Implications for Metabolite Quantification by Mass Spectrometry in the Absence of Authentic Standards

Panos Hatsis, Nigel J. Waters, and Upendra A. Argikar

Drug Metabolism & Pharmacokinetics, Novartis Institutes for Biomedical Research Inc., East Hanover, NJ (P.H), Syros Pharmaceuticals, Cambridge, MA (N.J.W), Analytical Sciences & Imaging, Novartis Institutes for Biomedical Research Inc., Cambridge, MA (U.A.A)

1

Short Communication   **DMD # 75259**

Running title: Mass spectrometric responses of drugs and metabolites

Address the correspondence to:

Upendra A. Argikar, Ph.D.

Novartis Institutes for BioMedical Research, Inc.

Analytical Sciences and Imaging, Biotransformation group,

250 Massachusetts Avenue, Cambridge, MA 02139, USA

Tel: 617-871-3583, 617-335-9110

E-mail: upendra.argikar@novartis.com

Number of text pages: 15
Number of tables: 0
Number of figures: 3
Number of references: 12
Number of words in the Abstract: 232
Number of words in the Introduction: 541
Number of words in the Results and Discussion: 1620

Abbreviations:

ESI-MS, electrospray ionization mass spectrometry; PCA, principal component analysis; RF, response factor; DDI, drug-drug interactions; LC/MS, liquid chromatography mass spectrometry; ADME; absorption, distribution, metabolism and excretion; NMR, nuclear magnetic resonance; ICP-MS, inductively coupled plasma mass spectrometry; SAR, structure activity relationship

2

ABSTRACT

Quantification of metabolites by mass spectrometry in the absence of authentic reference standards or without a radiolabel is often called 'semi-quantitative', which acknowledges that mass spectrometric responses are not truly quantitative. For many researchers, it is tempting to pursue this practice of semi-quantification in early drug discovery and even preclinical development, when radiolabeled ADME studies are being deferred to later stages of drug development. The caveats of quantifying metabolites based on parent drug response are explored in this investigation. A set of 71 clinically relevant drugs/metabolites encompassing common biotransformation pathways, was subjected to flow injection analysis coupled with electrospray ionization mass spectrometry (ESI-MS). The results revealed a large variation in ESI response even for structurally similar parent drug/metabolite pairs. The ESI response of each metabolite was normalized to that of the parent drug, to generate an ESI relative response factor. Overall, relative response factors ranged from 0.014 (> 70-fold lower response than parent) to 8.6 (8.6-fold higher response than parent). Various 2D molecular descriptors were calculated that describe physicochemical, topological and structural properties for each drug/metabolite. The molecular descriptors, along with the ESI response factors were used in univariate analyses as well as a principal components analysis to ascertain which molecular descriptors best account for the observed discrepancies in drug/metabolite ESI response. This investigation has shown that the practice of using parent drug response to quantify metabolites should be used with caution.

3

INTRODUCTION

Quantitative assessments are at the heart of pharmaceutical drug discovery and development. They usually involve the quantification of parent drug exposure since this informs absorption, distribution, metabolism and excretion (ADME) properties, as well as efficacy and toxicity. Quantification of drug or drug related material is thus vital to assessments of pharmacokinetics, efficacy, toxicokinetics, and toxicity. In addition to qualitative assessments of metabolic pathways, often called 'metabolite identification', and quantification of parent disappearance termed as 'metabolic stability', the potential benefits of metabolite quantification cannot be ignored. For example, metabolite quantification in drug discovery and development can help identify gaps and better define the pharmacological and toxicological properties of a drug *e.g.*, primary pathway/s of metabolic clearance, pharmacologically active metabolites, reactive/toxic metabolites, metabolite-mediated drug-drug interactions (DDI), *etc*. The routine application of metabolite quantification in drug discovery and early development is hindered by the absence of reference standards and/or radio-isotope labelled compound, both of which are typically available much later in the development lifecycle. Recent advancements in the analytical specificity and sensitivity of liquid chromatography mass spectrometry (LC/MS) approaches has also lessened the focus and need for radiolabeled ADME studies early in drug development (Obach et al., 2012).

