High Throughput Screening-ADME-Tox; Transitioning from Triple Quadrupole to High Resolution Accurate Mass Spectrometry

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INTRODUCTION

High throughput screening (HTS) is a routine and robust approach for early phase drug discovery. This is normally combined with the characterization of the ADME-Tox (absorption, distribution, metabolism, excretion and toxicity) attributes of potential drug candidates.

The goal of ADME-Tox is to better understand the safety and efficacy of a drug candidate as early as possible in the discovery phase. High attrition rates of new chemical entities are often being attributed to poor ADME-T characteristics [1]. Therefore having the confidence in the assays to measure these characteristics with good accuracy and throughput is highly beneficial.

In its nature one of the primary requirements for HTS-ADME-Tox is throughput. On triple quadrupole instruments one of the largest bottlenecks when analysing hundreds of new chemical entities every week is upfront compound optimization. MRM optimization has to occur prior to sample analysis. In recent times software solutions like DiscoveryQuant™ have significantly improved the optimization process by automation. However with the increased capacity this introduces it can once again become a bottleneck.

This is where the possibility of utilizing high resolution accurate mass spectrometry (HRAMS) has been scoped. One of the primary reasons for this is the no-to-very-little up-front optimization required to perform MS and MS/MS quantification on a quadrupole-time-of-flight instrument. This is very attractive where high throughput is required and can alleviate one of the major bottlenecks.

Having an instrument that shows good throughput is unhelpful if the software does not integrate into the large data flows in a HTS laboratory. This is where DiscoveryQuant™ software is utilized over the instrument control software. This allows for simple LIMS/ELN import/export functionality which can quickly import the compounds and their sample lists for acquisition. Followed by semi-automated data extraction and processing the processed peak areas and information can be passed back into LIMS/ELN.

One of the secondary reasons for using HRAMS is the extra information that can be ascertained from the same sample injection. For example from a typical microsomal incubation one can perform dedicated quantification to determine the parent intrinsic clearance. Subsequent software analysis of the same injection can generate its metabolite profile.

In this study we utilise high resolution accurate mass spectrometry to obtain the *in-vitro* metabolic profile and intrinsic clearance values for several pharmaceuticals.

MATERIALS AND METHODS

Sample Preparation: Human liver microsomes were incubated with the test compounds (Verapamil, Haloperidol,) at 37oC in the presence of the cofactor and NADPH. The reaction is terminated by the addition of methanol containing the internal standard (Metoprolol).

UHPLC Conditions: A Shimadzu Nexera UHPLC system was used. A Phenomenex Kinetex C18, 50x2.1mm, 1.7µm column at 60˚ C with a gradient of mobile phase A of 0.1% formic acid in water and mobile phase B 0.1% formic acid in acetonitrile was used at a flow rate of 600µL/min. The injection volume was set to 8µL. A 1.5 minute gradient elution profile was utilized with a total runtime of 3 minutes.

HRAMS Conditions: A SCIEX TripleTOF® 6600 high resolution accurate mass (HRAMS) LC-MS/MS system with the IonDrive™ Turbo V source and Electrospray Ionization (ESI) probe was used.

Software: Analyst® TF 1.7 control software, DiscoveryQuant™ 3.0 and MetabolitePilot™ 2.0α software were used.

RESULTS

As described earlier the primary requirement for HTS-ADME-Tox is throughput with the current bottleneck for very high throughput being compound optimization. DiscoveryQuant™ software has been established on triple quadrupole instruments for several years for automating the compound optimization process. This has introduced significant time saving with the possibility to optimize compound MRM conditions in around 1 minute via flow injection analysis.

However this still represents a significant amount of valuable instrument acquisition time spent on compound optimization. We have investigated the functionality of the TripleTOF® 6600 in combination with DiscoveryQuant™ to perform optimization free acquisition/quantification. The first key to unlock optimization free quantification is the ability to automatically create both ToF MS and dedicated ToF MS/MS (MRM^{HR}) acquisition methods. The acquisition methods are created dependent on the compounds imported for sample analysis. For all methods we have an initial ToF MS experiment across our selected mass range which is looped with dedicated MRM^{HR} experiments. Figure 1 shows two ways that DiscoveryQuant™ will create the MRM^{HR} experiments.

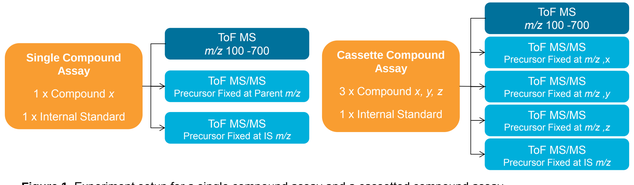


Figure 1. Experiment setup for a single compound assay and a cassetted compound assay

As shown in figure 1 there are two options for acquisition creation, either a single compound assay or a cassetted assay. Going into more detail with the ToF MS/MS experiments a generic collision energy and collision energy spread are utilised to produce a high quality MS/MS spectrum. This provides a choice of low, medium and high energy fragment ions for peak extraction.

