

LC-MS ADME/PK support in drug discovery

James J. Wang

MethylGene Inc., 7220 Frederick-Banting St., St-Laurent, QC H4S 2A1

LC-MS has become the must-have technology in supporting ADME/PK studies in drug discovery. This presentation will focus on the application of LC-MS in PK sample analysis, metabolic stability ranking and metabolite identification. Real-life examples from drug discovery studies at MethylGene would be used.

Agilent HPLC systems coupled with AB Sciex mass spectrometers were used for separation and detection. The Analyst software was used for data acquisition and quantitation. The Lightsight and MS Fragmenter software were used for elucidation of metabolite structures.

Case 1. An HPLC-MS/MS method to simultaneously determine MS-275 and M1 in rat plasma samples developed for PK studies.

100 μ L plasma was loaded onto Oasis HBL 3 cc cartridges. The sample eluted from the cartridge was reconstituted and injected onto a C18 analytical column (Keystone AQUASIL C18, 50x2.1 mm, 3 μ m) using an Agilent 1100 HPLC and detected by an AB SCIEX API2000 mass spectrometer. The analytical elution was isocratic (A/B: 65/35; A: H₂O with 0.1% formic acid, B: methanol with 0.1% formic acid) at a flow rate of 300 μ L/min. MRM in positive mode was used for MS detection (MS-275, 377 > 149; M1, 287 > 149; IS, a structural analogue, 405 > 297). The assay was established in the range of 0.01 - 50 μ M for both MS-275 and M1. Assay validation achieved satisfactory accuracy, precision and specificity. PK parameters were calculated from the rat PK study.

Case 2. Metabolic stability in liver microsomes

Clearance is the most important pharmacokinetic parameter. Both dosing frequency and dose amount depend on clearance. Considerable efforts have been made to correlate in vitro and in vivo clearance, so in vitro screening can be used to filter discovery compounds.

Test compounds (2 μ M) were incubated with human and rat liver microsome (0.6 mg/mL protein) at 37°C for 60 minutes. Incubation was quenched with cold acetonitrile. Each four individually incubated compounds were pooled before sample analysis. Samples were injected onto an Aquasil C18 column (50 x 2.1 mm). Separation was done with a generic gradient elution using an Agilent 1100 HPLC. Mass spectrometric detection was done with an API2000 instrument. Both incubation and sample analysis were carried out with 96-well plates. Compounds were classified into three categories: high, medium and low metabolic stability. Assays were successfully used in metabolic stability screening.

Case 3. Metabolite identification and structure elucidation of a Histone Deacetylase Inhibitor MG89911 Using Information Dependent Acquisition

MG89911 is an HDAC inhibitor. The compound (at 12.5 μ M) was incubated with dog liver microsomes (1.0 mg/mL protein) at 37C for 60 minutes. The incubation was stopped with cold acetonitrile. The mixture was analyzed on an Agilent 1200 HPLC with a Gemini Phenyl 150 mm x 2.1 mm column (5 μ m) using a gradient elution. The elution was detected with an AB Sciex 3200 QTRAP mass spectrometer. IDA methods were used with MRM, NL, PC and EMS as survey scans. Major metabolites were found to be formed by O-dealkylation, N-dealkylation, Oxidation and O-dealkylation plus oxidation.

“Sample Preparation in Method Development: A Streamlined Approach for Choosing the Right Technique for Optimal Detection”

By Victor Vandell, Ph.D., Lee Williams, Ph.D.

Biotage, 10430 Harris Oaks Blvd., Suite C., Charlotte, NC 28269, USA

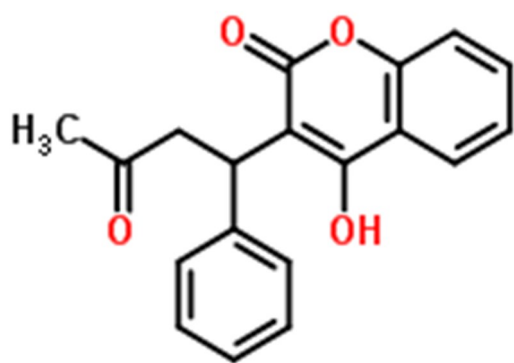
Biotage, GB Limited, Dyffryn Business Park, Ystrad Mynach, Mid Glamorgan, CF82 7RJ, UK.

