Available online at www.sciencedirect.com

SCIENCE DIRECT®

Journal of Pharmaceutical and Biomedical Analysis
34 (2004) 631–641

JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS

www.elsevier.com/locate/jpba

Quantitative analysis of squalamine, a self-ionization-suppressing aminosterol sulfate, in human plasma by LC–MS/MS

Austin C. Li*, Andrew M. Sabo, Timothy McCormick, Sean M. Johnston

Genaera Corporation, 5110 Campus Drive, Plymouth Meeting, PA 19462, USA

Received 28 July 2003; received in revised form 8 October 2003; accepted 11 October 2003

Abstract

The special physico-chemical property of squalamine enables the formation of intra- or inter-molecular non-volatile strong salt, which is difficult to ionize in a mass spectrometer's interface. A sensitive, accurate, precise, and specific method for the quantitative determination of this self ion-suppressing compound in human plasma has been developed and validated using high performance liquid chromatography (HPLC) coupled with positive electrospray tandem mass spectrometry (MS/MS). Solid phase extraction (SPE) technique was utilized to extract human plasma samples using the Waters Oasis HLB cartridges. Deuterated squalamine was used as the internal standard (IS). Positive multiple reaction monitoring (MRM) mode was used to achieve both sensitivity and selectivity. A quadratic linearity range over 5–1000 ng/ml, $R > 0.999$ was achieved. Performance of the method has been validated and met all the specifications set forth in the US Food and Drug Administration's May 2001 "Bioanalytical Method Validation Guidance for Industry". Different sample reconstitution solutions were found to have dramatic impact on sensitivity of mass spectrometer used to squalamine. This is the first quantitation method using a positive and true multiple reaction monitoring mode detection for squalamine.

© 2003 Elsevier B.V. All rights reserved.

Keywords: Squalamine; Bioanalytical; Quantitation; LC–MS/MS; Self-ionization–suppression

1. Introduction

Squalamine is a 7,24-dihydroxylated 24-sulfated cholestane steroid conjugated to spermidine at C-3, a natural product (Fig. 1) originally isolated from liver of the dogfish shark [1]. It has shown significant anti-angiogenic and antitumor, but not cytotoxic properties [2–5]. Squalamine lactate is currently in different phases of clinical trials as a new anticancer entity for the treatment of non-small cell lung cancer and ovarian cancer. Squalamine is used with other cytotoxic agents, such as paclitaxel or carboplatin, to achieve tandem anticancer effect. That is, squalamine is used to inhibit the growth of cancer tissue by limiting nutrition supply, while cytotoxic agents are used to directly kill the cancer cells. A bioanalytical method for quantitative determination of squalamine in patient plasma as well as in other animal plasma is needed to acquire toxicology and pharmacokinetic parameters. All previous methods [6,7] for the quantitation of squalamine in animal and human plasma

* Corresponding author. Present address: Covance Laboratories Inc., 3301 Kinsman Boulevard, Madison, WI 53704, USA.
Tel.: +1-608-242-7982; fax: +1-608-241-7412/242-2735.
E-mail address: cjli65@yahoo.com (A.C. Li).

0731-7085/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0731-7085(03)00556-9

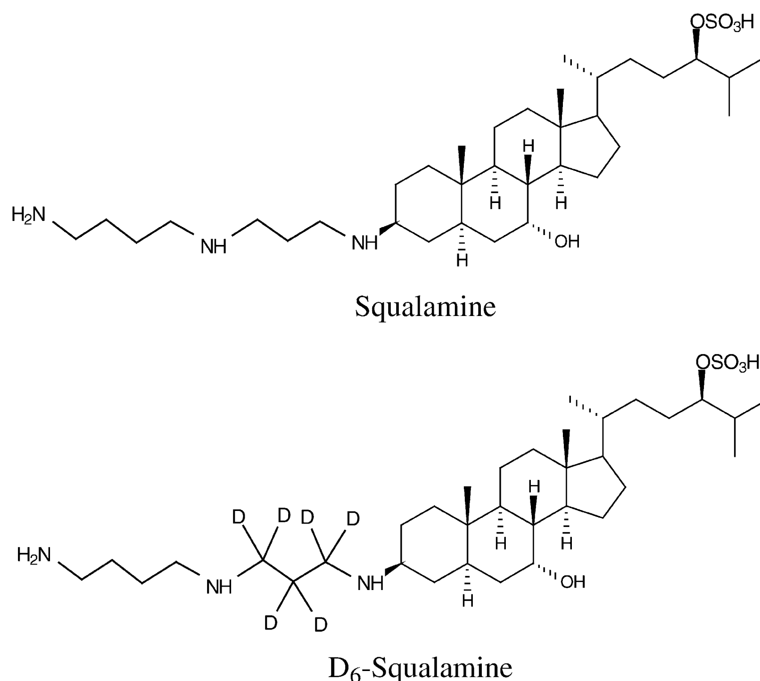

Fig. 1. Chemical structures of squalamine and deuterated squalamine.

utilized solid phase extraction (SPE) technique for sample preparation, and the extract was reconstituted in the solution of MeOH–1N HCl (80:20 (v/v)). The analyte was then chromatographed on a C-18 reverse phase LC column. The detection methods were all in negative modes, one used selected ion monitoring (SIM) and monitored the signal of negative molecular ion at *m/z* 626 with single quadrupole mass spectrometer [6]. Another method monitored the molecular ion (*m/z* 626) in both Q1 and Q3 quadrupoles. Even though the methods were validated as accurate and precise with the lower limit of quantitation (LLOQ) at 10 ng/ml and met the specifications as for a GLP bioanalytical method, it has poor selectivity. Significant endogenous interference was observed from some animal species, such as rabbit amniotic fluid, in routine analysis. Moreover, the noise level (chromatogram baseline) was high, ranging from 400 to 1200 counts/s.

