The Forensics 664 vMethod: Accurate and Specific Forensic Toxicology Screening

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As drug abuse continues to grow worldwide, the need for accurate and specific forensic toxicology screening increases. Mass spectrometry, specifically LC-MS/MS has become the preferred method for forensic drug screening and an ideal approach is quadrupole time-of-flight MS as it provides high-resolution, accurate-mass data for full-scan information of both precursor ion and all product ions. This article describes a method for forensic drug screening using the SCIEX X500R QTOF system, which generates data that offers more confirmation criteria than nominal mass triple quad workflows, and reduces the risk of reporting false positives and false negatives that could compromise an investigation.

Introduction

An estimated 183,000 drug-related deaths occurred worldwide in 2012, according to the United Nations Office on Drugs and Crime 2014 World Drug Report [1] and more than 300 million people (7% of the global population) used an illicit drug last year. These statistics clearly display a critical need for law enforcement agencies to undertake specific and accurate drug testing. Forensic toxicology screening identifies drugs and their metabolites, chemicals, volatile substances, gases and metals in human tissues for law enforcement purposes. It relies on the identification of unknown compounds from complex samples and information-rich data sets.

During recent years, mass spectrometry, and specifically LC-MS/MS has become the industry's preferred method for forensic toxicology screening due to its sensitivity, selectivity and accuracy over conventional methods such as GC, GC-MS and UV detection.

An ideal approach is quadrupole time-of-flight mass spectrometry (QTOF-MS) as it provides high-resolution, accurate-mass data for full-scan information of both precursor ions and all product ions. An LC-MS/MS-based toxicological screening method has recently been developed that includes the retention times for 664 forensic compounds. When combined with high-resolution mass spectrometry (HRMS) and HR-MS/MS information, the retention time enables more accurate compound identification and an enhanced ability to identify structurally similar isomers.

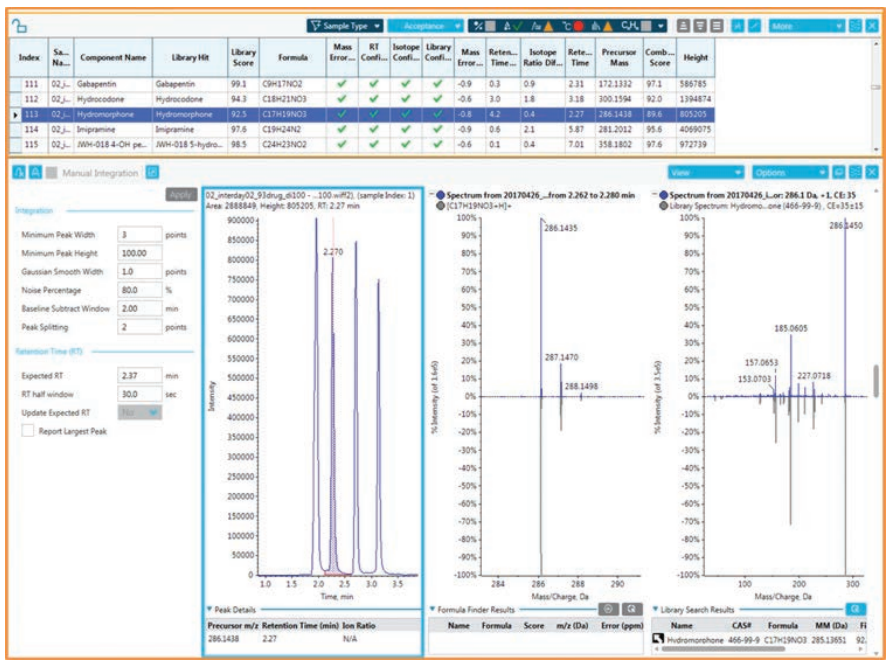

Figure 1.The parameters used for the integration of hydromorphone.

