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ANALYTICAL METHOD VALIDATION FOR DETERMINATION OF RELATED SUBSTANCES OF DEXMEDETOMIDINE (IMPURITY-1) IN DEXMEDETOMIDINE HYDROCHLORIDE INJECTION

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ABSTRACT: Dexmedetomidine, approved by the Food and Drug Administration (FDA) in 1999 as a sedative for use in the intensive care unit, is a potent and highly selective α2-adrenoceptor agonist with significant sedative, analgesic and anxiolytic effects mostly used in the intensive care units. This article describes validation for determination of related substances of dexmedetomidine (impurity-1) in dexmedetomidine hydrochloride injection by using a high performance liquid chromatography. The high performance liquid chromatography resolution was achieved on an Phenomenex Luna C18 (2) 150 x 4.6 mm, column with an isocratic elution at a flow rate of 1.0 mL/min using a mobile phase of75-25% of Buffer with Acetonitrile. The detection was performed by a photo diode array Detector. The method was validated in the concentration range of 0.003 ppm (Limit of quantification) to 0.06 ppm (150%). The intra and inter-day precision and accuracy were within Limit (10 % RSD). The overall mean recoveries of Dexmedetomidine were 97.5% for Limit of Quantitation and 95.6 % for 50% to 150%.

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].

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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].

MATERIAL AND METHOD

Standards Used: Dexmedetomidine HCl working standard: Use the standard as such and use % potency on as is basis for calculations. Keep the container tightly closed. Batch No. : 110613, Potency: 99.9%, Impurity 1 standard: Use the standard as such and use % potency on as is basis for calculations. Keep the container tightly closed. Batch No.: 1203114, Potency: 100.3%

Reagents and solvents used: Water (HPLC grade, Milli Q), Acetonitrile (HPLC grade, JT Baker) Methanol (HPLC grade, JT Baker), Triethylamine (GR grade), Ammonium acetate (GR grade), Glacial acetic acid (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

METHODOLOGY

Preparation of buffer: Weigh accurately 1.5g of Ammonium acetate and transfer into a 1000mL volumetric flask. Add 800mL of water and sonicate to dissolve. Add 1.0ml of triethylamine. Mix well and bring to pH 5.0 ± 0.1 with glacial acetic acid. Make up to the mark with water. Mix well.

Preparation of mobile phase: Buffer and Acetonitrile (75:25)

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

Blank: Diluent Chromatographic conditions:

Column	Phenomenex Luna C18 (2) 150 x 4.6 mm,
Wavelength	230 nm
Flow rate	1.0 mL/min
Injection volume	1000 µL
Column Temperature	40°C
Runtime	15mins for standard and 45mins for Blank, Placebo, Resolution solution and Sample

Preparation of Resolution solution**:** Weigh accurately about 2.3mg of Dexmedetomidine Hydrochloride standard and 2.3mg Impurity 1 standard and transfer into a 10.0 mL volumetric flask, add 7 mL of diluent and sonicate to dissolve. Make up to the mark with diluent& mix well. Then dilute 1 mL to 20 mL with diluent. . Further dilute 1 mL to 5mL with diluent. Mix well.

Preparation of Standard solution: Weigh about 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. Further dilute 1.0ml of thissolution to 100ml with diluent and mix well. . Further dilute 1 mL to 25 mL with diluent. Mix well.

Preparation of Sample solution: Use as such.

Preparation of Placebo Solution: Use as such.

Preparation of System suitability Solution: Inject separately Resolution solution and Standard solution into the chromatograph, record the chromatograms, and measure the peak responses. The resolution between Dexmedetomidine and impurity 1 should be more than 6.0. The Relative standard deviation for six replicate injections should not be more than 10%, for Standard solution.

