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Research Article

REVERSE-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY METHOD DEVELOPMENT AND VALIDATION FOR THE QUANTIFICATION OF SUGAMMADEX IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Objectives: A reproducible, precise, accurate method was developed for the analysis of sugammadex in pharmaceutical dosage form and bulk, using reverse-phase high-performance liquid chromatographic. This method is established in compliance with the International Conference of Harmonization (ICH) guidelines Q2R1.

Methods: The method development and method validation of sugammadex was performed using the instrument, SHIMADZU LC- 20 AD pumps with single pump drive- 20A UV detector. A Phenomenex C18 250 mm × 4.6 mm × 5 µm column was employed with a detection wavelength of 210 nm.

Results: The method validation was performed in accordance with ICH Q2R(1) guidelines where the calibration curve was found to be linear with r^2 as 0.9993. The limit of detection and limit of quantification were found to be 0.03784 µg/mL and 0.114667 µg/mL, respectively. The % relative standard deviation of the precision was observed to be within limits (≤2%).

Conclusion: All validation parameters, including linearity, range, detection limit, quantification limit, precision, and accuracy, were examined and found to be within the specified limits in accordance with ICH recommendations Q2(R1). In view of this, adopting this approach for routine quality control tests of both pure and pharmaceutical formulations can be done with ease.

Keywords: Sugammadex, Reverse-phase high-performance liquid chromatographic, High-performance liquid chromatographic acetonitrile, High-performance liquid chromatographic water, International conference of harmonization guidelines Q2.

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INTRODUCTION

Sugammadex sodium with brand name Bridion marketed by Merck Sharp and dohmecationobtained approval from the U.S. Food and Drug Administration in the year 2015 [1], utilized in anesthesia and surgery to counteract the effects of specific neuromuscular blocking agents (NMBAs) such as rocuronium and vecuronium. Sugammadex is a modified γ-cyclodextrin molecule with a central cavity that has hydrophobic properties, allowing it to encapsulate certain molecules, such as the NMBAs rocuronium and vecuronium [2,3]. The hydrophilic exterior of sugammadex helps it remain soluble in water [4-6].

Reverse-phase high-performance liquid chromatographic (RP-HPLC) involves hydrophobic interactions between the non-polar molecules in the sample and the non-polar stationary phase. Polar molecules move through the column more quickly, whereas non-polar compounds interact more strongly with the stationary phase and elute gradually. The stationary phase in reverse phase chromatography is non-polar, the most employed stationary phase material is a hydrophobic hydrocarbon chain bonded to a solid support, commonly referred to as C18 (octadecyl) or C8 (octyl) silica, and the mobile phase is polar [7-10].

MATERIALS AND METHODS

Materials

Chemicals

A gift sample of sugammadex from pharmaceutical industry, high-performance liquid chromatographic (HPLC) acetonitrile procured from SDFCL, and HPLC water procured from finer.

Instruments/equipment/glassware

Digital ultrasonic cleaner, Shimadzu LC 20D pump, SPD 20A detector, Phenomenex C18 column (150 mm × 4.6 mm × 3 µm) borosil pipettes, volumetric flasks (10 mL, 25 mL), Millipore Millex – GV hydrophilic PVDF 0.22 µm.

Methods

Chromatographic method development

A concentration of 1000 µg/mL was prepared by accurately weighing 10 mg of the drug sugammadex, then taken in a 10 mL volumetric flask, dissolved using HPLC water, and volume was made up to 10 ml using HPLC water as a diluent, using the prepared stock solution, a concentration of 75 µg/mL standard solution was prepared by pipetting 0.75 mL into a 10 mL volumetric flask, dissolving it with HPLC water, and volume was made upto 10 mL using HPLC water.

Optimized chromatographic conditions

- Mobile phase: HPLC water: HPLC acetone 85: 15
- Runtime: 15 min
- Retention time: 9.70 min
- Flow rate: 1 mL/min
- Column: 150 mm × 4.6 mm × 3 µm
- Detection wavelength: 210 nm.

