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Development and Validation of Stability-Indicating Reverse Phase HPLC Method for the Determination of Related Substances in Sugammadex Sodium Drug Substance

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ABSTRACT

A sensitive, precise, specific, linear, and stability-indicating gradient reverse-phase high-performance liquid chromatography (RP-HPLC) method has been developed and validated for the determination of related substances of Sugammadex sodium. The successful chromatographic separation of Sugammadex sodium from its related substances was achieved on octadecyl silane chemically bonded to porous silica particles stationary phase i.e Kromasil 100-3.5 C18, 250mm x 4.6mm, i.d., 3.5µ column maintained at 55°C using Orthophosphoric acid (OPA) as mobile phase A and mobile phase B composed of a mixture of Orthophosphoric acid (OPA): acetonitrile (ACN) (10:90 v/v) respectively. The flow rate was 0.6 mL/min, the detection wavelength was set at 205 nm and the injection volume: 10µl. The method was validated according to the ICH guidelines for specificity, linearity, accuracy, and precision, the limit of quantification and the limit of detection. Sugammadex was subjected to stress conditions of thermal, hydrolysis, humidity, peroxide and photolytic to observe the degradation products. The limit of detection of impurities were in the range of 0.013%–0.017% indicating the high sensitivity of the developed method. The method was proved to be reliable for the determination of related substances in Sugammadex sodium drug, which is essential and important in quality control. The experiment results are given in detail in this paper.

INTRODUCTION

Sugammadex sodium (ORG 25969, tradename *Bridion*) is a unique neuromuscular reversal drug; a novel cyclodextrin, the first in a new class of selective relaxant binding agents, which reverse neuromuscular blockade (NMB) with the aminosteroid non-depolarizing muscle relaxants rocuronium and vecuronium [1] in general anesthesia. Sugammadex is a synthetic cyclodextrin molecule, which acts by encapsulation of rocuronium [2-4]. Sugammadex sodium is used for prevention and treatment for the Anticoagulation. The Empirical formula of Sugammadex sodium is $C_{72}H_{104}Na_8O_{48}S_8$ and the molecular weight is 2178.01g/mol. Sugammadex sodium is chemically known as $6^A,6^B,6^C,6^D,6^E,6^F,6^G,6^H$ - octakis-*S*-(2-carboxyethyl)- $6^A,6^B,6^C,6^D,6^E,6^F,6^G,6^H$ octathio - γ-cyclodextrin sodium salt, which is a modified γ-cyclodextrin. Cyclodextrins are cyclic dextrose units joined through 1-4 glycosyl bonds that are produced from starch or starch derivates using cyclodextrin glycosyltransferase. The three natural unmodified cyclodextrins consist of 6, 7, or 8 cyclic oligosaccharides and are called α-, ß- or γ-cyclodextrin, respectively. α-cyclodextrin, ß-cyclodextrin and γ-cyclodextrin are naturally occurring compounds derived from the degradation of starch by the glycosyltransferase enzyme. They can be formed naturally from bacteria or manufactured synthetically. Sugammadex, a novel selective relaxant binding agent can reverse both shallow and profound aminosteroid-induced neuromuscular blockade and has a unique mechanism of action that distinguishes it from cholinesterase inhibitors. It exerts no effect on acetylcholinesterase or any receptor system in the body, thus eliminating the need for anticholinergic drugs and their undesirable adverse effects. Also, the unique mechanism of reversal by encapsulation is independent of the depth of neuromuscular block; thus the reversal can be accomplished even during profound neuromuscular block [5]. These modifications resulted in a sugammadex compound with a hydrophobic cavity large enough to encapsulate steroidal neuromuscular blocking drugs, especially rocuronium. The aqueous solution of sugammadex has a pH of approximately 7.5. Sugammadex exerts its effect by forming very tight complexes at a 1:1 ratio with steroidal neuromuscular blocking agents (rocuronium > vecuronium >> pancuronium) [5] The solubility of sugammadex in water is 26.6 mg/ml [6]. European public assessment report (EPAR) for Bridion.[7] Bridion is a solution for injection that contains the active substance sugammadex (100mg/ml).

Sugammadex is a modified γ-cyclodextrin, with a lipophilic core and a hydrophilic periphery. This gamma-cyclodextrin has been modified from its natural state by placing eight carboxyl thioether groups at the sixth carbon positions. These extensions extend the cavity size

allowing greater encapsulation of the rocuronium molecule. These negatively charged extensions electrostatically bind to the quaternary nitrogen of the target as well as contribute to the aqueous nature of the cyclodextrin. Sugammadex's binding encapsulation of rocuronium is one of the strongest among cyclodextrins and their guest molecules. The rocuronium molecule bound within sugammadex's lipophilic core is rendered unavailable to bind to the acetylcholine receptor at the neuromuscular junction. Sugammadex, unlike neostigmine, does not inhibit acetylcholinesterase so cholinergic effects are not produced and co-administration of an antimuscarinic agent (glycopyrronium bromide or atropine) is not needed. Sugammadex might therefore be expected to have fewer adverse effects than the traditional reversal agents.

When muscle relaxant with rapid onset and short duration of action is required, there has been little choice apart from succinylcholine but this drug has important contraindications; for example, it can trigger malignant hyperthermia in susceptible individuals, it has a prolonged duration of action in patients with pseudocholinesterase deficiency and it causes an increase in plasma potassium concentration which is dangerous in some circumstances. Rocuronium has a comparably quick onset in high dose (0.6 mg kg^{-1} to 1 mg kg^{-1}) and can be rapidly reversed with sugammadex (16 mg kg^{-1}), so this drug combination offers an alternative to suxamethonium.

'Recurarisation', a phenomenon of recurrence of neuromuscular block, may occur where the reversal agents wear off before a neuromuscular blocking drug is completely cleared. This is very unusual with all but the longest acting neuromuscular blocking drugs (such as gallamine, pancuronium or tubocurarine). It has been demonstrated to occur only rarely with sugammadex, and only when insufficient doses were administered. The underlying mechanism is thought to be related to the redistribution of relaxant after reversal. It may occur for a limited range of sugammadex doses which are sufficient for complex formation with relaxant in the central compartment, but insufficient for additional relaxant returning to central from peripheral compartments. [8-9]

Sugammadex has been shown to have an affinity for two other aminosteroid neuromuscular blocking agents, vecuronium and pancuronium. Although sugammadex has a lower affinity for vecuronium than for rocuronium a reversal of vecuronium is still effective because fewer vecuronium molecules are present in vivo for equivalent blockade: vecuronium is approximately seven times more potent than rocuronium. Sugammadex encapsulates with a

1:1 ratio and therefore will adequately reverse vecuronium as there are fewer molecules to bind compared to rocuronium. Shallow pancuronium blockade has been successfully reversed by sugammadex in phase III clinical trials.[10-11]

Sugammadex is a very novel and recently synthesized drug. It is UV inactive compound. It is not listed in Pharmacopoeias. Best our knowledge, HPLC literature methods and Pharmacopoeia methods are not available for the determination of Sugammadex and its related substances. However, a few methods have been reported in the literature for the Determination of Sugammadex in human plasma, urine and dialysate using a high-performance liquid chromatography/tandem mass spectrometry assay. [12]

The reported methods are related to Bioanalytical methods. Hence, the stability-indicating RP-HPLC method has been developed for the quantification of impurities related to Sugammadex.

