Simultaneous Targeted and Unknown Screening using the 3200 QTRAP® LC/MS/MS System and Cliquid® Software

Screening Workflows for Targeted and Unknown Analytes, using Cliquid® 3.2 Software with MS/MS Library Searching Confirmation

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Abstract

In this technical note, a novel methodology is described which combines targeted and unknown screening in a single LC-MS/MS analysis, using a QTRAP® hybrid tandem mass spectrometer / linear ion trap. In addition to providing screening for target and unknown analytes, this method also generates full-scan MS/MS spectra for the detected analytes, to be used for confirmation via searching against an MS/MS spectral library. The combination of targeted and unknown screening in a single LC-MS/MS analysis saves time, requires less sample volume, and affords the analyst with the opportunity to perform retrospective data mining to extract valuable information.

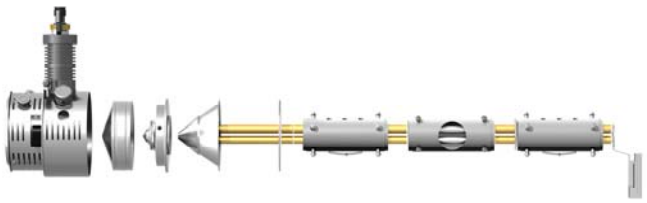
Introduction

Drug screening has traditionally been performed using immunoassay, liquid chromatography with ultra-violet detection (LC/UV), or gas chromatography-mass spectrometry (GC/MS). However these techniques are being increasingly supplanted by liquid chromatography-tandem mass spectrometry (LC-MS/MS) due to their various limitations, which may include lack of specificity, incomplete compound coverage, tedious sample preparation, long run-times, and difficulty in adapting the analyses to new drugs.

In contrast, LC/MS/MS allows detection of low level analyte concentrations using short chromatographic run times due to the sensitivity and selectivity of the technique. Sample preparation is also simpler because most biologically active compounds are readily ionized via LC/MS/MS, without any need for derivatization. A single-step protein precipitation or sample dilution is often sufficient prior to analysis. In addition to being convenient, LC/MS/MS sample preparation procedures are generic, making them ideal for both targeted screening and general unknown drug screening.

Targeted screening is a directed screening approach that analyzes samples for a specific list of drugs. This approach is often referred to as “multi-target screening”, or MTS, and currently constitutes the majority of the screening tests performed. The types of drugs used or abused are often limited to a few hundred compounds; therefore, most targeted screening methods are focused on detecting a subset of the most commonly used drugs. Restricting the analysis in this way allows the use of sensitive and selective workflows. When performed on an LC-MS/MS system, targeted screening methods typically employ the Multiple Reaction Monitoring (MRM) mode of operation, which provides superior sensitivity and selectivity, enabling detection of low concentrations of drugs in complex biological matrices. Since this approach detects only those compounds selected, *a priori*, it will not reveal the presence of a compound not included in the target drug list.

Figure 1. Ion Path of the 3200 QTRAP® LC/MS/MS System.



The 3200 QTRAP® LC/MS/MS system is a hybrid triple quadrupole / linear ion trap mass spectrometer. This instrument incorporates linear ion trap capabilities in Q3, allowing ion accumulation for full-scan MS or MS/MS analysis, which provides highly sensitive qualitative spectra.

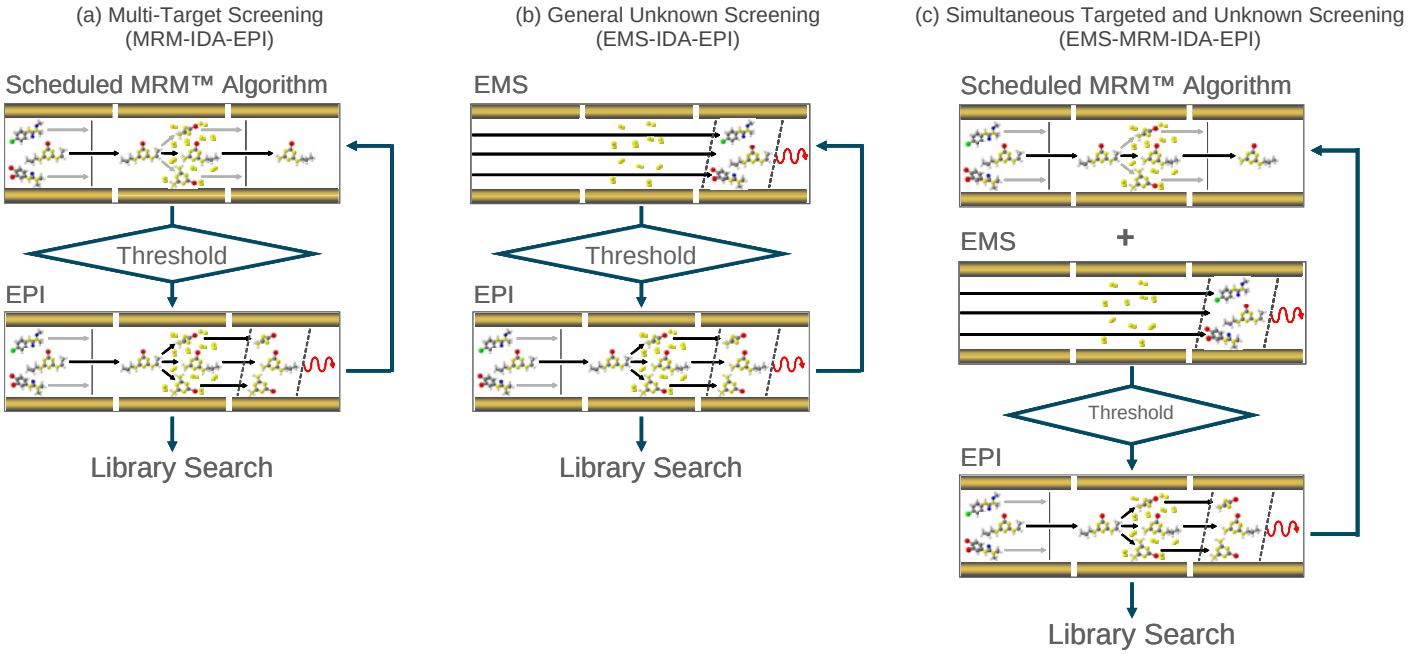
General unknown screening (GUS) does not rely upon a target compound list, so the analysis is sensitive to detection of unexpected pharmaceuticals, nutritional supplement-based analytes, and designer drugs. When performed on an LC-MS/MS system, unknown screening methods typically employ full-scan MS experiments in order to detect all major components present in a sample, including unexpected compounds. The downside to this approach is a slight compromise in the level of detection, primarily due to a reduction in selectivity when performing single-MS, rather than MS/MS, experiments. In many applications, this limitation is minor given the benefit of identifying unanticipated analytes.

