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Determination of Limaprost, an analogue of PGE1 in human plasma by QTRAP® 6500+ and SelexION®+ technology

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ABSTRACT

A more simplified 1D-LC/MS/MS assay based on a QTRAP® 6500+ system using SelexION®+ DMS technology is proposed and better usability and higher efficiency (~3 times increased) are obtained in comparison of current 2D-LC/MS/MS for the quantitation of limaprost in human plasma.

INTRODUCTION

Limaprost, an analogue of prostaglandin E1 analogue, 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 thromboangitis obliterans (TAO) and subjective symptoms associated with acquired lumber spinal canal stenosis (LCS).

Requirement of ultra-low limit of quantitation (sub-pg/mL level) and separation from endogenous interferences in human plasma becomes a big obstacle for its pharmacokinetic research. By now 2D-LC-MS/MS system [1-2] is the principal solution to reduce or separate the endogenous interferences. However, it suffers from complexity of 2D-system optimization and method development, especially the pretty long analytical time (>50 min). We present here 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 aimed at better usability and higher efficiency in comparison of current 2D-LC/MS/MS.

MATERIALS AND METHODS

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

- LC System: Shimadzu Prominence LC System (Shimadzu Corp)
- Column: Kinetex C18, 2.1mm*50mm, 1.7 µm (Phenomenex Inc.)
- Mobile Phase: A: 1 mM ammonium acetate (PH=4.5): Acetonitrile =95:5/B: Acetonitrile
- Flow Rate: 0.3 mL/min, Gradient program
- Injection Volume: 50 µL
- Run Time: 15 min
- Mass Spectrometry: SelexION®+ technology device interfaced with a QTRAP® 6500+ system
- Scan and Ion Mode: MRM Negative ESI (IonDrive™ Turbo V source) 379.2> 299.3 (COV=-7.5 V)
- Software (SCIEX): ♦ Acquiring: Analyst® 1.6.3 Software ♦ Processing: MultiQuant™ 3.0 Software

PRELIMINARY RESULTS

The desired LLOQ for pharmacokinetic research of limaprost in human plasma is 0.3~0.5 pg/mL. Even after the three-step SPE extraction and careful chromatographic optimization, a lot of endogenous co-eluting interferences and high background noise (>10^4 cps) are found, resulting in pretty low sensitivity on 1D-LC/MS/MS system. In consideration of complexity and long run time (over 50 min) for each sample on 2D-LC, SelexION® technology combined with LC can provide an orthogonal separation to make the quantitation of limaprost in complex matrices more simplified (Fig. 1 and Fig. 2).

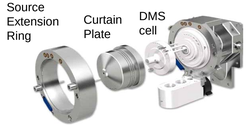
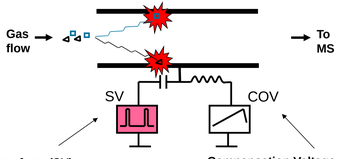


Figure 1. A differential ion mobility spectrometry (DMS) device interfaced with a mass instrument - DMS device is attached in front of the curtain plate and separates ions prior to entering the instrument orifice.



Separation waveform (SV)

Radially displaces ions towards one or the other electrode, depending upon high and low field mobility characteristics

Compensation Voltage (COV)

Restores the trajectory for a given ion to allow them to transmit through the DMS device and enter the mass spectrometer

Figure 2. Principle of DMS - Planar differential mobility device (DMS) separates ions based on differences in their chemical properties. A separation voltage (SV) is applied as the filtering voltage and the compensation voltage (COV) is applied as the restoring voltage, which can be tuned for the compound of interest. Other co-eluting species that tune with different COV will be filtered away.

SelexION®+ technology equips with a new DMS (Differential Mobility Spectrometry) cell designed to improve ion transmission by 2 folds without loss in selectivity or resolution compared with SelexION® technology (Fig. 3). 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 ion into the mass spectrometer and resolves molecules prior to MS based on their chemical and structural properties. Thus SelexION®+ has the ability to prevent isobaric and isomeric interferences from contributing to quantitation during MRM analyses, resulting in unparalleled selectivity, a general reduction in noise, which can improve the overall S/N and sensitivity of limaprost on 1D-LC system.

