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In 10 jaar van HPLC naar LC-MS/MS met een snelle toxscreen op een Thermo triple quadrupole

Annelies Egas
UMC Utrecht

Ontwikkeling lab apotheek

2006: eerste Triple Quadrupole LC-MS
(TSQ Quantum Access)

2018: 4 TSQ Quantum Access
1 TSQ Quantiva
1 TSQ Altis
1 QExactive-Orbitrap

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Ontwikkeling lab apotheek

Multicomponent methodes:

- Anti-epileptica (16)
- Cardiaca (16)
- Antiviralen (14)
- Psychofarmaca (9)
- TCA (7)
- Benzodiazepines (7)
- Neuroleptics (6)
- Antimycotica (6)
- Immunosuppressiva (4)
- Antibiotica (4)

Screening methodes:

- Laxantia/diuretica (18)
- Orale antidiabetica (8)
- Antihypertensiva (50)
- TOX (283)

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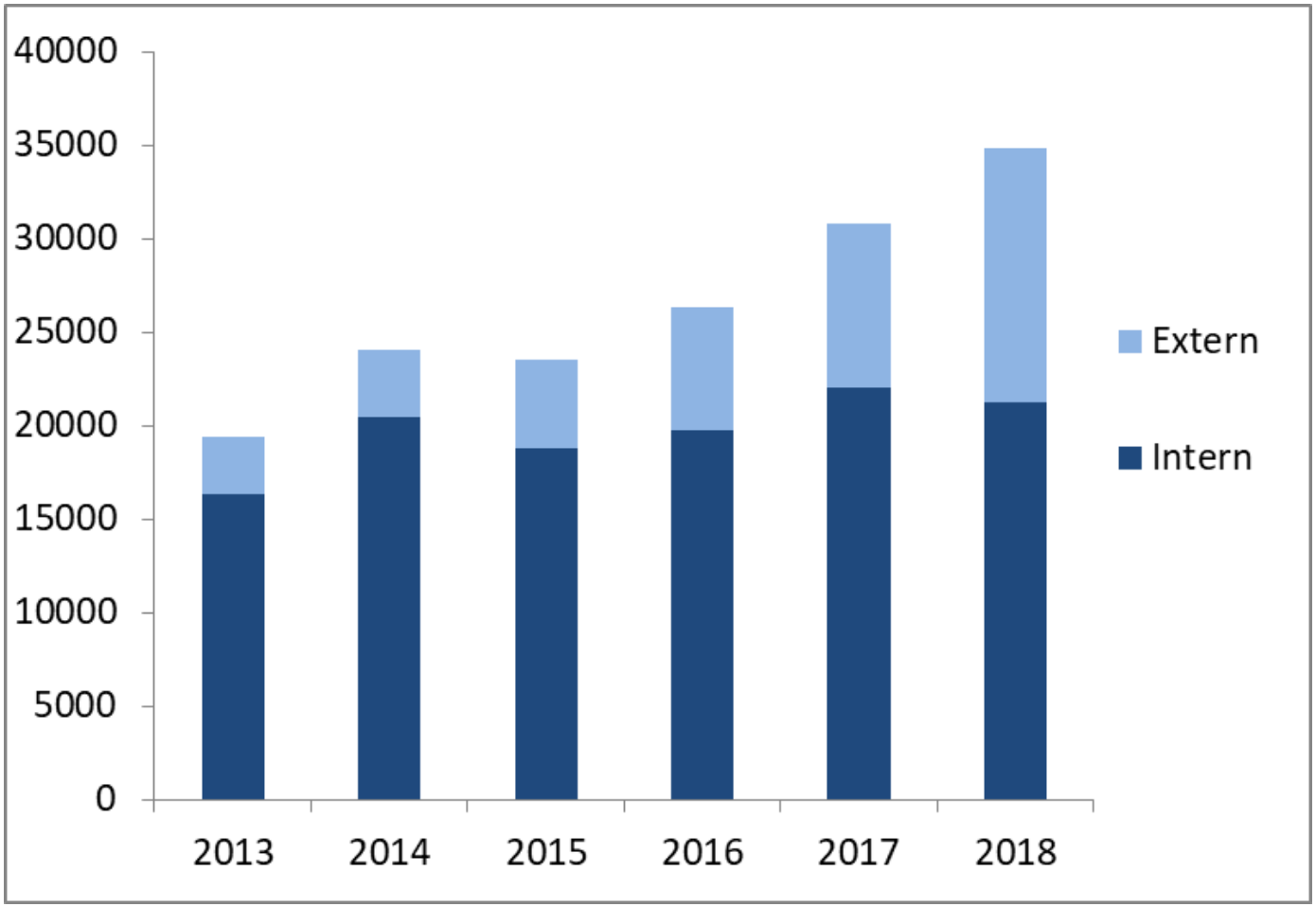
Ontwikkeling lab apotheek



