Drug Testing Advisory Board
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Update on Expanded Confirmatory Test

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This presentation was modified from its original format for 508 compliancy.

Expanded Confirmatory Test Technologies (ECTT) – Definition and Evaluation Projects

- Revised Mandatory Guidelines — Notice Fed. Regis. / Vol. 73, No. 228 / November 25, 2008
  - Allows expanded confirmatory test technologies (ECTT)
    - Liquid chromatography/mass spectrometry (LC/MS)
    - GC/MS/MS
    - LC/MS/MS
  - Two projects: I and II
    - I: ECTT forums
    - II: Comparison of GC/MS and LC/MS/MS

Mandatory Guidelines for Federal Workplace Drug Testing Programs

Federal Register Notice/ Vol. 73, No. 228 / November 25, 2008

- Section 11.13. What are the requirements for a confirmatory drug test?

  a) The analytical method used must combine chromatographic separation and mass spectrometric identification [e.g., GC/MS, liquid chromatography/mass spectrometry (LC/MS), GC/MS/MS, LC/MS/MS].

  b) A confirmatory drug test must be validated before the laboratory can use it to test specimens.

Mandatory Guidelines for Federal Workplace Drug Testing Programs

Federal Register Notice/ Vol. 73, No. 228 / November 25, 2008

- Section 11.14. What must an HHS certified laboratory do to validate a confirmatory drug test?
- An HHS-certified laboratory must demonstrate and document for each confirmatory drug test:
  - Linear range
  - Limit of Detection (LOD)
  - Limit of Quantitation (LOQ)
  - Carry over
  - Accuracy & precision (cutoff)
  - Accuracy & precision (40% cutoff)
  - Interfering substances
- An HHS-certified laboratory must re-verify its confirmatory drug test methods periodically or at least annually.

ECTT Project I: Expanded Confirmatory Test Technologies Forums

Forum Overview

- Objective
  - Convene stakeholders with MS/MS experience from the field of forensic toxicology to provide comments on issues and minimally acceptable criteria for the ECTT
- 3-hour format
  - Presentations from each of the 5 instrument manufacturers
  - Open discussion

Forum	Date	Topic
I	April 30	Chromatography
II	May 7	MS/MS Detector
III	May 14	Maintenance and validation
IV	TBD	Follow-up

Forum Participants

- RTI NLCP Staff
  - Michael Baylor
  - John Mitchell
  - Peter Stout
  - Jeri Ropero-Miller
  - Susan Crumpton
  - Jared Cooper
  - Erica Harbison
- Federal Representatives
  - Donna Bush
    - Division of Workplace Programs, Center for Substance Abuse Prevention, SAMHSA
  - Jason Schaff
    - FBI, Forensic Chemist, Chemistry Unit
  - Frances Scott
    - Sensors and Surveillance Portfolio Manager, National Institute of Justice

Forum Participants

Participant	Affiliation
Larry Bowers	Chief Science Officer, US Anti-Doping Agency
Jennifer Collins	Director, MedTox Laboratory
Anthony Constantino	President and CEO, DrugScan
Dennis Crouch	Associate Director for Sports Testing Services, Aegis Sciences
Richard Hilderbrand	Toxicology Consulting
Marilyn Huestis	Chief, Chemistry and Drug Metabolism, Intramural Research Program, NIDA, NIH
Graham Jones	Chief Toxicologist, Alberta Medical Examiner's Office
David Kuntz	Executive Director of Analytical Toxicology, Clinical Reference Laboratory
Rod McCutcheon	Chief Toxicologist, Bexar County Office of the Medical Examiner
Christine Moore	VP Toxicology, Research and Development, Immunoalysis Corp.
Timothy Robert	Laboratory Director, Aegis Sciences
Matthew Slawson	Assistant Director, Center for Human Toxicology

Forum Participates

- MS/MS manufacturers
  - Agilent Technologies
    - Doug McIntyre, John Hughes, MP George, Tom Gluodenis
  - Applied Biosystems
    - Tania Sasaki, Matthew Clabaugh
  - Thermo Fisher Scientific
    - Bori Shushan
  - Varian
    - Ed George
  - Waters Corporation
    - Donald Mason

