[APPLICATION NOTE]

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EVALUATION OF A LC/MS METHOD TO SCREEN FOR DRUGS IN POST-MORTEM WHOLE BLOOD SPECIMENS

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INTRODUCTION

Toxicological screening of post-mortem whole blood specimens is routinely performed to help determine the cause of death. Traditionally, screening is performed using either GC/MS, immunoassays, or HPLC with UV detection. Immunoassays can be cost prohibitive and often suffer from cross reactivity . HPLC with UV detection often lacks specificity and sensitivity. GC/MS requires extensive sample preparation and is not suitable for thermolabile compounds. An LC/MS approach can potentially overcome many of these limitations and provide a more thorough screening solution. The aim of the work described in this application note was to compare a new LC/MS screening method to an existing GC/MS method. A key element of the study was to evaluate the efficiency of ChromaLynx™ deconvolution and the library searching software utilized in the LC/MS screening method.

OVERVIEW OF LC/MS METHODOLOGY

The described method utilizes full scan mass spectra recorded at multiple cone voltages using in-source collision induced dissociation (CID). Using a full scan mass spectra results in a more extensive and thorough toxicological analysis when compared to MS/MS based targeted screening methods. Specimens are analyzed under multiple fragmentation conditions. The degree of fragmentation is controlled by varying the cone voltage in the mass spectrometer. Sample spectra are then compared to library spectra which have been acquired under the same conditions. A key element in this approach is a unique chromatographic data processing software program: ChromaLynx™.

ChromaLynx™ performs two key functions:

- It uses a unique algorithm to detects peaks in a chromatogram. This peak detection process enables detection and location of low intensity and closely eluting peaks that could be missed on a manual visual inspection. Deconvoluted mass spectra of these peaks are then automatically compared to library mass spectra.
- ChromaLynx™ also produces a list of "candidate" components and applies confidence factors to the identification.

Retention time data is also used in component identification process which increases confidence in the library search results. The results are then displayed in an easy to view browser format. The processed data browser is fully customizable and can contain an overlayed chromatogram of all functions, spectral information for every component and its corresponding library hit, a list of identified candidates, and other relevant information (Figure 1).

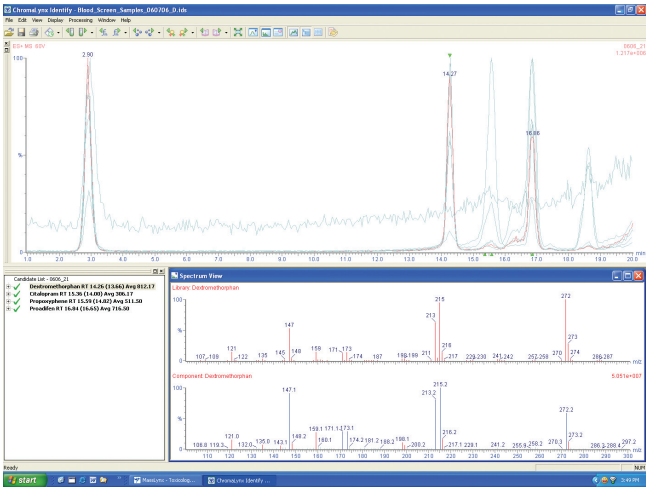

Figure 1 : ChromaLynx™ browser illustrating chromatograms recorded at different functions in the top window. Bottom left window lists compounds identified by ChromaLynx™ library search. Bottom right window compares mass spectra of a component with a library match

EXPERIMENTAL

The following sample preparation protocol was used for whole blood post-mortem samples. 20 µL of a 50 µg/mL of Proadifen internal standard solution was added to 2.5 mL whole blood. Three mL of 100 nM sodium acetate buffer, pH 4.5 was added and the sample centrifuged at 3000 RPM for 20 minutes. The supernatant was then further prepared using the following SPE protocol.

SPE PROCESS

a. Condition cartridge with 2 mL ethyl acetate.
b. Condition cartridge with 2 mL methanol.
c. Dry cartridge for 10 seconds.
d. Load sample onto cartridge and let flow through at 1.0 mL/minute.
e. Wash with 2 mL potassium carbonate buffer, pH 9.0.
f. Wash with 2 mL DI water.
g. Dry for 10 minutes.
h. Elute with 2 mL [98:2] ethyl acetate:ammonium hydroxide solution.
i. Dry down under nitrogen.
j. Reconstitute with 200 µL acetonitrile.

