Application Note: 536

Targeted Screening of Drugs of Abuse and Toxic Compounds with LC-MS/MS Using Triple Stage Quadrupole Technology

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Key Words

- TSQ Quantum Access MAX
- TraceFinder Software
- QED
- Forensic Toxicology

Introduction

Screening of biological samples for drugs of abuse and other toxic compounds is one of the main issues in forensic toxicology. The challenge is to provide rapid and accurate results despite the large number of targeted molecules and the complexity of biological matrices.

Here we present the workflow and results obtained by using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) timed selected reaction monitoring (T-SRM) method utilizing a triple stage quadrupole mass spectrometer. In a T-SRM experiment, the method is set to look for specific transitions only during the expected retention-time window. This increases the number of SRM transitions that can be monitored in a single experiment. It also increases the dwell time and duty cycle for monitoring individual compounds per experiment. Then, quantitation-enhanced data dependent (QED) MS/MS scan functions are used to trigger data dependent full scan MS/MS spectra from SRM transitions. When a particular SRM transition reaches a predefined intensity threshold, the instrument automatically triggers QED-MS/MS, using the reverse energy ramp (RER) scan function to increase the product ion sensitivity (Figure 1). Dynamic exclusion settings allow the maximum number of MS/MS collected for each compound to be specified, thus giving the ability to collect MS^2 spectra of coeluting molecules.

Goal

To evaluate a triple stage quadrupole mass spectrometer for targeted screening in human urine utilizing a LC-QED-MS/MS method for forensic toxicology laboratories. This screening technique is asked to be fast and reliable enabling high throughput screening.

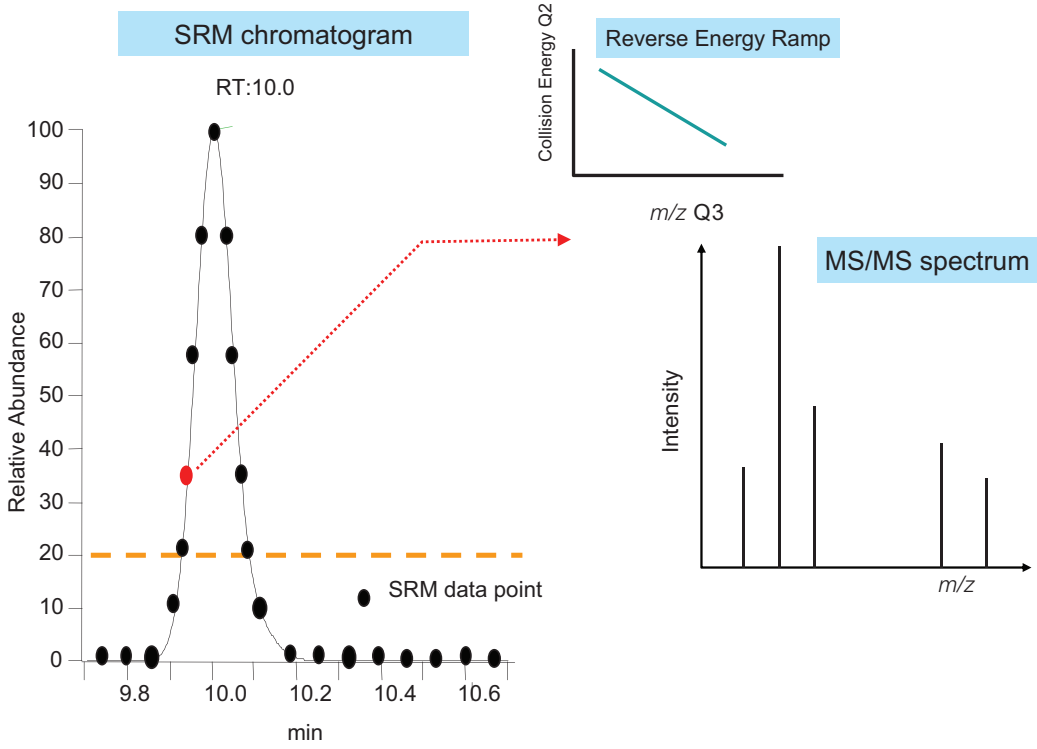

Figure 1: QED detection mode: when a monitored SRM transition reaches a targeted threshold, a full MS^2 spectrum is acquired using a Reverse Energy Ramp scan.

