Workflow and Proof of Concept for Non-Targeted Analysis of Environmental Samples by LC-MS/MS

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Advanced Analytical Methods for Contaminant Discovery

The Chemical Universe

We live in a chemical sea of continually changing composition – comprising both anthropogenic and naturally occurring chemical stressors.

Unlike biota, chemical pollutants have no boundaries in their global distribution – "*everything is everywhere*," only the concentrations vary.

Background/Problem

- Human ‘exposome’ – “At its most complete, the exposome encompasses life-course environmental exposures (including lifestyle factors) from the prenatal period onwards” Chris Wild, Cancer Epidemiology Biomarkers 2005.

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Office of Research and Development
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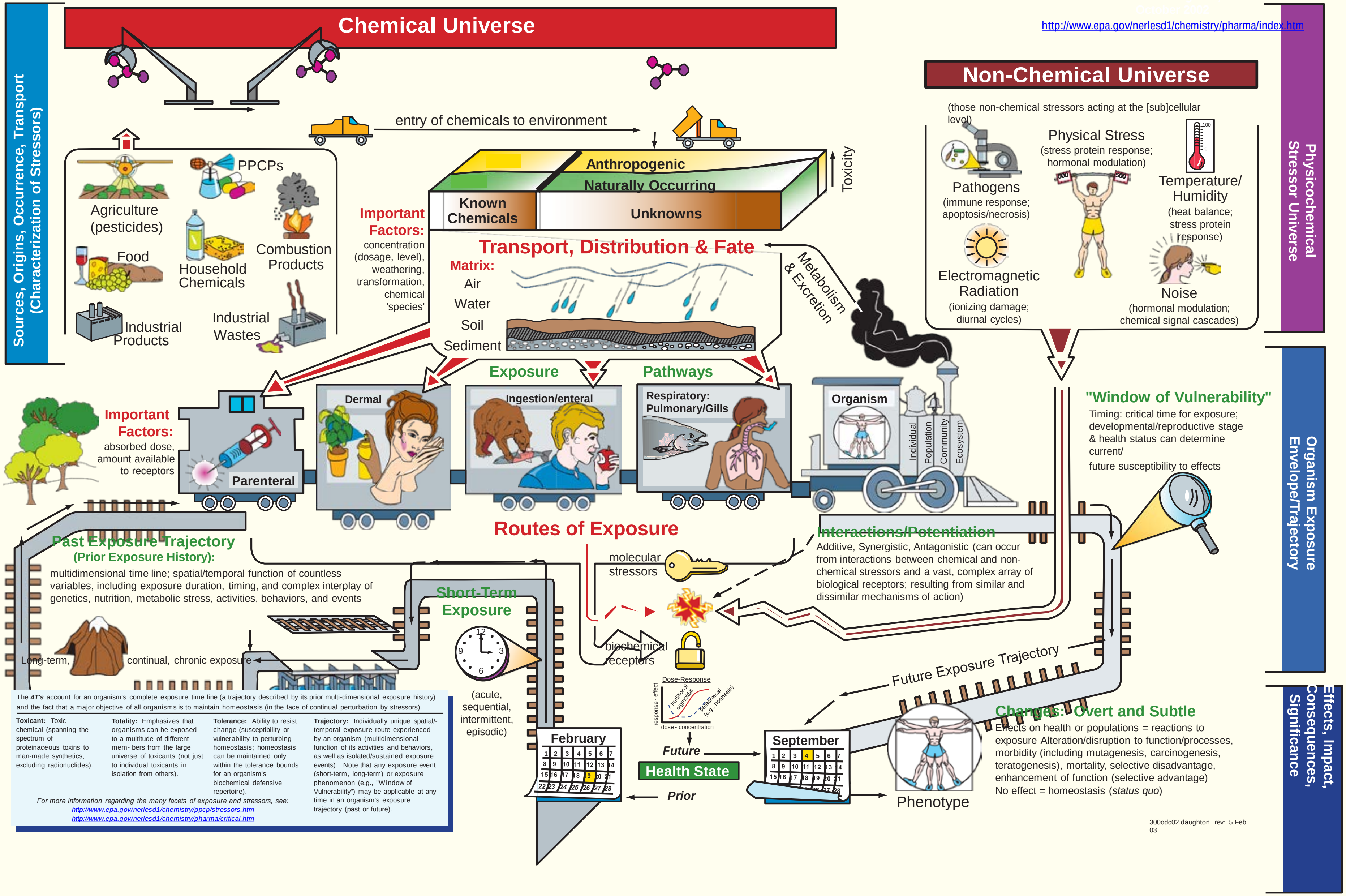
Environmental Chemistry Branch

