探索と開発における迅速な情報主導型意思決定を可能にする薬物代謝研究のための新しい化学的にインテリジェントなアプローチ

A Novel Chemically Intelligent Approach in Drug Metabolism Studies for rapid information-driven decisions in discovery and development

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INTRODUCTION

薬物代謝研究の現在の情報アプローチでは、多くの場合、事前に定義されたソフトウェアのワークフローの制限や、知識を共有することの難しさが、科学者の研究の進行を妨げています。組織内で普遍的に用いられる新しい薬剤クラスの生体内転換の発見や知識を共有するための機能、過去に発見された情報を利用するための機能によって、製薬業界の最も重要な資産である蓄積されたデータを含めた解釈が可能となります。本発表で紹介するアプローチは、正確に試料のスクリーニングを行い、確信を持ってデータセットを探索し、非常に柔軟な形式でデータを効率的に共有するための最新のハードウェアとソフトウェアを使用するシステムです。これらは、Waters UNIFI代謝物構造推定アプリケーションソリューションによって可能になりました。このシステムによって、研究者は代謝経路を解明し、お互いのデータを保存、共有、探索し、過去の研究から利益を得ることができます。

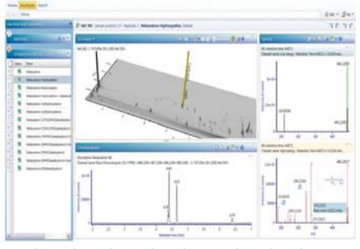
Oracleデータベースのアーキテクチャとワークグループ機能を使用して、UNIFI情報システムは、新たな代謝物同定および薬物代謝部門のワークフローを可能にします。

定量的および定性的な情報はひとつの分析に完全に統合されています。生データ、解釈されたデータ、完全なレポートが構造化されたプロジェクトフォルダ内に含まれています。

統合されたレビューやレポート機能は、科学者にデータセットのよりよい解析や、同僚や共同研究者とのより迅速で効果的な知識の共有をも可能にします。

DATA PROCESSING

All data (MS, MS/MS, MSE and analog traces) are passed through the Apex peak detection and alignment processing algorithms[1]. This enables related ion components to be grouped together and analyzed as a single entity. Charge species, salt adducts, and fragments (MS/MS and in-source species) are all automatically aligned and grouped. All of this information can be used to automatically (or manually) interpret the data.

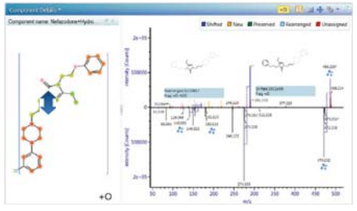


2D and 3D views for Nefazodone Hydroxylated Metabolite - MS^E spectral data is shown with automated strutural assignment.

Componentization allows the data to be represented in traditional formats (XICs, spectra, etc) but also in tabular format. This enables the intelligent filtering and sorting of data sets based on single or multiple criteria. Data can be filtered or searched by any given LC or MS property of the peak. Data is stored in a database driven by Oracle, enabling data to be interrogated in many unique ways.

Tabulated Properties of Compenentized Data for Nefazodone and Metabolites *Flags or Filters can be set on any parameter. e.g. Flagging of isotopic patterns deviating over 10% from expected intensities.*

Interchangeable properties table or visual display of data

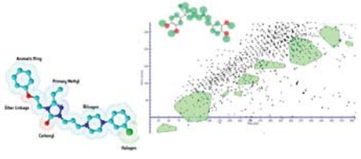


Automatically Interpreted Structural Data for the Alkyl Hydroxylated Metabolite of Nefazadone *– Structural heat map and mirror plot of automatically annotated Metabolite (top) and Parent spectra (bottom). The software automatically compares all metabolites detected to parent and uses the spectral evidence to provide a heat map the most likely site(s) of biotransformation.*

PREDICTION & INTERPRETATION

The automated approach is driven from structural knowledge of the parent molecule using chemically intelligent algorithms which perform an *in silico* cleavage of the molecule and identify possible functional groups. This algorithm is designed and optimized by Dotmatics Ltd.[2] Structural elucidation on all metabolites is performed prior to data review to both speed up and refine the results of the analysis.

UNIFI Chemical Intelligence *– Funtional group recognition and Intelligent Mass Defect Filters are used to help detect drug related components*



METHODS

Sample Preparation: Samples were prepared by spiking nefazadone, 10µM in rat liver microsomal preparations (1-2.5 mg/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 acquisitions. MS^E uses parallel low and elevated collision energy MS acqusitions to provide comprehensive precursor and product ion information for virtually every detectable component in 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. Binary comparison and detection of metabolites at t=60 was performed using the t=0 as the control. Intelligent filters were applied to the data in order to identify drug related components for further investigation.