The 3200 QTRAP® instrument is a hybrid triple quadrupole / linear ion trap mass spectrometer – a unique, flexible LC/MS/MS

system that can accommodate a wide variety of both quantitative and qualitative workflows. A schematic of the QTRAP® ion path is shown in Figure 1. This instrument is based on a triple quadrupole platform, using Q1 and Q3 as mass selective filters and Q2 as a collision cell for fragmentation. Because it is a triple quadrupole, true MRM experiments – the gold standard in highly selective and sensitive quantitative analysis– can be performed. In addition to triple quadrupole functionality, the QTRAP® instrument incorporates linear ion trap capabilities in Q3, allowing ion accumulation for full scan MS or MS/MS analysis, which provides high sensitivity qualitative spectra. Ion trap enhanced mass spectra (EMS) scans provide enhanced sensitivity and resolution, partially offsetting the reduced selectivity associated with full-scan GUS experiments. Furthermore, EMS scans in the linear ion trap can be acquired much more rapidly than conventional quadrupole MS scans, permitting the acquisition of full-scan mass spectra on a time scale that is compatible with LC analysis. Ion trap enhanced product ion (EPI), or full scan MS/MS, spectra can be triggered and rapidly acquired during an LC-MS/MS analysis, and searched against a spectral library to provide compound identification and/or structural information. It is the ability to use both triple quadrupole and linear ion trap scan functions on a single platform – and even within a single LC/MS/MS run – that makes the QTRAP® LC/MS/MS system adaptable to a wide variety of both screening and quantitative tests.

The ability to perform either targeted or unknown screening on the same platform makes the 3200 QTRAP® system a powerful and versatile instrument. However, the need to perform two separate analyses – one LC-MS/MS method for targeted screening, followed by a second LC-MS/MS method for unknown screening – is less attractive than the ability to perform a single all-in-one method. In this note, a QTRAP® LC/MS/MS system has been used to perform several different types of screening experiments: (i) multi-targeted screening (MTS), with MS/MS library searching confirmation of detected compounds, (ii) general unknown screening (GUS), with MS/MS library searching confirmation of detected compounds, and (iii) *simultaneous* targeted and unknown screening, with MS/MS library searching confirmation of detected compounds. The results obtained with these different methods are compared, with the aim of demonstrating that the 3200 QTRAP® LC-MS/MS system may be used to perform simultaneous targeted and unknown screening, in a single LC-MS/MS method, with no compromise in the number of compounds detected, or in the quality of the MS/MS data obtained for compound confirmation.

Figure 2. Three Different Approaches to Drug Screening using the 3200 QTRAP® LC/MS/MS System.



The 3200 QTRAP® LC/MS/MS system permits a wide variety of workflows to facilitate drug screening. Using Information Dependent Acquisition (IDA) criteria, methods can be built that will trigger the automated acquisition of Enhanced Product Ion (EPI) scans – high-quality MS/MS scans acquired in the linear ion trap – whenever an analyte is detected above a pre-determined threshold. EPI spectra are submitted for library searching to confirm the identity of the compound. (a) Multi-Target Screening uses an MRM survey scan to achieve the most sensitive detection of a pre-determined list of target compounds (b) General Unknown Screening uses an Enhanced Mass Spectrum (EMS) full-scan as a survey to detect all possible compounds, including unanticipated compounds. (c) Simultaneous Targeted and Unknown Screening is accomplished by looping both MRM and EMS survey scans in a single experiment.

Experimental

An LC-MS/MS method was developed to provide simultaneous targeted and unknown screening, using a 3200 QTRAP® LC/MS/MS system interfaced to a standard HPLC system. To assess the performance of the method, several drug-positive urine samples were obtained from a local hospital toxicology lab.