Biological Systems and Stressors

"Toxicant Totality Tolerance Trajectory" - 4T's



Sources, Origins, Occurrence, Transport (Characterization of Stressors)
Chemical Universe
http://www.epa.gov/nerlesd1/chemistry/pharma/index.htm
entry of chemicals to environment
Agriculture (pesticides)
PPCPs
Combustion Products
Food
Household Chemicals
Industrial Products
Industrial Wastes
Important Factors:
concentration (dosage, level), weathering, transformation, chemical 'species'
Anthropogenic
Naturally Occurring
Known Chemicals
Unknowns
Toxicity
Transport, Distribution & Fate
Matrix:
Air
Water
Soil
Sediment
Metabolism & Excretion
Non-Chemical Universe
(those non-chemical stressors acting at the [sub]cellular level)
Pathogens
(immune response; apoptosis/necrosis)
Physical Stress
(stress protein response; hormonal modulation)
Temperature/ Humidity
(heat balance; stress protein response)
Electromagnetic Radiation
(ionizing damage; diurnal cycles)
Noise
(hormonal modulation; chemical signal cascades)
Physicochemical Stressor Universe
Exposure
Pathways
Important Factors:
absorbed dose, amount available to receptors
Parenteral
Dermal
Ingestion/enteral
Respiratory: Pulmonary/Gills
Organism
Individual
Population
Community
Ecosystem
"Window of Vulnerability"
Timing: critical time for exposure; developmental/reproductive stage & health status can determine current/ future susceptibility to effects
Organism Exposure Envelope/Trajectory
Routes of Exposure
Past Exposure Trajectory
(Prior Exposure History):
multidimensional time line; spatial/temporal function of countless variables, including exposure duration, timing, and complex interplay of genetics, nutrition, metabolic stress, activities, behaviors, and events
Long-term, continual, chronic exposure
Short-Term Exposure
(acute, sequential, intermittent, episodic)
molecular stressors
biochemical receptors
Interactions/Potentiation
Additive, Synergistic, Antagonistic (can occur from interactions between chemical and non-chemical stressors and a vast, complex array of biological receptors; resulting from similar and dissimilar mechanisms of action)
Dose-Response
response - effect
dose - concentration
traditional sigmoidal
paradoxical (e.g., hormesis)
Future
Health State
Prior
February
September
Future Exposure Trajectory
Phenotype
Changes: Overt and Subtle
Effects on health or populations = reactions to exposure Alteration/disruption to function/processes, morbidity (including mutagenesis, carcinogenesis, teratogenesis), mortality, selective disadvantage, enhancement of function (selective advantage)
No effect = homeostasis (status quo)
Effects, Impact, Consequences, Significance

The ***4T's*** account for an organism's complete exposure time line (a trajectory described by its prior multi-dimensional exposure history) and the fact that a major objective of all organisms is to maintain homeostasis (in the face of continual perturbation by stressors).

Toxicant: Toxic chemical (spanning the spectrum of proteinaceous toxins to man-made synthetics; excluding radionuclides).

Totality: Emphasizes that organisms can be exposed to a multitude of different mem- bers from the large universe of toxicants (not just to individual toxicants in isolation from others).

Tolerance: Ability to resist change (susceptibility or vulnerability to perturbing homeostasis; homeostasis can be maintained only within the tolerance bounds for an organism's biochemical defensive repertoire).

Trajectory: Individually unique spatial/-temporal exposure route experienced by an organism (multidimensional function of its activities and behaviors, as well as isolated/sustained exposure events). Note that any exposure event (short-term, long-term) or exposure phenomenon (e.g., "Window of Vulnerability") may be applicable at any time in an organism's exposure trajectory (past or future).

For more information regarding the many facets of exposure and stressors, see:
http://www.epa.gov/nerlesd1/chemistry/ppcp/stressors.htm
http://www.epa.gov/nerlesd1/chemistry/pharma/critical.htm

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Background/Problem

- Human ‘exposome’ – “At its most complete, the exposome encompasses life-course environmental exposures (including lifestyle factors) from the prenatal period onwards” Chris Wild, Cancer Epidemiology Biomarkers 2005.
- Rapid assessment and screening of these chemicals is a difficult challenge facing EPA in its mission to protect public health and the environment.
- Need a variety of methods and models to understand and predict exposures.
  - Tools to advance knowledge of chemicals to which we are exposed, and at what concentrations.
  - “Ground truth” various high-throughput exposure models.

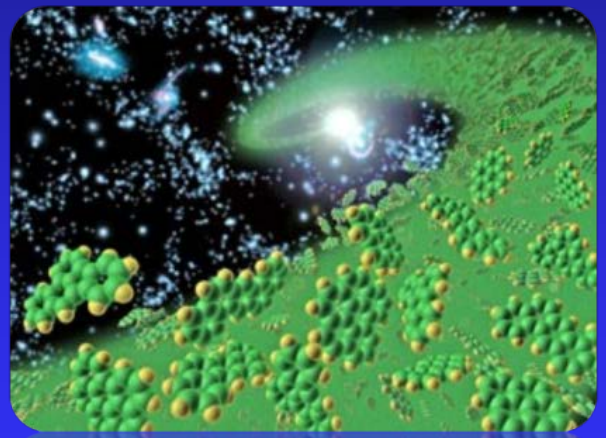
The Chemical Universe

The *KNOWN* Universe

As of October 2015, over 105 million commercially available chemicals.

(indexed by the American Chemical Society's Chemical Abstracts Service in their CAS Registry; excluding bio-sequences such as proteins and nucleotides: http://www.cas.org/content/chemical-substances/)

- Of these millions of known chemicals, only 344K+ are inventoried or regulated by government bodies worldwide - - representing only 0.3% of those that are commercially available.
- Approximately 15,000 new substances are added each day.



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Analytical Instrumentation

Objectives

- Develop a non-targeted analysis utilizing existing lab capabilities
  - Accurate mass- HPLC/TOF-MS
  - Fragmentation- UPLC-tandem MS

