Waters

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Meeting Analytical Challenges with ACQUITY UPLC™ /MS

Pittsburgh Conference 2005
Orlando, FL

For Complete Confidence

Outline

- Laboratories Objectives
- Questions you've heard
- Separation Methods
- Separation Results
- Conclusions

Analytical Objectives

- Improve Data Quality
- Identify peak impurities
- Increase throughput – improve efficiency, productivity
- Enhance qualitative detection and sensitivity
- Improve selectivity and resolution
- Raise laboratory capabilities and skill set

Pharmaceuticals
Waters Solutions

Waters solutions cross the entire pharmaceutical research, development and manufacturing process

Discovery

SCREENING AND CONFIRMATION—Medicinal chemistry, chemical library maintenance, physicochemical property analysis, ADME screening

PURIFICATION—Natural products, medicinal chemistry, metabolite ID, impurity analysis

IDENTIFICATION—Metabolites, impurities, contaminants, degradation products, biomarkers

Development

QUANTIFICATION—Pharmacokinetics, safety assessment, formulation, stability testing, degradation analysis, purity assessment

CHARACTERIZATION—Natural products, safety assessment, chemical and pharmaceutical development

METHOD DEVELOPMENT AND TRANSFER—Sample and method scouting, method development, optimization, transfer, validation

Manufacturing

MONITORING—Reaction processes, raw materials, active pharmaceutical ingredient quality, formulation, final product quality

Process Support

INFORMATION MANAGEMENT—Data acquisition, processing, reporting, maintenance, preservation, accessibility, electronic information management

REGULATORY COMPLIANCE—System qualification, calibration and maintenance, GXPs, software validation, and records management

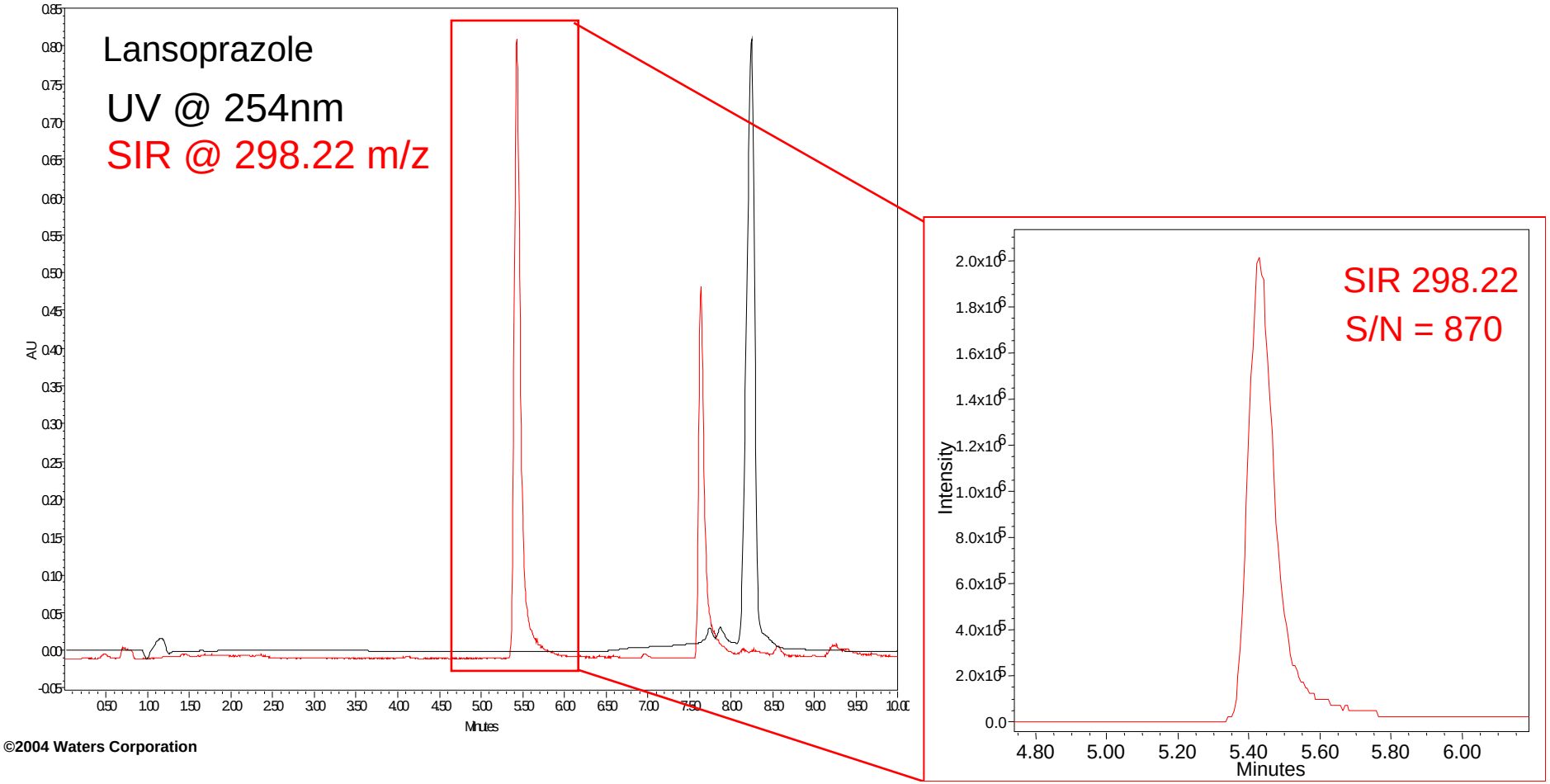
SOLUTIONS

Frequently Asked Questions

- I need to save time, money, but not at the sacrifice of performance, will this new ACQUITY UPLC™ help?
- Should I change one of my ZQ/Alliance systems to be a ZQ/ACQUITY UPLC™ system to improve my throughput, resolution, and sensitivity?
- Can I run my ZQ with an ACQUITY UPLC™ system?
- Do you have any data, you can share?

Enhance Sensitivity And Selectivity With MS Detection

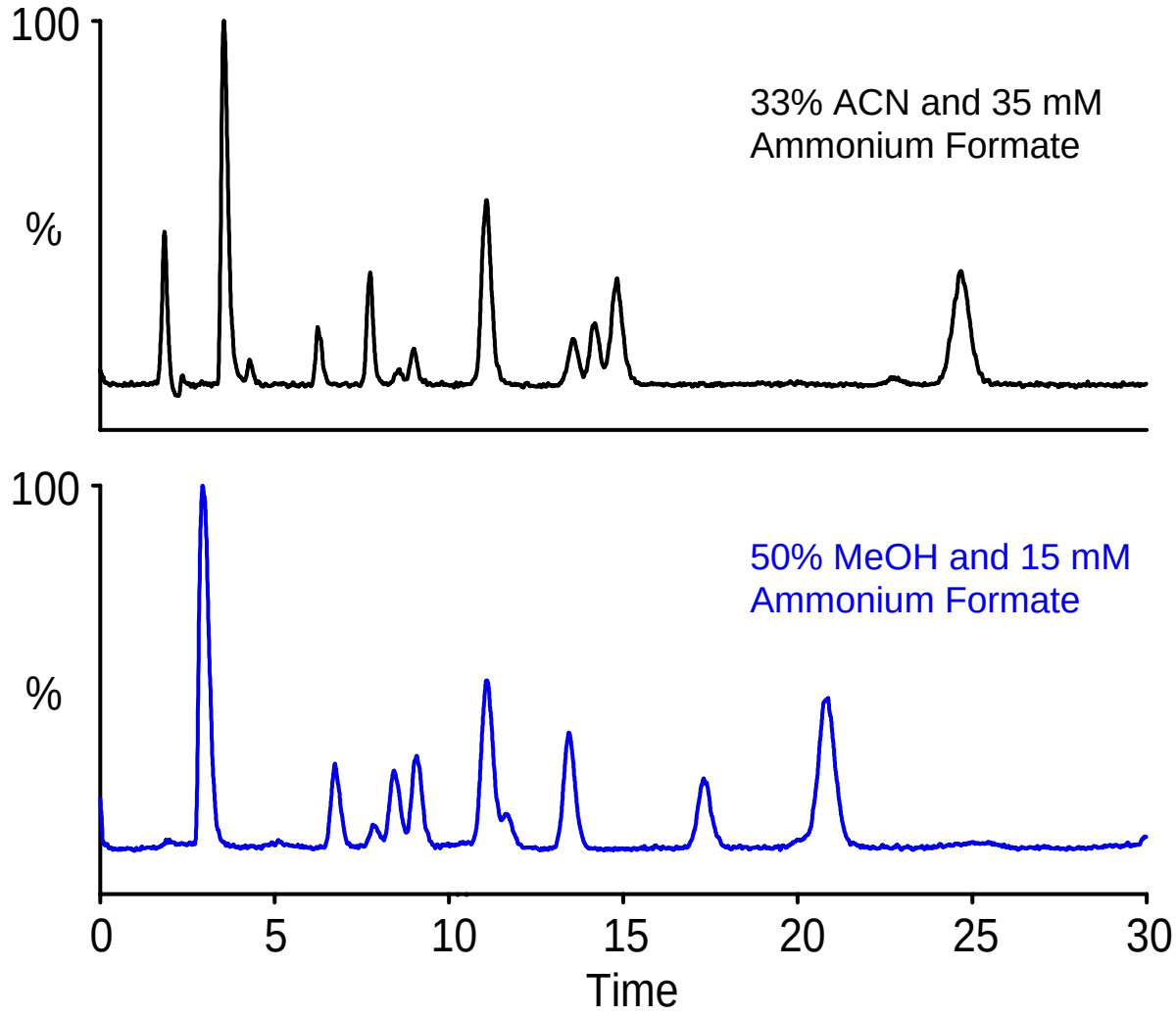
Expansion of region of 0.03% impurity by MS detection