Various analytical approaches, *e.g.*, equimolar detection based on nitrogen or sulfur chemiluminescence (Laks et al., 2004), charged aerosol and evaporative light scattering detection (Magnusson et al., 2015), inductively coupled plasma mass spectrometry (ICP-MS) (Zhang et al., 2016), as well as ultraviolet (UV) detection and nuclear magnetic resonance (NMR) spectroscopy (Vishwanathan et al., 2009) have been explored for quantification of analytes in the absence of reference standards. Nonetheless, LC/MS is the predominant technique for quantification of drugs in biological matrices (Lee and Kerns, 1999), making an approach based on LC/MS highly desirable. For this reason,

the practice of using LC/MS response of the parent drug, to calibrate the response of metabolites appears

attractive as a surrogate, to address the need for quantitative data, even though it is fraught with liabilities

and pitfalls. Electrospray ionization is sensitive to analyte structure and resulting molecular properties,

e.g., surface activity, non-polar surface area, *etc*., which can significantly affect relative signal intensity

between two seemingly similar molecules (Cech and Enke, 2001). This can have profound

consequences when using parent drug response to quantify a metabolite because large errors may result

that can lead project teams astray. Unfortunately, these liabilities are often forgotten by scientists when

pressured by the demands of project teams and tight timelines, and are therefore brushed aside by

qualifying data as 'semi-quantitative'.

This manuscript explores the relationship between parent drug and metabolite structures linked

by specific biotransformation pathways for a large and diverse number of compounds. The results

revealed a large variation in ESI response even for structurally similar parent drug/metabolite pairs,

analogous to that observed by Jones' group, for a small set of compounds (Dahal et al., 2011). Simple

univariate analyses as well as principal components analysis (PCA) with molecular descriptors were used

to investigate potential causes of the observed response variations. The results of this investigation

confirm for a wide range of compounds and their biotransformations that the practice of using parent

drug response to calibrate the response of metabolites is problematic at best, can frequently lead to

incorrect conclusions and should be avoided in its entirety.

MATERIALS AND METHODS

Chemicals and Reagents. Synthetic standards of 26 pharmaceutical drugs and 45 metabolites were

obtained from Toronto Research Chemicals (Toronto, Canada) or Sigma-Aldrich (St. Louis, MO) and are

listed in Supplemental Table 1. Dimethyl sulfoxide (DMSO, 99.7% purity) and formic acid (98% purity)

were obtained from Sigma-Aldrich (St. Louis, MO). Acetonitrile and water were both of LC/MS grade and were purchased from J. T. Baker (Center Valley, PA).

LC/MS Analysis of Drugs and Metabolites. LC/MS was performed using an Agilent 6530 quadrupole-time-of-flight (Qq-ToF) mass spectrometer with an Agilent Jet Stream electrospray ionization source. The Qq-ToF was coupled to an Agilent 1290 Infinity LC (Agilent, Santa Clara, CA). The Qq-ToF was calibrated daily in the 2 GHz Extended Linear Dynamic Range mode. Solutions of all drugs and metabolites were prepared at 1 mM in DMSO. These solutions were serially diluted using 50/50 ACN/water (v/v) to generate individual standard curves ranging from 1 to 1000 nM. All standards were analyzed in triplicate using the LC/MS system, but in flow injection analysis mode, *i.e.*, a chromatographic column was not used. This experimental setup was used to ensure all compounds entered the Qq-ToF dissolved in a consistent mobile phase composition. Flow injection analysis was performed using a mobile phase composition of 50/50 ACN/water at 0.3 mL/min flow rate and a temperature of 30 ^{o}C. All data was acquired in positive ion, full scan mode from *m/z* 100 to 1500 using a capillary voltage of 4500 V, drying gas temperature of 350 ^{o}C and a fragmentor voltage of 175 V. All compounds were detected as protonated molecule ions, *i.e.*, [M+H]$^+$, using high resolution accurate mass spectrometry on the Qq-ToF with the parameters listed above. This experimental setup enabled a consistent detection platform for all compounds, and eliminated the need for determining compound specific parameters, which is required for multiple reaction monitoring on a triple quadrupole mass spectrometer, *e.g.*, collision energy and gas setting.

