Journal of Chromatography B, 978–979 (2015) 151–156

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

A novel ultra performance liquid chromatography–tandem mass spectrometry method for the determination of sucrose octasulfate in dog plasma

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ARTICLE INFO

Article history:
Received 27 June 2014
Accepted 30 November 2014
Available online 19 December 2014

Keywords:
Sucrose octasulfate
Ion pairing
UPLC–MS/MS

ABSTRACT

A novel, specific and sensitive bioanalytical method has been developed for the determination of sucrose octasulfate (SOS) in dog plasma and urine using ion-pair reversed-phase ultraperformance liquid chromatography coupled with electrospray triple quadruple mass spectrometry (IPRP-UPLC ESI MS/MS). ^{13}C-labeled sucrose octasulfate-$^{13}C_{12}$ sodium salt is used as the internal standard. 200 μL of plasma or serum sample is extracted using weak anion exchange solid phase cartridge. In this method, a polar amide column is employed for the liquid chromatograph (LC) separation while the diethylamine and formic acid buffer is used as the ion-pairing reagent. The low limitation of quantitation of sucrose octasulfate is 0.20 ng on the column with a signal to noise ratio larger than 50. Parameters such as linearity, accuracy and precision have been validated in full compliance with the FDA guidelines for the bioanalytical method development and validation. A linear regression model fit the calibration curve very well with $R > 0.99$. The bias and coefficient of variation of all levels of QCs are within the range of 15%. The selectivity, matrix effect and stabilities of analytes in solution and matrix have also been evaluated and the results met the acceptance criteria according to the guidelines. Based on these results, the method has qualified to analyze sucrose octasulfate in dog plasma for clinic research. This method has been applied to 1000 preclinical samples.

1. Introduction

Polysulfated carbohydrates are an important class of compounds in terms of biological activity and pharmaceutical importance [1]. For example, heparin has been found to be involved in many biological processes such as blood coagulation, inflammation and even cancer disease [2,3]. Sucrose octasulfate is a synthetic polysulfated carbohydrate. An aluminum salt of sucrose octasulfate (SOS) is clinically used in the treatment of duodenal ulcers. It involves the stabilization of fibroblast growth factor, thus promoting wound healing [4,5]. New and important applications of sucrose octasulfate and its analogs in wound healing and in the treatment of cancer have been suggested from some study [6].

Challenges for the quantitative determination of sucrose octasulfate and its polysulfated carbohydrates family in biological matrix come from the lack of means of sensitive detection. The traditional UV absorption detection is not feasible due to its structure of carbohydrate with which the detection limit cannot meet the requirements of ng/mL, normally larger than 0.5 mg/mL. Fluorescence detection by derivatization usually is a technique to increase the detection sensitivity for HPLC. However, all the polar groups of sucrose octasulfate are sulfate, resulting in unlikely derivatization for fluorescence detection. Furthermore, most of the polysulfated carbohydrates carry many sulfate groups with formally negative charge under nearly all of the experimental conditions, which are labile in the gas phase due to coulomb repulsion. Therefore, their mass spectrum analysis is every challenging. Using mass spectrometer as a detector, the compounds are requested to possess high ionization efficiency and ionized species should be stable in mass spectrometry scale to achieve good sensitivity.

Soft ionization techniques of mass spectrometry have been explored with some success on polysulfated carbonates, such as fast atom bombardment mass spectrometry (FABMS) [7,8], matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) [9–12], and electrospray ionization mass spectrometry (ESI-MS) [13–17]. There is one common problem in these techniques: fragmentation of multiple-negatively charged

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http://dx.doi.org/10.1016/j.jchromb.2014.11.031
1570-0232/

polysulfate carbonates through loss of sulfo group and resulting in low sensitivity. One practical approach for preventing the sulfo loss and consequentially improving sensitivity is to add the counterions to form the stable complex positive ions. An early study of sucrose octasulfate in tetramethylammonium (TMA) matrix proved that stable and predominant $[SOS(TMA)_9]^+$ ion has been formed [7] on FABMS. A systematic approach has been done by Gunay and his coworkers to evaluate the electrospray ionization mass spectral (ESI-MS) analysis of sucrose octasulfate [18] by infusing the solutions. In a survey of ammonium counterions, quaternary ammonium and phosphonium salts were found to give the excellent response in the positive electrospray ionization mode. With cesium ion as counter ion, a sensitive method has been developed for the quantitation of glucose and glycerol [14].

In the present study, a simple, sensitive and rapid LC/MS/MS method for the quantitation of SOS in biological matrix is described. To authors knowledge, this is the first method ever reported for the quantitative analysis of sucrose octasulfate in biological samples using LC–MS/MS. Diethylammonium is capable of forming a cationic adduct with sucrose octasulfate in the positive ESI ionization mode. The stable and predominant doubly charged positive complex ions of sucrose octasulfate can be formed after diethylammonium attachment. The mass spectrometer is operated in MRM mode, and the transitions for analyte and internal standard are monitored. The total runtime is 10 min. This method is highly specific and sensitive, and does not require derivatization or post-column addition. It is ready for clinical research to measure sucrose octasulfate in biological fluids.

