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DEVELOPMENT AND VALIDATION OF A STABILITY INDICATING METHOD FOR ASSAY OF SUGAMMADEX SODIUM INJECTION BY RP HPLC METHOD

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ABSTRACT

A robust, specific, and stability-indicating reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the quantification of Sugammadex in Sugammadex sodium injection. Chromatographic separation was achieved using a Kinetex C18 100Å column (150 × 4.6 mm, 2.6 µm) operated in gradient mode. The mobile phase consisted of 0.1% v/v orthophosphoric acid as Mobile Phase A, and a mixture of Buffer: Isopropyl Alcohol: Acetonitrile (25:15:60) as Mobile Phase B, with a flow rate of 1.0 mL/min. Detection was carried out at 210 nm, and the retention time for Sugammadex was approximately 8.0 minutes.

The method demonstrated excellent linearity in the concentration range of 940–2800 µg/mL, with a mean %RSD for precision of 0.40% and mean % recovery ranging from 99.3% to 100.3%, confirming its accuracy and reproducibility. The stability-indicating capability of the method was established through forced degradation studies, ensuring reliable quantification in the presence of degradation products. The method was validated in accordance with ICH guidelines, confirming its suitability for routine quality control and stability testing of Sugammadex sodium injection.

Introduction

Sugammadex is a selective relaxant binding agent and a modified γ-cyclodextrin, chemically known as *6-per-deoxy-6-per-(2-carboxyethyl)thio-γ-cyclodextrin, sodium salt*. Its unique cyclic oligosaccharide structure contributes to high polarity and excellent water solubility, making it suitable for intravenous administration. Sugammadex is primarily indicated for the reversal of neuromuscular blockade induced by vecuronium bromide and rocuronium bromide, two commonly used non-depolarizing neuromuscular blocking agents during surgical procedures. These agents facilitate muscle relaxation for ventilation, general anesthesia, and tracheal intubation, but require effective reversal to ensure timely postoperative recovery. Sugammadex acts by encapsulating the steroidal neuromuscular blockers, forming stable complexes that are

rapidly eliminated from the body, thereby restoring neuromuscular function. This mechanism offers a novel and targeted approach compared to traditional cholinesterase inhibitors, with improved safety and efficacy profiles. The drug was approved by the United States Food and Drug Administration (FDA) on December 15, 2015, under the brand name Bridion, marking a significant advancement in anesthetic pharmacology [1,2].

The Sugammadex Injection 100 mg/mL is not official in United States Pharmacopoeia (USP) or any other pharmacopeia. This study was undertaken to validate an HPLC method according to the ICH guidelines to be performed for stress degradation of **Sugammadex sodium injection** in different conditions. An additional goal was to investigate the degradation kinetics of **Sugammadex sodium injection** in acidic, basic, oxidative conditions and heat conditions.

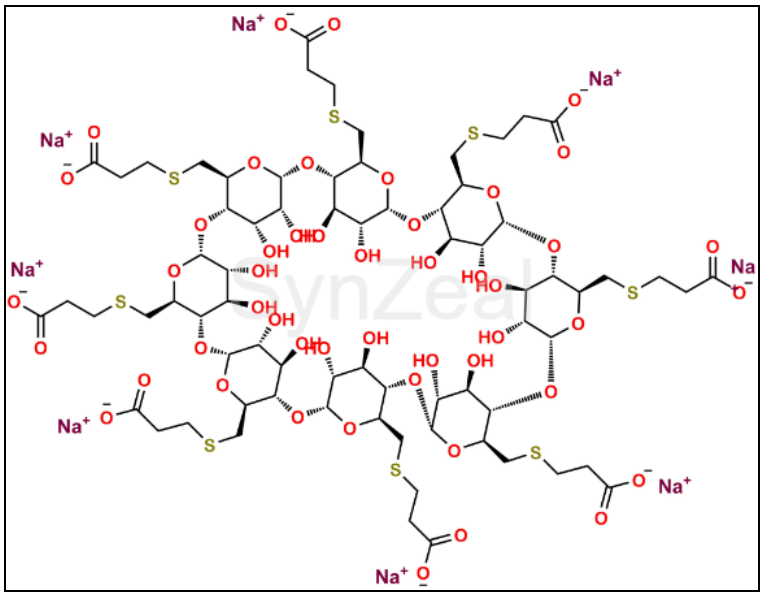

Figure 1: Chemical Structure of Sugammadex sodium

Material and MethodS

Sugammadex working standard, Sugammadex sodium injection , 2-Propanol, Orthophosphoric acid (85%), Acetonitrile. High purity water was prepared by Milli-Q purification system . All chemicals were of analytical grade. Chromatographic separation was carried out on a Waters HPLC system equipped with an Gradient pump , an autosampler and a variable UV-vis detector -PDA detector.

