# Breakthrough Productivity for ADME Studies Using The AB SCIEX TripleTOF™ 5600 System

***Speed, Sensitivity, Resolution, Mass Accuracy and Linearity - All Together in One Instrument for the First Time***

Hesham Ghobarah, Suma Ramagiri and Jim Ferguson
*AB SCIEX, Toronto, Ontario and Framingham, Massachusetts*


Increasing productivity in drug discovery and development continues to be a primary goal in pharmaceutical research. One of the most promising approaches to improving productivity is combining quantitative and qualitative analysis in a single analytical run (Quant / Qual). Quantitative information can be obtained on the parent compound while simultaneously acquiring qualitative information on metabolites in a completely automated fashion.

## The Challenge of Implementing Quant / Qual for Routine ADME Studies

Metabolic stability studies in microsomes, a classic example of in vitro studies, have traditionally been used for estimation of hepatic clearance. For compounds exhibiting rapid metabolism, additional qualitative studies are performed to identify the major metabolites in order to identify metabolic "soft spots". This information aids the medicinal chemist in optimizing the structure. However, performing a separate analysis for metabolite identification is time consuming. A major improvement in productivity can be achieved if both quantitative clearance and qualitative soft spot analysis can be performed in a single analytical run. A key requirement is to obtain accurate mass MS/MS spectra at high speed on a fast chromatographic time scale. In addition, due to the high throughput nature of in vitro assays, hundreds of compounds need to be analyzed weekly. Therefore, a generic data acquisition method without the need to optimize for individual compounds is highly desirable.

Due to their excellent sensitivity and high throughput, triple quadrupole instruments have been the workhorse instrument for the quantitative portion of this application. Accurate mass instruments such as Time of Flight (TOF) and orbital trapping analyzers have been used for metabolite identification due to their high mass accuracy and resolution. Unfortunately, orbital trapping involves a compromise between resolution and speed. Traditional TOF technology has shown limitations in linearity and requires internal calibration to maintain mass accuracy. Modifying study designs, sample preparation procedures, or chromatography in order to accommodate instrument limitations is not acceptable.

## Advanced Technology Enables True Quant / Qual without Compromises

The AB SCIEX TripleTOF™ 5600 System combines the best attributes of triple quadrupoles and accurate mass analyzers in a single instrument. The highly innovative design of the AB SCIEX TripleTOF™ 5600 System combines a proven high performance triple quadrupole front end with the Accelerator TOF™ Analyzer, a state of the art accurate mass analyzer with unprecedented performance and stability (Figure 1). This results in the linearity and sensitivity of a high performance triple quadrupole, combined with speed, high mass accuracy (2 ppm or less) and high resolution (30,000) of an accurate mass instrument, even at low mass for small molecules.

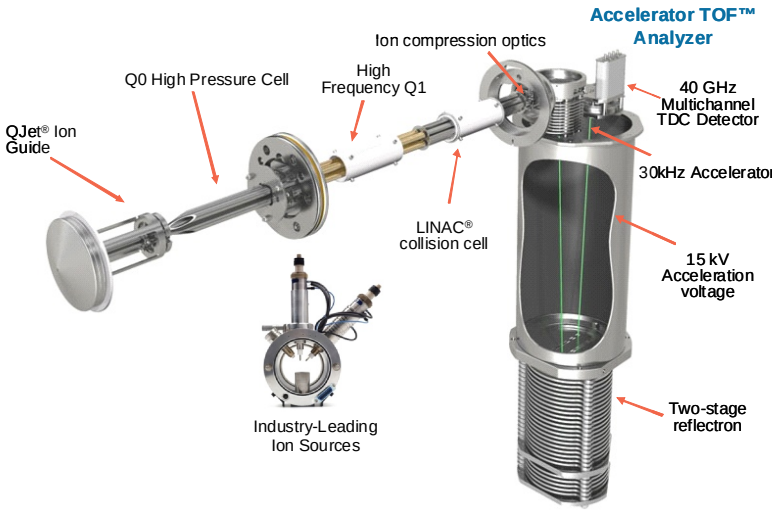


**Figure 1. AB SCIEX TripleTOF™ 5600 System Ion Path.** Proven front end technology coupled to a state of the art Accelerator TOF™ Analyzer resulting in unparalleled performance for both Quant and Qual.