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Experimental Conditions

Sample Preparation

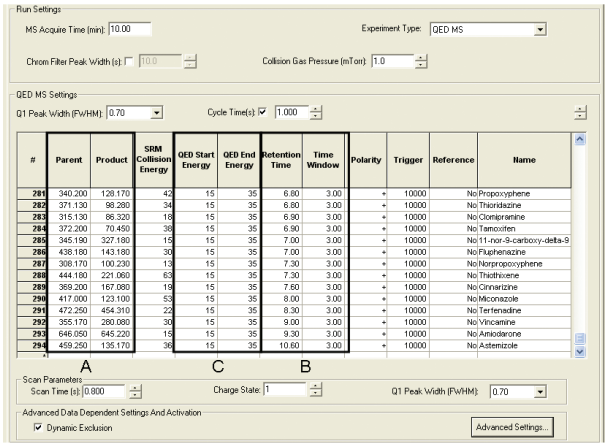
Urine was stored at -20 °C; for the analysis. After thawing, the urine was diluted 10 times with water. For the analysis, 10 µL of urine was directly injected into the LC-MS/MS.

Chromatography and Mass Spectrometry

A Thermo Scientific Hypersil GOLD PFP analytical column (50 x 2.1 mm, 5 µm) was used for separation of the compounds. A 15-minute gradient was set up using 10 mM ammonium formate and 0.1% formic acid in water for the mobile phase A and acetonitrile containing 0.1% formic acid for the mobile phase B.

The mass spectrometer was a Thermo Scientific TSQ Quantum Access MAX triple stage quadrupole with an Ion Max ion source. The instrument acquired SRM (Figure 2A) transitions of 294 compounds (drugs, toxic compounds, and metabolites) using T-SRM (Figure 2B). When an SRM transition reached 10,000 counts, QED detection was activated to collect full MS/MS spectra applying a ramp of collision energy from 15 to 35 eV (Figure 2C).

Data generated were processed with Thermo Scientific TraceFinder software for automated target screening. TraceFinder™ software can identify compounds based on their respective retention time, SRM transition, and full MS/MS spectra. The library contains 294 spectra of toxic and illicit compounds, and the corresponding SRM transitions are reported in the method.



#	Parent	Product	SRM Collision Energy	QED Start Energy	QED End Energy	Retention Time	Time Window	Polarity	Trigger	Reference	Name
281	340.200	128.170	42	15	35	6.80	3.00	+	10000	No	Propoxyphene
282	371.130	98.280	34	15	35	6.80	3.00	+	10000	No	Thioridazine
283	315.130	86.320	18	15	35	6.90	3.00	+	10000	No	Clomipramine
284	372.200	70.450	36	15	35	6.90	3.00	+	10000	No	Temoxifen
285	345.190	327.180	15	15	35	7.00	3.00	+	10000	No	11-nor-9-carboxy-delta-9
286	438.180	143.180	30	15	35	7.00	3.00	+	10000	No	Fluphenazine
287	308.170	100.230	13	15	35	7.30	3.00	+	10000	No	Norpropoxyphene
288	444.180	221.060	63	15	35	7.30	3.00	+	10000	No	Thiothixene
289	369.200	167.080	19	15	35	7.60	3.00	+	10000	No	Cinnarizine
290	417.000	123.100	53	15	35	8.00	3.00	+	10000	No	Miconazole
291	472.250	454.310	22	15	35	8.30	3.00	+	10000	No	Terfenadine
292	355.170	280.080	30	15	35	9.00	3.00	+	10000	No	Vincamine
293	646.050	645.220	15	15	35	9.30	3.00	+	10000	No	Amiodarone
294	459.250	136.170	36	15	35	10.60	3.00	+	10000	No	Astemizole

Figure 2: Method parameters used for LC-MS/MS screening of 294 compounds
Panel A: SRM transitions monitored
Panel B: Time segment used for Timed SRM
Panel C: When QED is activated an energy ramp from 15 to 35 eV is applied

Results and Discussion

The analysis time was 15 minutes. Figure 3A shows an example of an ion chromatogram of one of the monitored SRMs. Using QED-RER, the corresponding full MS^2 was recorded also (Figure 3B).

