Drug Discovery and Development

SCIEX

Quantitation of Limaprost, an Analogue of PGE1 in Human Plasma

A highly simplified and sensitive assay using the QTRAP® 6500+ LC-MS/MS system and the SelexION®+ differential mobility separation technology device

[1]Chao Zhang; [1]Dandan Si; [2]Xiangjun Meng; [2]Lei Yin; [2]Jingkai Gu; [1]Ting Liu; [1]Wenhai Jin
[1]SCIEX, China; [2]Research Center for Drug Metabolism, Jilin University, Changchun, China

Key Challenges in the Quantitation of Limaprost

- High sensitivity required with an LLOQ in the sub-pg/mL range

- Separation of endogenous interferences in human plasma to achieve desired LLOQ

- Lengthy and complex chromatography procedures which reduce sample throughput

Key Advantages of using the QTRAP® 6500+ LC-MS/MS System with the SelexION®+ Differential Mobility Separation Technology Device for the Quantitation of Limaprost

- Simplified LC-MS/MS method using only 1D chromatography on the QTRAP 6500+ system *vs.* a more complicated 2D LC-MS/MS assay.

- >3x faster run times: 15 minutes for current assay *vs.* >50 minutes for the previous 2D LC-MS/MS assay.

- High sensitivity with LLOQ of 0.3 pg/mL.

- Low background signal and separation of Limaprost from endogenous co-eluting interferences.

- SelexION+ technology is easy to maintain, and can be installed or removed in minutes with no need to break vacuum or use any tools.

- SelexION+ technology is highly robust, reproducible, and stable for use in regulated bioanalysis.

Introduction

Limaprost is an analogue of prostaglandin E1. It is a promising drug that has strong vasodilatory and antiplatelet activity for the treatment of various ischemic symptoms, such as ulcers, pain, and cold sensations associated with thromboangiitis obliterans

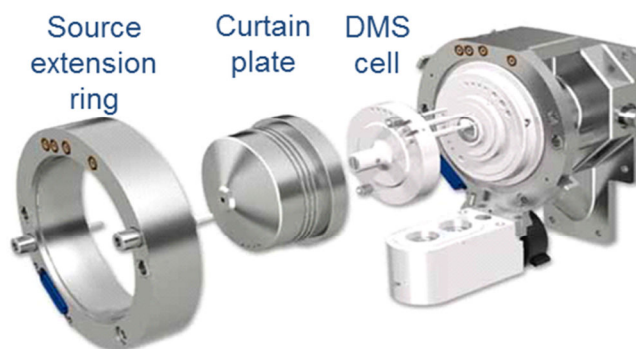**Figure 1.** The QTRAP® 6500+ System with SelexION®+ Technology.

Figure 2. Location of SelexION+ technology interfaced with a mass spectrometer. The SelexION+ differential mobility spectrometry (DMS) device is attached in front of the curtain plate and separates ions prior to entering the orifice of the QTRAP 6500+ instrument. It can be installed and removed in minutes with no need to break vacuum or use any tools.

Drug Discovery and Development

(TAO) and subjective symptoms associated with acquired lumber spinal canal stenosis (LCS).

The requirement for an ultra-low limit of quantitation (sub-pg/mL level) and separation from endogenous interferences in human plasma presents a challenge for its pharmacokinetic research. In order to reduce or separate the endogenous interferences, previous assays have been designed based on 2D-LC-MS/MS [1-2]. However, these assays suffer from the complexity of 2D-system method development and optimization, and include a long analysis time of more than 50 min.

Here we present a more simplified 1D-LC-MS/MS assay based on a SCIEX QTRAP® 6500+ LC-MS/MS system equipped with a SelexION®+ differential mobility separation technology device. The new assay provides excellent sensitivity with better usability and higher efficiency in comparison with previously published 2D-LC/MS/MS methods.

Materials and Methods

Samples Preparation: Human plasma samples were prepared according to the previously reported method [1].

Chromatography: A Shimadzu Prominence LC system (Shimadzu Corp) was used along with a Kinetex C18, 2.1mm x 50mm, 1.7 μm column (Phenomenex Inc.). Mobile phase A consisted of a 95/5 ratio of 1 mM ammonium acetate (pH=4.5) and acetonitrile. Mobile phase B was 100% acetonitrile. A gradient program was run over the course of 15 minutes at a flow rate of 0.3 mL/min. A 50 μL injection volume was used.