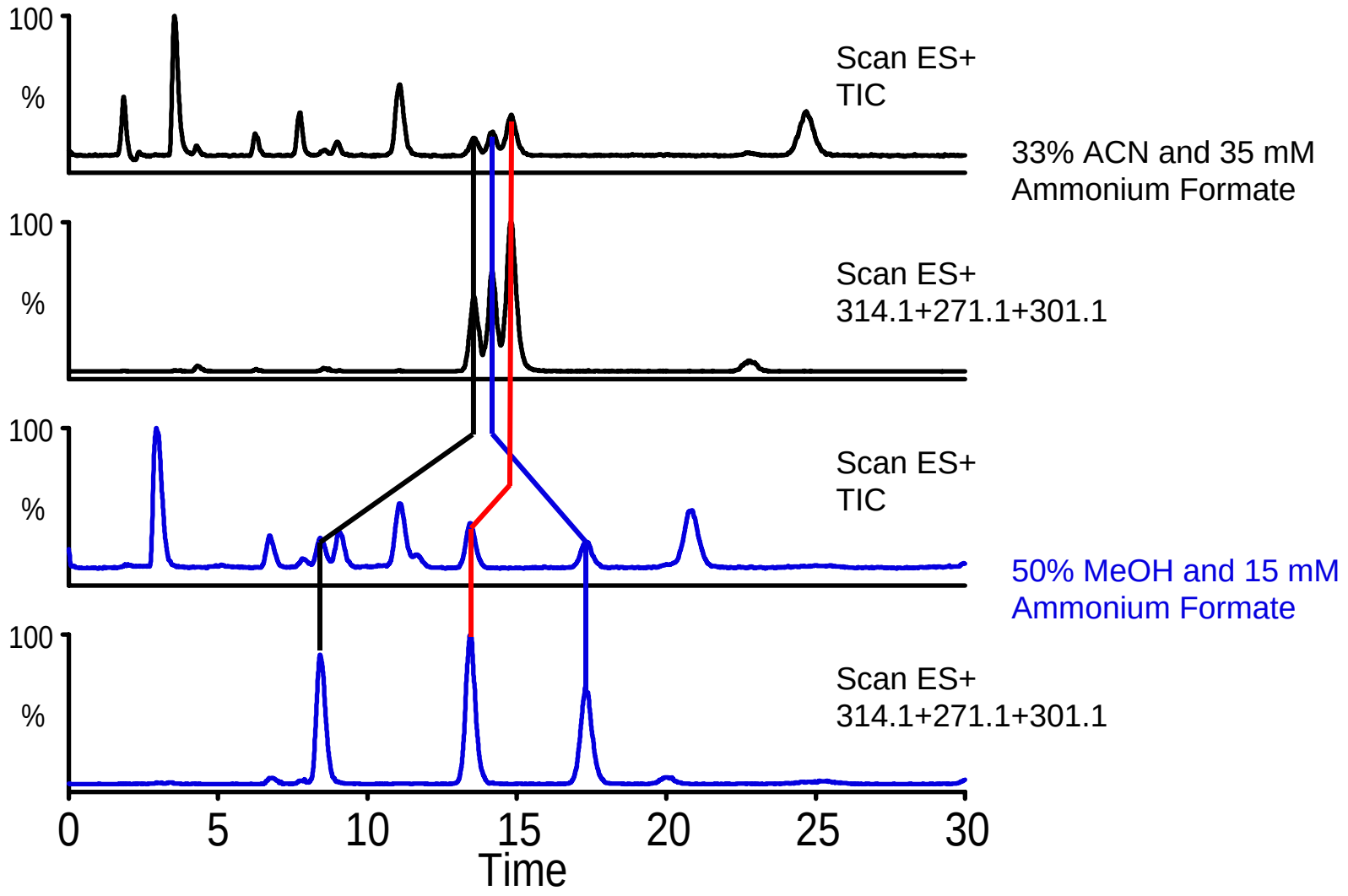
Peak Tracking In Methods Development

- What detector(s) do you currently use?
- How do you track peaks with changing chromatographic conditions?
- How much time does it take?
- How do you know when you are done?

Peak Tracking In Methods Development: Changing Solvent Conditions



Peak Tracking In Methods Development



Waters

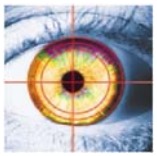
Ultra Performance LC™

Redefining separation science

Acquity™
Ultra Performance LC

Speed

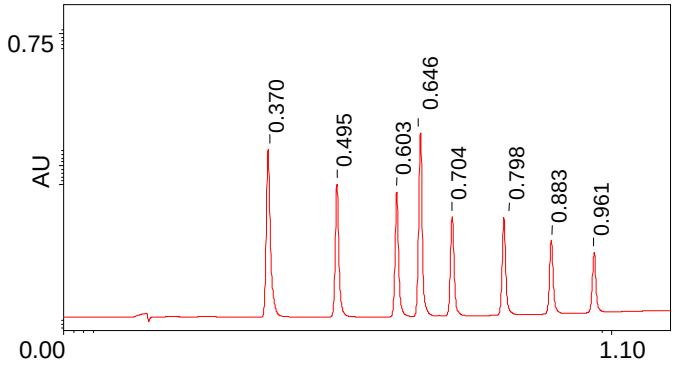
Sensitivity



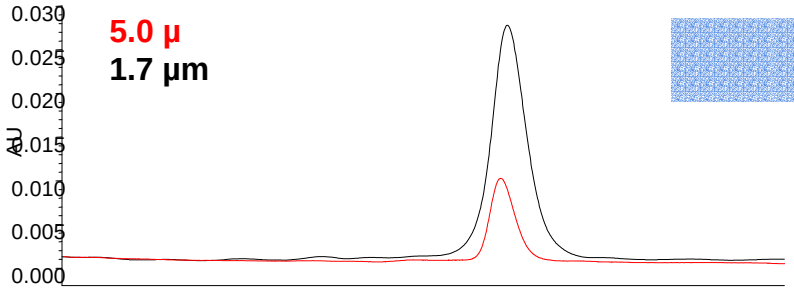
Resolution

Innovation

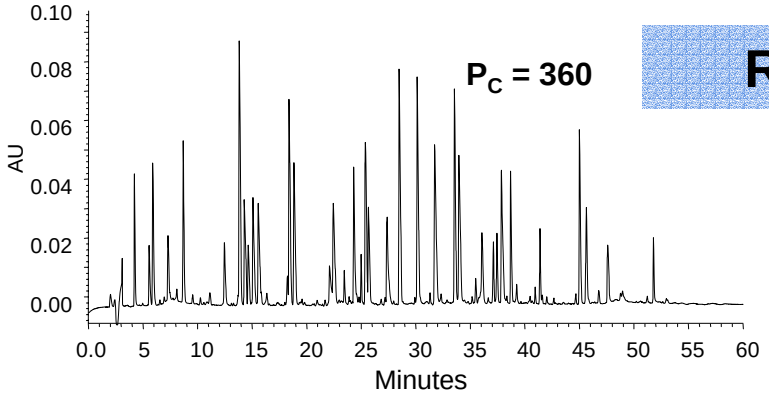
Ultra Performance LC™
Speed, Sensitivity and Resolution



Speed: 9X faster separations



Sensitivity: 3X increase



Resolution: 1.7X increase

Application of ZQ / ACQUITY UPLC™

ACQUITY UPLC™ Core System

Compounds:

- Acetaminophen (151.1)
- Lidocaine (234.2)
- Propranolol (259.1)
- Trimipramine (294.2)
- Terfenadine (471.3)

Method Conditions

- Column: 2mm x 50mm ACQUITY UPLC™ BEH C18
- Flow rate: 0.75ml/min
- Injection: 2ul
- Gradient: 0.5 min from 5% - 100% ACN
  - (mobile phase 0.1% formic in water and ACN)

