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Determination of Sugammadex in Human Plasma using Protein Precipitation and Ultra High Performance Liquid Chromatography/Tandem Mass Spectrometry

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Abstract

A simple LC-MS/MS method for the quantification of sugammadex in human plasma is described. Samples were prepared using protein precipitation with acetonitrile containing 0.37% hydrochloric acid as precipitator and Waters Ostra plate for protein and phospholipid removal. The analyte and its analog internal standard (IS) were chromatographed under reversed phase chromatography conditions on an Acquity UPLC BEH Shield RP18 column (2.1 × 50mm, 1.7µm) using a solution of methanol/water (50/50, v/v) containing 10mM ammonium format (pH3.0) and a solution of acetonitrile/water (85/15, v/v) containing 10mM ammonium format (pH3.0) as mobile phase A and B, respectively. Carryover was significantly reduced when linear gradient wash cycles were employed. The analytes were detected with a tandem mass spectrometer employing a turbo ion spray (TIS) interface in negative ionization mode. The multiple reaction monitoring (MRM) transitions were *m/z*999→963 for sugammadex and *m/z*1055→476 for IS. The lower limit of quantitation (LLOQ) for this method was 0.100µg/mL when 200µL of plasma is processed.

Keywords: Sugammadex; LC-MS/MS; Bioanalysis; Protein precipitation; Carryover

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Introduction

Sugammadex (tradename Bridion), per-6-(-2-carboxyethylthio)-per-deoxy-γ-cyclodextrin sodium (Figure 1a), is a modified γ-cyclodextrin with a lipophilic core and a hydrophilic periphery. The modification to γ cyclodextrin consists of the addition of a carboxyl thio ether group at the 6 carbon position of each of the 8 glucopyranose subunits. The molecule was designed to complex with neuromuscular blocking agents (NMBAs), such as rocuronium and vecuronium, reversing the depth of neuromuscular blockade by complexing with the agents and preventing them from binding to target receptors; the modification of the native cyclodextrin extends the cavity size, allowing greater encapsulation of NMBAs [1-3].

To support sugammadex pharmacokinetic studies in humans, an analytical method for the determination of sugammadex in human plasma has been reported [1]. This method required a custom-packed PEEK column in order to reduce carryover, presumably caused by binding of the analyte to stainless steel based column components. While the reported method has been used to support numerous studies [4-8], the need to use a custom-packed, non-commercially available column complicated assay application and increased assay cost. Hence, in order to support future studies, a method utilizing a commercially available column was highly desirable.

The purpose of the research described herein was to develop and validate a low carryover, high extraction recovery and high-throughput LC-MS/MS method for the quantification of sugammadex in human plasma that utilized a commercially available stainless steel column. The assay presented in this publication utilizes protein precipitation/filtration for sample preparation and a Waters Acquity UPLC system coupled to a Sciex API 5000 mass spectrometer for sample analysis. Results of assay validation, including assessment of intraday and interday precision and accuracy, quality control sample (QCs) stability, and incurred sample reproducibility are presented.

Experimental

Materials

Sugammadex (Figure 1a) was synthesized at MSD Laboratories, Oss, and The Netherlands and obtained as a 100 mg/mL stock solution in water. The internal standard (IS), Org 26265 (per-6-

