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Analytical Method Development and Validation for Dexmeditomedine HCL Injection by HPLC

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ABSTRACT:

Dexmedetomidine is a potent and highly selective α2-adrenoceptor agonist with significant sedative, analgesic and anxiolytic effects mostly used for patients in the intensive care units, Drug approved by the Food and Drug administration (FDA) in the year 1999.This Article describes a simple, accurate and precise new method development and validation for estimation of dexmedetomidine by using HPLC. The separation of the standard peak from baseline achieved by using Inertsil ODS-3V (250x4.6mm id, 5µm) column with mobile phase sodium dihydrogen phosphate buffer (pH 4.6) and Acetonitrile: buffer 80:20 ratio v/v%(pH adjusted by dilute orthophosphate), using a flow rate 1.5mL/min and column temperature was maintain at 30ºC.Detection was achieved at wavelength 215nm. The developed method was validated as per ICH guidelines the obtained results was the developed method was passed system suitability parameter, there is no interference in the standard peak ,hence the developed method is said to be specific for dexmedetomidine. The linearity results was obtained by injecting the concentration ranges from 1.04µg/ml to 6.23µg/mL with correlation coefficient (R^2) is 0.999 with slope 149098 and Y-intercept -1463.The %RSD of precision method was found to be 0.2. The % Recovery of the dexmedetomidine assay was found to be 100.3. The developed method for dexmedetomidine was also passed the robustness and ruggedness parameters.

Introduction: Dexmedetomidine [(S)-4-[1-(2,3- dimethylphenyl)ethyl]-1Himidazole(fig.no.1), Dexmedetomidine is a potent and highly selective α2-adrenoceptor agonist with significant sedative, analgesic and anxiolytic effects mostly used for patients in the intensive care units. Drug approved by the Food and Drug administration (FDA) in the year 1999. The advantage of D-MDT over other sedatives is the absence of respiratory depression. Dexmedetomidine Hydrochloride Injection has been continuously infused in mechanically ventilated patients prior to extubation, during extubation, and post-extubation. It is not necessary to discontinue Dexmedetomidine Hydrochloride Injection prior to extubation. There are several off label uses of dexmedetomidine like sedation for FOB (fiberoptic bronchoscopy) and intubation, sedation for Magnetic Resonance Imaging (MRI), endoscopies and ophthalmic surgeries, as an antishivering agent post operatively, for alcohol and opioid withdrawal.

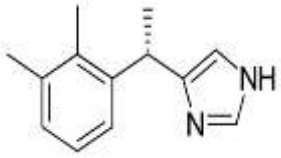

Fig.no.1.Struture of Dexmedetomidine

There is a lot of literature survey available for the method development and validation of dexmedetomidine in TLC,CE,LC-MS, but a very few methods was available on HPLC instruments. The aim of the present study was to develop and validated a simple, accurate and rapid analytical method suitable for estimation of dexmedetomidine in dexmedetomidine injections for routine quality test in pharmaceutical industry.

Materials and Methods:

Standard: Dexmeditomedine (Standard obtained from vendors), sample(Samples obtained from different batches of manufacturing in Neuheit pharma Ltd.)

Chemicals: HPLC grade solevnts like Orthophosphoric acid, Methanol, Acetonitrile are purchased from Rankem, AR grade reagents like Sodium dihydrogen phosphate, Disodium hydrogen phosphate, Sodium chloride are purchased from Merck and Ultrapure water form Rephile.

Instruments: Sonicator (Radwag, PS 600.R2), Semi micro balance (PCI analytics) 0.45 úm mdi membrane filter(Advanced micro devices) Ultra pure water system(Lab India, PICO+),HPLC (Waters Allaince separation module 2695 detector : PDA 2996/UV 2487 along with operating software Empower)

Preparation of Reagents:

Diluted Orthophosphoric Acid Preparation procedure: dissolve 5 ml of orthophosphoric acid (85%) in distilled water up to 100 ml and mix well.

Preparation of 0.1 molar sodium hydroxide: dissolve the 0.4g sodium hydroxide in 100 mL of unadulterated water and blend well.

Preparation of sodium chloride solution: Add 4.5 grams of sodium chloride to 500 ml of freshly filtered water and stir to dissolve.