Data Analysis. Data acquisition and post-acquisition peak integration and linear calibration of acquired data were performed using Agilent Mass Hunter software (v. B.05.01, Build 5.01.5125.3). Microsoft Excel (Microsoft, Redmond, WA) was used to perform any further post-processing data analysis. Chromatograms of individual drugs/metabolites were extracted from the total ion chromatogram using an extraction window of 5 ppm. Integrated peaks of all standards were used to prepare linear standard

curves with $1/x^2$ weighing. Points were excluded from the standard curve if their back-calculated

concentrations were not within +/- 30 % of their nominal concentration. This was done to ensure that all

subsequent data analysis was performed within the linear range of response for all drugs and metabolites.

It was determined that all drugs/metabolites in this study were within their linear range of response at a

concentration of 100 nM. The integrated peak area of metabolites was normalized to the integrated peak

area of its corresponding parent drug to generate an ESI RF, as shown in equation 1. These RFs were

used to facilitate visualization of the data, as well as for subsequent data analysis by principal

components analysis.

$$Equimolar\ Response\ Factor\ (RF) = \frac{Integrated\ peak\ area\ of\ metabolite}{Integrated\ peak\ area\ of\ parent\ drug} \qquad \text{Equation 1}$$

Calculation of Molecular Descriptors and Principal Components Analysis (PCA). All compounds

were coded as SMILES strings as input for the calculations. Physicochemical descriptors were

calculated using VolSurf+ (Molecular Discovery Ltd, UK) (Cruciani et al., 2000) and included 105

parameters describing physicochemical, topological and structural properties including molecular size,

shape, hydrophilicity and hydrophobicity. The physicochemical descriptors for each metabolite were

normalized to the equivalent set of parameters calculated for the corresponding parent drug in order to

derive a differential physicochemical 'fingerprint' for each metabolite. Differential $logD_{7.4}$ was

calculated as $logD_{7.4}$ metabolite minus $logD_{7.4}$ parent. The data matrix including metabolite-to-parent RF

and metabolite-to-parent physicochemical descriptor ratios were imported into Volsurf+ for the purposes

of principal components analysis. The data were then mean centered prior to PCA. With mean-

centering, the average value of each variable is calculated and then subtracted from the data, making it

possible to directly compare variables. This also negates a single variable from dominating the analysis.

PCA methods involve the calculation of linear combinations of the original descriptors, the PC's, such

that each PC is orthogonal to all others with the first PC (PC1) containing the largest amount of variance

with subsequent PC's containing progressively less variance. Thus, a plot of PC1 versus PC2 provides the most efficient 2D representation of the information contained in the data set.

RESULTS & DISCUSSION

The measured relative RF of metabolite-to-parent drug for 45 drug metabolites representing five categories of biotransformation (conjugation, carbon oxidation, heteroatom oxidation, dealkylation and hydrolysis) is shown in Figure 1. A relative RF of 1 indicates equal response of the metabolite compared to its parent drug. Overall, in the dataset, RF values ranged from 0.014 for resorufin glururonide (> 70-fold lower response than parent) to 8.6 (8.6-fold higher response than parent) for hydroxymethoxy diclofenac. Within specific categories, RF ranged between 0.014-2.7 for conjugations, 0.04-8.6 for carbon oxidations, 0.05-1.8 for dealkylations and 0.24-4.4 for heteroatom oxidations. Twenty one drug metabolites had a RF within 2-fold of unity, which is an error margin far greater than the 15% value based on bioanalytical regulatory guidance (Booth and Kadavil, 2013), or even the 30% value, which is commonly used in drug discovery. RF values from only 10 and 13 drug metabolites fell within the 15% and 30% bioanalytical limits, respectively. This means that more than half of the total number of data points were outside the limits represented by the 2-fold margin, and more than 75% were outside the limits represented by 15% error margin. Closer examination of these data points reveals that with the exception of desethyl resorufin ethyl ether, dealkylation seemed to be the most likely biotransformation that preserved the equivalence of response from parent drug. While it is observed that unmasking a polar group, which is the endpoint of dealkylation metabolic reactions, keeps the RF value within 2-fold, it should be noted that most of the metabolites in this study were a result of N-dealkylations, resulting in formation of corresponding primary and secondary amines, and not O-dealkylations that lead to primary or secondary alcohols, or phenols, for example. Thus, the evidence