Sample was then analysed using LC/MS method as below.

HPLC SEPARATION

A generic HPLC separation method was used to both generate library mass spectra and analyze post mortem blood samples. This enables retention time to be used in the library search process. Retention time filters can be automatically used by ChromaLynx™. HPLC separation was performed on a Waters Alliance® HPLC 2795.

Column: Waters XTerra®, MS C_{18}, 2.1 x 150 mm, 3.5 µm
Injection volume: 50 µL
Chromatographic run time 26 minutes

LC GRADIENT

Time (Min)	Mobile Phase A	Mobile Phase B	Flow (mL/min)	Curve
0	95	5	0.2	1
2	95	5	0.2	6
16	10	90	0.2	6
20	95	5	0.2	6
26	95	5	0.2	6

MASS SPECTROMETRY

A Waters Quattro micro™ API mass spectrometer was used in combination with the Waters Alliance® 2795 LC system. Electrospray ionisation was used under the following conditions:
Capillary Voltage: 3.2 kV
Source Temperature: 120 °C
Desolvation Temperature: 350 °C

Mass spectra of the whole blood samples were recorded using 7 different cone voltage functions. In this analysis 6 spectra were recorded in positive ion mode at cone voltages of 15, 30, 45, 60, 75 and 90 volts. In addition, mass spectra were also recorded at a negative ion voltage of 30 volts.

RESULTS

One hundred and twenty five post mortem blood samples were analyzed using the method described above. Results are shown in Tables 1 and 2. In many cases the GC/MS and LC/MS results were comparable. In the majority of cases, the LC/MS method was able to identify more analytes than the GC/MS method. Examples of these samples are given in Table 2.

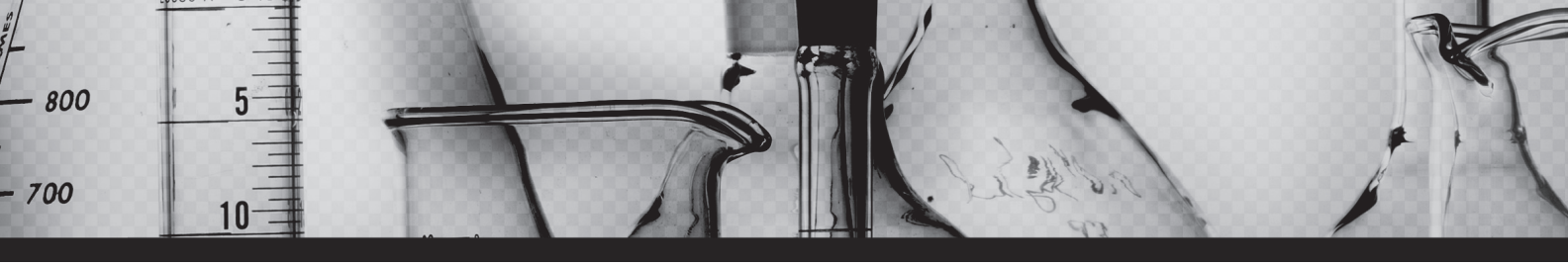

Table 1.

Sample	GC/MS Results	LC/MS Results	Confirmed Results
40007149	Amitriptyline Nortriptyline	Amitriptyline Nortriptyline	Amitriptyline Nortriptyline
40007687	Cotinine Lidocaine	Cotinine Lidocaine	Cotinine Lidocaine
40008731	Olanzapine Paroxetine	Olanzapine Paroxetine	Olanzapine Paroxetine
40008703	Bupropion Sertraline Desmethylsertraine*	Bupropion Sertraline	Bupropion Sertraline Desmethylsertraine*
40014159	Lamotrigine Diphenylhydramine	Lamotrigine Diphenylhydramine	Lamotrigine Diphenylhydramine
40014439	Cotinine Lidocaine	Cotinine Lidocaine	Cotinine Lidocaine
40014416	Chlorpromazine Sertraline	Chlorpromazine Sertraline	Chlorpromazine Sertraline

*Desmethylsertraline is currently not in the library used for these experiments, therefore it could not be positively identified by the LC/MS method. The library is fully user appendable, so the compound can easily be added.

