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Note d'application

Seized Drug Screening using the ACQUITY RDa Detector

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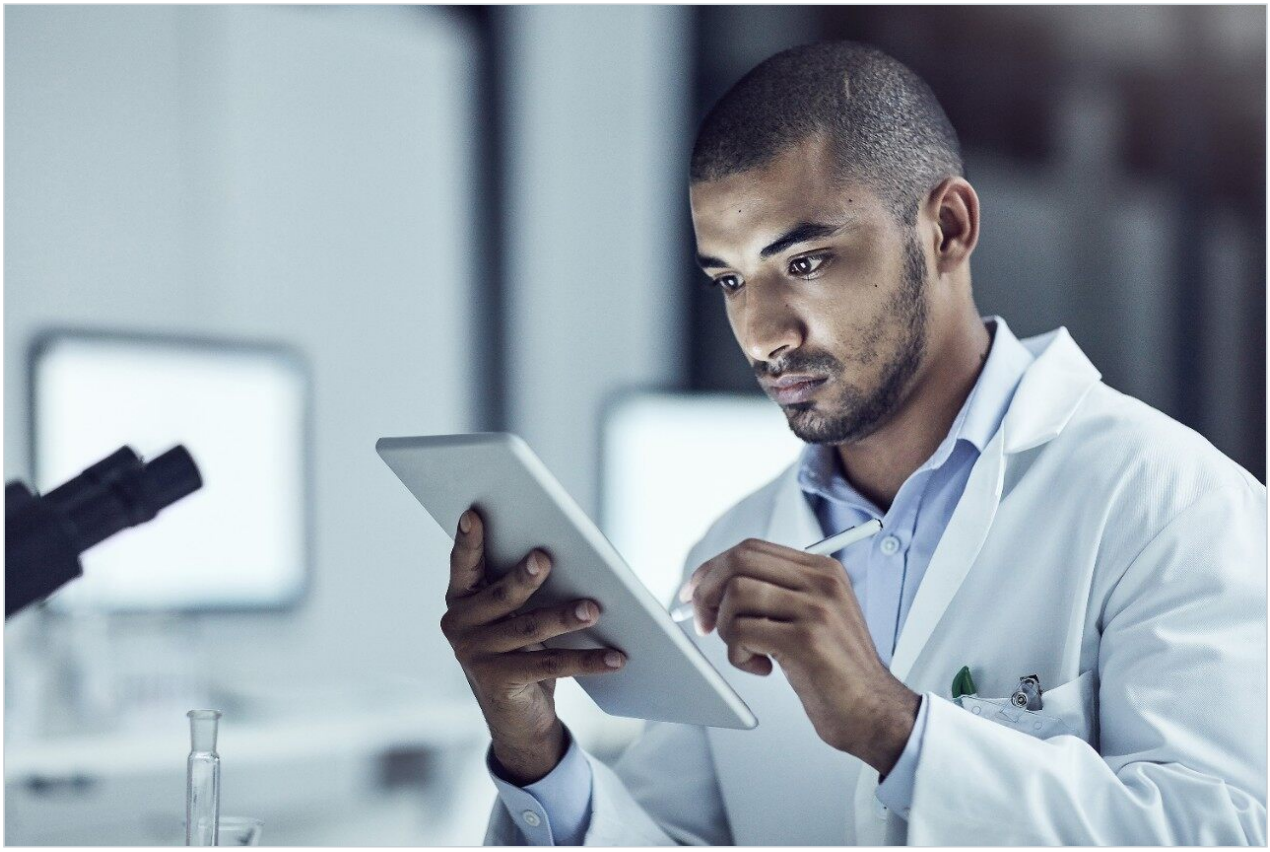

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Abstract

This application note details the use of Waters' new ACQUITY RDa Detector for seized drug screening with a ten minute analysis time utilizing a dissolve, filter, dilute, and shoot sample preparation.

Introduction

Illicit drug use and trafficking continue to be a significant problem for law enforcement agencies worldwide. Seized drug screening is a crucial first step in identifying sample evidence in controlled substance laboratories. Traditionally, seized drug screening techniques include color test and TLC; while GC-MS analysis, among other techniques, is used for confirmation.