The developed chromatographic method can resolve all these impurities with adequate resolution to achieve good chromatography and the optimized methodology has been validated to accomplish as per ICH guidelines [13-14]. The chemical structures of Sugammadex sodium (SGM) and its related impurities are shown in Fig.1 to Fig.6 in Table 1.

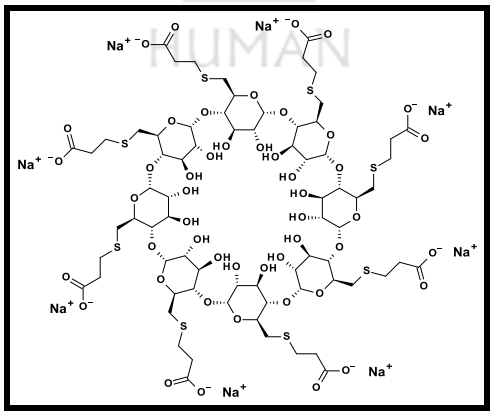
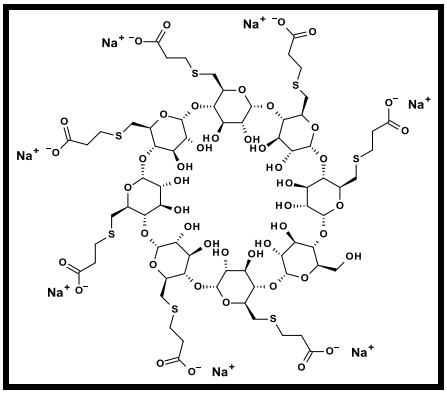
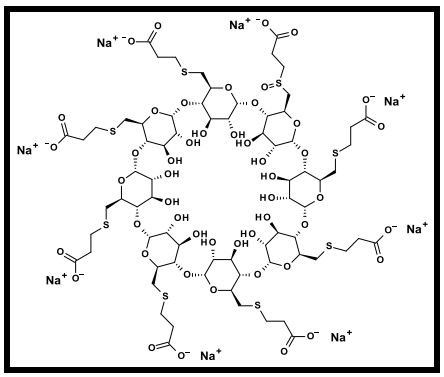
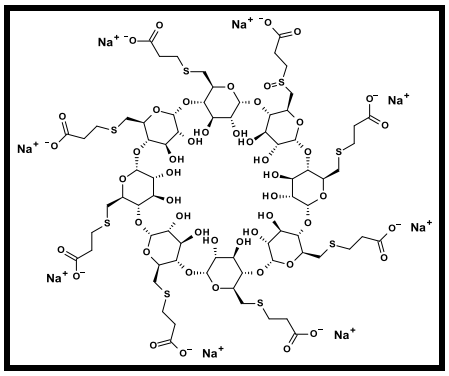
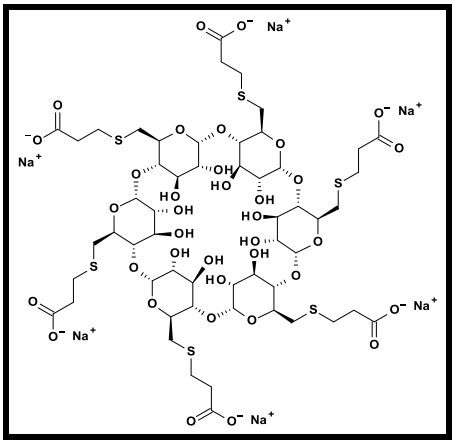
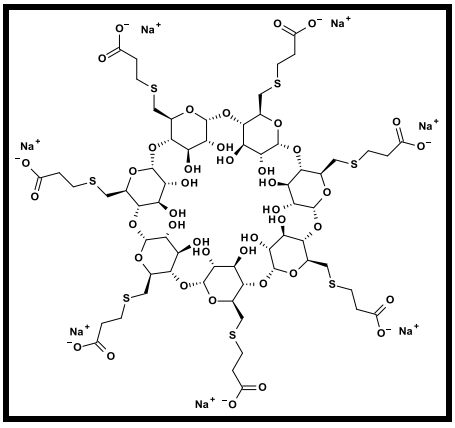


Figure No. 1: Chemical structure of Sugammadex sodium

Table No. 1: Chemical structures of related impurities:

SGM/ Monohydroxy impurity	$6^A,6^B,6^C,6^D,6^E,6^F,6^G$-heptakis-S-(2-carboxyethyl)-$6^A,6^B,6^C,6^D,6^E,6^F,6^G$-heptathio-γ-cyclodextrin sodium salt (or) Heptakis(6-deoxy-6-(2-carboxyethyl)thio)-gamma-cyclodextrin sodium salt Mol. Formula : $C_{69}H_{101}Na_7O_{47}S_7$ Mol. Weight : 2067.90	 **Figure No. 2: Monohydroxy impurity**
SGM/Sulfoxide impurity-1	Heptakis(6-deoxy-6-(2-carboxyethyl)thio)-mono(6-deoxy-6-(2-carboxyethyl)sulfinyl)gamma-cyclodextrin sodium salt Mol. Formula : $C_{72}H_{104}Na_8O_{49}S_8$ Mol. Weight: 2194.00	 **Figure No. 3: Sulfoxide impurity-1**

SGM/Sulfoxide impurity-2	Heptakis(6-deoxy-6-(2-carboxyethyl)thio)-mono(6-deoxy-6-(2-carboxyethyl)sulfinyl)gamma-cyclodextrin sodium salt Mol. Formula : $C_{72}H_{104}Na_8O_{49}S_8$ Mol. Weight: 2194.00	 **Figure No. 4**: **Sulfoxide impurity-2**
SGM/Alpha impurity	$6^A,6^B,6^C,6^D,6^E,6^F$-hexakis-S-(2-carboxyethyl)- $6^A,6^B,6^C,6^D,6^E,6^F$-hexathio-α-Cyclodextrin sodium salt. (or) 6-per-deoxy-6-per-(2-carboxyethyl) thio-α-cyclodextrin Sodium salt. Mol. Formula : $C_{54}H_{78}O_{36}S_6.6Na$ Mol. Weight: 1633.50	 **Figure No. 5: Alpha impurity**
SGM/Beta impurity	$6^A,6^B,6^C,6^D,6^E,6^F,6^G$-heptakis-S-(2-carboxyethyl)- $6^A,6^B,6^C,6^D,6^E,6^F,6^G$-heptathio- β -cyclodextrin sodium salt (or) 6-per-deoxy-6-per-(2-carboxyethyl) thio-β-cyclodextrin Sodium salt. Mol. Formula : $C_{63}H_{91}O_{42}S_7.7Na$ Mol. Weight: 1905.75	 **Figure No. 6: Beta impurity**

MATERIALS AND METHODS:

Chemicals, reagents, standards and samples:

The investigated samples of Sugammadex sodium drug substance, its related impurities, and Sugammadex for system suitability were arranged from Natco Research Centre (A division of Natco Pharma Ltd., Hyderabad). Acetonitrile procured from Merck, India. Orthophosphoric acid (~88%) was procured from Rankem and pure milli-Q water was used with the help of Millipore purification system (Millipore®, USA).