ZQ Parameters

Scan range: 150-700 da

Scan Time: 0.11 sec

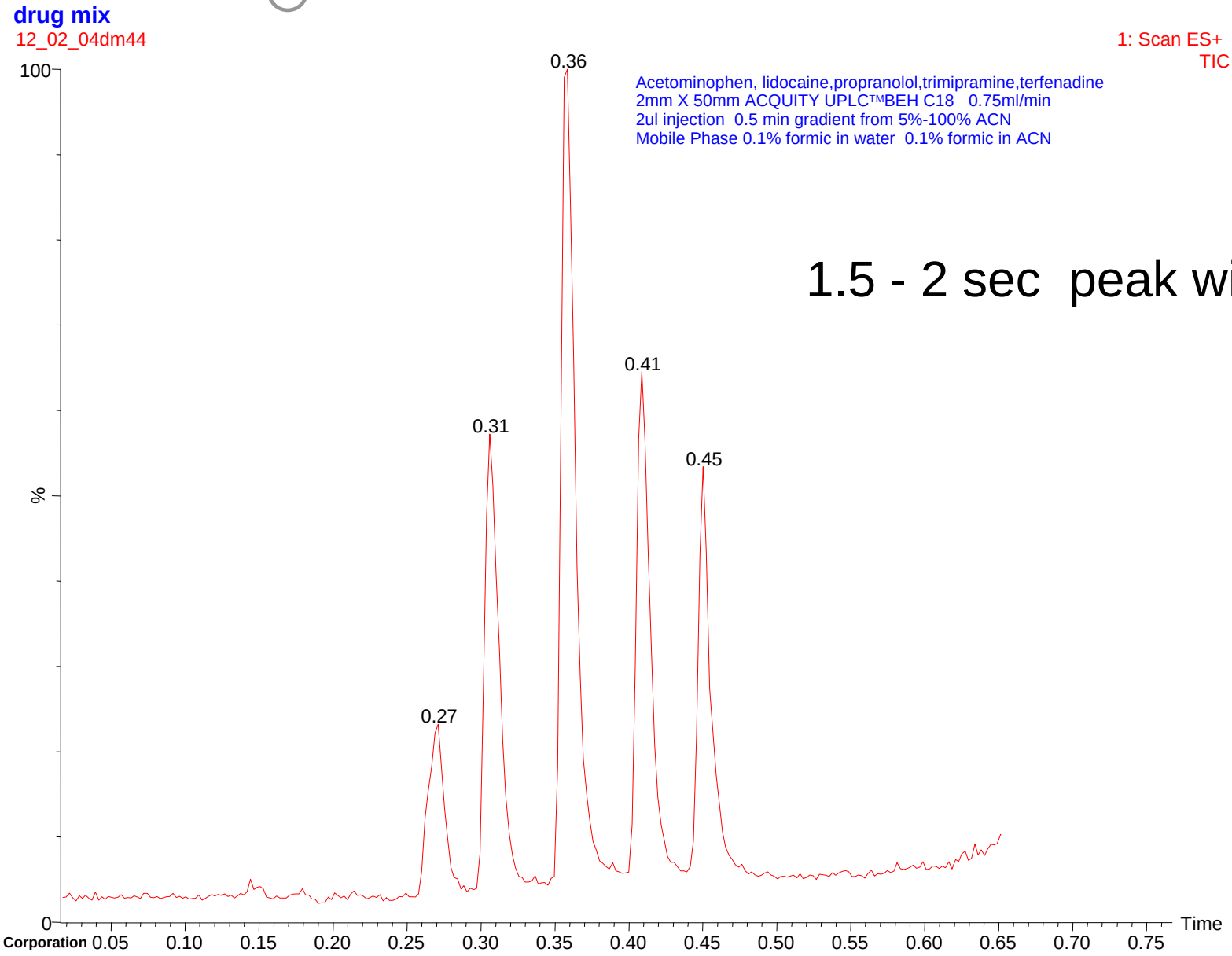
Interscan Delay: 0.02 sec*

Cycle time: 0.13 sec

*The short interscan delay of 20 ms can be achieved if there is no switching of polarity or switching of mode (ESI and APcI)

