

Toxicology



# Investigating Multiplexing a High Resolution, Accurate Mass Assay for a High Throughput Comprehensive Toxicology Urine Screening

AB SCIEX MPX™-2 High Throughput TripleTOF<sup>®</sup> 4600 LC/MS/MS System

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### **Overview**

Sample preparation and Liquid Chromatography (LC) separation are usually the most time consuming steps in a typical screening LC-MS analysis. LC separation is an effective way of separating out the compounds of interest from interfering components from the sample matrix and therefore reducing ion suppression effects as well as separating out isobaric compounds. LC therefore still remains a valuable tool in ensuring accurate and precise data quality for a broad compound analysis for both targeted and nontargeted screening. At the same time retention time can also be used as part of multiple points of selectivity to ensure confident identification of drugs in samples. For a comprehensive screening of hundreds of compounds LC separation can potentially have long run times. An integrated multiplex system [MPX<sup>™</sup>-2 High Throughput System] has been specifically designed to increase the throughput of an LC-MS/MS analysis and was evaluated as an alternative approach to traditional screening methods, to provide a high throughput, comprehensive drug screening analysis. Combining high resolution MS and MS/MS acquisition with LC separation, allows for retrospective compound identification with highest confidence based on accurate mass product ion specificity, mass spectral library matching as well as mass error, isotopic profile and molecular formula finding.

The AB SCIEX TripleTOF<sup>®</sup> 4600 LC/MS/MS system is a hybrid quadrupole/ time-of-flight (QqTOF) instrument allowing acquisition of highly sensitive full scan MS and MS/MS spectra with high resolution and mass accuracy. The combination of the MPX<sup>™</sup>-2 High Throughput System and TripleTOF® 4600 LC/MS/MS System meets the needs of a high throughput solution required to do qualitative screening/surveillance AND quantitation/confirmation in a single automated run, for generating and reporting results, while maintaining good precision and accuracy.



AB SCIEX TripleTOF® 4600 System

### Introduction

Laboratories performing drug screening analyses on samples often wish to identify as many compounds as possible from a single experiment. Furthermore, laboratories may also desire the capability and flexibility to perform retrospective data analysis, for the purpose of identifying compounds that were not included in the initial targeted screening experiments.

Recently, interest in high-resolution/accurate-mass MS systems as a potential tool for drug screening has grown, largely due to the ability to run simple and generic methods, and the possibility of performing retrospective data analysis.

With an increasing demand for retrospective and non-targeted analyses, full scan mass analyzers are gaining popularity. The AB SCIEX TripleTOF® 4600 LC/MS/MS system is a hybrid

quadrupole/ time-of-flight (QqTOF) instrument allowing acquisition of highly sensitive full scan MS spectra with high resolution and mass accuracy for accurate and reproducible quantification of targeted compounds. In addition, the speed of the TripleTOF® 4600 LC/MS/MS system allows 20-30 Information Dependent Acquisition (IDA) MS/MS spectra to be collected, in each scan, for confident compound identification based on MS/MS library searching and/or structural elucidation determinations. This allows the capability to perform targeted and non-targeted screening in a single LC-MS/MS run reducing the possibility of missing the detection of compounds.

The complexity of such data requires powerful data mining tools. PeakView® software with the MasterView<sup>™</sup> add-in allows targeted and non-targeted data processing. The MasterView<sup>™</sup> software manages large lists of compounds and performs extracted ion chromatogram (XIC) calculations, both targeted and non-targeted peak finding operations and library searching. MasterView<sup>™</sup> allows the ability to review results based upon retention times, accurate mass, isotopic pattern, MS/MS library searching and formula finding. The MasterView<sup>™</sup> software can further be used to aid in the structural elucidation, elemental composition determination and fragmentation interpretation to confidently identify unknowns.