In this technical note, we will highlight a true Quant / Qual application of the AB SCIEX TripleTOF™ 5600 system for ADME studies. Assay modifications are not necessary to realize the benefits of true quant and qual in a single experiment, a true breakthrough in productivity.

## Experimental Conditions

***Sample Preparation:*** Six compounds (clomipramine, diclofenac, imipramine, haloperidol, verapamil, and midazolam) were incubated in rat liver microsomes at an initial substrate concentration of 1 µM in 96-well format. Standard high throughput incubation conditions were used with time points of 0, 5, 15, 30, 60, and 120 minutes. Protein concentration was 1 mg/mL and NADPH was present at 4 mM. Reaction was quenched using an equal volume of acetonitrile then diluted 1:1 with water prior to analysis. The final substrate concentration at t=0 was 0.25 µM.

***Chromatography:*** Sample analysis was performed on the AB SCIEX TripleTOF™ 5600 system coupled with a Shimadzu Prominence UFLC-XR HPLC system. A generic acetonitrile / water / 0.1% formic acid gradient was used on a Phenomenex Synergi Polar-RP column 2.5 µm, 2 x 50 mm. Total run time was 3.7 minutes. Injection volume was 10 µL.

***Mass Spectrometry:*** A completely generic method was used for data acquisition on all compounds and all samples. The method consisted of a TOF MS survey scan followed by two IDA TOF MS/MS scans. The mass range was m/z 100 – 1000 for both MS and MS/MS. An accumulation time of 100 ms was used for each scan. Dynamic Background Subtraction was applied for IDA criteria and a collision energy of 35 eV with a spread of +/- 10 eV was used for the MS/MS scans. External mass calibration was performed automatically using a calibrant delivery system. Data processing was performed using MultiQuant™ and MetabolitePilot™ software (Figure 2).

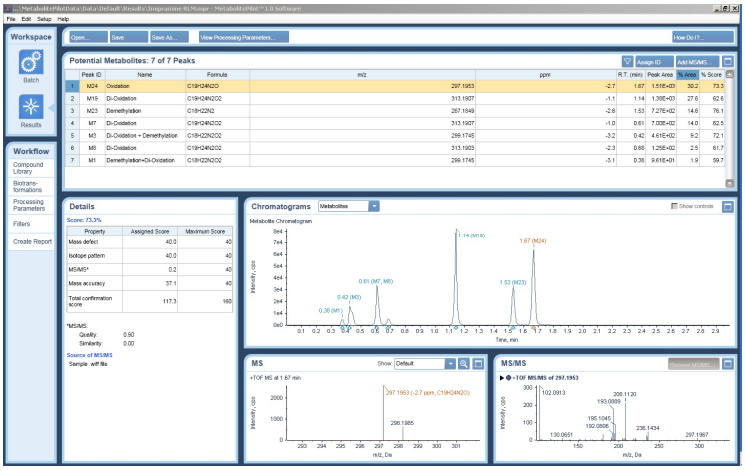

**Figure 2. Easy Data Processing in MetabolitePilot™ Software.** Next generation software simplifies accurate mass metabolite identification with automated data processing in batch mode.

## Results – Sensitivity and Speed

MultiQuant™ software was used to process the TOF MS data and generate all quantitative information. An XIC window of +/- 10 mDa was used for all compounds. Peak areas were used to plot the % remaining relative to t=0 (Figure 3) and MetabolitePilot™ software was used to process the data for metabolites.