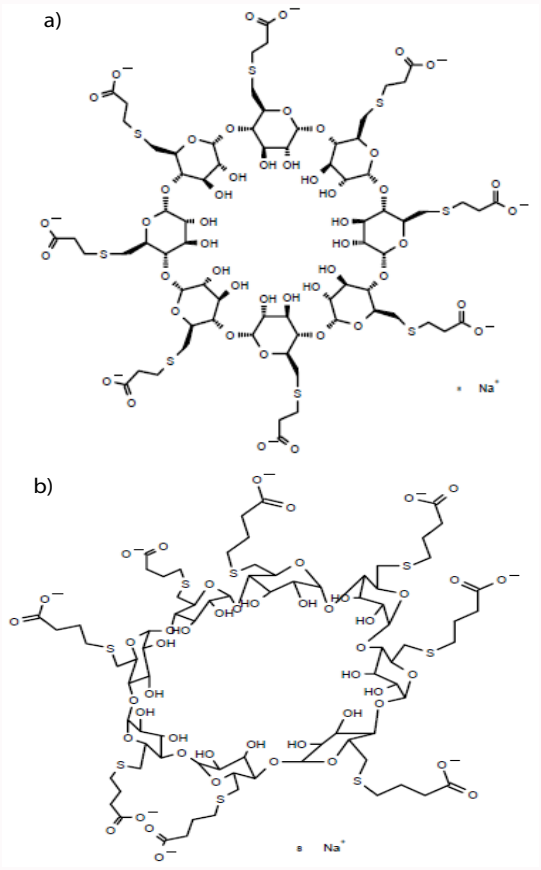

Figure 1: Chemical structures of sugammadex (a) and analog internal standard Org26265 (b); (a) Sugammadex, MW = 2002.18; (b) Org26265 (IS), MW = 2106.33.

(2-carboxypropylthio)-per-6-deoxy--cyclodextrin sodium), was also synthesized at MSD Laboratories, Oss, The Netherlands (Figure 1b). The purities of sugammadex and Org 26265 were 97.5% and 65.8%, respectively. HPLC grade acetonitrile (ACN), methanol (MeOH), ammonium format (NH_4FA), hydrochloric acid (HCl, 37%), formic acid (FA, 98 – 100%) and bovine serum albumin (37% solution) were purchased from Sigma-Adrich (St Louis, MO, USA). Control human plasma with sodiumheparin as the anticoagulant was purchased from Biological Specialty Corp. (Colmar, PA, USA). Ostroprotein precipitation and phospholipid removal plates were purchased from Waters (Milford, MA, USA).

Instruments

Chromatographic separation was carried out using a Waters Acquity ultra-performance liquid chromatograph (Waters Corporation, Milford, MA) consisting of a binary pump, auto sampler and column oven. The final assay utilized a Waters Acquity UPLC BEH Shield RP18 column (2.1 × 50mm, 1.7 µm). An Applied Biosystems Sciex API 5000 triple quadrupole mass spectrometer (Toronto, Canada) was used as the detector. Peak areas generated using SCIEX Analyst® software package (Sciex, Toronto, Canada) were exported to WATSON LIMS system (v 7.3) software (Thermo Fisher Scientific Inc., Pittsburgh, PA, USA) for quantitation.

Standard solutions and quality control (QC) samples

The intermediate stock solution of 1.00 mg/mL sugammadex was prepared in milli-Q water by appropriate dilution from the 100mg/mL stock solution provided. The internal standard, Org26265, stock solution was prepared at a concentration of 1.00mg/mL by weighing the solid compound and dissolving in appropriate volume of milli-Q water while correcting for purity. Working standard solutions from 0.400 to 160 µg/mL (0.400, 0.800, 2.00, 8.00, 20.0, 40.0, 128, and 160 µg/mL) in 0.1% BSA were prepared by appropriate dilution from the intermediate stock solution of sugammadex. The working internal standard (IS) solution was prepared at a concentration of 10.0µg/mL in 0.1% BSA by dilution from the stock solution of Org26265.

An intermediate stock solution of 1.00 mg/mL sugammadex, used for quality control (QC) preparation, was prepared in milli-Q water by appropriate dilution from the 100mg/mL stock solution. The QC working solutions (30.0, 400, and 3200µg/mL) in 0.1% BSA were prepared by appropriate dilution from QC stock and QC intermediate stock solutions. QC samples were prepared by adding appropriate volumes of QC working solutions into volumetric flasks and diluting to volume with control human plasma to achieve the desired concentrations of 0.300, 4.00, and 32.0µg/mL for low, middle, and high QCs, respectively. Aliquots (500µL) of each QC sample were transferred into Nunc Cryo vials, which were capped and stored at −20°C.

QC samples were processed along with the clinical samples during each analytical run. The minimum number of QC samples was at least

Table 1: Intra-day precision and accuracy for the determination of sugammadex in six lots of human plasma.

Nominal conc. (µg/mL)	Mean measured conc.[a] (µg/mL, n=6)	Precision (%RSD, n=6)	Accuracy[b] (%)
0.100	0.0979	2.2	97.9
0.200	0.207	3.7	104
0.500	0.503	5.3	101
2.00	2.03	2.5	102
5.00	5.12	2.7	102
10.0	10.3	3.6	103
32.0	31.2	1.7	97.5
40.0	37.7	3.2	94.2

[a]Calculated from the weighted linear least-squares regression curve using all six replicates at each concentration,
[b]Expressed as (mean measured conc./nominal conc.)×100%.

