routine quality control testing.

In clinical practice, desmopressin acetate injections are frequently diluted with intravenous diluents before administration, especially in hospital settings where individualized dosing is required. The compatibility of the drug with different diluents is an important quality attribute, as dilution may alter the chemical stability of the drug substance, leading to the formation of degradation products or related impurities. The evaluation of diluent compatibility is therefore essential to ensure that the diluted preparation maintains its identity, purity, potency, and safety throughout the intended period of use.

Although several analytical methods have been reported for the assay of desmopressin acetate in pharmaceutical formulations and biological matrices, limited information is available regarding stability-indicating methods specifically designed for the determination of related substances during diluent compatibility studies at very low concentrations, such as **0.2 µg/mL**.

The development of a sensitive and robust HPLC method capable of accurately detecting and quantifying trace impurities at this concentration is therefore of considerable analytical and regulatory importance.

The present study focuses on the development and validation of a stability-indicating reversed-phase HPLC method for the determination of related substances in Desmopressin Acetate Injection Diluent Compatibility Study (0.2 µg/mL)**.** The method was validated in accordance with the International Council for Harmonisation (ICH) Q2 (R2) guideline with respect to specificity, linearity, precision, accuracy, robustness, sensitivity, and solution stability. Furthermore, the validated method was applied to evaluate the compatibility of desmopressin acetate injection after dilution with commonly used intravenous diluents under specified storage conditions. The developed analytical method is intended to provide a reliable, sensitive, and reproducible approach for routine quality control, stability assessment, and regulatory compliance of desmopressin acetate injectable formulations.

TEST METHOD

The test method for the related substances in Desmopressin acetate injection diluent compatibility study 0.2 mcg/mL has been verified as per protocol No.: ARD/AMV/DES/FP/AS/009P/00.
The following experiments were carried out to determine the performance characteristics of the method: System suitability, System precision, Selectivity /Specificity, Linearity, Method precision, Accuracy

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For this analytical method validation, test method for the Related substances of Desmopressin acetate injection diluent compatibility study 0.2 mcg/mL was followed for instrumental setup, reagents, chromatographic conditions, chromatographic procedures, standard, sample and other solution preparations.
Test method: ARD/PRT/2019/005* (Annexure-1) (*Refer current version)

SAMPLES AND STANDARDS

S.No	Material Name	Batch.No./Lot.No.	Potency (%) (on as is Basis)	Use Before	LNB No & P.No
1	Desmopressin acetate	04WS20000010	97.6	10/02/2021	ARD/LNB/ VAL/2020/ 27 Page no- 001 to 003 & Page no - 007
2	DEHP	BCBX5578	99.7	July/2023	
3	Chlorobutanol	1HJ1030	99.5	31/05/2021	
2	Desmopressin Acetate injection-diluent 0.2 mcg/mL	1003-024	NA	NA	
3	Desmopressin Acetate injection-diluent placebo 0.2 mcg/mL	1003-028P	NA	NA	

EQUIPMENT / INSTRUMENT

S. No	Equipment / Instrument	Make / Specification	In-house ID
1.	HPLC	Agilent 1260 series with VWD / DAD	CPS-HPLC-003
2.	Column	Lichrospher RP-18, 125 mm x 4.0 mm, 5µm	ARD/LCC/475
3.	Balance	Sartorius	CPS-BAL-006
4.	Balance	Sartorius	CPS-BAL-130
5.	pH meter	Mettler Toledo	CPS-pH-059

REAGENTS AND CHEMICALS:

S. No	Name of the chemical	Make	Grade	Batch number
1.	Monobasic potassium phosphate	Merck	ACS	DB0D700254
2.	Sodium 1-Heptanesulphonic acid anhydrous	Thomas Baker	AR	H2056
3.	Ortho-phosphoric acid	Fischer scientific	AR	29905
4.	Acetonitrile	Merck	HPLC	DE0DF70501
5.	Water	NA	Milli-Q	NA

