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Novel Bioanalytical Method Development and Validation for Sugammadex in the Biological Matrix Samples Using Analytical Instrument

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Abstract: Sugammadex is a compound characterized by its cyclodextrin structure, which plays a crucial role in reversing the effects of neuromuscular junction blockers during surgical procedures and has FDA approval in the year 2015. This drug possesses the ability to dissolve in water and demonstrates its highest absorption capacity at around 200 nm. In compliance with the M10 ICH guidelines, a bioanalytical method was meticulously developed and validated for the analysis of this particular compound. The ELICO SL-210 UV-Visible spectrophotometer was employed for this purpose. To extract the compound from human blood plasma, a protein precipitation technique utilizing acetonitrile as a precipitating agent was employed. Various validation parameters, such as extraction efficiency, calibration curve, accuracy, and precision, were calculated to assess the method's performance. The results indicated that the method was precise, accurate, uncomplicated, and easily executed, without the necessity of employing any additional advanced techniques.

Keywords: Sugammadex, Protein precipitation, Method validation, Recovery, M10 ICH guidelines, UV-Visible spectrophotometry

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Introduction

Sugammadex sodium salt has significantly impacted the medical field since its approval in 2015 (Anonymous). It has revolutionized the approach to reversing neuromuscular blockade caused by steroidal NMBDs. By selectively binding to relaxants, Sugammadex ensures that only the NMBD is reversed, while leaving other muscle relaxants unaffected. The aqueous IV dosage form of Sugammadex, known as Bridion https://www.cancer.gov/publications/

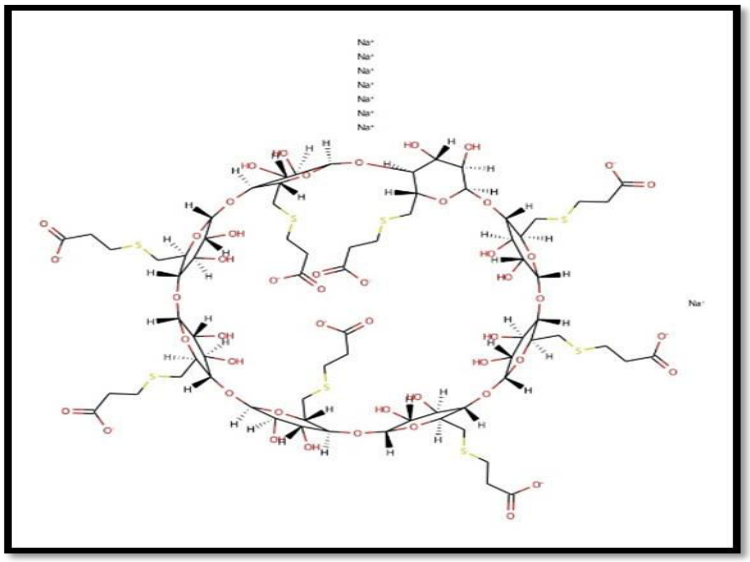

Fig. 1: Chemical structure of Sugammadex.

dictionaries/cancer-drug/def/sugammadex-sodium), is available in two different strengths for easy and efficient administration through bolus injection. The single-dose vials come in 200 mg/2 ml and 500 mg/ml, and water solubility, making preparation and administration convenient (https://go.drugbank. com/salts/DBSALT000556). This is facilitated by the hydrophilic exterior and hydrophobic core of Sugammadex's modified gamma cyclodextrin (Fig. 1) derivative (Jansook *et al*., 2018), making it an ideal candidate for selective relaxant binding. Bridion is generally well-tolerated, with minimal reported side effects. However, healthcare providers should be vigilant about the potential for rare cases of anaphylaxis, particularly in patients with a history of allergies or hypersensitivity, and closely monitor them during administration (Cada *et al*., 2016).

The protein precipitation technique (Reddy *et al*., 2016) is commonly used as the method of sample preparation for analyzing Sugammadex in human plasma (de Zwart *et al*., 2011). This technique is favored for its straightforwardness and ease of use. In this method, acetonitrile (ACN) is utilized as the precipitating agent, while hydrochloric acid is employed to create an acidic environment (Bhavyasri, *et al*., 2019; Shelke *et al*., 2021; Kagga and Parelli, 2021; https://database.ich.org/sites/default/files/M10_Guideline_Step4_2022_0524.pdf).