For ESCi work the interscan delay was increased to 100ms

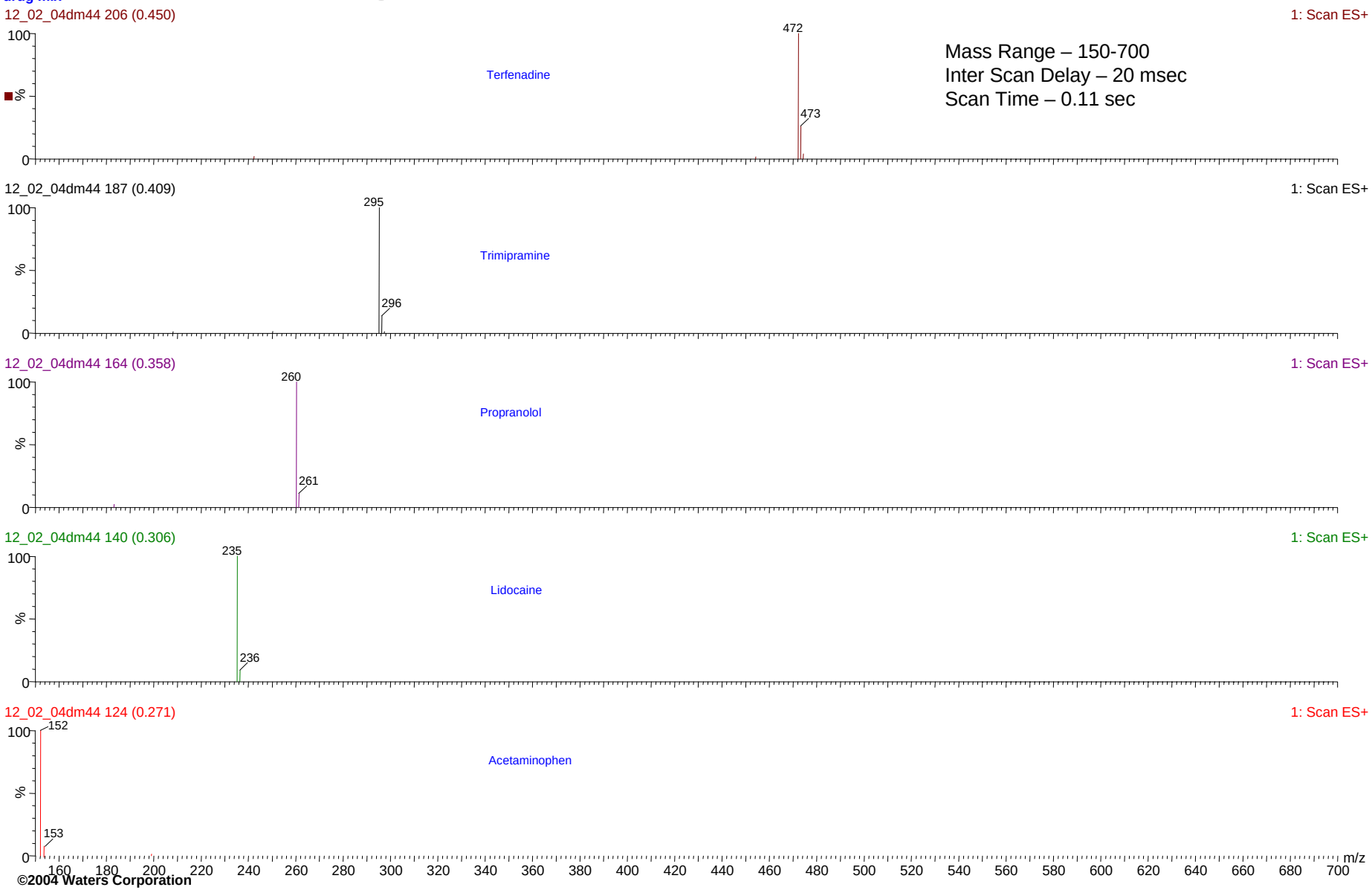
Drug Mix with ZQ / ACQUITY UPLC™



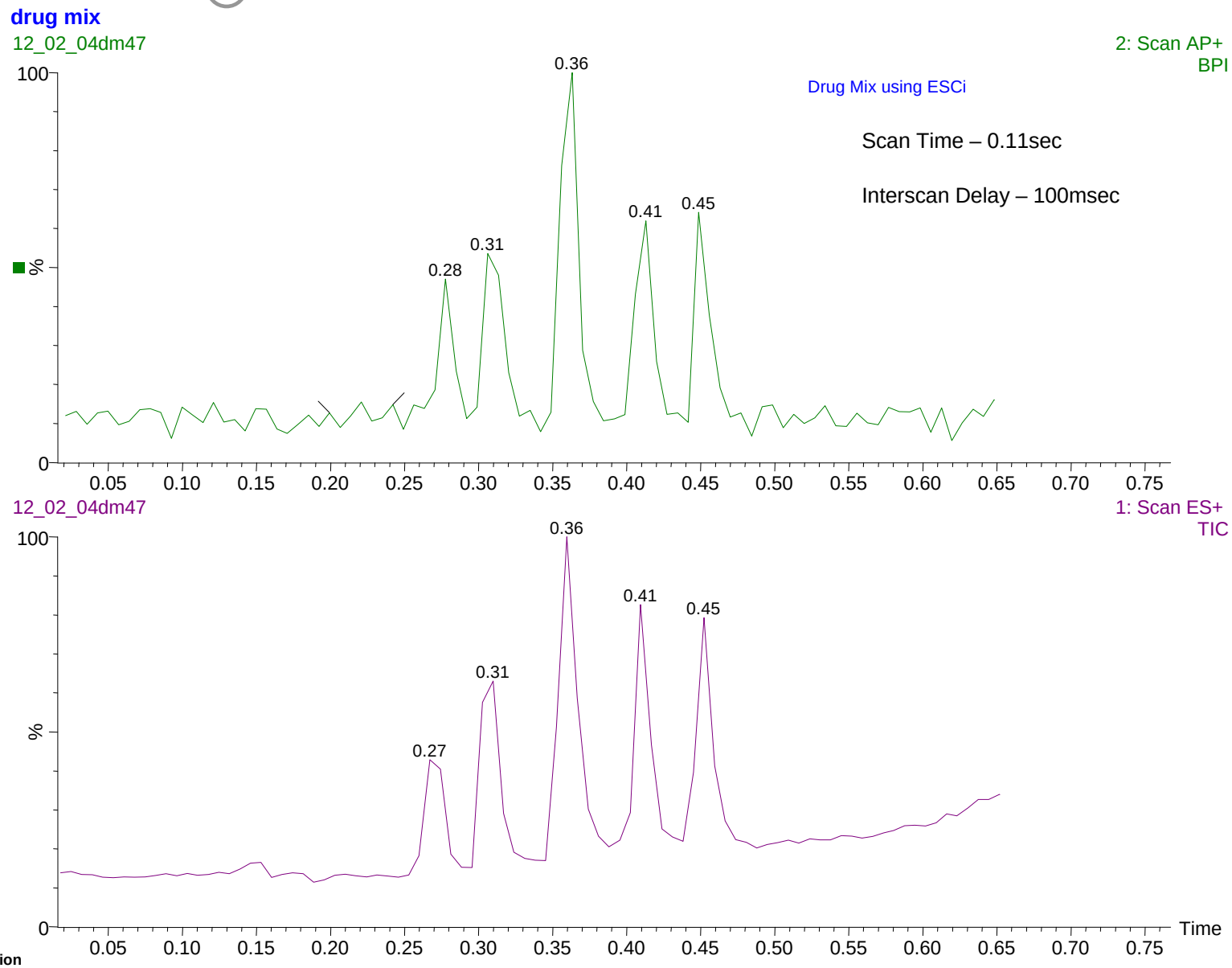
1.5 - 2 sec peak width

Mass Spectra from Drug Mix

drug mix

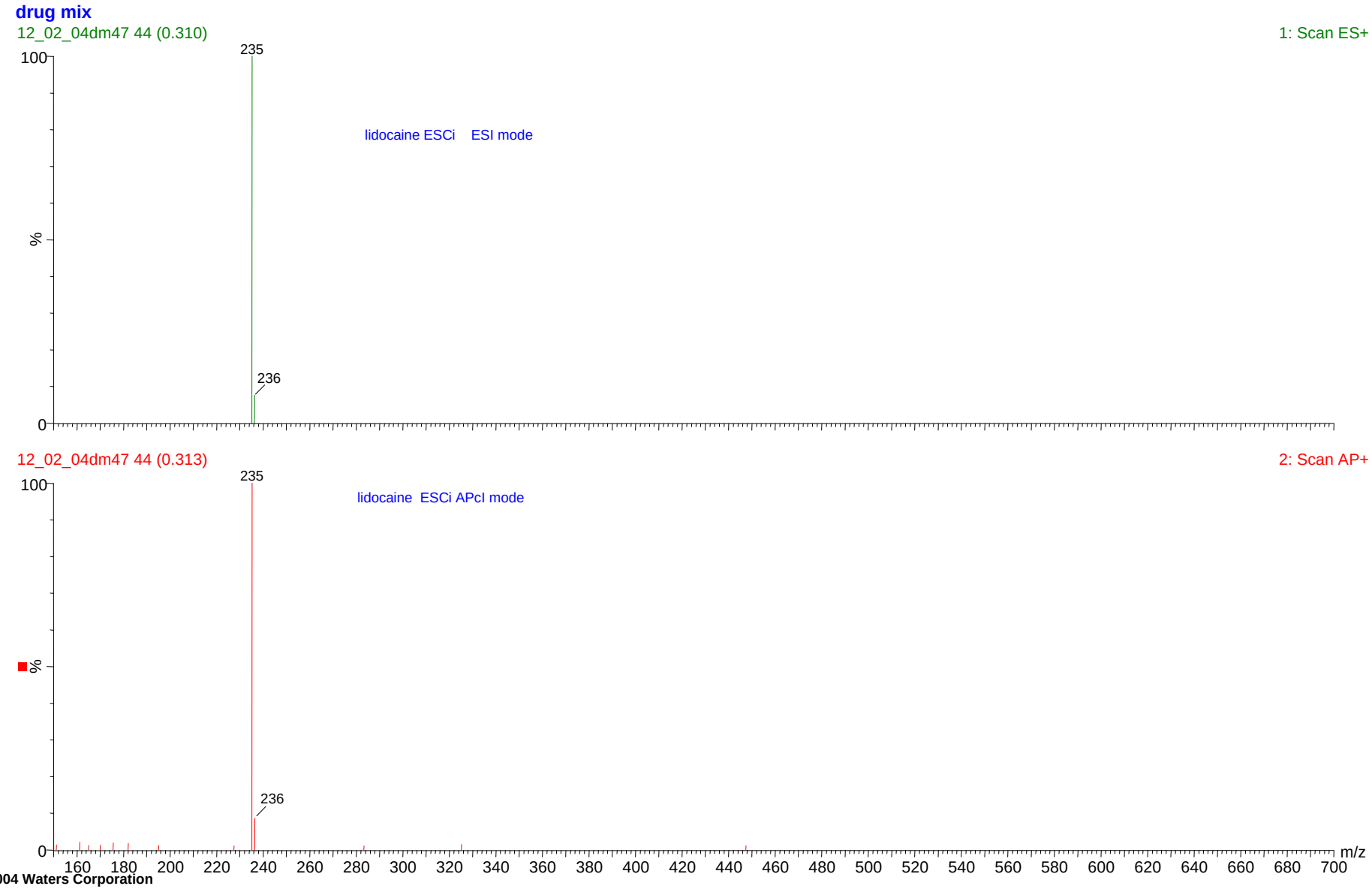