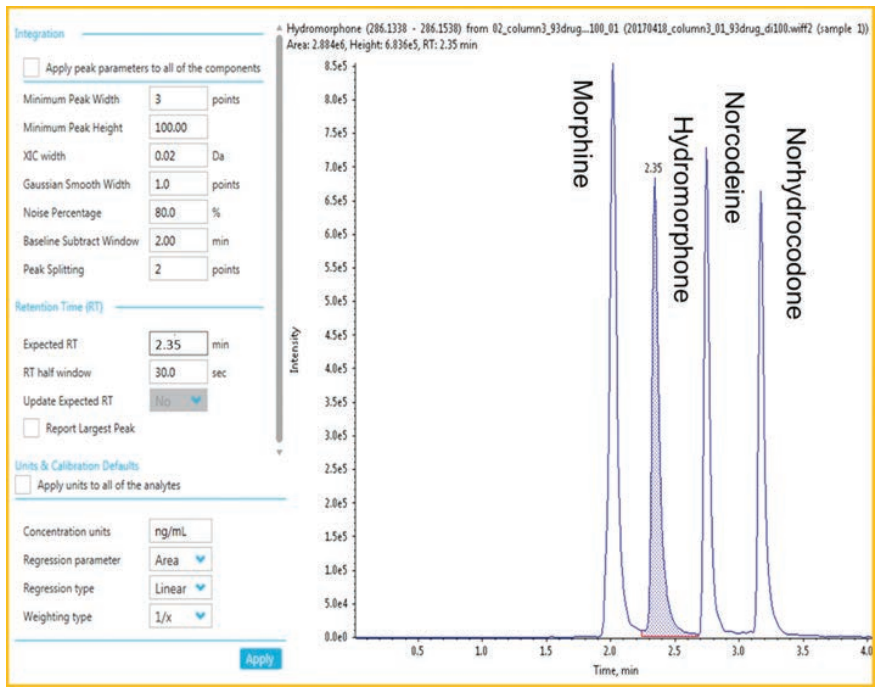

Figure 2. Representative search results obtained after using a targeted screening approach to identify compounds in urine samples.

This is the first verified method for forensic drug screening using the X500R QTOF system and data generated from this method offer more confirmation criteria than nominal mass triple quad workflows. The unique high-resolution spectra deliver unrivalled confidence in reporting the detected analyte. Cross-referencing the data with a forensic library gives added confirmation and the method reduces the risk of reporting false positives and false negatives that could compromise an investigation.

Materials and Methods

Sample preparation

The stock standard mixtures in neat solutions were diluted with methanol: water (20:80, v/v) to appropriate concentrations. These diluted solutions were used to determine the retention time of the 664 compounds using a SCIEX X500R QTOF system. Subsequently, the urine and whole blood samples were prepared to confirm the retention times in matrix. For urine samples, stock standards solutions (10.0 µL) were added into human urine matrix (90.0 µL) and then diluted 10-folds with methanol:water (20:80, v/v). After centrifugation at 8,000 rpm for 5 min, the supernatant was used for LC-MS analysis. For whole blood

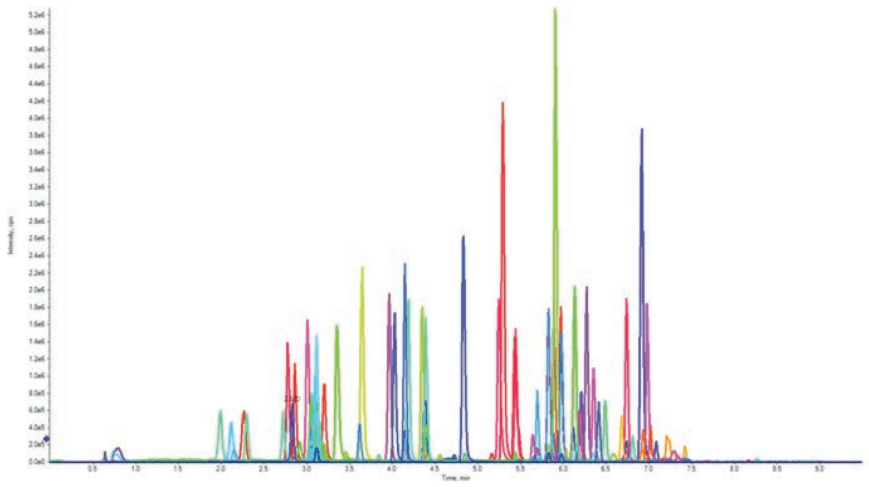

Figure 3. Extracted ion chromatograms (XICs) for multiple analytes (80 out of 600) show optimal peak separation.