Instrumentation and Methodology:

The HPLC system used for method development, method validations as well as forced degradation studies on Waters Alliance 2695 separation module equipped with 2996 photodiode array detector and UV detector with Empower data handling system i.e. Empower 3 software, Build No: 2154 [Waters Corporation, MILFORD, MA 01757, USA] was used. HPLC column: Kromasil 100-3.5 C18, 250mm x 4.6mm, i.d., 3.5µ (Make: Akzonobel), column oven temperature: 55°C, Sample cooler: 5°C, Mobile phase A: Transfer 2.0mL of Orthophosphoric acid in 1000 mL of Milli-Q-water, mix well and filter through 0.45µ membrane. Mobile phase B: Mixture of Mobile phase A and Acetonitrile in the ratio of 100:900 (v/v), mix well and sonicate to degas. Diluent: Water, Flow rate: 0.6 ml/min, injection volume: 10µl, data acquisition time: 70 min, test concentration: 2.5mg/mL and UV detection: 205 nm. The retention time of Sugammadex is about 18 minutes. The pump is in gradient mode and the program is as follows: Time (min)/ A (v/v): B (v/v); T0/80:20, T30/78:22, T35/78:22, T40/70:30, T48/60:40, T60/50:50, T62/80:20, T70/80:20.

Preparation of solutions:

Standard solution: Dissolve 10mg of Sugammadex sodium standard into a 50mL volumetric flask, dissolve in 20mL of diluent, then makeup to mark with diluent.

Reference solution:

Transfer 0.375mL of standard solution into a 20 mL volumetric flask, dilute and make up to the mark with diluent.

Impurity stock solution:

Dissolve each 5mg of SGM/Mono hydroxy, SGM/Sulfoxide impurity-1, SGM/Sulfoxide impurity-2, SGM/Alpha impurity and SGM/Beta impurity standards into a 10mL volumetric flask, dissolve in 2.0mL of diluent, then make up to mark with diluent.

System suitability solution:

Dissolve 25mg of Sugammadex sodium standard into a 10mL volumetric flask, add 0.25ml of impurity stock solution to dissolve in 2mL of diluent, then make up to mark with diluent.

Evaluation of system suitability:

Inject duplicate injections of blank followed by six replicate injections of the reference solution and system suitability solution once into the chromatograph and record the chromatograms. The system is suitable for analysis if and only if,

1. The relative standard deviation for Sugammadex peak in reference solution should be no more than 10.0%.

2. Resolution between SGM/Beta impurity and SGM peaks in system suitability solution should be not less than 1.5.

3. Theoretical plates for SGM peak in system suitability solution should be not less than 5000.

4. USP Tailing for SGM peak in system suitability solution should be not more than 2.0.

Test solution:

2.5 mg/ml concentration of solution using Sugammadex sodium sample in diluent.

RESULTS AND DISCUSSION

Method Validation

Specificity:

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present for the determination of specificity, injection of blank and all individual impurities solutions were prepared and injected to confirm the retention times. The solutions of Sugammadex sodium drug substance (Control Sample) and

Sugammadex sodium spiked with known related substances at specification level (Spiked Sample) were prepared and injected into HPLC. Peak purity was established by using Empower Software. The purity angle should be less than the purity threshold for all known related substances from spiked sample. The specificity results are tabulated in Table 2. A typical representative HPLC chromatogram of Sugammadex sodium drug substance spiked with all impurities is shown in Fig.7&8.

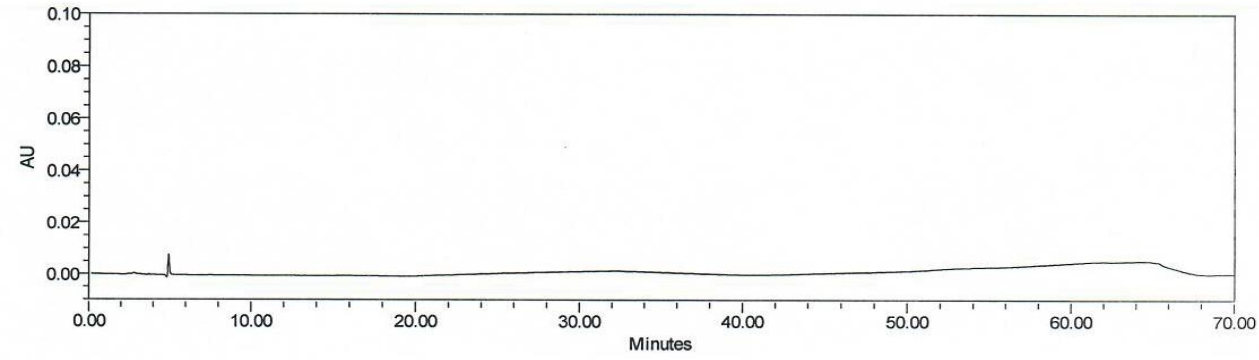


Figure No. 7: A typical representative HPLC chromatogram of blank

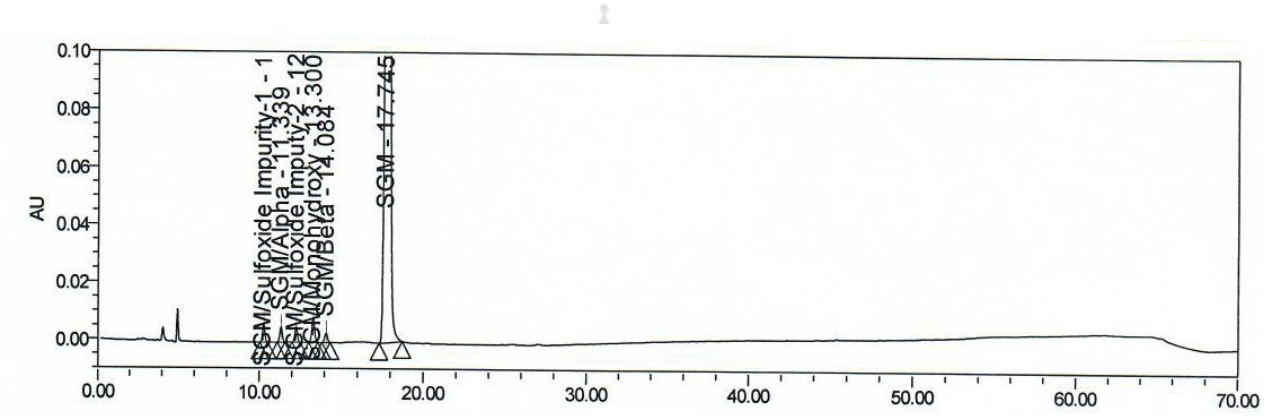


Figure No. 8: A typical representative HPLC chromatogram of spiked with all impurities