Drug Mix using ESCi



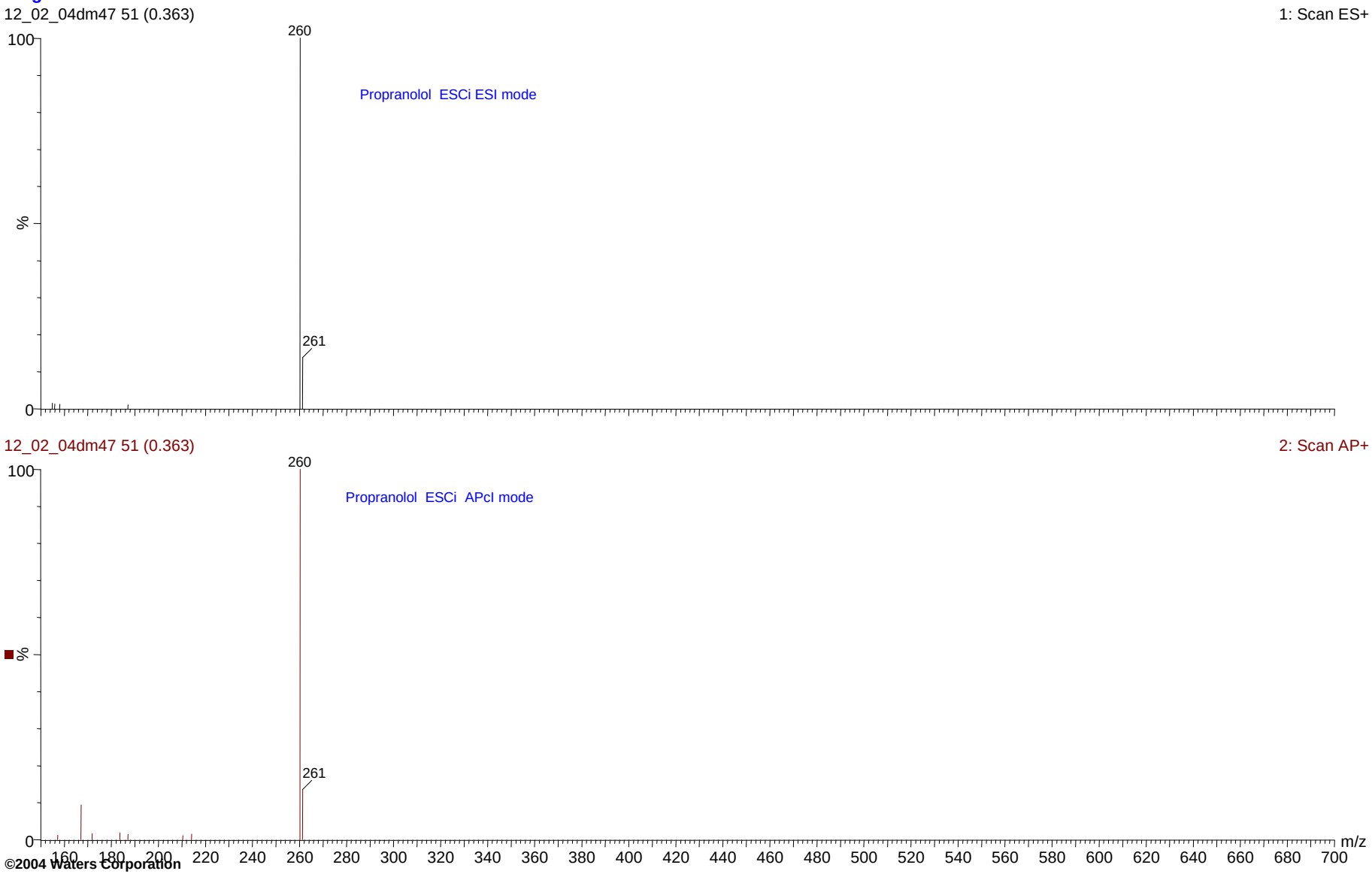
Mass Spectra from ESCi

Mass Spectra from ESCi

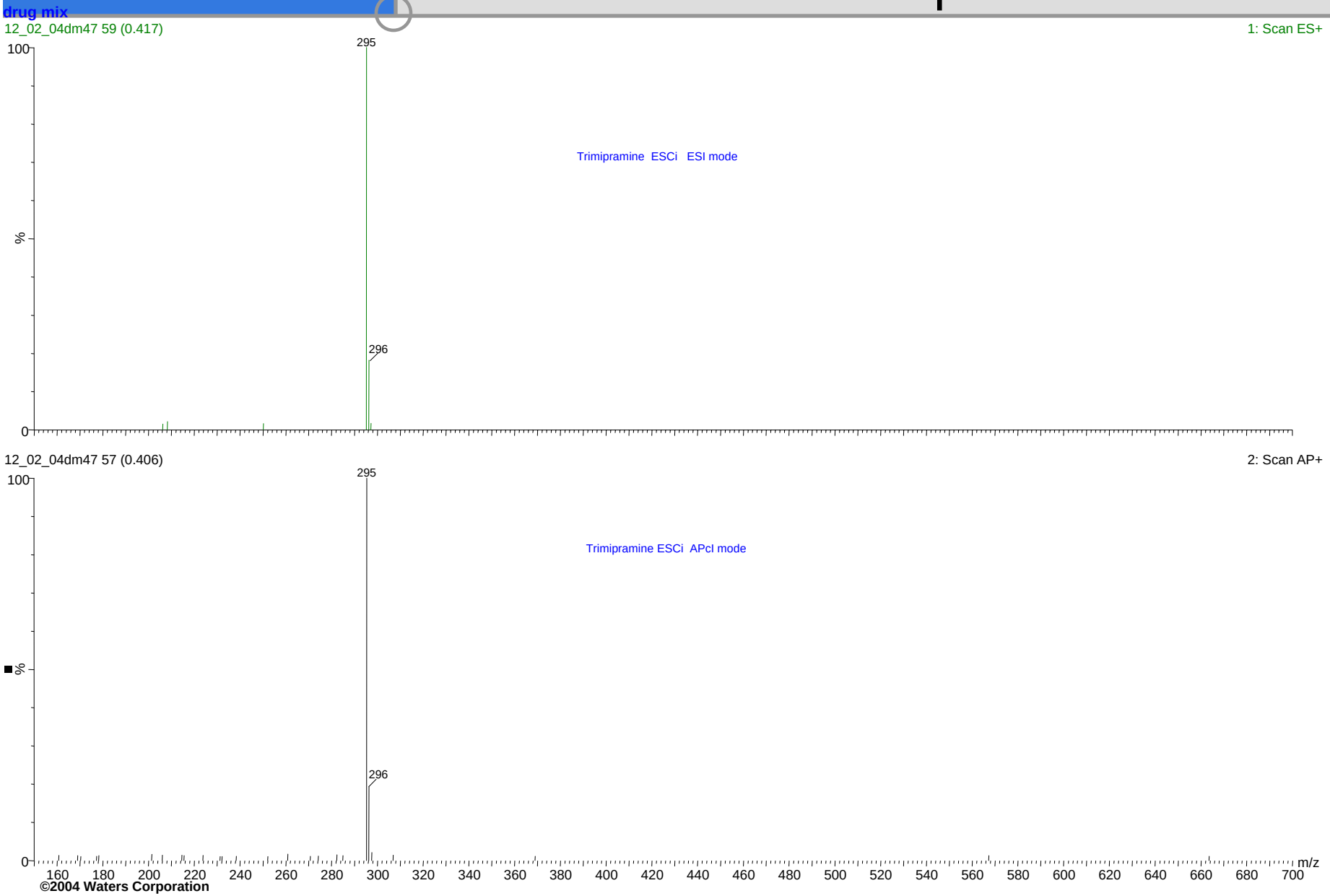


Mass Spectra from ESCi

drug mix

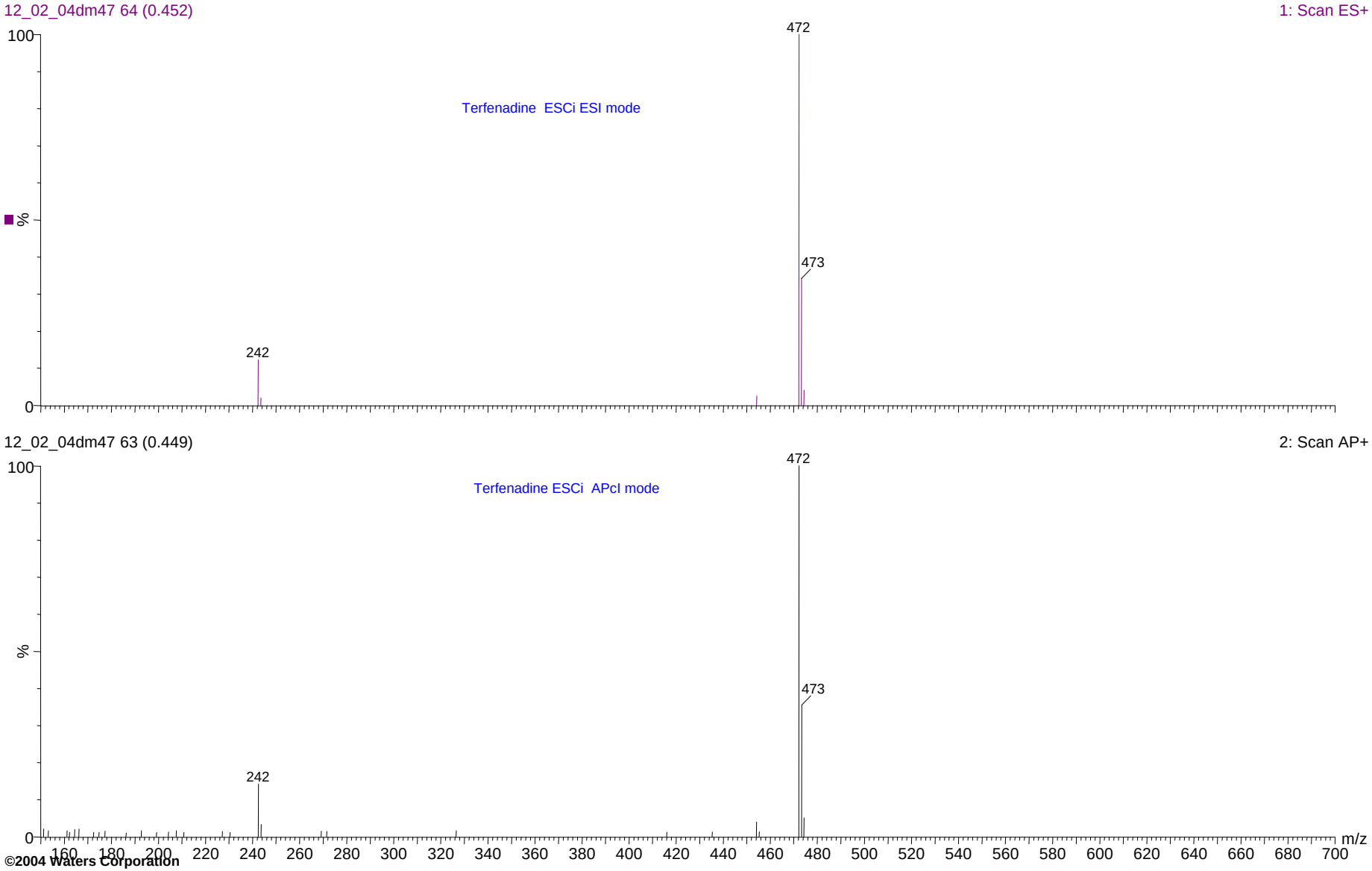


Mass Spectra from ESCi



Mass Spectra from ESCi

drug mix