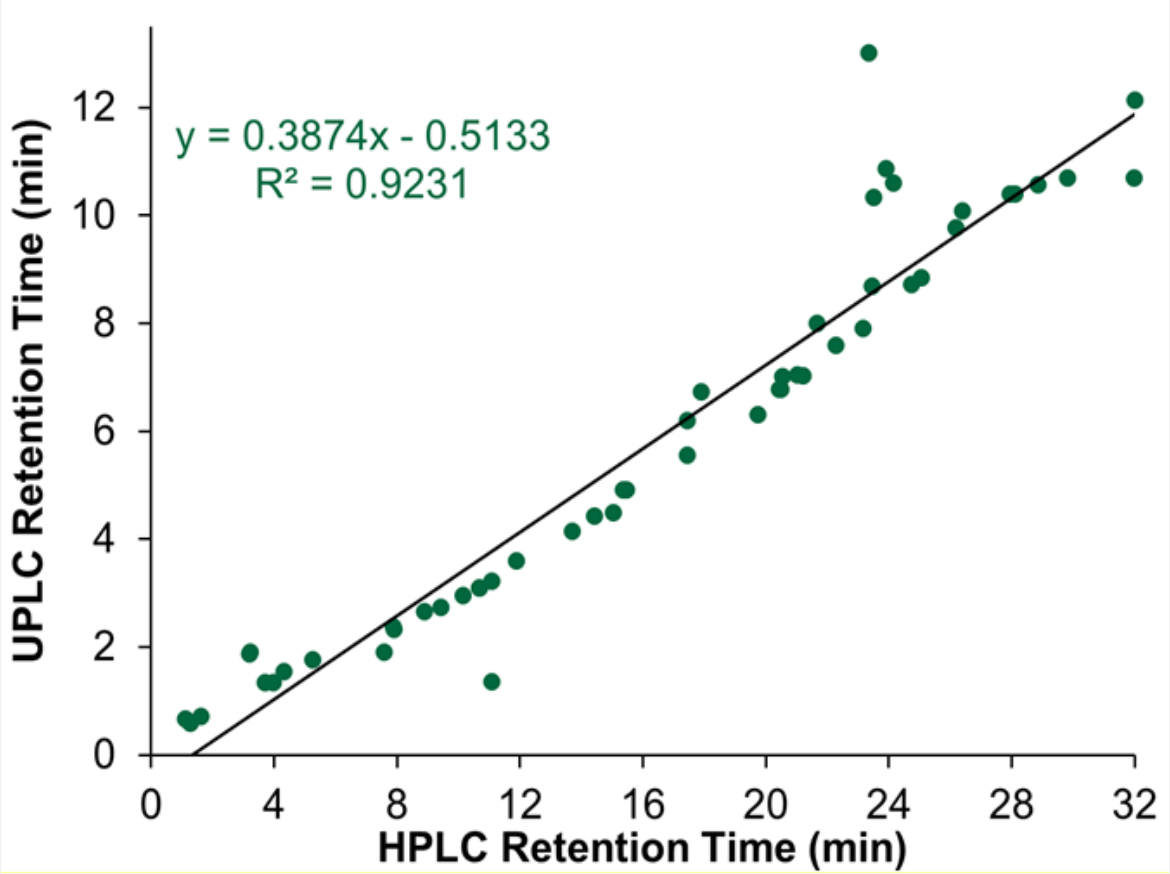
- Demonstrate the usefulness of this analysis in identifying non-targeted compounds.

UPLC conditions

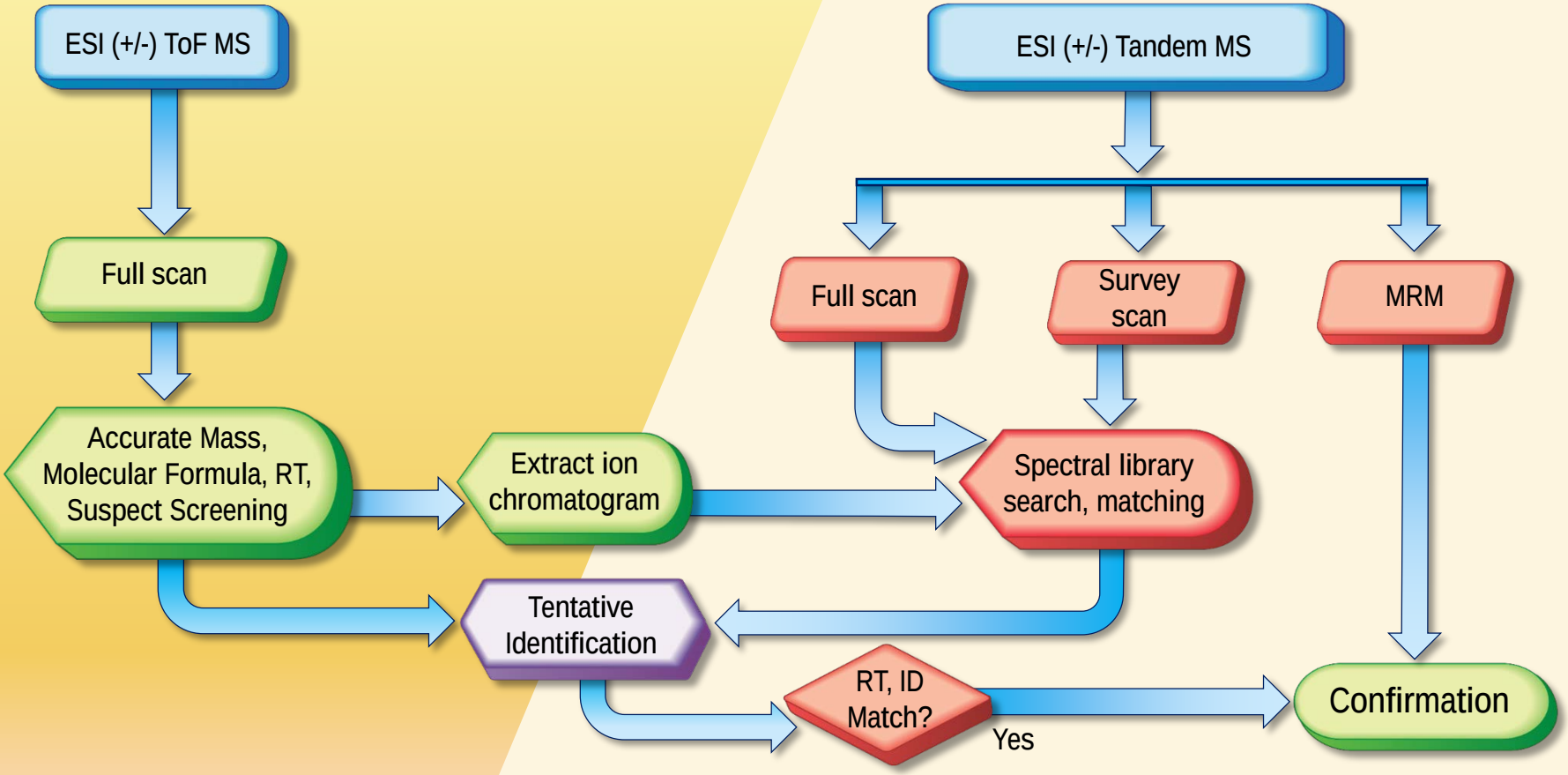
- Acquity® UPLC BEH C_8 1.7 µm, 2.1 x 50 mm analytical column
- Acquity® UPLC BEH C_8 1.7 µm, 2.1 x 5 mm Vanguard pre-column
- Mobile phase: A – 5:95 MeOH/DI water 0.4 mM ammonium formate
  B – 95:5 MeOH/DI water 0.4 mM ammonium formate
- Flow rate – 0.41 mL/min
- Gradient translated from HPLC/ToF method