Table No. 2: Specificity of impurities from system suitability solution

Peak Name	Retention time (Minutes)	Relative retention time (RRT)	Peak Purity	
			Purity angle	Purity Threshold
SGM/Sulfoxide impurity-1	10.277	0.579	0.679	0.831
SGM/Alpha impurity	11.339	0.639	0.838	0.982
SGM/Sulfoxide impurity-2	12.256	0.691	0.676	0.916
SGM/Mono hydroxy	13.300	0.749	0.652	0.698
SGM/Beta impurity	14.084	0.794	1.169	1.440
Sugammadex sodium (SGM)	17.745	1.000	0.103	0.261

Forced degradation:

The degradation behavior of Sugammadex sodium has been studied by performing forced degradation experiments. Sugammadex sodium was subjected to different stress conditions [13] i.e acid/base hydrolysis [2N HCl/RT/6hrs/70°C/9 hrs & 2N NaOH/ RT/6hrs/70°C/9 hrs], peroxide degradation under oxidative stress [0.1% w/v H_2O_2 solution, 6hrs], UV solution/24 hours, thermal degradation [105°C/24Hours] and UV Solid/24 hours. Peak purity of Sugammadex sodium peak was established by using a PDA detector in these stress samples. The Mass balance calculated and achieved in the acceptance range of 95% to 105%. The forced degradation results are tabulated in Table 3 and Table 4. The typical representative HPLC chromatograms of forced degradation experiments are shown in Fig.9-17.

Table No. 3: Degradation study Results

Name of the impurity	RRTs at	Control sample (%)	2N HCl		2N NaOH		0.1%	UV solution
			RT	70°C	RT	70°C	RT	
			6 hr	9 hr	6 hr	9 hr	6 hr	24hr
SGM/Sulfoxide imp-1	0.579	0.07	0.08	0.09	0.09	0.56	7.85	0.08
SGM/Alpha impurity	0.639	ND	ND	ND	ND	ND	ND	ND
SGM/Sulfoxide imp-2	0.692	0.05	0.06	0.07	0.07	0.56	8.50	0.07
SGM/Mono hydroxy	0.749	0.35	0.34	0.33	0.36	0.35	0.28	0.35
SGM/Beta impurity	0.794	ND	ND	ND	ND	ND	ND	ND
Unknown 1	0.36	0.01	0.01	0.02	ND	0.25	0.08	0.01
Unknown 2	0.40	0.01	0.01	0.01	0.01	0.02	0.41	0.01
Unknown 3	0.436	ND	ND	ND	ND	0.01	0.28	ND

Unknown 4	0.476	ND	0.01	0.05	ND	0.02	0.14	ND
Unknown 5	0.506	ND	ND	3.57	ND	0.16	0.10	ND
Unknown 6	0.528	ND	ND	1.80	0.04	0.09	ND	ND
Unknown 7	1.524	0.07	0.06	0.02	0.07	0.06	0.04	0.08
Unknown 8	1.933	0.02	0.04	0.03	0.04	0.06	ND	0.05
Unknown 9	2.392	0.11	0.12	0.11	0.11	0.11	0.08	0.11
Purity	1.000	99.03	99.03	93.47	99.07	97.40	82.11	98.92
Mass balance	NA	100.0	100.83	99.97	100.54	101.34	100.84	100.12

Table No. 4: Degradation study Results

Name of the impurity	RRTs	Control sample (%)	UV Solid 24 Hours	Heat at 105°c 24 Hours
SGM/Sulfoxide imp-1	0.574	0.07	0.63	0.08
SGM/Alpha impurity	0.634	ND	0.01	ND
SGM/Sulfoxide imp-	0.687	0.05	0.15	0.05
SGM/Mono hydroxy	0.745	0.35	0.32	0.35
SGM/Beta impurity	0.790	ND	0.02	ND
Unknown 1	0.366	ND	2.84	ND
Unknown 2	1.430	ND	0.14	0.02
Unknown 3	1.522	0.07	2.70	0.07
Unknown 4	1.933	ND	3.40	0.03
Unknown 5	2.395	0.11	0.07	0.11
Unknown 6	2.567	ND	0.16	0.03
Purity	1.000	99.17	88.64	99.13
Mass balance	NA	100.0	99.41	100.01