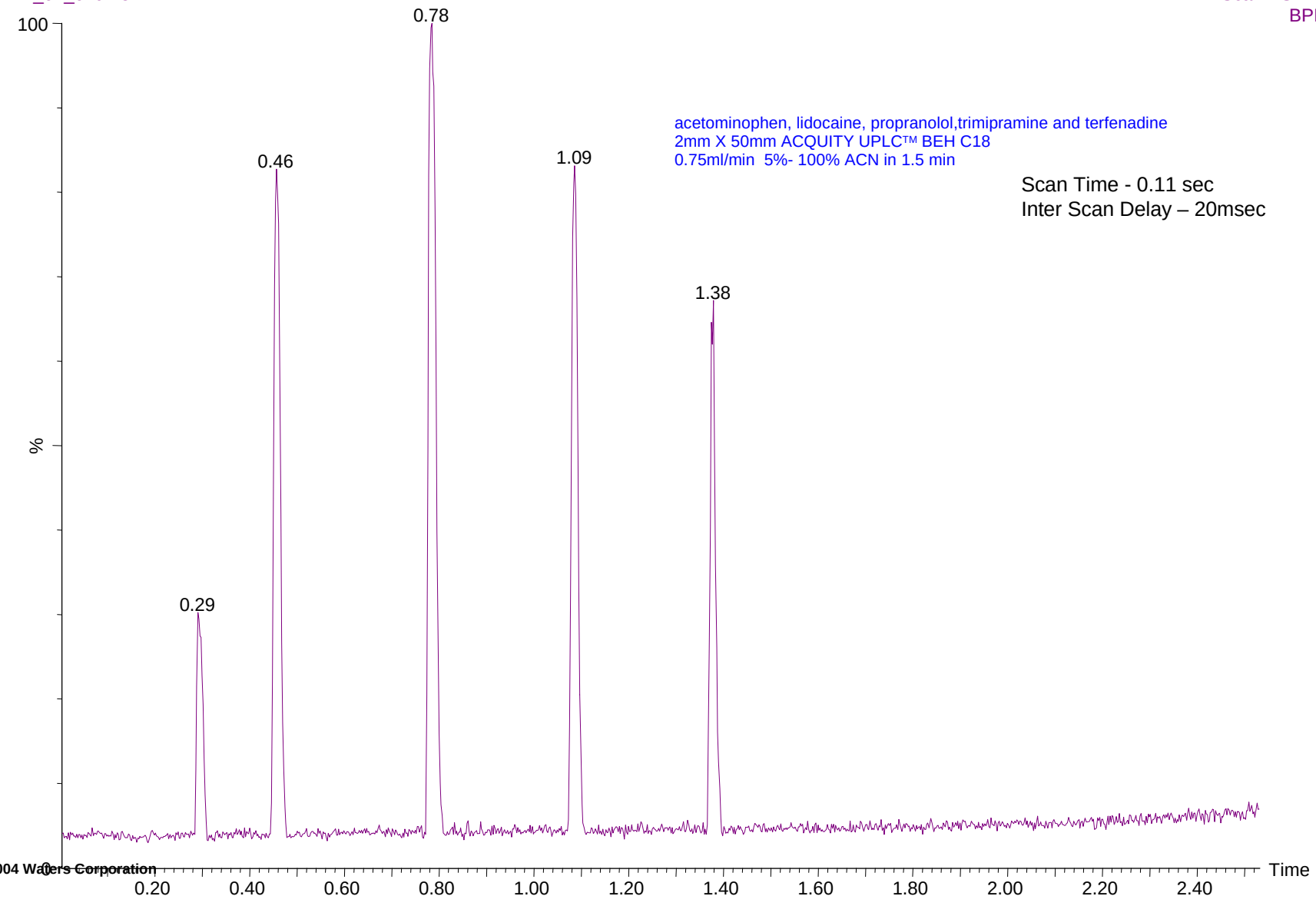


ZQ / ACQUITY UPLC™

drug mix

12_02_04dm51

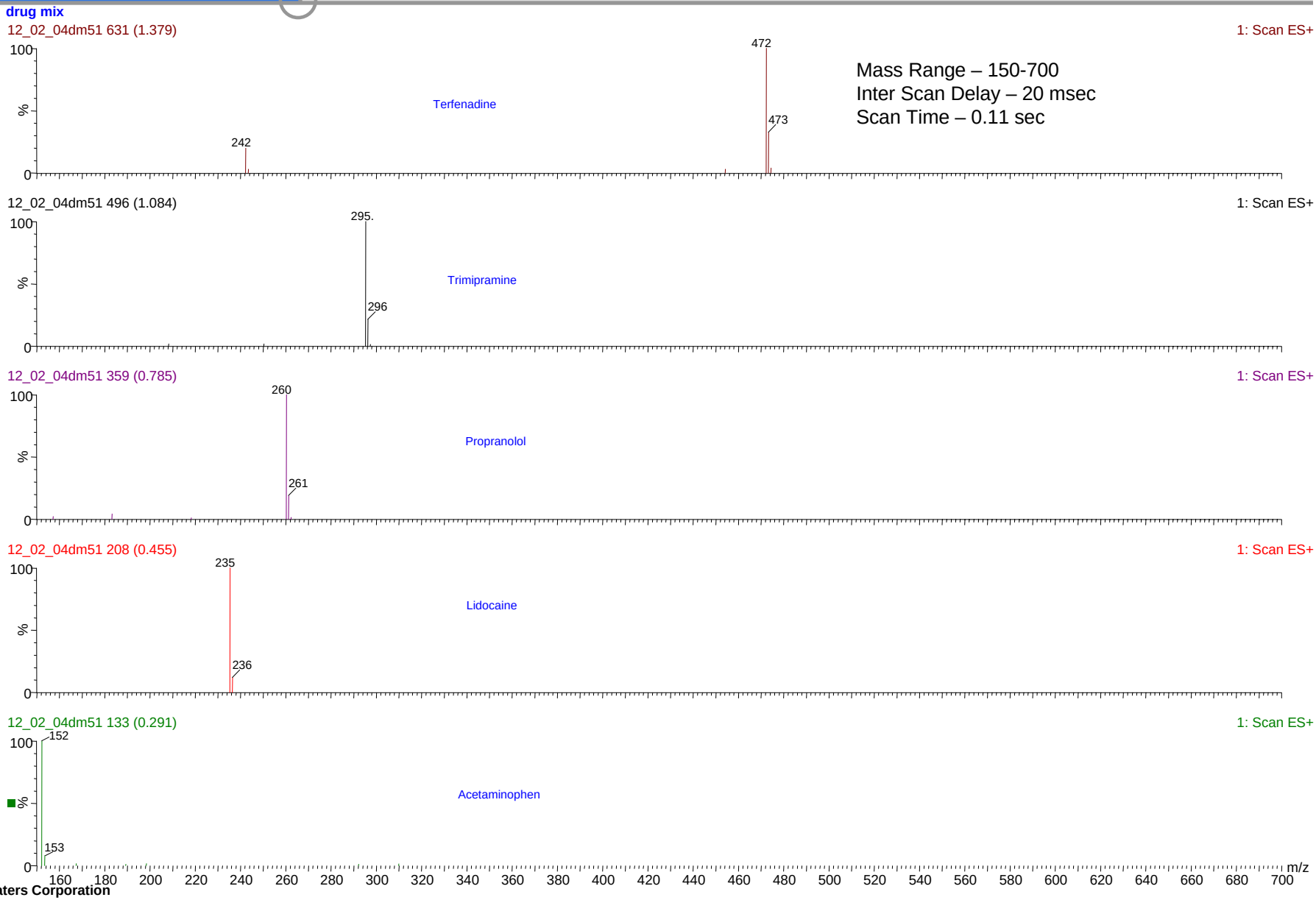
1: Scan ES+
BPI



acetominophen, lidocaine, propranolol,trimipramine and terfenadine
2mm X 50mm ACQUITY UPLC™ BEH C18
0.75ml/min 5%- 100% ACN in 1.5 min