Manual for Urine Laboratories (1.1.09)

Confirmatory Testing – Current GC/MS Minimal Acceptance Criteria

- Section L: Chromatography (L-8)
  - "Narrow, Gaussian-shaped, well separated from other ion peaks, and have an acceptable signal-to-noise (S/N) ratio"
  - Peak shape acceptability
    - Percent skew allowed, symmetry, criteria for peak width at half height, tailing/fronting calculated at 10% of peak height
  - Peak resolution acceptability
    - 10% valley/peak ratio

Manual for Urine Laboratories (1.1.09)

Confirmatory Testing – Current GC/MS Minimal Acceptance Criteria

- Section L: QC and drug positive specimens (L-12)
  - Ion peaks for analytes and internal standard (IS)
  - Retention Time (RT) and Relative Retention Time (RRT)
    - Ion ratio ranges
    - Outlined in the standard operating procedures (SOP)
    - Pre-determined for each batch
    - Uniformly applied to all specimens in all batches
    - Change requires recalibration and reinjection

Manual for Urine Laboratories (1.1.09)

Confirmatory Testing – Current GC/MS Minimal Acceptance Criteria

- Section L: QC and drug positive specimens (L-12)
  - Relative IS abundance (≥50% and <200%)
    - Outside established range — re-extract
    - Similar results — assume no IS addition error and report
  - Blind Controls
    - Not required
    - Inclusion — same acceptance criteria as for open controls

Manual for Urine Laboratories (1.1.2009)
Confirmatory Testing – Current GC/MS Minimal Acceptance Criteria

- Section L: Corrective action (L-13 & L-14)
  - Failed Acceptance Criteria
    - Must be stated in SOP to re-inject or re-extract
  - Potential Carryover in the subsequent specimen(s)
    - Must be stated in SOP
    - Determined in validation process
    - Analyte concentration cannot be greater than upper limit of linearity (ULOL)
    - Re-extraction of specimen immediately following a specimen with a concentration higher than the carryover limit

ECTT Project II: Comparison of GC/MS to LC/MS/MS Using Same Urine Workplace Drug Testing (WPDT) Samples

Objective

- Explicitly demonstrate that LC/MS/MS as a technology can produce results at least as valid as GC/MS

Study	Results
Linearity, precision, accuracy (LPA) w/ constructed urine samples	Reproducibility of RT Product ion ratios Responses
Interference (GC/MS & LC/MS/MS) and matrix effects (LC/MS/MS)	Interference as described in the Manual Matrix effect(s) between and within lots of urine
Archived, previously confirmed samples	Reproducibility of RT Product ion ratios Responses

Project Time Line

Date	Project
August 2008	New instrument
August-November 2008	Method Development Extraction & sample prep Instrument optimization Sample manufacture
November-February 2009	Running samples Morphine (MOR), codeine (COD), 6-acetylmorphine (6-AM), benzoylecgonine (BZE), phencyclidine (PCP), amphetamines (AMPs), and tetrahydrocannabinol carboxylic acid (THCA)
March 2009	Manuscript submitted to JAT for BZE, MOR, COD, and 6-AM
May 2009	1st manuscript accepted Working on second manuscript for PCP, AMPs, THCA

Validation Samples – Linearity, Precision, and Accuracy

- Manufactured in human urine
- RTI
  - 10 replicate analyses by LC/MS/MS
- 5 NLCP HHS-certified Laboratories
  - 5 replicate analyses by GC/MS
- Included past PT samples as controls

		Concentration of Drug (ng/mL)			
Sample #	% Cutoff	COD	MOR	6-AM	BZE
1	10	200	200	1	15
2	20	400	400	2	30
3	40	800	800	4	60
4	75	1500	1500	8	113
5	100	2000	2000	10	150
6	125	1500	2500	13	188
7	200	4000	4000	20	300
8	500	10000	10000	50	700
9	1000	20000	20000	100	1500
10	2000	40000	40000	200	3000