RESULT AND DISCUSSION:

Linearity: A series of Standard preparations (minimum of five preparations) in duplicate of Dexmedetomidine and Impurity 1 working standards were prepared over a range of the LOQ to 150% of specification limits (taken as 1.0% of Impurity 1 and 1.0 % of Dexmedetomidine). The Correlation coefficient for Dexmedetomidine and impurity 1 is more than 0.99. Therefore, HPLC Method for the determination of related substances of Dexmedetomidine (Impurity-1) in Dexmedetomidine Hydrochloride Injection is linear. Linearity reported in **Table 2-3**.

TABLE 2: TABLE FOR LINEARITY OF DEXMEDETOMIDINE

Level	Concentration (µg/ml)	Response (Area 1)	Response (Area 2)
LOQ	0.004	4522	4522
Lin-1	0.008	7704	7580
Lin-2	0.020	17058	16943
Lin-3	0.032	27596	28237
Lin-4	0.040	32768	33619
Lin-5	0.048	39699	39704
Lin-6	0.060	49406	50456
	Slope	800378	817616
	Intercept	1296	1104
	Correlation Coefficient	0.99975	0.99949

TABLE 3: TABLE FOR LINEARITY OF IMPURITY 1

Level	Concentration (µg/ml)	Response (Area 1)	Response (Area 2)
LOQ	0.003	7340	7340
Lin-1	0.008	22573	21190
Lin-2	0.020	57935	59254
Lin-3	0.032	93166	94154
Lin-4	0.040	118311	118365
Lin-5	0.048	139749	141262
Lin-6	0.060	177683	176514
	Slope	**2973720**	**2924366**
	Intercept	**-1528**	**-687**
	Correlation Coefficient	**0.99992**	**0.99970**

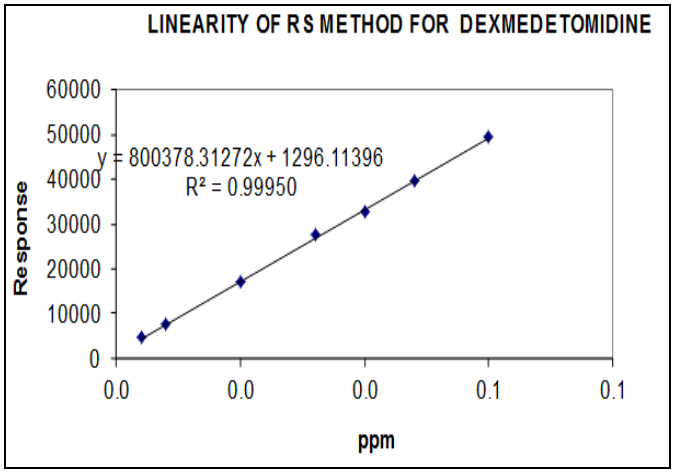

FIG.1: LINEARITY GRAPH OF DEXMEDETOMIDINE

Specificity: Blank (diluent), sensitivity solution, system suitability solution, placebo solution, diluted standard solution, all known impurity solutions individually, sample solution and sample solution spiked with all known impurities at specification level were prepared and injected into the HPLC equipped with a photodiode array detector and analysed. Peak purity passed for Dexmedetomidine and Impurity 1 in control sample and spiked sample. Data is reported in **Table 4** and **5** and **Fig. 2, 3** and **4**. Prepared Placebo solution of Dexmedetomidine Hydrochloride Injection. Injected diluent and Placebo preparations in an

HPLC using a photodiode array detector. No interference was observed from Blank and Placebo at the retention time of Dexmedetomidine and Impurity 1 peak.

TABLE 4: PEAK PURITY OF STANDARD AND CONTROL SAMPLE

Sample	Dexmedetomidine		Retention time (min)
	Purity angle	Purity Threshold	
Standard solution	4.544	6.084	11.588
Control sample	0.654	1.282	28.645

TABLE 5: PEAK PURITY OF SPIKED SAMPLE

Sample	Purity angle	Purity Threshold
Dexmedetomidine	0.397	1.188
Impurity 1	8.343	12.026