Method validation

Preparation of standard stock solution

A 1000 µg/mL concentration stock solution was prepared by taking 25 mg of the drug and dissolving it in a 25 mL volumetric flask filled

with HPLC water. The volume was then made up to 25 mL using the same HPLC water.

Preparation of serial dilutions

The aliquots of 0.75, 2, 3, 4, and 5 mL, were taken from 1000 µg/mL into respective 10 mL volumetric flasks, dissolved using diluent, and made up with the same diluent resulting in concentrations of 75, 200, 300, 400, and 500 µg/mL, respectively.

Preparation of sample solution

To prepare a sample solution with a concentration of 200 µg/mL, 1 mL of the solution was withdrawn from the formulation of label claim 200 mg/mL and added to a 100 mL volumetric flask. Then it is dissolved using HPLC water and made up to the mark whose concentration turns out to be 100 mg/100 mL or 1000 µg/mL. From the above-prepared solution 2 mL is pipetted into a 10 mL volumetric flask, dissolved using HPLC water, and made up to the mark using HPLC water whose concentration is determined to be 200 µg/mL it was then filtered through 0.22 µm.

To prepare a sample solution with a concentration of 100 µg/mL, 1 mL of the solution was withdrawn from the formulation of label claim 200 mg/mL and added to a 100 mL volumetric flask. Then, it was dissolved using HPLC water and made up to the mark whose concentration turns out to be 100 mg/100mL or 1000 µg/mL. From the above-prepared solution 1 mL was pipetted into a 10 mL volumetric flask, dissolved using HPLC water, and made up to the mark using the same whose concentration is determined to be 100 µg/mL, it was then filtered using 0.22 µm filter.

RESULTS AND DISCUSSION

Linearity and range

The prepared serial dilutions of concentrations 75 µg/mL, 200 µg/mL, 300 µg/mL, 400 µg/mL, and 500 µg/mL were then injected into the HPLC system; their linearity data are discussed in Table 1, calibration curve graph is depicted in Fig. 2 and the overlay of chromatograms is shown in Fig. 3.

Table 1: Linear response data of sugammadex

Injection concentration (ppm)	Retention time (min)	Peak area	Theoretical plate count	Tailing factor
75	9.839	379909	104325.239	0.935
200	9.880	652543	104823.396	0.957
300	9.849	922760	105535.684	1.050
400	9.812	1209945	107164.108	0.926
500	9.815	1515584	74329.201	0.939

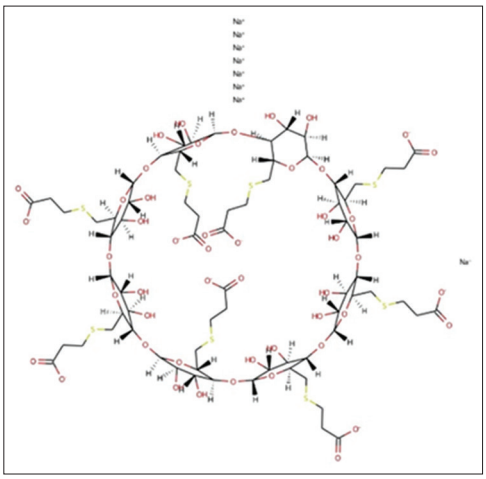

Fig. 1: Structure of sugammadex sodium

Lower range limits: Using the standard deviation (SD) method, limit of detection (LOD), and limit of quantification (LOQ) is established as shown below

a. The LOD = 3.3 σ/S
b. The LOQ = 10 σ/S [11-15].

Here, σ represents the SD of responses, while S signifies the slope of the calibration curve. The results of the LOD and LOQ of performed sugammadex quantification are observed to be 0.03784 and 0.114667, respectively, using the below-mentioned formulae and the calculation discussed below with the results as shown in Table 2.

Limits: $r^2 \geq 0.999$.