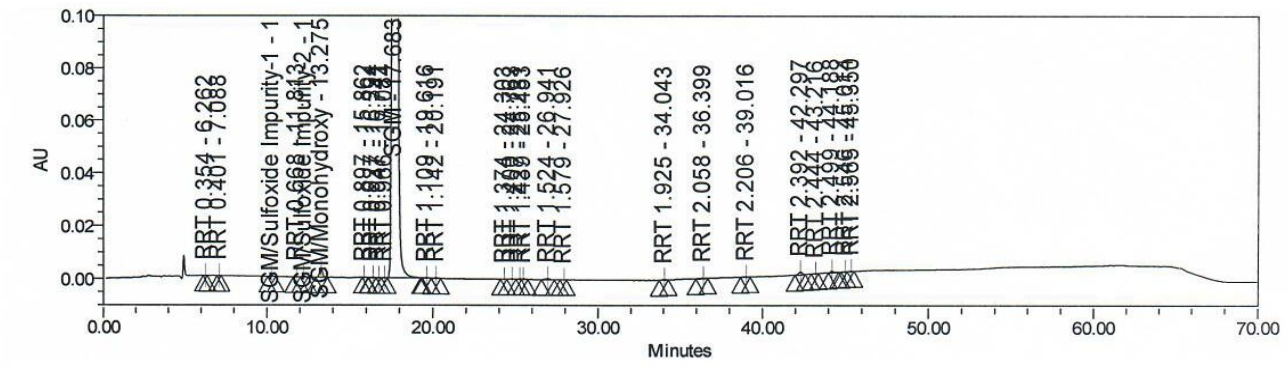


Figure No. 9: Typical chromatogram for Control sample

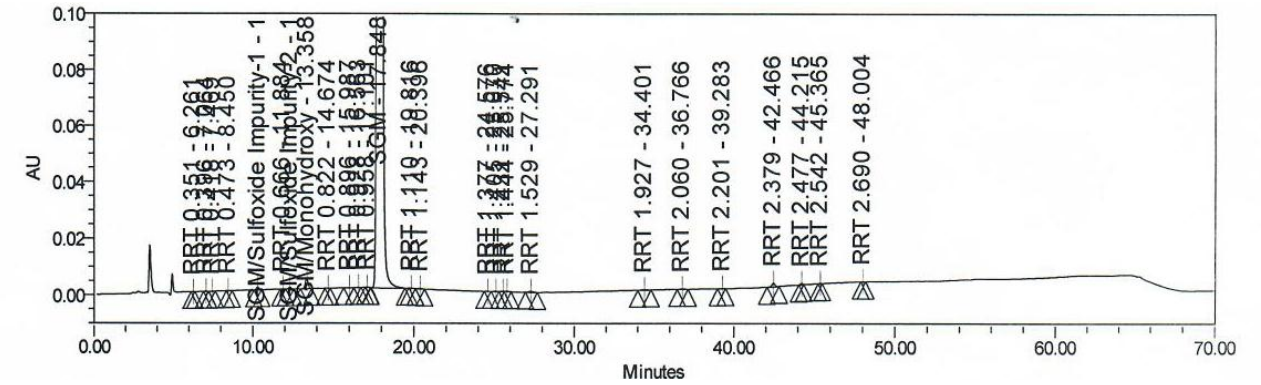


Figure No. 10: Typical chromatogram for 2N HCl RT 6 hrs

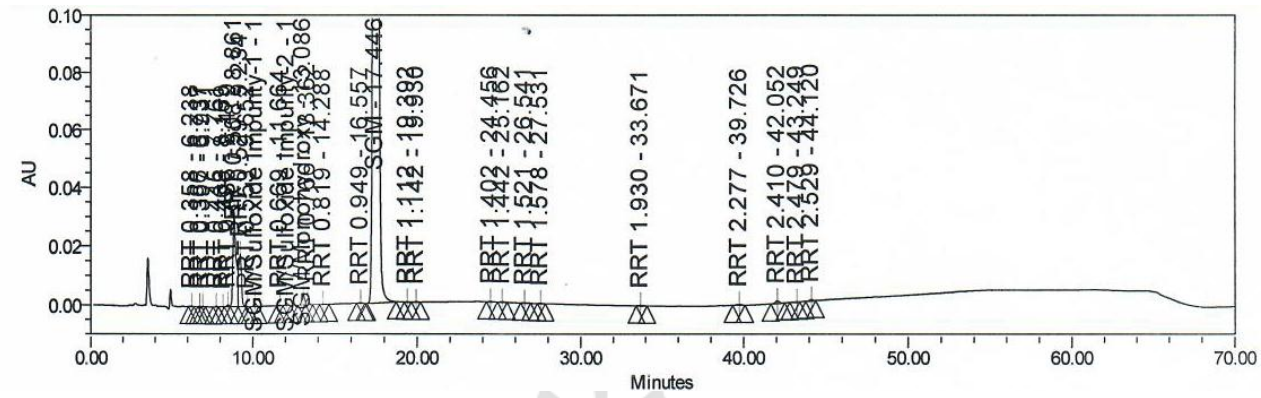


Figure No. 11: Typical chromatogram for 2.0 N HCl at 70°C 9 hrs

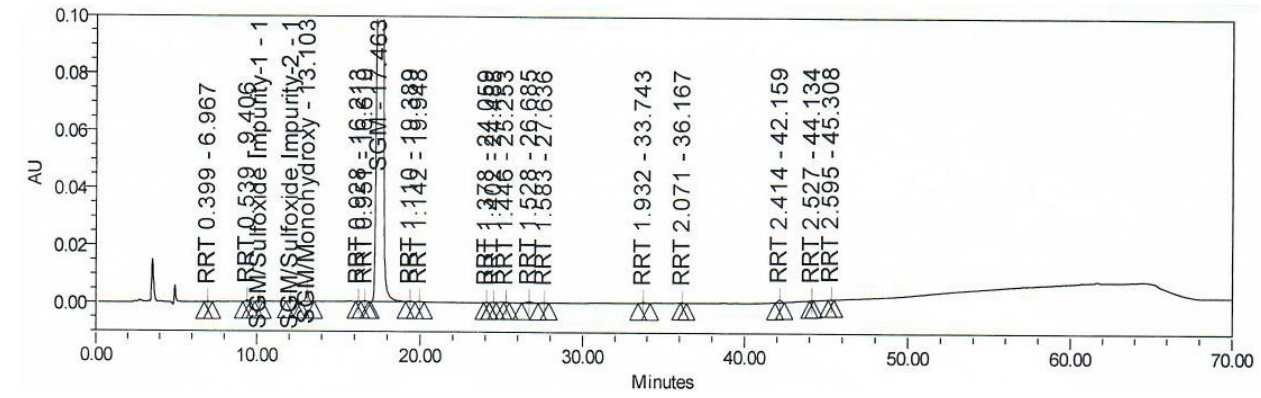


Figure No. 12: Typical chromatogram for 2N NaOH RT 6 hrs

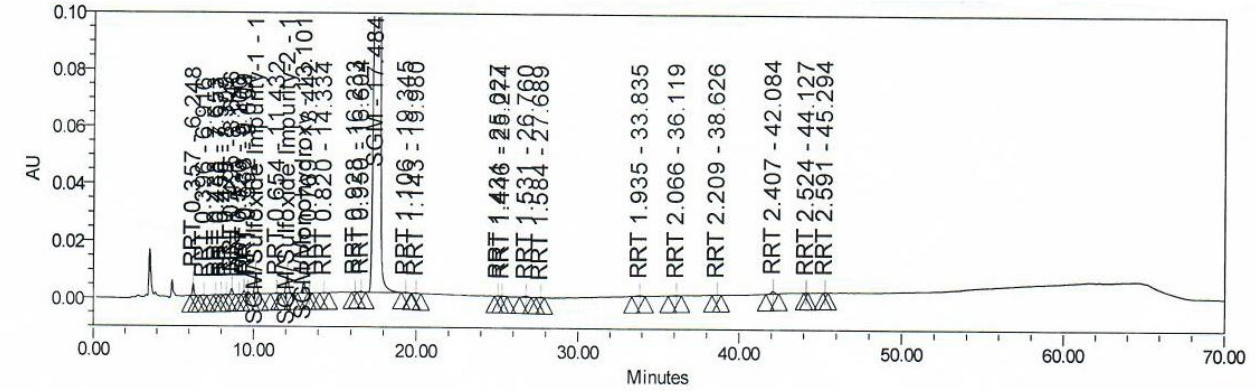


Figure No. 13: Typical chromatogram for 2N NaOH at 70°C 9 hrs

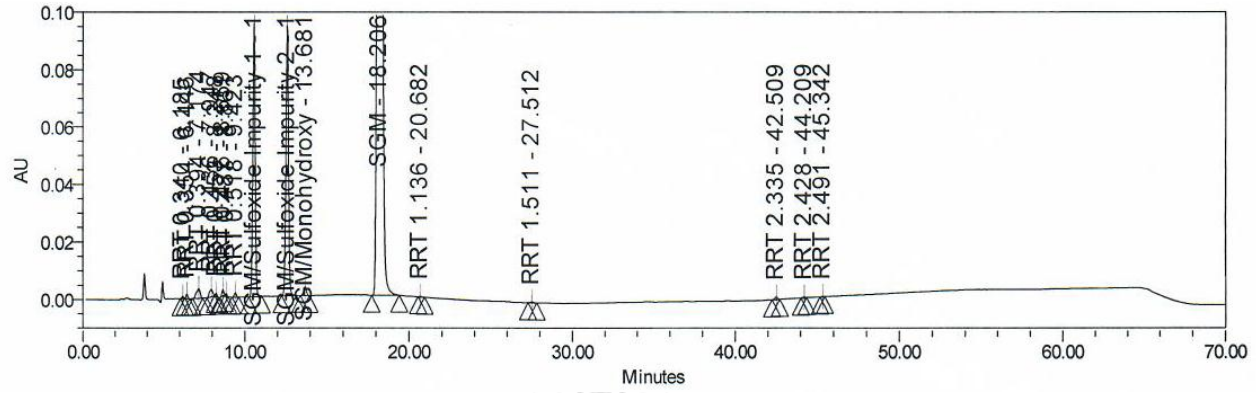


Figure No. 14: Typical chromatogram for 0.1% H_2O_2 RT 6hrs

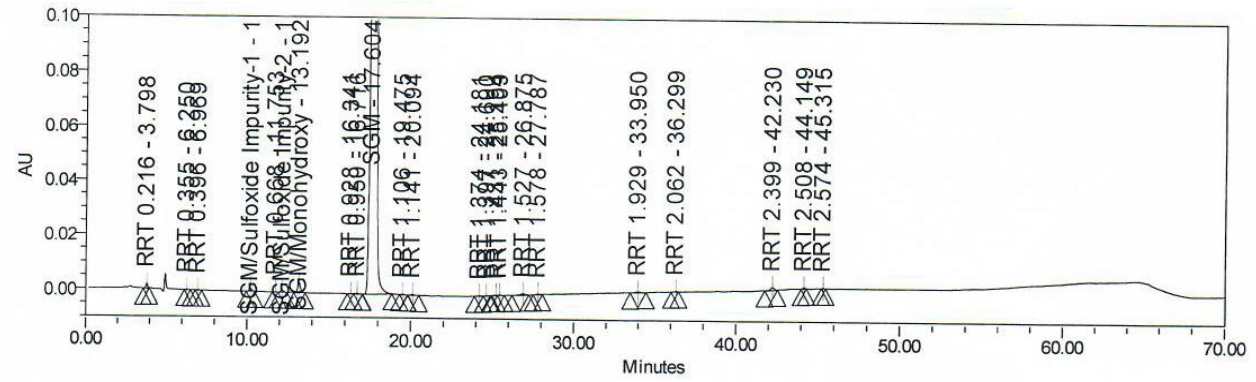


Figure No. 15: Typical chromatogram for Solution at UV 24 hrs

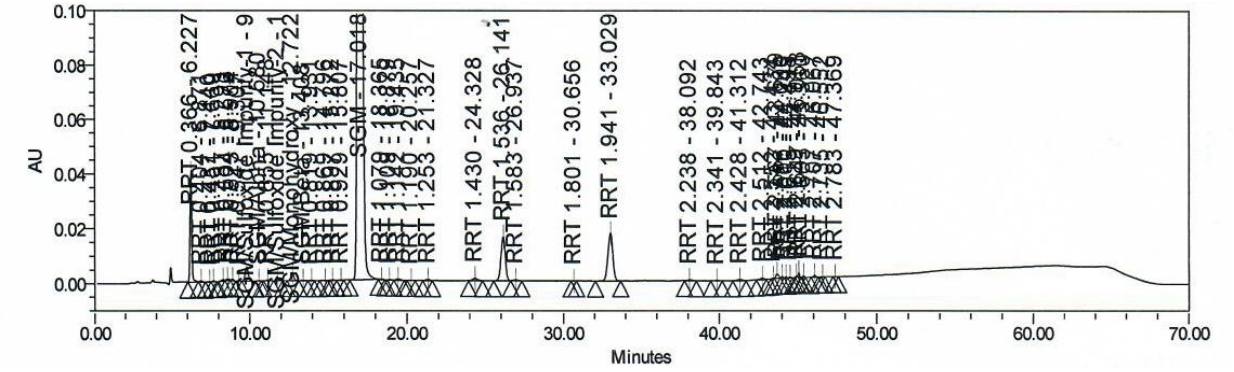


Figure No. 16: Typical chromatogram for Solid at UV 24 hrs