presented for dealkylation reactions in this investigation may inherently be biased by formation of metabolites that ionize readily under the present conditions. Also, the corresponding aldehydes, which are the other products formed in the dealkylation reactions were not a part of this investigation, due to the unstable chemical nature of aliphatic aldehydes. Heteroatom oxidation, *e.g.*, N- and S-oxides, sulfides and sulfoxides, with the exception of the sulfone metabolite of fenbendazole, was the next most likely biotransformation that keeps the RF value within 2-fold. Perusal of the data for the other 24 data points whose RF was not within a factor of 2 of unity, revealed 42% with RFs < 2 and 11% with RFs > 2, with conjugative metabolism, *e.g.*, glucuronidation and sulfation, resulting in the most variable RFs as a group. This observation makes intuitive sense since conjugative metabolism represents a significant increase in size and hydrophilicity of a molecule. Somewhat surprisingly, oxidation of carbon atoms also resulted in significant changes in RF, even though this biotransformation is not as impactful on structure and molecular properties as conjugative metabolism. For instance, just under half of the drugs undergoing oxidation at a carbon atom had RFs close to unity. Hydrolytic cleavage metabolites, e.g., nafamostat resulted in RFs close to unity, however this is not conclusive given the small sample size.

Figure 1 offers conclusive evidence that the practice of using LC/MS response of a parent drug to calibrate the response of its metabolite(s) is most likely to lead to significant errors, and accordingly incorrect conclusions concerning the importance of a particular biotransformation pathway on the overall metabolic clearance and drug interaction potential of a pharmaceutical drug. This is exemplified with the case of resorufin ethyl ether where the response for resorufin glucuronide and the ethyl ether parent is > 1300-fold different. Although changes in RF have been categorized according to biotransformation, Figure 1 does not show which molecular (or physicochemical) property may be most responsible for driving the observed changes in RF. In this analysis, $logD_{7.4}$ was determined to be the single most important physicochemical property for describing the change in RF. This is consistent with the observation that compound polarity correlates with ESI response (Cech and Enke, 2001), and with results

from a different study that found logD$_{7.4}$ to be a key parameter in predicting relative RF of amino acids/metabolites through multivariate calibration (Chalcraft and Britz-McKibbin, 2009). Figure 2 shows the change in RF as a function of the differential logD$_{7.4}$ between metabolite and parent drug categorized by the type of biotransformation. The goodness of fit (R^2) is low at 0.24 but it does illustrate a trend that the more hydrophilic a metabolite is relative to parent, the lower the relative RF is likely to be. Furthermore, in this dataset, logD$_{7.4}$ unit differences of less than 1 unit lower led to relative RF values of 0.1 or lower. And conjugative metabolites incorporating significant changes in logD$_{7.4}$ (4-5 units lower) can lead to relative RF values of 0.01-0.1. The trend with logD$_{7.4}$ was apparent for each category of biotransformation with the exception of carbon oxidation, where there was no trend at all between logD$_{7.4}$ and relative RF. This may suggest other physicochemical determinants are important for these types of metabolites, since this is a relatively small change in chemical structure (introduction of 1 hydrogen bond donor) but led to the widest range in RF values (after conjugative reactions). To this point, the structure activity relationship (SAR) was taken a step further by analyzing a broader set of calculated physicochemical descriptors together with RF values. Figure 3 is the PCA scores plot showing the clustering of biotransformations based on relative RF and relative difference in physicochemical properties. It shows that different types of biotransformations tend to cluster together, based on their RF values and physicochemical properties. This suggests that a computational modeling approach may provide a means to predict the likely RF based on structural descriptors that could be used in the early stages of drug discovery, prior to availability of authentic metabolic standards or radiolabeled material.

Very limited information, if any, can be obtained from downstream applications of semi-quantification approaches. Calculation of metabolite formation kinetics based on relative peak areas, in the absence of a reference standard is also incorrect. Kinetic rates such as maximal velocity of metabolic reaction cannot be truly ascertained without an authentic reference standard of a metabolite (Argikar and Nagar, 2014). Michaelis-Menten kinetics describe metabolic processes at steady state, and are

10

represented by Km, the substrate concentration at half-maximal velocity, (concentration at Vmax/2, with units of concentration) and Vmax, maximal rate (with units of concentration per unit time, sometimes normalized to concentration of enzyme used in the experiment). To obtain Vmax, reaction velocity (v) has been plotted versus substrate concentration [S] (Seibert and Tracy, 2014). A true Vmax cannot be obtained by plotting relative peak areas of metabolites to parent, thus rendering the subsequent kinetic analysis fundamentally incorrect. In this case, the analysis is further compromised by the assumption that the linearity of metabolite MS response with concentration is equivalent to the parent drug. Similarly, in vivo pharmacokinetics cannot be accurately assessed either with the so-called 'semi-quantification' results. Elimination half-life is typically independent of a true concentration and may be calculated by plotting mass spectrometric response over the terminal phase, with the assumption of linearity within a detector's dynamic range. However, primary pharmacokinetic parameters such as area under the concentration-time curve, clearance, and volume of distribution at steady state obtained for metabolites in the absence of an authentic reference standard or a radiometric detector would be considered meaningless.