Procedure for Dexmedetomidine standard stock solution Preparation (About 200 ppm): Weigh out 20 mg of dexmedetomidine hydrochloride reference/standard and pour into a 100 mL container. Add 30ml of ultrapure water and stir for 5 minutes until dissolved. Fill the container with diluent and mix well. **Procedure for Dexmedetomidine Working standard solution preparation (about 4ppm):** Pipette out 2 mL volume solution from Dexmedetomidine standard stock preparation into 100 mL volumetric flask. Add the required amount Diluent up to mark and mix well.

Sample preparation for Assay: Transfer 2 mL of 100 mcg mL dexmedetomidine injection sample to a 50 mL vial, add the required volume of diluent to the mark, and mix well.

Optimized Chromatographic conditions: After several trails with sodium dihydrogen phosphate buffer (optimized condition was achieved by changing pH of the buffer from pH7.8 to pH 4.6 pH was adjusted with dilute orthophosphate). The separation of the standard peak from baseline achieved by using Inertsil ODS-3V (250x4.6mm id, 5µm) column with mobile phase sodium dihydrogen phosphate buffer (pH 4.6) and Acetonitrile: buffer 80:20 ratio v/v%(pH adjusted by dilute orthophosphate), using a flow rate 1.5mL/min and column temperature was maintain at 30ºC.Detection was achieved at wavelength 215nm.

Results and Discussion:

Method validation: Validation of proposed analytical method involves linearity and range, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ) and robustness study. It was validated according to ICH Q2 (R1) guideline.

System Suitability: System suitability is an integral part of chromatographic system. The calculation and comparison of verified resolution, capacity factor, tailing factor, theoretical plate count with standard specification of system. The column was equilibrated with mobilehase for 30min with flowrate1.5mL/min and dexmedetomidine standard with 4 ppm concentration was injected sixes times into HPLC system after the injecting of one blank.

Table.no.1. System suitability Data of Dexmedetomidine

Column Description	Name of Component	Evaluation parameters				
		Avg. Area	% RSD	Retention time (t_r)	Tailing factor (Tf)	Plate count (N)
Inertsil ODS 3V (250 X 4.6 mm) 5 µm	Dexmedetomidine	508272	0.6	7.739	1.1	8738

Acceptance Criteria: When calculating the peak area of Dexmedetomidine using six injections of the standard solution, the %RSD should not exceed 0.85. For dexmedetomidine spikes, the USP tail factor should not be greater than 2.0.Column efficiency: More than 2000 theoretical plates are required on top of the dexmedetomidine column.

Conclusion: System suitability parameters were acceptable and met the criteria.

Specificity: Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. The determination of the excipients effect and other additives which are present in formulation can be determined by using analytical method i.e. specificity. Prepared placebo and blank are injected into HPLC system along with a standard of 4ppm concentration.