UPLC gradient

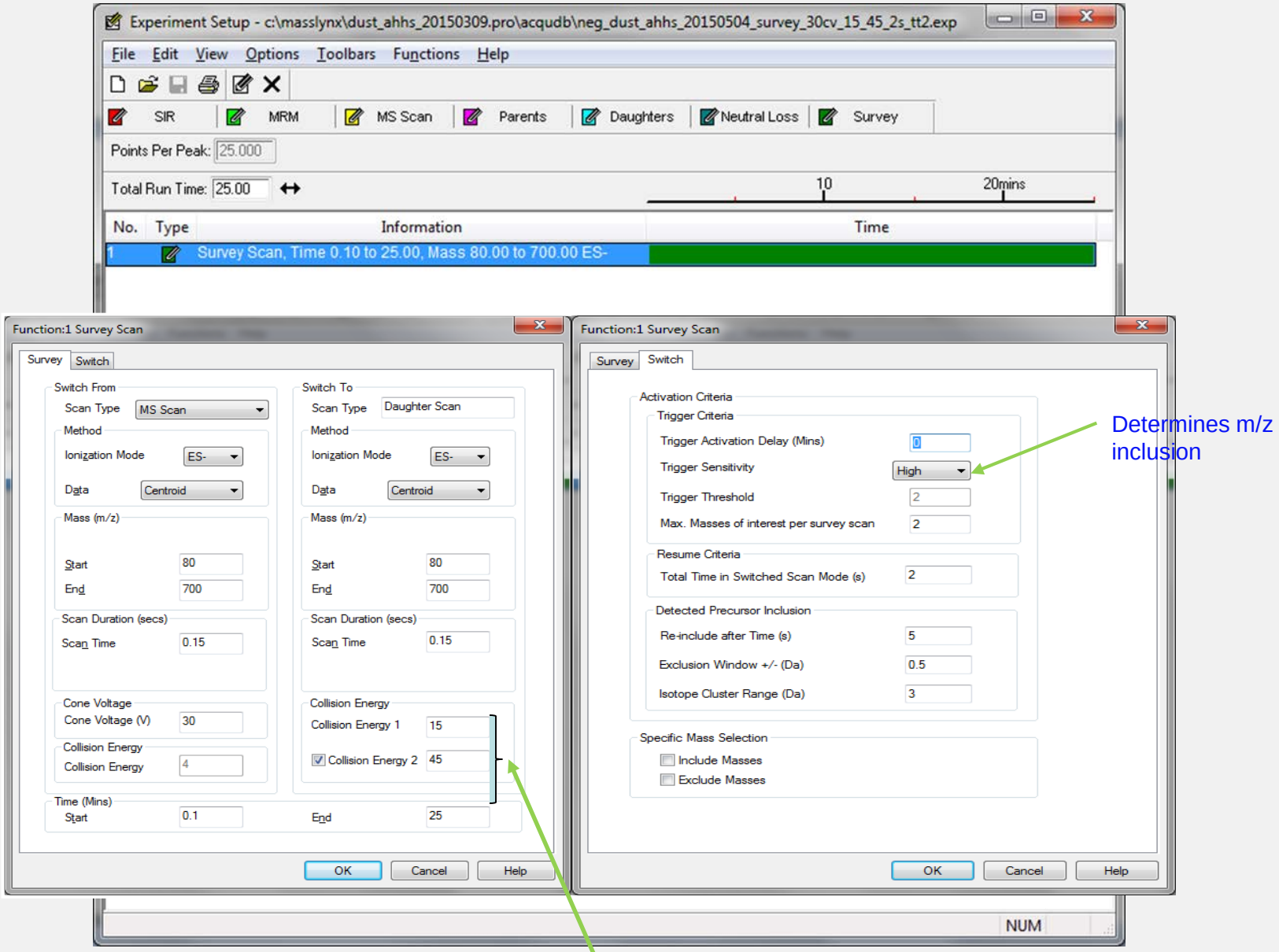
Time (min)	%A	%B
Initial	75	25
12.14	15	85
19.43	0	100
21.86	0	100
23.31	75	25
25.0	75	25



