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Application Note # LCMS-72

Toxtyper™ – a Comprehensive LC-MS^n Screening Solution for Clinical and Forensic Toxicology

Abstract

A major task for clinical and routine toxicology screening is the reliable, fast and comprehensive identification of drugs and drugs of abuse (DOA). Current techniques such as GC-MS, LC-UV/DAD and immunoassays have several limitations. Liquid chromatography tandem mass spectrometry (LC-MS/MS) is an emerging technology that is more specific than immunoassays, provides more information than LC-UV/DAD detection, and covers a broader range of compounds than GC-MS.

Here we describe a robust and automated screening solution based on the latest LC-MS^n ion trap technology that promises to provide a combination of the highest performance LC-MS/MS and easy-to-use screening with the greatest transferability of results from lab to lab.

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Drug Screening	DA 4.1
Drugs of Abuse	Ion Trap

Introduction

Comprehensive clinical and forensic toxicology screening makes high demands on analytical solutions in terms of sensitivity, robustness, reliability and ease-of-use. In the clinic and the routine lab, fast and unambiguous identification of toxic compounds is the primary task. The screening solution presented here is based on ultrahigh performance liquid chromatography (UHPLC) coupled to an LC-MS^n ion trap system (amaZon speed) and uses a comprehensive spectral library of 830 drugs and drugs of abuse. The spectral library contains over 2600 spectra from MS, MS^2 and MS^3 experiments, as well as retention time information.

Retention time and compound mass information are used for a sophisticated data-dependent MS^n acquisition using a scheduled precursor list. The unique ion trap feature of generating multiple fragmentation stages (MS^n) from the same precursor is used to reach high levels of confidence for compound ID. The complete LC-MS^n method for one screening round takes less than 11 min and includes continuous positive/negative switching to cover acidic and basic compounds in a single run. The fast acquisition cycles are achieved by the latest amaZon speed ion trap technology, which offers a 100% increase in MS/MS speed and zero time loss polarity switching (Zero Delay Alternating, ZDA™). To our knowledge, this solution provides the fastest screening method for such a comprehensive library of 830 cpds.

Current LC-MS/MS instruments, workflows and software often require expert MS knowledge, which is not always available in routine clinical laboratories. The Toxtyper solution was developed with this in mind and provides a number of features to meet the needs of clinical and routine labs. Firstly, the unique patented SmartFrag™ technology substantially removes variation and the need for tuning from the MS/MS process. Secondly, the complete data acquisition and post-processing software is hidden by Compass OpenAcess, which provides a wizard for easy web-based access, even for MS non-experts. Finally, automated ID reporting enables fast inspection of results and automatic sending of reports by email to senior laboratory personnel (see LCMS-62).

Experimental

Sample preparation

A mixture of 9 different compounds of forensic and clinical interest at therapeutic / toxic levels was spiked into a blank human serum matrix. Sample preparation was carried out using the following liquid-liquid extraction (LLE) protocol: serum (1 mL) was spiked with 50 ng of D5-Diazepam as an internal standard and then mixed with 0.5 mL borate buffer (pH 9) and 1.5 mL 1-chlorbutane. After a 3 min mixing step the solution was centrifuged at 4000 × g for 5 min. The organic phase was separated and evaporated at 40°C with N_2. The residue was redissolved in 25 µL solvent A/B (50:50; v/v; see Table 1).

This method proved to be well-suited for the extraction of a wide range of analytes; such as hypnotics, neuroleptics, antidepressants and many others.

LC-MS^n conditions

Two microliters of the redissolved solution was separated on an Ultimate3000 RSLC system using the settings described in Table 1. The amaZon speed ion trap system was used for generation of MS and MS^n spectra in continuous polarity switching mode (for details refer to Table 2). Data were acquired using a data-dependent scheduled precursor list approach.