This investigation has shown that the practice of using parent drug response to quantify metabolites should be used with caution. There are many examples of this practice in the literature, but these will not be cited in this manuscript. The RF values obtained in the present investigation ranged from 0.014 to 8.6. The acceptable error margin of 15% based on regulatory guidance for bioanalysis in drug development should yield a theoretical RF range of 0.85 to 1.15. It is incomprehensible how such a large discrepancy as seen in this study or even literature examples of RF values of 2-fold can be considered acceptable. In the context of the MIST guidance (Metabolites in Safety Testing guidance, FDA, 2016), where disproportionate metabolites are considered as 10% or greater of total drug related exposure at steady state, such a RF range makes the use of mass spectrometric responses far from favorable. From a drug discovery perspective, the current technology limits the use of mass

spectrometric responses because, it is impossible to predict RF as a vector for unknown metabolites, i.e., it is not possible to calculate the directionality or magnitude of the change in RF even with in silico approaches. It is evident from our investigation that large discrepancies in parent drug/metabolite RF can result from seemingly small biotransformations, i.e. errors in metabolite quantification can far exceed 2-fold (or greater) based on our results. The nature of these discrepancies makes it difficult to predict when a mass spectrometric response for metabolite is over-estimated, equal or under-estimated, thus casting doubt on when semi-quantification can be or cannot be reliably used. ESI response of drugs and their metabolites are thought to be a function of chemical structure along with molecular properties, as well as the constituents and pH of the mobile phase, flow rates, parameters of the ESI source, protonation/deprotonation, resonance and inductive effects in drugs and their metabolites which affect chemical reactivity for ionization, *etc*. Until an exact understanding of how one or more of these variables affects ionization individually or multi-factorially, predictive algorithms for semi-quantification of metabolites will be limited at best. While it is beyond the scope of the present investigation to demonstrate a predictive model, future work will involve increasing the data set for higher statistical significance, modeling different response parameters, *e.g*., raw peak area and work toward building a greater understanding of the SAR and potentially predictive models.

ACKNOWLEDGEMENTS

AUTHORSHIP CONTRIBUTIONS

Participated in research design: Hatsis and Argikar

Conducted experiments: Hatsis, Waters, and Argikar

Contributed new reagents or analytic tools: Hatsis and Waters

Performed data analysis: Hatsis, Waters, and Argikar

Wrote or contributed to the writing of the manuscript: Hatsis, Waters, and Argikar

REFERENCES

Argikar UA and Nagar S (2014) Case Study 2. Practical analytical considerations for conducting in vitro enzyme kinetic stdies. *Methods in Molecular Biology* **1113:**431-439.

Booth B and Kadavil J (2013) *Bioanalytical Method Validation*. Center for Drug Evaluation and Research, United States Food & Drug Administration, United States Department of Health and Human Services, Rockville, MD.

Cech NB and Enke CG (2001) Practical implications of some recent studies in electrospray ionization fundamentals. *Mass Spectrom Rev* **20:**362-387.

Chalcraft KR and Britz-McKibbin P (2009) Newborn screening of inborn errors of metabolism by capillary electrophoresis-electrospray ionization-mass spectrometry: A second-tier method with improved specificity and sensitivity. *Anal Chem* **81:**307-314.

Cruciani G, Pastor M, and Guba W (2000) Vol Surf: a new tool for the pharmacokinetic optimization of lead compounds. *Eur J Pharm Sci* **11:**S29-S39.

Dahal UP, Jones JP, Davis JA, and Rock DA (2011) Small molecule quantification by liquid chromatography-mass spectrometry for metabolites of drugs and drug candidates. *Drug Metab Dispos* **39:**2355-2360.