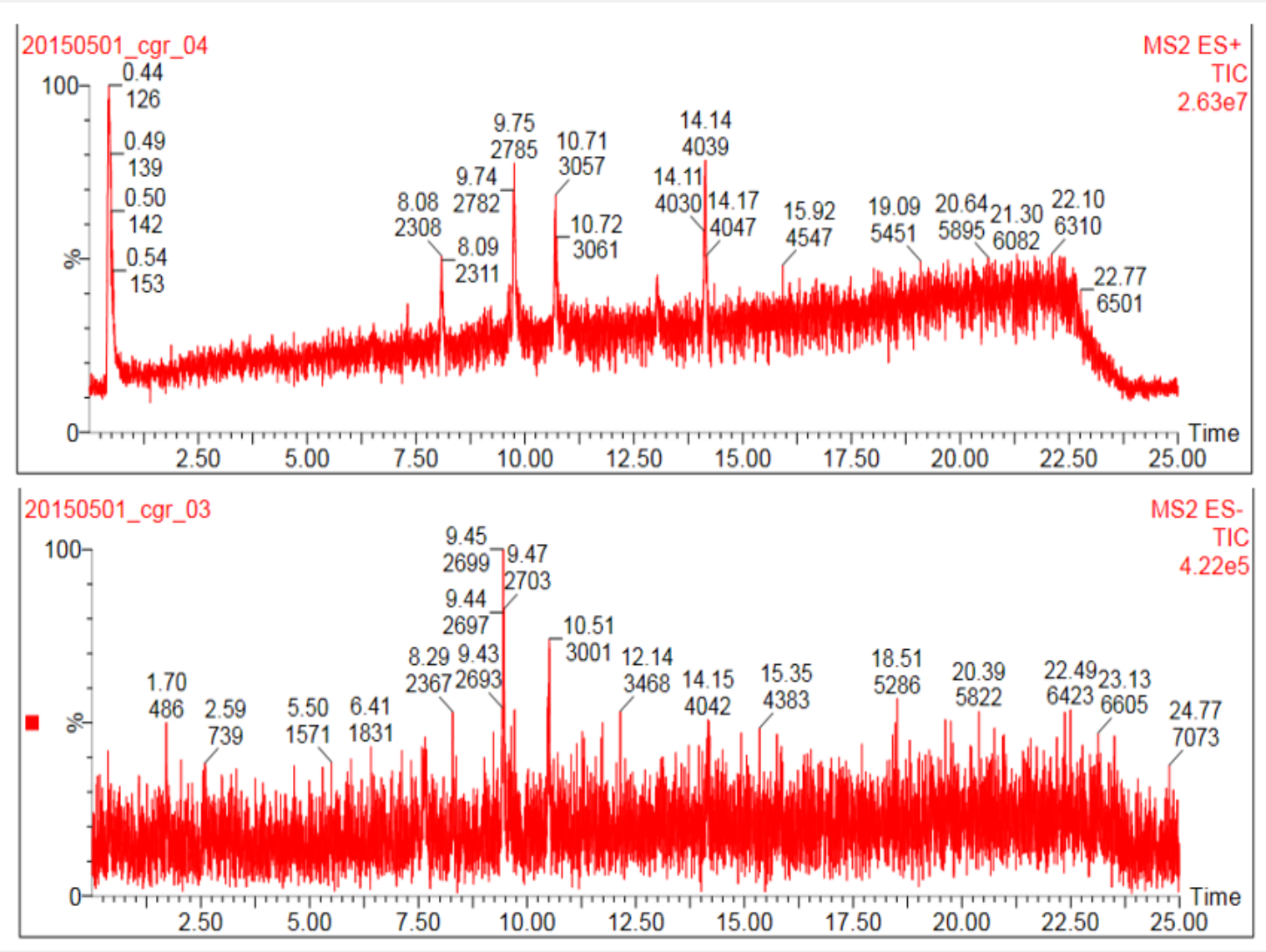
Workflow


ESI (+/-) ToF MS
Full scan
Accurate Mass, Molecular Formula, RT, Suspect Screening
Extract ion chromatogram
Tentative Identification
RT, ID Match?
Yes
ESI (+/-) Tandem MS
Full scan
Survey scan
MRM
Spectral library search, matching
Confirmation