Chromatographic Conditions

A Kinetex C18 column (2.6 µm, 150×4.6mm i.d.) was used for chromatographic separation. Chromatographic separation was performed on a Kinetex C18 column . The mobile phase consisted of 0.1% v/v orthophosphoric acid as Mobile Phase

A, and a mixture of Buffer: Isopropyl Alcohol: Acetonitrile (25:15:60) as Mobile Phase B, with a flow rate of 1.0 mL/min. The UV detection was performed at 210 nm.

Standard Solution

Stock standard solution of Sugammadex sodium was prepared in Diluent (Milli Q water :Acetonitrile, 80:20 to reach a final concentration of 2000 µg/ml.

Test Solution

Sugammadex sodium injection test solution was prepared in Diluent (Milli Q water :Acetonitrile, 80:20 to reach a final concentration of 2000 µg/ml.

Method Validation

The developed analytical method was further subjected to validation in accordance to the current ICH guidelines. The evaluated parameters like system suitability, specificity, linearity, accuracy, precision, accuracy, limit of detection, limit of quantification, ruggedness and robustness. [3-5,8,9,10]

Specificity:

Specificity is a key feature of HPLC, enabling accurate detection of an analyte without interference from related substances like degradants, impurities, or matrix components.

Identification tests ensure differentiation between structurally similar compounds.

Purity tests confirm accurate quantification of impurities.

Assay provides precise measurement of analyte content or potency, supporting reliable reporting and quality control.

System Suitability

System Suitability Testing (SST) ensures the HPLC system is functioning properly before sample analysis. It must be performed prior to every run, following guidelines from CDER and ICH.

Key parameters include:

- Retention time
- Tailing factor
- Column efficiency

SST confirms that the system meets performance criteria and will fail if any issue is present, preventing unreliable results.

Linearity and Range

Linearity refers to the method's ability to produce results directly proportional to the analyte concentration. It is assessed by measuring responses at multiple concentrations and analyzing the data using linear least squares regression.

Key outputs:

- Slope
- Intercept
- Correlation coefficient (R^2)

For example, linearity of Sugammadex is tested using % level of 50, 80, 100, 120, and 150 % of the target concentration.

Precision

Precision reflects the closeness of repeated measurements under similar conditions. As per ICH guidelines, it includes:

- Intraday Precision: 3 replicates at 3 concentrations levels (50, 100, 150 % of target concentrations) within a day.

- Interday Precision: Same setup across different days.
- Repeatability: 6 replicates at 100 % target concentration under identical conditions.

Accuracy

Accuracy measures how close the test results are to the true value. It is often expressed as percent recovery and can be determined by:

- Spiking analyte into a blank matrix
- Comparing with a reference standard
- Standard addition method

Limit of Detection (LOD) in HPLC

LOD is the lowest concentration of an analyte that can be detected but not necessarily quantified. It is typically expressed in ppm and determined using samples with known concentrations.

According to ICH guidelines, acceptable approaches for LOD determination include:

- Signal-to-noise ratio (typically 3:1)
- Standard deviation of the response
- Standard deviation of the slope from the linearity plot
- Visual evaluation

Formula:

$LOD=3.3\times\sigma/S$

Where:

- σ = standard deviation of the response
- S = slope of the calibration curve

Limit of Quantitation (LOQ) in HPLC

LOQ is the lowest concentration of an analyte that can be quantified with acceptable accuracy and precision under validated conditions.

Formula:

$LOQ=10\times\sigma/S$

Where:

- σ= standard deviation of the response
- S = slope of the calibration curve

LOQ is typically determined using methods recommended by ICH, such as signal-to-noise ratio, standard deviation of response, or calibration curve analysis.