samples, 10.0 µL of stock standard solutions were spiked into 90.0 µL of human whole blood matrix. The samples were extracted by using a protein precipitation procedure. Basically, 900 µL of Methanol: MeCN (50:50, v/v) were added into the above mixture and vortexed for 1 min then follow by 3 min sonication and another 1 min vortex. Then the samples were centrifuged for 5 min at 8,000 rpm. The supernatant was transferred out and completely dried down under nitrogen gas. The residues were reconstituted with 500 µL methanol: water (20:80, v/v).

LC separation

Analytes (10 µL sample injection volume) were chromatographically separated using a Phenomenex Kinetex® 2.6 µm phenyl-hexyl (50 x 4.6 mm) column. 10 mM ammonium formate in water was used as mobile phase A and 0.05% formic acid in methanol was employed as mobile phase B. The mobile phases were replaced every 2 days. A linear gradient (600 µL/min) from 10% B to 98% B in 7.0 min followed by 1.5 min of 98% B and 1.0 min of 10% B was employed.

Processing method settings

To identify compounds in the analysed samples, a targeted screening approach was employed using SCIEX OS software version 2.0. Samples were evaluated against a list of parameters containing the names, molecular formulas and retention times (RTs) for all targeted compounds. Appropriate integration parameters were defined for each component. For example, the compound, hydromorphone, was defined as the peak at 2.35 min (*Figure 1*) with a 30 second half time window. An MS/MS library [2] was used for MS/MS library matching.

The confidence criteria used for screening were mass error, RT error, isotope ratio difference, and library score. A traffic light system where different colours were assigned to different performance levels provided a way to assess the quality of the match. For example, in the case of mass error, green represented mass errors less than 5 ppm; orange, mass errors between 5 and 10 ppm; and red, mass errors larger than 10 ppm. A representative search result is shown in *Figure 2*.

Results and Discussion

For forensic analysis, it is imperative to be able to quickly test whole blood and urine samples with straightforward sample preparation to deliver clear accurate data, quickly. To this end, a single-injection method for screening 664 of the most up-to-date forensic compounds has been developed.

The method, denoted vMethod, allows for screening of 664 compounds in HR-MS/MS mode on a QTOF System.

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The confidence criteria used for screening were mass error, retention time error, isotope ratio difference, and library score. A traffic light system where different colours were assigned to different performance levels provided a way to assess the quality of the match. For example, in the case of mass error, green represented mass errors below 5 ppm; orange, mass errors between 5 and 10 ppm; and red, mass errors larger than 10 ppm A representative search result is shown in *Figure 2*.

The injection volume for the vMethod is 10 µl and sample matrices that can be analysed include whole blood and urine. The sample preparation protocol details all the steps required for the clean-up of both blood and urine samples and in just 10 minutes, detailed, verified LC separation conditions are achieved with retention times.

Figure 1 shows an example of full chromatographic separation for 4 isomers, including Morphine, Hydromorphone, Norcodeine and Norhydrocodone, with the optimised LC condition. *Figure 3* show example extracted ion chromatograms for 80 out of the 664 compounds using the optimised LC condition.

Retention time is a critical element for accurate identification of each forensic analyte using this screening method. Therefore, retention time reproducibility tests were conducted for each compound to evaluate the robustness of the LC condition in this method. These tests indicate that the retention times generated from the optimised LC conditions are consistent and reproducible. Retention times measured on three separated analytical columns all have % coefficient of variation (CVs) of less than 5% for each of the 664 compounds.