Mass Spectrometry and Differential Mobility: A SelexION®+ differential mobility technology device was interfaced with a QTRAP® 6500+ system equipped with the IonDrive™ Turbo V source. Acquisition was performed using MRM analysis in negative-ion mode by monitoring *m/z* 379.2 > 299.3. The compensation voltage (CoV) of the SelexION+ DMS cell was set to -7.5V.

Software: Analyst® 1.6.3 software was used for data acquisition. MultiQuant™ 3.0 software was used for data processing.

Results

The desired LLOQ for pharmacokinetic research of Limaprost in human plasma is sub pg/mL levels. Even after a three-step SPE extraction and careful chromatographic optimization, a lot of endogenous co-
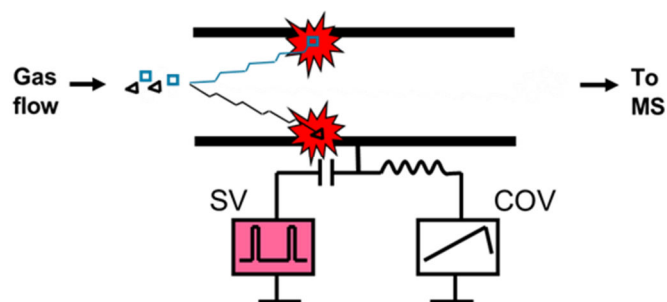

Figure 3. Principles of DMS. The planar differential mobility device (DMS) separates ions based on differences in their chemical properties using a combination of a separation voltage (SV) that filters unwanted ions away and a compensation voltage (COV) that only allows transmission of the ions of interest.
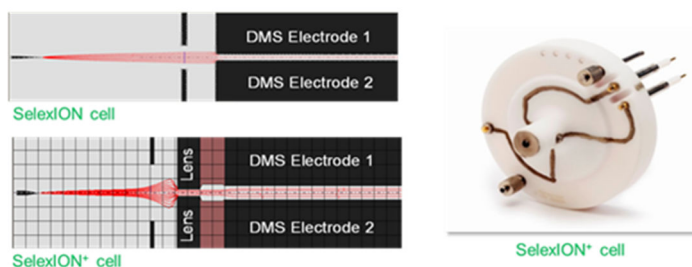

Figure 4. Comparison of SelexION and SelexION+ technology. **SelexION+ technology improves ion transmission by 2 fold without loss in selectivity or resolution and reduces transit time through detrimental fringing fields compared with the original SelexION technology**.

eluting interferences and high background noise (>104 cps) are found, resulting in fairly low sensitivity on a 1D-LC-MS/MS system. In order to avoid the complexity and long run time (over 50 min) for each sample using a 2D-LC method, the SelexION+ technology was combined with 1D-LC.

The SelexION+ technology is based on a planar differential mobility device (DMS). DMS separates ions based upon differences in their chemical properties and provides an orthogonal separation mechanism. In combination with 1D-LC, it makes the quantitation of Limaprost in complex matrices more simplified. As shown in Figure 3, a separation voltage (SV) is applied as the filtering voltage. It radially displaces ions towards one or the other electrode, depending upon high and low field mobility characteristics. The compensation voltage (COV) is applied as the restoring voltage, which can be tuned for the compound of interest. It restores the trajectory for a given ion to allow them to transmit through the DMS device and enter the mass spectrometer. Other co-eluting species that tune with different COV will be filtered away.

The SelexION+ technology is equipped with a new DMS (Differential Mobility Spectrometry) cell designed to improve ion transmission by 2 fold without loss in selectivity or resolution compared with the original SelexION technology (Figure. 4). It delivers a new dimension of selectivity and separation for Limaprost quantitation in complex plasma matrices. A discrete, Limaprost-specific compensation voltage (COV, -7.5 V) is used to filter ions into the mass spectrometer and resolve molecules prior to MS analysis based on their chemical and structural properties. Thus SelexION+ has the ability to prevent isobaric and isomeric interferences from contributing to quantitation during MRM analysis, resulting in unparalleled selectivity and a general reduction in noise, improving the overall S/N and sensitivity compared with 1D-LC.SelexION+ Technology is compatible with fast cycle times required for monitoring multiple MRM transitions combined with narrow HPLC peaks.