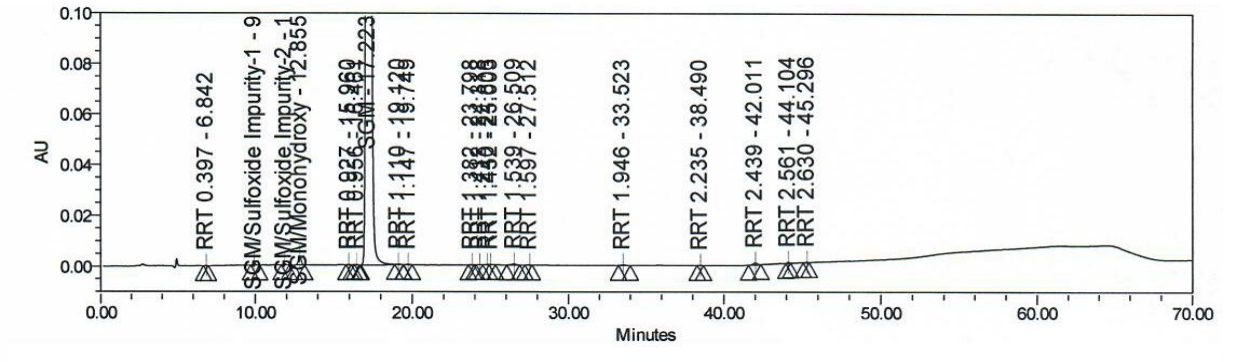


Figure No. 17: Typical chromatogram for Heat at 105 °C 24 hrs

Limit of Detection (LOD)/ Limit of Quantification (LOQ):

The limit of detection and limit of quantification is determined by calculating the signal to noise ratio method. By comparing test results from samples with known concentrations of analyte with those of blank samples and establishing the minimum level at which the analyte can be reliably detected. The results obtained for each impurity is listed in Table 5. These are determined from the formula S/N ratio is 3:1 for LOD and 10:1 for LOQ respectively. The LOD and LOQ results are tabulated in Table 5. The typical representative HPLC chromatograms of Blank, LOD and LOQ experiment are shown in Fig.18 and Fig.19 and Fig.20.