Liquid Chromatography coupled to Tandem Mass Spectrometry (LC-MS/MS) is a widely used analytical tool for the screening of drugs. LC separation is an effective way of separating out the compounds of interest from interfering components from the sample matrix and therefore reducing ion suppression effects as well as separating out isobaric compounds. LC therefore still remains a valuable tool in ensuring accurate and precise data quality for a broad compound analysis for both targeted and nontargeted screening. A comprehensive drug screening approach however often requires long LC run times. These times can be reduced by multiplexing LC systems, and synchronizing to a single MS, generating the high throughput needed by maximizing the efficiency of the MS. An integrated multiplex system has been specifically designed to synchronize two LC systems and a mass spectrometer, allowing injection of samples into two LC streams in parallel (Figure 1). The overlapping LC runs and efficient use of MS detection, achievable with the MPX<sup>™</sup>-2 High Throughput System, is shown to result in an overall higher throughput system for LC-MS/MS analyses.

The effectiveness of the high resolution and accurate mass AB SCIEX TripleTOF® 4600 LC/MS/MS system in combination with the MPX<sup>™</sup>-2 High Throughput System to screen for drugs from urine, and to quantify drug compounds with excellent accuracy and high reproducibility, was investigated. Here we present examples of using the AB SCIEX MPX<sup>™</sup>-2 High Throughput

TripleTOF® 4600 system for the screening and identification of drugs in urine. Features of the MasterView<sup>™</sup> software for targeted and non-targeted screening are highlighted.



Figure 1. Multiplex LC system Designed to Support Two Parallel LC Streams into a Single MS

# **Experimental Conditions**

#### **Sample Preparation**

Urine samples were prepared by spiking analytical standards (Cerilliant) into blank urine (Lot 1) to create 100, 30, 10, 4, 1, 0.5 and 0.25 ng/mL concentration calibrants. 4 ng/mL and 10 ng/mL urine samples were also prepared in urine Lot 2 and 3 respectively. 50  $\mu$ L of each urine sample was transferred to a micro-centrifuge tube and 20  $\mu$ L of internal standard mix added and vortexed. The samples were then diluted with 300  $\mu$ L 10% MeOH in water with 0.1% formic, vortexed and centrifuged before being transferred to autosampler vials for LC-MS/MS injection.

### Multiplex Software Configuration and Operation

An integrated multiplex LC MS-MS System was used, consisting of an AB SCIEX TripleTOF<sup>®</sup> 4600 LC/MS/MS system, two Shimadzu Prominence XR LC systems with a shared CTC PAL autosampler, a pump containing a four solvent selection valve for sample loading and 3 switching valves for flow path control (Figure 2).

The two chromatographic channels were not independent as they share a single high pressure loading pump which provides additional flexibility for injection and wash solvent composition. All hardware modules are controlled by Analyst® TF 1.6 software with MPX<sup>™</sup> TF Driver 1.0 add-on. Precise timing of the switching valves allowed each LC stream to perform interleaved injection and LC gradient elution. Targeted MS data acquisition was enabled by selecting a retention time window around the peaks of interest in the MPX<sup>™</sup> Method Editor. Figure 3 shows common flow paths for a typical LC-MS analysis.

After running the multiplex software installer, the user may easily configure and activate the multiplex option from the Hardware Profile dialog within the Analyst<sup>®</sup> program. MPX<sup>™</sup> Driver provides an easy-to-use interface for the user to modify the system configuration in the Settings Pane (Figure 4) and to create or update a multiplex LC method in the Method Pane (Figure 5).



Figure 2. MPX<sup>™</sup>-2 High Throughput System Configuration and Module Arrangement Used in this Application.



Figure 4. Settings Workspace of the MPX<sup>™</sup> TF Version 1.0. Easy Instrument Configuring

An example chromatogram can simply be loaded into the software and the region that should be directed to the mass spectrometer for data acquisition is highlighted. The timing is automatically calculated and throughput increased displayed in the gauge. The interface also allows monitoring the real-time acquisition and system status (including pressure, flow rate, temperature, flow path, and system state for both LC streams) in the Status Pane (Figure 6).