SUMMARY OF RESULTS

Table number 1: Summary of results

SUMMARY CHART OF THE VALIDATION RESULTS				
Sr.No	**TEST**	**ACCEPTANCE CRITERIA**	**RESULTS**	**REMARKS**
1.	• **System suitability: System analysis and system precision**			
	Tailing factor for Desmopressin	NMT 2.0	1.02	Nil

	peak			
	%RSD for replicate injections of Desmopressin acetate peak and bracketing standard Desmopressin peak area.	NMT 10.0	0.7	
	The agreement between standard solution-1 and standard solution-2	Should be between 0.90 and 1.10.	1.00	
2.	**Selectivity/Specificity:**			
	Interference study	There should not be any interference from diluent & Placebo for Desmopressin Peak.	Desmopressin peak was free from any interference from diluent, in sodium chloride bag and placebo.	Nil
3.	Method Precision	RSD for six samples should not be more than 10.0%.	Sample Preparation-1 : ND Sample Preparation -2 : ND Sample Preparation -3 : ND Sample Preparation -4 : ND Sample Preparation -5 : ND Sample Preparation -6 : ND	Nil

			%RSD : NA	
4.	Accuracy	The individual recovery obtained for each sample should be within 75.0% and 125.0%.	**Accuracy at:** 50% : 90.3% (%RSD : 3.9) 100%: 81.9% (%RSD: 1.5) 150% : 79.0% (%RSD: 1.5)	Nil

SYSTEM SUITABILITY

System analysis

Tailing factor of Desmopressin acetate peak were reported from first injection of standard and the % RSD of Desmopressin acetate peak area was reported from the five standard injections.

(Refer LNB No. ARD/LNB/VAL/2020/26, Page No: 013).

The results are summarised in Table 2.

Table 2: System analysis results

Parameter	Based upon	Results
Tailing factor (T): Desmopressin acetate peak	Standard-1 injection	1.02
%RSD of Desmopressin acetate peak in standard solution	Standard 1 to 5 injections	0.7
Agreement between standard solution-1 and standard solution-2	Should be between 0.90 and 1.10	1.00

Acceptance Criteria:

- Tailing factor (T) not more than 2.0 for Desmopressin acetate peak
- % RSD for Desmopressin acetate peak in replicate standards not more than 10.0%.
- The agreement between standard solution-1 and standard solution-2 should be between 0.90 and 1.10.

Data Interpretation:

- Test method met the criterion for system suitability.

System precision:

Six consecutive injections of the standard solution were made. The results are summarised in Table 3. (Refer LNB No. ARD/LNB/VAL/2020/26, Page No: 014).

Table 3: System precision results

Injection	Standard area
1	283559
2	281010
3	280975
4	285242
5	281587
6	282048
Mean	**282404**
Std. dev	**1683.31634**
RSD (%)	**0.6**

Acceptance criteria:

- % RSD should not be more than 10.0 for Desmopressin acetate peak area response.

Data interpretation:

- Test method met the criterion for system precision.

SELECTIVITY / SPECIFICITY

Interference study

The Blank, placebo, standard solution and sample were injected to demonstrate non-interference. The results are summarised in the below table 4.

(Refer LNB No. ARD/LNB/VAL/2020/26, Page No: 018).

Table 4: Specificity results

S.No	Solution	Retention Time (in min)
1.	Blank (diluent)	1.42,3.48,5.02
2.	Blank (0.9% NaCl)	0.99, 1.32, 1.73, 2.33, 2.72, 2.94, 3.55, 3.92 ,4.35, 4.82, 5.31, 5.75, 7.84, 9.62, 14.56, 16.72, 22.95, 29.18, 36.13, 38.64, 46.08
3.	Placebo	0.95, 1.30, 6.33, 6.64, 7.36, 7.89, 8.46,

		8.86, 9.62, 10.41, 13.22
4.	Standard solution	12.27
5.	Sample solution	12.32
6.	Chlorobutanol	13.10
7.	DEHP	1.15

Acceptance criteria:

- There should not be any interference at the region of active peak.