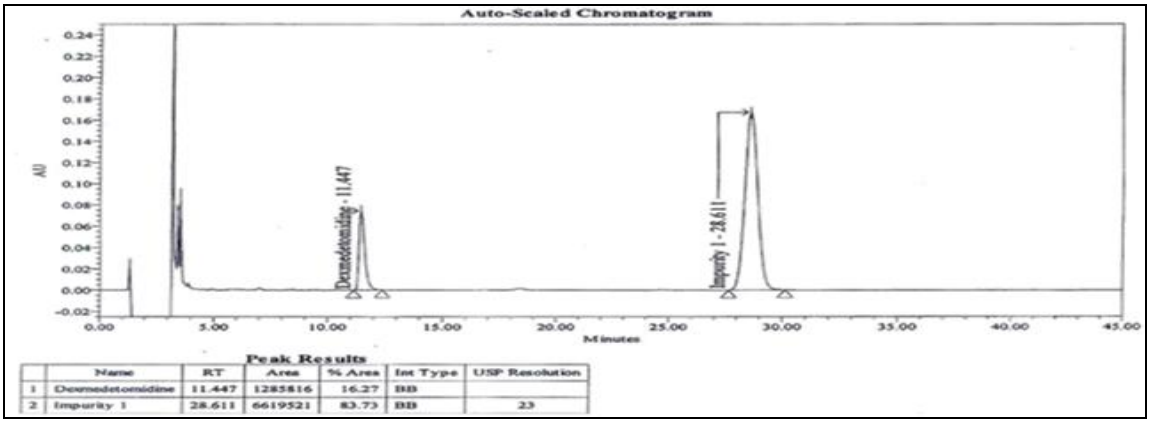

FIG.2: RESOLUTION SOLUTION

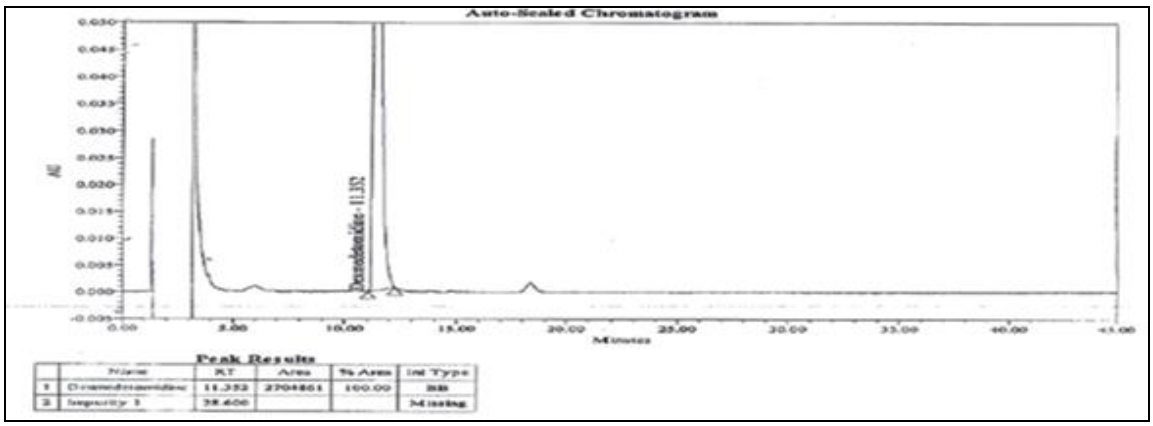

FIG.3: CONTROL SAMPLE

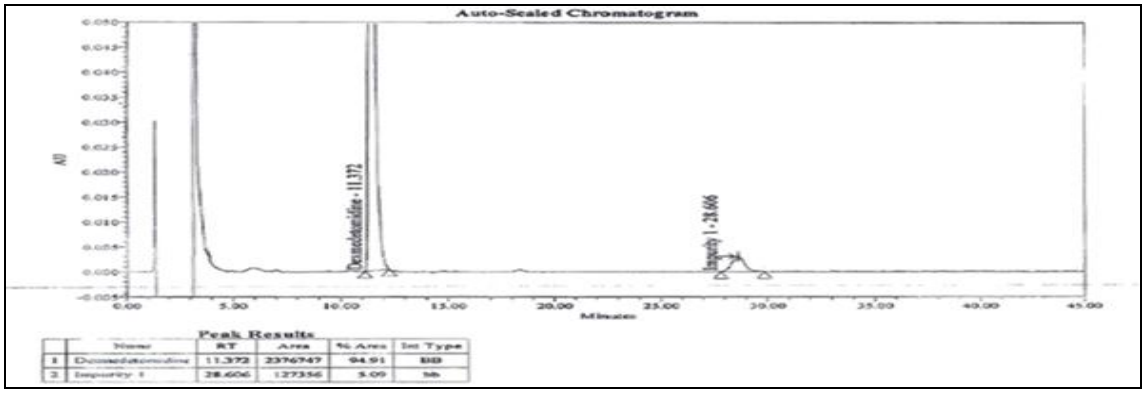

FIG.4: SPIKE SAMPLE

Forced Degradation Studies:

Acid Degradation (5N HCl/70°C/3hours): Pipetted out 10ml of sample solution, to a 50-mL stopper test-tube, added 1 ml of 5N HCl and heated on water bath at 70°C for 3 hrs. Cooled to room temperature. Added 1 mL of 5N NaOH to neutralize the solution and injected.

Base Degradation (2N NaOH/ 70°C/3 hours): Pipetted out 10ml of sample solution, to a 50-mL stopper test-tube, added 1 ml of 2N NaOH and heated on water bath at 70°C for 3 hrs. Cooled to room temperature. Added 1 mL of 2N HCl to neutralize the solution and injected.

Peroxide Degradation (50% H_2O_2/70°C/3hours): Pipetted out 10ml of sample solution, to a 50-mL stopper test-tube. Added 1 ml of 50% H_2O_2 and heated on water bath at 70°C for 3 hrs. Cooled to room temperature and injected.

Thermal Degradation (60°C/72 hours):
Samples were exposed at 60°C for 72h and analysed.

Humidity Degradation (25°C/92%RH/72 hours.): Samples were exposed at 25°C/92%RH humidity condition for 72 hrs & analysed.

Photolytic Degradation (1.2 million lux hours): Samples were exposed to 1.2 million lux hours of light and analysed. Simultaneously subjected the placebo to all the above finalized stress conditions and prepared the solutions in a similar manner followed for test sample and injected into HPLC. The peak purity data of Dexmedetomidine peak in every