Figure 3. Plumbing Diagrams and Solvent Flow Paths at Typical States of a Multiplex Run.



Figure 6. Status Workspace of the MPX<sup>™</sup> TF Version 1.0. Allows monitoring the system and multi-method equilibration

For this high throughput comprehensive screening method a Phenomenex Kinetex 2.6  $\mu$ m C18 Column, 100 Å, 50 x 2.1 mm was used, with a 6 minute gradient of water and methanol with formic acid buffer.



Figure 5. Methods Workspace of the MPX<sup>™</sup> TF Version 1.0. Easy to use method editor

Automated multiplex data acquisition for a batch of samples is very similar to performing a regular LC-MS data acquisition by creating and submitting a batch with Batch Editor in Analyst® software. During a multiplex run, a staggered injection timing schedule is calculated in real-time by the MPX<sup>™</sup> TF Driver 1.0. All switching valves and synchronization between LC components and the mass spectrometer are also controlled by the MPX<sup>™</sup> TF Driver 1.0

#### **Mass Spectrometric Conditions**

The AB SCIEX TripleTOF® 4600 system was operated with DuoSpray<sup>™</sup> source and Electrospray (ESI) probe.

An IDA method was used containing a TOF-MS survey of 100 ms and up to 10 dependent TOF-MS/MS scans of 50 ms accumulation time; sufficient to acquire enough data points across the LC peak to allow reproducible and accurate quantitation with a total cycle time of 0.65 seconds. Collision Energy (CE) of 35 V and Collision Energy Spread (CES) of 15 V were used.

For mass calibration of the AB SCIEX TripleTOF® 4600 system, an on-column injection of an AB SCIEX APCI calibration solution via the LC autosampler was performed.

Acquired MS/MS spectra were searched against an accurate mass drug library.

### **Results and Discussion**

Enabling Rapid, High Resolution Screening with the MPX<sup>™</sup>-2 High Throughput TripleTOF® 4600 System with High Confidence Identifications

The effectiveness of the MPX<sup>™</sup>-2 High Throughput System with MPX<sup>™</sup> TF Driver 1.0 software in combination with the TripleTOF® 4600 System in a comprehensive drug screening method was investigated. Using the MPX<sup>™</sup>-2 High Throughput System increased the throughput of a comprehensive drug screening assay by 1.5 times compared to the singleplex method. The cycle time using MPX<sup>™</sup>-2 is 4.53 min, compared to 6.7 min in single-stream.

The method uses full advantage of the high resolution, accurate mass capabilities of the TripleTOF® system using multiple points of selectivity for identification based on mass error, isotope matching, retention time, MS/MS library searching and formula finding. Relying solely on accurate mass and isotopic matching can potentially lead to false negative conclusions as illustrated in Figures 7 and 8. Figures 7 and 8 show examples where, due to a co-eluting unknown compound, the isotopic ratio for the targeted compound was off. This may cause a false negative conclusion but because MS/MS data was collected for the compound and a good library match was generated, the compound was successfully identified. False negative rates can therefore be reduced by collection of MS/MS data during these fast screening workflows.



Figure 7 Reduction of false negative rates with MS/MS data collection for unknown urine sample



Figure 8 Interference caused isotopic ratio change, but MS/MS confirmed identity and eliminated possible false negative

Defining an XIC List and Processing Options in the XIC Manager for Targeted Qualitative Screening and Identification

After opening a data file in PeakView® software, MasterView™ can be launched from the 'MasterView' menu in the PeakView® toolbar.

The table contains a number of columns with values that can be edited, including name, formula, adduct/modification, retention time, width and more. To define an XIC, a mass must be entered. This can be done by directly editing the cell, having the software calculate the value based on formula, isotope, and adduct provided, or by pasting values from a spreadsheet. The generated XIC list can be saved for future processing (Figure 9).

A number of processing and display settings can be adjusted in the 'Options' dialog. This includes intensity, signal-to-noise threshold, and confidence settings for mass error, retention time, isotope matching, library searching and formula finding (Figure 10).