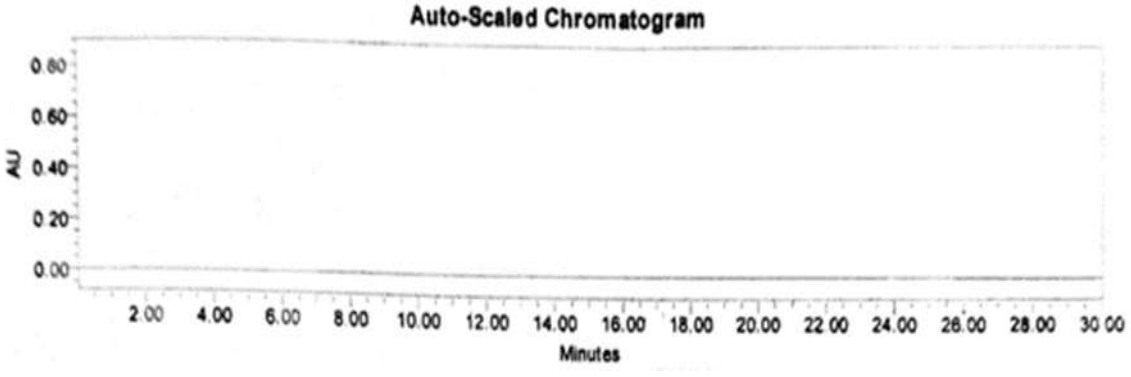

Fig.no.2. Chrmatogram of Blank

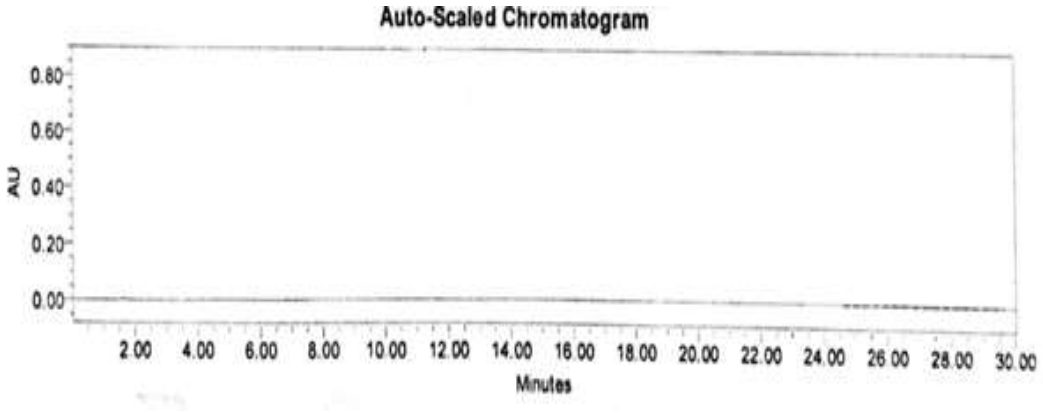

Fig.no.3. Chrmatogram of Placebo

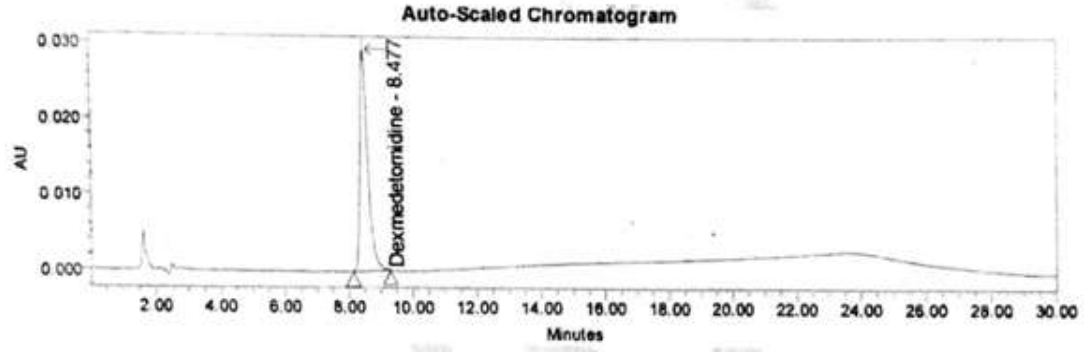

Fig.no.4.Chromatogram of Dexmedetomidine Standard

Table.no.2. Interference table

Injection ID	**Name of Impurities**	**Retention time(t_r)**	**Resolution**	**Purity angle**	**Purity Threshold**	**Interference (Yes/No)**
Blank	Dexmedetomidine	Not detected	NA	NA	NA	No
Placebo	Dexmedetomidine	Not detected	NA	NA	NA	No
Standard	Dexmedetomidine	8.456	NA	0.262	0.506	NA
Individual Impurities (1%)	Ketone impurity	11.824	NA	0.754	0.777	No
	ethyl Dexmede tomidine impurity	13.006	NA	0.748	1.045	No
	Olefine impurity	14.997	NA	0.597	0.937	NA
Sample	Dexmedetomidine	8.462	NA	0.270	0.543	No
Spiked sample	Dexmedetomidine	8.456	NA	0.202	0.673	No
	Ketone impurity	11.824	8.9	0.641	0.934	No
	Ethyl Dexmede tomidine impurity	13.006	3.6	0.540	1.721	No
	Olefine impurity	14.997	6.1	0.323	1.017	No

Acceptance Criteria: Each peak needs to be properly resolved. Do not interfere with the dexmedetomidine peak of other peaks. NLT 0.99 is the recommended maximum purity for dexmedetomidine.

Conclusion: No interference peak was observed in the RT of standard peak.

Linearity: Linearity of an analytical method is carried out to demonstrate that concentration of an analyte is directly proportional to the peak area of analyte. The evaluation of linearity is done by visual inspection of plot of signal as a function of analyte concentration in sample. The linearity was determined by analysing six solutions over the concentration range of 1.04-6.23 µg/mL.