Laks S, Pelander A, Vuori E, Ali-Tolppa E, Sippola E, and Ojanpera I (2004) Analysis of street drugs in seized material without primary reference standards. *Anal Chem* **76:**7375-7379.

Lee MS and Kerns EH (1999) LC/MS applications in drug development. *Mass Spectrom Rev* **18:**187-279.

Magnusson LE, Risley DS, and Koropchak JA (2015) Aerosol-based detectors for liquid chromatography. *J Chromatogr A* **1421:**68-81.

Obach RS, Nedderman AN, and Smith DA (2012) Radiolabelled mass-balance excretion and metabolism studies in laboratory animals: are they still necessary? *Xenobiotica* **42:**46-56.

Seibert E and Tracy TS (2014) Fundamentals of enzyme kinetics. *Methods in Molecular Biology* **1113:**9-22.

Vishwanathan K, Babalola K, Wang J, Espina R, Yu LN, Adedoyin A, Talaat R, Mutlib A, and Scatina J (2009) Obtaining exposures of metabolites in preclinical species through plasma pooling and quantitative NMR: Addressing metabolites in safety testing (MIST) guidance without using radiolabeled compounds and chemically synthesized metabolite standards. *Chem Res Toxicol* **22:**311-322.

Zhang T, Cai S, Forrest WC, Mohr E, Yang QH, and Forrest ML (2016) Development and validation of an inductively coupled plasma mass spectrometry (ICP-MS) method for quantitative analysis of platinum in plasma, urine, and tissues. *Appl Spectrosc* **70:**1529-1536.

FIGURE LEGENDS

Figure 1. Metabolite-to-parent relative RFs for 45 drug metabolites categorized based on biotransformation reaction. From left to right, hydrolysis (black), conjugation (gray), aliphatic or aromatic C-oxidation, (black), dealkylation (gray) and heteroatom oxidation (black). The dotted lines indicate unity and a 2-fold change in RF.

Figure 2. Relationship between metabolite-to-parent relative RFs and the differential $logD_{7.4}$ values (defined as the difference in $logD_{7.4}$ between parent and metabolite) for 45 drug metabolites. Types of biotransformations include hydrolysis (closed circles), conjugation (gray circles), aliphatic or aromatic C-oxidation (open circles), dealkylation (open triangles) and heteroatom oxidation (closed diamonds).

