Analysis of Prostaglandin Isomers using the SelexION® Device

Enhanced Selectivity using Differential Mobility Separation Technology

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) has become increasingly popular as an analytical technique in large part due to the improved selectivity that it provides in comparison to other techniques including LC-UV, immunoassay, GC-MS, and LC-MS. In many instances, this enhanced selectivity allows analysts to remove diverse sources of interferences, which may include isobaric analytes or persistent chemical background. However, there are also many application areas where still greater selectivity is desired, primarily due to the structural similarities of target compounds. The use of accurate-mass / high-resolution mass spectrometry systems cannot aid with the differentiation between the interfering species when the exact mass of each target compound is identical.

The analysis of the eicosanoid family of molecules, including the prostaglandins (Figure 1), is just such an application area, as many of these compounds are very similar in structure and may have identical chemical composition, making it virtually impossible to separate these species by tandem mass spectrometry. The analysis of Prostaglandin F2α (PGF2α) and its isomer 8-iso-Prostaglandin F2α (8-iso-PGF2α) presents such a challenge, as the chemical formula for each is $C_{20}H_{34}O_5$, and so the exact mass of the deprotonated parent ion for each is identical, m/z 353.2334. Typically, very long chromatographic gradients are used in order to separate the interfering compounds prior to detection by the mass spectrometer for these compounds.

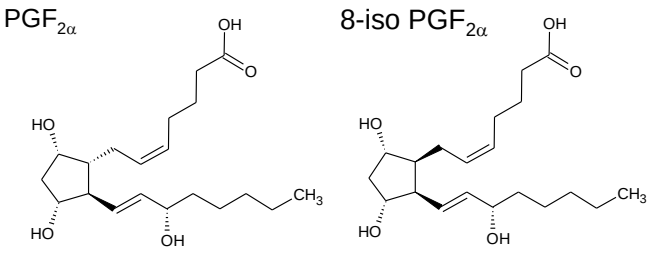

Figure 2. Structural isomers Prostaglandin F2 alpha (PGF2α) and 8-iso-Prostaglandin F2 alpha (8-iso-PGF2α). Due to the identical masses and the very similar chemical properties of these molecules, they are difficult to analyze by LC-MS alone.

Here, differential mobility separation (DMS)[1] is to resolve the isobaric species PGF2α and 8-iso-PGF2α, prior to analysis by LC-MS/MS. Long chromatographic separation of these compounds is no longer strictly required, since complete separation can be achieved post-LC using DMS. The significance of this result is that analysts may dramatically decrease chromatographic run-times used in traditional LC-MS/MS methods, thus increasing the throughput and efficiency of the laboratory, while providing higher confidence in the accuracy of results.

Key Characteristics of the SelexION Technology for Prostaglandin Separation

- QTRAP® 5500 LC-MS/MS system equipped with the SelexION device provides the enhanced selectivity required to separate the prostaglandin isomers PGF2α and 8-iso-PGF2α
- SelexION device provides an easy to implement solution for separation of eicosanoid isomers:
  - Easily installed and removed in <2 minutes, without breaking vacuum. No tools or cables are required;
  - Rapid switching of voltages is compatible with multi-component MRM analysis and fast LC;
  - Option to add chemical modifiers to the transport gas allows users to dramatically enhance the efficacy of ion mobility separations.

Methods

Sample Preparation: Samples of prostaglandins were received by donation.

HPLC Conditions: Liquid chromatography was performed using a Shimadzu Prominence HPLC system. Reversed-phase chromatographic separation was accomplished using a Phenomenex Kinetex column (2.6 µm, 100 x 2.1 mm) with mobile phases consisting of water (mobile phase A) and acetonitrile (mobile phase B). The HPLC run consisted of a linear gradient from 10-98-10% mobile phase B in 12.5 minutes.

Mass Spectrometry: The QTRAP 5500 LC-MS/MS system was operated in the Multiple Reaction Monitoring (MRM) mode, using negative electrospray ionization. 68 different MRM transitions were monitored, each with a dwell time of 5 msec, to simulate a multi-component method for targeting a large number of compounds from the eicosanoid family.[2] The Compensation Voltage (CoV) parameter was optimized for each of the target analytes, PGF2α and 8-iso-PGF2α, and comparative experiments were performed with the SelexION device "on", and in "transparent" mode.