degradation sample shows that Dexmedetomidine peak and Impurity 1 peaks are homogeneous and there are no co-eluting peaks indicating that the method is stability indicating and specific. Forced degradation data is given in **Table 6**.

TABLE 6: TABLE FOR IMPURITIES IN FORCED DEGRADATION STUDIES

Sr. No.	Experiment	Degradation Condition	% Impurity 1
1	Control Sample	--	ND
2	Acid Degradation	5N HCl – 70°C/3 hours	ND
3	Base Degradation	2N NaOH– 70°C/3 hours	ND
4	Peroxide Degradation	50% H_2O_2 – 70°C/3 hours	1.751
5	Thermal Degradation	60°C – 72 hours	ND
6	Photolytic Degradation	1.2 million lux hours	ND
7	Humidity Degradation	25°C/92%RH – 72 hours	ND

Limit of Detection and Limit of Quantification: Based on determination of Prediction linearity, six replicate injections were made for LOD & LOQ. Datais summarizedin the given **Table 7**.

TABLE 7: LIMIT OF DETECTION AND LIMIT OF QUANTITATION

	Dexmedetomidine	Impurity 1
	LOD	
(%)	0.05	0.04
(µg/mL)	0.002	0.0015
% RSD	8.64	10.69
	LOO	
(%)	0.10	0.08
(µg/mL)	0.004	0.003
% RSD	6.12	6.49

Accuracy:

Sample of Dexmedetomidine Hydrochloride Injection, were spiked with Impurity 1 at four different levels: LOQ, 50%, 100%, and 150% of specification limits (taken as 1.0% of Impurity 1) in triplicate (in total twelve determinations) and analysed. The Mean Recovery for known impurities is within limits. Therefore, the HPLC Method for the determination of related substances of Dexmedetomidine (Impurity-1) in Dexmedetomidine Hydrochloride Injection is accurate. Accuracy reported in **Table 8**.