Materials and Methods

Chemicals used were-- ACN (AR grade), HCl 98%v/v, double distilled water, Sugammadex, human plasma (procured from blood bank). The instrument used was ELICO SL-210 UV double-beam spectrophotometer and the used Software was Spectral treats.

Preparation of standard Sugammadex stock solution:

Accurately weighing 10 mg of Sugammadex sodium (API), was carried out using a calibrated weighing balance. The measured quantity was then transferred into a 10 ml calibrated volumetric flask, and a concentration of 1000 µg/ml was prepared using double distilled water as the diluent.

Preparation of serial dilutions 25 ppm, 35 ppm, 75 ppm, 200 ppm and 400 ppm:

From the above prepared stock solution 0.25 ml, 0.35 ml, 0.75 ml, 2 ml, 4 ml were pipetted out into five different 10 ml volumetric flasks whose concentrations were 25 ppm, 35 ppm, 75 ppm, 200 ppm and 400 ppm, respectively.

Extraction of drug from the human plasma matrix:

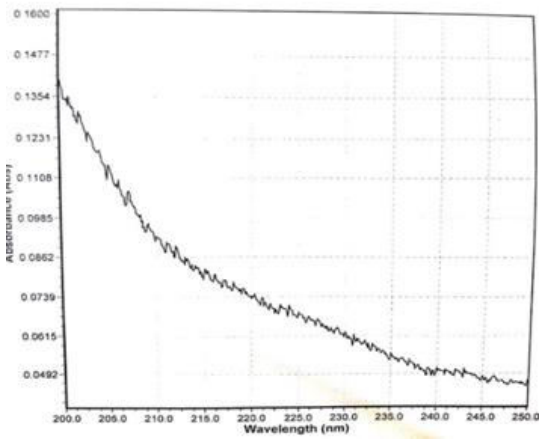

Fig. 2: UV spectrum of Blank.

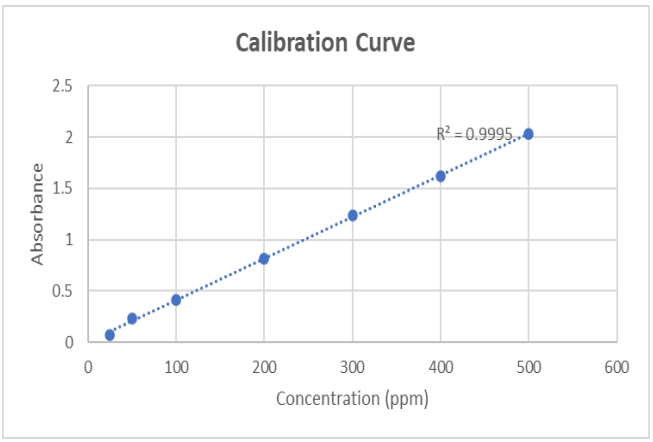

Fig. 3: Calibration curve.

The process of extracting the drug from the human plasma matrix involved 0.5 ml of a prepared drug solution placed in a centrifuge tube. To this, 2 ml of human plasma, 6 ml of acetonitrile, and 1 ml of 0. 37% HCl were added. The mixture was then subjected to centrifugation at 1700 rpm three times, with a 10 min interval between each centrifugation. Afterward, the supernatant layer was separated and further diluted using acetonitrile. The resulting dilution was then filtered and used for absorbance scanning.

(i) *Calibration curve*:

The calibration curve illustrates the connection between the expected concentration of the analyte and the response of the analytical platform toward the analyte. Calibration standards are created by adding a known amount of analyte(s) to the matrix, covering the calibration range, and forming the calibration curve.

The calibration curve is established within the concentration range of 25 ppm to 400 ppm. Absorbances at 210 nm, which is the maximum wavelength (λmax), were measured for each concentration.

(ii) *Accuracy*:

During method validation the QCs for accuracy were prepared at a minimum of 4 concentration levels within the calibration curve range: the LLOQ, within five times of the LLOQ (low QC), around 30 - 50% of the calibration curve range (medium QC) and at least 75% of the ULOQ (high QC), ULOQ.

(iii) *Precision*:

During method validation the QCs for accuracy were prepared at a minimum of 4 concentration levels within the calibration curve range: the LLOQ, within three times of the LLOQ (low QC), around 30 - 50% of the calibration curve range (medium QC) and at least 75% of ULOQ (high QC).