Table 2: Intra-day and interday precision and accuracy of LLOQ and QC samples for the determination of sugammadex in human plasma.

		Concentration (µg/mL)			
Run	Nominal conc. (µg/mL)	0.100	0.300	4.00	32.0
1	Mean measured conc.[a](µg/mL)	0.0965	0.308	4.14	31.6
	Precision (%RSD)	4.7	2.3	2.6	3.2
	Accuracy[b] (%)	96.5	103	104	98.7
2	Mean measured conc.[a](µg/mL)	0.0928	0.292	4.17	31.9
	Precision (%RSD)	5.0	4.2	0.70	1.3
	Accuracy[b] (%)	92.8	97.3	104	99.7
3	Mean measured conc.[a](µg/mL)	0.107	0.298	4.11	33.2
	Precision (%RSD)	5.8	2.4	2.2	1.4
	Accuracy[b] (%)	107	99.3	103	104
	Inter-day mean measured conc. (n=3)	0.0989	0.300	4.14	32.2
	Inter-day precision (%RSD)	8.1	3.6	1.9	3.0
	Inter-day accuracy[b](%)	98.9	100	104	101

[a]Calculated from the weighted linear least-squares regression curve,
[b]Expressed as (mean measured conc./nominal conc.)×100%.

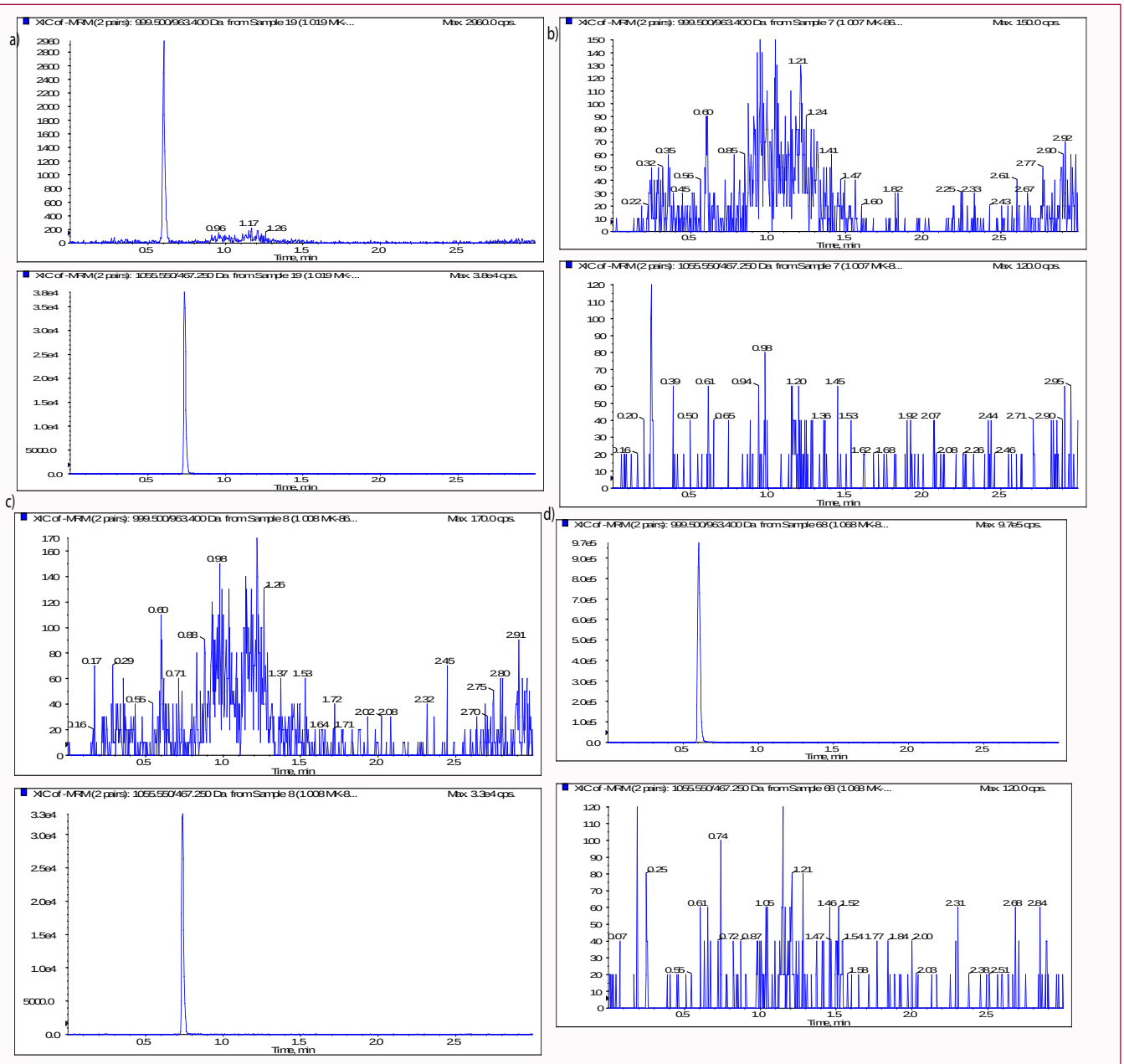

Figure 2: Chromatograms of the plasma extract from (a) 0.100µg/mL standard sample (LLOQ), (b) double blank, (c) single blank, and (d) 40.0µg/mL sugammadex in the absence of IS. The top and bottom panels in each figure are the MRM of analyte and IS, respectively.

5% of the number of unknown samples analyzed in a given run or six total QCs, whichever was greater [9].

Sample preparation

Plasma standards were prepared by mixing 50 µL of each working standard with 200µL aliquots of control human plasma pipetted into individual wells of a 96-well plate. Aliquots (200µL) of QC and subject samples were pipetted into individual wells of the assay plate. A 50µL aliquot of make-up solvent (0.1% BSA) was added to the wells containing the QC and subject samples. IS working