Data interpretation:

- The Desmpressin aceate peak was free from any interference, and met the acceptance criteria.

LINEARITY

A series of solutions containing Desmopressin acetate at concentrations listed in below Table 6 were analysed to determine the linearity of the method. Single injection of each solution was performed. (Refer LNB No. ARD/LNB/VAL/2020/26, Page No: 015).

Linearity preparations: Weighed accurately about 10.70 mg of Desmopressin acetate in 250 mL volumetric flask, dissolved and diluted to the mark with diluent.

Further pipetted out 1 mL of the above solution in to 100 mL volumetric flask, diluted to the mark with diluent and mixed well. Furhter dilutions are prepared as per table number 5.

(Refer LNB No. ARD/LNB/VAL/2020/26, Page No: 005-006).

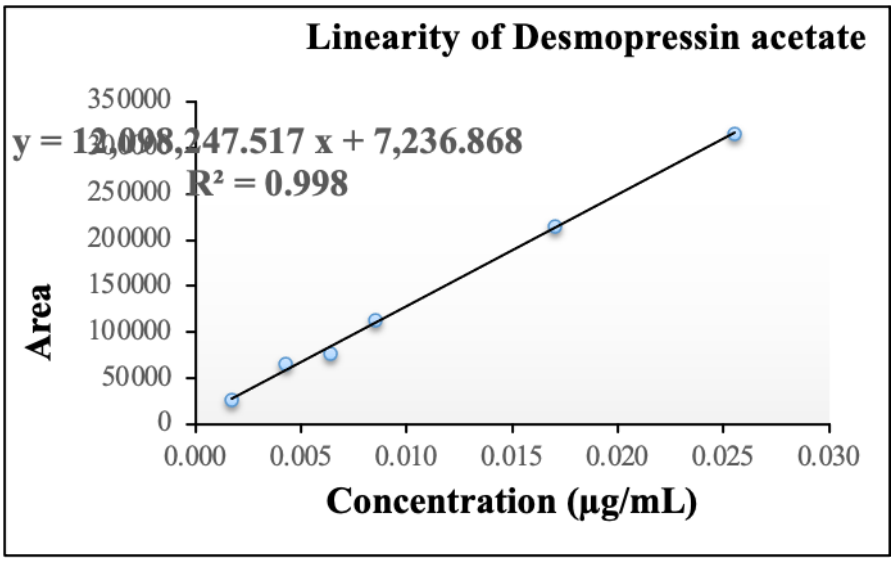
Table 5: Linearity preparations:

% Levels	Volume pipetted out (mL)	Final volume (mL)
10	0.4	100
25	1.0	100
40	1.5	100
50	1.0	50
100	1.0	25
150	1.5	25

Table 6: Linearity results

% Levels	Concentration (µg/mL)	Area Response
10	0.002	26166
25	0.004	65277
40	0.006	76000
50	0.009	113483
100	0.017	214492
150	0.026	315675
Slope		**12098247.52**
Intercept		**7236.86776**
Regression coefficient (r^2)		**0.998**
Correlation coefficient		**0.999**
Y- intercept		**3.4**

Figure 1



Acceptance criteria:

Correlation and Regression coefficient is not less than 0.995.

Data interpretation

Test method was linear for Desmopressin acetate in the concentration ranges listed above and correlation coefficient is 0.999 and regression coefficient 0.998.

METHOD PRECISION

The method was assessed by analysing a series of six samples for related substances at 100% sample concentration on a single day.

(Refer LNB No. ARD/LNB/VAL/2020/26, Page No: 007).

Note: In Method precision, unknown impurities were not observed, hence calculation was not performed.

Acceptance criteria: RSD for six samples should not be more than 10.0%.

