

AB SCIEX SelexION™ Technology Used to Improve Mass Spectral Library Searching Scores by Removal of Isobaric Interferences

Differential Mobility Used as a Tool to Address Selectivity Challenges

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Overview

A highly selective differential mobility spectrometry device has been used to enhance the quality of multi-targeted screening analysis by LC/MS/MS. This has been achieved by pre-separating ions of similar mass prior to tandem mass spectrometric analysis; therefore removing these isobaric interferences and improving mass spectral library searching scores.

Introduction

Rapid and reliable screening methods for drugs of abuse are required for the detection of xenobiotics in forensic intoxication cases. Tandem mass spectrometry has become an increasingly popular analytical technique used in screening of drugs, due to the additional selectivity that is provided compared to single-stage MS and other techniques. Multi-targeted screening by LC/MS/MS uses Multiple Reaction Monitoring (MRM) triggered Information Dependent Acquisition (IDA) MS/MS spectra which are used to confirm the identity of detected drugs based on mass spectral library searching.

Matching MS/MS spectra generated from real samples to library spectra can be impeded by the presence of interfering isobaric compounds in the sample matrix. These isobaric interferences having similar m/z to the analyte will fragment producing extra peaks in the sample spectrum not present in the library spectrum. This results in a reduced score and reduced confidence rating. For these challenging analyses an orthogonal separation technique, such as differential mobility spectrometry (DMS), may be used to resolve isobaric species that cannot be separated by tandem mass spectrometry. In this work presented here, the new SelexION™ ion mobility technology has been utilized to enhance the quality of mass analysis by pre-separating ions of similar mass thereby removing these isobaric interferences, improving the mass spectral library searching scores.

Hardware and Methods

Experiments were performed using a QTRAP® 5500 LC/MS/MS system equipped with the novel SelexION™ differential mobility device, to provide enhanced selectivity.

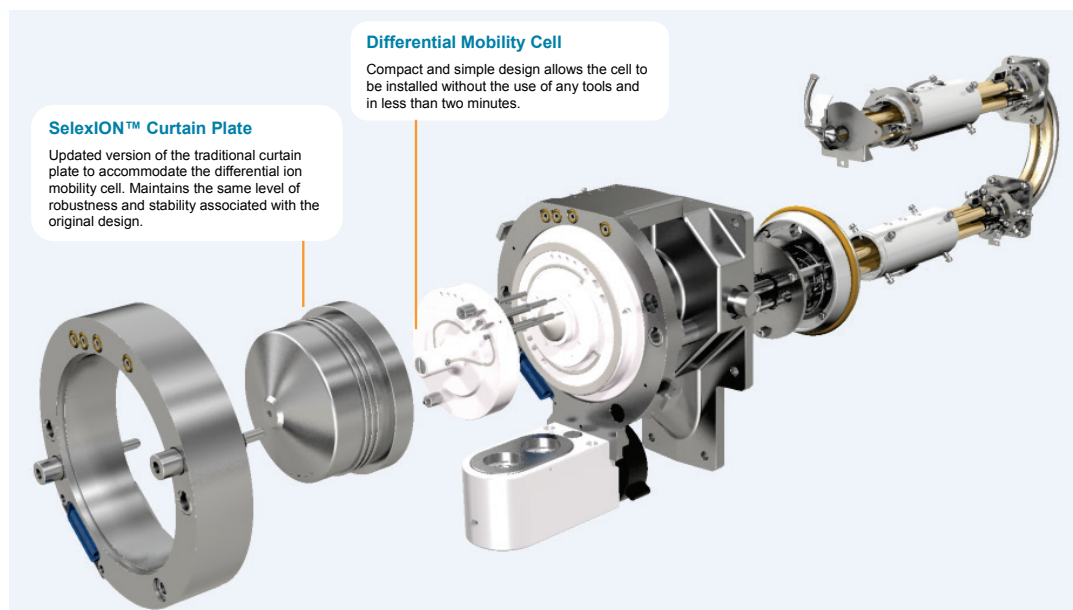


Figure 1. SelexION™ Technology. The DMS interface is directly coupled to the orifice plate. A modified curtain plate accommodates the DMS cell which can be easily installed and removed without the use of any tools and without venting the system. The source extension ring enables use of the standard AB SCIEX Triple Quad™ and QTRAP® system sources.