Tandem mass spectrometric detection used in quantitative analysis has proved to be more selective, even specific, for target analytes than single quadrupole mass spectrometers [8]. This technique offers low noise baseline as well. To improve the selectivity of the method, efforts were made to develop a true multiple reaction monitoring (MRM) detection method. Therefore, scanning of product ions of squalamine was carried out in both positive and negative modes. By comparing the respective intensities of both parent ions and daughter ions, determination was made to use the positive ion mode to achieve better sensitivity (see Section 3). Meanwhile, the sample preparation procedure and high performance liquid chromatography (HPLC) conditions were also optimized.

This paper presents the collision induced dissociation of squalamine in both positive and negative ion modes, the optimization of sample reconstitution solution as well as optimized parameters of the mass spectrometer. The validation data including matrix standard curve, accuracy, precision, storage stability and specificity are also presented.

2. Experiemntal

Standards and chemicals: Squalamine lactate and deuterated squalamine lactate were supplied by the

Manufacturing Department of Genaera Corporation, with certificates of analysis.

Stock solutions of squalamine lactate and deuterated squalamine lactate of 1 mg/ml were prepared in solution of methanol–1N HCl in water (80:20 (v/v)). By series dilution of squalamine lactate stock solution with this solution, working standard solutions at 20, 10, 4, 2, 1, 0.4, 0.2 and 0.1 μg/ml and working quality control fortification solutions at 16, 8, 0.3 and 0.1 μg/ml were prepared. Working internal standard solution was prepared by a 1000-fold dilution of the deuterated squalamine lactate stock solution. All these stock solutions and working solutions were stored refrigerated (4 °C) for up to 1 month. The working solutions must be warmed up to room temperature unassisted and sufficiently vortex mixed before being spiked to control plasma.

All solvents and modifiers used were of analytical or HPLC grade.

2.1. Sample extraction procedure

Ten microliter working standards or working quality control fortification solutions were spiked to 200 μl blank plasma sample, 10 μl working internal standard was also spiked to the sample. The sample was vortex mixed and then diluted with 200 μl of 0.15N NaOH in water, and again vortex mixed. Since the residue of spermidine is a strong base, the purpose of using 0.15N NaOH to basify the sample was to convert squalamine to its free base form, and therefore to increase its hydrophobicity and be better retained in the SPE cartridge. The mixture was then loaded to conditioned Waters Oasis HLB cartridge (30 mg, 1 cc). After the plasma sample passed the column, the column was washed with 0.5 ml of water followed by 0.5 ml of MeOH–water (5:95 (v/v)). Vacuum was applied to the cartridge manifold to remove the residual washing solution in the cartridge. The analytes were eluted from the cartridge with 1.5 ml solution of MeOH–1N HCl (80:20 (v/v)). The eluent was concentrated to dryness with N_2 on a 35 °C water bath and the residue was reconstituted in 200 μl reconstitution solution for LC–MS/MS analysis. The sample reconstitution solution is a complex mixture of MeCN–water–MeOH, 33.25:62.75:4 (v/v/v), containing 0.024% formic acid and 0.01N HCl.

Table 1
HPLC gradient elution of squalamine

Time (min)	A (%)	B (%)	Flow (ml/min)
0.0	100	0	0.21
3.2	100	0	0.21
3.5	0	100	0.40
5.5	0	100	0.40
5.8	100	0	0.40
8.0	100	0	0.40
8.4	100	0	0.21

A: acetonitrile–water–formic acid (35:65:0.025 (v/v/v)); B: acetonitrile–water–formic acid (90:10:0.025 (v/v/v)).

2.2. Liquid chromatography

Chromatographic separation was performed on an Agilent 1100 LC system that includes a binary pump, a column thermostat and an autosampler with temperature control. The system was programmed at 35 °C for the column thermostat, 4 °C for the sample oven (autosampler), 20 μl for injection volume. A gradient elution (Table 1) was used to elute the analytes of interest and possible late-eluting endogenous components from the column. A MacMod Ace5C_{18} LC column in the size of 2.1 mm × 150 mm was used with a MacMod Ace5C_{18} guard column of 2.1 mm × 10 mm. This chromatographic condition results in good separation between squalamine and its diastereomer impurity 24S-squalamine, which elutes at about 2.2 min whereas squalamine elutes at about 2.5 min. Separation of these two diastereomers was important for quantitation since different batches of drug substance of squalamine (active ingredient, API) contained different amounts of 24S-squalamine. The flow from the LC column was diverted to the mass spectrometer only from 2.3 to 3.0 min. Other than this period of time the flow was diverted to waste, while at the same time, a second LC pump delivered isocratic mobile phase A at 210 μl/min to the mass spectrometer.

