METABOLITE ANALYSIS USING A NOVEL RELATIONSHIP AND DATABASE DRIVEN SOFTWARE PLATFORM APPROACH FOR SCREENING AND UNDERSTANDING METABOLISM

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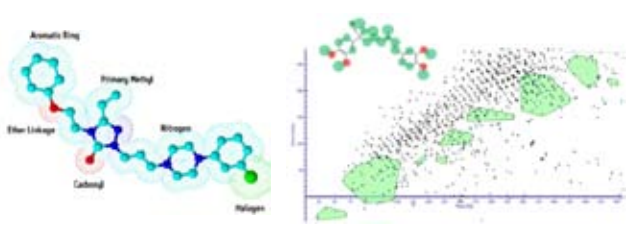
INTRODUCTION

Current informatics approaches for xenobiotic studies limit the progress of scientists due to the restrictions of predefined software workflows and the difficulty on passing on one's knowledge to peers. The ability to share discoveries and knowledge on the biotransformations of drug candidates for universal use in an organization and to feed previously discovered information into today's experiments holds the potential to unlock the potential of historical data. The approach defined here is a system that uses the latest generation hardware and informatics to accurately screen samples, confidently interrogate datasets and efficiently disseminate data in a highly flexible format. This system allows users to elucidate metabolic pathways, store, access and interrogate each other's data. This capture and ability to retrieve scientific knowledge allows the organization's best scientists to apply a growing body of work towards future challenges.

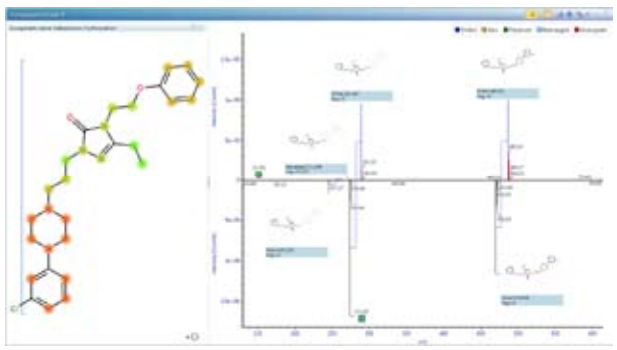
ADOPTING CHEMICALLY INTELLIGENT ALGORIHMS

In order for the system to integrate effectively with today's DMPK scientist there is a much greater pressure to provide accurate, confident data with minimal user intervention. The automated approach we have implemented is driven from structural knowledge of the parent molecule using chemically intelligent algorithms that will identify functional groups, predict enzymatic cleavage and perform structural elucidation prior to data review to speed the analysis



We have also introduced the concept of "soft" filtering. This new technique shows all of the information required by the scientist to make a decision if a metabolite is real and will promote metabolites for which ID has not been made but shows evidence of being drug related, such isotopic pattern, common fragment ions, common neutral loss, compliance with Mass Defect Filters etc.

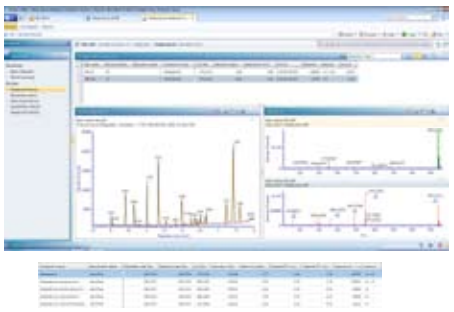
These soft filters reduce false positives while ensuring that no data is thrown away. In this manner, we don't have to direct the data collection upfront and potentially miss something based upon incomplete scientific assumptions at the time of the analysis.



Biotransformation localization is automatically performed and saved as part of the process method generating decision driving information faster and in a format that is easily shared amongst scientists.

FLEXIBILITY IN WORKFLOW

In a single LC/MS experiment we can collect a wealth of information regarding the drug and its metabolites. We obtain m/z and RT for virtually all detectable ions in the same experiment, for both precursors and products and also intensity related information such as the isotopic pattern and the overall abundance of each ion in the sample.



However, it is becoming impractical to manually interrogate these increasingly complex datasets. The real goal of the experiment is to answer a relevant biological question. Informatics has the power to both simplify and enrich the process for the user. In UNIFI, the user can visualize and review the data in workflows customized to be relevant to the question being asked.

For example, the scientist may choose to show:

A) only fragment information to elucidate the structures of their metabolites, a typical met ID experiment.
B) Use the quantitative data provided by MS^E to determine the absolute levels of parent
C) Process multiple datasets and look at only the relative quantitation of parent and metabolites to look for trends in metabolic stability.
D) or all of the above

$UPLC/MS^E$ methods excel at providing this information and now, collating and processing the data in one software package gives the user the power to define their own workflows to answer the questions relevant to their metabolism studies.

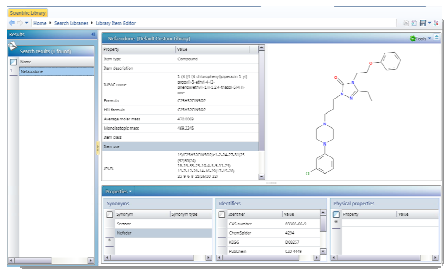
STORING AND COMMUNICATING INFORMATION

All over the world, DMPK scientists and labs are constantly striving to enhance collaborative drug discovery and development.

With this in mind this solution allows scientists to drastically compress not only the data acquisition and interpretation timelines but also, critically, those for reporting. The ability to communicate reports effectively and produce them in a format that easily integrated into current processes is a key bottleneck. With UNIFI users can generate reports in a concise and highly customizable manner to address this need.