Process

To start data processing, simply click the button in the lower right of the table. MasterView™ will automatically calculate XICs, perform compound identification and display results.

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MPX_52_LC8	_Lot3C5_2I Sample 1		7	✓		Clonazepam	C15H10CIN3D3	0	315.04107	+H		316.04835	0.02	63.281	3.5	1	
			8	✓		Codeine	C18H21ND3	0	299.15214	*H		300.15942	0.02	66.631	1.91	1	
			9	✓		Diazepam	C16H13CIN2O	0	284.07164	*H		285.07892	0.02	70.156	3.28	1	
			10	✓		EDOP	C20H23N	0	277.18305	+H		278.19033	0.02	71.893	2.75	1	
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			12	<ul> <li>Image: A second s</li></ul>		Flunitrazepam	C16H12FN3O3	0	313.08627	+H		314.09355	0.02	63.675	3.09	1	
			13	<ul> <li>Image: A second s</li></ul>		Flurazepam	C21H23CIFN3O	0	387.15137	+H		388.15865	0.02	51.525	2.76	1	
			14	<		Hydrocodone	C18H21NO3	0	299.15214	+H		300.15942	0.02	66.631	1.91	1	
			15	$\checkmark$		Hydromorphone	C17H19NO3	0	285.13649	+H		286.14377	0.02	69.895	0.28	1	
			16	$\checkmark$		Hydroxyalprazolam	C17H13CIN4O	0	324.07779	+H		325.08507	0.02	61.522	3.1	1	
			17	$\checkmark$		Lorazepam	C15H10Cl2N2O2	0	320.01193	+H		321.01921	0.02	62.302	3.15	1	
			18	$\checkmark$		MDA	C10H13NO2	0	179.09463	+H		180.10191	0.02	111.048	2.1	1	
-			19	$\sim$		MDEA.	C12H17NO2	0	207.12593	+H		208.13321	0.02	96.092	2.23	1	
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Figure 9. PeakView® software with the MasterView<sup>™</sup> add-in; the bottom pane shows an XIC table loaded for target drug screening and p 5 identification used to search 6 samples (left hand pane) that have been loaded in PeakView® RUO-MKT-02-0670-B p 5 identification used to search 6 samples (left hand pane) that have been loaded in PeakView®



Figure 10. Confidence settings for compound identification

#### **Results Display in MasterView™**

After processing, the results are displayed to show the mass error (ppm or mDa), found at retention time, isotopic pattern, MS/MS library search results and formula finder score. XICs above a defined intensity threshold are highlighted in green and confidence data for compound identification is visualized using traffic lights (Figure 14). The extracted compounds that are displayed can be customized based on the strength of the hit (Figure 15). For example the software can be set up to only display compounds with 5 green traffic lights.

The example data presented in Figures 11 and 13 shows the automatically generated XICs for each targeted analyte and compared against the user defined threshold. High resolution and accurate mass LC-MS/MS chromatograms contain comprehensive information of all molecules present in the sample that are amenable to the ionization techniques and polarity used. Narrow XICs can be generated to selectively screen for targeted compounds. The AB SCIEX TripleTOF® 4600 system provides high resolution of up to 35,000 dependent on the mass detected (Figure 11) and stable mass accuracy of ~2 ppm at fast acquisition speed in MS and MS/MS mode. This allows the generation of narrow XICs to achieve both selectivity and increased S/N when screening for a large set of targeted drug compounds in complex samples (Figure 12).

Figure 13 shows XICs of 41 drug compounds that were identified from a spiked urine sample using information of the isotopic pattern of the detected molecular ion, retention time, accurate mass and MS/MS spectral searching against an accurate forensic dug MS/MS library. A fifth identification criterion that is automatically calculated during the extraction is a formula finding. The accurate mass TOF-MS/MS product ion scan of a drug compound is shown as a mirror view with the matched library spectrum for easy comparison. The bottom right panel of Figure 13 shows an example of the spectral comparison of library versus urine sample of methadone. The sample spectrum is the top blue trace and the library is the grey trace.