2.3. Mass spectrometer conditions

The LC eluent was delivered to a PE Sciex API-2000 triple quadrupole mass spectrometer, equipped with an electrospray ionization interface. The instrument was calibrated with a solution of polyprolyene glycol (PPG) according to the Applied Biosystems training instructions. To find out the op-

timal parameters for ion path, the mass spectrometer was tuned for both compounds by direct infusion of a mixture solution, each at 1 μg/ml concentrations. Based on the findings of positive Q1 scan and product ion scan, the following ion transitions are used for final MRM detection: *m/z* 628 → *m/z* 530 for squalamine, *m/z* 634 → *m/z* 536 for deuterated squalamine.

The parameters of ion path were optimized as following: declustering potential (DP): 26 V; focusing potential (FP): 350 V; entrance potential (EP): −10 V; collision cell entrance potential (CEP): 30 V; collision energy (CE): 44 eV; collision cell exit potential (CXP): 15 V.

The parameters of ion source were optimized by flow injection analysis as following: ion spray voltage (IS): 5500 V; Nebulizer gas (Gas 1): 20 psi; Turbo gas (Gas 2): 40 psi; Turbo gas temperature (TEMP): 550 °C; curtain gas (CUR): 35 psi; collision gas (CAD): 2 psi.

Quantitation was done with the Analyst 1.1 software (Applied Biosystems) using the above mass transitions. Further average accuracy and precision were calculated using Microsoft Excel spread sheet.

2.4. Method validation

The proposed method was fully validated by the parameters that are defined in the FDA bioanalytical method validation regarding linearity range, intra-day accuracy and precision, lower limit of quantitation (LLOQ), selectivity and storage stability.

The linearity of the method was evaluated by analysis of matrix standard curve samples, which were prepared by fortifying blank human plasma with the working standard and working internal standard solutions. The fortification resulted in the concentrations of squalamine in the final prepared sample at 5, 10, 20, 50, 100, 200, 500 and 1000 ng/ml. The concentration of internal standard in the final sample was 50 ng/ml. Calibration curve was generated by plotting peak area ratios between squalamine and deuterated squalamine against the concentration ratios. The correlation efficient (R) was determined.

The intra-day accuracy and precision were evaluated by analyzing six replicates of fortified blank plasma samples at each of four concentration levels, including LLOQ (5 ng/ml), QC-low (15 ng/ml), QC-medium (400 ng/ml) and QC-high (800 ng/ml). The QC samples were prepared the same procedure as for standard curve samples. The LLOQ was defined as the lowest point on the standard curve, which presents the lowest concentration that the method is capable of quantitating the amount of squalamine in the matrix accurately and precisely.

The selectivity of the method was evaluated by analyzing blank plasma samples. A total of six lots of control human plasma were screened for endogenous interferences and were spiked at LLOQ level for accuracy and precision analysis.

Storage stability of squalamine in different matrix was also evaluated. Squalamine stock solution stored in refrigerated conditions was analyzed in comparison with freshly prepared stock solution. Plasma samples spiked with squalamine at 15 and 800 ppb, in triplicate, were stored in a −80 °C freezer for different periods of time. Another set of samples for a three-cycle freeze and thaw testing were prepared. These samples were analyzed at different time points with fresh QC samples. The reconstituted QC sample solutions were kept in the refrigerated autosampler for 24 h and analyzed with fresh matrix STDs and QCs.

The extraction efficiency was obtained by comparison between responses of post extraction spiked matrix samples and spiked matrix samples at the nominal concentration of 500 ng/ml.

3. Results and discussion

3.1. Product ions of positive mode versus negative mode

Squalamine lactate dissolved in diluent at 10 μg/ml was directly infused to the API-2000 mass spectrometer at a flow rate of 10 μl/min using a syringe pump. Q1 scan was performed to scan the mass range of *m/z* 400–*m/z* 700 for both positive and negative modes. Squalamine gave its molecular and adduct ions at *m/z* 628 $[M+H]^+$, 664 $[M+H+HCl]^+$ and 626 $[M-H]^-$, 662 $[M-H+HCl]^+$, respectively (Fig. 2). When the parent ion at *m/z* 628 $[M+H]^+$ was selected at Q1 and collided in Q2, the most intense product ion at *m/z* 530 $[M-H_2SO_4]^+$ and other less intense ones at *m/z* 548 $[M-SO_3]^+$, 459, 442 were generated (Fig. 3). This shows that the elimination of the sulfate group is the most dominant CAD reaction. When the parent ion

