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STABILITY INDICATING HPLC METHOD VALIDATION FOR THE ASSAY OF DEXMEDETOMIDINE IN DEXMEDETOMIDINE HYDROCHLORIDE INJECTION

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ABSTRACT

A simple, accurate, rapid and precise High performance liquid chromatographic (HPLC) method was validated for the determination of Assay of dexmedetomidine in dexmedetomidine hydrochloride injection. The method employs Waters HPLC system on LiChrospher, 100 RP-18 end capped, 4mm x 12.5 cm, 5µm column with an isocratic elution at a flow rate of 1.0 mL/min using a mobile phase of 60-40% of methanol and Buffer. The detection was performed by a photo diode array Detector. In Linearity over concentration range of 50% to 150% correlation observed was 0.999. The intra and inter-day precision are with in limit (overall % RSD not more than 2.0 %). The overall mean recovery over a range 80,100, 120 % of Dexmedetomidine was 101.1%. The method is robust even for slight change in chromatographic conditions. Dexmedetomidine in this study complied with the pharmacopeial limits. The validated method was Specific, Linear, Precise, Accurate, Rugged and Robust for Assay of Dexmedetomidine in Dexmedetomidine HCl injection. The method validated as per ICH guideline by High performance liquid chromatography.

Keywords – *Dexmedetomidine, Validation, Assay, High performance Liquid Chromatography.*

1. INTRODUCTION

Dexmedetomidine is a highly selective α-2 adrenergic receptor agonist with several diverse actions like sedation, anxiolysis, sympatholysis, analgesia, and decreased intraoperative anesthetic requirements (narcotic, inhalational), cardiovascular stability, smooth recovery when used as an adjunct to general anesthesia, and above all, preserves respiratory function. It was approved by United States Food and Drug Administration (US FDA) in 1999 for use in humans for short term sedation and analgesia in Intensive Care Unit (ICU) for less than 24 hours[1,2]. 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 anti-shivering agent post operatively, for alcohol and opioid withdrawal [3,5]. Though not approved for use in pediatric patients, especially infants, there is a lot of literature available in the form of case reports and a review article that describes successful use of dexmedetomidine in this group of patients as well. It is been rigorously explored as an adjunct to local anesthetic in spinal and epidural anesthesia [6,9]. But there is some reluctance in using dexmedetomidine by anesthesiologists in parturients; the reason being possible

uteroplacental transfer and untoward effects on the baby [10]. Dexmedetomidine has many advantages over more commonly used hypnotics. Although it produces sedative, analgesic, and anxiolytic effects unlike other sedatives, it provides respiratory stability in that it does not cause ventilatory depression[8-9]. Dexmedetomidine is well suited for use in the intensive care environment, allowing sedated patients to be quickly aroused and oriented upon demand. Interestingly, this agent does not require discontinuation prior to weaning from mechanical ventilation [11,12].

The objective of the present research work was to validate a developed assay method for determination of Dexmedetomidine in dexmedetomidine hydrochloride injection by using a high performance liquid chromatography.

2. MATERIALS AND METHOD

2.1 Materials

Standards Used: Dexmedetomidine HCl working standard: Use the standard as such and use % potency on as is basis for calculations.

Batch No. : 110613, Potency: 99.9%,

Reagents and solvents used: Water (HPLC grade, Milli Q), Acetonitrile (HPLC grade, JT Baker) Methanol (HPLC grade, JT Baker), Sodium Phosphate dihydrate monobasic (GR grade), Sodium Phosphate dihydrate dibasic (GR grade). Apparatus and instruments used in experiment are listed in table 1.

Table 1 : List of Instrument Used

Sr No	Instrument	Make	Software	Detector/Model No.
1	HPLC	Waters	Empower Software	2489 dual wavelength
2	HPLC	Waters	Empower Software	2998 PDA Detector
3	Sonicator	Lab India	NA	NA
4	Weight balance	Mettler Toledo	NA	ML204
5	Oven	Thermo lab	NA	GMP
6	Photolytic Chamber	Thermo lab	NA	GMP

2.2 Methodology

2.Preparation of Sodium Phosphate dihydrate monobasic solution (Solution A): Weigh accurately 16.0 g of Sodium Phosphate dihydrate monobasic and transfer into a 1000 mL volumetric flask, add 800 mL of water and sonicate to dissolve. Make up to the mark with water & mix well.

2.2.1 *Preparation of buffer:* Weigh accurately 0.89g of Sodium Phosphate dihydrate dibasic and transfer into a 1000 mL volumetric flask. Add 900 mL of water and sonicate to dissolve, adjust with solution A to a pH of 7.0, and dilute with water to volume. Mix well.