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Toxscreen methode

Doel:

Opzetten van een snelle en betrouwbare methode op een TSQ Quantum Access

Nadelen STIP:

- Hoge detectie limiet
- Tijdrovende monstervoorbewerking (zuur/base LLE)
- Veel monstervolume nodig
- Lange runtijd (2 x 30 min)

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Thermo Application Note 536

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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For Forensic Toxicology Use Only.

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.

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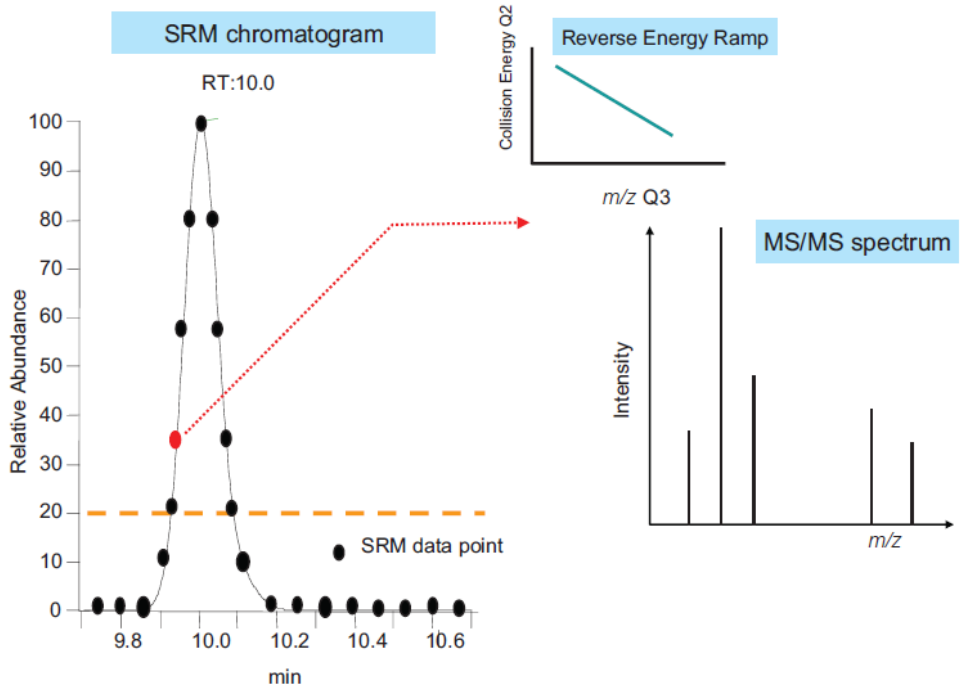
Principe t-SRM QED

- Timed selected reaction monitoring (T-SRM)
- Quantitation enhanced data dependent (QED-MS/MS)
- Reverse energy ramp (RER)



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Methode ontwikkeling

Component	Concentratie (mg/L)	Log P	Eiwitbinding (%)	Zuur / Base / Neutraal
Metformine	5	-2.6	-	Base
Allopurinol	10	-0.55	17	Base
Caffeine	50	-0.07	35	Base
Paracetamol	1	0.5	8-40 (>60 mg/L)	Neutraal
Fenobarbital	50	1.5	50	Zuur
Clobazam	0.5	2.12	85	Base
Fenytoine	25	2.5	90	Zuur
Diclofenac	0.5	4.5	>99	Zuur
Clomipramine	0.3	5.2	90-95	Base
Amiodarone	5	7.57	>96	Base

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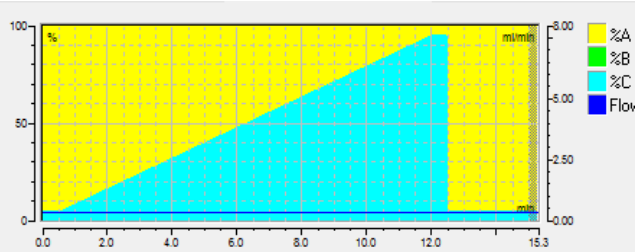
LC methode

Kolom	Hypersil GOLD™ aQ C18 Polar Endcapped (150 x 2.1mm, 1.9 µm)
Kolomtemp	50°C
Eluens	A: 10mM ammoniumformaat in 0.1% mierenzuur C: 0.1% mierenzuur in acetonitril
Injectievolume	10 µl
Runtime	15 min