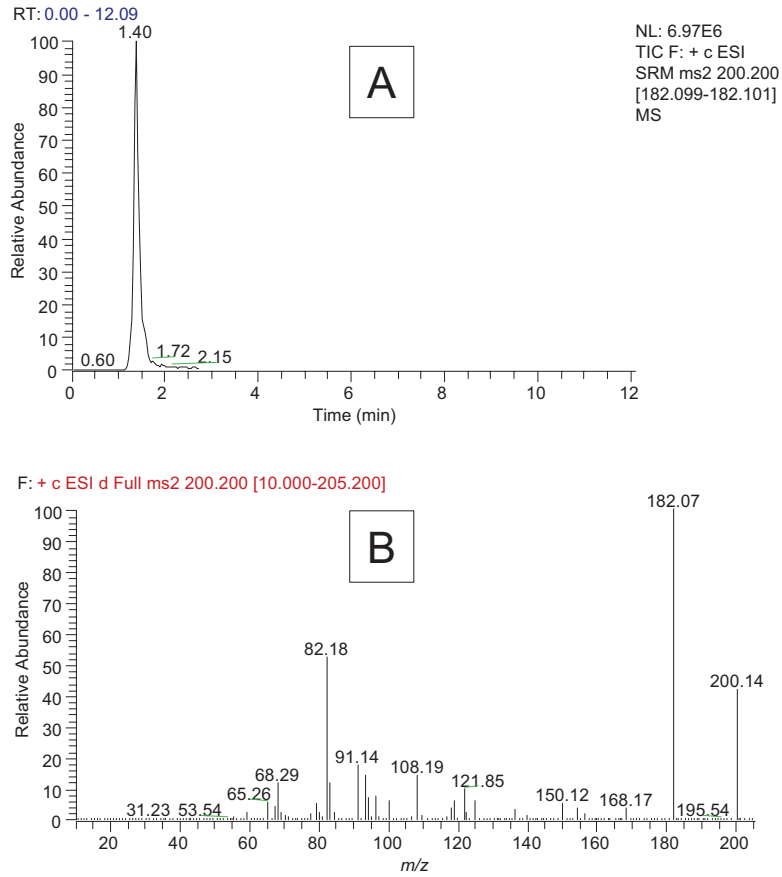


Figure 3: Example of ion chromatogram of transition 200 → 182 (A) and corresponding full MS^2 spectra collected (B)

Analyses were then processed with TraceFinder software using the Target Screening option (Figure 4), which allows the identification of target compounds present in the sample. Data obtained are highly specific and reliable because the identification of compounds is based on three parameters: retention time of the molecule, SRM transition, and MS/MS spectra.

Figure 5 shows an example of a summary report generated by TraceFinder software after the analysis of a urine sample that tested positive for cocaine. In addition to cocaine, *in vivo* metabolites such as benzoylecgonine, ecgonine methyl ester, and cocaethylene were also identified. The same sample was found positive for methadone – its metabolite, EDDP, was also identified.