2.2.2 Preparation of mobile phase: Methanol and Buffer (60:40)

2.2.3 *Preparation of diluent:* Dissolve 0.9g of Sodium chloride in 100ml of water.

2.2.4 *Blank:* Diluent

2.3 Chromatographic conditions

Column	LiChrospher, 100 RP-18 end capped, 4mm x 12.5 cm, 5µm
Wavelength	220 nm
Flow rate	1.0 mL/min
Injection volume	200 µL
Runtime	15minutes

2.3 Preparation of standard solution

Weigh 11.8 mg of Dexmedetomidine Hydrochloride standard into 5 mL volumetric flask, add 3mL diluent and vortex till dissolve. Dilute to volume with diluent and mix well. Then dilute 1.0ml of this solution to 20ml with diluent and mix well. Then dilute 1.0ml of this solution to 25ml with diluent and mix well.

2.4 Sample solution: Use as such.

2.5 System Suitability

The Relative standard deviation for five replicate injections should not be more than 2.0% for Standard solution.

3. RESULTS AND DISCUSSION

3.1 Linearity

A series of solutions of Dexmedetomidine HCl Standard were prepared over a range of 50% to 150% of the working concentration of Dexmedetomidine in Dexmedetomidine HCl injection (Minimum Five points should be in the range 80-120% of sample concentration for Assay). Since the working concentration of Dexmedetomidine is 4 µg per mL, the range proposed is about 2 µg per mL to 6 µg per mL. Correlation coefficient was 0.99999. Therefore, the HPLC method for the determination of Assay of Dexmedetomidine in Dexmedetomidine HCl injection is linear. Linearity reported in Table no 2.

Table 2: Table for Linearity of Dexmedetomidine

% Level	Concentration(µg/ mL)	Response (Area)	Statistical analysis	
50%	1.988	1282116	Slope	729149
80%	3.180	2162024		
90%	3.578	2449665	Intercept	-162864
100%	3.976	2731658		
110%	4.373	3027821	Correlation Coefficient	0.99999
120%	4.771	3314222		
150%	5.963	4183942		

3.2 Specificity

No interference was observed from Blank and Placebo at the retention time of Dexmedetomidine peak. Also, The Dexmedetomidine peak is pure in Standard solution and Sample solution. Specificity Data reported in table no 3.

Table 3: Table for Specificity

Sr. No.	Name	Purity Angle	Purity Threshold
1	Standard solution	0.547	1.621
2	Sample solution	0.516	1.601

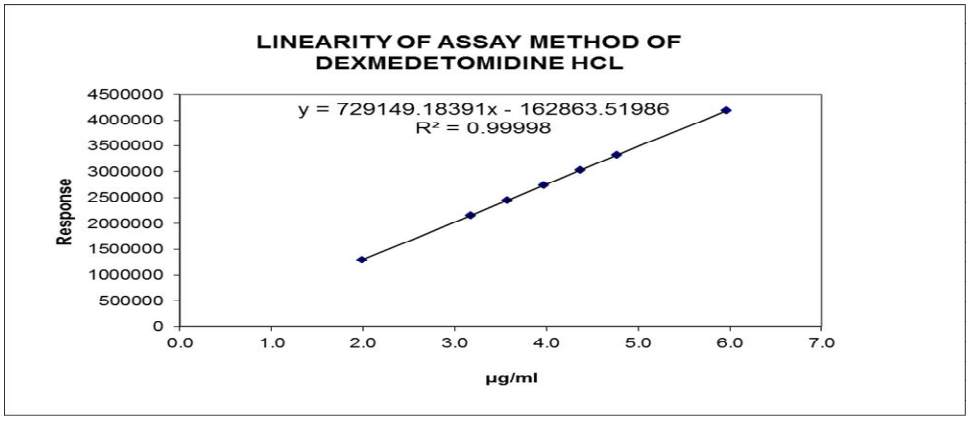

Figure 1 : Linearity graph of Dexmedetomidine

3.3 Accuracy (Recovery)

Placebo of Dexmedetomidine HCl injection was spiked with Dexmedetomidine HCl at three different levels: 80%, 100% and 120% of the label claim in triplicate (total nine determinations). Each of the sample preparations were injected in duplicate and the average area count to be taken for calculation. Mean recovery was 100.1 % & %RSD was 1.27 %. Therefore, the HPLC method for the determination of Assay of Dexmedetomidine in Dexmedetomidine HCl injection is accurate. Accuracy reported in Table no 4.