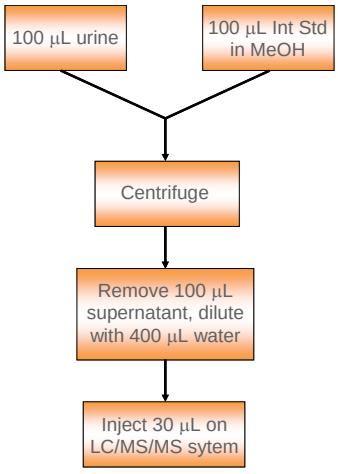
Figure 3. LC-MS/MS Experimental Setup

Experiments were performed using a 3200 QTRAP® LC/MS/MS System (left)and a Shimadzu Prominence HPLC System (right).

Sample Preparation

Samples were prepared by adding 100uL of internal standard solution in methanol to 100uL of urine, followed by centrifugation at 14,000g for 10 minutes. The supernatant was removed, and HPLC-grade water was added to achieve a final dilution factor of 10 times. Since true quantitative results were not necessary, the samples were not hydrolyzed in order to simplify sample preparation for high throughput analysis.

Figure 4. Sample preparation protocol.



Sample preparation consisted of a simple "dilute and shoot" protocol. Since absolute quantitative results were not required, the lengthy hydrolysis step was omitted for simplicity.

Mass Spectrometry Conditions

Mass spectrometric analysis was performed using a 3200 QTRAP® hybrid triple quadrupole / linear ion trap mass spectrometer. Screening and confirmation was accomplished using different scanning modes for targeted and general unknown screening.

- **Targeted screening** was accomplished using a Multiple Reaction Monitoring (MRM) survey scan to identify up to 51 target analytes. Since MRM scans are extremely sensitive and selective, this survey scan can detect drugs present at low concentrations in complex biological matrices.
- **General unknown screening** was accomplished using an MS survey scan from m/z 60-1000 to identify any major components in the samples, including unexpected compounds. For optimal sensitivity, the Enhanced Mass Spectrum (EMS) scan-type was used to acquire this data in the linear ion trap of the QTRAP® system. Since the EMS survey scan is not biased to look for a pre-determined list of target analytes, the general unknown screening workflow can be used to detect pharmaceuticals, metabolites, designer drugs, and degradation products.
- **Simultaneous targeted and unknown screening** was accomplished by using looped MRM and EMS survey scans to detect both target analytes and unanticipated analytes. The MRM experiment was set up to detect up to 51 target analytes. The EMS experiment was set up to detect any analytes with m/z in the range of 60-1000.
- For all of the experiments performed, **Information Dependent Acquisition** (IDA) criteria were employed in order to automatically trigger the acquisition of full-scan MS/MS spectra for any compounds that were detected by the survey scans. The Enhanced Product Ion (EPI) scan-type was used to acquire full-scan MS/MS spectra in the linear ion trap of the QTRAP® system, since it provides improved data quality, better sensitivity, higher resolution, and faster acquisition speed compared to conventional quadrupole MS/MS scans. After acquisition, the EPI spectra were searched against a comprehensive library of MS/MS spectra to provide additional confirmation of compound identifications.

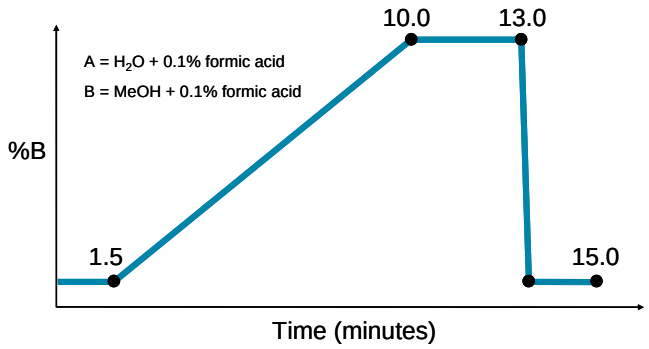
Each of the drug-positive urine samples was analyzed using (i) the multi-targeted screening method (MRM-IDA-EPI), with MS/MS library searching confirmation, (ii) the general unknown screening method (EMS-IDA-EPI), with MS/MS library searching confirmation, and (iii) the simultaneous targeted and unknown screening method (EMS-MRM-IDA-EPI), with MS/MS library searching confirmation. The results from these experiments were compared with the aim of demonstrating that the 3200 QTRAP® LC/MS/MS system may be used to perform simultaneous

targeted and unknown screening (iii), in a single LC-MS/MS method, with no compromise in the number of compounds detected, or in the quality of the MS/MS data obtained for compound confirmation.

LC Conditions

Liquid chromatography was performed using a Shimadzu Prominence HPLC system. Reversed-phase chromatographic separation was accomplished using a Restek Allure PFP Propyl column (5um, 50 x 2.1mm), with mobile phases consisting of 0.1% formic acid in water (mobile phase A) and 0.1% formic acid in methanol (mobile phase B). The HPLC run consisted of a linear gradient from 10-95% mobile phase B in 8.5 minutes, followed by a hold at 95% mobile phase B for 3 minutes, and finally a re-equilibration at 10% B for 2 minutes. The total chromatographic run-time was 15 minutes.

Figure 5. HPLC Gradient for Screening Methods.