Key SelexION™ Technology Innovations

- Planar geometry results in short residence times, high speed and minimal diffusion losses for maximum sensitivity and UPLC compatibility.
- Easy to maintain and can be installed or removed in minutes with no need to break vacuum or use any tools.
- The ability to introduce chemical modifiers to the homogenous fields of the SelexION™ cell allows the amplification of the separation capacity and adds a new dimension to selectivity.

The separation of ions in the SelexION™ device is based upon differences in their migration rates under high versus low electric fields. A high field is applied between the electrodes for a short period of time, and then a low field is applied in the opposite direction for a long period of time (see Figure 2). Any difference between the low-field and high-field mobility of an analyte ion causes it to migrate towards one of the electrodes. The ion is steered back towards the center-line of the device by the application of a second voltage offset, known as the Compensation Voltage (CoV); a compound-specific parameter that can be used to selectively filter out all other ions. Rapid switching of the Compensation Voltage parameter allows the user to concurrently monitor many different compounds.

Innovative Planar Design; SelexION™ Ion Mobility Cell.

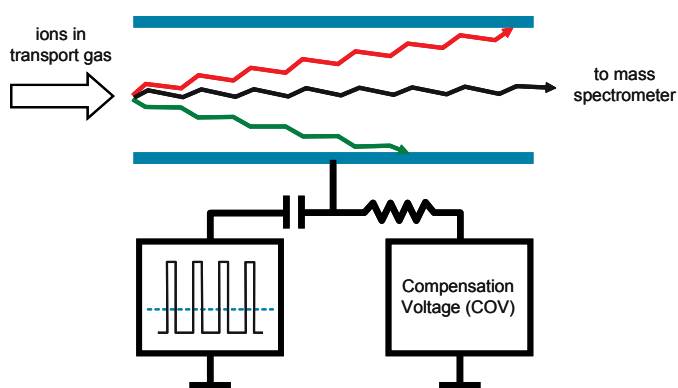


Figure 2. Separation Process of the Differential Ion Mobility Device; ions are separated on the basis of the difference in their migration rates under high versus low electric fields, migrating towards the walls of the cell. The application of a compound specific, intense DC electric field, known as the DMS separation field, steers the ions back to the center line.

Experimental

Sample Preparation:

200 μL of acetonitrile was added to 100 μL spiked urine sample. The vial was mixed and centrifuged and the supernatant removed and diluted with 700 μL of water. The diluted urine sample was directly injected into the LC/MS/MS system.

HPLC Conditions:

A Shimadzu Prominence LC system with a Restek 5 μm 60 $\text{\AA}$, PFP Propyl Column, 50 x 2.1 mm at 40°C with a gradient of eluent A water + 2 mM ammonium formate + 0.2 % formic acid and eluent B acetonitrile + 2 mM ammonium formate was used at a flow rate of 600 $\mu\text{L}/\text{min}$. The injection volume was set to 10 μL .

MS/MS Conditions:

Data was obtained using an AB SCIEX 5500 QTRAP® LC/MS/MS system, equipped with the SelexION™ interface, coupled with a Shimadzu Prominence LC system. The mass spectrometer analysis consisted of an MRM detection using scheduled MRM algorithm; using compound specific CoVs and Enhanced Product Ion (EPI) Dependent scans using the linear ion trap, automatically triggered to collect full scan MS/MS fragmentation spectra. The EPI data were collected using the Collision Energy Spread (CES) and contained low, medium and high energy fragment ions. A 1250 compound Forensic Drug Library was searched to provide identification and confirmation.