Table No. 5: Limit of detection and Quantification for Sugammadex sodium (SGM) and its Impurities

Component	LOQ		LOD		LOQ (%)	LOD (%)
	Concentration (mg/ml)	Signal to noise ratio (S/N)	Concentration (mg/ml)	Signal to noise ratio (S/N)		
Sulfoxide impurity-1	0.0010104	15.2:1	0.000333432	4.9:1	0.040	0.013
Alpha-SGM	0.0010136	13.7:1	0.000334488	4.7:1	0.041	0.013
Sulfoxide impurity-2	0.0010192	12.3:1	0.000336336	4.0:1	0.041	0.013
Mono hydroxy	0.0012680	13.9:1	0.000418440	4.4:1	0.051	0.017
Beta-SGM	0.0012785	11.3:1	0.000421905	3.5:1	0.051	0.017
SGM	0.0010076	10.2:1	0.000332508	3.5:1	0.040	0.013

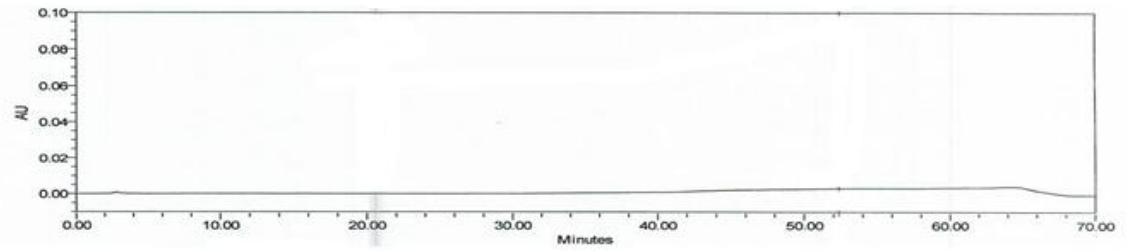


Figure No. 18: A typical representative HPLC chromatogram of Blank

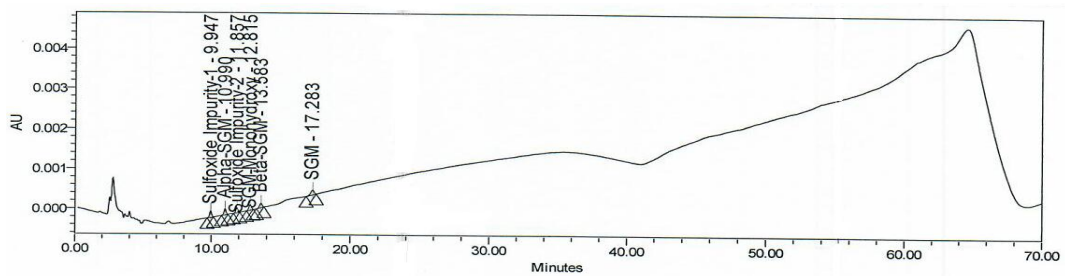


Figure No. 19: A typical representative HPLC chromatogram of LOD

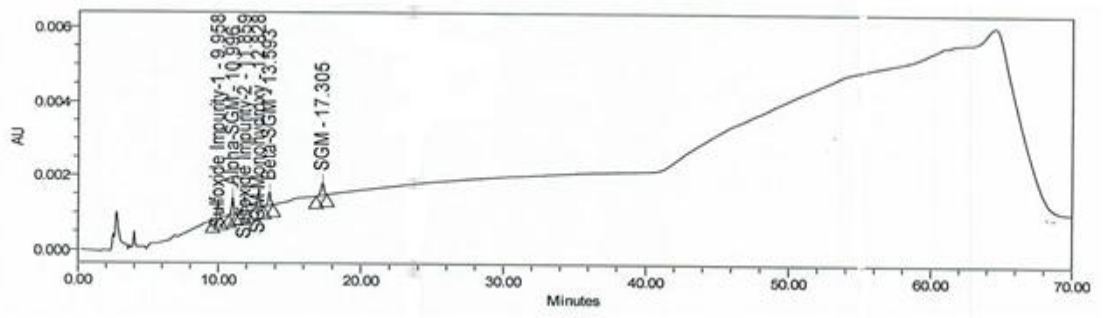


Figure No. 20: A typical representative HPLC chromatogram of LOQ

Linearity

A series of solutions were prepared using Sugammadex sodium and its impurities at concentration levels from LOQ to 150% of specification level and each solution was injected and calculated the statistical values like slope, intercept and correlation coefficient from linearity plot drawn for concentration versus area. Linearity graphs Fig. 21-26 and Tables 6-11 are shown below. The statistical values are presented in Table 12.

Table No. 6: **Linearity Table for Sugammadex**

Level	Concentration (mg/mL)	Average Peak area
LOQ	0.0010076	5007
25%	0.0018893	10953
50%	0.0037785	22117
80%	0.0060456	36051
100%	0.0075570	45027
120%	0.0090684	53610
150%	0.0113355	66915

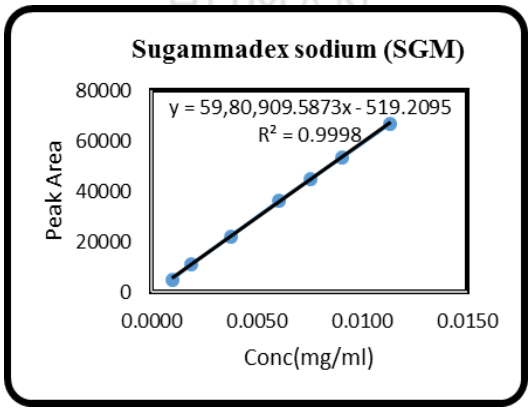


Figure No. 21: Linearity graph for Sugammadex

Table No. 7: Linearity Table for Sulfoxide impurity-1

Level	Concentration (mg/mL)	Average Peak area
LOQ	0.0010104	4506
25%	0.0031575	14091
50%	0.0063150	28601
80%	0.0101040	46315
100%	0.0126300	58226
120%	0.0151560	69652
150%	0.0189450	87333

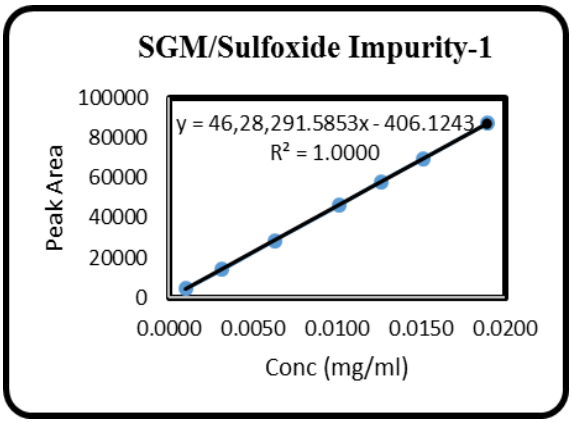


Figure No. 22: Linearity graph for Sulfoxide impurity-1

Table No. 8: Linearity Table for Alpha impurity

Level	Concentration (mg/mL)	Average Peak area
LOQ	0.0010192	4445
25%	0.0031850	14499
50%	0.0063700	29232
80%	0.0101920	46745
100%	0.0127400	58867
120%	0.0152880	70519
150%	0.0191100	87990