SelexION®+ Technology is compatible with fast cycle times required for monitoring multiple MRM transitions combined with narrow HPLC peaks. Over 10 points are acquired within <0.1 min peak width during which 12 MRM transitions are simultaneously monitored in the quantitation of limaprost.

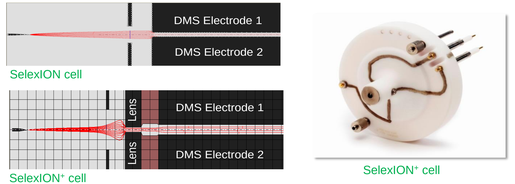


Figure 3. SelexION®+ technology equips with a new DMS (Differential Mobility Spectrometry) cell designed to improve ion transmission by 2 folds without loss in selectivity or resolution and reduces transit time through detrimental fringing field compared with SelexION® technology

With the pretty high MRM sensitivity of the QTRAP® 6500+ system, an LLOQ of 0.33 pg/mL in plasma (LLOQ of 0.5 pg/mL for neat standard solution) is achieved using our orthogonal method, which meets the requirement of pharmacokinetic research of limaprost in human plasma (Fig. 4 and Fig. 5). The overall analysis time for each sample has been shortened to 15 min, leading to 3 times efficiency increased than 2D-LC/MS/MS method (15 min vs. 50 min).

Sample Name	Sample Type	Acquisition Date & Time	Component Name	Actual Concentration	Area	Retention Time	Used	Calculated Concentration	Accuracy
0.5 pg sd	Standard	2015/10/30 16:47:18	299.3	0.50	5.442e2	9.58	☑	0.49	97.40
1pg sd	Standard	2015/10/30 14:11:55	299.3	1.00	1.092e3	9.59	☑	1.05	105.34
10pg sd	Standard	2015/10/30 14:27:24	299.3	10.00	9.631e3	9.58	☑	9.89	98.87
100pg sd	Standard	2015/10/30 14:42:51	299.3	100.00	9.519e4	9.59	☑	98.40	98.40

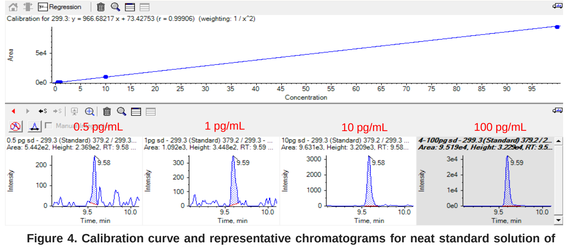


Figure 4. Calibration curve and representative chromatograms for neat standard solution of Limaprost from 0.5 pg/mL~100 pg/mL (1/x² weighting linear regression).

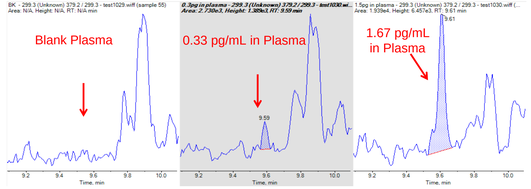


Figure 5. Representative chromatograms of Limaprost in human blank plasma and standard samples at 0.3 pg/mL and 1.5 pg/mL(without Internal Standard correction).

CONCLUSIONS

■ SelexION®+ technology has the ability to prevent isobaric and isomeric interferences from contributing to quantitation of Limaprost in human plasma, resulting in unparalleled selectivity, a general reduction in noise

■ A simplified 1D-LC-MS/MS assay based on a SCIEX QTRAP® 6500+ LC-MS/MS system equipped with a SelexION®+ device provides better usability and higher efficiency in comparison of current 2D-LC-MS/MS for the quantitation of Limaprost in human plasma.

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