solution (50µL) was then added to each well. The contents of the wells were mixed, after which 600µL of ACN with 0.37% HCl was added to each sample to precipitate the protein. Following centrifugation, the supernatants were filtered through a 96-well Ostro protein and phospholipid removal plate. The filtered samples were then further diluted using milli-Q water so that the final acetonitrile content of the samples was less than 25%.

Chromatographic conditions

The chromatographic separation was accomplished using an Acquity UPLC BEH Shield RP18 column (2.1 × 50mm, 1.7µm, Waters)maintained at 35°C. A solution of MeOH/water (50/50, v/v) containing 10mM ammonium format (pH3.0) and a solution of ACN/water (85/15, v/v) containing 10mM ammonium format (pH3.0) were used as mobile phase A and B, respectively. A linear mobile phase gradient with a flow rate of 0.6mL/min was selected, starting at 100% mobile phase A and raising mobile phase B content from 0 to 80% in one minute. To decrease carryover, three wash cycles were used, each cycle consisted of the following steps: linear gradient from 100% B to 100% A over 0.1 minute, hold at 100% A for 0.4 minute, followed by a linear gradient from 100% A to100% B again over 0.1 minute, hold at 100% B for 0.4 minute. Under these

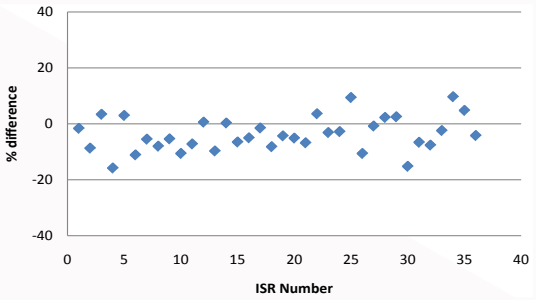

Figure 3: Incurred samples reanalysis. The difference is expressed as {(repeat measured conc.- initial measured conc.)/[(initial measured conc. + repeat measured conc.)/2]}×100%.

conditions, the retention times for both sugammadex and internal standard Org26265 were about 0.6 and 0.7 minutes, respectively. The total run time was 6.0 minutes.