Table 1: HPLC conditions used for the Toxtyper screening workflow

LC settings	
LC System	Thermo Dionex Ultimate 3000 RSLC
Eluent A	H_2O, 0.1% formic acid, 2 mM ammonium formiate, 1% acetonitrile
Eluent B	Acetonitrile, 0.1% formic acid, 2 mM ammonium formiate, 1% H_2O
Analytical column	Acclaim® RSLC 120 C18 2.2 µm, 120A, 2.1 x 100 mm
Flow rate	500 µl/min
Gradient	0.0 to 1.0 min: 1% B
	1.0 to 8.0 min: 1% B to 95% B, linear
	8.0 to 9.0 min: 95% B
	9.0 to 9.06 min: 95% B to 1% B, linear
	9.06 to 11 min: 1% B

Library search and reporting

The data set was post-processed using DataAnalysis (DA) 4.1 and the processed spectra were submitted to the DA 4.1 library module. The whole process up to final report generation and display of results in the web-based Compass OpenAccess interface is driven by a predefined Toxtyper automation script.

Results

The Toxtyper screening workflow is shown schematically in Figure 1. An essential part of the solution is the spectral library, which consists of more than 830 compounds of clinical and forensic interest and more than 2600 spectra (see Table 3). The library was generated in close collaboration with the Forensic Institute in Freiburg, Germany.

To demonstrate the workflow, spiked serum samples were processed by the Toxtyper workflow in a completely automated fashion under Compass OpenAccess as part of an interlaboratory test.

Figure 2 shows how users are guided through the web-based Compass OpenAccess interface until final report display. When a screening sample is prepared and ready for injection it can be accessed via Compass OpenAccess. The sample data are submitted by an intuitive graphical user interface. The user inputs a sample name, a description and chooses the predefined Toxtyper method. The next available rack position is indicated, and after the sample is placed in the requested location, the sample queue can be started. The easy-to-use client interface provides information about instrument status as well as about length of the sample queue.

Table 2: amaZon speed ion trap MS and MS^n parameters

MS settings	
Scan mode	UltraScan 32.500 m/z sec^{-1}
Scan range	70 - 800 m/z
Source	Electrospray ionisation (ESI)
Polarity	Zero Delay Alternating polarity
MS^n Acquisition	Data dependent using a Scheduled Presursor List with 830 cpds
	Active exclusion after 1 spectrum, reconsider if intensity increase by factor 5
Target mass	300 m/z
ICC	200.000

Table 3: Characteristics of the Toxtyper library

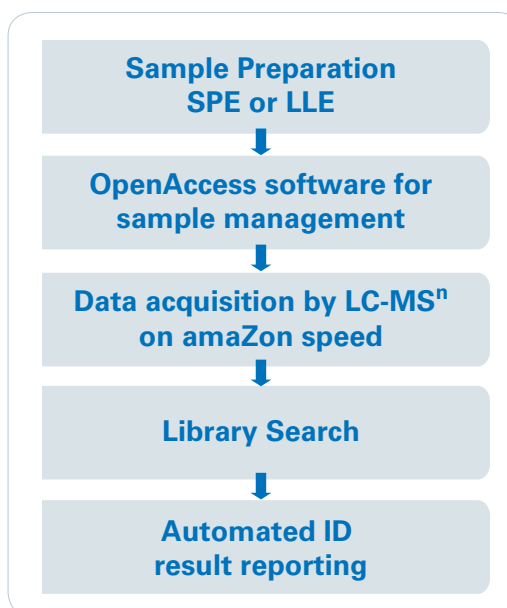| Toxtyper library	Description
Content	Neuroleptics, antidepressants, hypnotics, benzodiazepines and metabolites
Number of compounds	> 830
Number of spectra	> 2600
Characterization of spectra	Ion Trap, MS, MS^2 and MS^3, positive
HPLC	Retention time information included