TABLE 8: TABLE FOR RECOVERY OF IMPURITY 1

Sample No.	Amount added (mg)	Amount recovered (mg)	% Recovery		
Acc. LOQ-1	0.000061	0.000064	105.1	Mean	97.5
Acc. LOQ-2	0.000061	0.000055	90.8	SD	7.192
Acc. LOQ-3	0.000061	0.000059	96.6	% RSD	7.38
Acc. 50% -1	0.000406	0.000378	93.1		
Acc. 50% -2	0.000406	0.000386	95.1	Mean	95.6
Acc. 50% -3	0.000406	0.000390	96.1		
Acc. 100% -1	0.000812	0.000761	93.7	SD	1.908
Acc. 100% -2	0.000812	0.000795	97.9		
Acc. 100% -3	0.000812	0.000800	98.5		
Acc. 150% -1	0.001218	0.001165	95.6	% RSD	2.00
Acc. 150% -2	0.001218	0.001141	93.7		
Acc. 150% -3	0.001218	0.001179	96.8		

Precision: System Precision: Six replicate injections of the standard solution were made & injected. RSD should not be more than 10.0%. The RSD of system precision is 2.43 %. Therefore, the HPLC Method for the determination of related substances of Dexmedetomidine (Impurity-1) in Dexmedetomidine Hydrochloride Injection is precise.

Method Precision:

Six Sample solutions of Dexmedetomidine Hydrochloride Injection spiked with Known impurity was prepared and injected into the HPLC, along with standard solution. RSD should not be more than 10.0%. RSD is less than 10.0%. Therefore, the HPLC Method for the determination of related substances of Dexmedetomidine (Impurity-1) in Dexmedetomidine Hydrochloride Injection is precise.

Ruggedness (Intermediate Precision): Six Sample solutions of the same lot of Dexmedetomidine Hydrochloride Injection, spiked with Known Impurity was made by a different analyst and analysed using different column on a different day and injected into a different HPLC, along with Standard solution. Overall RSD is less than 10.0%. Therefore, the HPLC Method for the determination of related substances of Dexmedetomidine (Impurity-1) in Dexmedetomidine Hydrochloride Injection is rugged. Precision and ruggedness data summarized in **Table 9**.

TABLE 9: PRECISION & RUGGEDNESS

Sr No		1	2	3	4	5	6
Precision	% impurity 1	1.009	1.005	1.016	0.994	1.005	0.990
Ruggedness	% impurity 1	1.091	1.101	1.089	1.095	1.076	1.069
	Mean	1.045		**SD**	0.0045	**% RSD**	4.30

System Suitability: Recorded resolution between Dexmedetomidine and impurity 1 Recorded Relative standard deviation for six replicate injections for Standard solution. System suitability given in **Table 10**.

TABLE 10: SYSTEM SUITABILITY

Sr. No.	Experiment	% RSD	Resolution between Dexmedetomidine and impurity 1
1	Accuracy, Precision, Solution Stability	2.43	22.88
4	LOD & LOQ, Linearity	0.77	23.33
5	Specificity, FD	3.57	23.00
6	Ruggedness	1.74	23.21

SUMMARY AND CONCLUSION: The test method was validated for Specificity, LOD/LOQ, Linearity and range, Precision, Accuracy (Recovery), Ruggedness and found to be meeting

the predetermined acceptance criteria. The validated method is Specific, Linear, Precise, Accurate and Rugged for Related substances of Dexmedetomidine (Impurity-1) in Dexmedetomidine Hydrochloride Injection. Hence this method can be introduced into routine use for the related substances of Dexmedetomidine (Impurity-1) in Dexmedetomidine Hydrochloride Injection.

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