INTEROFFICE COMMUNICATION

WASHINGTON STATE PATROL

TO: Dr. Fiona J. Couper, Toxicology Laboratory Division

FROM: Ms. Amanda Black, Toxicology Laboratory Division

SUBJECT: Doxepin/desmethyldoxepin confirmation/quantitation by LC-MS

DATE: August 19, 2015

Currently, the Laboratory does not have a formalized, validated method for confirmation/quantitation of the tricyclic antidepressants (TCAs) doxepin and metabolite desmethyldoxepin . In order to provide quantitative results for doxepin and desmethyldoxepin to our customers, a test method was created that uses an extraction procedure adapted from the United Chemical Technologies (UCT) Solid Phase Extraction Applications Manual and analysis by liquid chromatography – mass spectrometry (LC-MS).

A comprehensive liquid chromatography – tandem mass spectrometry (LC-MSMS) test method for confirmation/quantitation of TCAs (including doxepin/desmethyldoxepin) is now in the development phase. This new method is expected to be validated and implemented into the Laboratory's routine testing at some time in 2015.

I have reviewed the UCT/LC-MS sample preparation procedure, acquisition method, and the calibration/control performance for four representative test batches (140821, 141021RF, 150206CJKH and 150609). I find this method to be fit-for-purpose and request that it be considered for use in confirmation/quantitation of doxepin and metabolite desmethyldoxepin, pending the approval and implementation of the forthcoming TCA LC-MSMS confirmation assay.

Copies of the extraction procedure (including UCT), acquisition method and the four test batches are attached for your review. A list of recent cases is attached, with the reported doxepin results obtained from this test method.

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AB:ab

cc: Mr. Brian Capron, Toxicology Laboratory Division
Dr. Brianna Peterson, Toxicology Laboratory Division
Ms. Brianne O'Reilly, Toxicology Laboratory Division
Ms. Lisa Noble, Toxicology Laboratory Division

Reviewed procedure, method, batches & cases (including 3 dilution cases). This method continues to be fit-for-purpose and is acceptable for the confirmation and quantitation of doxepin and nordoxepin.

8-21-15

Doxepin/Desmethyldoxepin Confirmation in Biological Specimens by LC-MS

1. Add 1 mL of DI H_2O to each tube
2. Add 0.5 mL of blood to each tube for the calibrators and controls
3. Follow the table below for the spiking of calibrators and controls:

Level & Concentration	Working solution	Amount
Cal 1 – 50 ng/mL	1 ng/µL	25 µL
Cal 2 – 100 ng/mL	1 ng/µL	50 µL
Cal 3 – 250 ng/mL	1 ng/µL	125 µL
Cal 4 – 500 ng/mL	10 ng/µL	25 µL
Cal 5 – 1000 ng/mL	10 ng/µL	50 µL
Negative		
Control 1 – 150 ng/mL	1 ng/µL	75 µL
Control 2 – 800 ng/mL	10 ng/µL	40 µL

4. Add 125 µL of ISTD, Protriptyline 1ng/µL (250 ng/mL final concentration).
5. Add 2 mL of 0.1 M phosphate buffer (pH 6.0) to each tube
6. Vortex the tubes and centrifuge for 10 minutes at 3000 rpm
7. Condition the UCT Columns
    a. 3 mL Methanol
    b. 3 mL Deionized water
    c. 1 mL 0.1 M phosphate buffer pH 6.0
8. Apply samples to their columns
9. Wash columns
    d. 2 mL DI H_2O
    e. 1 mL 0.1 M acetic acid
    f. 3 mL Methanol
10. Dry columns for 10 minutes under full vacuum
11. Elute samples in to 10 mL conical tubes with 3 mL DCM:IPA:NH_4OH (78:20:2)
12. Evaporate the extracts to dryness in a 50°C turbo vap.
13. Reconstitute the extracts with 100 µL of mobile phase (75% 0.1% formic acid/25% ACN). Vortex. Centrifuge for 1 minute.
14. Transfer to autosampler vials for analysis.