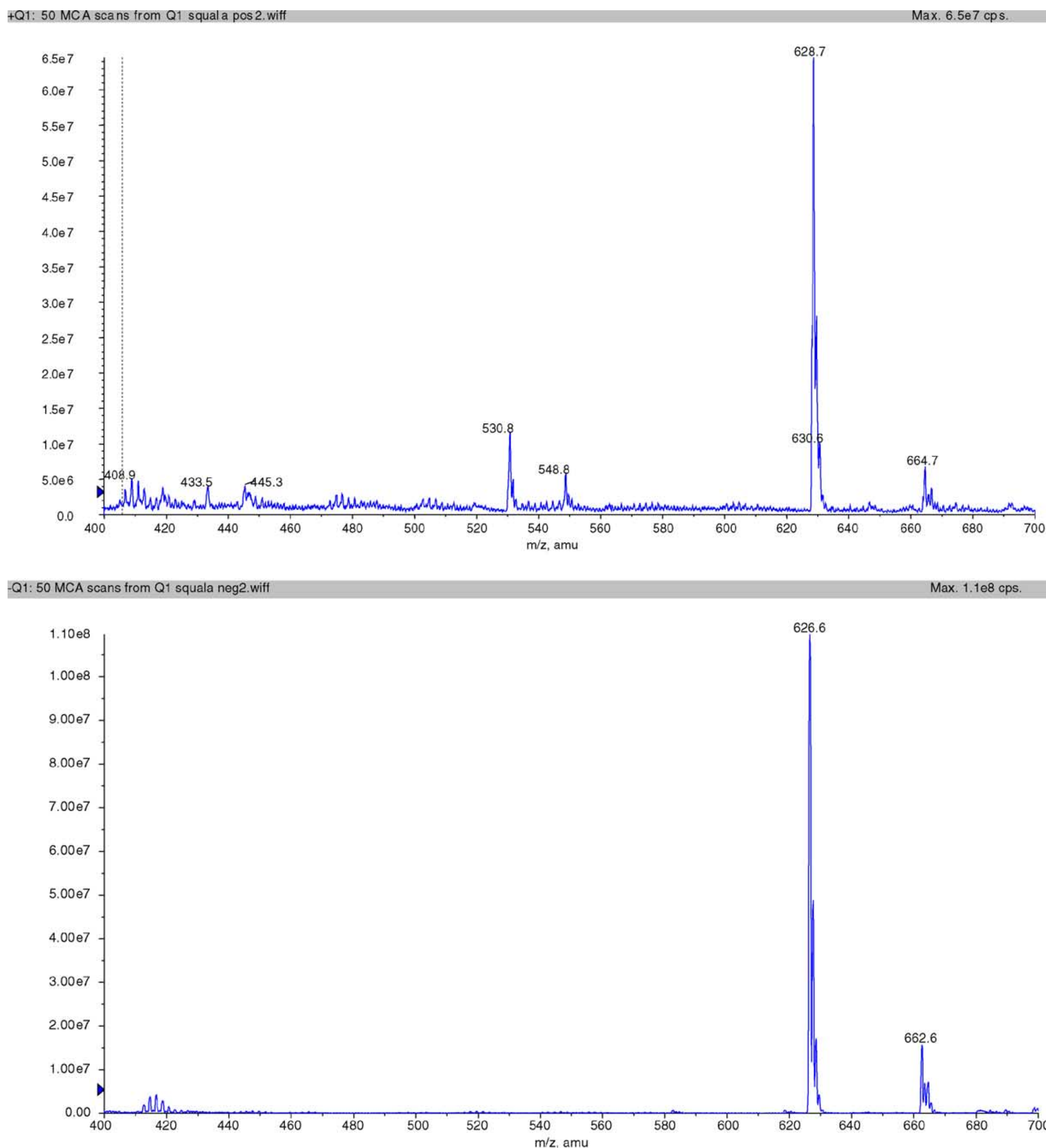

Fig. 2. Q1 Scan of squalamine lactate in positive and negative modes.

at m/z 626 $[M-\mathrm{H}]^-$ was selected at Q1 and collided in Q2, no other product ion can be unambiguously identified except the one at m/z 97 $[\mathrm{HSO_4}]^-$ (Fig. 3). This is because after losing a molecule of sulfuric acid the residual mass is incapable of carrying a negative charge since there is no acidic proton.

Even though the m/z 626 $[M-\mathrm{H}]^-$ was about two-fold more intense than m/z 628 $[M+\mathrm{H}]^+$, the