Results

A mixture containing Prostaglandin F2α (PGF2α) and 8-iso-Prostaglandin F2α (8-iso-PGF2α) was analyzed using a QTRAP 5500 LC-MS/MS system equipped with the SelexION device. Since these analytes are structural isomers, they will both have the same precursor ion (m/z 353.2) when subjected to electrospray ionization in negative mode. Similarly, these

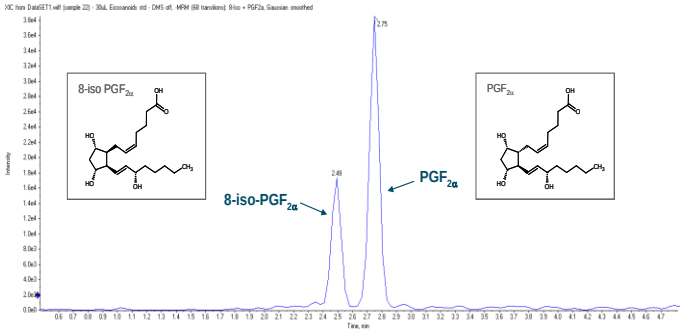

Figure 2. Chromatographic Trace with the SelexION Device Operating in "Transparent" Mode (DMS Off). The MRM 353.2/309.2 displays chromatographic peaks for both PGF2α and 8-iso-PGF2α when the SelexION device is operated in "transparent" mode, because these isomers cannot be resolved by MS/MS alone. An orthogonal separation technique, such as ion mobility separation, is required in order to distinguish between these analytes.

analytes have virtually the same fragmentation pattern, and share a common MRM transition, 353.2/309.2. When the SelexION device was operated in "transparent" mode, these two

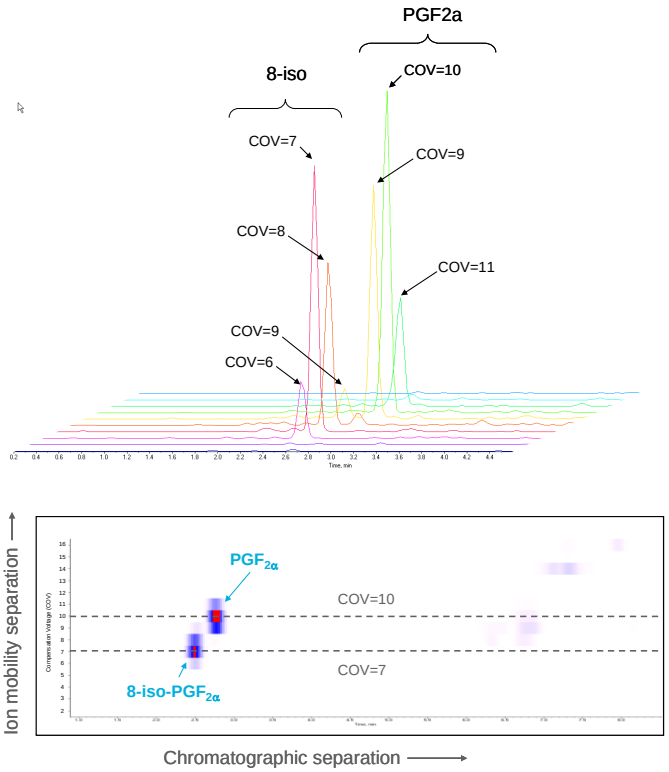

Figure 2. Tuning of Compensation Voltages (CoV) for PGF2α and PGF2α. (Top) The same sample was injected multiple times, with a different fixed value of Compensation Voltage (CoV) for each injection. As can be seen from the overlaid traces, the optimal value for PGF2α is CoV=10, while the optimal value for 8-iso-PGF2α is CoV=7. (Bottom) A heat map view of the CoV tuning experiment shows that the separation of the isomers PGF2a and 8-iso-PGF2a can be achieved equally effectively by using either chromatography (x-axis) or ion mobility (y-axis).

analytes could not be resolved by the mass spectrometer alone, and so it was necessary to use an extended LC gradient in order to chromatographically resolve the analytes, as shown in Figure 2.