Data interpretation: Test method met the criteria for method precision.

ACCURACY

The accuracy of the method was assessed by spiking Desmopressin acetate standard to placebo solution in triplicates in 50% & 150% and six preparations in 100% level.

The results are summarised in Table 8.

(Refer LNB No. ARD/LNB/VAL/2020/26, Page No: 016 & 017)

Accuracy preparation

Weighed accurately about 10.70 mg of Desmopressin acetate in 250 mL volumetric flask, dissolved and diluted to the mark with diluent. Further pipetted out 1 mL of the above solution in to 100 mL volumetric flask, diluted to the mark with diluent and mixed well. Furhter dilutions are prepared as per table number 7.

(Refer LNB No. ARD/LNB/VAL/2020/26, Page No: 008 & 009)

Table 7: Accuracy preparations

Sample Name	Standard solution (mL)	Dilution made with placebo (mL)
50% level -Preparation-1	0.5	25
50% level -Preparation-2	0.5	25
50% level -Preparation-3	0.5	25
100% level -Preparation-1	1.0	25
100% level -Preparation-2	1.0	25
100% level -Preparation-3	1.0	25
100% level -Preparation-4	1.0	25
100% level -Preparation-5	1.0	25
100% level -Preparation-6	1.0	25

150% level -Preparation-1	1.5	25
150% level -Preparation-2	1.5	25
150% level -Preparation-3	1.5	25

Table 8: Accuracy results

Sample Name	Added(mg)	Found(mg)	% Recovery	% mean	% RSD
50% level -Preparation-1	0.0084	0.0079	94.4	**90.3**	**3.9**
50% level -Preparation-2	0.0084	0.0074	88.8		
50% level -Preparation-3	0.0084	0.0073	87.8		
100% level -Preparation-1	0.0167	0.0138	82.5	**81.9**	**1.3**
100% level -Preparation-2	0.0167	0.0138	82.8		
100% level -Preparation-3	0.0167	0.0137	82.2		
100% level -Preparation-4	0.0167	0.0137	82.3		
100% level -Preparation-5	0.0167	0.0136	81.5		
100% level -Preparation-6	0.0167	0.0134	79.9		
150% level -Preparation-1	0.0251	0.0201	80.3	**79.0**	**1.5**
150% level -Preparation-2	0.0251	0.0198	78.9		
150% level -Preparation-3	0.0251	0.0195	78.0		

Acceptance criteria:

- The individual recovery obtained for each sample should be within 75.0% and 125.0%.
- **Data interpretation:** The individual recovery obtained for each sample level (50% to 150%) met the acceptance criteria.

DEGRADATION PRODUCTS METHOD

Instrument: HPLC, pH meter, analytical balance.

NOTE: Ensure for 500 µL loop to the HPLC system prior to start the analysis and ensure calibration for the same.

Critical points

- Flush the system with Hot water for 20 minutes.

- Flush the system with Water: ACN (1:1) for 15 minutes and followed by column wash with the same solvent with 1 mL/min.
- Stabilize the column with mobile phase for 1 hour before starting the sequence.
- Desmopressin acetate peak is sensitive to acetonitrile composition in the mobile phase, adjust the mobile phase accordingly to achieve the desired retention time.

Reagents

Monobasic potassium phosphate	: AR grade or equivalent
Sodium 1-Heptanesulphonic acid anhydrous	: AR grade or equivalent
Ortho-phosphoric acid	: AR/HPLC grade or equivalent
Sodium hydroxide	: AR grade or equivalent
Acetonitrile	: HPLC grade or equivalent
Water	: Milli-Q/HPLC water or equivalent

Buffer solution

Dissolve about 3.4 g of monobasic potassium phosphate and about 2.0 g of Sodium 1heptanesulfonic acid in 1000 mL of water. Adjust the pH to 3.0 ± 0.05 with phosphoric acid or Sodium hydroxide, as needed. Pass through 0.45 µm nylon filter.