The retention time determined by the optimised LC condition combined with high-resolution mass spectrometry (HRMS) and HR-MS/MS information, enable more accurate compound identification than regular triple quad workflows. For example, the Noroxycodone (*Figure 4A*) and Oxymorphone (*Figure 4B*) have the exact same precursor ion and very similar MS/MS spectra. However, these two compounds were fully resolved by using the LC condition in this method. The retention time is 3.05 min for Noroxycodone and 2.10 min for Oxymorphone. Therefore, it is easier and more accurate to distinguish these two compounds by using retention time combined with MS and MS/MS information.

In addition, because the data was acquired in a non-targeted approach, the processing method designed here for screening targeted compounds can be quickly adjusted and used for unknown compound identification using non-targeted data processing. Users can retrospectively analyse previously acquired MS and MS/MS data sets to screen for new compounds without having to re-inject samples, allowing data sets to be re-processed when newly identified forensic targets are discovered. For retrospective data analysis, a new process method was built for 10 compounds including 5 initial compounds and 5 new compounds by using search parameters that included the compound's name, formula and retention times. The updated processing method was then used to re-analyse data sets for the new compound.

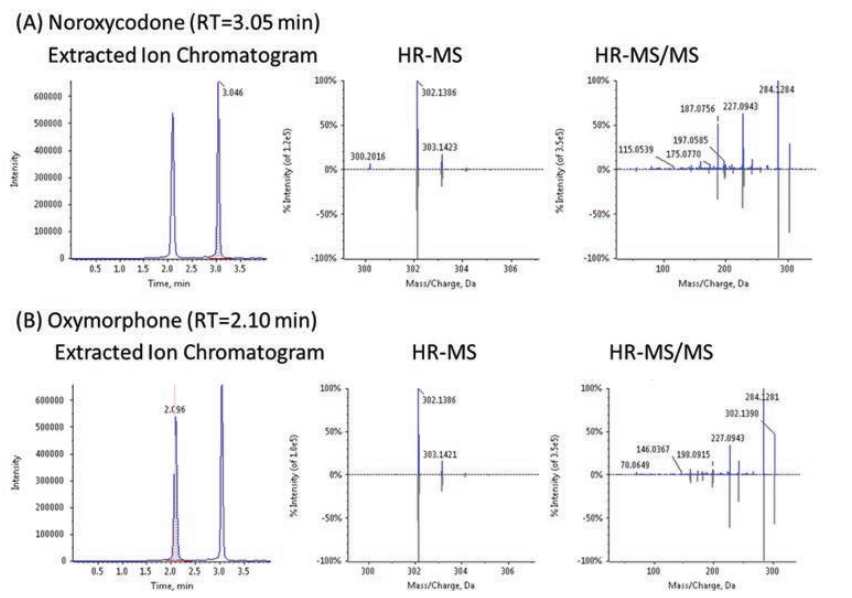

Figure 4. Representative XIC HR-MS and HR-MSMS spectra for Noroxycodone and Oxymorphone

The vMethod gives data that provide both structural information and retention times to enhance identification accuracy, especially for structurally similar isomers. The sample preparation procedures for urine and whole blood samples and library-search settings recommended in the method can help automate and confidently establish the identification of unknowns in an efficient, all-in-one workflow.

Overall, the ability to identify structurally similar isomers was largely enhanced using the vMethod. In addition, because the data were acquired in a non-targeted approach the processing method designed here for screening targeted compounds can be quickly adjusted and used for unknown compound identification using a non-targeted data processing. Users can retrospectively analyse previously acquired MS and MS/MS data sets to screen for new compounds without having to re-inject samples, allowing for data sets to be re-processed when newly identified forensic targets are discovered.

Conclusions

This broad-based drug screening method offers a good balance between chromatographic separation and analysis time. The mass acquisition is designed to acquire analytes of interest in a semi-non-targeted fashion, allowing for high specificity in tracing fragment ions back to precursor ions. In addition, it has the potential for more wide-ranging retrospective data analysis for determination of analytes not within the current scope of data processing.

References

1. *The United Nations Office on Drugs and Crime 2014 World Drug Report http://www.unodc.org/wdr2014/*

2. *SCIEX Forensics High Resolution MS/MS Spectral Library 2.1, part number: 5059566*