To demonstrate the tuning of the CoV parameters using the SelexION device, the same sample was injected multiple times, with a different fixed value of CoV used for each injection. The traces for each injection are overlaid and displayed in Figure 3 top.

When the CoV parameter was set to a value less than or equal to 5 or greater than or equal to about 12, neither PGF2α or 8-iso-PGF2α was allowed to pass into the orifice of the mass spectrometer – both were filtered out DMS. For CoV values ranging from 6 to 8, only 8-iso-PGF2α was selected, and for CoV values ranging from 9 to 11 only PGF2α was selected. From Figure 3 bottom, it is clear that by selecting the appropriate values of the CoV parameter – 7.0 for 8-iso-PGF2α, and 10.0 for PGF2α – it is possible to completely resolve these analytes.

Using the LC-MS/MS workflow with DMS on to apply gas phase separation, it was possible to completely resolve the PGF2α and 8-iso-PGF2α isomers, as shown in Figure 4. Since the voltages can be rapidly switched throughout the analysis, both compounds may be measured simultaneously and completely independently of one another. The CoV parameter for each compound is simply added to the MRM table in the instrument acquisition method.

Conclusions

With appropriate optimization of the Compensation Voltage (CoV) parameter, SelexION differential mobility separation technology was able to completely resolve the isomers Prostaglandin F2α and 8-iso-Prostaglandin F2α. This is a significant result, since it implies that chromatographic separation is no longer strictly required for a robust and accurate LC-MS/MS method. This is clearly shown in the Figure 3 heat map, where equivalent separation is achieved in the time and CoV dimensions. Significant shortening of the chromatographic run times can now be explored in combination with DMS separation, offering analysts the potential to dramatically increase the throughput and efficiency of the laboratory, while maintaining confidence in the accuracy of results.

References

1. SelexION® Technology: The Solution to Selectivity Challenges in Quantitative Analysis - Differential Mobility Separations Enhanced with Chemical Modifiers: A Higher Dimension in Selectivity. SCIEX Technical note RUO-MKT-02-3251-C.
2. Targeted Profiling of Lipid Mediators - Multiplexed Assay using the SCIEX QTRAP® 6500+ LC-MS/MS System. SCIEX Technical note RUO-MKT-02-10787-A.

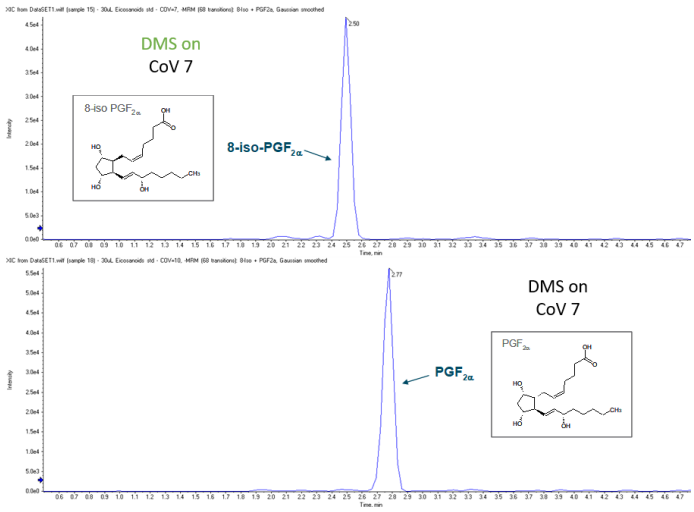

Figure 4. Chromatographic Trace with the SelexION Device Turned "on". When the DMS is activated and the compensation voltages are tuned for each of the compounds, complete separation is achieved as observed from monitoring the same MRM transitions. With the Compensation Voltage (CoV) parameter set to a value of 7.0, only 8-iso-PGF2α is transmitted by the ion mobility device (top). Similarly, with CoV=10.0, only PGF2α is transmitted (bottom).

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Headquarters
500 Old Connecticut Path | Framingham, MA 01701 USA
Phone 508-383-7700
sciex.com

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