Mobile phase preparation:

Mix 800 mL of Buffer solution with 200 mL of Acetonitrile, and degas. Make adjustments if necessary.

Chromatographic conditions:

Instrument	: HPLC system equipped with UV-Detector/PDA
Column	: Lichrospher RP-18, 125 mm x 4.0 mm, 5µm
Flow rate	: 1.2 mL/minute
Wavelength	: 220 nm

Column temperature	: 45℃
Injection volume	: 500 µL
Runtime	: 55 minutes.
Sample compartment	: 5℃
Needle wash	: Diluent
Retention time	: Desmopressin acetate is about 12 minutes.
Diluent	: Use Mobile phase as diluent.

Standard solution preparation: (In case of using working standard)

Weigh accurately about 10.0 mg of Desmopressin acetate in 250 mL volumetric flask, Dissolve and dilute to the mark with diluent. Further pipette 1.0 mL from this and dilute to 100 mL with diluent and mix well.

Again pipette 1.0 mL from the above solution and dilute to 25 mL with 0.9% sodium chloride (which is used for diluent compatibility study) and mix well.

(Or prepare a solution containing 0.02 µg/mL Desmopressin acetate in diluent).

Prepare in duplicate.

Standard solution preparation: (In case of using Desmopressin acetate USP Reference Standard)

Take reference standard vial and reconstitute with 2 mL of water and mix well. From this solution pipette out 1 mL in to 250 mL volumetric flask and dilute to the mark with diluent and mix well. Further again pipette out 5 mL in to 50 mL volumetric flask and dilute to the mark with diluent and mix well. Again pipette 1.0 mL from the above solution and dilute to 25 mL with 0.9% sodium chloride (which is used for diluent compatibility study) and mix well. Prepare in duplicate.

(**Additional Information:** Reconstitution: carefully open the vial, add 2.0 mL of water.

Place stopper back and screw the cap on tightly. Mix by inverting. Make further dilution with a suitable diluent if necessary. Protect solution from light. Each vial contains 2.01 mg of Desmopressin).

Sample solution preparation

Directly take sample into a new HPLC vial and use. (Desmopressin acetate injection 0.2 Mcg/mL sample concentration).

Injection sequence:

Name	umber of injections
Diluent	1
Standard solution-1	6
Standard solution-2	1
Placebo solution	1
Sample solution	1
tandard solution (Bracketing standard)	1

System suitability criteria

- Tailing factor for standard solution should NMT 2.0
- Relative standard deviation for replicate injections of Desmopressin acetate peak is not more than 10.0%.
- The agreement between standard solution-1 and standard solution-2 should be between 0.90 and 1.10.

System suitability Criteria for bracketing standard:

- Tailing factor for standard solution should NMT 2.0

- Relative standard deviation for replicate injections of Desmopressin acetate peak and bracketing standard Desmopressin peak area response is not more than 10.0%

Calculation

Standard agreement calculation:

$$\text{Agreement} = \frac{\text{Avg Area of Std solution} - 1}{\text{Area of std solution} - 2} \times \frac{\text{Weight of std solution} - 2}{\text{Weight of std solution} - 1}$$

Disregard the peaks due to blank (diluent) & placebo and integrate the peak of impurities and Desmopressin acetate peak. Respective placebo should be injected along with the samples. Calculate the percentage of each impurity in the portion of Desmopressin Acetate injection taken by the formula:

In case of using working standard:

$$\%\ \text{Impurities} = \frac{\text{AT}}{\text{AS}} \times \frac{\text{WS}}{\text{DS}} \times \frac{\text{DT}}{\text{VL}} \times \frac{\text{P}}{100} \times \frac{100}{\text{LC}}$$

Where,

AT : Area of unknown impurity peak from sample solution.

AS : Average area of Desmopressin Acetate peak from standard solution.