Results: The correlation coefficient $r^2 = 0.9993$ and was found to be in limits.

a. LOQ

$$DL = 3.3\frac{\sigma}{S}$$

σ = 32.43712
S = 2828.8
= 0.03784 µg/mL

b. LOQ

$$QL = 10\frac{\sigma}{S}$$

σ = 32.43712
S = 2828.8
= 0.114667 µg/ml

Precision

Repeatability (method precision)

Six replicates of the standard concentration of 300 µg/mL were performed and relative SD (% RSD) was calculated as discussed below. The % RSD of the repeatability precision is found to be 0.00351% which is considered to be within the limits given by ICH guidelines Q2R1 as is shown in Tables 3 and 4.

% RSD = (SD)/Mean × 100

Where,

$$Standard\ Deviation = \sqrt{\frac{\Sigma(x-\mu)^2}{N-1}}$$

Where,
x = individual absorbance
µ = mean of peak areas
N = number of peak areas taken [16].

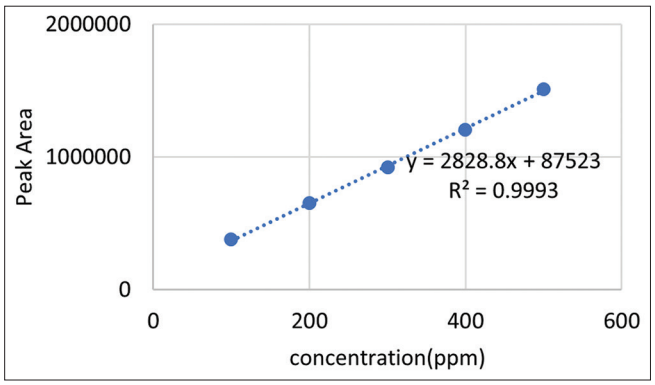

Fig. 2: Calibration curve of sugammadex

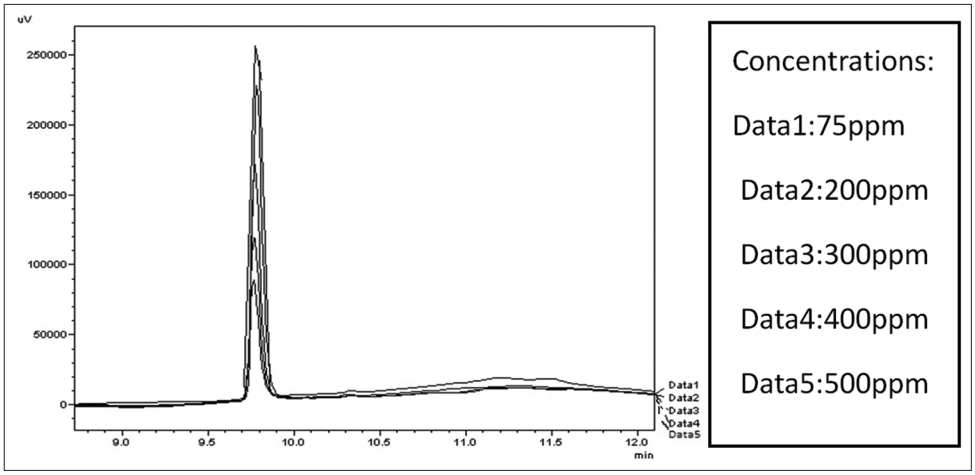

Fig. 3: Overlay of linear response chromatogram of sugammadex

Table 2: LOD and LOQ of sugammadex

LOD	0.03784
LOQ	0.114667

LOD: Limit of detection, LOQ: Limit of quantification

Table 3: Peak table of precision of sugammadex

Retention time	Peak area	Theoretical plate	Tailing factor
9.870	922760	105593.271	0.951
9.845	922714	105613.512	0.956
9.793	922790	105653.245	0.967
9.984	922798	105653.678	0.962
9.845	922774	105553.271	0.953
9.877	922735	105653.271	0.961