	Retention [min]	Flow [ml/min]	%B	%C	Curve
1	0.000	0.300	0.0	5.0	
2	0.000	0.300	0.0	5.0	
3	0.500	0.300	0.0	5.0	
4	12.000	0.300	0.0	95.0	
5	12.480	0.300	0.0	95.0	
6	12.500	0.300	0.0	5.0	
7	15.000	0.300	0.0	5.0	

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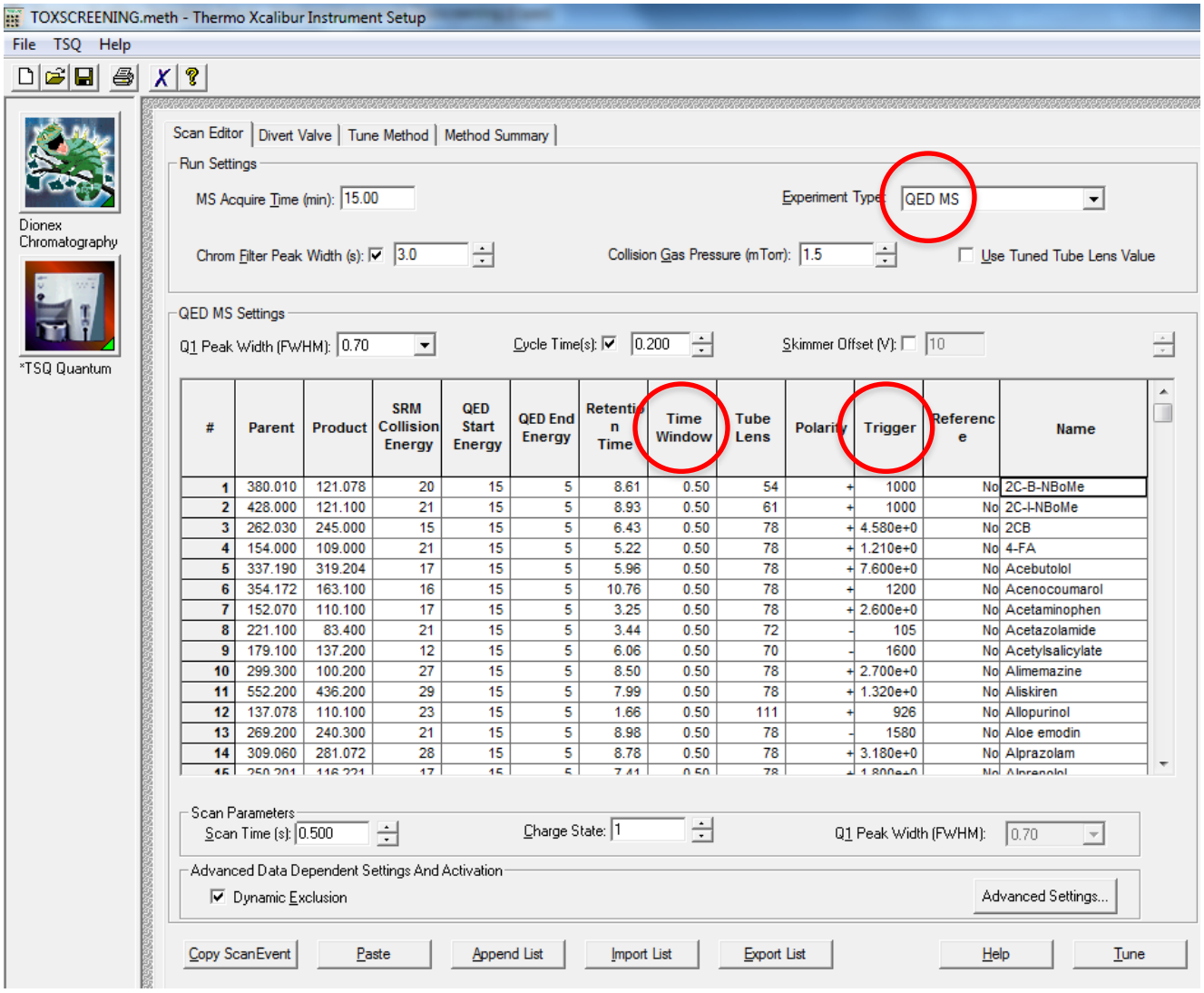
MS methode