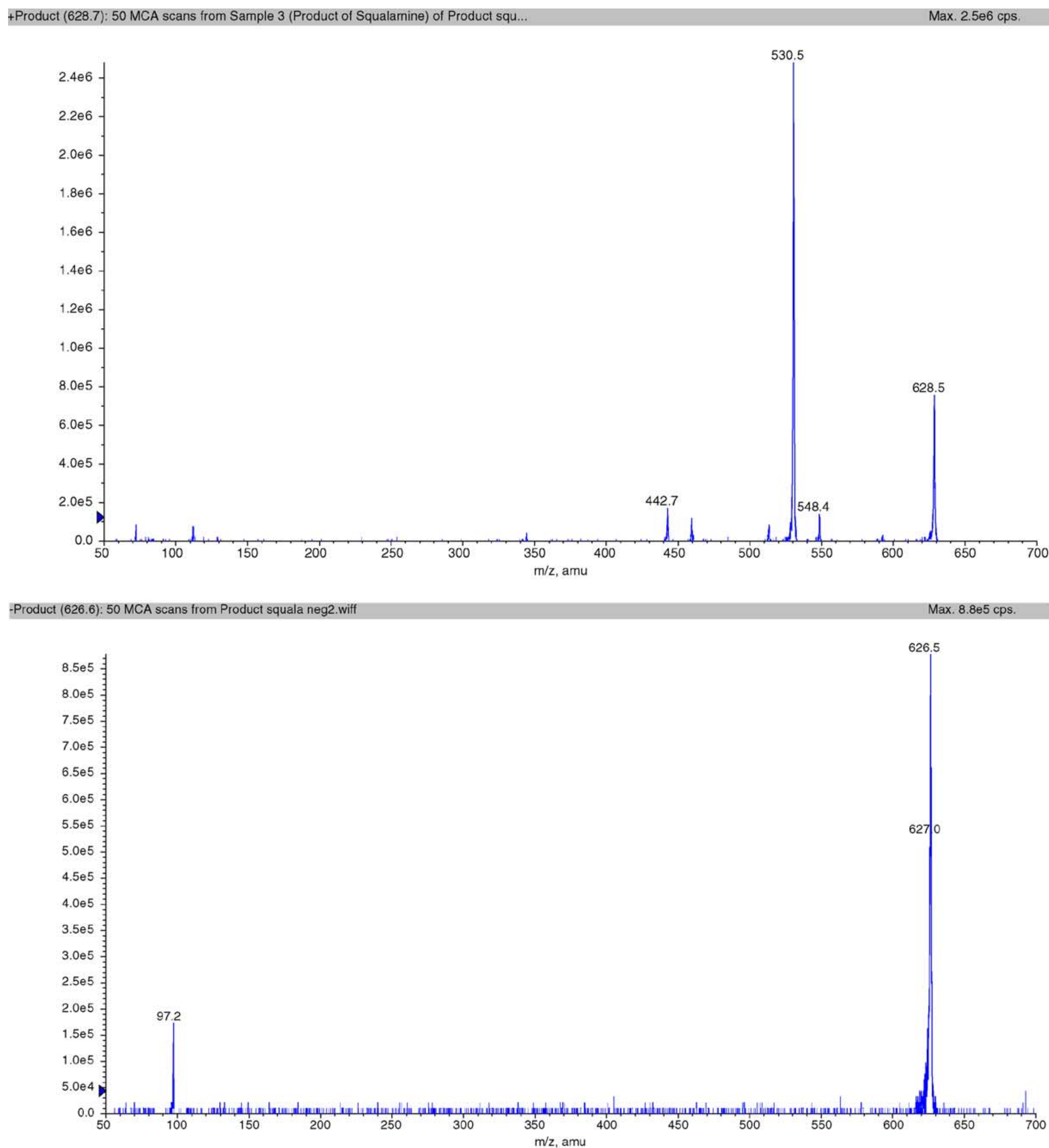

Fig. 3. Product ion scan of squalamine lactate in positive and negative modes.

daughter ion m/z 97 $[HSO_4]^-$ was about 10-fold less intense than the positive counterpart at m/z 530 $[M - H_2SO_4]^+$. Therefore, in order to more specifically detect squalamine using multiple reaction monitoring mode and achieve better sensitivity, the positive mode was selected. The collision activated dissociation in positive ion mode is proposed as illustrated in Fig. 4.

Squalamine is a fairly insensitive compound for mass spectrometers due to its chemical composition. The insensitivity was obvious even at the very ini-

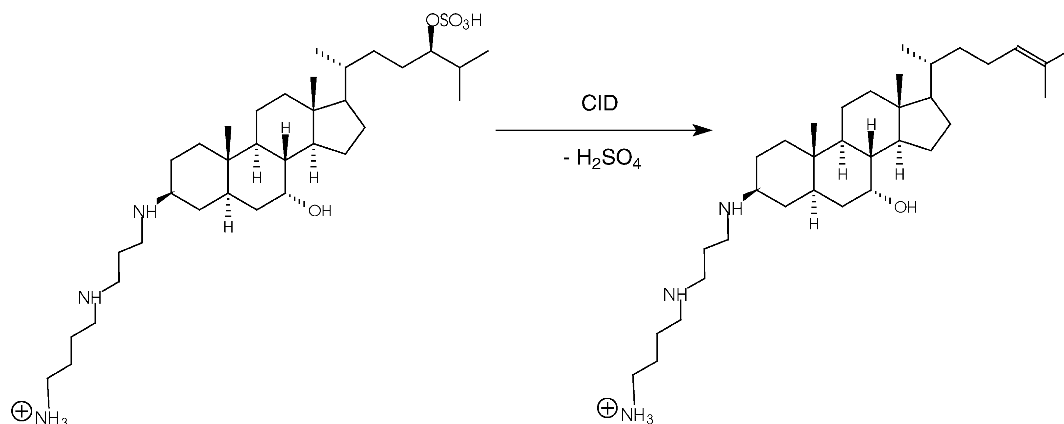

Fig. 4. Proposed collision activated dissociation of squalamine.

tial Q1 scan infusion test. Most compounds could show significant molecular ion signals at about 200–500 ng/ml, but squalamine could not generate a molecular ion peak well standing-out of the baseline noise unless the concentration was raised to 10 μg/ml. The molecule has a strong acidic sulfate group and a strong basic polyamine (spermidine) residue. Therefore, it is believed to form an inter- or intra-molecular salt in neutral condition. This strong acid–strong base salt is theoretically difficult to get ionized in the ionization interface due to its non-volatile ion-pair. To get better ionization efficiency, strong acid or strong base must be used to "free" this inter- or intra-molecular salt before the ionization interface. When the positive ion mode was considered for the method, hydrochloric acid was chosen to free the salt. Even though the hydrochloric acid forms another strong acid–strong base salt, squalamine responded more than 2 times better than it did in neutral condition. Fig. 5 shows the direct comparison, where the concentration in both solutions was 50 ng/ml.