Results

The SelexION™ mobility cell's ability to enhance the selectivity of mass analysis by pre-separating ions of similar mass was demonstrated by the separation of the isobaric compounds indomethacin and bendamustine (Figure 3). The ability to perform this separation of compounds of similar mass aids in confident identification of either of these compounds in drug screening MRM IDA triggered Enhanced Product Ion (EPI) work flows. It also eliminates the need to chromatographically separate the compounds.

Using SelexION™ technology, we have shown the separation of isobaric interferences from urine matrix that, in the absence of the device, would have been transmitted into the mass spectrometer and fragmented at the same time as the analyte. Comparisons of product ion spectra generated from urine samples both with and without the use of the SelexION™ device show that fragment ions are detected and falsely represented in the resulting product ion spectrum in those experiments performed without the use of the SelexION™ device. Figure 4B shows an example of a poor purity spectral library score obtained for indomethacin using a product ion spectrum, of an $[\text{M}+\text{H}]^+$ ion at m/z 358, generated without the use of the

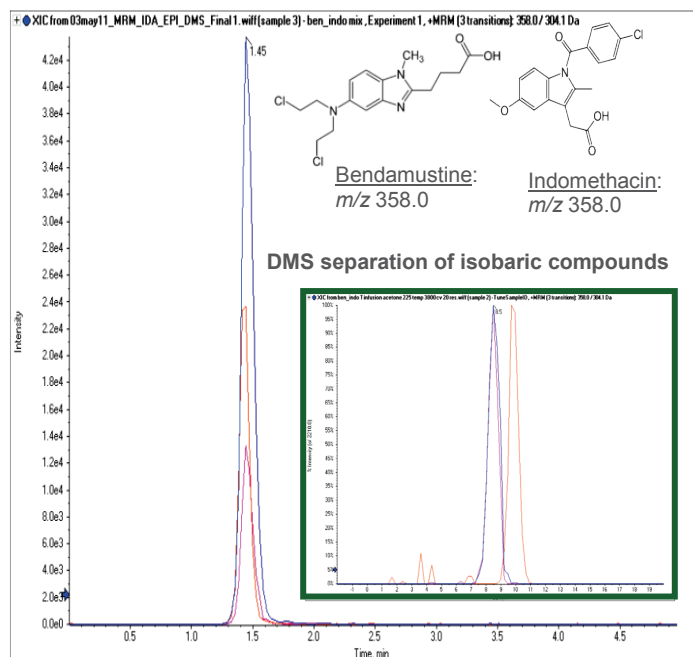


Figure 3: The selectivity obtained by introducing a chemical modifier to the homogenous fields of the SelexION™ cell is demonstrated by the separation of the isobaric compounds bendamustine and indomethacin.

SelexION™ technology and matched to the library spectrum for indomethacin (Figure 4A) with a purity score of 14%. Figure 4C shows a product ion spectrum of the $[M+H]^+$ ion at m/z 358 triggered from an MRM survey scan generated with the SelexION™ technology installed. The figure shows that interfering isobaric compounds in the sample have been pre-separated and removed reducing the number of interfering peaks in the product ion spectrum and improving the mass spectral library searching score from 14% (without SelexION™ technology) to 78% (with SelexION™ technology) for indomethacin.

Another example of an improvement in a library score is shown in Figure 5 in which the use of the SelexION™ technology has removed the interfering ion at m/z 192 that was present in the product ion spectrum of the $[M+H]^+$ ion at m/z 235 for lidocaine, generated without the use of the SelexION™ technology. The improvement in the library score from 79 to 90 % by removal of this one interfering ion allows for increased confidence in the match and identification of lidocaine.

Figure 6 shows a 100% confidence hit for the identification of amphetamine when using the SelexION™ technology. Without the use of the SelexION™ technology, a number of interfering ions are observed at m/z 77, 107 and 109 in the product ion spectrum of the $[M+H]^+$ ion at m/z 136, that were generated from a matrix isobaric interfering ion giving a purity score of only 41%.