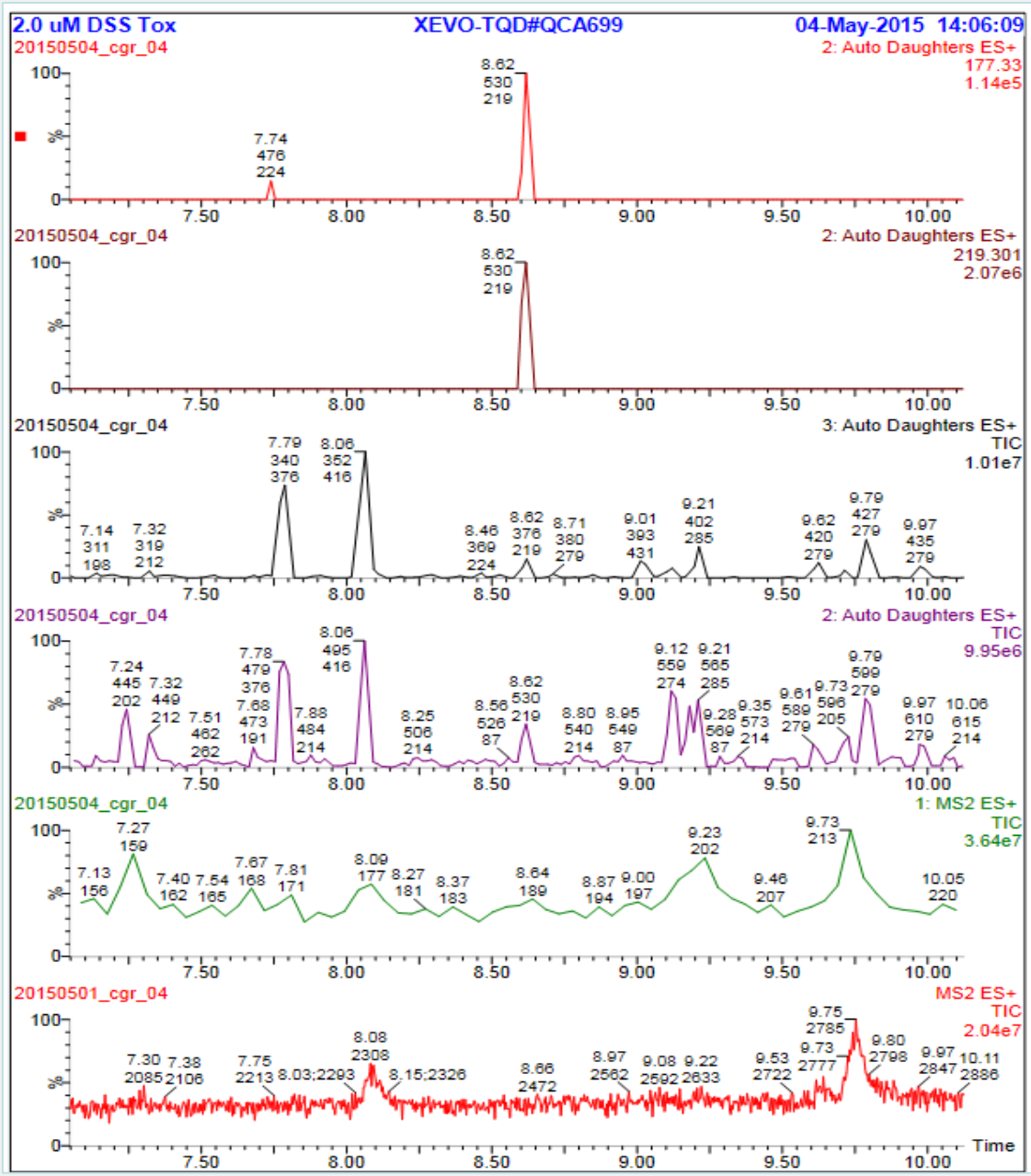
MS Parameters



MS Scan of Blinded Sample



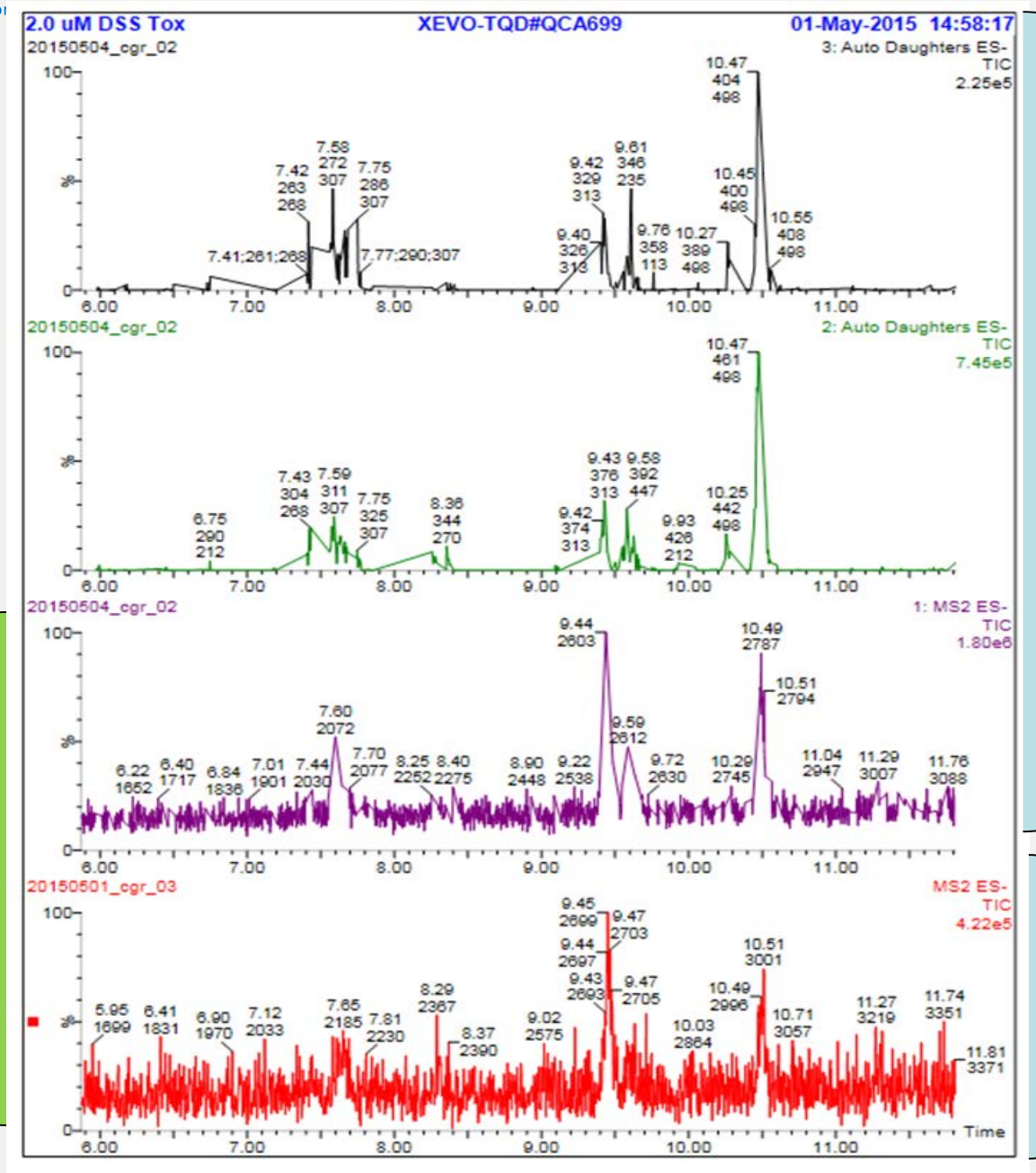
ESI Positive



+ Survey scan

+ MS scan

ESI Negative

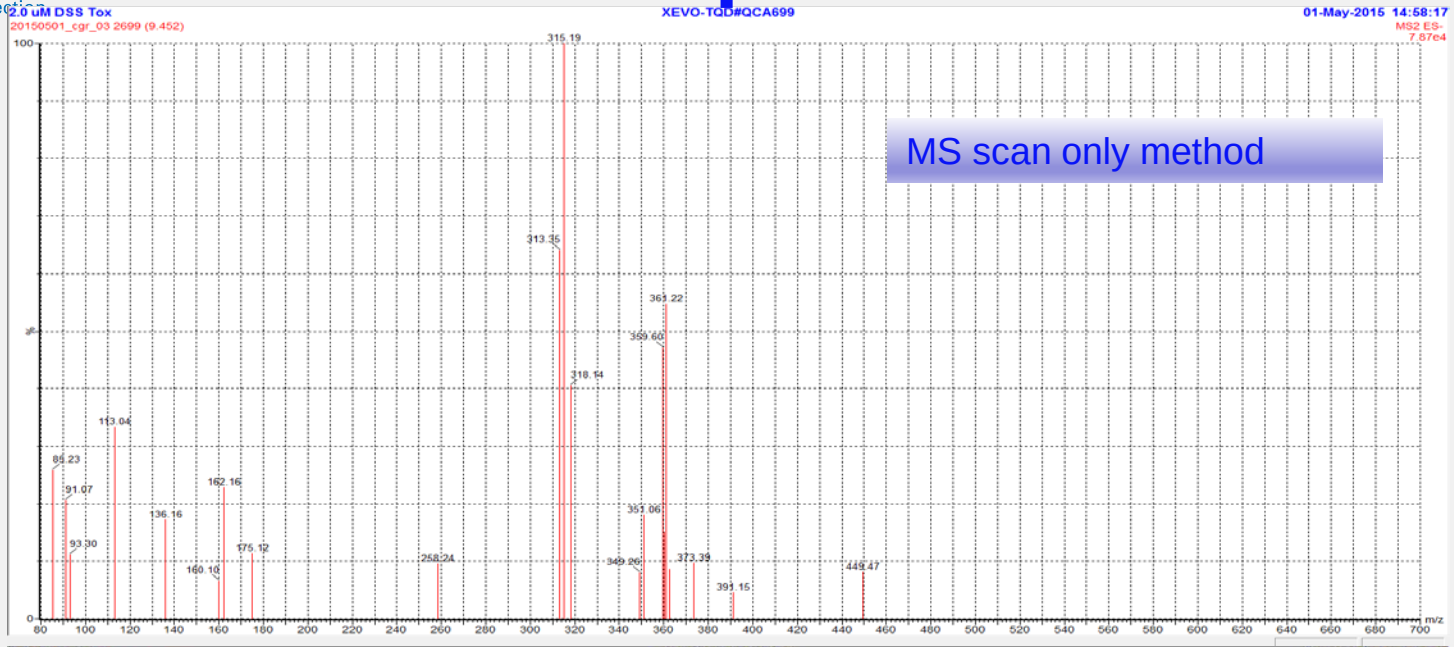


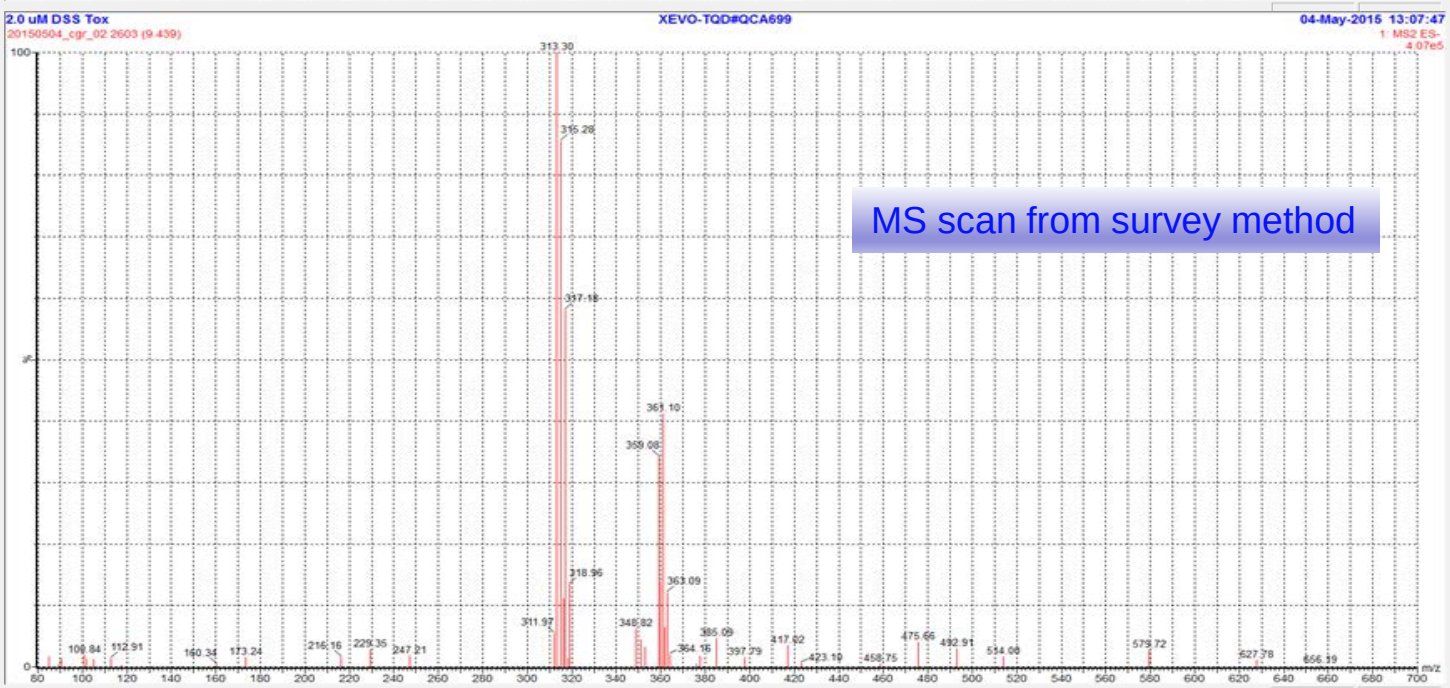
- Survey scan

MS scan

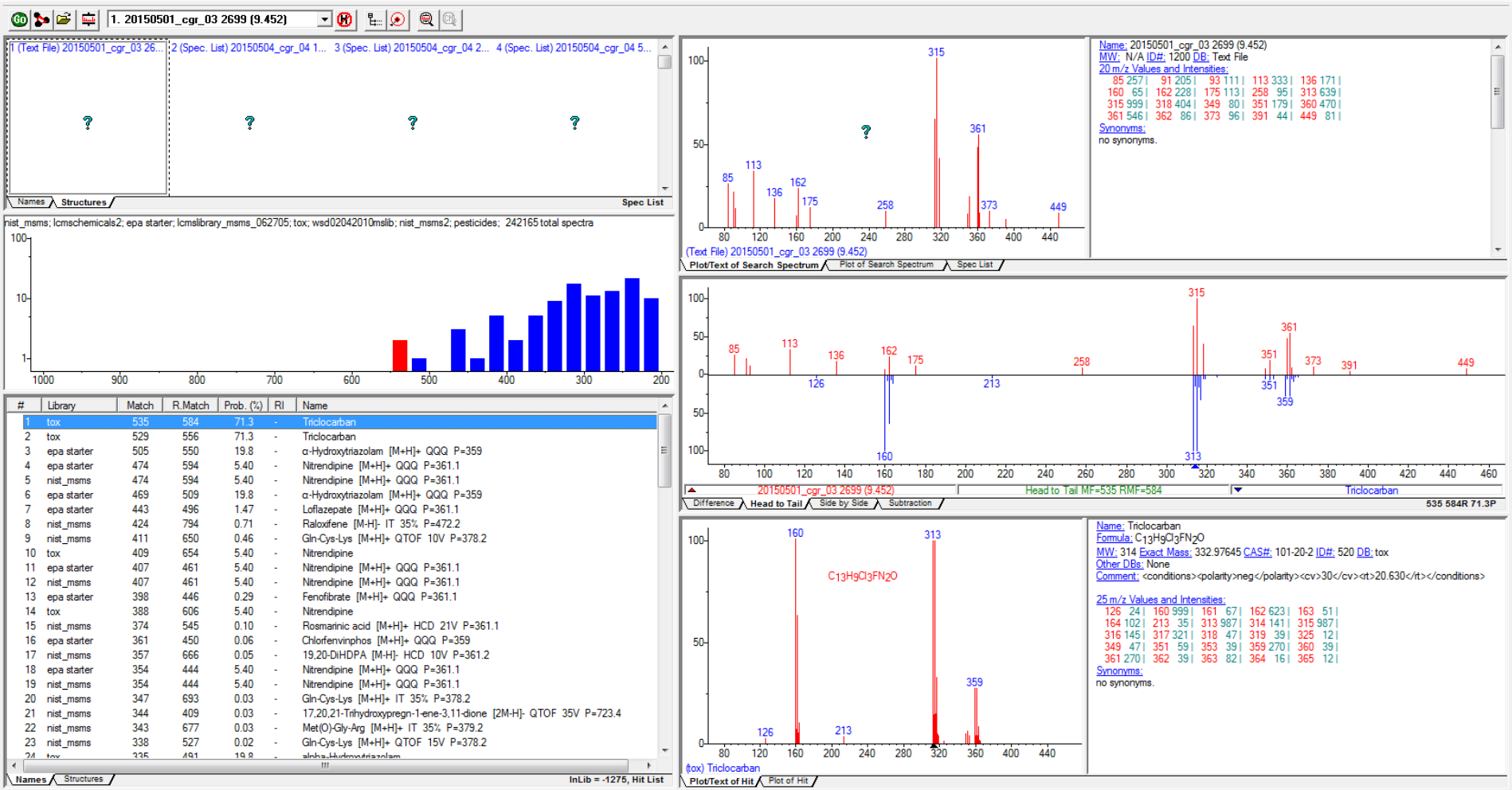
- MS scan

Mass Spectra





Library Search and Match



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Compounds from the blinded sample confirmed using LC-MS/MS with accurate mass from ToF, RT and spectral library matches.

Accurate mass	Retention Time (min) (+ or - ESI)	Compound name
266.163	0.67 (+)	Atenolol
162.1157	0.72 (+)	Nicotine
306.1041	1.35 (±)	Fluconazole
218.1055	1.36 (+)	Primidone
152.0473	1.77 (-)	Methyl paraben
351.0347	1.91 (±)	Meloxicam
206.1518	2.33 (+)	Ethanol, 2-[2-(2-Butoxyethoxy)ethoxy]-
221.1052	2.96 (+)	Carbofuran
276.1209	3.10 (+)	Triethyl citrate
201.079	3.23 (+)	Carbaryl
236.095	3.60 (+)	Carbamazepine
180.0786	4.15 (-)	Propyl paraben
191.131	4.43 (+)	Diethyltoluamide