Sample Preparation SPE or LLE

⬇

OpenAccess software for sample management

⬇

Data acquisition by LC-MS^n on amaZon speed

⬇

Library Search

⬇

Automated ID result reporting

Figure 1: Schematic of the Toxtyper library-based screening workflow.
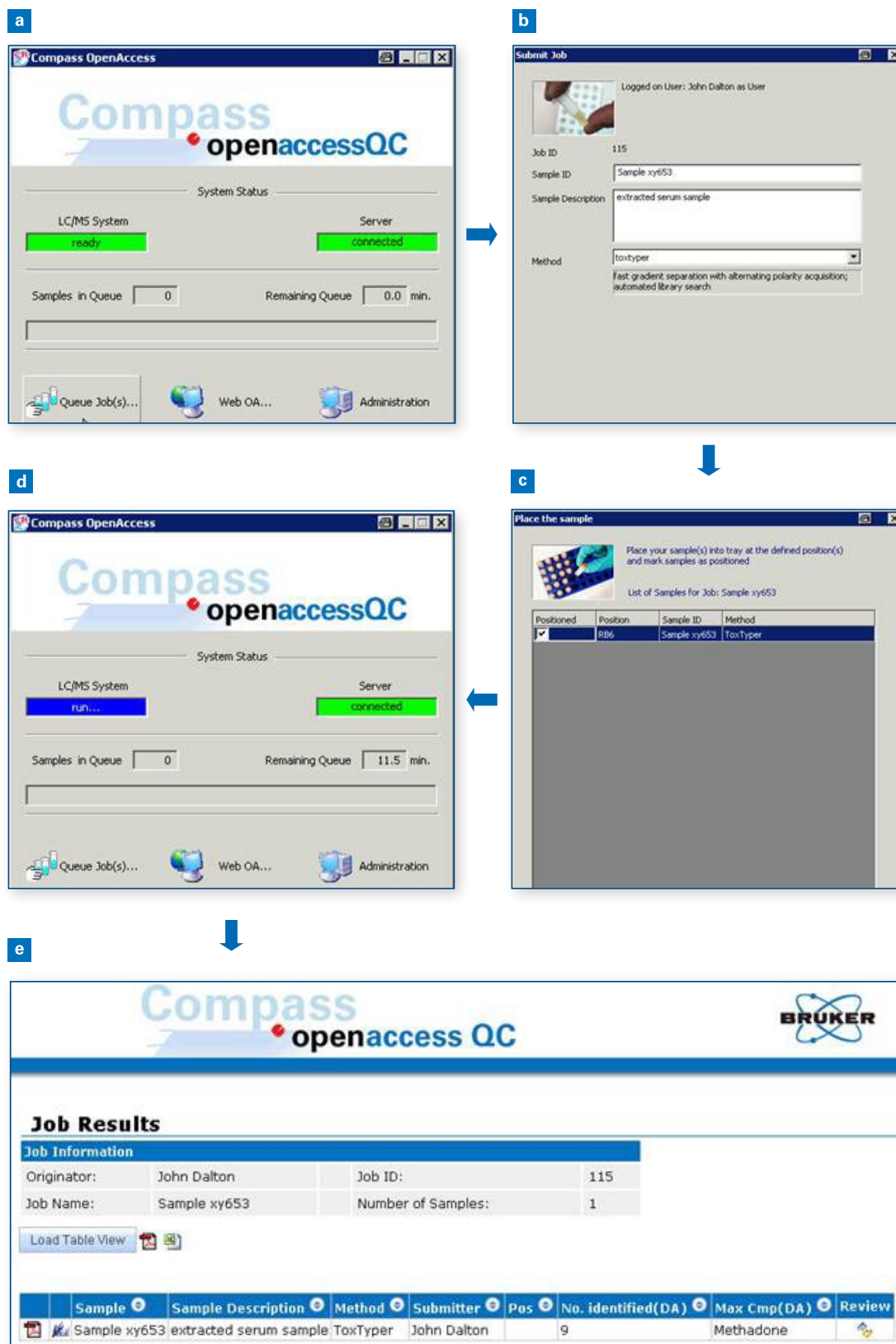
Figure 2: Users are guided through the Toxtyper workflow by a simple wizard based graphical user interface.