In a separate study for accelerated degradation of squalamine lactate, des-sulfate reaction was shown as the primary degradation of squalamine in acidic solution [9]. This phenomenon again showed that the sulfate group was the most labile part for squalamine molecule. Moreover, the new compound resulted from the des-sulfate reaction, namely 24-dessulfate squalamine, showed much greater sensitivity on mass spectrometer in either Q1 full scan or MRM scan modes. This finding again confirmed that the presence of inter- or intra-molecular salt in squalamine reduced the ionization efficiency of squalamine.

3.2. Optimization of reconstitution solution

It was discovered that the sample reconstitution solution had a tremendous impact on peak intensity, which directly affected the sensitivity of the method. Table 2 shows the peak heights and peak areas for different sample reconstitution solutions tested. No matrix effect, such as extraction efficiency and ionization suppression, involved in the test. Solvent solution of squalamine was aliquot to test tubes and dried down with nitrogen stream. Then the residue was reconstituted in different solutions as shown in Table 2, and sonicated for 5 min before analysis. Squalamine dissolved in the complex reconstitution solution (MeCN–water–MeOH, 33.25:62.75:4 (v/v/v), containing 0.024% formic acid and 0.01N HCl) gave the best signal-to-noise ratio, and the intensity was at least 2 times stronger than the response from in solution of MeOH–1N HCl (80:20 (v/v)), which was used as reconstitution solution in previous methods. The exact reason why squalamine responded best in this complicated solution remains unknown, but could be speculated as that the sulfate in intra- or inter-molecular salt of squalamine may have been replaced by volatile HCl or formic acid, similar to the well-known "TFA Fix" practices.

Data in Table 2 also showed that the retention time of squalamine in reconstitution solutions without methanol (nos. 8, 9, 10 and 11) was relatively

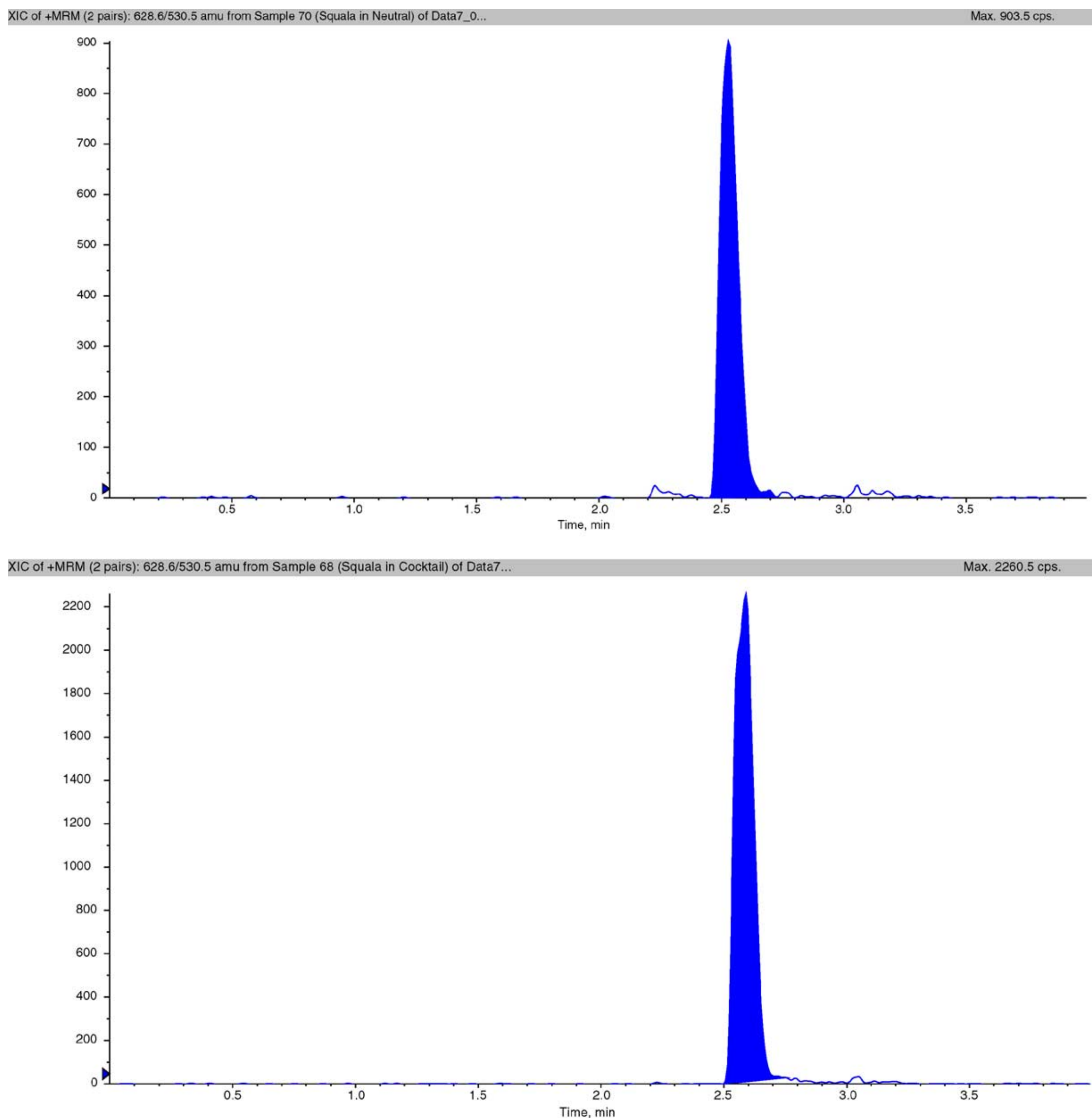

Fig. 5. Squalamine responses in neutral and acidic solutions (concentration 50 ng/ml). *Note*: (1) Injection volume: 20 μl. squalamine area—in neutral solution: 903; in acidic reconn: 2253. (2) Integration parameters—bunching factor: 2; noise threshold: 5; area threshold: 10; smooth times: 3.