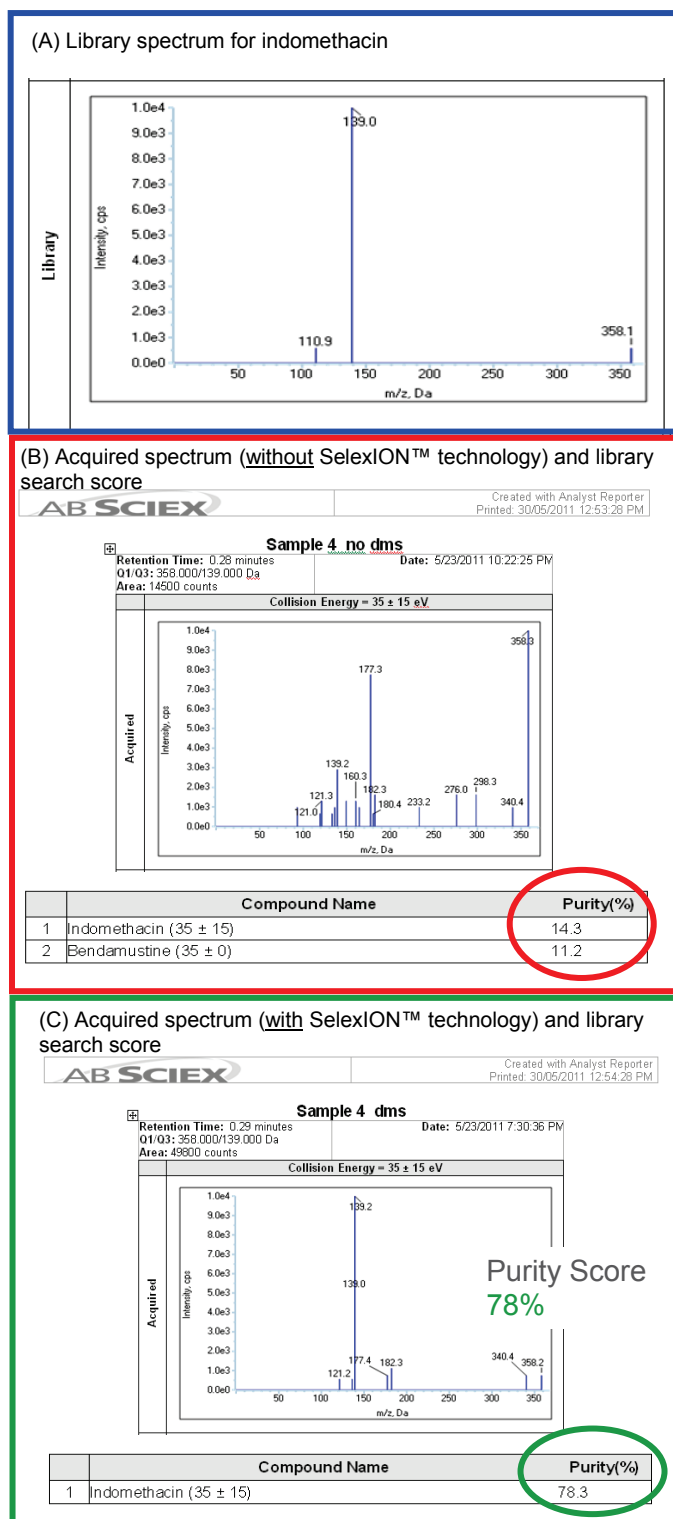
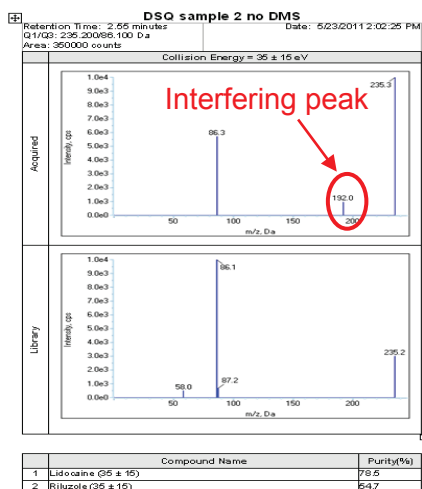