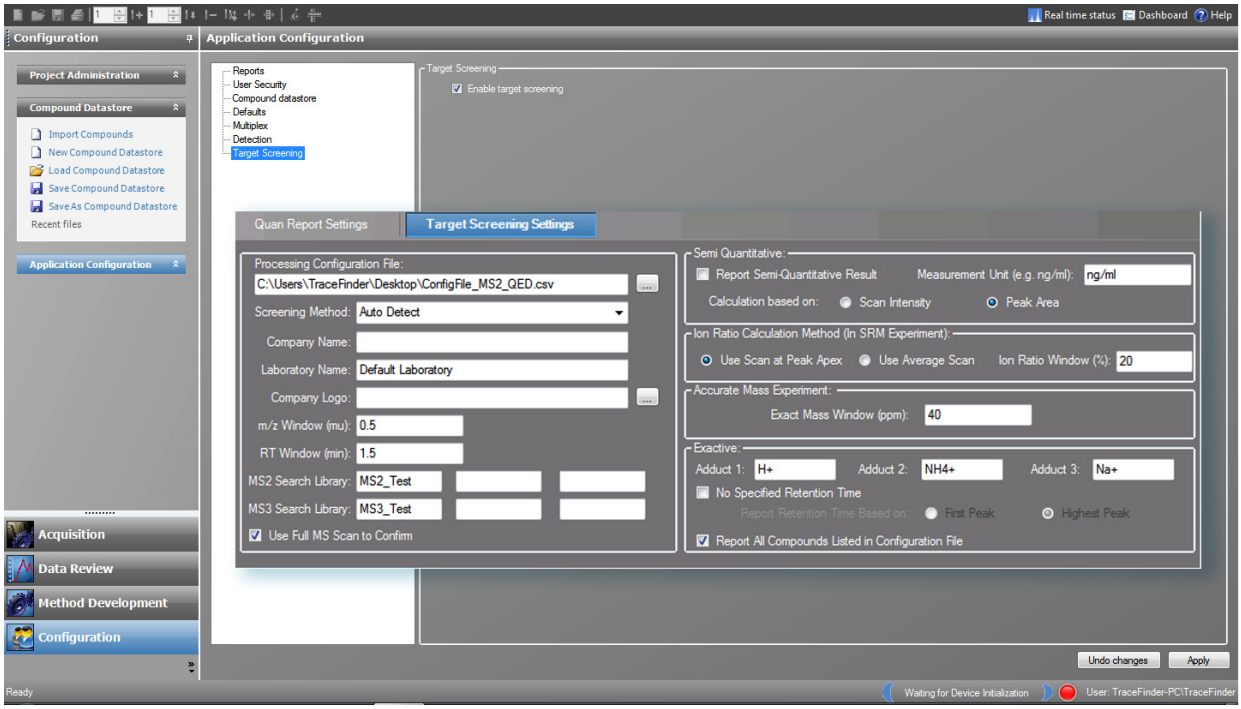
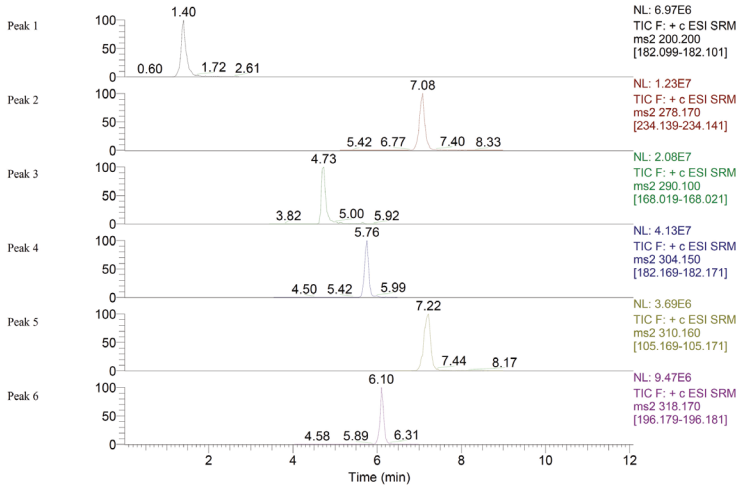


Figure 4. Selection of the Target Screening option in the configuration panel of TraceFinder software and settings used



Peak Number	Compound Name	Code	SI	RSI	m/z	Expected RT	Actual RT	Intensity	Library Name
1	Ecgonine-Methyl-Ester	m	758	784	200.200	1.20	1.40	6974863	MSSEARCH_08
2	EDDP	p	767	774	278.167	6.60	7.08	12298266	MSSEARCH_08
3	Benzoylecgonine	p	827	832	290.200	3.60	4.73	20833310	MSSEARCH_08
4	Cocaine	p	799	817	304.145	5.00	5.76	41325020	MSSEARCH_08
5	Methadone	p	770	777	310.163	6.70	7.22	3686206	MSSEARCH_08
6	Cocaethylene	p	742	758	318.166	5.50	6.10	9467052	MSSEARCH_08

Figure 5: TraceFinder Target Screening Short Report showing ion chromatograms and a list of compounds detected in urine positive for cocaine and methadone

Figure 6 shows an extract of the long report generated by TraceFinder software, showing the comparison between experimental spectra and library spectra for each compound. All of the spectra showed a high matching score confirming the presence of cocaine, methadone, and their metabolites in the urine sample.