(iv) *Extraction efficacy*:

Evaluation of recovery is necessary for methods that utilize sample extraction. Recovery is expressed as a percentage, representing the amount of an analyte that is retained throughout the sample extraction and subsequent processing steps. To determine recovery, the response of the analyte in a biological sample that has been spiked with the analyte and processed is compared to the response of a standard analyte solution. While it is not essential for the analyte recovery to reach 100%, it is important for the recovery to be consistent.

% recovery= spiked absorbance×100÷standard absorbance

Results and Discussion

Bioanalytical method validation: According to M10 ICH guidelines the following validation parameters are discussed:

(i) *Specificity*: The UV range scan of the blank (water) was performed (Fig. 2).

Table 1: Accuracy of Sugammadex 25 ppm

S. No.	Absorbance
1	0.0769
2	0.0775
3	0.0779
4	0.0778
5	0.0765
mean	0.07732
SD	0.000601664
%RSD	0.77814842

Table 2: Accuracy of Sugammade x 35 ppm

S. No.	Absorbance
1	0.1259
2	0.127
3	0.1164
4	0.1269
5	0.1262
mean	0.12448
SD	0.000020617
%RSD	0.0165625

Table 3: Accuracy of Sugammadex 75 ppm

S. No.	Abs
1	0.8746
2	0.8754
3	0.8749
4	0.8751
5	0.8747
mean	0.87494
SD	0.000320936
%RSD	0.03668093

Table 4: Accuracy of Sugammadex 200 ppm

S. No.	Absorbance
1	0.3126
2	0.3119
3	0.3121
4	0.3122
5	0.3124
mean	0.31224
SD	0.000270185
%RSD	0.086531233

Table 5: Accuracy of Sugammadex 350 ppm

S. No.	Abs
1	1.6459
2	1.6461
3	1.6466
4	1.6458
5	1.6462
mean	1.64612
SD	0.000311448
%RSD	0.018920141

Table 6: Accuracy of Sugammadex 400 ppm

S. No.	Abs
1	1.9265
2	1.9253
3	1.9261
4	1.9259
5	1.9263
mean	1.92602
SD	0.000460435
%RSD	0.023906012

(ii) *Calibration curve* (*response function*): The calibration curve is established within the concentration range of 25 µg/ml, 75 µg/ml, 100 µg/ml, 200 µg/ml, 300 µg/ml and 400 µg/ml at 210 nm, which is the maximum wavelength (λmax), were measured for each concentration (Fig. 3).

(iii) *Accuracy:* Tables 1, 2, 3, 4, 5 and 6 represent accuracy data that was performed by scanning each prepared concentration i.e., 25 ppm, 35 ppm, 75 ppm, 200 ppm, 350 ppm and 400 ppm for 5 times at 210 nm in UV-Visible spectrophotometer, then the relative Standard deviation was calculated and observed to be below 2% as mentioned in ICH guidelines.

% RSD = (Standard Deviation / Mean) x 100

(iv) *Precision*: Evaluated by analyzing each QC concentration level in at least 3 analytical runs over at least two days (Tables 7-12). The absorbance of the six standard solution triplicates were measured at a wavelength

Table 7: Precision of 25 ppm Sugammadex

S. No.	Absorbance	
	Day 1	Day 2
1	0.0769	0.0769
2	0.0775	0.0775
3	0.0779	0.0779
Mean	0.077433	0.07743
SD	0.000503	0.000503
%RSD	0.650073	0.650073

Table 8: Precision of 35 ppm Sugammadex

S. No.	Absorbance	
	Day 1	Day 2
1	0.1259	0.1260
2	0.127	0.1172
3	0.1264	0.1252
mean	0.126433	0.1228
SD	0.000550	0.00002368
	0.004356	0.000192834
%RSD	0.435610	0.019283388

Table 9: Precision of 75 ppm Sugammdex

S. No.	Absorbance	
	Day 1	Day 2
1	0.3126	0.3129
2	0.3119	0.3121
3	0.3121	0.3127
mean	0.3122	0.312566667
SD	0.0003605	0.000416333
%RSD	0.1154885	0.133198208

Table 10: Precision of 200 ppm Sugammadex

S. No.	Absorbance	
	Day 1	Day 2
1	0.8781	0.8746
2	0.8792	0.8754
3	0.8771	0.8769
mean	0.878133333	0.875633333
SD	0.001050397	0.001167619
%RSD	0.119617	0.133345616