Table.no.3.Linearity results of Dexmedetomidine

S. No	%Level	Concentration(ppm)	Peak Area
01	25	1.04	154450
02	50	2.08	307186
03	75	3.11	465390
04	100	4.15	613691
05	125	5.19	771711
06	150	6.23	929125
Correlation coefficient(r)			**1.000**
Regression coefficient (R^2)			**0.999**
Slope			**149098**
Y-Intercept			**-1463**
Blas at 100%			**-0.2**
Residual sum of squares			**29184240.79**

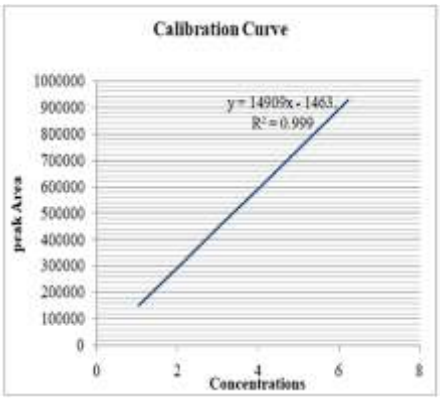

Fig.no.5. Linearity Curve of Dexmedetomidine

Acceptance Criteria: Correlation coefficient should not be less than 0.999.

Conclusion: The Correlation coefficient is 0.999 with slope 149098 and Y-intercept -1463. Therefore, the HPLC method for Dexmedetomidine is linear.

Accuracy: Accuracy of the analytical method is the capability of method to determine the correct assay value. Accuracy of analytical method can be determined by adding the known amount of standard drug to placebo preparation and then same shall be analysed by proposed method. The accuracy of the method was determined by calculating recovery of Dexmedetomidine by adding additional standard in different levels as 50% to 150%.

Table.no.4.% Recovery Data of Dexmedetomidine

S. No	Recovery level	Sample	Added "ppm"	Found "ppm"	% Recovery	Average % recovery	%RSD
1	50%	Sample-1	2.022	2.030	100.4	100.3	0.8
		Sample-2	2.022	2.043	101.0		
		Sample-3	2.022	2.012	99.5		
2	100%	Sample-1	4.044	4.093	101.2	100.2	0.9
		Sample-2	4.044	4.032	99.7		
		Sample-3	4.044	4.032	99.7		
3	150%	Sample-1	6.067	6.167	101.7	101.6	0.1
		Sample-2	6.067	6.162	101.6		
		Sample-3	6.067	6.169	101.7		

Acceptance criteria: It should be between 98% and 102% of the recovery level.

Conclusion: According to the results of this evaluation, the accuracy of this method is 50 to 150% level

Precision: The closeness of agreement between a series of measurements obtained from multiple sampling of similar homogenous sample under the prescribed condition. Precision was determined by injecting the 6 injections of standard preparation.

Table.no.5. Precision data of Dexmedetomidine

S. No	Sample Name	%Assay
1	**Sample-1**	**99.5**
2	**Sample-2**	**99.4**
3	**Sample-3**	**99.2**
4	**Sample-4**	**99.4**
5	**Sample-5**	**99.1**
6	**Sample-6**	**99.5**
	Average	**99.4**
	STDEV	**0.16**
	%RSD	**0.2**

Acceptance Criteria: The % RSD should not be more than 2%

Conclusion: % RSD of precision was found to be 0.2

Stability solution: To obtain accurate and reliable results, it is important to keep the test solutions, indicators and reagents used in HPLC methods for a specific period of time, such as a day, a week or a month, according to special requirements. These methods aim to ensure the proper maintenance of the workload. Stability of solution was determined by injecting prepared standard and sample solutions into an HPLC system at initial stage, after 24 hours and after 48 hours (with freshly prepared standard). Similarity factor for the initial standard after 24 hours and 48 hours stranded area response of Dexmedetomidine peak from the standard preparation. Difference of % assay between initial stage, after 24 hrs and 48 hrs solutions was calculated.

Table.no.6. Solution Stability data of Dexemedetomidine

Condition	Area	% Assay	Similarity Factor
Initial	523743	101.2	--
Room temperature (After 24hrs) (25±3ºC)	529478	100.2	1.01
Refrigerator (After 24hrs)(2-8ºC)	523614	100.7	0.99
Initial	521917	100.2	--
Room temperature (After 48hrs)(25±3)	535870	100.4	1.02
Refrigerator (After 48hrs)(2-8ºC)	526328	99.3	1.01

Acceptance Criteria: The % standard deviation from the peak area is limited to a maximum of 2.0.