#	Parent	Product	SRM Collision Energy	QED Start Energy	QED End Energy	Retention Time	Time Window	Tube Lens	Polarity	Trigger	Reference	Name
1	380.010	121.078	20	15	5	8.61	0.50	54	+	1000	No	2C-B-NBoMe
2	428.000	121.100	21	15	5	8.93	0.50	61	+	1000	No	2C-I-NBoMe
3	262.030	245.000	15	15	5	6.43	0.50	78	+	4.580e+0	No	2CB
4	154.000	109.000	21	15	5	5.22	0.50	78	+	1.210e+0	No	4-FA
5	337.190	319.204	17	15	5	5.96	0.50	78	+	7.600e+0	No	Acebutolol
6	354.172	163.100	16	15	5	10.76	0.50	78	+	1200	No	Acenocoumarol
7	152.070	110.100	17	15	5	3.25	0.50	78	+	2.600e+0	No	Acetaminophen
8	221.100	83.400	21	15	5	3.44	0.50	72	-	105	No	Acetazolamide
9	179.100	137.200	12	15	5	6.06	0.50	70	-	1600	No	Acetylsalicylate
10	299.300	100.200	27	15	5	8.50	0.50	78	+	2.700e+0	No	Alimemazine
11	552.200	436.200	29	15	5	7.99	0.50	78	+	1.320e+0	No	Aliskiren
12	137.078	110.100	23	15	5	1.66	0.50	111	+	926	No	Allopurinol
13	269.200	240.300	21	15	5	8.98	0.50	78	-	1580	No	Aloe emodin
14	309.060	281.072	28	15	5	8.78	0.50	78	+	3.180e+0	No	Alprazolam
15	250.201	116.221	17	15	5	7.41	0.50	78	+	1.800e+0	No	Alprenolol

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Toevoegen component

Stap 1 Selecteren van m/z parent ion → m/z product ion + collision energie (CE)

Stap 2 Toevoegen aan MS methode

Stap 3 Bepalen van retentietijd en intensiteit bij hoog therapeutische concentratie

Stap 4 Toevoegen van volledig MS2 spectrum aan bibliotheek

Stap 5 Optimaliseren methode voor retentietijd en trigger

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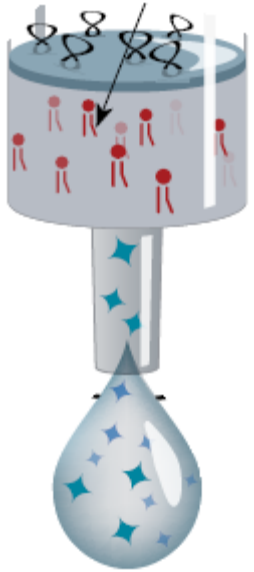
Monstervoorbewerking met Phree Phospholipid Removal Tubes (Phenomenex)