Figure 3. Principal components analysis scores plot showing the clustering of biotransformation reactions based on relative RF and relative difference in physicochemical properties between parent and metabolite. Types of biotransformations include hydrolysis (closed circles), conjugation (gray circles), aliphatic and aromatic C-oxidation (open circles), dealkylation (open triangles) and heteroatom oxidation (closed diamonds). PC1 and PC2 describe 52% of the variance in the dataset.
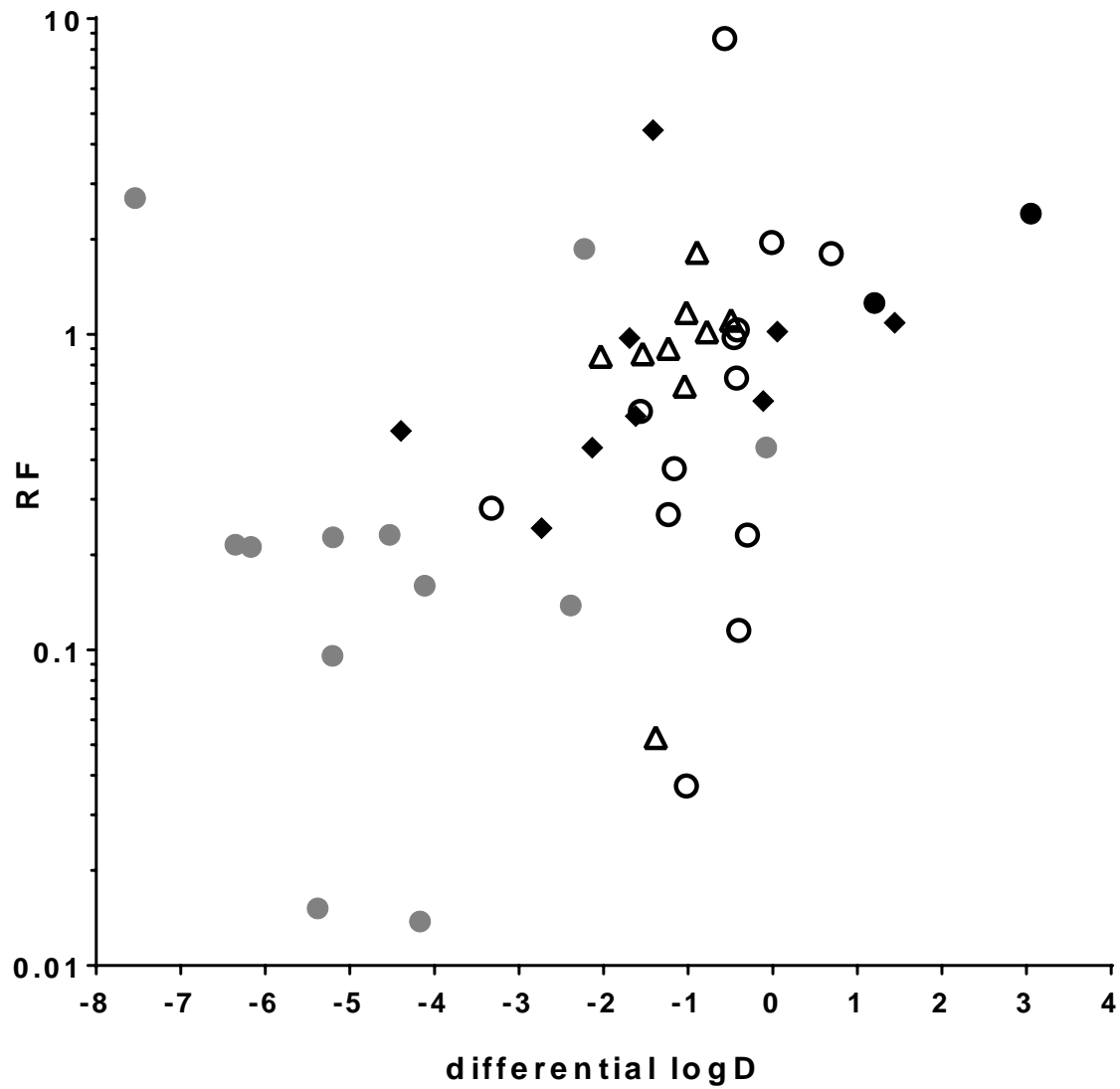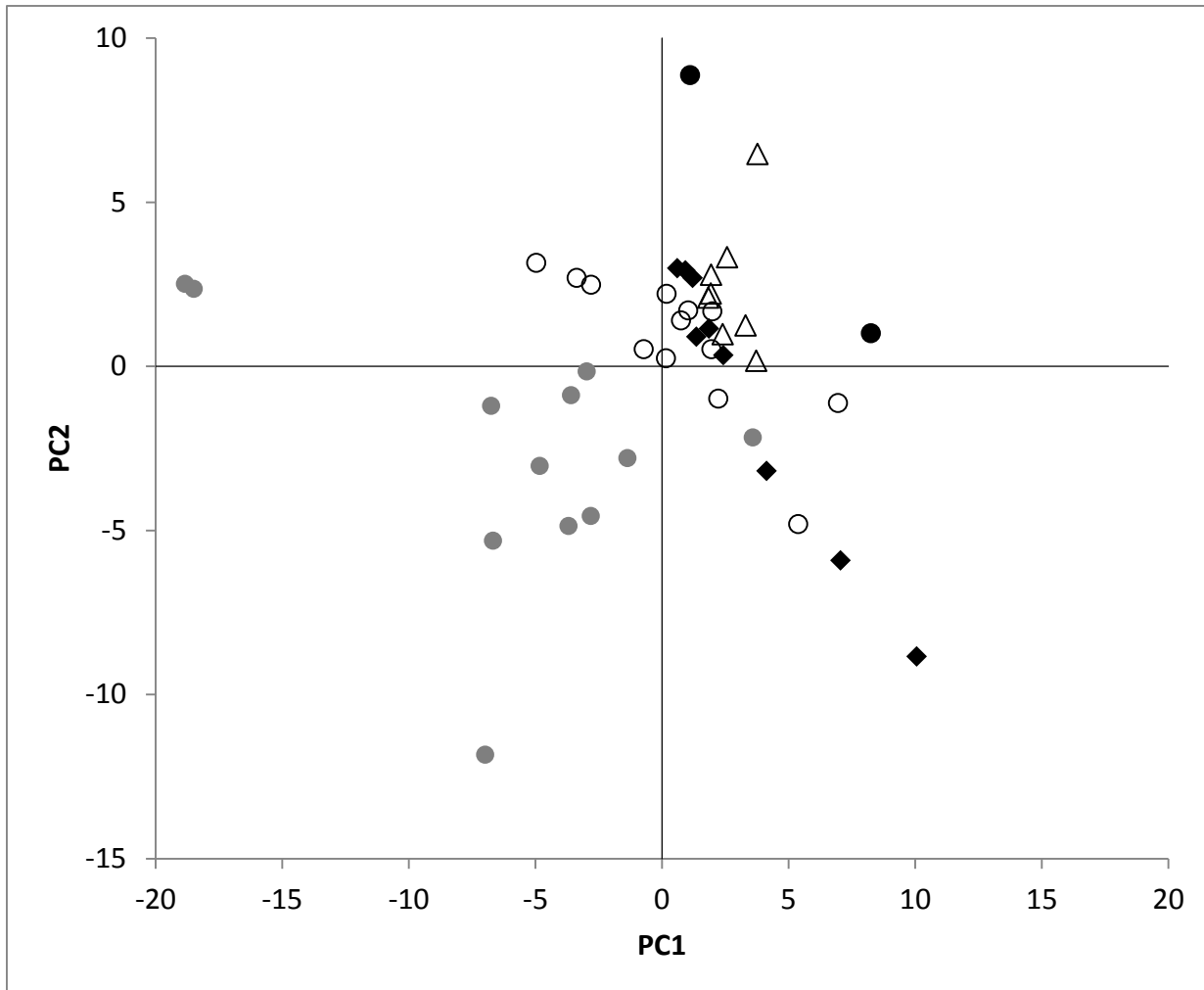
Figure 1