constant (at ∼2.40 min). This seemed to assume that the concentration of hydrochloric acid in reconstitution solution did not affect the retention time for squalamine, even though it affected the responses of squalamine. For the other group of reconstitution solutions containing methanol (nos. 4, 5, 6, 7 and 1), the retention time kept steadily increasing as the concentration of methanol increased in the reconstitution solutions. The explanation for this phenomenon could be that squalamine molecule formed charged parti-

Table 2
Responses of squalamine in different reconstitution solutions

Reconstitution solution	Peak height (cps)	Peak area (counts)[a]	Retention time (mm)
(1) MeOH–1N HCl (80:20 (v/v)) (diluent)	2715	13600	2.97
(2) Mobile phase A	2285	15500	2.35
(3) MeCN–NHOAc (pH 3, 10 mM) (35:65 (v/v))	2230	18200	2.48
(4) Mobile phase A:diluent (95:5 (v/v))	6100	24100	2.60
(5) Mobile phase A:diluent (7:1 (v/v))	5730	22600	2.65
(6) Mobile phase A:diluent (3:1 (v/v))	5805	23200	2.75
(7) Mobile phase A:diluent (1:1 (v/v))	4730	19700	2.82
(8) Mobile phase A containing 0.01N HCl	2795	13100	2.40
(9) MeCN–water (35:65 (v/v)) containing 0.2N HCl	915	4900	2.40
(10) MeCN–water (35:65 (v/v)) containing 0.05N HCl	1665	7800	2.42
(11) MeCN–water (35:65 (v/v)) containing 0.01N HCl	2320	11500	2.40

Mobile phase A: MeCN–water–formic acid (35:65:0.025 (v/v/v)).
[a] Peaks were integrated using the same integration parameters. Injection volume: 40 μl of 50 ng squalamine/ml.

cles with different number of methanol molecules, and therefore this solvated material had less polarity (more hydrophobic) than non- or less-solvated squalamine molecules, and hence retained better in reversed phase column.

3.3. Ionization suppression and chromatography

The matrix extract had about 33% suppression on squalamine ion signal. The assay was performed by analysis of pure solvent and reconstituted blank plasma extract both spiked with the same amount of squalamine. Direct comparison was made between the peak responses. It was found that gradient elution was necessary to run the analysis since a more significant ionization suppression effect on analytes was observed when isocratic mobile phase was used. The isocratic mobile phase was MeCN–water–formic acid (35:65:0.025 (v/v/v)) with the flow set at 0.21 ml/min. This ionization suppression effect could probably be caused by late-eluting components, which co-eluted with sqalamine of next injection. The possible late-eluting components also build up contamination of the column resulting in varying retention times for squalamine. This would also result in a GLP compliance issue. Therefore, the gradient elution had to be used to wash out all late-eluting materials and clean-up the column for next injection. Columns of different batches but of the same brand were used in the validation, nearly the same chromatograms were obtained. This indicates the ruggedness of the chromatographic conditions.

3.4. Method validation

3.4.1. Standard curves

Eight concentrations of squalamine, from 5 to 1000 ng/ml, in plasma were prepared to calibrate the instrument. The matrix standards were analyzed in duplicate to bracket the quality control samples, generating a 16-point standard curve. Peak area ratios of squalamine to internal standard were used for regression. The standard curves were fitted to a $1/X$ weighted quadratic regression, where x represents the concentration of squalamine. Table 3 shows the results of the quadratic regression analysis of typical standard curves achieved in the validation process. The accuracy and precision of the back-calculated concentrations were excellent. For all calibration curves,

Table 3
Quadratic regression analysis results of typical standard curves for quantitative determination of squalamine in human plasma

Spiked concentration (ng/ml)	Calculated concentration (ng/ml)		Deviation (%)	
5.00	4.94	4.38	−1.2	−12
10.0	10.9	10.0	9.0	0.0
20.0	19.8	19.7	−1.0	−1.5
50.0	51.9	50.1	3.8	0.2
100	100	97.7	0.0	−2.3
200	209	206	4.5	3.0
500	498	481	−0.4	−3.8
1000	1000	1000	0.0	0.0

$Y = 3.92e^{-6}X^2 + 0.021X + 0.049$, $1/X$ weighting, $R = 0.9998$.

Table 4
Intra-day and inter-day accuracy and precison for quantitative determination of squalamine in human plasma

	Spiked concentration (ng/ml)	Intra-day accuracy (%)	Intra-day precision (CV (%))	Number assays (n)
Day-1	15.0	91.7	5.4	6
	400	102	1.3	6
	800	98.5	2.2	6
Day-2	15.0	90.5	4.6	6
	400	102	0.82	6
	800	101	1.9	6
Day-3	15.0	97.2	5.8	6
	400	103	3.3	6
	800	103	1.6	6
Overall	15.0	93.1	6.0	18
	400	102	2.1	18
	800	101	2.7	18

the correlation coefficient (R) of each calibration fell in specified range ($R > 0.999$), and the y-intercepts were virtually zero, indicating the absence of endogenous interference.