100µl plasma

50µl interne standaard oplossing

300µl 1% mierenzuur in acetonitril



Vortex

Filtratie onder vacuum

Droogdampen onder stikstof

Heroplossen in 100µl 5/95 (v/v) 0.1% mierenzuur in acetonitril/water

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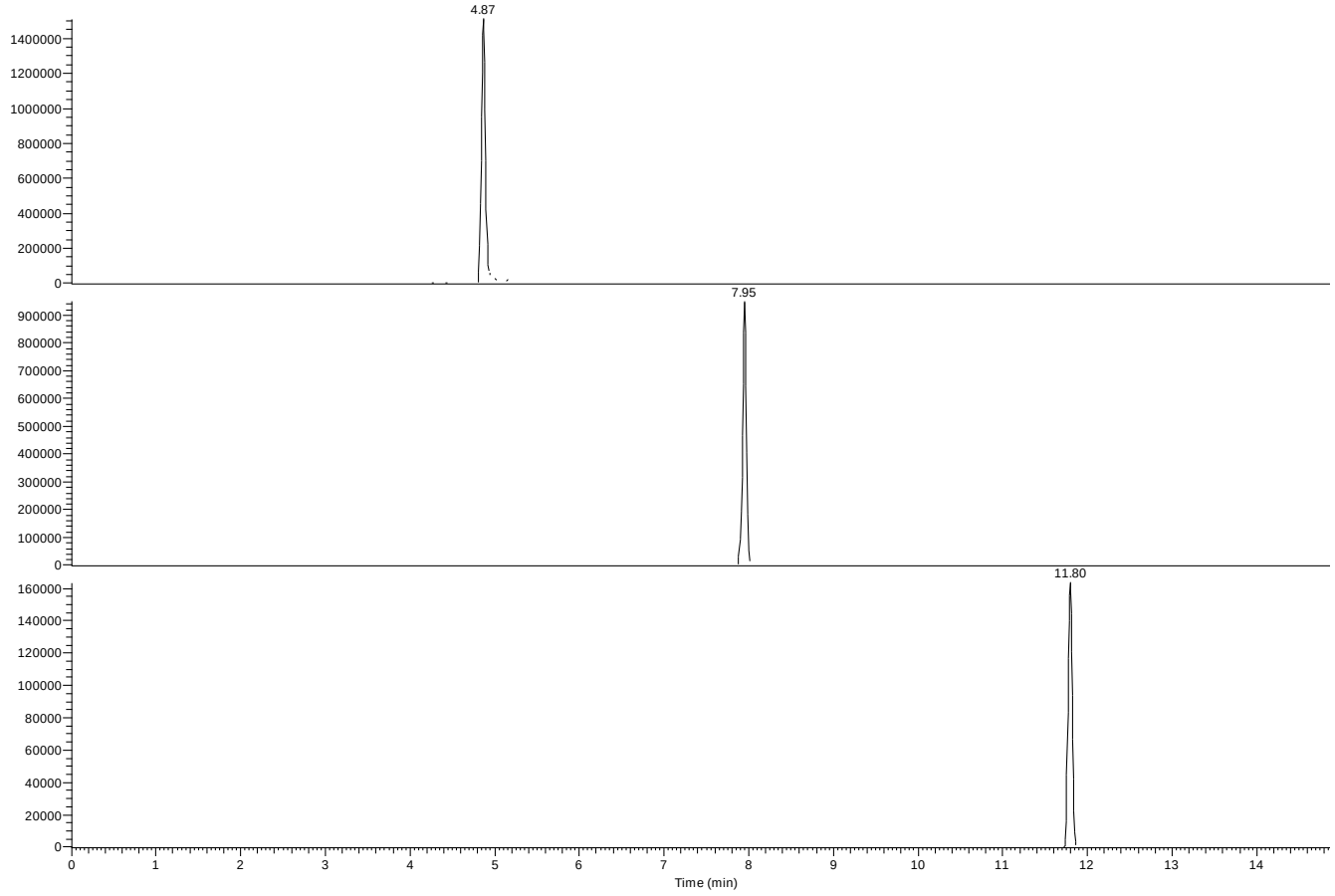
Interne standaard oplossing



Caffeine-$^{13}C_3$

Haloperidol-D_4

Amiodaron-D_4

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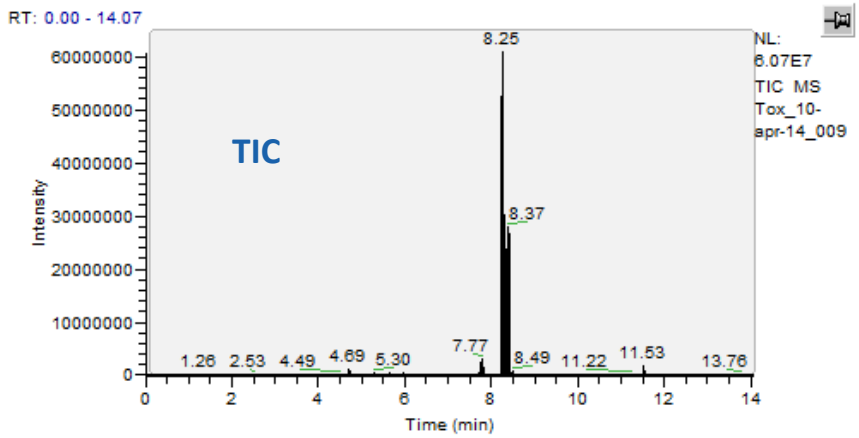
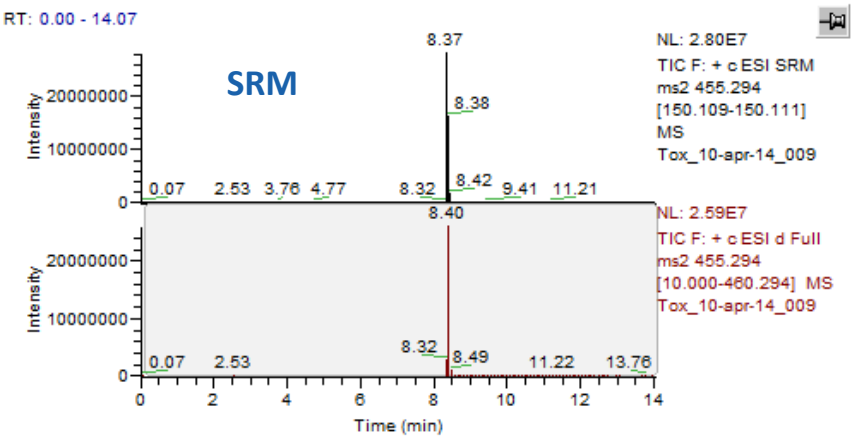
Resultaten