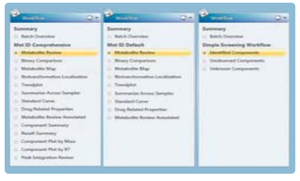
REVIEWING DATA

In a single LC/MS high resolution, full scan 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 have the power to both simplify and enrich the process for the user. In UNIFI Software, the user can visualize and review the data in workflows customized to be relevant to the question being asked. One example of this would be to show top 3 metabolites in one view (providing a way to provide rapid metabolic hot spot information to the medicinal chemistry groups) while another workflow step may involve a thorough review of all metabolism present and full biotransformation characterization. A third workflow step may focus purely on quantitation of the parent molecule. Each of these workflow steps can also be tied to the same report, or three different reports depending on the experimental purpose.

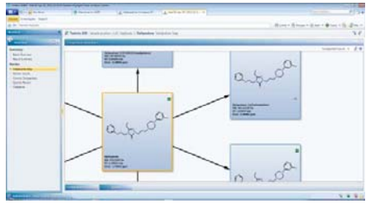
The concept of "soft" filtering is key to reviewing data in UNIFI. This new technique shows all of the information required by the scientist to make a decision. All evidence , such as isotopic pattern, common fragment ions, common neutral loss, compliance with Mass Defect Filters, etc. can be displayed and flagged (soft filtered) to allow the user to take all evidence supporting (or against) a particular peak being parent related.

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 analysis. This also presents assumptions at the time of the analysis. This also presents a powerful approach for archiving data that can be re-interrogated at a future point in time.

Workflows – The relevant data is displayed at each given step in the process depending on the workflow needed, views and filters can be added or removed to simplify the process.



Metabolite Map - UNIFI incorporates a structural interrogation view, that automatically takes identified metabolites and arrays them around the parent molecule and annotates them with much of the information that was used in order to perform the identification, giving a single screen from which to view major metabolic routes. Each structure can be clicked to access information such as spectra, metabolite localization prediction and fragmentation data. Metabolites and structures can then be exported into the scientific library.

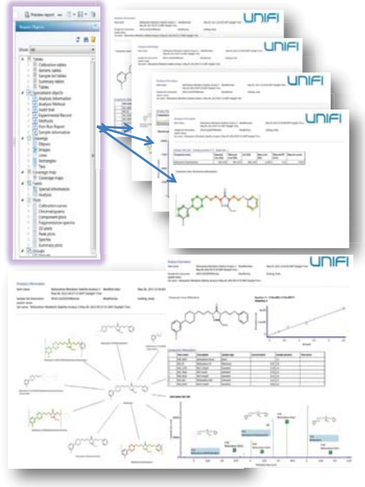


Workflows – *Other example displays*

REPORTING DATA

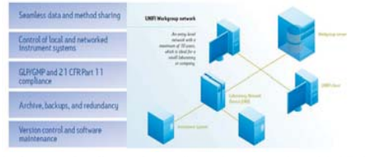
The concepts utilized in reviewing data which allow the user to visualize and present data in the best way for a given task have also been applied to reporting. The ability to communicate reports effectively and produce them in a format that easily integrates into the current lab practices is a key bottleneck. With UNIFI users can generate reports in a concise and highly customizable manner to address this need. The ability to generate multiple reports from a single analysis is also possible.

Customized Reports - *Full control of report templates*



NETWORK / INFORMATICS

UNIFI Software is network ready, meaning scientists no longer have to worry about backing up or archiving data. Metabolite identification can be performed on a system built around informatics and sharing data. The system is also built to be compliant with FDA regulatory demands and has full audit trail capabilities.



Workgroup Configuration *- allows the connection of 2 QTof instruments and up to 10 users across a lab,. Instriuments, methods and data can be shared seamlessly.*

CONCLUSION

Using an Oracle database architecture and workgroup functionality, the **UNIFI Scientific Information System** enables new metabolite identification and drug metabolism departmental workflows.

Quantitative and qualitative information is contained entirely in the same analysis. Raw data, interpreted data and full reports are contained within a structured project folder.

Integrated reviewing and reporting enable scientists to analyze their data sets better and also share this knowledge more quickly and effectively with colleagues and collaborators.

UNIFI Met ID compatibable systems:

Vion IMS QToF – *Next generation IMS Qtof for routine ion mobility mass allowing the analysis of samples beyond mass resolution.*

Xevo G2-XS QToF – *Next generation QTof technology allowing the analysis of samples with the highest sensitivities possible.*

References

1. Geromanos et al., `The detection, correlation, and comparison of peptide precursor and product ions from data independent LC-MS with data dependant LC-MS/MS`, *Proteomics*, 2009; **9**: 1683-1695
2. Mortishire-Smith et al.,`Generic dealkylation: a tool for increasing the hit-rate of metabolite rationalization, and automatic customization of mass defect filters`, *Rapid Commun Mass Spectrom.*, 2009; **23**: 939-948
3. Bateman et al., `$MS^E$ with mass defect filtering for in vitro and in vivo metabolite identification`, Rapid Commun Mass Spectrom. 2007;**21** :1485-1496.

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