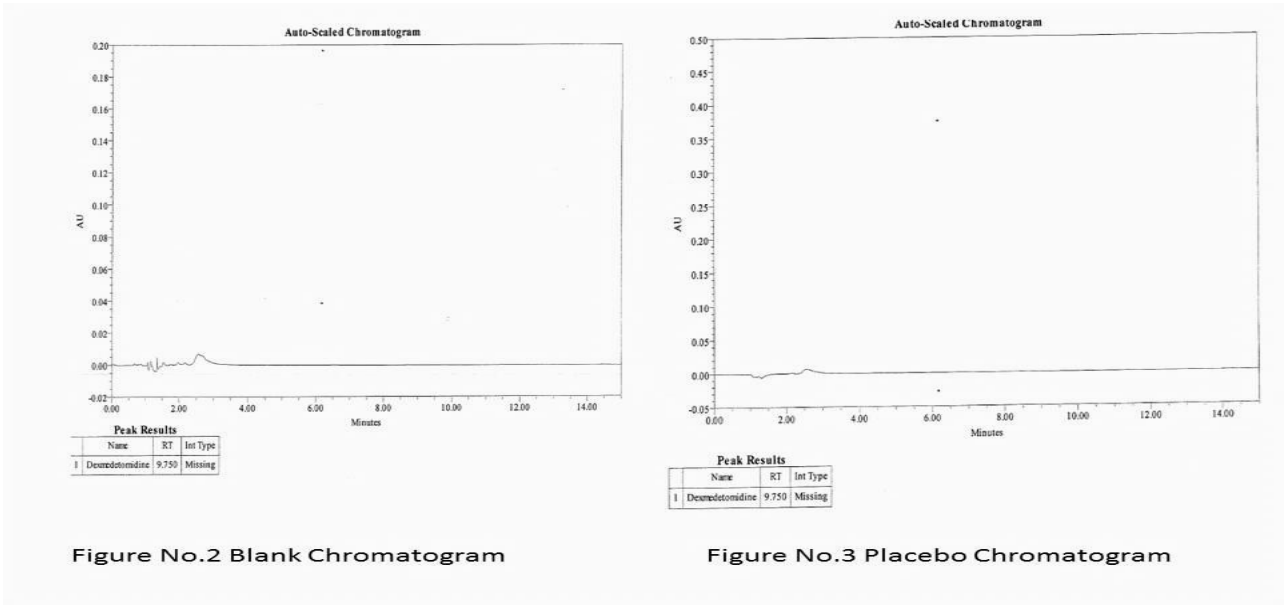

Figure No.2 Blank Chromatogram

Figure No.3 Placebo Chromatogram

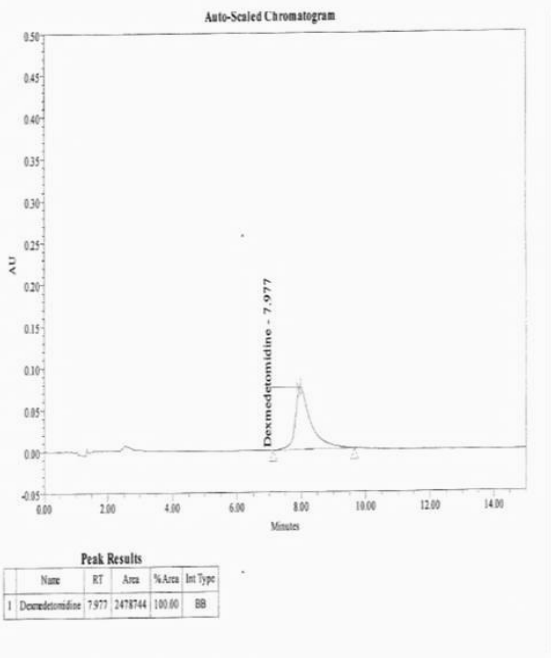

Figure No.4 Standard solution

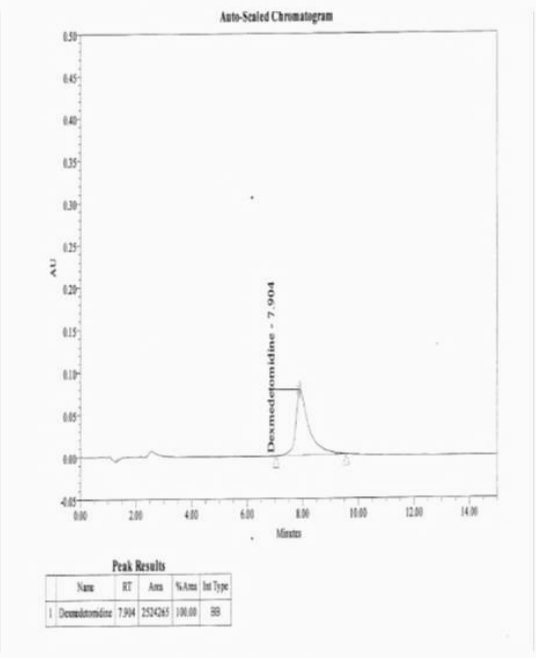

Figure No.5 Sample solution

Table No.4: Table for Accuracy

Sample No.	Amount added (mg)	Amount recovered (mg)	% Recovery
Acc. 80% -1	0.06567	0.06463	98.4
Acc. 80% -2	0.06567	0.06467	98.5
Acc. 80% -3	0.06567	0.06481	98.7
Acc. 100% -1	0.08208	0.08245	100.5
Acc. 100% -2	0.08208	0.08228	100.2
Acc. 100% -3	0.08208	0.08215	100.1
Acc. 120% -1	0.09850	0.09973	101.2
Acc. 120% -2	0.09850	0.10038	101.9
Acc. 120% -3	0.09850	0.09962	101.1
Mean		100.1	
SD		1.276	
% RSD		1.27	

3.4 Precision

3.4.1 *System Precision:* five replicate injections of the standard solution were made & injected. RSD should not be more than 2.0%. The RSD of system precision was 0.14 %. Determination of Assay of Dexmedetomidine in Dexmedetomidine HCl Injection was precise. System precision reported in table no.5.

Table 5: Table for System Precision

Injection	Area
1	2548524
2	2553781
3	2551994
4	2556155
5	2547400
Mean	2551571
SD	3631.719
%RSD	0.14

3.4.2 *Method Precision:* Six sample solutions of Dexmedetomidine HCl Injection are to be prepared and injected into the HPLC. The RSD of method precision was 0.42 %. Therefore, the HPLC method for the determination of Assay of Dexmedetomidine in Dexmedetomidine HCl Injection was reproducible.

3.5 Ruggedness (Intermediate Precision)

Six sample solutions of the same lot of Dexmedetomidine HCl injection were made by a different analyst, using different column on a different day and injected in duplicate into a different HPLC (other than that used in precision). The overall %RSD of ruggedness is 0.36 %. Therefore, the HPLC method for the determination of Assay of Dexmedetomidine in Dexmedetomidine HCl injection is rugged. Precision and ruggedness data summarized in table no 6.

Table No.6: Data of Precision and Ruggedness

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	

3.6 Robustness

System suitability should meet as per the test method at each variable condition. Overall RSD should not be more than 2.0% for the results obtained at control and variable conditions. Data Summarized in table no.7-9.