2. Material and methods

2.1. Chemicals and materials

Potassium sucrose octasulfate was from USP (Rockville, MD, USA). Phosphoric acid, diethylamine (DEA), formic acid (FA) were from Sigma (Sigma–Aldrich Canada, Oakville, Canada) and sucrose octasulfate-$^{13}C_{12}$ Sodium was custom synthesized. Acetonitrile and methanol were purchased from Fisher Scientific (Fisher Scientific limited, Nepean, Canada). The Strata X-AW 33U Polymeric Weak Anion 30 mg/1 mL cartridges were purchased from Phenomenex (Torrance, CA, USA). Milli-Q water was provided in house by Milli-Pore system (Billerica, MA). Dog plasma (K_2EDTA) was purchased from Biochemed Services (Winchester, VA, USA). All the reagents were used without further purifications.

2.2. LC–MS/MS instrumentation setup

The chromatography is obtained on Waters ACQUITY UPLC system (Waters Canada, Mississauga, Canada) with Waters ACQUITY UPLC column (BEH Amide, 1.7 μm, 2.1 × 100 mm assembled with Waters in-line pre-column filter). Deionized water with 15 mM DEA/FA is used as mobile phase A (MPA) while ACN with 15 mM DEA/FA is used as mobile phase B (MPB). The gradient starts with 1% MPA and increases MPA to 40% over 5 min, then quickly switches MPA to 1% and keeps it to 10 min. Flow rate is 0.40 mL/min and column compartment temperature and sample compartment temperature are maintained at 35 °C and 5 °C, respectively. Under this analytical condition, the retention time of sucrose octasulfate is ∼5.0 min while the retention time of internal standard (IS) (sucrose octasulfate-$^{13}C_{12}$) is ∼5.0 min as well. Mass detector is Waters triple quadrupole (TQD) with ESI positive mode and MRM is employed for monitoring the analyte and internal standard. The analyte MRM transition is 858 → 74 while the IS MRM transition is 864 → 74. Instrument parameters were optimized, such as cone voltage, electrospray voltage, desolvation temperature and desolvation gas to achieve the optimal sensitivity. Milli-Q water was used as strong wash and 80% acetonitrile as weak wash while 10% acetonitrile was used as seal wash.

2.3. Stock preparation, calibration standards and quality controls

About 65.43 mg potassium sucrose octasulfate was weighted into 50 mL of volumetric flask and dissolved with Milli-Q water to make the final concentration of SOS stock solution as 1.0 mg/mL. Internal standard was prepared in the same manner but in much small volume due to the small amount of internal standard obtained. The stock solutions were stored in the refrigerator (5 ± 3 °C) for use or after use. The spiking solutions were diluted from stock solution (1.0 mg/mL) using Milli-Q water to intended concentrations.

The calibration curve range is 50–5000 ng/mL and the spiking solution is not greater than 5% in total of the plasma. The spiked plasma samples of calibration curves with eight calibrants, i.e. STD1 through STD 8, and quality controls (QC), i.e. QC LLOQ, QC Low (QC L), QC Medium (QC M) and QC High (QC H) were stored in −20 °C freezer. Working internal standard of 1000 ng/mL was prepared in deionized water.

2.4. Sample processing

Unknown samples, calibration curve (including blank without internal standard and blank with internal standard) and QC samples are thawed in ice-water bath and vortexed for ∼15 s after completely thawed. A typical extraction procedure is as follows:

- Aliquot 200 μL of plasma sample into 1.5 mL plastic tube and add 100 μL of working internal standard solution except for the blank without internal standard and vortex briefly. Instead, 100 μL of Milli-Q water is added into the blank without internal standard.
- Add 300 μL of 2% phosphoric acid to all samples and vortex briefly to mix well.
- Fix the Strata X-AW 33U Polymeric Weak Anion 30 mg/mL cartridges into the vacuum manifold.
- Add 1.0 mL of methanol followed by 1 mL of water into cartridges and let it drain at gravity flow.
- Load the prepared samples and let them drain slowly under gentle vacuum.
- Wash the cartridges with 1.0 mL of 2% FA in water; let it drain under 2–5 psi vacuum.
- Wash the sample with 1.0 mL of methanol; let it drain fast with vacuum.
- Elute with 0.5 mL of 10:4:100 of DEA:FA:(50/50ACN/H_2O) to the cartridge and let it drain under gentle vacuum.
- Collect the eluent to the tube and transfer 0.3 mL into HPLC insert vial. Inject 10.0 μL into the UPLC–MS/MS system.

2.5. Matrix effect and hemolysis effect

Six individual lots of K_2EDTA dog plasma were spiked at low and high QC level, respectively, i.e., QC L and QC H for matrix effect. Hemolysed plasma was prepared from regular K_2EDTA dog plasma spiked with 2% of whole blood. Three replicates from each level of each lot along with calibration curve were processed and analyzed by following the sample processing procedure described above. Low and high QCs of Hemolysis effect were prepared and processed in the same manner as for matrix effect QCs.

2.6. Stability evaluation

Stabilities in solution and biological matrix were assessed. The area ratio of analyte to internal standard was used to calculate the