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LC/MS Method
Runs under method DOXEPIN

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8-20-15

Method Information

Method: C:\CHEM32\1\METHODS\DOXEPIN.M
Modified: 8/20/2014 at 10:25:17 AM

Doxepin Confirmation by LC-MS
Agilent Zorbax Eclipse Plus C18
(75 x 4.6 mm ID, 3.5um)
0.1% Formic Acid/ACN Gradient

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method: C:\CHEM32\1\METHODS\DOXEPIN.M
Modified on: 8/20/2014 at 10:25:17 AM

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Agilent 1100/1200 Quaternary Pump 1
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Control
Column Flow : 0.500 ml/min
Stoptime : 9.00 min
Posttime : 5.00 min

Solvents
Solvent A : 75.0 % (0.1 % Formic Acid in DI H2O)
Solvent B : Off
Solvent C : 25.0 % (ACN)
Solvent D : Off

PressureLimits
Minimum Pressure : 0 bar
Maximum Pressure : 400 bar

Auxiliary
Maximal Flow Ramp : 100.00 ml/min^2
Primary Channel : Auto
Compressibility : 100*10^-6/bar
Minimal Stroke : Auto

Store Parameters
Store Ratio A : Yes
Store Ratio B : Yes
Store Ratio C : Yes
Store Ratio D : Yes
Store Flow : Yes
Store Pressure : Yes

Timetable

Time	Solv.B	Solv.C	Solv.D	Flow	Pressure
0.00	0.0	25.0	0.0	0.500	400
8.00	0.0	60.0	0.0	0.500	400

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Agilent 1100/1200 Diode Array Detector 1
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Signals

Signal	Store	Signal,Bw	Reference,Bw	[nm]
A:	No	254 4	360 100	
B:	No	254 16	360 100	
C:	No	210 8	360 100	
D:	No	230 16	360 100	
E:	No	280 16	360 100	

Spectrum
Store Spectra : None

Time

method: C:\CHEM32\1\METHODS\DOXEPIN.M
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Stoptime : As pump
Posttime : Off

Required Lamps
UV lamp required : No
Vis lamp required : No

Autobalance
Prerun balancing : No
Postrun balancing : No
Margin for negative Absorbance: 100 mAU

Peakwidth : > 0.1 min
Slit : 4 nm

Analog Outputs
Zero offset ana. out. 1: 5 %
Zero offset ana. out. 2: 5 %
Attenuation ana. out. 1: 1000 mAU
Attenuation ana. out. 2: 1000 mAU

Timetable is empty

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Mass Spectrometer Detector

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General Information
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Use MSD : Enabled
Tune File : atunes.tun
StopTime : 8.00
Time Filter : Enabled
Data Storage : Condensed
Peakwidth : 0.05 min
Fast Scan : Disabled
Fast Scan Data Reconstruction: Disabled
Polarity Switch Delay : 300 ms
Ionization Switch Delay : 50 ms

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Signals

[Signal 1]

Ionization Mode : API-ES
Polarity : Positive
Fragmentor Ramp : Not Applicable
Percent Cycle Time : 100.00 %
Sim On Target Mass : Disabled

Sim Parameters

Time (min)	Group Name	SIM Ion	Frag-mentor	Gain EMV	Actual Dwell	Compound Name	ISTD
0.00	doxepin/Pro	107.00	220	1.0	47		1

method: C:\CHEM32\1\METHODS\DOXEPIN.M
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191.00	240	47	0
235.00	200	47	0
264.00	110	47	1
266.00	90	47	0
280.00	100	47	0

[Signal 2]

Not Active

[Signal 3]

Not Active

[Signal 4]

Not Active

Spray Chamber
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[MSZones]

Gas Temp	: 350 C	maximum 350 C
DryingGas	: 13.0 l/min	maximum 13.0 l/min
Neb Pres	: 40 psig	maximum 60 psig
VCap (Positive)	: 4000 V	
VCap (Negative)	: 4000 V	

[Time Table]

Time Table is empty.

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END OF MS ACQUISITION PARAMETERS

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FIA Series
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FIA Series in this Method : Disabled

Time Setting
Time between Injections : 0.73 min
Injection Loop Flush Time : 0.17 min

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Agilent 1100 Autosampler 1
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Injection
Injection Mode : Needle Wash

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Injector volume	:	2.00 µl
Wash Vial	:	81
Optimization	:	none

Auxiliary

Drawspeed	:	100 µl/min
Ejectspeed	:	100 µl/min
Draw position	:	0.0 mm

Time

Stoptime	:	As Pump
Posttime	:	Off

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Agilent 1100/1200 Column Thermostat 1

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Temperature settings

Left temperature	:	40.0°C
Right temperature	:	Same as left
Enable analysis	:	When Temp. is within setpoint +/- 0.8°C
Store left temperature	:	Yes
Store right temperature	:	No

Time

Stoptime	:	As pump
Posttime	:	Off

Column Switching Valve : Column 1

Timetable is empty

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