Introduction:

Sample preparation during method development for new compounds of interest can be challenging for analytical scientist when trying to employ ultra sensitive methods of detection like Atmospheric Pressure Ionization Mass Spectrometry (API-MS). Sample analysis can be further complicated by the need for chromatographic separation using High Pressure Liquid Chromatography (HPLC). Qualitative and quantitative method development can be achieved at unprecedented lower limits of detection as a direct result of today’s Liquid Chromatographic Mass Spectrometry (LC-MS) systems. More often than not, some form of sample clean-up (i.e., Solid Phase Extraction, Supported Liquid Extraction) from a complex matrix (e.g. plasma, urine, serum, waste water) prior to LCMS analysis is necessary in order to achieve the desired levels of sensitivity for the target analytes. While most would rather avoid sample clean-up steps, it is often unavoidable and can be the limiting factor for optimal detection method performance. Hence, the thought process governing the “what”, “why” and “how” of sample prep begins. This presentation will address the sample prep thought process and aid the method development scientist in narrowing down viable options for dealing with samples in complex matrices while employing generic methodologies as a guide tool towards optimizing your final methods.

Experimental Procedure: Analyte Assessment

The evaluation of the physical and chemical properties of an analyte or class of analytes to aid in the development of analytical methods for qualitative and quantitative analysis is key to the success of finding an optimized method. Several basic properties should be initially researched prior to method development. These properties include pKa, log P, solubility, molecular weight and other relevant chemical structure properties. These properties aid in deciding on solvents to test in developing chromatographic techniques (e.g. HPLC) and choosing an effective detector (e.g. MS/MS). These properties also help in deciding the type of sample clean-up technique (e.g. solid phase extraction, supported liquid extraction, protein precipitation, etc) that could be used



- Warfarin
- Acidic (pKa= 4.5)
- Log P=3.1
- Solubility: ACN, MeOH
- Molecular Wt.: 308.3
- Density: 1.308 g/mL
- Matrix: Biological

for complex matrices. An example of an analyte assessment is shown in Figure 1.

Warfarin is just one of several compounds to be discussed in this presentation. A comparison and contrast of different classes of compounds will be presented to demonstrate the versatility of following a standard assessment process. Some of the types of compounds to be discussed are shown in Figure 2.