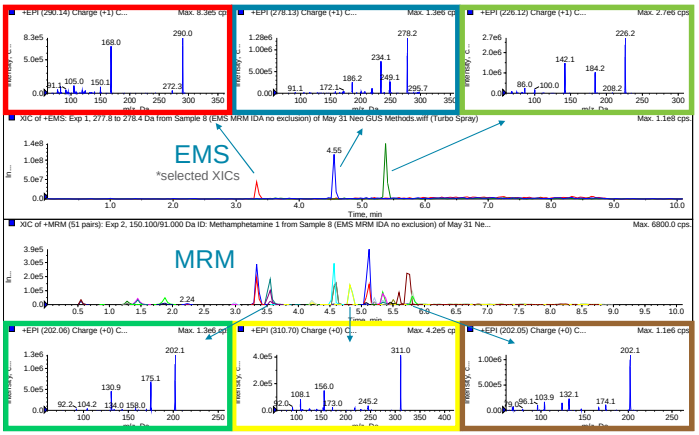


The chromatographic run time for the three LC-MS/MS screening methods was 15 minutes, consisting of a linear gradient from 10% to 95%B over 8.5 minutes, followed by a 3 minute hold, and then re-equilibration. Chromatographic separation was accomplished using a Restek Allure PFP Propyl (5um, 50 x 2.1mm) column.

Results

Each of the drug-positive urine samples was analyzed using (i) a multi-targeted screening method (MRM-IDA-EPI), with MS/MS library searching confirmation, (ii) a general unknown screening method (EMS-IDA-EPI), with MS/MS library searching confirmation, and (iii) a simultaneous targeted and unknown screening method (EMS-MRM-IDA-EPI), with MS/MS library searching confirmation. Example data for the simultaneous targeted and unknown screening method is shown in Figure 6 below, which demonstrates that EPI spectra (full-scan MS/MS) were acquired for both the target analytes detected by the MRM survey scan and the unknown analytes detected by the EMS full-scan.

Figure 6. Example Data from the Simultaneous Targeted and Unknown Screening Method.



The simultaneous targeted and unknown screening method consisted of 2 looped survey scans: an Enhanced Mass Spectrum (EMS) scan to detect any unanticipated compounds, and a Multiple Reaction Monitoring (MRM) scan to detect target compounds with maximum sensitivity. Both survey scans triggered the automated acquisition of confirmatory MS/MS spectra.

Multi-Target Screening (MRM-IDA-EPI)

As described in detail in the Experimental section, the Multi-Target Screening approach leveraged the superior sensitivity of the MRM survey scan to detect target compounds at low concentrations, even in complex biological matrices. The QTRAP® system was operated in Information-Dependent Acquisition (IDA) mode, in order to automatically trigger the acquisition of Enhanced Product Ion (EPI) full-scan MS/MS spectra, which were used for confirmation of compound identifications via searching against a comprehensive library of MS/MS spectra.

Table 1. Advantages and Disadvantages of the Multi-Target Screening Approach (MRM-IDA-EPI)

Advantages	Disadvantages
o MRM detection provides ultimate sensitivity and selectivity	o Only compounds on the target list are detected
o MS/MS library searching provides unambiguous confirmation	
o Screening for 100's of target compounds is feasible	

Upon analysis of the 15 drug-positive urine samples using the targeted screening method, 42 different compounds were detected, and are tabulated in Table 2. Compound identifications were only deemed to be positive if the compound was both (a)

detected by the MRM survey scan and (b) confirmed via library-searching of the automatically-triggered EPI spectrum against a comprehensive MS/MS spectral library.

Example data for a positive identification is shown in Figure 7, including the chromatogram from the MRM survey scan, the acquired EPI spectrum, and the matching library spectrum.

Since the multi-target screening method employs an MRM survey scan, it is expected that this method will provide superior sensitivity and selectivity – and ultimately lower detection limits – when compared to a general unknown screening method.

Table 2. Compounds Detected Using a Targeted Screening Method (MRM-IDA-EPI) on the 3200 QTRAP® LC/MS/MS System

Compound Names		
3,4-MDMA	Ecgoninemethylester	Norfentanyl
6-Monoacetylmorphine	Fentanyl	Nortriptyline
7-Aminoclonazepam	Gabapentin	Olanzapine
Amitriptyline	Heroin	Oxycodone
Atenolol	Hydrocodone	Paroxetine
Benzoylecgonine	Hydroxyzine	Pseudoephedrine
Brompheniramine	Irbesartan	Quetiapine
Bupivacaine	Lidocaine	Ranitidine
Cetirizine	Lorazepam	Risperidone
Citalopram	Metformin	Temazepam
Cocaine	Morphine	Tramadol
Codeine	Morphine-β-glucuronide	Trazodone
Dextromethorphan	Norbuprenorphine	Venlafaxine
Diphenhydramine	Nordiazepam	Zopiclone

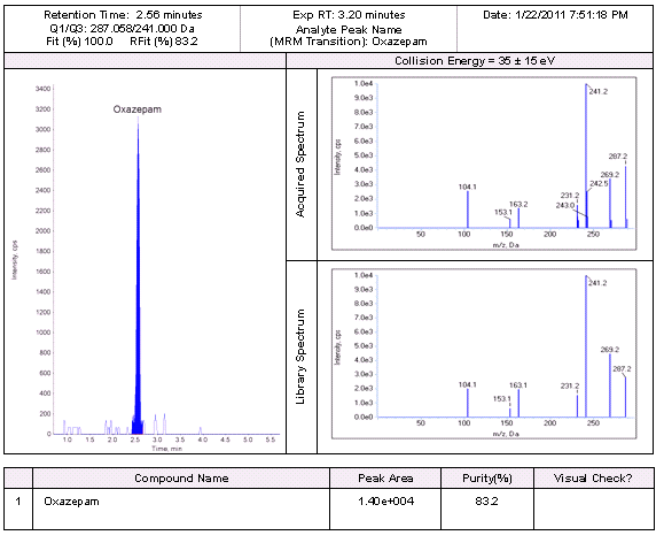
Compounds were detected using an MRM survey scan, and confirmed using library searching of the automatically-triggered Enhanced Product Ion (EPI) full-scan MS/MS spectra.