Tox_10-apr-14_009 Start S#: 21997 End S#: 22708
Start Time: 8.15 End Time: 8.32

```
+ c ESI d Full ms2 280.150 [10.000-285.150]
+ c ESI d Full ms2 441.177 [10.000-446.177]
+ c ESI d Full ms2 455.294 [10.000-460.294]
+ c ESI SRM ms2 267.175 [72.435-72.437]
+ c ESI SRM ms2 278.100 [191.068-191.070]
+ c ESI SRM ms2 278.163 [91.295-91.297]
+ c ESI SRM ms2 280.150 [107.220-107.222]
+ c ESI SRM ms2 295.186 [100.279-100.281]
+ c ESI SRM ms2 296.200 [222.769-222.771]
+ c ESI SRM ms2 296.201 [58.099-58.101]
+ c ESI SRM ms2 296.202 [223.099-223.101]
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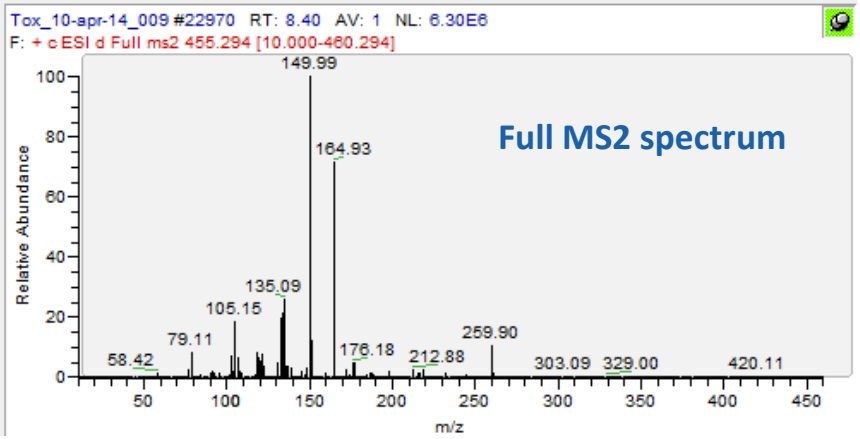

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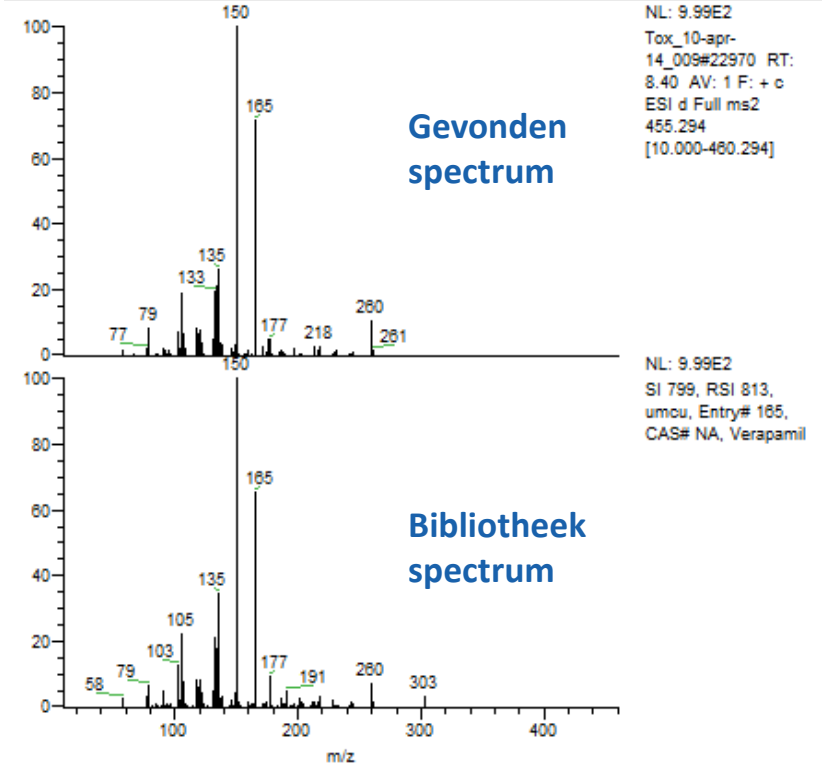
Resultaten

Hit	SI	RSI	Prob	Name	Library Name
1	799	813	98.98	Verapamil	umcu
2	547	609	0.98	Norverapamil	umcu
3	260	265	0.00	Ethosuximide	umcu
4	244	644	0.00	Pioglitazon-d4	umcu
5	243	253	0.00	Codeine	umcu



Hit is gebaseerd op een volledig MS2 spectrum

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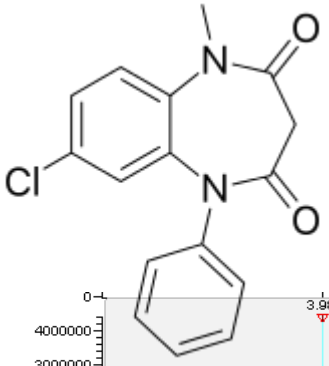
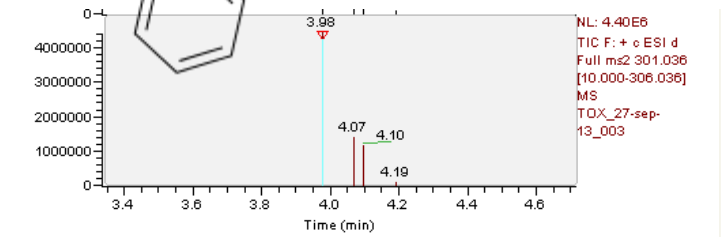
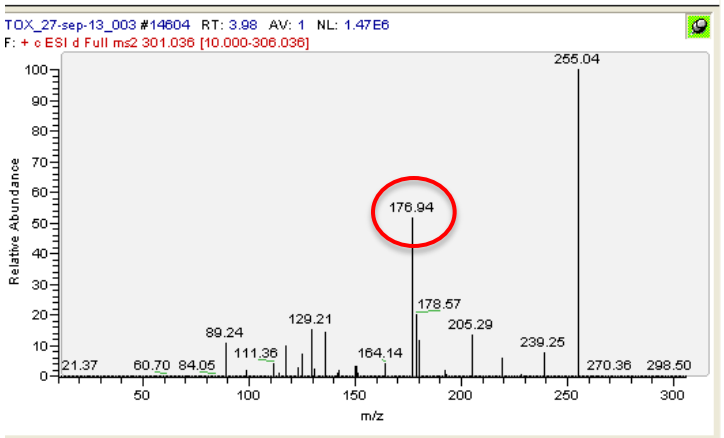
Clobazam

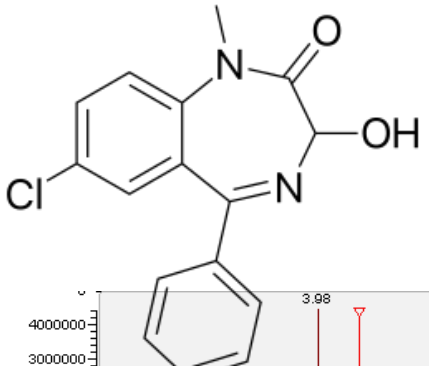


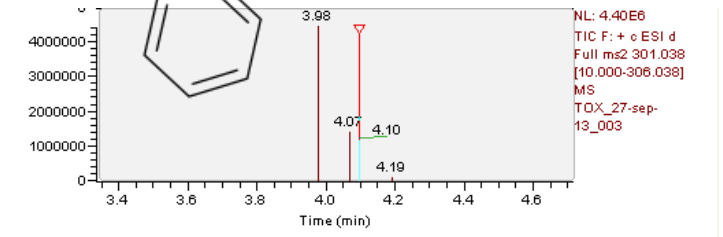
Isomeren
MW = 300,74 g/mol

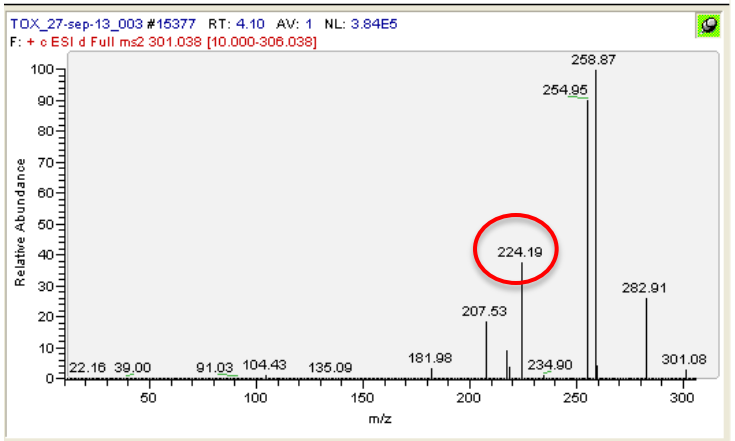