Mass spectrometry detection conditions

The MS was operated in negative electrospray ionization (ESI) mode with Nitrogen used as nebulizer, curtain, and collision gas. The curtain gas was set at 40 Psi and collision gas was set at 10 Psi. The setting of nebulizer gas (Gas 1) and heater gas (Gas 2) were 45 and 75 Psi, respectively. Precursor ions for sugammadex and internal standard Org26265 were determined from the Q1 negative ion spectra that were obtained during the infusion of a neat solution of each compound, via the TurboIonSpray™ (TIS) source, into the mass spectrometer. Under these conditions, the analyte and internal standard yielded predominately deprotonated doubly charged molecular ions of *m/z*999 and *m/z*1055, respectively. Each of the precursor ions was subjected to collision induced dissociation (CID) in order to generate product ions. Sugammadex gave rise to a predominant product ion of *m/z*963 and the internal standard Org26265 gave the corresponding product ion of *m/z*476. Based on these spectra, the ion transitions of *m/z* 999 → 963 (sugammadex) and *m/z*1055 → 476 (IS) were used for MRM. MS parameters were further optimized by infusing a solution of sugammadex through the TIS interface. The ion spray voltage was optimized at -4500V and the TIS interface temperature was maintained at 500˚C. The declustering potential was -130V, entrance potential was -10V, collision energy was -45V for sugammadex and -90V for IS, and collision cell exit potential was -20V.

Results and Discussion

Chromatography – column and mobile phase selection

The sole published bioanalytical method for sugammadex utilizes a custom-packed PEEK column [1]. This column is not widely available, requires an extensive lead time from the manufacturer, and is considerably more costly than standard stainless steel columns. Hence, development of a method using a commercially available column was highly desirable.

Table 4: Absolute matrix effect of sugammadex and analog internal standard Org 26265 in protein precipitated human plasma with the corresponding recovery.

Standard concentration in plasma (µg/mL)	Mean IS normalized matrix factor [a] (%, n=6)	Mean recovery[b] (%, n=3)
0.100	1.02	105
40.0	1.00	92.0
(IS)[c]		107

[a]IS normalized matrix factor was calculated as mean of (the analyte matrix factor for a specific matrix/the IS matrix factor for that same matrix).
[b]Expressed as (the mean peak area of analyte spiked before extraction / the mean peak area of analyte spiked after extraction) ×100%.
[c](n=6).

Table 5: Inter-day precision and accuracy of eight run's standard samples for the determination of sugammadexin human plasma during study support.

Nominal conc. (µg/mL)	Mean measured conc.[a] (µg/mL, n=8)	Precision (%RSD, n=8)	Accuracy[b] (%)
0.100	0.102	1.1	102
0.200	0.194	2.3	97.0
0.500	0.487	3.1	97.4
2.00	1.97	2.5	98.5
5.00	5.06	2.1	101
10.0	9.93	3.1	99.3
32.0	33.3	3.9	104
40.0	40.2	3.7	100

[a]Calculated from the weighted linear least-squares regression curve using all eight replicates at each concentration,
[b]Expressed as (mean measured conc./nominal conc.)×100%.

Initially, an assessment of a stainless steel column packed with the material specified in the published method was conducted. When such a column was used, carryover, assessed by the analyte signal observed from the blank injection immediately following the highest standard, was greater than 400% of the LLOQ signal. These results confirmed that the use of a stainless steel column with the packing material specified in the published method yields unacceptable results. Furthermore, additional experiments confirmed that the primary source of carryover was the column as opposed to the auto sampler.

Various approaches including the addition of EDTA into the mobile phase as well as the use of strong column washes with solvents such as 100% acetonitrile or Tetrahydrofuran (THF) did not reduce carryover with the original packing material. Therefore, the need to evaluate an alternate chromatographic system for sugammadex analysis became apparent.

Two important factors were considered during method development. Firstly, a packing/hardware configuration for the column that minimized carryover was required. Secondly, chromatographic conditions were required under which sharp symmetrical peaks were obtained, thus permitting the use of lower injection volumes and/or eliminating the need for complex sample pre-concentration. Several different types of columns were investigated, including: Atlantis

Table 3: Freeze-thaw, room temperature (5h) and longterm (29 Days) stability of sugammadex QC samples in human plasma.