The calibration curve for Limaprost standard in human plasma is shown in Figure 5. The calibration extends from 0.3 pg/mL to 15 pg/mL using a $1/x^2$ weighting linear regression. No manual modification was used for integration. Representative chromatograms of Limaprost standard are displayed in Figure 6. As shown, the sensitivity and S/N are excellent even for the lowest level standard at 0.3 pg/mL. Three QC levels were used at 0.6 pg/mL, 3 pg/mL, and 12 pg/mL. The QC samples have excellent reproducibility as shown in Figure 7 for 0.6 pg/mL and 3 pg/mL with CVs less than 2%. With the high MRM sensitivity of the QTRAP 6500+ system, an LLOQ of 0.3 pg/mL in plasma is achieved using the orthogonal 1D-LC with DMS method. This meets the requirement for pharmacokinetic research of Limaprost in human plasma. The overall analysis time for each sample has been shortened to 15 min, leading to an increase in efficiency of more than 3x *vs.* the previous 2D-LC/MS/MS method (15 min vs. 50 min).

Conclusions

A more simplified 1D-LC/MS/MS assay based on a QTRAP 6500+ system equipped with a SelexION+ DMS technology device provides better usability and higher efficiency (>3x improvement) in comparison with a previously published 2D-LC-MS/MS assay for the quantitation of Limaprost in human plasma.

SelexION+ technology has the ability to prevent isobaric and isomeric interferences from contributing to the quantitation of Limaprost in human plasma, resulting in unparalleled selectivity, and a general reduction in noise. SelexION+ technology can be installed or removed in minutes with no need to break vacuum or use any tools and is highly robust, reproducible, and stable for use in regulated bioanalysis.

References

1. Komaba J, Masuda Y, Hashimoto Y, Nago S, Takamoto M, Shibakawa K, Nakade S, Miyata Y. Ultra sensitive determination of limaprost, a prostaglandin E1 analogue, in human plasma using on line two-dimensional reversed-phase liquid chromatography– tandem mass spectrometry. J Chromatogr B. 2007;852:590–7.

2. Park YS, Park JH, Kim SH, Lee MH, Lee YS, Yang SC, Kang JS. Pharmacokinetic characteristics of a vasodilatory and antiplatelet agent, limaprost alfadex, in the healthy Korean volunteers. Clin Appl Thromb Hemost. 2010;16:326–33.

Drug Discovery and Development

SCIEX

Index	Sample Name	Sample Type	Component Name	Actual Concentration	Area	Retention Time	Modified	Used	Calculated Concentration	Accuracy
1	std1	Standard	299	0.30	2.065e3	10.23	☐	☑	0.31	102.32
2	std2	Standard	299	0.60	3.080e3	10.22	☐	☑	0.58	95.88
3	std3	Standard	299	1.50	6.339e3	10.22	☐	☑	1.44	95.76
4	std4	Standard	299	3.00	1.288e4	10.21	☐	☑	3.17	105.52
5	std5	Standard	299	9.00	3.563e4	10.20	☐	☑	9.18	101.97
6	std6	Standard	299	15.00	5.685e4	10.19	☐	☑	14.78	98.55
7	QC1	Quality Control	299	0.60	3.082e3	10.20	☐	☑	0.58	95.97
8	QC2	Quality Control	299	3.00	1.244e4	10.20	☐	☑	3.05	101.59
9	QC3	Quality Control	299	12.00	4.399e4	10.19	☐	☑	11.39	94.89

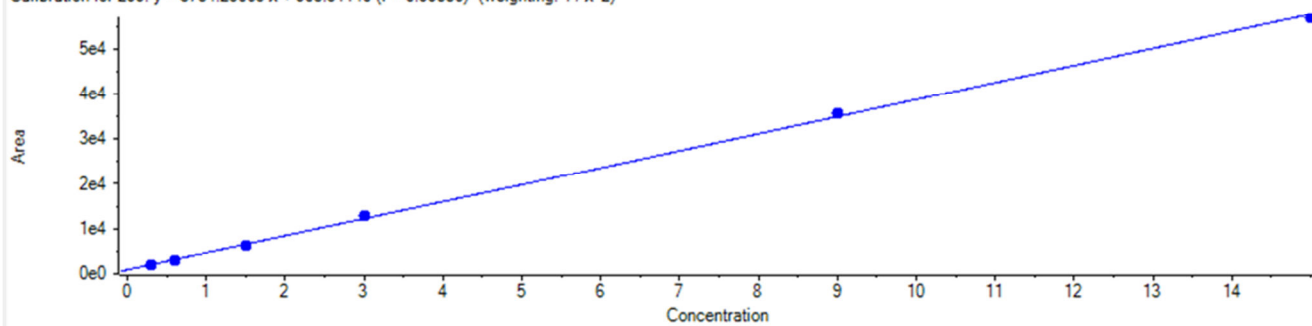Calibration for 299: y = 3784.25065 x + 903.51140 (r = 0.99886) (weighting: 1 / x^2)