After completion of the run, the user can receive a PDF report of the LC-MS^n results either by logging onto the system again, by email or by requesting a printout. The first page of the result report is shown in Figure 3. The user receives an overview of the screening result consisting of base peak chromatograms (for positive and negative ionization mode) and a table that summarizes the identification results using purity score, intensity, and mass/retention time shifts.

For further result evaluation, each identified compound is listed on a separate report page that displays the extracted ion chromatogram of the substance as well as its MS, MS^2, and if required, MS^3 spectrum (see Figure 4) and the corresponding library spectra. This enables potentially critical IDs – for example false positives – to be ruled out very quickly.

Figure 4 shows the identification of methadone from serum. It reveals a very good match of MS and retention time between experimental results and library data. The MS^2 spectrum shows only one major peak whereas the MS^3 spectrum provides the important spectral information enabling an unambiguous match of experimental result and library spectrum.

The complete workflow can be tested using a quality control sample consisting of 8 library compounds that cover a broad retention time and mass window (Figure 5). In addition one of the QC compounds (hydrochlorothiazide) is well-suited for testing the negative ionization mode. The results of this QC sample are part of the automatic result reporting and can be used for a daily check of the workflow.
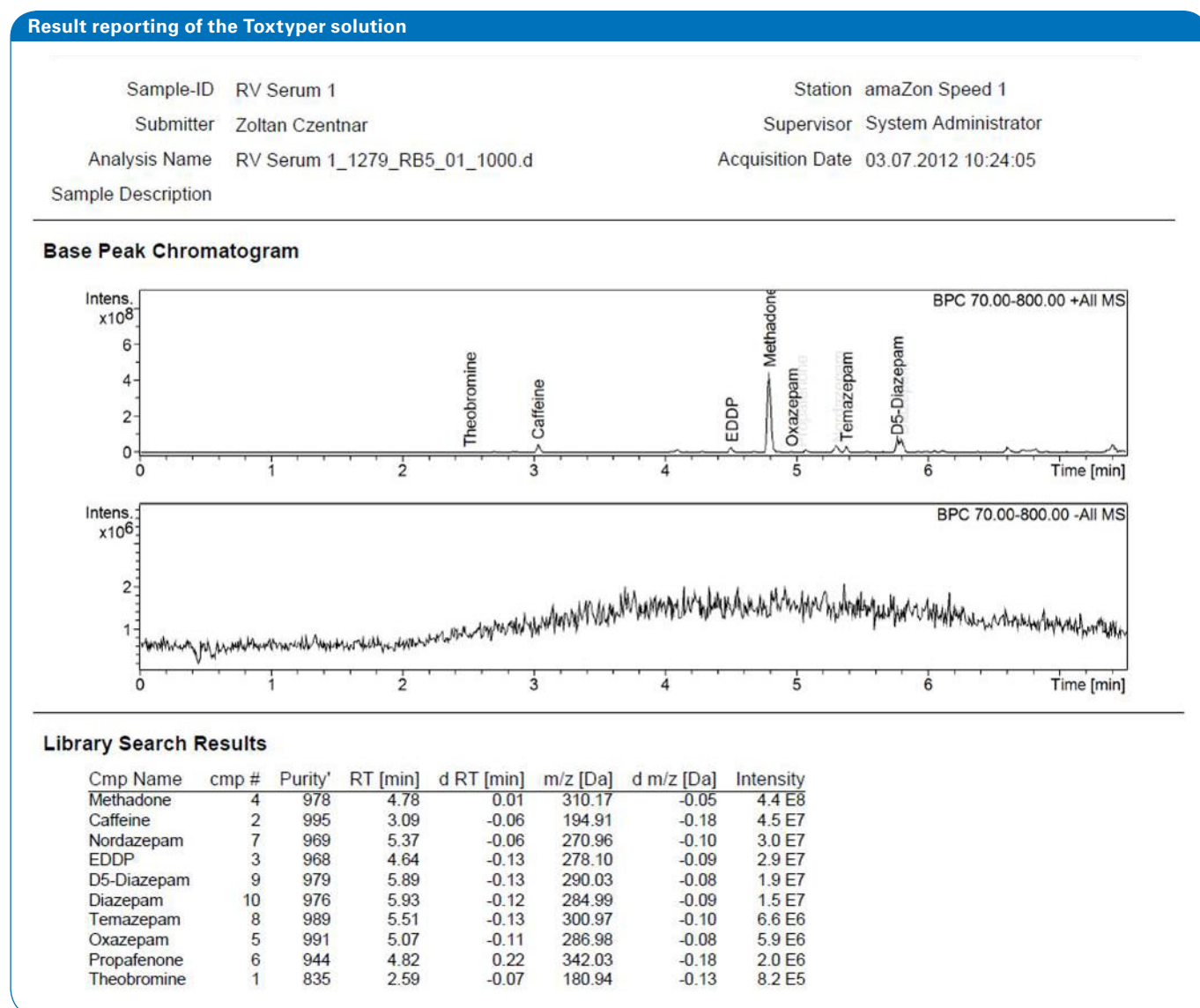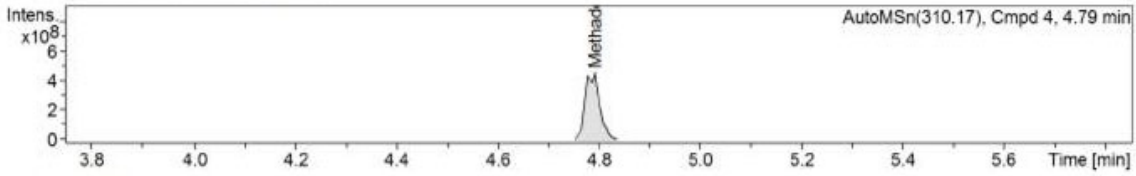
Result reporting of the Toxtyper solution