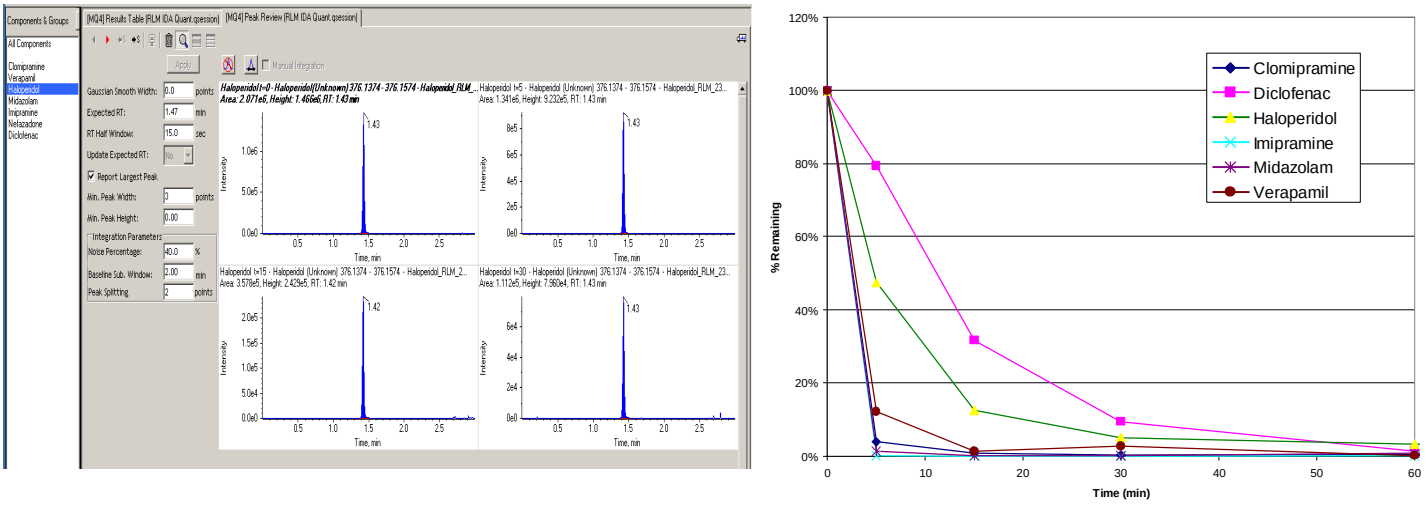


**Figure 3. Data Processing in MultiQuant™ Software.** (a) TOF MS data was easily processed for multiple analytes to obtain quantitative data. (b) Metabolic stability profiles of 6 common substrates incubated at 1 µM and analyzed using TOF MS with IDA triggered MS/MS.

Metabolic stability studies are best performed at a lower substrate concentration (1 µM) which is more physiologically relevant. Enough sensitivity is required to detect at least 1% of parent remaining in order to obtain meaningful kinetic data. The AB SCIEX TripleTOF™ 5600 system demonstrated excellent sensitivity and speed. For example, 0.1 % of midazolam remaining was easily detected (Figure 4). This represents a concentration of 0.25 nM or 0.8 pg on column.

Sensitivity does not mean compromising speed. A minimum of 8 points was obtained in IDA mode (with two dependent MS/MS scans) across a 4 second peak while maintaining 30,000 resolution in both MS and MS/MS mode. In fact, each data point consisted of 3 scans (TOF MS plus 2 MS/MS scans) for a total of 24 scans.

## High Resolution and Mass Accuracy

Excellent mass accuracy and resolution were achieved in both MS and MS/MS scans (Figures 5). High mass accuracy in MS/MS greatly facilitates structure elucidation as it allows unambiguous assignment of elemental composition.

Using MetabolitePilot™ software, data from multiple samples and compounds was processed unattended in batch mode. The software reported metabolites detected and elemental compositions in a concise and powerful yet user friendly interface (Figure 2).

Excellent separation, metabolite coverage, and speed were demonstrated using a generic IDA method (Figure 6).