Spot color tests and TLC may flag common drugs of abuse that are previously characterized, but may not detect novel psychoactive substances. Seized drug screening utilizing an LC-Tof instrument provides a quick and efficient method to screen a customizable target list of drugs and facilitates identification of unidentified compounds.

Experimental

New Instrumentation

The new ACQUITY RDa Detector, coupled to an ACQUITY UPLC I-Class PLUS FTN System, was used to develop this assay. This is a compact, benchtop Tof mass spectrometer that has a mass resolution of >10,000 FWHM for routine accurate mass detection. The system can acquire both full scan data and full scan data with fragmentation (data independent acquisition). The seized drug screening experiment utilizes the full scan data with fragmentation acquisition mode, which provides confirmatory fragmentation data to facilitate compound identification. The waters_connect Software is an easy-to-use and customizable platform that utilizes accurate mass, retention time, and compound fragmentation data to quickly search a customizable

library of application specific compounds to identify case work samples. Figure 1 shows the schematic of the ACQUITY RDa Detector.

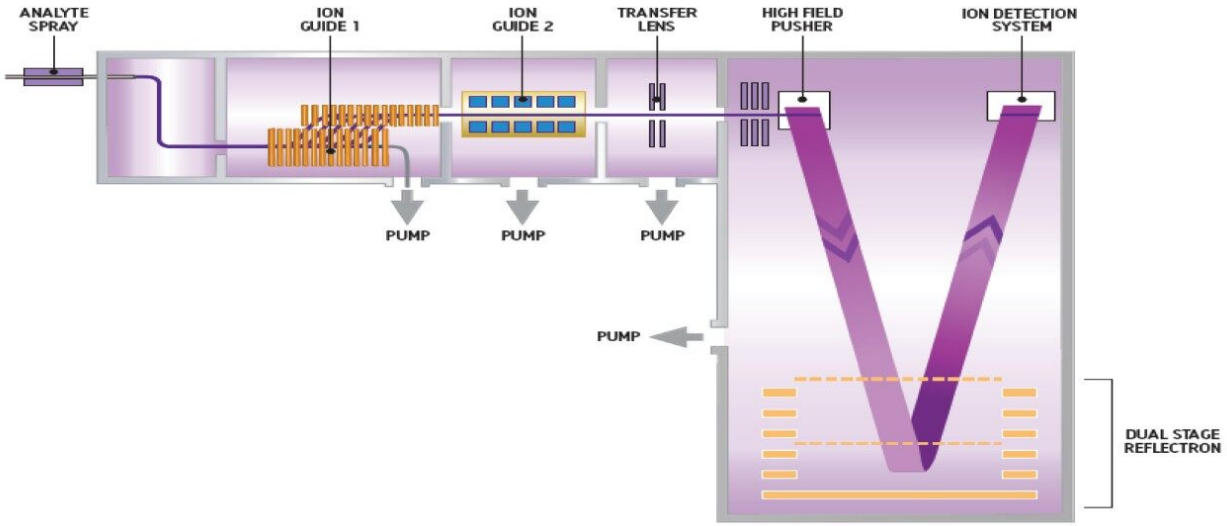

Figure 1. ACQUITY RDa Detector schematic.

Standards were acquired from Cerilliant (Round Rock, TX). Prescription drug samples were obtained from Safeway pharmacy (Livermore, CA). A baggie of presumed street drug(s) was discovered on the streets of Livermore, CA.

A custom target list of 100 compounds was created from an informal survey and was used for the screening experiment. The compounds are listed in Appendix A.