Sample-ID	RV Serum 1	Station	amaZon Speed 1
Submitter	Zoltan Czentnar	Supervisor	System Administrator
Analysis Name	RV Serum 1_1279_RB5_01_1000.d	Acquisition Date	03.07.2012 10:24:05
Sample Description			

Base Peak Chromatogram

Library Search Results

Cmp Name	cmp #	Purity'	RT [min]	d RT [min]	m/z [Da]	d m/z [Da]	Intensity
Methadone	4	978	4.78	0.01	310.17	-0.05	4.4 E8
Caffeine	2	995	3.09	-0.06	194.91	-0.18	4.5 E7
Nordazepam	7	969	5.37	-0.06	270.96	-0.10	3.0 E7
EDDP	3	968	4.64	-0.13	278.10	-0.09	2.9 E7
D5-Diazepam	9	979	5.89	-0.13	290.03	-0.08	1.9 E7
Diazepam	10	976	5.93	-0.12	284.99	-0.09	1.5 E7
Temazepam	8	989	5.51	-0.13	300.97	-0.10	6.6 E6
Oxazepam	5	991	5.07	-0.11	286.98	-0.08	5.9 E6
Propafenone	6	944	4.82	0.22	342.03	-0.18	2.0 E6
Theobromine	1	835	2.59	-0.07	180.94	-0.13	8.2 E5

Figure 3: Result reports can be accessed by the web or sent by e-mail.