WS : Weight of Desmopressin acetate standard taken in mg.

VL : Volume of the sample taken in mL

DT : Dilution of sample preparation.

DS : Dilution of standard preparation.

P : Potency of Desmopressin Acetate standard on as is basis.

LC : Label claim of Desmopressin Acetate injection. (i.e, 0.0002 mg/mL)

(**In case of using USP reference standard**)

$$\% \text{ Impurities} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{VL} \times \frac{P}{100} \times \frac{100}{LC} \times \frac{1183.3}{1069.2}$$

Where,

AT : Area of unknown impurity peak from sample solution.

AS : Average area of Desmopressin Acetate peak from standard solution.

WS : Weight of USP reference standard in mg (i.e 2.01 mg).

VL : Volume of the sample taken in mL

DT : Dilution of sample preparation.

DS : Dilution of standard preparation.

P : Potency of USP reference standard.

LC : Label claim of Desmopressin Acetate injection. (i.e, 0.0002 mg/mL)

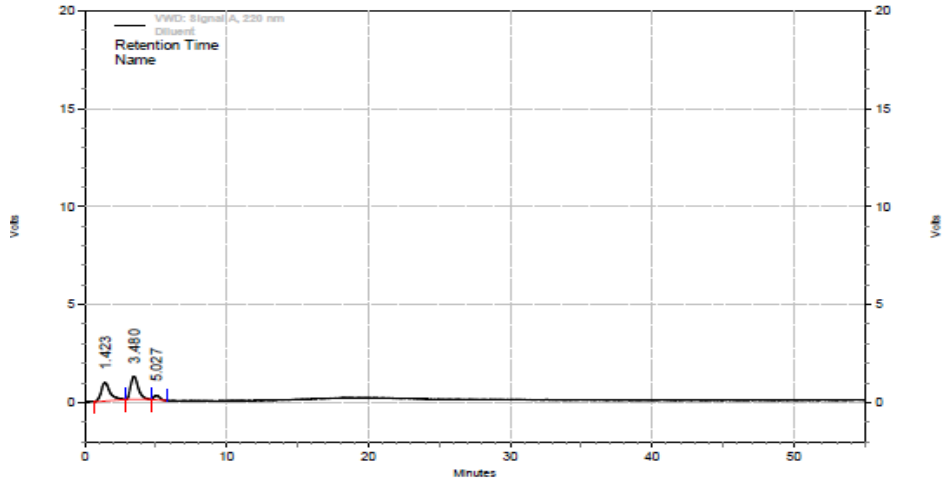
1183.3 : Desmopressin acetate molecular weight

1069.2 : Desmopressin molecular weight.