Nominal conc. (µg/mL)	3 F/T Cycles mean measured conc.[a] (n=5)	Difference from nominal[b] (%)	Room temp. mean measured conc.[a] (n=5)	Difference from nominal[b] (%)	Long term mean measured conc. a (n=5)	Difference from nominal[b] (%)
0.3	0.304 (2.8)	1.3	0.302 (5.1)	0.67	0.279 (2.6)	-7.0
32	31.7 (1.4)	-0.9	31.4 (3.2)	-1.9	33.0 (2.6)	3.1

Numbers in parentheses are coefficients of variation (% RSD).
[a]Calculated from the weighted linear least-squares regression curve,
[b]Expressed as ((mean measured conc. - nominal conc.)/nominal conc.)×100%.

Table 6: Inter-day precision and accuracy of eight run's QC samples for the determination of sugammadex in human plasma during study support.

	Concentration (µg/mL)		
	0.300	4. 00	32.0
Inter-day mean measured conc. (n=16)	0.295	4.08	33.2
Inter-day precision (%RSD)	4.6	3.7	3.1
Inter-day accuracy[a](%)	98.3	102	104

[a]Expressed as [(mean measured conc.)/(nominal conc.)] ×100%.

dC18, Atlantis T3, Atlantis HILIC Silica, X bridge Amide, X bridge shield RP 18, Onyx Monolithic C18, Bio basic C18, X terra RP 18, X Terra MS C18, Symmetry Shield RP18, Polaris 5 C18-A, and Scherzo SM-C18.

When acetonitrile combined with different additives at different pH were used as mobile phase, sugammadex had no retention on any of the tested columns even when low organic content mobile phases were employed. When methanol was used as mobile phase, sugammadex was retained on the X bridge shield RP 18, BEH Shield RP18, and Atlantis dC18 columns. The carryover for these three columns was 100 to 150% of LLOQ. To further decrease the carryover, it was necessary to introduce a stronger solvent such as ACN into the MeOH/water mobile phase system. For all three columns, it was determined that if MeOH/water (50/50, v/v) containing 10 mM NH_4FA (pH3.0) as mobile phase A and ACN/water (85/15, v/v) containing 10 mM NH_4FA (pH3.0) as mobile phase B were used, the carryover could be decreased by about 50%. With this mobile phase combination, the BEH Shield RP18 column exhibited the most efficient chromatography, hence this column was selected for further work to minimize carryover.

Column wash optimization

To further reduce the carryover, two approaches were evaluated. The first approach was to use a step gradient and wash the column using 100% of mobile phase B (ACN/water (85/15, v/v) containing 10mM NH_4FA (pH3.0) for periods of 1, 5, and 10 minutes following the elution of the target analyte. The second approach was to wash the column using a linear gradient wash following sugammadex elution. Each gradient wash cycle included two steps. The first step was a linear gradient from 100% B to 100% A over 0.1 minute and then a hold at100% A for 0.4 minute. The second step was a linear gradient from 100% A to100% B over 0.1 minute and then a hold at 100% B for 0.4 minute.

Carryover was not significantly reduced with the step gradient approach using mobile phase B to wash the column for periods up to10 minutes. However, when the linear gradient wash was employed the level of carryover was reduced by about 33% with each wash cycle.

A possible reason that the first approach was ineffective to reduce carryover could be that under these conditions a thin layer of acetonitrile was quickly formed on the surface of the stationary phase. As a result, although acetonitrile kept flowing through the stationary phase, the sugammadex on the surface of the stationary phase may have been shielded by the acetonitrile layer and thus could not be removed from the column. In contrast to the first approach, when a linear gradient wash cycle was employed, stagnant solvent layers were disrupted and any analyte on the column was effectively removed, thus reducing carryover.

Sample preparation

Typically, in order to improve assay ruggedness, pretreatment of biological samples is required to remove potentially interfering components (e.g. proteins, lipids , salts, etc.). Solid-phase extraction (SPE) [11], liquid-liquid extraction (LLE) [12], solid-liquid extraction (SLE) [13] (using diatomaceous earth plate), protein precipitation (PP) [14], and online extraction [15,16] are commonly used sample clean-up methods.

Since sugammadex and its internal standard are both polar compounds due to the polycarboxylic groups present, the recovery of LLE was found to be nearly zero regardless of solvent or pH.