Table 3. Additional Compounds Detected Using a General Unknown Screening Method (EMS-IDA-EPI) on the 3200 QTRAP® LC/MS/MS System

Compound Names		
Acetaminophen	Caffeine	Nizatidine
Benzododecinium	Metoclopramide	Tetramethrin

Compounds were detected using an Enhanced Mass Spectrum (EMS) survey scan, and confirmed using library searching of the automatically-triggered Enhanced Product Ion (EPI) full-scan MS/MS spectra.

Figure 7. Positive Compound Identification for Oxazepam Using a Targeted Screening Method (MRM-IDA-EPI)



	Compound Name	Peak Area	Purity(%)	Visual Check?
1	Oxazepam	1.40e+004	83.2	

Left: The targeted screening method provides the most sensitive and selective detection of target compounds.

Right: MS/MS library searching unambiguously confirms the compound identification.

General Unknown Screening (EMS-IDA-EPI)

The General Unknown Screening (GUS) approach employed the Enhanced Mass Spectrum (EMS) scan as survey scan, enabling the identification of any major components in samples, including unanticipated compounds. While this approach is free of bias, and requires no *a priori* knowledge of the compounds to be detected, it is not as sensitive to the presence of low-abundance compounds due to the reduced selectivity associated with single-stage MS (compared to MS/MS) experiments. As with the Multi-Target Screening approach, the QTRAP® system was operated in Information-Dependent Acquisition (IDA) mode, to automatically trigger the acquisition of Enhanced Product Ion (EPI) full-scan MS/MS spectra, which were used for compound confirmation via library searching.

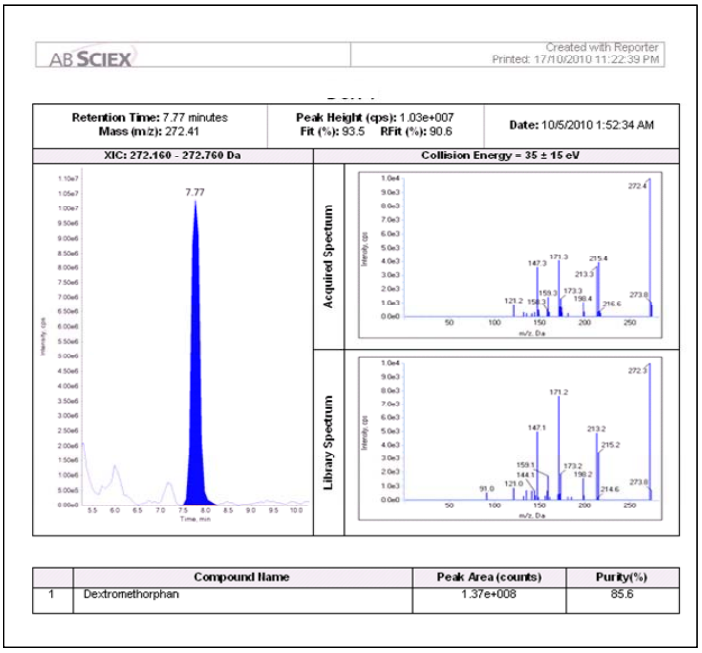
Table 4. Advantages and Disadvantages of the General Unknown Screening Approach (EMS-IDA-EPI)

Advantages	Disadvantages
o Full-scan EMS detection is not biased, and can therefore identify all compounds and metabolites	o Sensitivity and selectivity of single-MS survey scan is worse than MRM
o The analyst may perform retrospective data mining of full-scan EMS data	o Data mining is laborious. Automated software is very helpful.
o MS/MS library searching provides unambiguous confirmation	

Upon analysis of the urine samples using the general unknown screening method, fewer compounds were identified compared to the targeted screening method. This was as anticipated, since it is expected that the sensitivity of this method will not be as good as the multi-target screening method which uses the MRM mode of operation as a survey scan. More importantly, however, the general unknown screening method identified several compounds that were not detected by the multi-target screening method (see Table 3), because these were not included in the list of target compounds. This observation highlights the benefit of using a non-biased screening approach for drug screening, to complement the more sensitive targeted screening approach. As with the Multi-Target screening approach, compound identifications were only deemed to be positive if the compound was both (a) detected by the EMS survey scan and (b) confirmed via library-searching of the automatically-triggered EPI spectrum against a comprehensive MS/MS spectral library.

Example data for a positive identification using the general unknown screening approach is shown in Figure 8, including the chromatogram from the EMS survey scan, the acquired EPI spectrum, and the matching library spectrum. The elevated background in the chromatogram is evidence of the fact that the method using EMS-based detection suffers from a reduction in selectivity compared to the method using MRM-based detection.