Table 7 : Change in organic phase composition (± 2% absolute)

	Control	(+2% absolute)	(-2% absolute)
	101.4	101.0	100.8
	100.6	100.7	101.7
	100.7	100.5	101.4
Mean	100.9	100.7	101.3
Sd	0.436	0.252	0.458
%RSD	0.43	0.25	0.45
Overall Mean of Control and Variable conditions		100.8	101.1
Overall % RSD of Control and Variable conditions		0.33	0.45

Table 8: Change in flow rate (± 0.1 ml/min)

	Control	(+0.1mL/min)	(-0.1mL/min)
	101.4	101.2	100.5
	100.6	101.0	100.5
	100.7	100.5	100.6
Mean	100.9	100.9	100.5
Sd	0.436	0.361	0.058
%RSD	0.43	0.36	0.06
Overall Mean of Control and Variable conditions		100.9	100.7
Overall % RSD of Control and Variable conditions		0.35	0.34

Table 9 : Change in wavelength (± 5 nm)

	Control	(+5nm)	(-5nm)
	101.4	101.8	101.4
	100.6	100.7	100.3
	100.7	100.9	101.0
Mean	100.9	101.1	100.9
Sd	0.436	0.586	0.557
%RSD	0.43	0.58	0.55
Overall Mean of Control and Variable conditions		101.0	100.9
Overall % RSD of Control and Variable conditions		0.47	0.44

3.7 System Suitability

%RSD of five replicate injections for Dexmedetomidine HCl in standard solution was within the limit as per method on everyday. The relative standard deviation of five replicate injections should not be more than 2.0%.

4. SUMMARY AND CONCLUSION

The test method is validated for Specificity, Linearity and Range, Precision, Accuracy (Recovery), Ruggedness, and Robustness found to be meeting the predetermined acceptance criteria. The validated method was Specific, Linear, Precise, Accurate, Rugged and Robust for Assay of Dexmedetomidine in Dexmedetomidine HCl injection.

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6. COMPETING INTERESTS

This study was performed in Glenmark Pharmaceutical limited Pithampur. The authors have no financial or proprietary interest in the subject matter or material discussed.

7. AUTHORS' CONTRIBUTION

Ranjith Reddy: Designed and conducted the study, procured the medicines, analysed, recorded the results and wrote the paper. Meghana Nadre & Anirudhha Sherikar: Contributed in reviewing the results. Muralee Krishna: Approved the results.

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