Conclusion: From the observations, it was concluded that this pattern is stable for 48 hours at room temperature (25 ± 3ºC) and refrigerated (2ºC to 8ºC).

Robustness: The robustness of analytical method is a measurement of its capacity to remain unaffected by small changes but deliberate changes in procedure parameters and provides an indication of its reliability during normal usages.

Table.no.7. Robustness data of Dexmedetomidine

Parameteres	Changes	Avg. Area	RT	Tailing Factor	USP Plate	%RSD
Flow rate	1.3mL/min	596798	9.418	1.1	16351	0.1
	1.5mL/min	511241	8.451	1.1	11452	0.3
	1.7mL/min	449782	7.519	1.1	8039	0.3
Column Temperature	25ºC	504511	8.653	1.5	5183	0.3
	30ºC	511241	8.451	1.1	11452	0.3
	35ºC	498589	8.362	1.4	6304	0.3
Mobile phase Composition	Low (76:24)	545660	10.824	1.8	15730	0.5
	Organic(80:20)	511241	8.451	1.1	11452	0.3
	High (88:12)	540887	7.568	1.2	8542	0.8
Mobile phase pH	pH.4.4	503368	8.451	1.2	8013	0.3
	pH.4.6	511241	7.934	1.1	11452	0.4
	pH.4.8	500960	9.227	1.2	15760	0.2
Acceptance criteria		NA	NA	NMT 2.0	NLT 2000	NMT 0.85

Acceptance Criteria: The % amount found should be between 98% to 102%. % relative standard deviation should not be more than 2.0%

Conclusion: The test method was found to be robust against changes in flow rate, buffer pH, column temperature, and organic ratio.

Ruggedness : From stock solutions, sample solution of 4 µg/mL of dexmedetomidine was prepared and analyzed by two different analysts using similar operational and environmental conditions. Peak area was measured for same concentration solutions, six times.

Table.no.8. Ruggedness data of Dexmedetomidine

Sample	Precision % Assay	Ruggedness % Assay
1	102.5	102.9
2	102.6	102.8
3	102.9	102.9
4	103.1	103.4
5	103.4	103.4
6	103.6	103.5
Mean	103.0	103.2
SD	0.436	0.315
%RSD	0.42	0.31
Overall Mean	103.1	
Overall SD	0.369	
Overall %RSD	0.36	

Acceptance Criteria: % amount found should be between 98% to 102%. % relative standard deviation should not be more than 2.0%

Ruggedness Conclusion: From the observation, we conclude that the HPLC method developed for determining the concentration of dexmeditomidine and dexmeditomidine HCl injection is effective.

Conclusion:

For the purposes of regular analysis, it is desirable to develop a method that can reliably, precisely and accurately analyze a large number of samples in a short time without prior separation. HPLC methods produce large amounts of high-quality data that serve as very powerful and useful analytical tools.

A simple, precise, rapid, and accurate high-performance liquid chromatography HPLC method for the analytical determination of dexmedetomidine in dexmedetomidine hydrochloride injection was developed and validated. This process was done by using inertsil ODS-3V 5(250 × 4.6 mm)5µm,mobile phase A, pH 4.6, sodium dihydrogen phosphate buffer pH adjusted with dilute orthophosphoric acid, and mobile phase B containing Acetonitrile: buffers in 80:20 ratio respectively. It was tested in simple gradient mode with a flow rate of 1.5 ml min. The column temperature was set to be 30 °C and the injection volume was 50 µL. Detection was achieved at a wavelength of 215 nm. The retention time of dexmedetomidine is 7.739. The total time of the procedure is 30 minutes.

The consistency, order, accuracy, precision, independence, reliability and reliability of the process system were tested. The method showed linearity with an R^2 correlation coefficient of 0.999, a slope of 149098, an intercept of -1463, and a concentration range of 16 g/ml. The recovery rate of dexmedetomidine is 100.1 to 101.6. The precision of this method was a standard deviation of 0.1789 and an RSD of 0.18. The products are unique because the mobile units and accessories are less efficient. Small differences in test results between analyzers and specific flux and wavelength changes indicate that the method is robust and reliable.

Therefore, this analytical method is strong in terms of reliability, accuracy, precision, and specificity, and is considered suitable for continuous and extensive analysis.

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