Figure 8. Positive Compound Identification for Dextromethorphan Using a General Unknown Screening Method (EMS-IDA-EPI)



Left: The general unknown screening method is capable of detecting unanticipated compounds, however the reduced selectivity results in elevated chromatographic background.

Right: MS/MS library searching unambiguously confirms the compound identification.

Simultaneous Targeted and Unknown Screening (EMS-MRM-IDA-EPI)

The simultaneous targeted and unknown screening approach employed both a targeted MRM survey scan and a non-targeted EMS survey scan, enabling the simultaneous detection of target analytes at low concentration levels, as well as unanticipated compounds. In this method, the two survey experiments were looped, and the QTRAP® system was operated in Information-Dependent Acquisition (IDA) mode, to automatically trigger the acquisition of Enhanced Product Ion (EPI) full-scan MS/MS spectra, which were used for compound confirmation via library searching.

The advantage of this approach is that it is possible to simultaneously screen for a target list of those drugs that are most commonly used and abused, using the most sensitive and selective mode of detection available, while still obtaining information about unanticipated compounds that were not included in the target list. Furthermore, since full-scan EMS data is collected throughout the chromatographic run, the analyst is afforded with the opportunity to perform retrospective data mining at a later date, to identify unanticipated compounds. The only trade-off of using this approach is a slight increase in the overall cycle time required to perform each measurement, which translates into a slight reduction in the number of data points collected across each chromatographic peak.

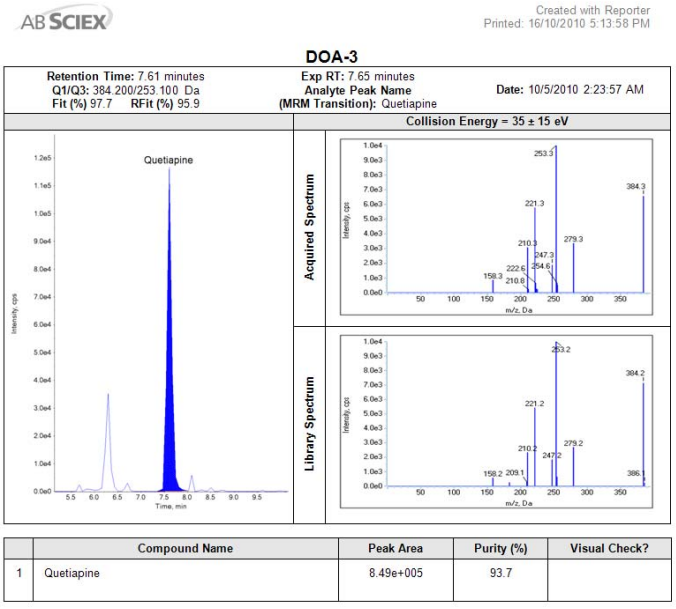
Table 5. Advantages and Disadvantages of the Simultaneous Targeted and Unknown Screening Approach (EMS-MRM-IDA-EPI)

Advantages	Disadvantages
o MRM detection provides ultimate sensitivity and selectivity for target analytes	o Slight reduction in the number of data points collected across each chromatographic peak
o Full-scan EMS detection is not biased, and can therefore identify all compounds and metabolites	o Data mining for unknowns is laborious. Automated software is very helpful.
o Screening for 100's of target compounds is feasible	
o The analyst may perform retrospective data mining of full-scan EMS data	
o MS/MS library searching provides unambiguous confirmation	

When the drug-positive urine samples were analyzed using the simultaneous targeted and unknown screening method, the list of positive identifications included all 42 compounds that were detected previously by the multi-targeted screening method, as well as all 6 compounds that were detected previously by the

general unknown screening method. Example data for a positive identification of a target compound using the simultaneous targeted and unknown screening method is shown in Figure 9 below, including the chromatogram from the MRM survey scan, the acquired EPI spectrum, and the matching library spectrum. Note that due to the slight increase in the cycle time for this method, which uses looped EMS and MRM survey scans, there are slightly fewer data points across the chromatographic peak. Nevertheless, there are more than enough data points for the purposes of this screening LC-MS/MS method, since absolute quantitative results are not required.

Figure 9. Positive Identification of a Target Compound, Quetiapine, Using a Method for Simultaneous Targeted and Unknown Screening (EMS-MRM-IDA-EPI)



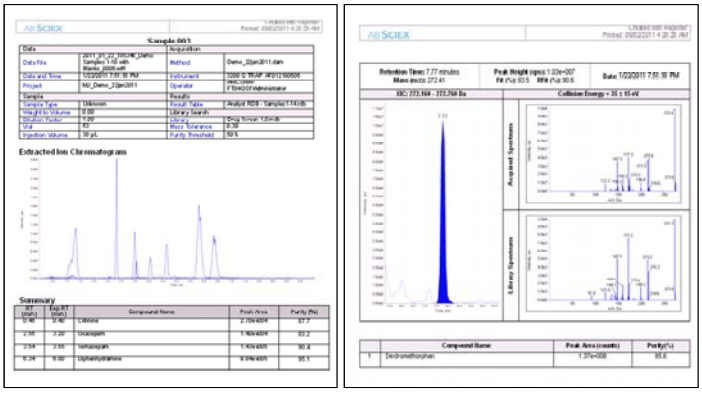
	Compound Name	Peak Area	Purity (%)	Visual Check?
1	Quetiapine	8.49e+005	93.7	

Left: Quetiapine, a target compound included in the MRM target list, is detected by the Simultaneous Targeted and Unknown Screening method.