Robustness in HPLC

Robustness is the ability of an analytical method to remain unaffected by small, deliberate variations in method parameters, ensuring consistent results under varied conditions.

Common parameters tested include:

- Flow rate
- Column temperature
- Mobile phase composition

Testing involves varying one parameter while keeping others constant, using standard solutions (e.g., Sugammadex). This helps identify factors that may affect method performance and ensures reliability across laboratories.

Ruggedness in HPLC

Ruggedness refers to the reproducibility of test results under varied normal conditions, such as:

- Different instruments
- Different analysts
- Different days

It demonstrates the stability and reliability of the method across diverse environments, helping ensure consistent performance in real-world applications.

RESULTS AND DISCUSSION

Specificity

The specificity of the method (analytical) for the assay of Sugammadex Sodium injection is demonstrated by injecting Diluent, Standard solution and Test Solution into the HPLC system.

Table 1: Specificity Summary

Sr. No.	Sample Name	Component Name	Specificity
1	Blank	No peak	-
2	Standard	Sugammadex	Specific
3	Test	Sugammadex	Specific

System Suitability

System Suitability Parameters were monitored by preparing 2000µg/mL standard solution of Sugammadex and the solution were injected into 5 replicates and measure parameter like retention time, theoretical plate, peak tailing. Then calculate %RSD and data shows that the system functioning correctly as %RSD observed within acceptable limit (table 2).

Table 2: System suitability data of Sugammadex

Parameters	Mean (n=5)	%RSD
RT (min)	8.1	0.5
Theoretical plates	22125	0.89
Tailing Factor	1.4	1.35

Linearity

The linearity of sugammadex was determined by analyzing at 5 independent level. The range of 50% – 150% of target concentration. The calibration curve of AUC of sugammadex vs concentration was plotted and correlation coefficient and regression line equation was calculated. The method considered to be linear as the correlation coefficient was found within acceptance criteria.

Table 3: Linearity data of sugammadex

µg/mL	Mean Area	Correlation Coefficient r ≥ 0.995
1000	1982946	1.0000
1600	3142346	
2000	3934518	
2400	4562365	
3000	5756528	

Table 4: Regression data analysis RP-HPLC

Method Parameters	Sugammadex
Wavelength	210nm
Range	1000 -3000 µg/mL
Regression coeeficient (r^2)	0.9999
Slope (m)	2004.4840
Intercept (c)	+2.3%
Correlation coefficient	1.0000

Precision and Intermediate Precision

Repeatability expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the sample at test concentration under the same operating conditions over a short interval of time.

Precision of the method was carried out by preparing and analysing six sets of Test solution from a homogenous sample of a single batch and injected Test solution and calculated the RSD (%) of the obtained % Assay of the six sets of Test Solution**.**

Intermediate precision expresses within-laboratory variations: different days and different analysts, different column (Different Lot No. and Serial No.) and different Instrument.

Precision of the system was carried out by performing System suitability as per analytical procedure and results were evaluated against the acceptance. Prepared and analysing six sets of Test solution from a homogenous sample of a single

batch and injected Test solution and calculated the RSD (%) of the obtained % Assay of the six sets of Test Solution and Cumulative % RSD of obtained % Assay of Sugammadex values of the twelve sets of Test Solution following the same analytical procedure by different analyst, on different day.

Table 5: Intraday precision data of sugammadex Assay

Occasion	**Precision**	**Intermediate Precision**
Analyst Name	Mr. Vaibhav Jain	Mr. Kamal K
Date of Analysis	18/01/2024	22/01/2024
Set No.	**% Assay of Sugammadex Sodium**	
Parameter	**Precision**	**Intermediate Precision**
1	97.3	97.1
2	97.6	98.2
3	97.1	98.0
4	97.0	98.3
5	97.1	98.4
6	97.4	98.2
Mean	97.3	98.0
% RSD	0.23	0.49
Overall % RSD	0.55	

Accuracy

Accuracy expresses the closeness of agreement between the value, which is accepted either as a conventional true value or an accepted reference value and the value found.