Figure 1. Critical chemical/physical parameter assessment for Warfarin

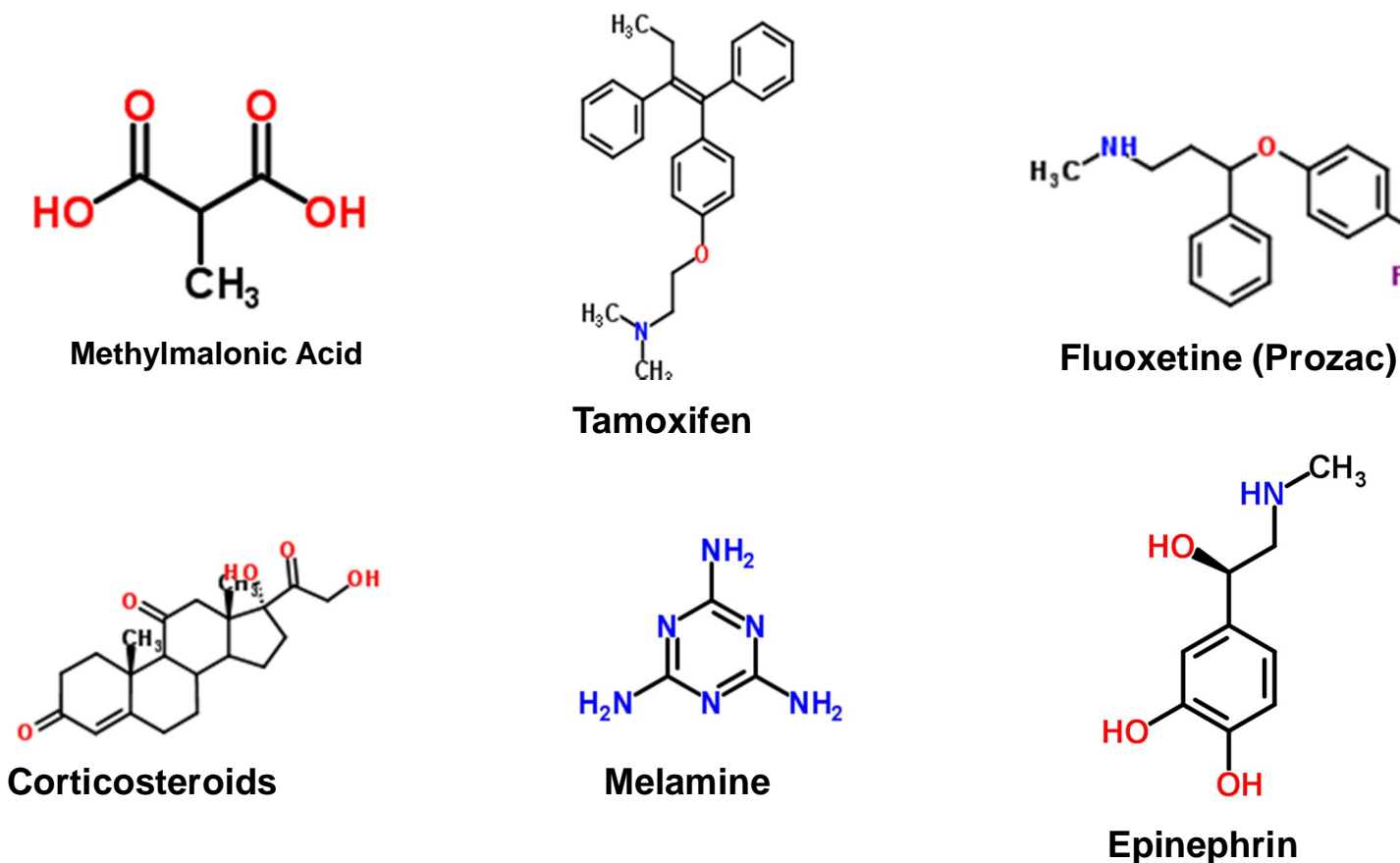


Figure 2. Examples of target analytes where analytical methodologies have been developed in Biotage laboratories

Results:

The successful development of optimized analytical methods for a variety of compound classes is the ideal scenario for the analytical chemist tasked with “finding a way”. The effectiveness of sophisticated analytical instrumentation like the Waters Quattro Premier XE triple quadrupole and Applied Biosystems 4000-Qtrap triple quadrupole in our laboratories is directly related to the sample preparation.

Target analyte detection at ultralow sensitivities can be dramatically affected by the wrong choice in sample clean-up during sample preparation. Figure 3 is an example of the LC-MSMS analysis of a suite of catecholamine analytes in plasma at subnanogram detection levels (0.8ng/ml). Sample preparation using a weak cation exchange mechanism (chromatogram A) affords detection of targets compared to protein precipitation as the sample preparation method (chromatogram B).