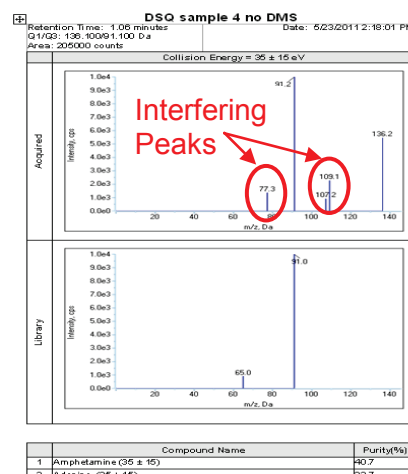
Figure 4 Library searching results for a spiked urine sample. (A) Library spectrum of indomethacin; Library search scores without (B) and with (C) the use of the SelexION™ device

(A) NO SelexION™ Technology



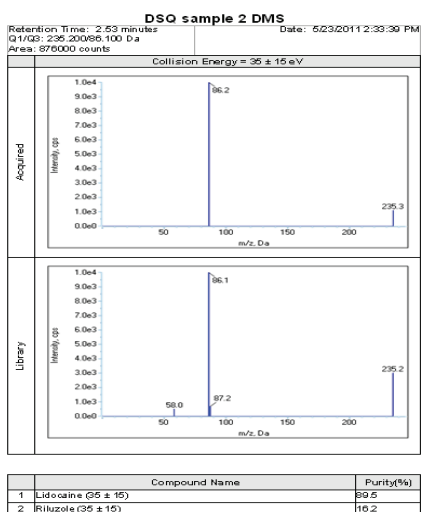
Purity Score
78.5%

(A) NO SelexION™ Technology



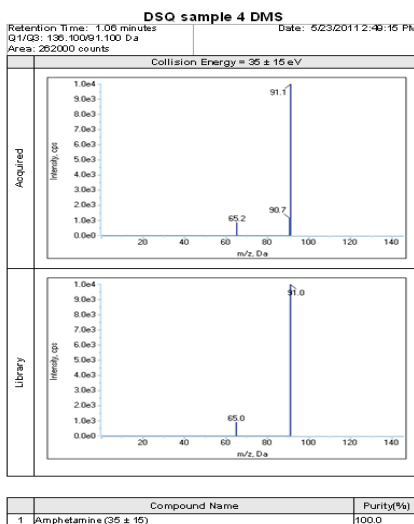
Purity Score
40.7%

(B) WITH SelexION™ Technology



Purity Score
89.5%

(B) WITH SelexION™ Technology



Purity Score
100%

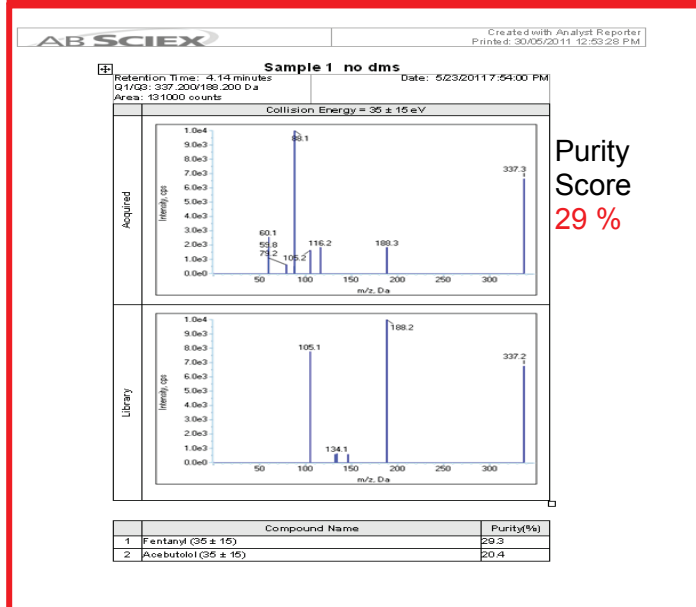
Figure 5 Library searching results for a spiked urine sample; Library search scores for lidocaine without (A) and with (B) the use of the SelexION™ device