Temazepam







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Rapport TOXID™

Een positieve hit is gebaseerd op retentietijd en volledig MS2 spectrum

De concentratie kan semi-kwantitatief berekend worden m.b.v. de intensiteit en de concentratie van het spectrum in de bibliotheek

Toxicologische Screening
Summary Report

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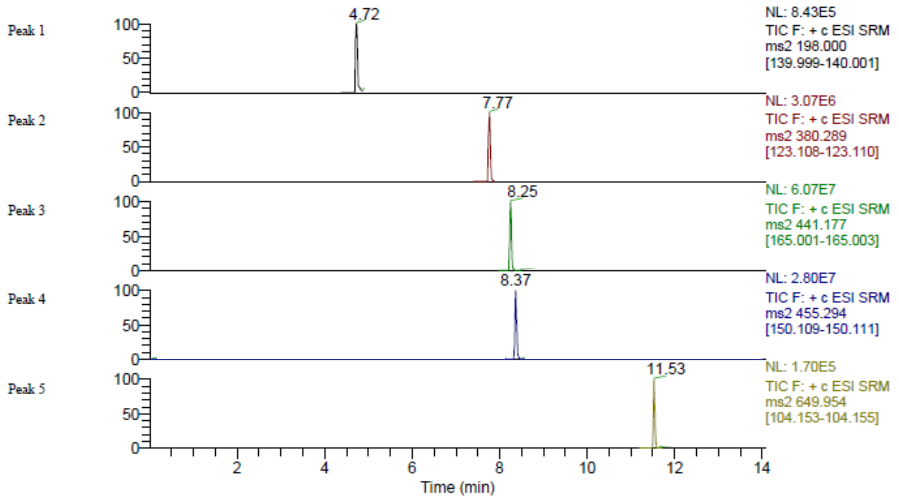
Raw File Name: C:\TSQ Access\Data\2014\Tox_10-apr-14_009.raw
Config File Name: C:\Users\Quantum\Desktop\TOX screening\csv f...\UMCU_MS2_QED_C18 kolom.csv
Sample Name: KKGT TOX 2014.1 Laboratory: Laboratorium Apotheek
Acquistion Start Time: April 10,2014 3:50:29 PM
Screening Conditions: Based on SRM and MS2 scans. m/z window(amu): 0.40, RT window (min): 0.20, MS2 Search libraries: Use full MS scan to confirm.



Peak Number	Compound Name	Code	SI	RSI	m/z	Expected RT	Actual RT	Intensity	Library Name
1	Caffeine-13C3	i	772	817	198.000	4.69	4.72	843410	umcu
2	Haloperidol-d4	i	791	854	380.289	7.64	7.77	3066411	umcu
3	Norverapamil	p	762	774	441.177	8.25	8.25	60707728	umcu
4	Verapamil	p	799	813	455.294	8.37	8.37	28022516	umcu
5	Amiodaron-D4	i	864	886	649.954	11.45	11.53	170308	umcu

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Methode validatie

- STIP vs LC-MS/MS met 10 onbekende monsters