Figure 16 shows the details of the library search and formula finder results that are automatically performed during the extraction of the ion chromatograms. Both results are based on MS and MS/MS results. The Formula Finder uses high resolution accurate mass information of the molecular ion, adducts, isotopic pattern and fragment ion information to empirically calculate potential molecular formula which can only be achieved by combining all available MS and MS/MS data.



Figure 11. Resolution and mass accuracy across the mass range for selected drug compounds



Figure 12. Increasing selectivity and S/N using narrow extracted ion chromatograms (XICs)



Figure 13. 41 drugs were detected in a targeted approach and identified using retention time, mass error, isotopic pattern and confirmed with MS/MS library matches and formula finder results in a urine sample at 10 ng/mL.

Data Review Functionality

• Confidence in identification is visualized using <u>five</u> 'traffic lights' (including RT error, mass error, isotope matching, library search, and formula finding)

	Name
	Alprazolam
	Amphetamine
	Benzoylecgonine
	Buprenorphine
$\blacksquare \checkmark \bullet \checkmark \bullet$	Carisoprodol
$\checkmark$	Clonazepam

#### Figure 14. Data Review

• XIC highlighted in green:

XIC above a user defined intensity threshold will be highlighted in green as specified by the criteria chosen in 'Select combination for positive result (equal or better)' box (Figure 4). The identification criteria above defined thresholds can be displayed.

# Positive ID with high confidence



# Identified compounds to review



Figure 15. Identifying which components to review

Display highlighted XIC only



Show MS and MS/MS spectra



· Show details of library searching and formula finding



Figure 16. Library Search and Formula Finder Results are automatically generated using both MS and MS/MS information

All the drugs identified are quantifiable using the MultiQuant<sup>™</sup> software, as represented in Figure 17.



Figure 17. Example quantification of a selected drug compounds using MultiQuant<sup>™</sup> software.

MasterView<sup>™</sup> has flexible reporting features showing the compound identifications using both high resolution and accurate mass MS and MS/MS information. Customizable report templates can be used to generate word, csv text or PDF reports (Figure 18).



Figure 18. Flexible Reporting Features in MasterView™

### Comparative Screening and Identification - Quantitative Comparison; Finding Compounds greater than a cutoff concentration

Another approach for targeted analysis is through a comparative screening where we can directly compare our unknown sample, in this case urine sample, to a compound standard solution containing our targeted list of compounds at the cutoff concentration level we'd like to report to. In this example a threshold for the ratio of the control is set to 1, and the urine standard (blank urine spiked with 10 ng/mL concentration of each targeted compound) is used as the control and the control concentration inputted. In this type of analysis, all drug

compounds present in our unknown sample that exceed the level detected in our 10 ng/mL standard are flagged as positives exceeding the limit we entered. This is a great way to quickly survey samples to identify targeted compounds and perform a semi-quantitative analysis to see how much of those compounds are there. Figure 19 shows an example of this workflow; only compounds having 10 ng/mL concentrations or higher will be extracted and highlighted in green. Eight compounds were identified that were above the cutoff concentration and their concentrations calculated.



Figure 19. Comparative Screening and Identification - Quantitative Comparison Finding Compounds > cutoff

### Non-Targeted General Unknown Comparative Screening and Unknown Identification – Simultaneous targeted screening and identification of unknowns (unexpected) compounds