Right: MS/MS library searching unambiguously confirms the compound identification.

The simultaneous targeted and unknown screening analysis was performed using the Cliquid® 3.2 software, which automatically generated reports summarizing the target compounds that were detected in each sample. An example report for the target compounds is shown in Figure 10.

Figure 10. Example Report for Target Compounds Detected by the Simultaneous Targeted and Unknown Screening Method.



After data acquisition was completed, and the reports for all detected target compounds had been automatically generated, the Cliquid® software was used to interrogate the data for unknown compounds. As has been mentioned several times, the mining of full-scan EMS data to identify unknown compounds can be extremely laborious; however this process can be facilitated by the use of intelligent, automated data-mining software. Many experiments that have claimed to be a general unknown screen only extract ion chromatograms from a list of M+H values that correspond to known or suspected drugs. As a result, even though data for drug analogs, metabolites, or unanticipated compounds may be acquired, a corresponding XIC may not be extracted in the processing step if the M+H value is not “expected”. In reality, this type of data processing results in an experiment that more closely resembles a targeted rather than a general unknown screen. In contrast, by using the Cliquid® software the analyst may set up several criteria to direct the automated mining of full-scan data for the identification of unknown compounds, as demonstrated in Figure 11 below. Since this approach does not make use of a pre-determined target list, it is truly a general unknown screen.

In addition to allowing the user to direct the automated identification of unknown compounds, the Cliquid® software also permits the analyst to define the library search parameters, as displayed in Figure 12 below. These parameters will affect the results of the library search, and allow the user to define a minimum acceptable purity score for compound confirmations via MS/MS library searching.

Figure 11. User-Defined Parameters in Cliquid® Software for Intelligent and Automated Data Mining of Unknown Compounds.

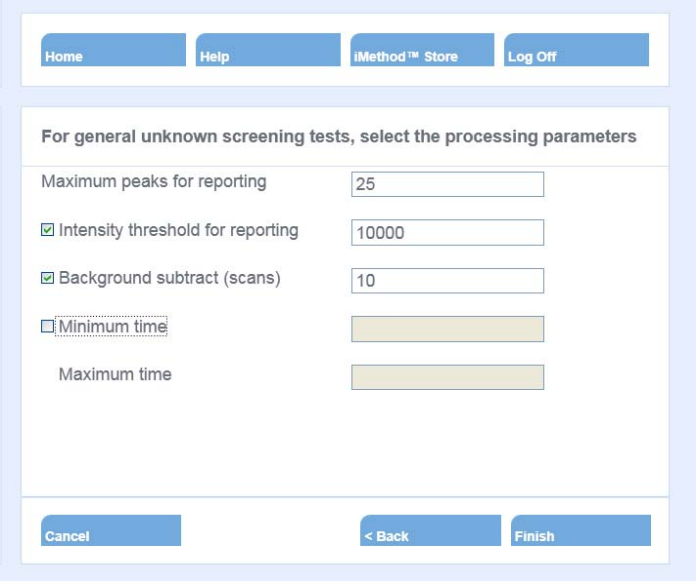
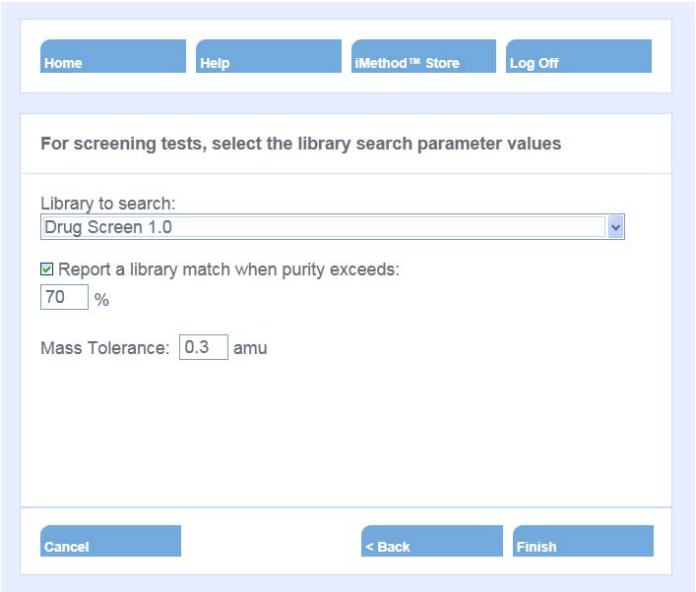


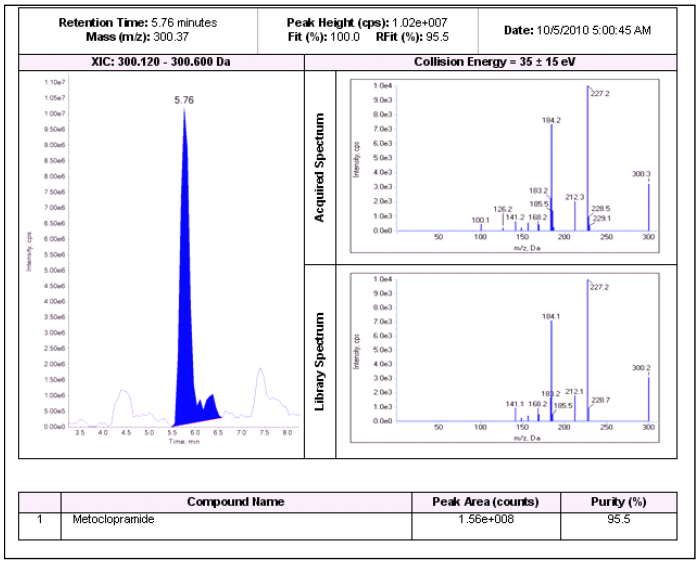
Figure 12. User-Defined Parameters in Cliquid® Software for MS/MS Library Searching Confirmation.