Given the inapplicability of LLE, we assessed SPE for sample clean-up and extraction of the analyte from the matrix. Initial experiments using SPE focused on the use of Isolute HAX (Biotage) material, since this plate was utilized by the published method [1]. Isolute HAX is a silica-based mixed mode strong anion exchange hydrophobic sorbent for extraction of acidic compounds. Attempts to use this phase yielded low recoveries of about 20-30%; additionally, the flow rate through each well was highly variable on this plate.

Initially, the low recovery on the Isolute plate was believed to be due, at least in part, to either the silica based packing irreversibly binding the analyte or the variable flow rates observed on this plate during wash and elution steps. To address these possibilities, a polymer-based mix mode anion exchange sorbent (Waters Oasis MAX) was evaluated. While recovery was greater than the silica based plate, it was still lower than desired; about 50% recovery was observed when the sample was applied following treatment with 0.1 M sodium hydroxide and elution with ACN/water (80/20, v/v) containing 5% FA.

As an alternative to mixed-mode based SPE phases, use of a hydrophilic-lipophilic balanced reversed-phase macroporous copolymer sorbent (Waters Oasis HLB) was next evaluated. Formic acid and phosphoric acid were assessedat 1, 5, and 10%concentration to acidify the plasma sample before loading it on the plate. Under acidic conditions, the carboxylic acid functional groups on the analyte would be expected to be protonated and hence exhibit highest retention on the HLB phase. Results showed that the recovery was about 10% when 1% formic acid was used and recovery was about 50% for all other conditions; the analyte was eluted from the column with ACN/water (80/20, v/v) containing 2% NH_4OH in each case.

Given the low recoveries observed with both mixed-mode and HLB SPE phases, a protein precipitation approach for sample preparation was next assessed. The commonly used precipitators, acetonitrile (ACN) and acetonitrile combined with formic acid (FA), were used initially. However, results were unacceptable. When ACN or 0.1% formic acid ACN was used as precipitator, recovery was almost zero. When the content of FA in the ACN increased to about 1%, recovery was increased to about 10%. When ACN was replaced by methanol, the results were almost identical.

The low recovery was believed to be due to the co-precipitation of sugammadex with the plasma proteins. A possible reason for the co-precipitation is that the sugammadex tightly binds with protein and precipitates with protein when precipitator was added. This is also a possible reason that the recovery of SPE was always lower than 50%.

To increase the recovery of analyte via protein precipitation, disrupting the binding of sugammadex with protein was believed to be essential. Strong acids have been used historically to break such binding. When ACN containing 0.37% HCl was used as precipitator, the recovery of protein precipitation increased to almost 100%.

Therefore, protein precipitation was selected as the sample clean-up method for the determination of sugammadex and 0.37% HCl with ACN was selected as the precipitator.

Significant ionization suppression was observed when this method was used to clean-up the samples. A possible reason for the ion suppression was thought to be the presence of phospholipids in the final samples since this is known to be a major contributing source of matrix effects in LC-MS/MS based bioanalytical methods [17-20]. To remove phospholipids, the filtration of the supernatants was performed using a 96-well Ostro sample preparation plate. With this approach, ion suppression was reduced considerably, suggesting that phospholipids in the sample were contributing to the suppression.

Assay validation

Selectivity: Selectivity is the ability of an analytical method to differentiate and quantify the analyte in the presence of other components in the sample [9]. To demonstrate that no interfering components elute at the retention times of sugammadex or internal standard, the assay selectivity was assessed using samples from 6 different lots of human control plasma. The peak response of the analyte and IS in the lower limit of quantification (LLOQ) samples (Figure 2a) was compared with the peak response at the expected retention time of the analyte and IS of blank samples (Figure 2b). No interfering peak was observed at the retention times of sugammadex and its internal standard in any of the 6 lots of matrix evaluated.

The absence of "cross-talk" between channels used for monitoring the analyte and the internal standard was confirmed by the analysis of plasma samples containing internal standard at the working concentration in the absence of sugammadex (Figure 2c) and the analysis of plasma samples containing sugammadex at 40µg/mL in the absence of internal standard (Figure 2d). No "cross-talk" was observed in either experiment.

Accuracy and precision: A calibration standard curve is the relationship between instrument response and known concentration of analyte [9]. Weighted ($1/x^2$) least-squares linear regression calibration curves were constructed by plotting the peak area ratio of analyte to internal standard versus concentrations of calibration standards. The range of the standard curve was 0.100 to 40.0 µg/mL. The lower limit of quantification (LLOQ) was 0.100µg/mL.