Another example of data processed using MasterView<sup>™</sup> in PeakView® Software using a Sample-Control comparison workflow is illustrated in Figures 20-26. Here we are quickly identifying compounds in a sample that are at or above a cutoff level. The General Unknown Comparative Screening determines any features that differ from that found in the control and are automatically extracted. The MasterView<sup>™</sup> displays the results by comparing the TOF MS and MS/MS spectra from both the unknown sample and control. Figure 20 shows an unknown sample data file and a urine standard (10 ng/mL) data file being uploaded into PeakView®. In this example a threshold for the ratio of the control is set to 1, and the urine standard (blank urine spiked with 10 ng/mL concentration of each targeted compound) is used as the control and the control concentration inputted. An XIC list containing a list of targeted compounds was used to extract any targeted drugs present in the unknown sample. All ions with the same and higher intensity in the unknown sample than in the control are reported and automatically searched

against the accurate mass drug library. Three compounds, Lorazepam, Merperidine and Temazepam were identified that were at or above the 10 ng/mL cutoff concentration and their concentrations calculated. All three compounds were successfully identified by isotopic pattern of the detected molecular ion, retention time, accurate mass, calculated molecular formula as well as a library match. All compounds showed five green traffic lights for each identification criteria giving high confidence in the identifications (Figure 22).



Figure 20. General Unknown Comparative Screening; An unknown sample and 10 ng/mL standard are opened in PeakView™ Software

Sample - unknown	* Contr 300 Contr 4 100 0.007	ol – 10 ng/mL standard 1775 1202 1775 1202 1075 100 1075 100 1000 1000 10	100
	The Legalshired Probing Produced Difference and Blanch Mar- Manname Performance and Blanch Mar- Manname Performance and Blanch Mar- Maramen Performance and Blanch Mar- Produce Performance and Blanch Mar- Produced Performance and Blanch Mar- Performance and Blanch Mar- Performance and Blanch Mar- Performance and Blanch Mar- Performance and Blanch Mar- Marameter and Blanch Marameter and Blanch Mar- Marameter and Blanch Marameter and Blanch Marameter and Blanch Mar- Marameter and Blanch Marameter and Blanch	Entirgent and a second a s	
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Figure 21. Setting up the simultaneous targeted and non-targeted peak finding workflow. Comparison of an unknown with 10 ng/mL standard control was made to only extract compounds in the sample that were at a cutoff concentration of 10 ng/mL or higher. A small list of targeted compounds was initially screened for with any unknown peaks added to the XIC list after the extraction using the Non-Targeted Peak Finding.

At the same time as extracting out targeted compounds a Non-Targeted Peak Finding algorithm is used and any unknown (unexpected), non-targeted peak is added to the XIC list of targeted compounds. The table will populate with a list of nontargeted mass peaks that were found by adding the masses to the XIC list after the extraction. It will also include the associated retention times for those peaks. Figure 21 shows the setup of this simultaneous extraction of targeted and untargeted compounds. MasterView<sup>™</sup> automatically empirically calculates potential molecular formula for the extracted mass of the unknown compound. The Formula Finder uses high resolution accurate mass information of the molecular ion, adducts, isotopic pattern and fragment ion information to empirically calculate potential molecular formula which can only be achieved by combining all available MS and MS/MS data. By setting the threshold of the ratio of the control to 10, the list of chemical characteristics from reduced from 1452 to 63. Figure 23 shows a sample in which the "Enhance Peak Find" and sample-control comparative screening has identified an unknown, significant feature at an accurate m/z of 282.1852 at retention time of 2.45 min that is present in the sample. A calculated formula of C<sub>19</sub>H<sub>23</sub>NO, derived from both MS and MS/MS information was automatically generated with the highest score.



Figure 22. Sample-Control Comparison in a General Unknown Comparative Screening. The drugs Lorazepam, Meperidine and Temazepam were identified above the 10 ng/mL cutoff concentration, using retention time, mass error, isotope pattern, formula finding and confirmed with MS/MS library matches in a sample-control comparison workflow. The spectral comparisons between the sample and control are shown for Merperidine



Figure 23. Unknown Structural Elucidations - Non-targeted peak finding. Unknown (unexpected) non-targeted peaks are added to the original XIC list of targeted compounds. MasterView<sup>TM</sup> automatically calculated potential molecular formula for the extracted accurate mass of 282.1852 at RT 2.45 min of the unknown compound using both MS and MS/MS data. The formula calculated with the highest score is C<sub>19</sub>H<sub>23</sub>NO

The calculated formula was then automatically searched against ChemSpider to find matching structures. ChemSpider Session opens to show results of potential structures for the calculated formula and a proposed structure is confirmed using fragment ion predictions (Figure 24). The ChemSpider search lists all possible structures that could be associated with the suggested molecular formula, and sorts them by their frequency of publications and relevance. Hundreds of structures match to this calculated formula.