LC Conditions

LC system:	ACQUITY UPLC I-Class PLUS (FTN)
Column:	ACQUITY HSS T3 1.8 µm, 2.1 x 100 mm (P/N 186003539)
Column temp.:	45 °C
Sample temp.:	10 °C

Injection volume:	10 µL
Flow rate:	0.5 mL/min
Mobile phase A:	5 mM NH_4 formate pH 3.0
Mobile phase B:	ACN + 0.1% FA
Purge solvent:	50:50 MeOH:H_2O
Wash solvent:	25:25:25:25 MeOH: H_2O:IPA:ACN + 1% FA

UPLC Gradient Program

Time (min)	Flow (mL/min)	%MPA	%MPB	Curve
0.0	0.5	95	5	Initial
7.0	0.5	2	98	6
8.0	0.5	2	98	6
8.05	0.5	95	5	1
9.5	0.5	95	5	1

MS Conditions

MS system:	ACQUITY RDa Detector
Ionization mode:	ESI positive
Desolvation temp:	550 °C

Acquisition mode:	Full scan with fragmentation
Mass range:	Low (50–2000 *m/z*)
Scan rate:	10 Hz
Cone voltage:	20 V
Fragmentation cone voltage:	70–130 V
Capillary voltage:	0.8 kV
Intelligent data capture:	Off
Dynamic lockmass correction:	On
Acquisition time:	0–8 min

Data management

waters_connect v1.9.12

Sample Preparation

Pills are crushed to a powder and prepared as below:

- Tip of spatula ~1 mg
- Dissolve in 1 mL EtOH
- Filter 1 mL with 0.2 µm Teflon filter (p/n 186009327)
- Dilute filtered stock 100x in 20% MeOH
- Inject 10 µL and results <10 min

Liquids are diluted 100x in 20% MeOH and analyzed.

Results and Discussion

The typical workflow for seized drug screening on the ACQUITY RDa Detector would be as follows:

1. Document sample and prepare as shown above.
2. Place prepared labeled sample vial into autosampler and analyze.
3. Analyze solvent blank between samples to confirm/illustrate no sample cross contamination.

This allows more samples to be prepared and analyzed during a shift while maintaining evidence chain of custody.

As an example, Figure 2 shows a picture of a presumed drug baggie found on the street in California.