222.0892	4.55 (+)	Diethyl phthalate
346.2144	4.92 (±)	Corticosterone
298.1933	5.82 (+)	Norethindrone
285.1365	6.36 (+)	Piperine
250.1205	6.93 (+)	Dipropyl phthalate
313.978	8.72 (±)	Triclocarban

Results/Conclusions

- Gradient successfully migrated from HPLC to UPLC decreasing run time from 45 to 25 minutes (linear relationship between RTs).
- While MS Full Scan mode was necessary to acquire for every sample, Survey Scan mode provided an easy way to automatically find fragment ions (threshold setting plays an important role in method development).
  - ~20 compounds were confirmed using this workflow (slide #11).
- TOF accurate masses were very helpful for finding peaks through extracted ion chromatograms.
  - Additional 30 compounds were tentatively identified but not confirmed due to a limited database.

Conclusions (*cont'd)*

- Commercial MS/MS libraries are very limited and are instrument – software specific.
- This process is not automated and can be very time consuming.
- LC-MS/MS has a place in non-targeted analysis/suspect screening.
  - Cannot do the job alone (accurate mass is important).
  - Will be useful to develop targeted methods allowing high resolution instruments to focus on more difficult aspects.
  - Excellent instrument for confirming with authentic standards.

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References

Wild CP "Complementing the Genome with an 'Exposome': The Outstanding Challenge of Environmental Exposure Measurement in Molecular Epidemiology." Cancer Epidemiology Biomarkers & Prevention 2005, 14(8):1847-1850.

Daughton CG "Biological Systems and Stressors: 'Toxicant Totality Tolerance Trajectory' - 4T's," U.S. EPA, Las Vegas, NV; illustrated poster, October 2002; available: http://www.epa.gov/nerlesd1/bios/daughton/stressor.pdf - first published in Daughton CG "'Emerging' Chemicals as Pollutants in the Environment: a 21st Century Perspective," Renewable Resources Journal, 2005, 23(4):6-23.

Acknowledgement

Thank You!

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