Validation Samples – Interference Study

- Interference Analytes: opioid structural analogs
- Target Analytes (40% cutoff)
- RTI: analyzed by LC/MS/MS
- 5 NLCP HHS-certified labs: analyzed by GC/MS

Drug	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Codeine	800	----	800	----	800	----
Morphine	800	----	800	----	800	----
6-AM	4	----	4	----	4	----
Hydrocodone	----	----	5000	5000	----	----
Hydromorphone	----	----	5000	5000	----	----
Oxycodone	----	----	----	----	5000	5000
Oxymorphone	----	----	----	----	5000	5000
Norcodeine	5000	5000	----	----	----	----

Validation Samples - LC/MS/MS Matrix Effect

- Matuszewski et al. (Anal Chem 2003)
- Analyzed by RTI
- 10 lots of urine
  - B/A = matrix effect
  - C/B = extraction efficiency
  - C/A = process efficiency (all x100 to convert to %)

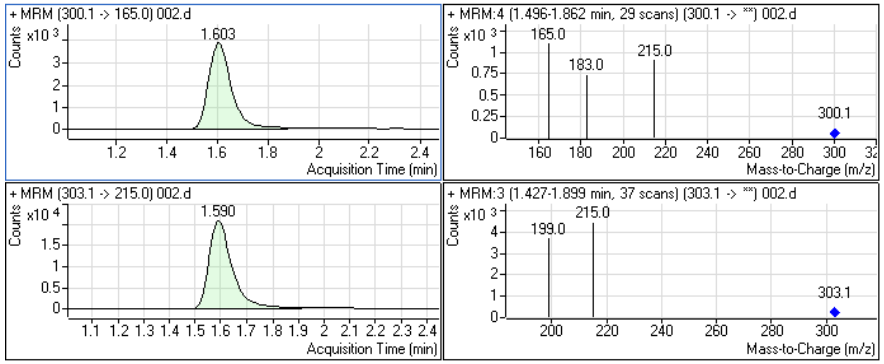
LC/MS/MS Ions

Drug	Precursor (*m/z*)	Product 1 (*m/z*)	Product 2 (*m/z*)	Product 3 (*m/z*)
COD	300.1	165	183	215
COD-d3	303.1	215	199	----
MOR	286.1	155	201	165
MOR-d3	289.0	201	157	----
6-AM	328.3	268	210	165
6-AM-d3	331.2	271	191	----
BZE	290.1	168	105	----
BZE-d3	293.1	171	105	----