Upon interrogation of the full-scan data from the EMS-MRM-IDA-EPI experiments, using the parameters shown in Figures 11 and 12 above, a number of unanticipated compounds were identified in the drug-positive urine samples. In fact, all of the unanticipated compounds that had been previously detected (see Table 2) by the *dedicated* general unknown screening (EMS-IDA-EPI) method were once more detected and confirmed using the data collected acquired by the *simultaneous* targeted and unknown screening method (EMS-MRM-IDA-EPI). This observation suggests that although the instrument was performing more looped experiments per cycle while running the EMS-MRM-IDA-EPI experiments, the rapid scanning capability of the QTRAP® system allows acquisition of the data at a rate that is sufficiently fast to enable the simultaneous screening for both target and unknown compounds, with no compromise in the quality of the acquired confirmatory MS/MS spectra.

Example data for a positive identification of an unknown compound, metoclopramide, using the simultaneous targeted and unknown screening method is shown in Figure 13 below, including the extracted ion chromatogram from the EMS survey scan, the acquired EPI spectrum, and the matching library spectrum. This compound was not detected by the targeted MRM experiment, because it was not included in the list of target compounds. Note that due to the slight increase in the cycle time for this method, which uses looped EMS and MRM survey scans, there are slightly fewer data points across the chromatographic peak, however there are more than enough data points for the purposes of this screening LC-MS/MS method.

Figure 13. Positive Identification of an Unknown Compound, Metoclopramide, Using a Method for Simultaneous Targeted and Unknown Screening (EMS-MRM-IDA-EPI).



Left: Metoclopramide, an unknown compound, is detected by the Simultaneous Targeted and Unknown Screening method.

Right: MS/MS library searching unambiguously confirms the compound identification

Once the Cliquid® software has performed the extraction of ion chromatograms for all unknown compounds, based upon the user-defined data-mining parameters, a report is automatically generated summarizing the unknown compounds that were detected in each sample.

As expected, the unknown screening component of the EMS-MRM-IDA-EPI method detected fewer drugs overall compared to the targeted component of the method, due to the reduced sensitivity of the EMS survey scan, however the inclusion of the unknown screening aspect in the same LC-MS/MS run enabled the concurrent detection of compounds that were not included in the list of target compounds. This observation affirms the complementary nature of targeted and non-targeted screening approaches.

Conclusions

The ability to perform simultaneous targeted and unknown screening on the same platform, and most importantly in a single LC-MS/MS method, makes the 3200 QTRAP® system a powerful and versatile tool for forensic toxicology screening.

The novel methodology described in this note consisted of an EMS-MRM-IDA EPI experiment. The Multiple Reaction Monitoring (MRM) scan provided superior sensitivity and selectivity, enabling the detection of very low concentrations of a pre-determined subset of target drugs in complex biological matrix. Since this scan detected only those compounds selected in advance, it did not reveal the presence of those compounds which were not included in the target drug list. The Enhanced Mass Spectrum (EMS) scan provided detection of all major components present in the samples, including any unexpected compounds.

By combining both MRM and EMS scans in a single LC-MS/MS method, it was possible to achieve (i) highly sensitive detection of those drugs which are most commonly used or abused, and (ii) the simultaneous detection of high-abundance, but unanticipated compounds. Information Dependent Acquisition (IDA) criteria were employed in order to automatically trigger the acquisition of full-scan Enhanced Product Ion (EPI) spectra for any compounds that were detected by the survey scans, and these were searched against a spectral library to confirm the compound identifications.

To test the efficacy of the novel method for simultaneous targeted and unknown screening, a group of drug-positive urine samples were analyzed using three different screening methods:

1. A multi-target screening (MRM-IDA-EPI) method, with MS/MS library searching confirmation for detected compounds,
2. A general unknown screening (EMS-IDA-EPI) method, with MS/MS library searching confirmation for detected compounds, and
3. A novel method for *simultaneous* targeted and unknown screening (EMS-MRM-IDA-EPI), with MS/MS library searching confirmation for detected compounds, described in detail above

The latter method for simultaneous targeted and unknown screening successfully detected all of the compounds that were detected by methods 1 and 2 combined – only in a single LC-MS/MS run, thus saving both time and money, and requiring less sample volume. Furthermore, this approach afforded the analyst with the opportunity to perform retrospective data mining to extract valuable information.

The results of this work highlight the complementary nature of the targeted screening and unknown screening workflows, since neither workflow alone is capable of identifying all of the components present in a complex mixture. These results demonstrate that the 3200 QTRAP® LC-MS/MS system is capable of performing simultaneous targeted and unknown screening, in a single LC-MS/MS analysis, with no compromise in the number of compounds detected, or in the quality of the MS/MS data obtained for compound confirmation.

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