Scan Time - 0.11 sec
Inter Scan Delay – 20msec

Mass Spectra from Drug Mix



Quantitation with ZQ / ACQUITY UPLC™

Fast Gradient

Column: ACQUITY UPLC™ BEH C18 2.1x50mm 1.7um

Mobile Phase A: 0.1% Formic Acid in H_2O

Mobile Phase B: 0.1% Formic Acid in Acetonitrile

Gradient:10% B to 90% in 3 min

Flow Rate: 0.85 ml/min; 400 ul split into MS

Injection Volume: 2ul

Ionization Mode: ESI+

Analytes: Trimipramine (m/z 295) and Terfenadine (m/z 472)

Waters ZQ Parameters

SIR mode for two masses: 295 and 472

Dwell time: 50ms

Interchannel delay: 20ms

Interscan delay: 20ms

Cycle time: 0.14 sec

The interchannel and interscan delay was set to 20 ms (since there was no mode or polarity switching)

Both masses were in one function

Flow was split to allow 400 ul/min into the source

Quantitation-5pg/ul to 1000 pg/ul

Quantify Compound Summary Report
Printed Tue Dec 21 16:34:05 2004
Compound 1: trimpiramine

	Name	Type	Std. Conc	RT	Area	ug/ml	%Dev
1	120804UPLC_ZQ_12	Standard	0.005	1.11	829	0.006	11.2
2	120804UPLC_ZQ_13	Standard	0.007	1.11	1043	0.007	-7.8
3	120804UPLC_ZQ_14	Standard	0.01	1.11	1528	0.01	-0.2
4	120804UPLC_ZQ_15	Standard	0.066	1.11	9564	0.061	-7.4
5	120804UPLC_ZQ_16	Standard	0.075	1.11	12109	0.077	2.4
6	120804UPLC_ZQ_17	Standard	0.1	1.12	15795	0.1	0.1
7	120804UPLC_ZQ_18	Standard	0.65	1.12	109000	0.689	6
8	120804UPLC_ZQ_19	Standard	0.75	1.12	115389	0.729	-2.8
9	120804UPLC_ZQ_20	Standard	1	1.12	155889	0.985	-1.5

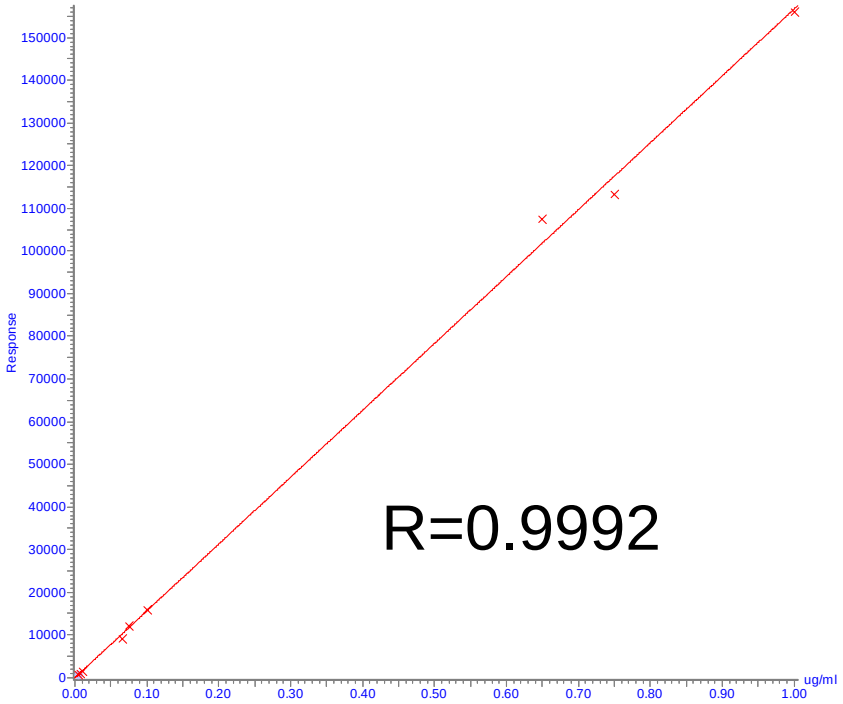
Quantitation-5pg/ul to 1000 pg/ul

Quantify Compound Summary Report
Printed Tue Dec 21 16:30:18 2004
Compound 2: terfenadine

	Name	Type	Std. Conc	RT	Area	ug/ml	%Dev
1	120804UPLC_ZQ_12	Standard	0.005	1.4	1612	0.006	13.1
2	120804UPLC_ZQ_13	Standard	0.007	1.4	1919	0.007	-9.7
3	120804UPLC_ZQ_14	Standard	0.01	1.4	2742	0.01	-2.2
4	120804UPLC_ZQ_15	Standard	0.066	1.4	16691	0.061	-7.4
5	120804UPLC_ZQ_16	Standard	0.075	1.4	20907	0.076	1.5
6	120804UPLC_ZQ_17	Standard	0.1	1.4	28335	0.103	3.3
7	120804UPLC_ZQ_18	Standard	0.65	1.4	187475	0.685	5.3
8	120804UPLC_ZQ_19	Standard	0.75	1.4	200666	0.733	-2.3
9	120804UPLC_ZQ_20	Standard	1	1.4	269359	0.984	-1.6

Quantitation-Linearity

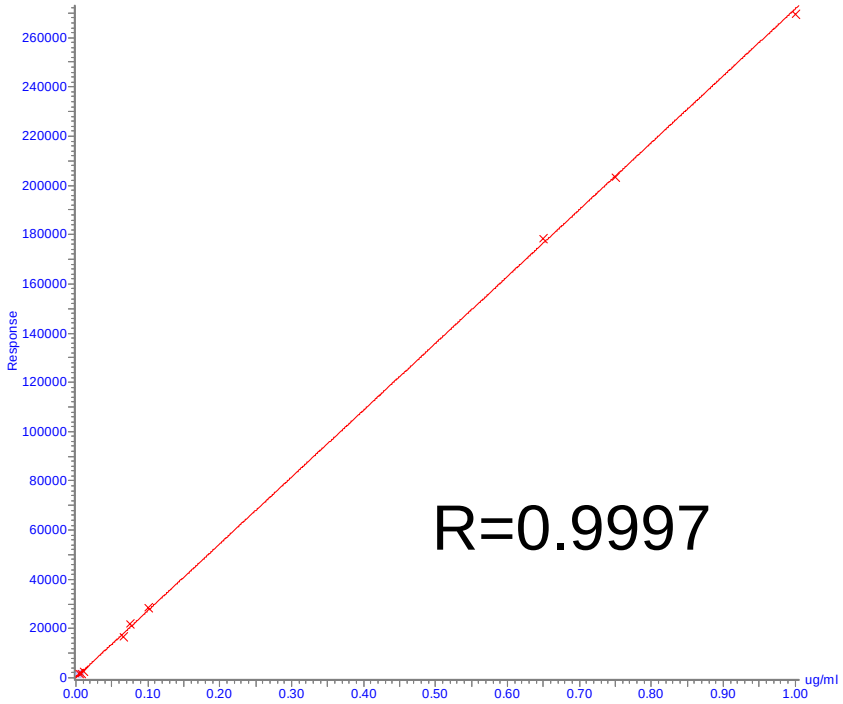
Compound name: trimpiramine
Correlation coefficient: r = 0.999242, r^2 = 0.998484
Calibration curve: 156745 * x + -45.7211
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