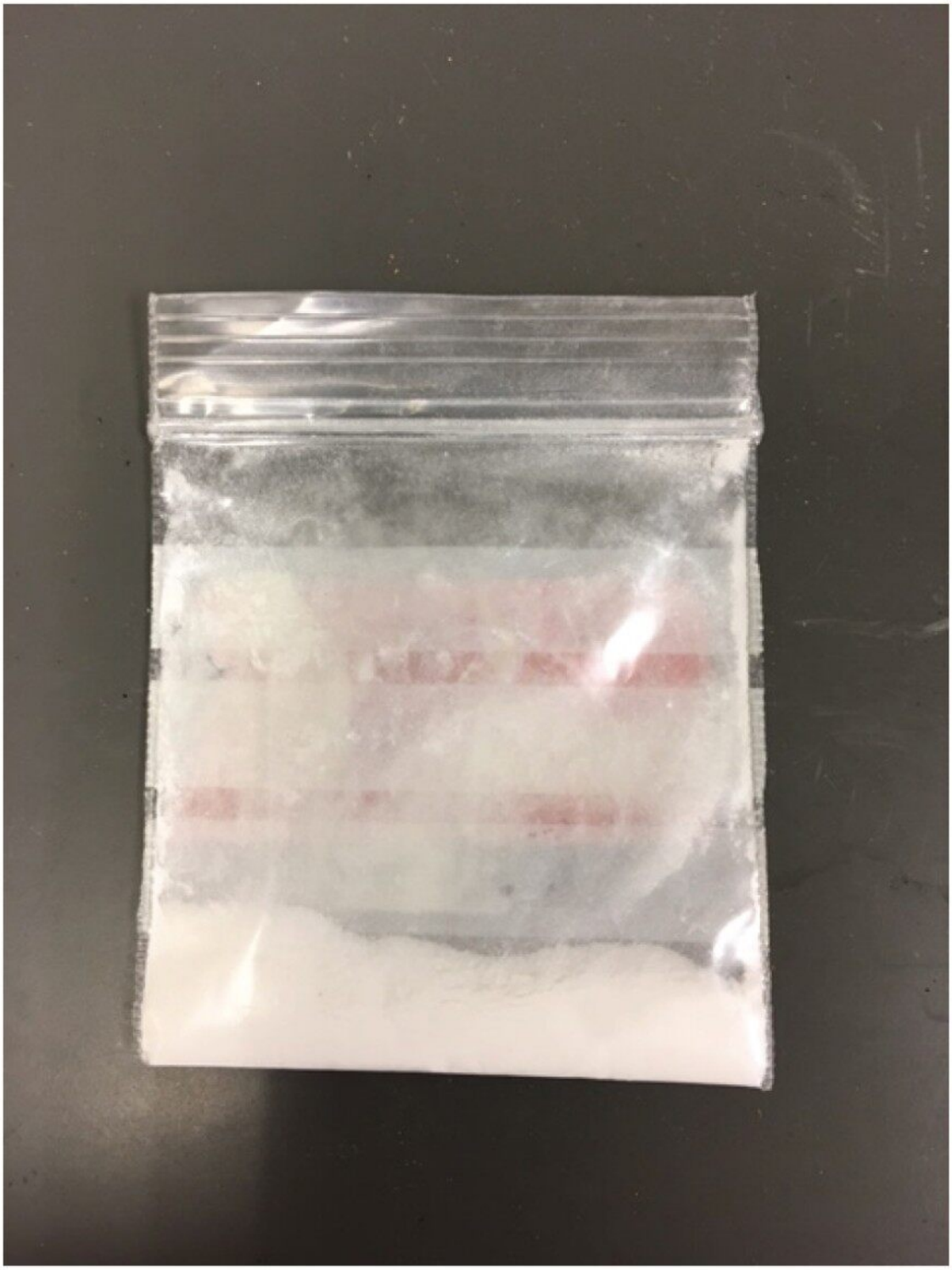

Figure 2. Baggie found on street.

Figure 3 illustrates the results obtained from the screening experiment of the baggie contents on the ACQUITY RDa Detector in a ten-minute analysis.

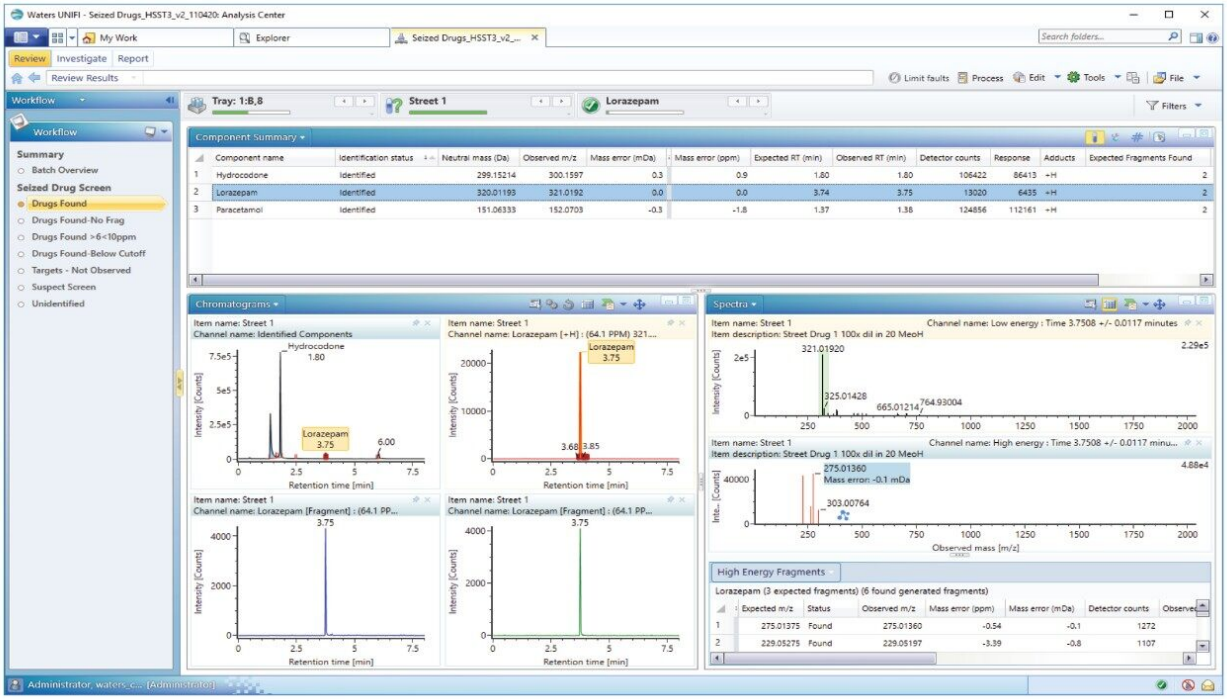

Figure 3. Seized drug screening results from baggie in Figure 2.