Figure 5. Calibration curve for Limaprost in human plasma. Excellent linearity is obtained for the calibration of Limaprost from 0.3 pg/mL to 15 pg/mL using 1/x2 weighting linear regression without manually modifying the integration.
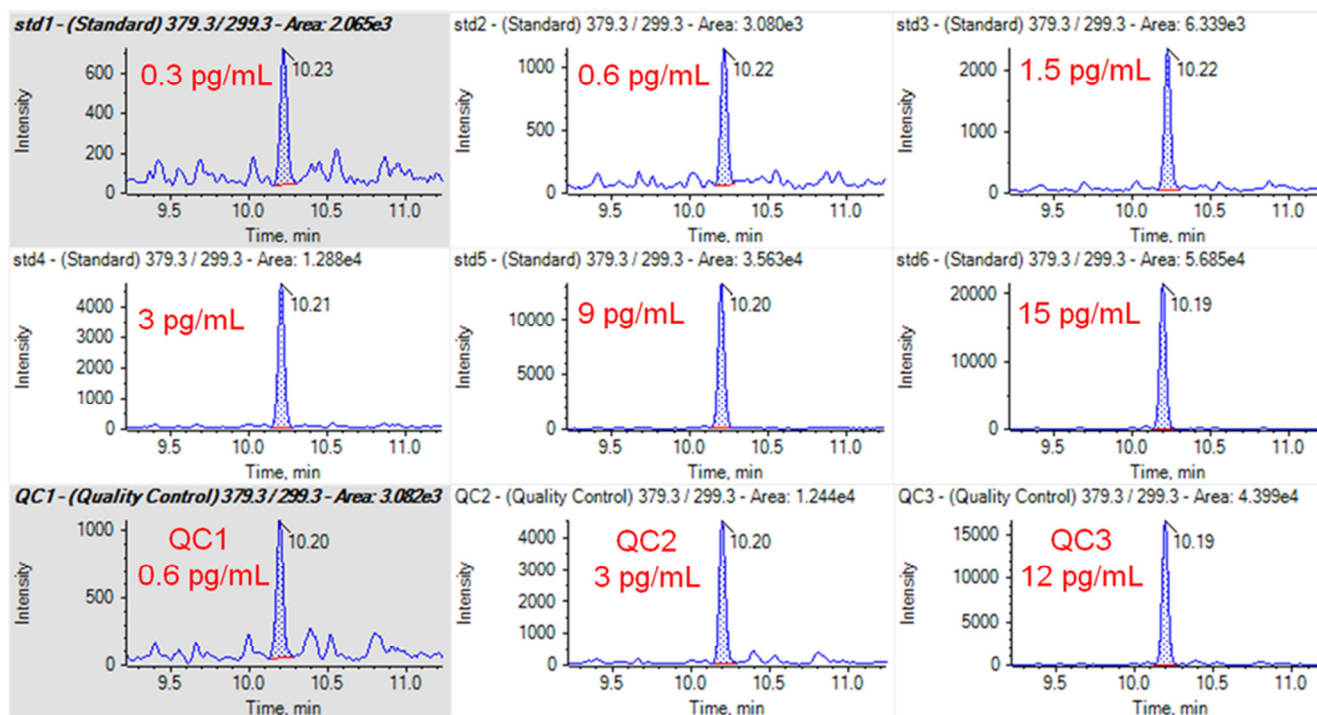

Figure 6. LC/MS Chromatograms. Representative chromatograms of Limaprost in human plasma samples from 0.3 pg/mL to 15 pg/mL and three QC levels.

For Research Use Only. Not for Use In Diagnostic Procedures.