Result reporting of the Toxtyper solution

Cmpd 4, AutoMS^n (310.17), 4.79 min, Methadone
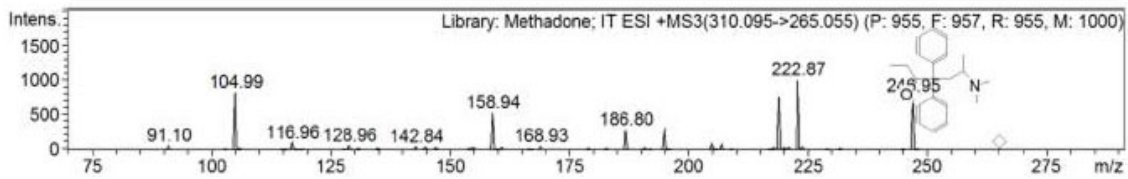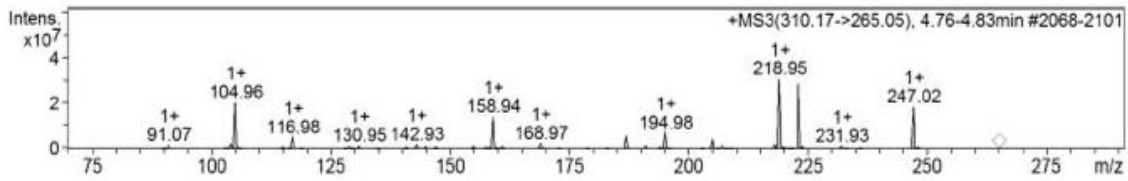
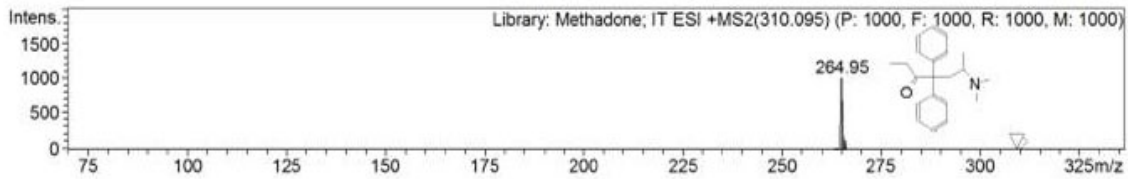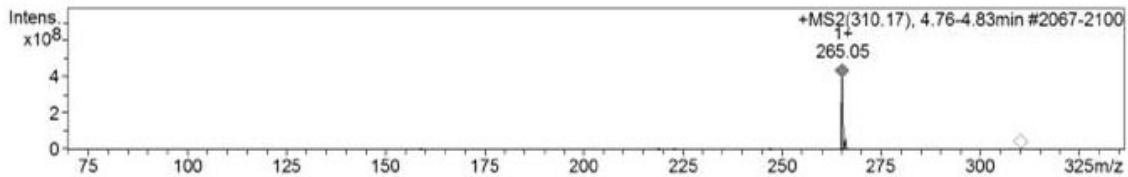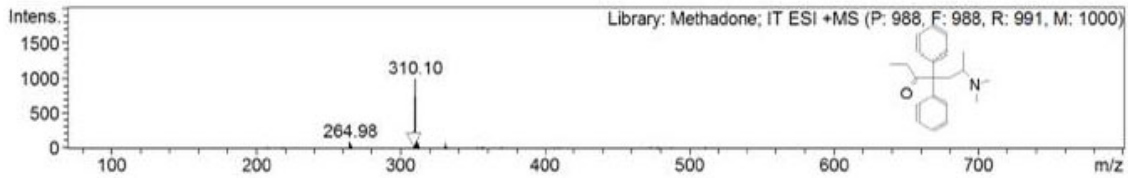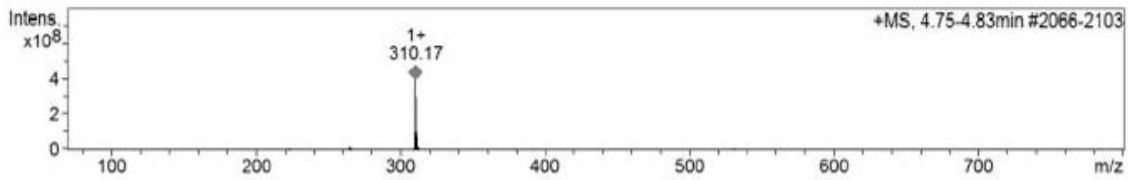Extracted Ion Chromatogram

Compound Spectra

Figure 4: Result reports can be accessed via the web or sent by e-mail.

Summary and Conclusion

The Toxtyper workflow is based on the latest generation of the reliable amaZon speed ion trap instruments. It is a spectral library approach for reliable identification and confirmation of toxicologically relevant compounds using MS, MS^2 and MS^3 spectral information in combination with UHPLC retention times.