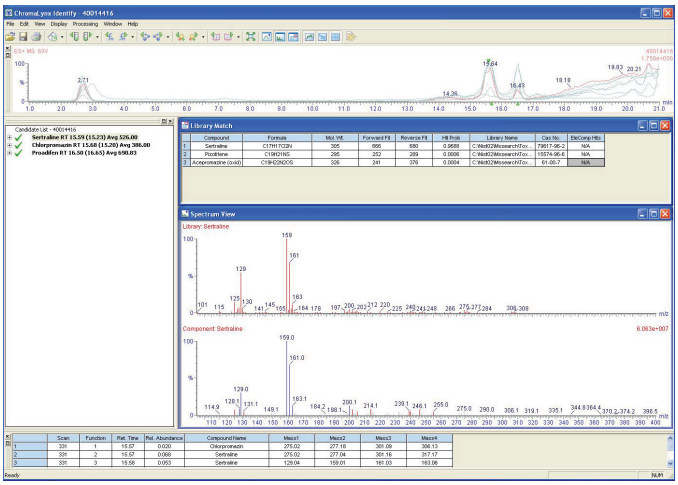

Figure 2: ChromaLynx™ Browser showing results from 40014416 (from Table 1), illustrating identification of Sertraline and Chlorpramazin.

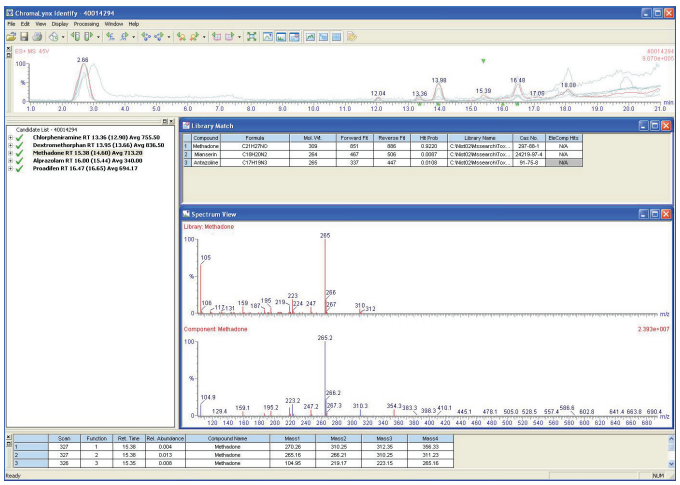

Figure 3: ChromaLynx™ browser showing results from sample 40014294 (Table 2), illustrating identification of chlorpheniramine, dextromethorphan, methadone and alprozolam. Note: The LC/MS method identified two compounds, methadone and alprazolam, that were not identified by the GC/MS screening method.

CONCLUSION

The evaluated library* is comprehensive and includes the majority of compounds encountered in forensic toxicology laboratories in the USA and Europe. The automated software provided is easy to use and the ChromaLynx™ deconvolution process is very effective. The LC/MS method identified more components than the GC/MS method, in particular the LC/MS method was more effective at identifying polar and basic drugs such as benzodiazepines and opiates. The method uses a full scan spectra approach and therefore enables the use of lower cost single quadrupole technology. LC/MS technology provides an excellent additional tool for toxicology screening

*Library developed by Calmette Hospital, Lille, France

Table 2. Illustrating sample analysis in which the LC/MS screening method identified several compounds that were missed by the GC/MS screening method.

Sample Number	GC/MS Results	LC/MS Results	Comfirmed Results
40007649	Diphenhydramine	Diphenhydramine Fentanyl	Diphenhydramine Fentanyl
40008528	Negative	Cocaine Methadone Diazepam	Cocaine Methadone Diazepam
40014237	Negative	Diazepam Nordiazepam	Diazepam Nordiazepam
40007175	Acetaminophen	Acetaminophen Propoxyphene	Acetaminophen Propoxyphene
40009423	Promethazine	Promethazine Diazepam Nordiazepam Citalopram Desmethylcitalopram	Promethazine Diazepam Nordiazepam Citalopram Desmethylcitalopram
40014294	Chlorpheniramine Dextromethorphan	Chlorpheniramine Dextromethorphan Methadone Alprazolam	Chlorpheniramine Dextromethorphan Methadone Alprazolam
4007302	Negative	Amiodarone Desmethylamiodarone	Amiodarone Desmethylamiodarone

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