Drug Discovery and Development

SCIEX

Sample Name	Sample Type	Component Name	Area	Retention Time	Modified	Used
0.6	Quality Control	299	2.973e3	10.20	☐	☑
0.6	Quality Control	299	3.008e3	10.19	☐	☑
0.6	Quality Control	299	3.082e3	10.20	☐	☑
3	Quality Control	299	1.288e4	10.21	☐	☑
3	Quality Control	299	1.244e4	10.20	☐	☑
3	Quality Control	299	1.247e4	10.20	☐	☑

Group by Sample Name for QCs — Area

Row	Compo_	Sample Na_	Num. Val_	Mean	Standard Devia_	Percent CV
1	299	0.6	3 of 3	3.021e3	5.588e1	1.85
2	299	3	3 of 3	1.260e4	2.494e2	1.98

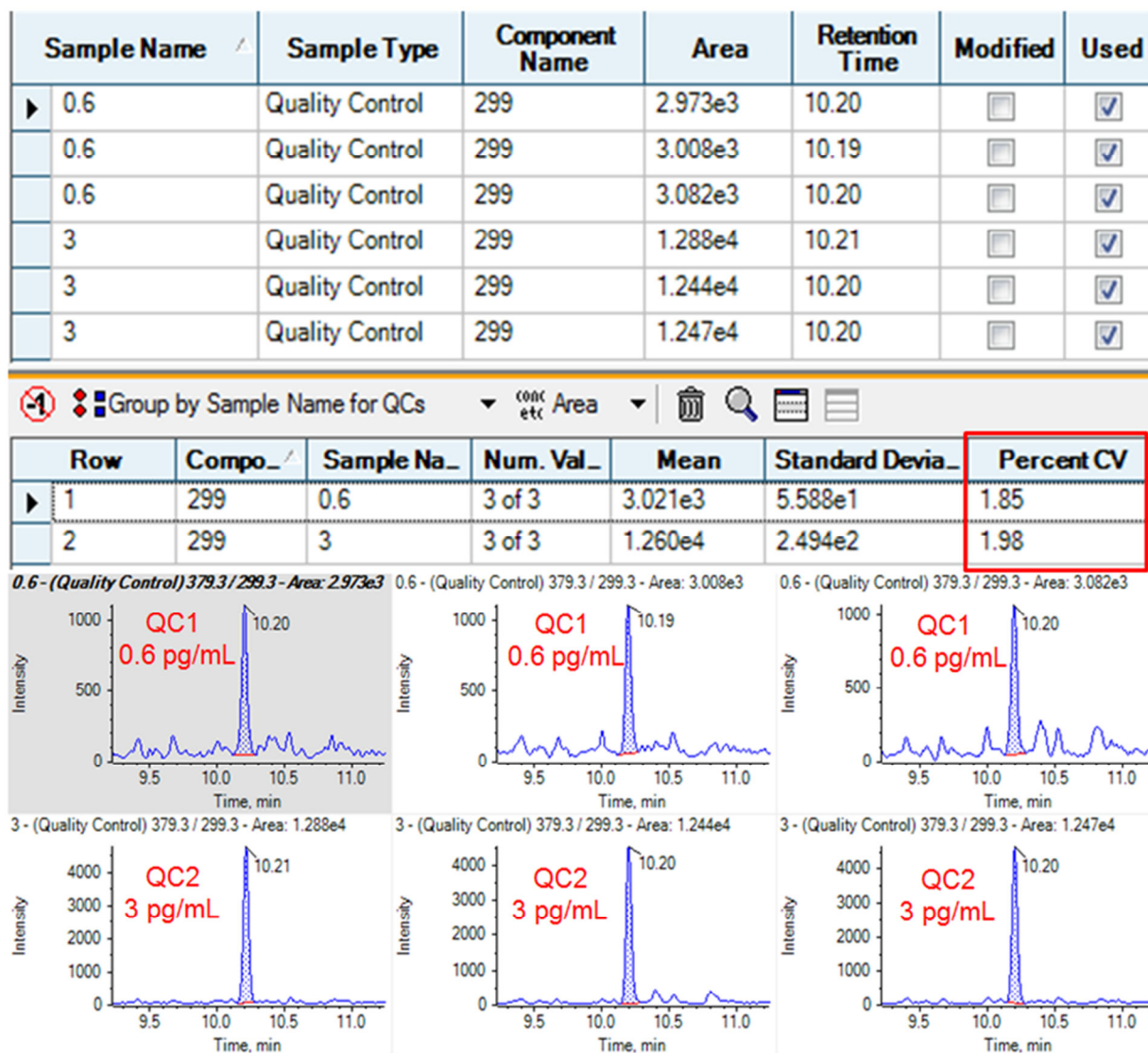**Figure 7.** Reproducibility of assay. Excellent reproducibility of Limaprost in human plasma samples is shown at two QC levels.