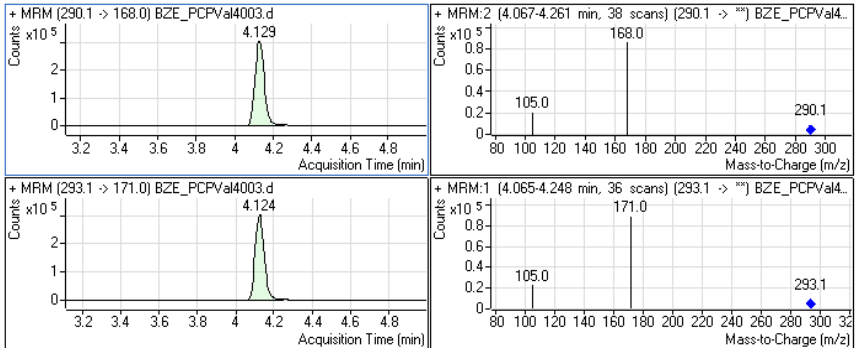
- d3 – deuterated internal standard

LC/MS/MS Chromatography and Ions

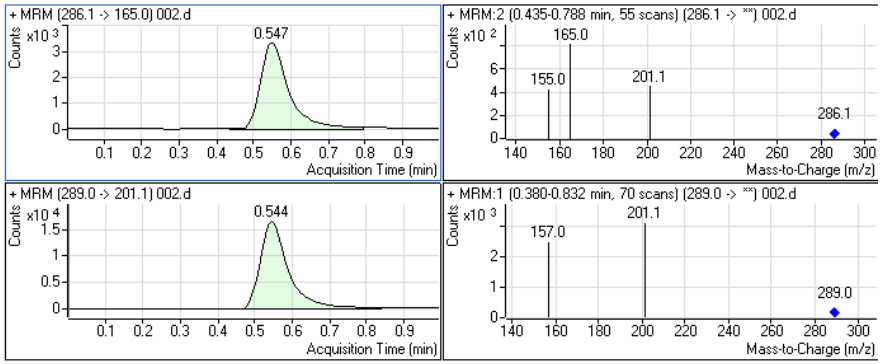
- Morphine



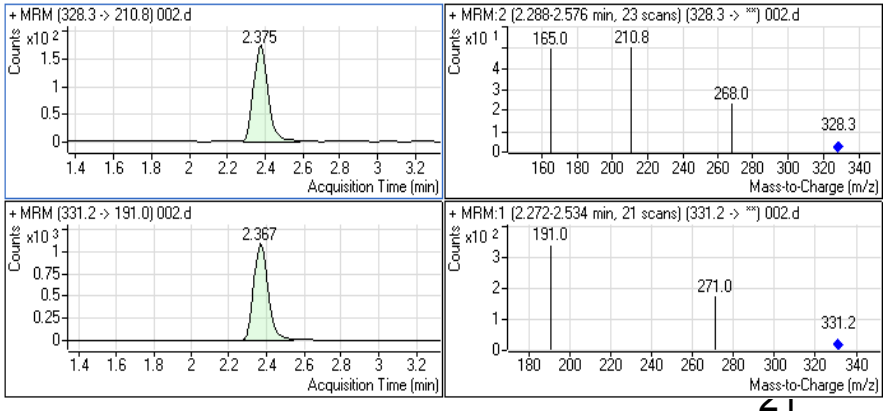
- Benzoylecgonine



- Codeine

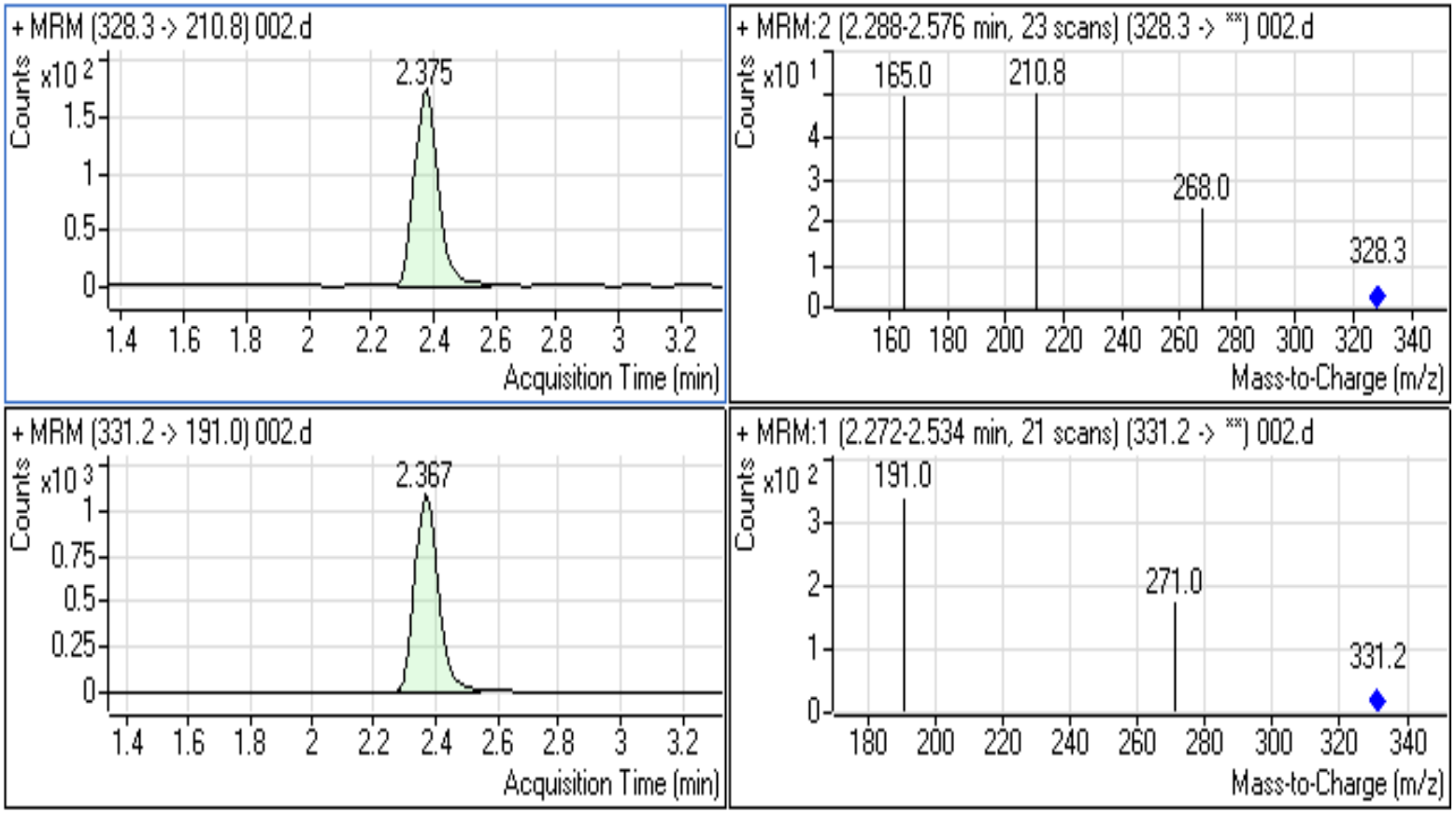


- 6-acetylmorphine



LC/MS/MS Chromatography and Ions

- 6-AM



LC/MS/MS Criteria Comparison

- Manufactured samples
- 100 analyses per target analyte
- Qualifier ratios for target analytes and IS consistent
- Average RT for target analytes and IS consistent

Drug	n	Average Target Qualifier Ratio	%CV	Average IS Qualifier Ratio	%CV	Average Target RT	%CV	Average IS RT	%CV
Morphine	100	49.6	4.81	53.2	2.05	0.54	0.90	0.54	1.02
Codeine	100	75.3	4.52	64.1	2.16	1.58	1.78	1.56	1.99
6-AM	100	38.8	11.6	47.8	7.51	2.36	0.24	2.36	0.24
BZE	100	25.4	1.09	24.8	1.06	4.12	0.21	4.12	0.21

Comparison Results

- Accuracy and precision of GC/MS and LC/MS/MS results were similar
- Linear range for LC/MS/MS using a 5-point calibration was broader than for GC/MS
- No significant interference from opioid structural analogs

Archived Sample Comparison

- GC/MS to LC/MS/MS
- Regression analysis unity — except COD
  - No significant difference — 6-AM & BZE
  - Significant difference — COD & MOR
- LC/MS/MS ion ratios & RTs — similar to manufactured samples

Drug	n	r^2	Regression Slope	t-test result (p-value >0.05)	Target Ion ratio	%CV	RT	%CV
	LC/MS/MS to GC/MS				LC/MS/MS			
Morphine	40	0.994	1.02	0.003	40.04	2.65	0.53	1.07
Codeine	21	0.993	0.679	0.003	71.21	1.81	1.57	1.65
6-AM	5	0.979	1.01	0.497	34.09	7.66	2.35	0.17
BZE	60	0.980	1.05	0.870	23.60	1.41	4.12	0.14

Where Do We Go From Here?

- Complete GC/MS and LC/MS/MS validation comparison for PCP, amphetamines and THCA
- Forum 4: summarize the information from previous forums
- Update NLCP Manual for laboratories and inspectors with inclusion of ECTT minimally accepted criteria
- Review comments and finalize revised NLCP Manual
- Continue to prepare for May 1, 2010 and beyond