- Sensitiviteit (% positieve hits) en selectiviteit (% vals positieve hits) voor 67 componenten
  - Andere kolom batch
  - Ander TSQ systeem (back-up)

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Validatie resultaten

STIP vs LC-MS/MS:

- 2 positieve hits met STIP
- 7 positieve hits met LC-MS/MS

Oorzaken:

- MS gevoeliger dan DAD
- b.v. baclofen en enalapril elueren onder elutiepiek
- verschil in bibliotheek

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Validatie resultaten

Sensitiviteit en selectiviteit voor 67 componenten

- Sensitiviteit: % positieve hits
- Selectiviteit: % vals positieve hits

	Sensitiviteit (%)	**Selectiviteit (%)**
TSQ Access max A	96	10
TSQ Access max A en andere kolom batch	91	10
TSQ Access max B (back-up)	84	6

- Altijd blanco meenemen

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Conclusie & discussie

Snelle en robuuste methode met 283 componenten

Nadelen:

- Geen screening mogelijk buiten de bibliotheek
- Niet alle componenten zijn detecteerbaar (digoxine, ibuprofen)
- Vergelijken met blanco noodzakelijk

Voordelen:

- Snelle monstervoorbewerking met laag monstervolume
- Korte runtijd
- Lage detectielimiet
- Hoge specificiteit
- Bibliotheek is uitwisselbaar

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Bedankt voor uw aandacht!

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