A further demonstration of the use of the SelexION™ technology to improve mass spectral library scores by removal of isobaric interferences is shown in Figure 7. The figure shows two separate library search result reports generated from two different experiments on the same drug mix spiked urine sample. Both reports show a hit for fentanyl. The first report was generated from an MRM IDA triggered EPI experiment performed without the use of the SelexION™ technology (Figure 7A). The second report was generated from a similar experiment

Figure 6 Library searching results for a spiked urine sample; Library search scores for amphetamine without (A) and with (B) the use of the SelexION™ device

on the same sample but with the use of the SelexION™ technology (Figure 7B). By using the SelexION™ device the library hit score for fentanyl improved from 32 to 89 %. The use of the SelexION™ device has allowed the successful removal of the interfering ions that were present in the product ion spectrum of the $[M+H]^+$ ion at m/z 337 for fentanyl; generated without the use of the SelexION™ device.

(A) NO SelexION™ Technology



(B) WITH SelexION™ Technology

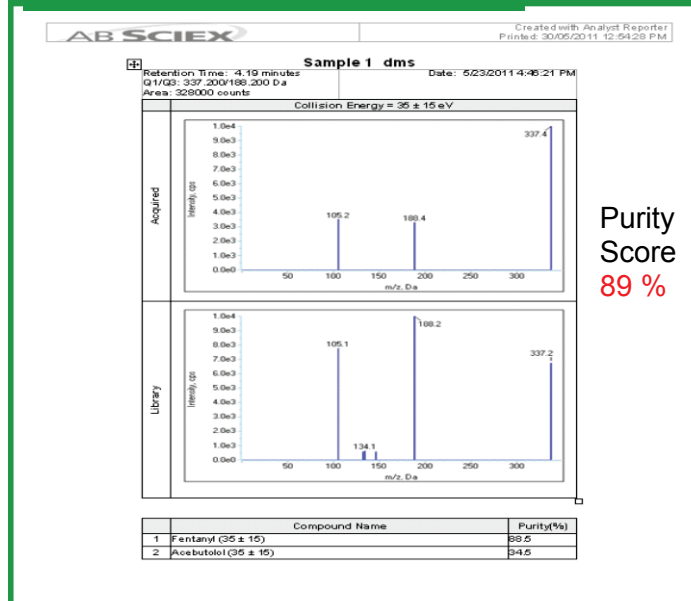


Figure 7. Library searching results for a spiked urine sample. Library search scores for fentanyl without (A) and with (B) the use of the SelexION™ device

Conclusions

The SelexION™ device has been shown to be successful in separating out analytes from isobaric interferences in urine matrix, to allow improvements in mass spectral library searching scores.

An improvement in the MS/MS spectrum generated with the use of the SelexION™ device was seen for amphetamine detected in urine, by removal of the interfering ions at m/z 77, 107 and 109. This improved the quality of the spectrum for searching against the forensic drug library producing 100% purity score for an amphetamine match.

Improvements obtained in library hit scores for compounds from spiked urine samples was achieved by pre-separating ions of similar mass using the SelexION™ technology for lidocaine, indomethacin, amphetamine and fentanyl.

SelexION™ Ion Mobility Technology

- Easily installed in less than two minutes
 - No tools or cables
- On-demand operation, can be turned on or off without removing the device
- Powerful ion mobility separations can be further enhanced by chemical modifiers

- Useful for separation of isobaric interferences, or reduction of high background noise

References

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Publication number: 3460111-01



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