Rapid Metabolite Tof Screening, Structure Elucidation and Clearance Estimates Using Informatics-HRMS Approaches

AAPS Poster # W4297, Wednesday 9:30-12:30 Yun W. Alelyunas, Mark D. Wrona, and Paul Rainville Waters Corporation, Milford, Massachusetts, USA

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Purpose:

Demonstrate comprehensive reporting of metabolite identification and quantification using HRMS QTof data for both phase I and phase II metabolism using glyburide, buspirone, and clozapine. Viewing data across multiple species for verapamil is also demonstrated.

Methods:

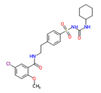
Test compounds were incubated using human, rat, dog, or monkey liver microsomes in the absence or presence of GSH for trapping of potential reactive metabolites. After quenching using 2 volume of ACN the precipitated protein was removed through centrifugation and the supernatant was transferred to a 2mL vial. The LC system was Acquity I-Class UPLC and Acquity BEH C18 1.7um 2.1 x 100 mm column. A generic gradient from 5-60% in 8 minutes was used (ACN + 0.1% formic acid, H_2O + 0.1% formic acid). The flow rate was 0.6 ml/min and column temperature was 60°C. The MS system was Xevo G2-S QTof running in MS^E mode in ESI^+ at 32,500K resolution. The low CE was 2.0 and high CE ramp was 10-30 V. Scan time was 0.1 s. Data acquisition, automated charge state deconvolution and data processing was performed using UNIFI software.

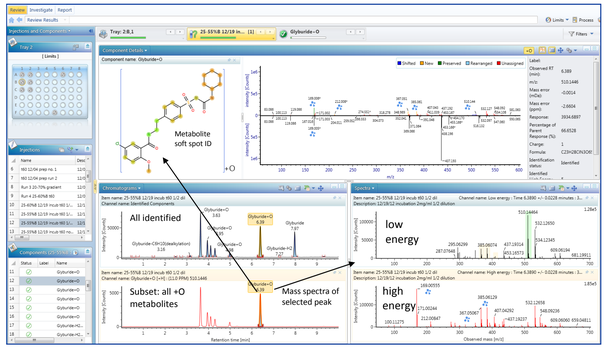
Results:

Metabolites are summarized across samples and across species for added confirmation of the metabolites detected and cross sample comparisons. In the case of GSH adduct the software automatically detects, deconvolutes and groups charge states and adducts related to the parent compound simplifying detection and analysis. Specifically, for glyburide, the structure heatmap, binary plot and chromatogram of all identified metabolites and +O subset are displayed. For buspirone, the summary table, chromatograms of all identified metabolites, subset +O2 metabolites, and subset +O metabolites, precursor and fragment ion mass spectra of +O metabolite at r.t. = 2.38 min are displayed. For clozapine, phase I metabolites and GSH adducts, trend plotting using overlaid line plots are demonstrated. For Verapamil, fragmentation map, trend plotting across species and time using bar plots are displayed.

Phase I Examples

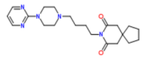
Glyburide





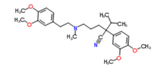
Glyburide Metabolite ID, showing structure heatmap, binary plot, chromatogram of all identified metabolites and +O subset.

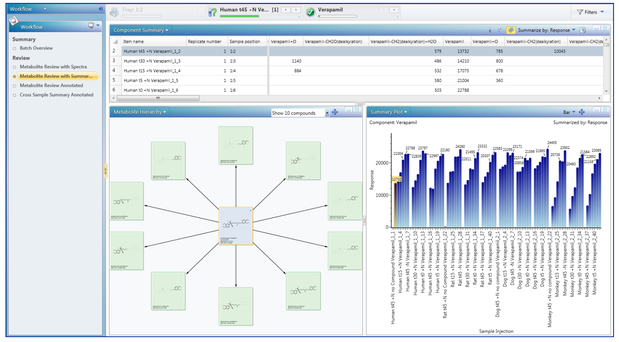
Buspirone



Buspirone metabolite ID, showing summary table, chromatograms of all identified metabolites, subset +O2 metabolites, and subset +O metabolites, high energy and low energy mass spectra of +O metabolite at r.t. = 2.38 min.

Verapamil





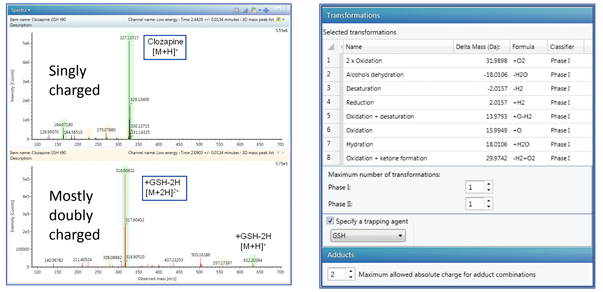
Verapamil metabolite ID and quantification, showing summary table, fragmentation pattern of parent compound, bar plot of verapamil concentration change across species and time. The data is exported to enable clearance and other statistics calculation using Winnonlin or other programs.

Phase II Example

Clozapine

Clozapine Phase I and II metabolite ID, showing summary table, chromatogram of all identified metabolites, +GSH and +O+GSH subset, overlaid line plots showing concentration of several GSH adducts change over time.

Automatically extract multiple charged species



(Left) Clozapine ESI^+ low energy spectra obtained from MS^E experiment, (top) clozapine, (bottom) +GSH metabolite. (Right) Generic biotransformation used for metabolites ID, Detection of GSH adduct was enabled by choosing GSH as the trapping reagent. The number of charge state for charge state deconvolution is selected in "Adduct" panel.

Conclusion:

We demonstrate the use of a novel informatics platform for the simultaneous metabolite identification of HRMS data across species, time, and other variables. The added feature of trend plot and data calculations enable the software to be a comprehensive DMPK tool for HRMS data acquisition and processing of large multivariate datasets.

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