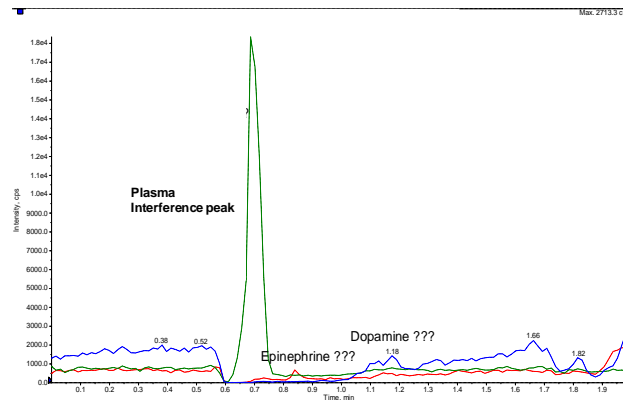
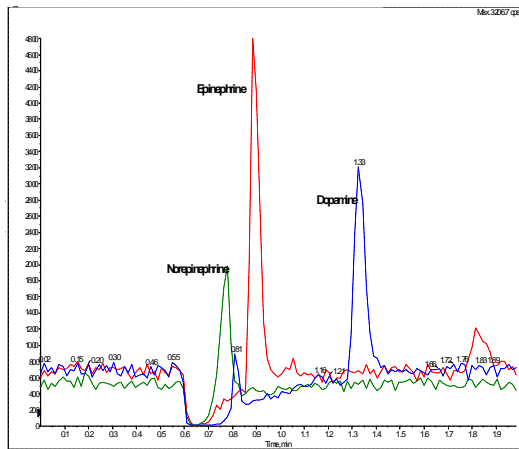


Figure 3. LCMSMS of low levels of catecholamines in plasma. Sample prep of weak cation exchange (A) vs. protein precipitation (B).

Comparative assessment of sample preparation techniques that are similar in principal can aid in increasing the efficiency of an analytical method during development. A side by side comparison of limit of detection results can be viewed in Figure 4 which shows the total ion chromatograms for the analysis of a suite of β -blocker compounds using a liquid-liquid

extraction (LLE) clean-up procedure and supported liquid extraction (SLE). Both techniques are similar in their application principals but SLE is more efficient and affords the option of automation as a possible route in final method implementation. Comparative LLE and SLE data extracted from the TICs for the β -blockers in Figure 4 and a group of NSAIDs from another study is shown in Table 1.

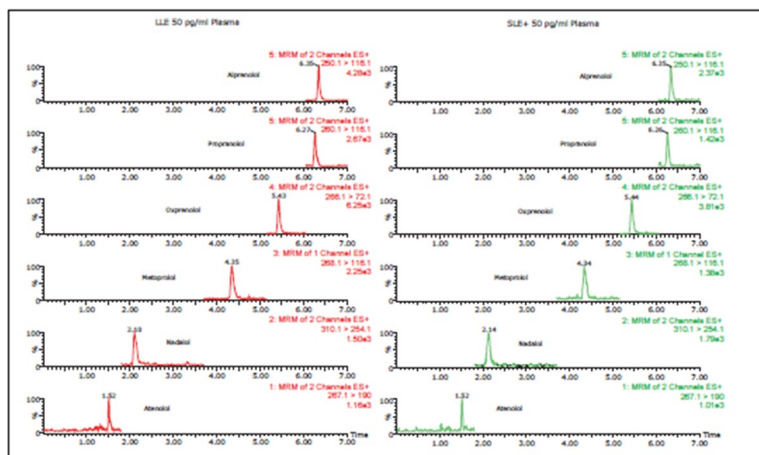


Figure 4. Total Ion Chromatograms (TICs) for β -blocker at the LOD prepared from LLE and SLE

	100	100	200	200
Atenolol	100	100	200	200
Nadolol	50	50*	50	50*
Metoprolol	<50 (25)	<50 (20)	<50 (25)	<50 (20)
Oxprenolol	<50 (25)	<50 (15)	<50 (25)	<50 (15)
Propranolol	<50 (25)	<50 (15)	<50 (25)	<50 (15)
Alprenolol	<50 (25)	<50 (15)	<50 (25)	<50 (15)

Table 2. LLE/SLE+ NSAID LoQ comparison.