The screening experiment performed with the waters_connect Software suite allows one to create and utilize a custom 100-compound drug library which can be updated at any time.

The software deploys the power of accurate mass, retention time and full scan with fragmentation data collection to identify and display sample data in a user customizable format.

This sample screened presumptive positive for hydrocodone, lorazepam, and paracetamol. It can now be transferred to confirmatory testing (GC-MS or LC-MS) for positive identification.

Another advantage to screening seized drug samples with the ACQUITY RDa Detector is the ability to perform retrospective data analysis since a full data set is collected with a single injection. Figure 4 illustrates the analysis of a prescription pill with no drugs from the target list detected.

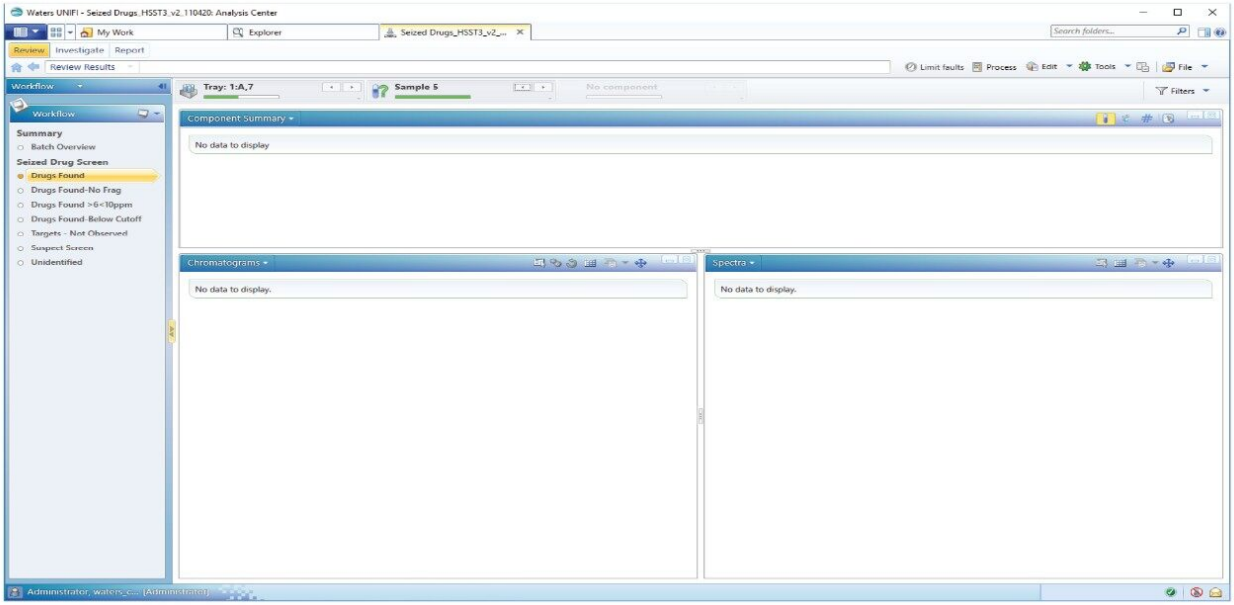

Figure 4. Prescription pill. No drugs detected on first pass screen with 100 compound drug target library.

We can use the Discovery tools built into the waters_connect Software to identify the unknown peak at RT of 2.19 min as shown in Figure 5.