Table 4: Results of repeatability precision of sugammadex

Mean	922761.8
Standard deviation	32.43712
% RSD	0.003515

RSD: Relative standard deviation, Limits: % RSD ≤2%

$$Mean = \frac{922760 + 922714 + 922790 + 922798 + 922774 + 922735}{6}$$

Mean (μ) = 922761.8

$$Standard\ Deviation = \sqrt{\frac{\Sigma(x-\mu)^2}{N-1}}$$

Where, $\Sigma(x-\mu)^2$ = 5260.833 and N = 6

SD = 32.43712

$$\%RSD = \frac{Standard\ deviation}{Mean} \times 100$$

$$= \frac{32.43712}{32.43712} \times 100$$

% RSD = 0.00351.

Robustness

Deliberate changes were made to the flow rate, i.e., the flow rate was changed to 0.95 mL/min and 1.05 mL/min, and the obtained peak area for respective flow rates is discussed in Table 5.

Table 5: Robustness of sugammadex

S. No.	0.95 mL/min	1 mL/min	1.05 mL/min
1	902760	922760	922760
2	902714	922714	922714
3	902790	922790	922790
4	902798	922798	922798
5	902774	922774	922774
6	902735	922735	922735
Mean	902761.833	922761.8	922761.833
SD	32.4371187	32.43712	32.4371187
% RSD	0.0035931	0.003515	0.00351522

RSD: Relative standard deviation, SD: Standard deviation

The % RSD after deliberate changes being made are found to be within the limits (<2%) as mentioned in ICH guidelines Q2R1.

Accuracy

The standard drug concentrations selected for the study are 50 ppm, 100 ppm, and 150 ppm and the sample concentration is 200 ppm.

Spiking Procedure: At three distinct concentration levels

- 50%: 2 mL of standard concentrations of 50 ppm is spiked to the 2 mL of sample solution, of concentration 200 μg/mL
- 100%: 2 mL of standard concentrations of 100 ppm is spiked to the 2 mL of sample solution, of concentration 200 μg/mL
- 150%: 2 mL of standard concentrations of 150 ppm is spiked to the 2 mL of sample solution, of concentration 200 μg/mL.

The % recovery was found to be 98.71, 99.41, and 99.5%, and is discussed in Table 6.

Assay

The assay of sugammadex was performed was performed with the marketed formulation and the obtained calculated data are shown below.

The sample peak area was found to be 922760

The standard concentration taken was 100 μg/mL and its peak area was observed to be 923465.

$$Sample\ concentration = \frac{Sample\ peak\ area}{Standard\ peak\ area} \times Standard\ concentration$$

[17,18].

The sample concentration was found to be 99.23 μg/mL.

$$\%Assay = \frac{Peak\ area\ of\ sample}{Peak\ area\ of\ standard} \times \frac{Concentration\ of\ standard}{Concentration\ of\ sample} \times 100$$

Table 6: Accuracy of sugammadex

% Level	Concentration (ppm) (spiked sample+standard)	Sample peak area	Total peak area	Mean % recovery	% recovery
50	200+50	652543*	840045*	97.67	98.71*
100	200+100	652543*	1050100*	98.07	99.41*
150	200+150	652543*	698450*	99	99.5*

*Triplicates were taken as per ICH guidelines. Limits: % recovery should be 98–102%. Results: % recover was found to be 97–99%. ICH: International conference of harmonization

$$= \frac{922760}{923465} \times \frac{100}{99.23} \times 100 = 98.46\%$$

% assay was determined to be 98.46%.

CONCLUSION

RP-HPLC was advised for determining the concentration of sugammadex in bulk and pharmaceutical preparation since it is a straightforward, precise, reproducible, and even sensitive approach. All validation parameters, including linearity, range, detection limit, quantification limit, precision, and accuracy, were examined and found to be within the specified limits in accordance with ICH recommendations Q2(R1). In view of this, adopting this approach for routine quality control tests of both pure and pharmaceutical formulations can be done with ease.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report.

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