Accuracy was demonstrated in by spiking of Sugammadex Sodium Reference Standard into the placebo by covering the known concentration levels from 80.0 % to 120.0 % of the target concentration. Prepared each Accuracy solution of each level in triplicate set and injected each solution in duplicate into the liquid chromatograph and % Recovery calculated and evaluated against acceptance criteria by measuring the practical value against the theoretical value of each accuracy solutions. The results are tabulated in below table:-

Table 6: Accuracy: Summary of Assay of Sugammadex

Sr. No.	Accuracy Level (%)	Sets	Practical Value (ppm)	Theoretical Value (ppm)	(%) Recovery	Average Recovery (%)	% RSD
1	80	Set 1	1609.19	1609.38	100.0	100.1	0.10
		Set 2	1610.67	1608.07	100.2		
		Set 3	1595.45	1593.71	100.1		
2	100	Set 1	1999.23	2010.20	99.5	100.0	0.46
		Set 2	2007.42	2002.37	100.3		
		Set 3	2020.78	2014.12	100.3		
3	120	Set 1	2272.17	2281.33	99.6	99.6	0.25
		Set 2	2266.21	2270.89	99.8		
		Set 3	2238.18	2253.04	99.3		
Overall Mean						99.9	
Overall % RSD						0.37	

Robustness

Robustness is a measure of the method's capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

It is the ability of method to derive accurate results even when there is slight change in the condition such as Flow Rate, Mobile Phase Ratio and column oven temperature etc.

The Robustness of the analytical method was studied by performing the system suitability and determined the % Assay of Sugammadex by changing in method parameters as described in below table.

Table 7: System suitability Summary

Validation Parameter	With Standard Solution		
	Tailing Factor (Maximum)	Theoretical plate (Minimum)	% RSD for Area of Sugammadex
Robustness 1 (As such)	1.5	22077	0.1
Robustness 2 – Variation in Flow Rate: 0.9 mL/minute	1.5	20078	0.1
Robustness 3- Variation in	1.4	19053	0.2

Flow Rate: 1.1 mL/minute			
Robustness 4- Variation in Column Oven: 40°C	1.5	18073	0.1
Robustness 5- Variation in Column Oven: 44°C	1.4	19655	0.1
Robustness 6- Variation in Mobile Phase B: Buffer: 2-Propanol (IPA) : (502:28)	1.4	18632	0.1
Robustness 7- Variation in Mobile Phase B: Buffer: 2-Propanol (IPA) : (498:32)	1.4	19663	0.0
Minimum	1.4	18073	0.0
Maximum	1.5	22125	0.3
Average	1.4	20134	0.1
Acceptance Criteria	**Not more than 2.0**	**Not less than 2500**	**Not more than 2.0 %**

Degradation Study

The goal of the present study is to provide information about condition for stress testing and to establish the stability of drug substances and product. There is different condition to observed degradation of drug.

Table 8: Forced degradation Summary

Stress Study	**Stress Condition**
Acid Degradation	2.0 mL of 1 M Hydrochloric Acid Solution, kept it at Room Temperature for 96 hours, After 96 hours neutralized it with 2.0 mL of 1 M Sodium Hydroxide Solution.
Base Degradation	2.0 mL of 1 M Sodium Hydroxide Solution, kept it at Room Temperature for 96 hours, After 96 hours neutralized it with 2.0 mL of 1 M Hydrochloric Acid Solution.

Oxidative Degradation	0.1 mL of 0.5 % Hydrogen Peroxide solution, kept it at Room
Heat Degradation	Heat at 80°C for 96 hours.

Table 9: Results of Forced Degradation

Stress condition	**% Assay of Sugammadex**
As Such	97.69
Acid Degradation	97.59
Base Degradation	97.55
Oxidative Degradation	91.56
Heat Degradation	97.23

Conclusion

Sugammadex is selective relaxant binding. A simple, precise, specific, accurate, sensitive RP-HPLC method have been developed and validated. Accuracy was observed. The analytical method for Assay of Sugammadex by High Performance Liquid Chromatography in Sugammadex Injection 100 mg/mL is found specific, precise, linear, accurate, robust and stable within the studied range for finished product stage. The result of the study follows the protocol of ICH guideline. The procedure described is suitable for the routine estimation of sugammadex.

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