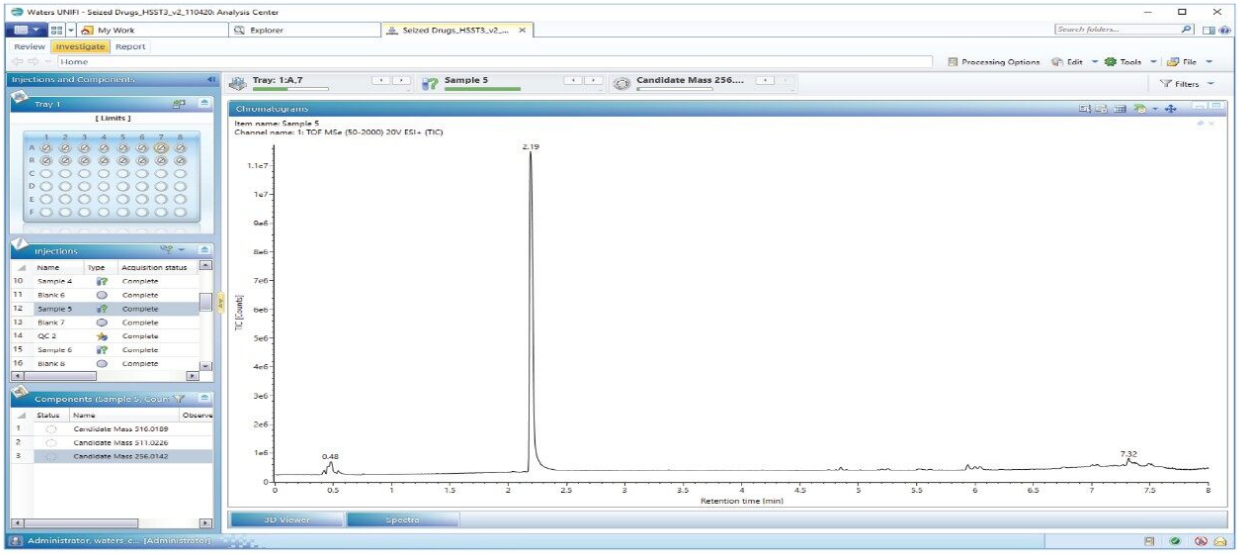

Figure 5. Unknown peak at RT of 2.19 minutes.

The Discovery toolkit will take the accurate mass measured at 2.19 minutes, propose elemental formulae and

submit that information to the selected Chemspider libraries (550 to choose from) to search for possible candidates. The analyst can select a favorite subset of libraries to search, or search all of those available. In this case, nine possible database matches were found. The Discovery tool also performs an *in-silico* bond-breaking experiment to yield theoretical fragment ions for the database match candidates and compares that spectrum to the acquired fragment spectrum. The first candidate found is lamotrigine which matches the spectral composition of the "unknown" pill in sample 5 that was prepared and analyzed in Figure 6.

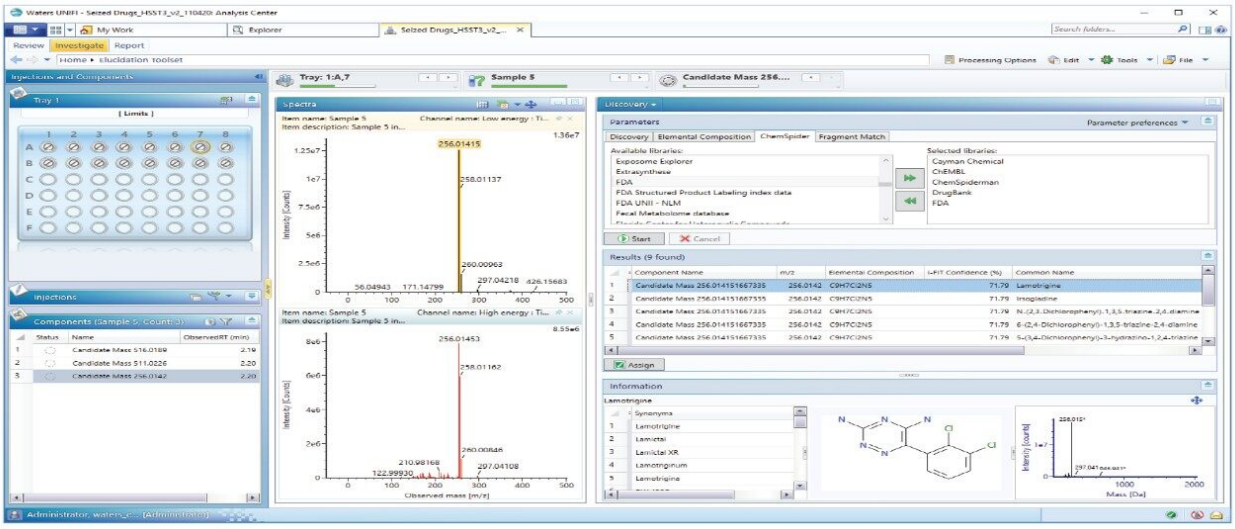

Figure 6. Discovery tool showing "presumptive" identification of "unknown" pill.