This comprehensive and ultra-fast screening solution is available as a push-button solution with powerful algorithm and visualization tools. Fast and automated informative reporting of the screening results is provided, and the easy-to-use interface enables operation by MS non-experts.
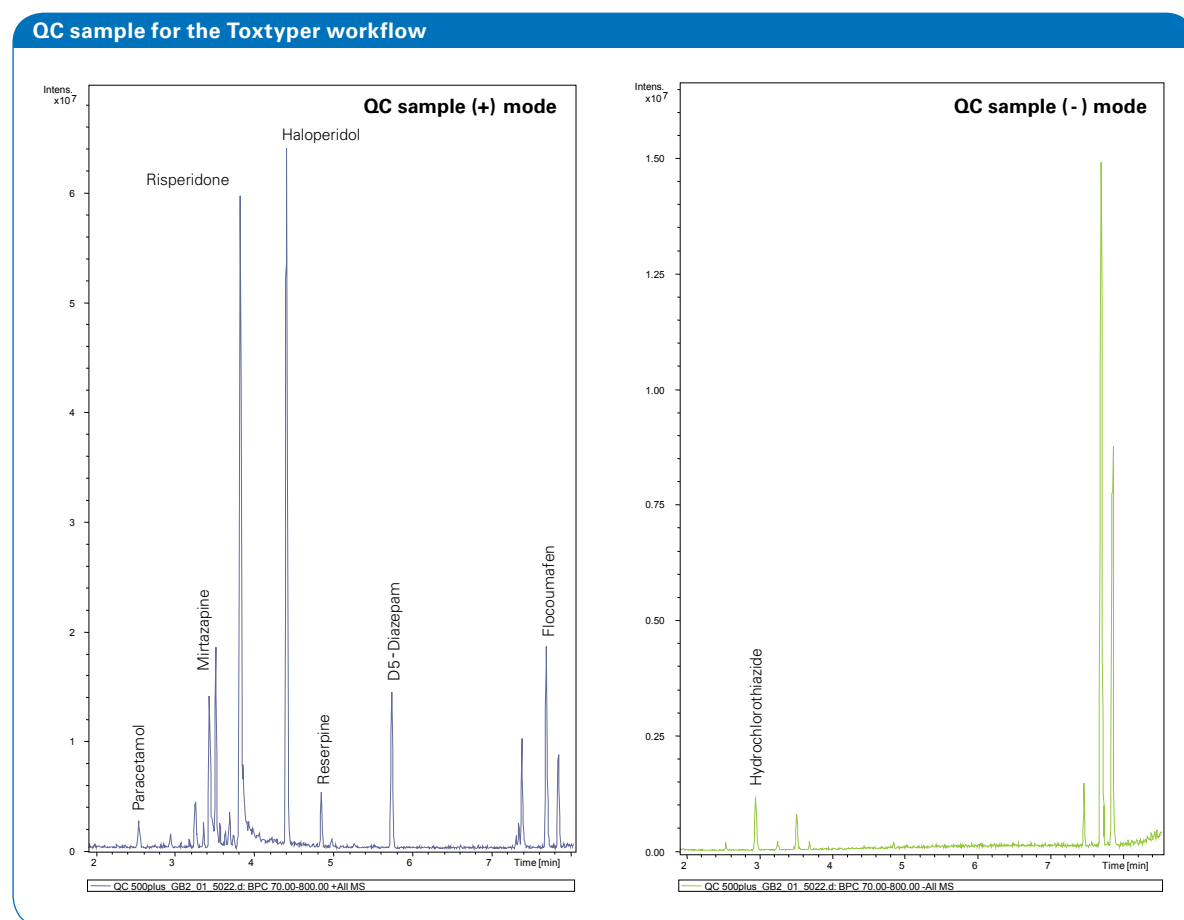
QC sample for the Toxtyper workflow

Figure 5: Base peak chromatogram of the Toxtyper QC sample. Data were acquired in positive and negative ionization mode.

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