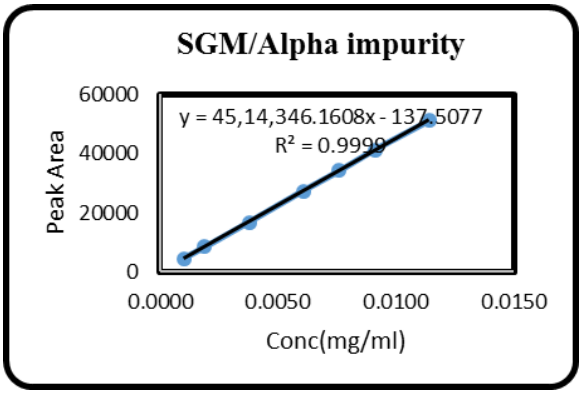


Figure No. 23: Linearity graph for Alpha impurity

Table No. 9: Linearity Table for Sulfoxide impurity-2

Level	Concentration (mg/mL)	Average Peak area
LOQ	0.0010192	4445
25%	0.0031850	14499
50%	0.0063700	29232
80%	0.0101920	46745
100%	0.0127400	58867
120%	0.0152880	70519
150%	0.0191100	87990

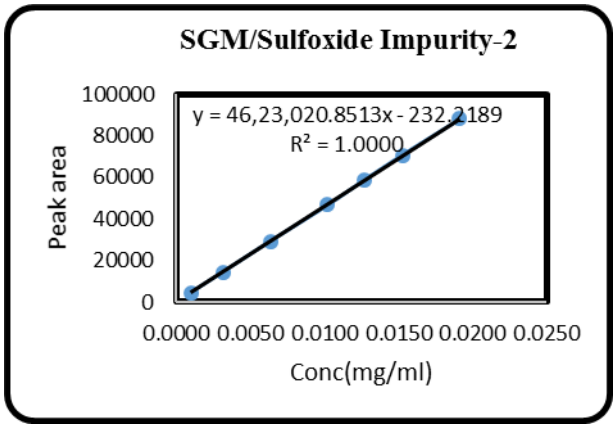


Figure No. 24: Linearity graph for Sulfoxide impurity-2

Table No. 10: **Linearity Table for Mono hydroxy impurity**

Level	Concentration (mg/mL)	Average Peak area
LOQ	0.0012680	5036
25%	0.0190200	74556
50%	0.0380400	151360
80%	0.0608640	242019
100%	0.0760800	302121
120%	0.0912960	362695
150%	0.1141200	449914

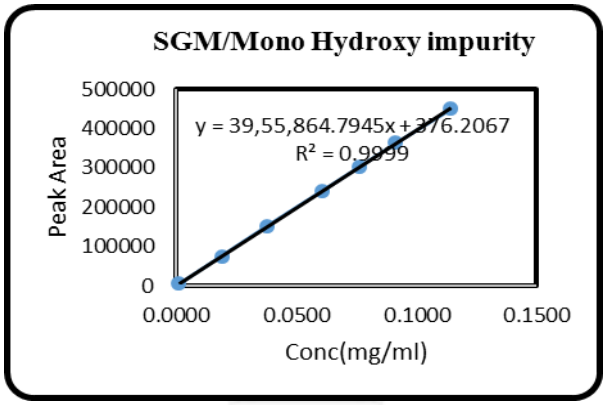


Figure No. 25: Linearity graph for Mono hydroxy impurity

Table No. 11: **Linearity Table for Beta impurity**

Level	Concentration	Average
LOQ	0.0012785	4285
25%	0.0019178	6287
50%	0.0038355	12572
80%	0.0061368	20312
100%	0.0076710	25295
120%	0.0092052	30131
150%	0.0115065	37359

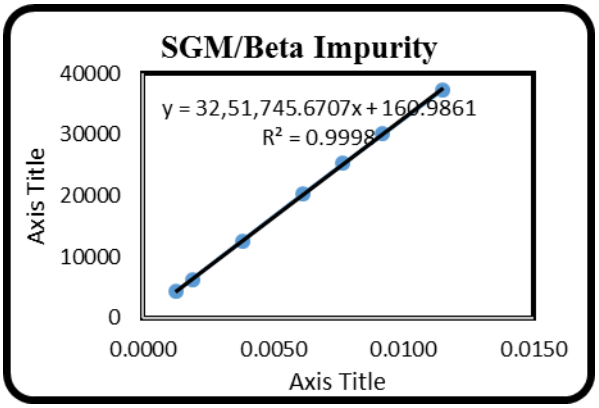


Figure No. 26: Linearity graph for Beta impurity

Table No. 12: Statistical evaluation of Linearity

Component	Slope	Intercept	correlation coefficient (R)	R^2	Intercept value w.r.to 100% conc. standard response
Sulfoxide impurity-1	4628291.5853	-406.1243	1.0000	1.0000	-0.70
Alpha-SGM	4514346.1608	-137.5077	1.0000	0.9999	-0.40
Sulfoxide impurity-2	4623020.8513	-232.2189	1.0000	1.0000	-0.40
Mono hydroxy	3955864.7945	376.2067	1.0000	0.9999	0.1
Beta-SGM	3251745.6707	160.9861	0.9999	0.9998	0.6
Sugammadex sodium (SGM)	5980909.5873	-519.2095	0.9999	0.9998	-1.16

Accuracy

The accuracy of the method is determined by using the solutions containing Sugammadex sodium samples spiked with the respective impurities at approximately LOQ to 150% of the specification limit. The percentage recovery calculated should be in the range of 80 to 120 and at the LOQ level, the % recovery calculated should be in the range of 70-130. The percentage recovery values for all the impurities are calculated and tabulated in Table 13.

Table No. 13: Statistical evaluation of Accuracy

Accuracy				
Name of the impurity /Compound	**Spiked levels**			
	LOQ Level	50% Level	50% Level	50% Level
Sulfoxide impurity-1	103.0	99.6	100.4	100.5
Alpha-SGM	97.0	97.4	98.7	98.1
Sulfoxide impurity-2	100.9	100.2	100.2	100.1
Mono hydroxy	98.7	101.5	100.5	99.9
Beta-SGM	104.6	101.4	101.0	99.1

Stability of solutions:

Standard solution and sample solution spiked with impurities were prepared and analyzed initially and at different time intervals for 24hrs by keeping the solutions at room temperature (~ 25°C) and refrigerator condition (~2-8°C) and found the solutions were stable.

CONCLUSION

The reverse-phase stability-indicating HPLC method was developed and validated for the quantitative determination of impurities of Sugammadex sodium. The present research work will help the manufacturers and suppliers of Sugammadex sodium to determine known and unknown impurities based on above data. Thus the HPLC method can be used for routine analysis and during the stability studies of pharmaceutical analysis.

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