Conclusion

The TSQ Quantum Access MAX™ with T-SRM and QED-RER acquisition mode was used to screen toxic compounds and their metabolites in urine. This screening approach provides rapid sample preparation, ease-of-use, sensitivity, specificity, and a low cost per sample analysis for forensic toxicology laboratories.

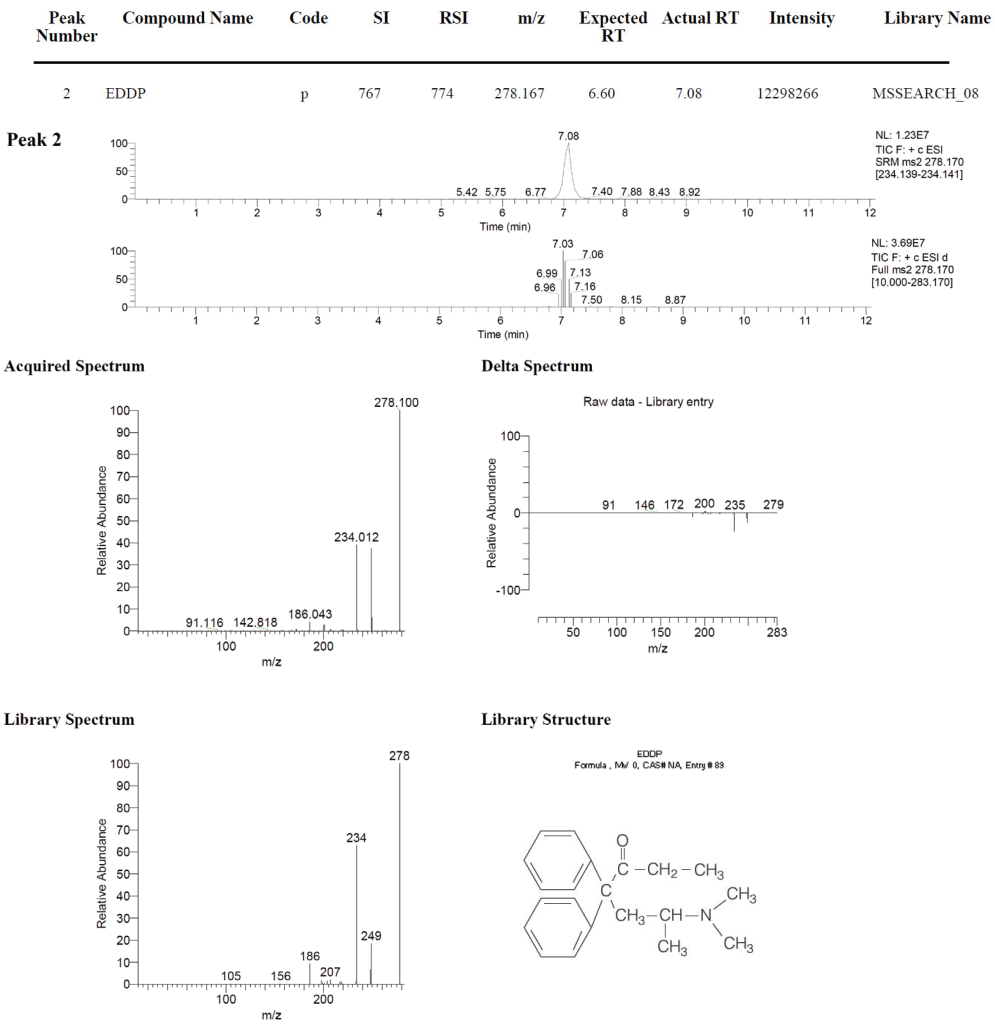


Figure 6. Extract of a TraceFinder Target Screening Long Report showing ion chromatograms and MS/MS spectra of EDDP detected in urine

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