Intra-day accuracy and precision of the standard was evaluated by analyzing six sets of calibration standards, each prepared in a different lot of control matrix (Table 1). The precision (RSD%, n=6) ranged from 1.7% to 5.3% and the accuracy from 94.2% to 104% of the nominal values.

Low, middle and high QC samples containing sugammadex were prepared at concentrations of 0.300, 4.00, and 32.0µg/mL in human plasma. The intraday precision (RSD%, n=5) for QCs varied from 0.70% to 4.2% and accuracy from 97.3% to 104% of the nominal value (Table 2).

Precision and accuracy at the LLOQ were evaluated by analyzing five freshly prepared LLOQ samples along with a calibration curve. The intraday precision (RSD%, n=5) at the LLOQ varied from 4.7% to 5.8% and accuracy from 92.8% to 107% of the nominal values (Table 2).

The interday precision (RSD%, n=3) at the LLOQ, calculated based on results from three independent runs on three days, was 8.1% and accuracy was 98.9% of the nominal value (Table 2); interday precision (RSD%, n=3) for the QCs obtained from three independent runs on three days was 1.9% to 3.6% and accuracy was 100% to 104% of the nominal value (Table 2).

Stability: Freeze-thaw stability of sugammadex in human plasma was evaluated using five sets of low and high QCs that underwent three freeze-thaw cycles; QC samples at each concentration were thawed at room temperature for more than four hours and then frozen at –20°C overnight for each cycle. No significant concentration differences were observed for the QC samples that were subjected to three freeze-thaw cycles compared to the nominal concentrations (Table 3).

Room temperature stability was evaluated by analyzing five sets of low and high QC samples exposed to ambient temperature for 5 hours before being extracted. No significant calculated concentration differences were observed compared to the control QC concentrations (Table 3).

The long term freezer storage stability of sugammadex in human plasma was evaluated at -20°C. The results indicated that sugammadex in human plasma was stable for at least 29 days at -20°C (Table 3).

Matrix effect and recovery: To evaluate possible matrix suppression/enhancement of ionization, the response of the analyte spiked into extracted blank matrix was compared with the response of the analyte spiked into matrix-free reconstitution solution. Using this method, the matrix effect for the determination of sugammadex was evaluated at two concentration levels and using six different lots of plasma. No significant matrix effects were observed (Table 4).

Recovery was determined by comparing the response of the analyte spiked into matrix with the response of the analyte spiked into extracted blank matrix [21]. The recovery of sugammadex at two concentration levels and the IS utilizing protein precipitation/filtration for sample preparation was near 100% (Table 4).

Application of the method and incurred sample reanalysis

This method was successfully applied to the analysis of samples from a sugammadex clinical study. Samples from the study were analyzed in eight runs; all runs met pre-established acceptance criteria. Interday accuracy and precision of the standards analyzed over the course of the study were calculated and are presented in Table 5. The precision (RSD%, n=8) ranged from 1.1% to 3.9% and the accuracy from 97.0% to 104% of the nominal values. Interday accuracy and precision of the QCs analyzed during the study were computed based on the mean calculated QC concentrations from the eight runs (Table 6). The precision (RSD%, n=16) ranged from 3.1% to 4.6% and the accuracy from 98.3% to 104% of the nominal values.

Incurred samples reanalysis (ISR) [22] was performed with 10% of the patient plasma samples from the study; these samples were randomly selected and were re-assayed in a separate batch run. The ISR results, shown in Figure 3, indicate that the differences in concentrations between the repeat and the initial values for all the tested samples were less than 20%, indicating good reproducibility of the method.

Conclusions

A simple, high throughput LC-MS/MS method using a commercially available column for the determination of sugammadex in human plasma was developed and validated. The LLOQ of this method was 0.100µg/mL using a 200µL aliquot of plasma and the linear dynamic range of the calibration curve was 0.100 to 40.0µg/mL.

Carryover was significantly decreased with the addition of gradient wash cycles. Sample preparation, using protein precipitation is simple and fast. Applying an Ostra phospholipid removal plate significantly reduced matrix effect and thus improved assay ruggedness.

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