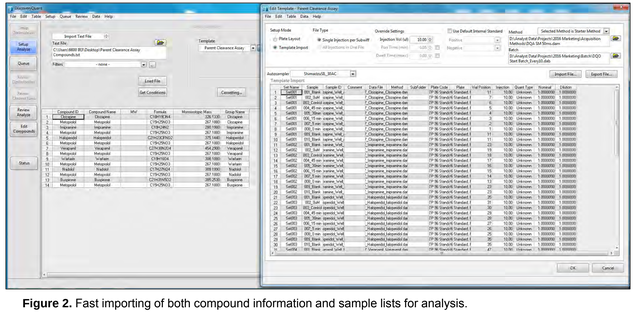


Figure 2. Fast importing of both compound information and sample lists for analysis.

The second half of the throughput key is the ability to integrate the workflow into the dataflow of laboratory information management systems (LIMS). With LC-MS/MS instrumentation and software this normally translates into text file import/export. The strategy of DiscoveryQuant™ is to import all of our compound information; compound identifier, molecular formulae and polarity amongst others. At the same time we will import our sample information which is shown in figure 2. This quick process for compound selection and batch submission allows for a walk up and analyse approach.

Following the acquisition of the sample batch the next potential bottleneck becomes the data processing and the mass peak extraction. With the proposed workflow this is where DiscoveryQuant™ makes full use of the supplied compound information. As we have specified the molecular formula of the compound and therefore the monoisotopic accurate mass this allows for the semi-automated mass extraction from the ToF MS data. This is shown below in figure 3.

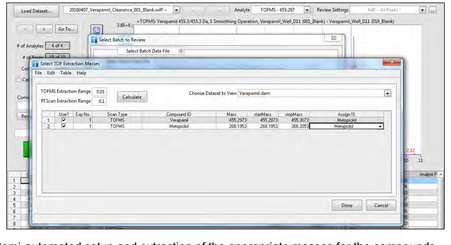
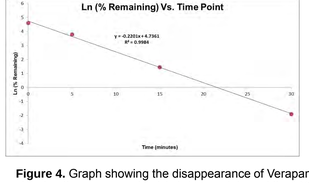


Figure 3. Semi-automated setup and extraction of the appropriate masses for the compounds.

With the semi-automated extraction of the masses from the ToF MS data we can quickly extract, integrate and export the data back into our LIMS/ELN. To calculate the intrinsic clearance (CL_{int}) and t½ for the test compounds the peak area for both analyte and internal standard have been exported. Once exported the peak areas for both the analyte and internal standard are used to calculate the intrinsic clearance (CL_{int}) and t½ for the test compounds (only Verapamil shown in figure 4).



	CL_{int} (µL/min/mg protein)	T½ (min)
TT6600	220.12	3.15
Reference	204.28	3.39

Figure 4. Graph showing the disappearance of Verapamil with time in the presence of microsomes and a table showing the clearance in µL/min/mg protein and the t ½ in minutes.

Figure 4 shows the solution proposed for optimization free quantification produces high quality data which is in close alignment to current reference values based on triple quadrupole quantification.

The secondary reason for utilizing HRAMS is the extra information that can be ascertained from the same injection. The microsomal stability assay which was chosen as the test assay for the HTS-ADME-Tox workflow is a prime candidate for extracting extra information.

Our primary goal was to determine quantitatively the intrinsic clearance and half life for the test molecule(s). However by simply processing the same data files through MetabolitePilot™ this allows us to generate a quick screen of the metabolites formed *in-vitro*.

MetabolitePilot™ is a software package for metabolite profiling for data acquired on the TripleTOF® platform [2]. Within the software there are three workspaces results, interpretation and correlation. Each of the time points were processed against a standard processing method for each compound. The main filtering options employed to detect potential metabolites included the mass accuracy, isotope, mass defect filtering, predicted biotransformation's and cleavage metabolites. Once each time point has been processed it was moved into the correlation workspace and the metabolic profile for the parent generated. This is shown in figure 5.

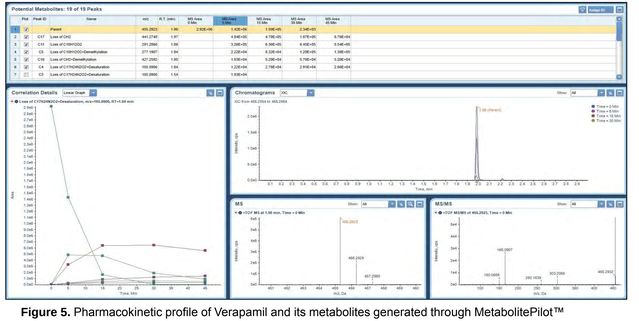


Figure 5. Pharmacokinetic profile of Verapamil and its metabolites generated through MetabolitePilot™

The workflow described is a combination of the acquisition and quantification through DiscoveryQuant™ and the metabolite profiling through MetabolitePilot™. This delivers multi-level HTS-ADME data from only one injection.

CONCLUSIONS

The benefits for HTS-ADME-Tox have been explored for some time already, but the solution described here is in its functionality enabling the use of HRAMS in a true HTS-ADME-Tox environment for the first time. The ease-of-use workflows enabled by DiscoveryQuant™ on the TripleTOF® generates high quality multi-leveled data allowing for confidence in the final result of clearance and metabolite profiles.

REFERENCES

1 Kola I and Landis J. (2004) *Nat. Rev. Drug. Discov*. 3(8). 711-715
2 Breakthrough Productivity for ADME Studies Using The SCEIX TripleTOF™ 5600, Ghobarah H, Ramigiri S and Ferguson J. SCIEX Publication Number: P-0480110-01

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