3.4.2. Accuracy and precision

Table 4 shows the results of inter- and intra-day accuracy and precision. Two different lots of human plasma were used, respectively, in Day-1 and Day-2 validation, while for Day-3 validation, one lot plasma was used for preparation of matrix standard curve samples, and the other lot plasma was used for quality control samples. All the accuracy and precision of inter-day (within Run Batch) and intra-day (Overall) fell well within the specified ranges.

3.4.3. Limit of quantitation

Besides the accuracy and precision validation above, the lower limit of quantitation was further assayed for six different lots of human plasma, to evaluate the accuracy, precision and endogenous interferences. Table 5 shows the accuracy and precision results for all six lots of human control plasma spiked at 5 ppb. The overall accuracy and precision were at 96 and 9.8%, respectively.

3.4.4. Storage stability

The stability of squalamine in human plasma was investigated with fortified QC samples. Triplicate QC

Table 5
Determination of LLOQ for squalamine in human plasma

	Blank plasma lot					
	1	2	3	4	5	6
Average accuracy (%)	101	91.3	105	99.2	91.5	92.1
With-in-lot precision (%)	5.8	5.3	14	8.2	8.1	6.2

Overall percent accuracy: 96.7%; overall precision (CV): 9.8%.

samples at 15 ppb (low) and 800 ppb (high) levels were assayed after being stored at −80 °C for different periods of time. Squalamine in plasma was found to be stable for at least 2 months under this condition, for at least 16 h at room temperature as well as 7-day three freeze and thaw cycles. The reconstituted QC samples were also stable for at least 24 h in the refrigerated (4 °C) autosampler.

Squalamine in solution of MeOH–1N HCl (80:20 (v/v)), including stock solution, working standards and working quality control fortification solutions, were found stable for at least 2 months at refrigerated condition (4 °C).

3.4.5. Specificity and sensitivity

The accuracy of LLOQ test for the six lots of human control plasma (Table 5) represent good selectivity and acceptable sensitivity for the method. The interference observed from all blank plasma was minor. Comparison of the response between LLOQ and blank samples show a signal to noise ratio of around 10:1. The previously mentioned questionable rabbit amniotic fluid was analyzed using this new method, and no interference was observed. This test again confirmed the new method is specific for squalamine detection. Moreover, the noise level is very low, maintaining at less than 20 counts/s, which is indicative of good sensitivity for squalamine.

3.4.6. Application

This analytical method for quantitation of squalamine in human plasma has better selectivity, longer stability and more sensitivity over the previous method, and has been applied in our laboratory to the routine analysis of non-clinical and clinical samples. The method has also been used to analyze preclinical samples of mouse, rat and rabbit plasma as well as rabbit amniotic fluid.

4. Conclusions

A more selective and sensitive LC–MS/MS bioanalytical method has been developed for the quantitative determination of squalamine in human plasma, and has been validated with excellent accuracy and precision. The study also presented the tremendous impact on mass spectrometer's response by the final sample reconstitution solution. Primary investigation has been carried out to optimize the ionization efficiency for squalamine, which represents the aminosterol sulfate species being difficult to ionize in mass spectrometer's ionization interface.

References

[1] K.S. Moore, S. Wehrli, H. Roder, M. Rogers, J.N. Forest Jr., D. McCrimmon, M. Zasloff, Proc. Natl. Acad. Sci. U.S.A. 90 (1993) 1354–1358.
[2] A.K. Sills Jr., J.I. Williams, B.M. Tyler, D.S. Epstein, E.P. Sipos, J.D. Davis, M.P. McLane, S. Pitchford, K. Cheshire, F.H. Gannon, W.A. Kinney, T.L. Chao, M. Donowitz, J. Laterra, M. Zasloff, H. Brem, Cancer Res. 58 (1998) 2784–2792.
[3] B.A. Teicher, J.I. Williams, H. Takeuchi, G. Ara, R.S. Herbst, D. Buxton, Cancer Res. 58 (1998) 2567–2792.
[4] J.A. Schiller, G. Bittner, Clin. Cancer Res. 5 (1999) 4287–4294.
[5] D. Li, J.I. Williams, R.J. Pietras, Oncogene 21 (2002) 2805–2814.
[6] Taylor Technologies Inc., Validation report "The quantitation of squalamine (MSI-1256) in human plasma", Report No. 1150.012400, 6 September 2000, Princeton Press, NJ.
[7] Communication with ClinTrials BioResearch (CTBR), 87 Senneville Road, Senneville, Quebec, Canada H9X 3R3, Tel.: +1-514-630-8200, fax: +1-514-630-8320.
[8] G.K. Poon, G. Kwei, R. Wand, K. Lyons, Q. Chen, V. Didolkar, C.E.C.A. Hop, Rapid Commun. Mass Spectrom. 13 (1999) 1943–1950.
[9] C.-J. Li, U.P. Kari, L.A. Noecker, S.R. Jones, A.M. Sabo, T.J. McCormick, S.M. Johnston, J. Pharm. Biomed. Anal. 32 (2003) 85–96.