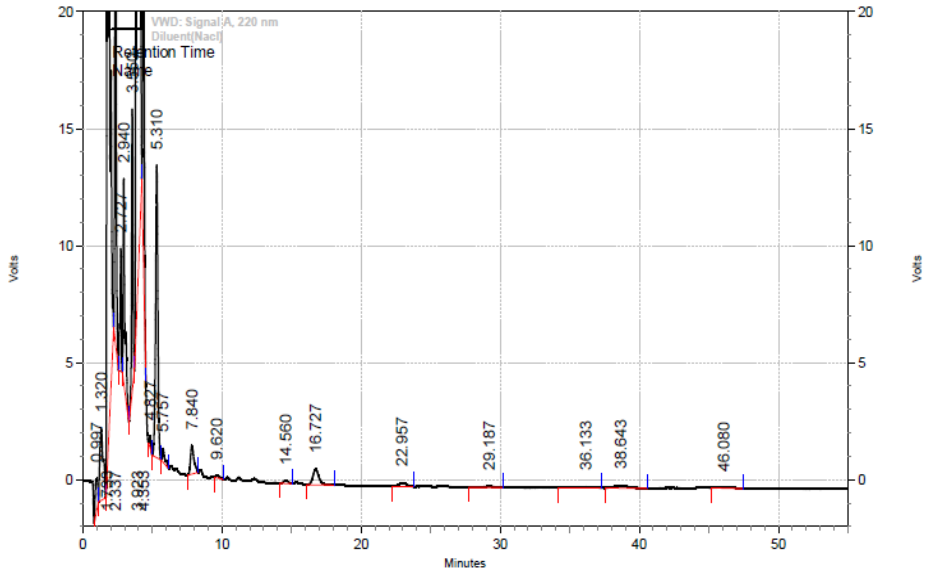
APPENDIX -2

Typical Chromatograms:

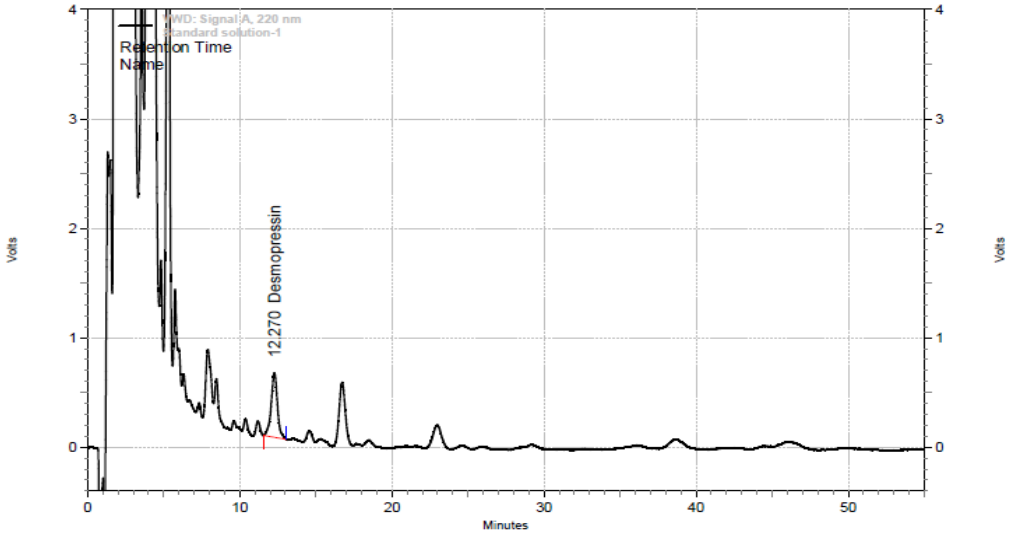
1. **Blank chromatogram**



2. **Diluent (0.9% NaCl bag) chromatogram**

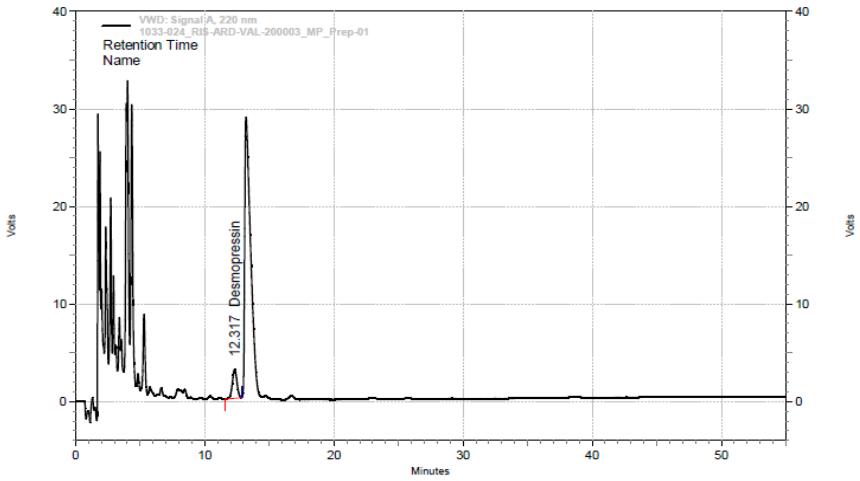


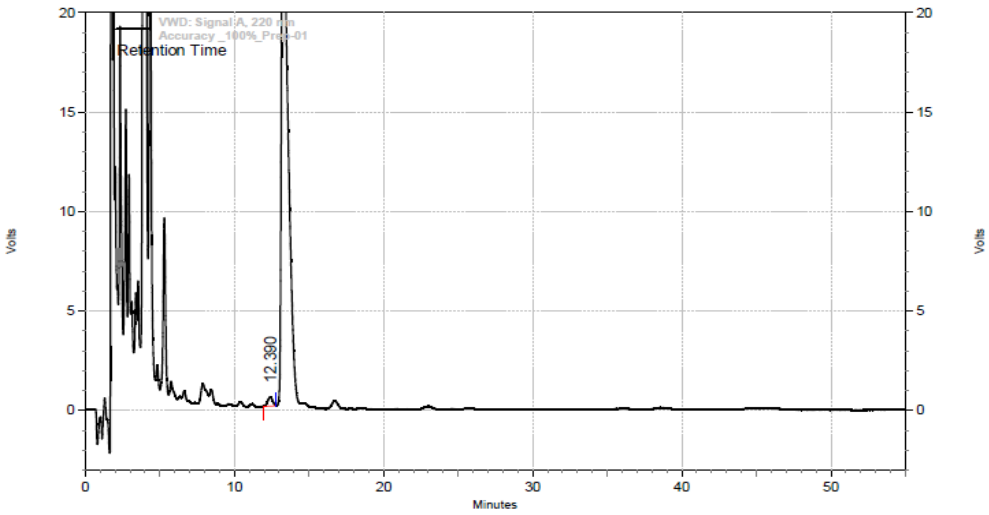
Standard chromatogram



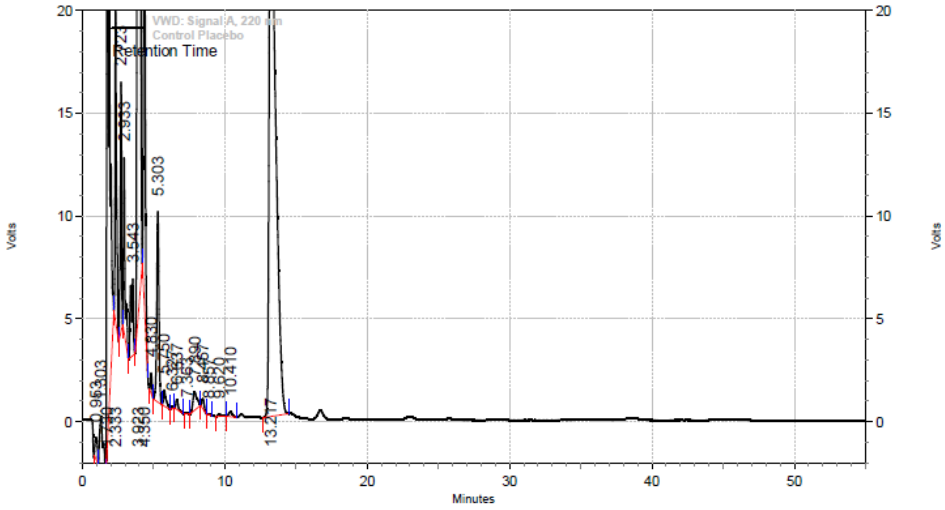
3. Control sample chromatogram

Spiked sample chromatogram





4. Placebo chromatogram



CONCLUSION

The Desmopressin acetate related substances method by HPLC for Desmopressin acetate injection, diluent compatibility study 0.2 mcg/mL has been verified and the method was found to be specific, precise, linear and accurate from 50% to 150% level with respect to the working concentration. Hence, the method is suitable for indented use.

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