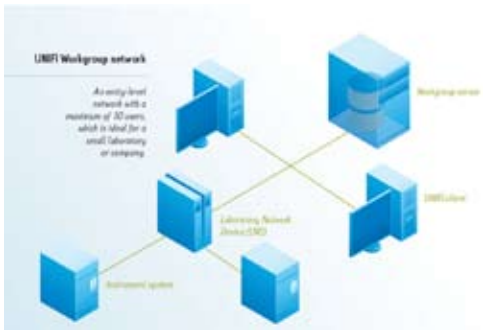
Should an unexpected metabolic event be discovered, UNIFI records the occurrence and stores that information in its scientific library.

This repository of information builds as each scientist uses and contributes to it – allowing the entire laboratory to work in the context of a growing body of information. The consequence of this is that every scientist can make better decisions based upon their own work and the work of their peers.

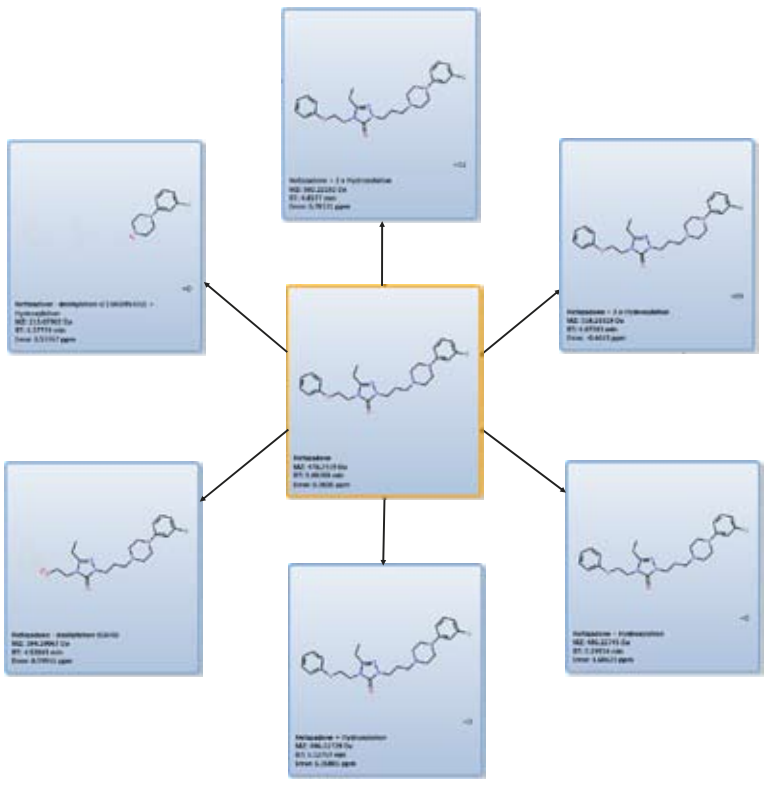


UNIFI supports a workgroup configuration allowing the connection of 2 QTof instruments and up to 10 users, so that across a lab, methods and data can be shared seamlessly.

Also, the means that scientists no longer have to working about archiving and backup of data, and for the first time metabolite identification can be performed on a system built to be compliant with FDA regulatory demands.



METABOLITE IDENTIFICATION



Sample Preparation

Samples were prepared by spiking nefazodone, 10µM in rat liver microsomal preparations (1-2.5mg/mL protein). Incubations were performed using a NADPH regenerating system in Na_2CO_3 buffer at pH 7.4. Time points at t=0 and t=60 were taken. Samples were quenched with an equal volume of cold acetonitrile, were centrifuged and supernatant was taken for further analysis.

$UPLC/MS^E$

Comprehensive datasets are collected using $UPLC/MS^E$ data acquisition. MS^E uses parallel low and elevated collision energy MS acquisition to provide comprehensive precursor and product ion information for virtually every detectable component of a mixture. Proprietary processing provides clean and accurate fragment spectra enabling confident automatic structural elucidation.

Identification

Detection and automated structure driven identification of metabolites was performed in UNIFI, the figure above shows the output as it is displayed to the user. Control comparison was performed using the t=0 sample as control. Intelligent filters were applied to the data in order to identify drug related components for further investigation.

METABOLITE IDENTIFICATION APPLICATION SOLUTION

We have brought together UPLC, MS, Informatics and customized reagents and solvents in a single package. The benefits of this reside in the fact that the system is designed to work optimally together to generate the highest quality data and to solve drug metabolism problems.

The components of this system include:

Analytical Standards and Reagents
Precisely formulated calibrators, controls, and standards ensure that you achieve quality and consistency.

ACQUITY UPLC I-Class System
Gives excellent peak capacities for enhanced MS sensitivity – well suited for complicated matrices such as plasma, bile, urine, and feces.

Xevo G2-S QTof
UPLC®-compatible mass resolution, matrix-tolerant dynamic range, quantitative performance, mass accuracy and speed of analysis – simultaneously.

UNIFI Scientific Information System
Built upon a scalable architecture to migrate from a single workstation to a collaborative enterprise deployment with ease. It easily stores, make use of, and allows sharing of all data, all methods, all reports – everything associated with analytical results across an organization. UNIFI uniquely enables leveraging today's information to share knowledge and solve for tomorrow's challenges.

The Metabolite Identification Application Solution with UNIFI

CONCLUSION

With this application solution, the breadth of quantitative and qualitative information generated coupled with the specificity of user defined reporting and the speed at which information can be shared could very well impact critical decisions regarding a drug or class of drugs.

With data independent analysis, chemically intelligent processing and a informatics platform that will redefine the way we think about collaboration and sharing of data, scientists have the tools to proliferate information through their organization to drive key decisions to make safer, more effective drugs.