Analyte	Plasma		Urine	
	SLE+ LoQ (ng/mL)	LLE LoQ (ng/mL)	SLE+ LoQ (ng/mL)	LLE LoQ (ng/mL)
Acetaminophen	5	10	-	-
Sulindac	<1 (0.5)	<1 (0.5)	<1 (0.5)	<1 (0.3)
Ketoprofen	3	2	3	2
Naproxen	10	10	10	5
Flurbiprofen	10	10	10	5
Indomethacin	2	2	3	2
Diclofenac	1 (0.5)	1 (0.5)	1 (0.4)	1 (0.2)
Mefenamic Acid	<1 (0.2)	<1 (0.2)	<1 (0.1)	<1 (0.05)

*RSD > 10%.
- acetaminophen detected in urine.
Parentheses - estimated levels based on S/N at previous level

Conclusion:

Method development for analytes of interest can be a straightforward process if the chemistry of the molecule is understood. Standard information can be gathered from a variety of sources to help inform the analytical chemist on the physical/chemical properties of a compound. Understanding the important parameters of the

chromatography and detection method chosen as the means of analysis and the influence of sample preparation on these methods ultimately can lead to finding an optimized sensitive method.

Table 1. Limit of Detection (LOD) data from TICs gathered during the analysis of suites of analytes prepared by LLE and SLE.

Measurements of Legacy and Current Commercial Fluorinated Chemicals in Human Sera Using High Performance Liquid Chromatography-Tandem Mass Spectrometry (HPLC-MS/MS)

Holly Lee and Scott A. Mabury*

*Corresponding author: Phone: (416) 978-1780; Email: smabury@chem.utoronto.ca

Department of Chemistry, University of Toronto

80 St. George St. Toronto, ON M5S 3H6

Biomonitoring of fluorinated chemicals in humans has traditionally focused on the perfluorinated carboxylic acids (PFCAs) and perfluorinated sulfonic acids (PFSAs), although total organofluorine analysis has demonstrated the presence of other unknown fluorinated chemicals in human blood. In this work, fifty human sera samples ($n = 50$) collected in 2009 from United States donors were analyzed by high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) for forty fluorinated analytes that included the polyfluoroalkyl phosphate diesters (diPAPs), N-ethyl perfluorooctanesulfonamidoethanol-based polyfluoroalkyl phosphate diester (SAmPAP), one fluorotelomer mercaptoalkyl phosphate diester congener (FTMAP), fluorotelomer sulfonates (FTSs), perfluorophosphonates (PFPAAs), and perfluorophosphinates (PFPIAs). These target analytes range from those present as active or residual chemicals in the final consumer products to their potential degradation intermediates and products. DiPAP concentrations were lower than those reported in human sera samples collected in 2004, 2005, and 2008. PFPIAs were detected for the first time in human sera. The detection of various fluorinated analytes in human sera, such as the diPAPs and PFPIAs, is evidence of human exposure to commercial fluorinated products. The human fluorochemical contamination observed here will be discussed with respect to the regulations, industrial practices, and pharmacokinetic data concerning these fluorinated chemicals.

Clinical use of the Symbiosis-Quattro Premier LC-MS/MS system at the Montreal University Hospital Center (CHUM)

Dr. Luce Boulanger, PhD CSPQ FCACB

Clinical Biochemist

Centre Hospitalier De L'Universite De Montreal

Hopital Saint-Luc, Pavillon Principal (Port 3247)
1058, rue Saint-Denis
Montreal (Quebec) H2X 3J4

High performance liquid chromatography (HPLC) coupled to tandem mass spectrometry is almost standard technology in a research environment. In routine clinical hospital laboratories, it is still considered high technology reserved for those who deal in “rare and exotic” analysis deserving high sensitivity and specificity. Some of the main reasons for the refractivity to that specific technology in hospitals, besides the cost, are the time and expertise needed to develop methods for specific analyte determination and the technical expertise required of a daily staff on rotating schedule. The turn-around time of analysis considering the volume of testing needed and the complexity of the technology in times of troubleshooting can also hinder the choice of that technology in an hospital lab. The Montreal University Hospital Center (CHUM) being a teaching hospital administers tertiary and quaternary care for the great Montreal area. Its biochemistry laboratory provides service to a large population and handles a great volume of the rarer analysis in the province of Quebec. It is in that optic that we decided to acquire the LC-MS/MS technology for the metabolic section of our biochemistry laboratory. This talk will describe the processes leading to the selection and acquisition of the Symbiosis-Quattro Premier and the implementation of two analyses on that system: plasmatic metanephrines and urinary cortisol. Encountered problems and applied solutions will also be discussed.