This "presumptive" identification was performed with the data from a single injection of the sample and less than five minutes of additional analyst time. The next step would be to purchase the relevant certified reference material and analyze it under the same conditions, for unequivocal confirmation of this tentative data.

Conclusion

The Waters ACQUITY RDa Detector provides a quick and efficient method to perform a comprehensive screen on seized drug samples. The combination of accurate mass, short analysis time, a customizable, and expandable, library, and a full data set acquisition with fragmentation, provides a high confidence result for the drug screening experiment. The ability of the Tof analyzer to acquire a full data set facilitates

retrospective data analysis when a result is not obtained from the target library search. The integrated Discovery tool allows the user to interrogate unidentified peaks and quickly search hundreds of databases for presumptive identifications of the unknown compound.

Accurate mass screening with the ACQUITY RDa Detector provides a superior level of data and confidence in the screening results for seized drug case work that spot color tests and TLC cannot achieve. In addition, since a full data set is acquired with the first injection, retrospective data analysis can be performed if a compound is not identified from the target list library.

Appendix A	
Component name	
1-Benzylpiperazine (BZP)	Guaiphenesin
25B-NBOMe (2C-B-NBOMe)	Heroin
25C-NBOMe (2C-C-NBOMe)	Hydrocodone
25I-NBOMe (2C-I-NBOMe)	Hydromorphone
2C-H	Ketamine
2-Ethylamino-1-(3,4-Methylenedioxyphenyl)propan-1-one (bkMDEA, ethylone)	LAMPA
2-Methylamino-1-(3,4-Methylenedioxyphenyl)butan-1-one (bkMBDB, butylone)	Lidocaine
2-Methylamino-1-(3,4-Methylenedioxyphenyl) propan-1-one (bkMDMA, methylone)	Lisdexamphetamine
3,4-DMMC (3,4 Dimethylmethcathinone)	Lorazepam
4-ANPP/despropionylfentanyl	LSD
4-Ethylmethcathinone (p-EMC)	MDA
4-Methylethcathinone (4-MEC, NRG-2)	MDMA
AB-FUBINACA	Meperidine
AB-PINACA	Methadone
Acetylfentanyl	Methamphetamine
Acrylfentanyl	Methoxyacetylfentanyl
Alfentanil	Midazolam
Alpha-PVP (alpha Pyrrolidinopentiophenone)	Mitragynine
Alprazolam	Morphine
AM-2201	Morphine, 6 Monoacetyl
Amphetamine	MT-45
APINACA (AKB-48	Naloxone
Benzocaine	N-Ethylpentylone
Bufotenine (5-HO-DMT)	Nicotine
Buprenorphine	Noscapine
Butyrfentanyl	Oxazepam
Caffeine	Oxycodone
Carbamazepine	Oxymorphone
Carfentanil	Papaverine
Carisoprodol	Paracetamol
Chlorpheniramine	Pentylone (bkMBDP)
Clonazepam	Phenazepam
Clonazolam	Phencyclidine (PCP)
Cocaine	Phentermine
Codeine	Procaine
Cyclopropyl fentanyl	Pseudoephedrine
Dextromethorphan	Psilocin
Diazepam	Psilocybin
Dibutylone (bk-DMBDB)	Sildenafil
Diclazepam	Sufentanil
Dimethyltryptamine (DMT)	Tadalafil
Ephedrine	Temazepam
Estazolam	Thebaine
Etizolam	Tramadol
Fentanyl	U-47700
Flualprazolam	U-49900
Flubromazepam	Valerylfentanyl
Flubromazolam	W-15
Flurazepam	Xylazine
Gabapentin	Zolpidem