Table 11: Precision of 350 ppm Sugammadex

S. No.	Absorbance	
	Day 1	Day 2
1	1.6452	1.6466
2	1.6412	1.6478
3	1.6471	1.6469
mean	1.6445	1.6471
SD	0.0030	0.0006245
%RSD	0.183134	0.037915111

Table 12: Precision of 400ppm Sugammadex

S. No.	Absorbance	
	Day 1	Day 2
1	1.9465	1.9278
2	1.9253	1.9275
3	1.9261	1.9299
mean	1.93263	1.9284
SD	0.01201	0.00130767
%RSD	0.62171	0.067811122

of 210 nm, and the relative standard deviation (% RSD) was subsequently calculated using the formula: % RSD = (Standard Deviation / Mean) x 100

(v) *Extraction efficiency*:

% recovery = spiked bsorbance × 100 ÷ standard absorbance

The absorbance of the spiked sample: 0.8126

The absorbance of the standard: 0.9394

% Recovery= 86.5%

A straightforward approach for bioanalysis of Sugammadex spiked in human plasma using UV spectrophotometry was developed. The extraction procedure involved was protein precipitation. The validation process involves parameters like response function A standard calibration curve was plotted by taking the concentration of the Sugammadex spiked in human plasma on the X- axis and absorbance on Y-axis shown in Figure3. The concentrations chosen ranges from 25ppm to 400ppm. The regression coefficient was determined to be 0.999, five replicates for each concentration was carried out for accuracy whose % RSD's was measured at a wavelength of 210 nm were determined to be <2% and precision was determined at a wavelength of 210 nm, by taking three replicates of each concentration where their % RSD's were found to be <2%. The extraction efficacy (%recovery) of the drug from the biomatrix (human plasma) was determined to be 86.5%.

Conclusion

A fast, precise, and accurate protein precipitation approach using UV spectroscopic detection was used to determine the presence of Sugammadex in spiking human plasma. The method was validated. The results demonstrated the technique's applicability along with a substantial recovery rate. This technique is employed for the quantitative analysis of sugammadex in biological matrices and can be used for routine bioanalytical purposes.

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References

Bhavyasri K, Surekha T and Rambabu D. (2019) Bioanalytical method development and validation of atorvastatin in human plasma by using UV -visibile spectrophotometry. J Pharmaceut Sci Res. 11(6): 2243-2246.

Cada DJ, Levien TL and Baker DE. (2016) Sugammadex. Hospital Pharmacy 51(7): 585-596.

de Zwart MA, ten Bruggencate-Broeders J, van Hal HJ, Megens RH and Frasa HW. (2011) Determination of sugammadex in human plasma, urine, and dialysate using a high-performance liquid chromatography/ tandem mass spectrometry assay. J Chromatogr B Analyt Technol Biomed Life Sci. 879(19): 1573-1586.

Guideline ICH. (2022) Bioanalytical method validation and study sample analysis M10. ICH Harmonised Guideline: Geneva, Switzerland. 2022. https://database.ich.org/sites/default/files/M10_Guideline_Step4_2022_0524.pdf

Jansook P, Ogawa N and Loftsson T. (2018) Cyclodextrins: Structure, physicochemical properties and pharmaceutical applications. Int J Pharmaceut. 535(1-2): 272-284

Khagga BS and Parelli K. (2021) Bioanalytical method development and validation of UV spectrophotometric method for estimation of Bumetanide spiked in human urine. World J Adv Res Rev. 11(2): 241-248.

NCI Drug Dictionary [Internet]. National Cancer Institute. https://www.cancer.gov/publications/dictionaries/cancer-drug/def/sugammadex-sodium

PubChem. Sugammadex Sodium [Internet]. PubChem. https://pubchem.ncbi.nlm.nih.gov/compound/Sugammadex-Sodium

Reddy K and Yachawad A. (2016) Overview on recent extraction techniques in bioanalysis. Int Res J Pharm. 7(2): 15-24.

Shelke A, Someshwar M and Kolhe M. (2021) A review on analytical methods for estimation of apremelast in bulk, pharmaceutical formulation and in biological samples. Res J Sci Technol. 13(2):142-146.

Sugammadex sodium | DrugBank Online [Internet]. https://go.drugbank.com/salts/DBSALT000556