16

Figure 2

Figure 3

Implications for Metabolite Quantification by Mass Spectrometry in the Absence of Authentic Standards

Panos Hatsis, Nigel J. Waters, and Upendra A. Argikar

SUPPLEMENTAL DATA

Supplemental Table 1. A list of pharmaceutical drugs and their corresponding metabolites used in the present study.

Pharmaceutical Drug	Metabolite(s)
Sulfadiazine	N-Acetyl sulfadiazine
Fenbendazole	Fenbendazole Sulfone; Fenbendazole Sulfoxide
Dextropmethorphan	Dextrorphan; Dextrorphan Glucuronide; N-Desmethyl Dextrorphan Glucuronide
Clarithromycin	N-Desmethyl Clarithromycin
Ofloxacin	Desmethyl Ofloxacin
Amiodarone	N-Desethyl Amiodarone
Abacavir	Abacavir Glucuronide
Prazosin	6,7-Dimethoxy Quinazoline 2,4-diamine
Duloxetine	4-OH Duloxetine
Resorufin	Resorufin Ethyl Ether, Resorufin Glucuronide
Ranitidine	Ranitidine N-Oxide; Ranitidine N,S-Dioxide; Ranitidine S-Oxide
Phenolphthalein	Phenolphthalein Glucuronide
Metaclopramide	O-Desmethyl Metoclopramide
Lansoprazole	5-OH-Lansoprazole; Lansoprazole Sulfide; Lansoprazole Sulfone; Lansoprazole Sulfone N-Oxide
Imipramine	2-OH-Imipramine; 10-OH-Imipramine
Nafamostat	Nafamostat Acid; Nafamostat Phenol
Terfenadine	Fexofenadine

Diclofenac	Diclofenac Acyl Glucuronide; Hydroxy, Methoxy Diclofenac
Clozapine	N-Desmethyl Clozapine; Clozapine N-Oxide
Acetaminophen	Acetaminophen Glucuronide; Acetaminophen Glutathione; Acetaminophen Sulfate
Dexamethasone	17-Oxo-Dexamethasone
Mianserin	8-OH-Mianserin
Metoprolol	alpha-OH-Metoprolol
7-Ethoxycoumarin	7-OH-Coumarin; 7-OH-Coumarin Glucuronide
Warfarin	4-OH-Warfarin; 6-OH-Warfarin
Midazolam	1-OH-Midazolam; 1-OH-Midazolam Glucuronide; 4-OH-Midazolam