Figure 24. ChemSpider Session. Calculated formula are automatically searched against ChemSpider to find matching structures which are then confirmed by fragment ion predictions using acquired MS/MS data of the unknown peak. Six of six peaks matched with explainable MS/MS fragment ions for the Naphyrone cathinone structure. By automatically comparing the MS/MS pattern with potential fragmentations of each of the structures, a structure can tentatively be assigned to the unknown peak. In this example the closest match gave six of six peaks matching with explainable MS/MS fragment ions for the Naphyrone cathinone designer drug structure.

For another unexpected peak the combination of simultaneous Non-Targeted Peak Finding, formula finding, ChemSpider searching and fragment ion predictions has allowed the tentative identification of Methylenedioxypyrovalence (MDPV) for this unknown peak (Figures 25 and 26).



Figure 25. Identification of an unknown. Enhance Peak Find generated a list of most intense peaks and Formula Finder automatically aided in matching elemental compositions to the accurate mass using MS and MS/MS data for the extracted accurate mass of 276.1596 at RT 2.15 min; calculating a formula with the highest score to be  $C_{16}H_{21}NO_3$ 



Figure 26. ChemSpider Session. Calculated formula are automatically searched against ChemSpider to find matching structures which are then confirmed by fragment ion predictions using acquired MS/MS data of the unknown peak. Ten of ten peaks matched with 100% explainable MS/MS fragment ions for the MDPV cathinone structure.

## Conclusion

- The AB SCIEX TripleTOF® 4600 LC/MS/MS System was shown to be an effective tool in screening for, quantifying and confidently identifying drugs in urine.
- The high sensitivity, resolution and scan speed of the Accelerator TOF<sup>™</sup> analyzer enables reproducible and accurate quantitation.
- Accurate mass MS/MS spectra were searched against an accurate mass LC-MS/MS library of drugs to confirm the identity of quantified analytes.
- The acquired MS and MS/MS data can be used in a General Unknown Comparative Screening in an easy, fast and reliable workflow to quickly identify drugs present at or above a cutoff concentration in an unknown sample and allowed the calculation of their concentrations.
- Targeted screening assays can be performed while at the same time also surveying for 'unknowns' and unexpected compounds in the sample in a single injection.
- The TripleTOF<sup>™</sup> 4600 LC/MS/MS system in combination with PeakView<sup>®</sup> and MasterView<sup>™</sup> Software, with Formula Finder and Fragment Ion Predictor, allows the confident identification of unknowns using both acquired MS and MS/MS data.

We have shown that Multiplexing LC systems, and synchronizing to a single MS, can potentially generate the high throughput needed by modern day laboratories to analyze increasing numbers of samples but maintain or improve turn-around times.

The above features are achieved by the ability to automatically adjust program overlapping runs. By simply inputting the times or highlighting the analyte retention area of a sample chromatogram the mass spectrometer acquisition period is automatically updated.

The combination of the MPX<sup>™</sup>-2 SP High Throughput System and TripleTOF® 4600 LC/MS/MS System meets the needs of a high throughput solution required to do qualitative screening/surveillance AND quantitation/confirmation in a single automated run, for generating and reporting results, while maintaining good precision and accuracy.

LC separation is an effective way of separating out the compounds of interest from interfering components from the sample matrix and therefore reducing ion suppression effects as well as separating out isobaric compounds. LC therefore still remains a valuable tool in ensuring accurate and precise data quality for a broad compound analysis for both targeted and non-targeted screening. MPX<sup>TM</sup>-2 SP High Throughput System allows this robust means of analysis to be used in a high throughput environment.

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