R=0.9992

trimipramine

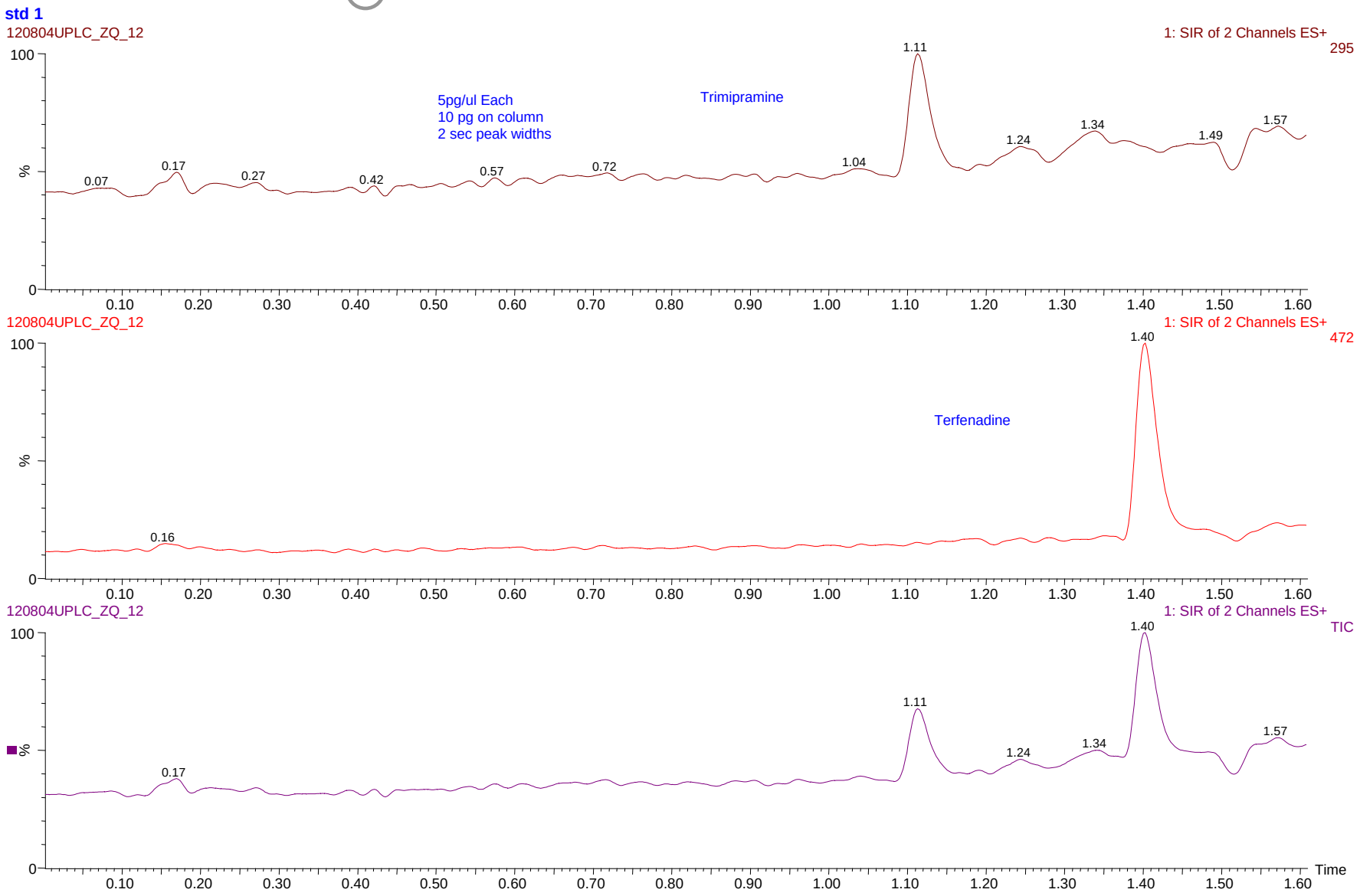
Compound name: terfenadine
Correlation coefficient: r = 0.999678, r^2 = 0.999356
Calibration curve: 271527 * x + 121.102
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



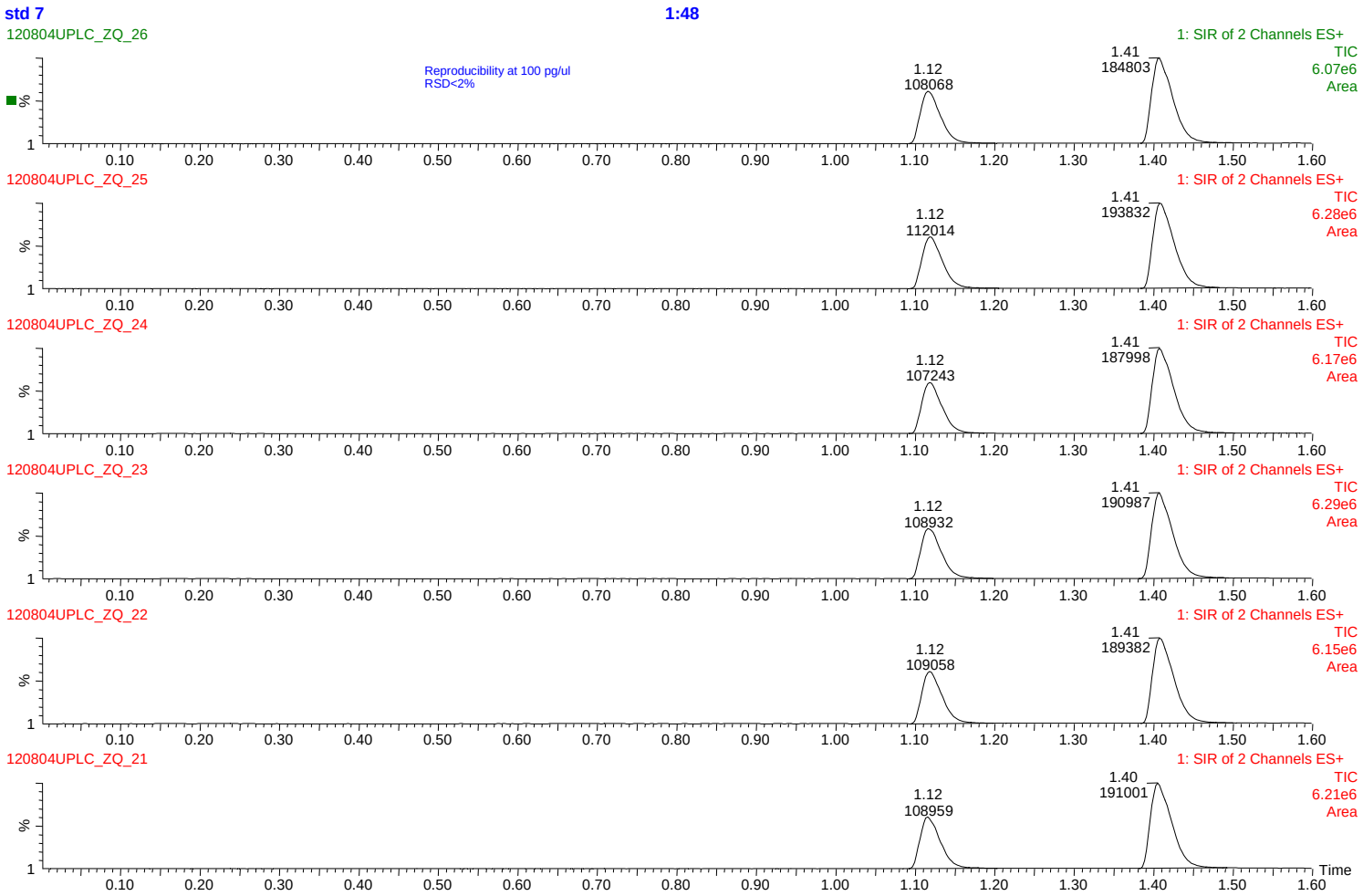
R=0.9997

terfenadine

Limits of Detection (LOD)
10 pg on Column



Reproducibility: Six Replicate Injections



Reproducibility

Quantify Compound Summary Report

Printed Thu Dec 09 09:18:32 2004

Compound 1: trimpiramine

	#	Name	Sample Text	Type	Std. Conc	RT	Area	ug/ml	%Dev
1	1	120804UPLC_ZQ_21	std 7	Standard	0.65	1.12	108959	0.649	-0.1
2	2	120804UPLC_ZQ_22	std 7	Standard	0.65	1.12	109058	0.65	0
3	3	120804UPLC_ZQ_23	std 7	Standard	0.65	1.12	108932	0.649	-0.1
4	4	120804UPLC_ZQ_24	std 7	Standard	0.65	1.12	107243	0.639	-1.7
5	5	120804UPLC_ZQ_25	std 7	Standard	0.65	1.12	112014	0.668	2.7
6	6	120804UPLC_ZQ_26	std 7	Standard	0.65	1.12	108068	0.644	-0.9
						Ave:	109046	0.650	
						Std dev:	1615	0.010	
						%RSD:	1.5	1.5	

Quantify Compound Summary Report

Printed Thu Dec 09 09:19:28 2004

Compound 2: terfenadine

	#	Name	Sample Text	Type	Std. Conc	RT	Area	ug/ml	%Dev
1	1	120804UPLC_ZQ_21	std 7	Standard	0.65	1.4	191001	0.655	0.7
2	2	120804UPLC_ZQ_22	std 7	Standard	0.65	1.41	189382	0.649	-0.2
3	3	120804UPLC_ZQ_23	std 7	Standard	0.65	1.41	190987	0.655	0.7
4	4	120804UPLC_ZQ_24	std 7	Standard	0.65	1.41	187998	0.644	-0.9
5	5	120804UPLC_ZQ_25	std 7	Standard	0.65	1.41	193832	0.664	2.2
6	6	120804UPLC_ZQ_26	std 7	Standard	0.65	1.41	184803	0.633	-2.6
						Ave:	189667	0.650	
						Std dev:	3079	0.011	
						%RSD:	1.6	1.6	

Frequently Asked Questions

- I need to save time, money, but not at the sacrifice of performance, will this new ACQUITY UPLC™ help?
- YES!
- Should I change one or more of my ZQ/Alliance systems to be a ZQ/ACQUITY UPLC™ system to improve my throughput, resolution, and sensitivity?
- YES!
- Can I run my ZQ with an ACQUITY UPLC™ system?
- YES!

Waters

Questions