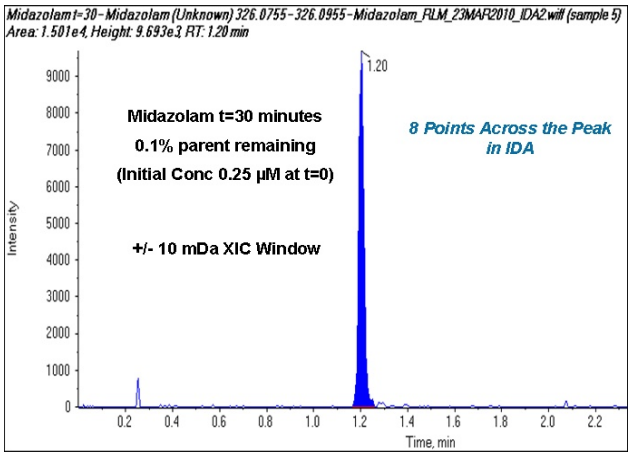


**Figure 4. Sensitivity and Speed.** The 30 minutes time point from Midazolam microsome incubation with 0.1% of parent remaining (0.8 pg on column). Analyte is easily detected in TOF MS IDA mode with 8 data points. Each point consists of a survey scan and two MS/MS dependent scans.

## No Compromises

Successful application of quant / qual technology to in vitro ADME was demonstrated using the AB SCIEX TripleTOF™ 5600 System. No compromises were made in resolution in order to achieve speed or sensitivity. A relatively low substrate concentration of 1 µm was successfully used. Standard incubation and HPLC conditions were used with no need to make any modifications in order to gain the benefits of quant / qual.

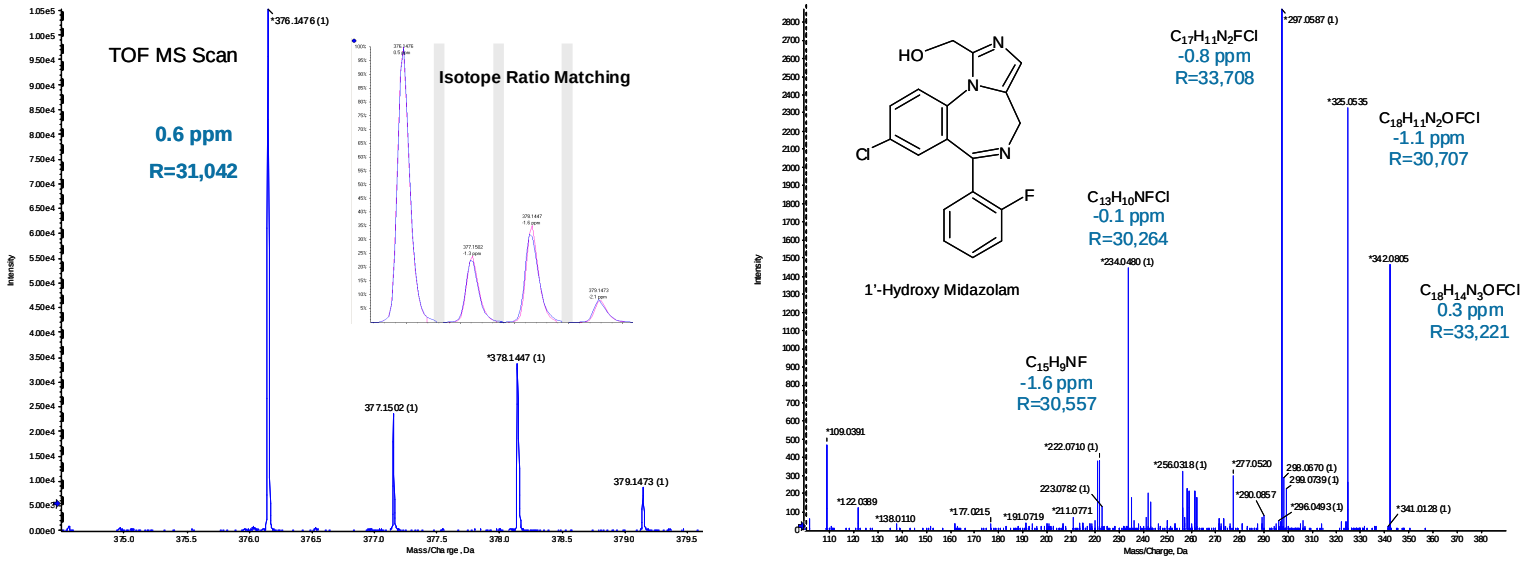


**Figure 5. Mass Accuracy and Resolution in TOF MS and MS/MS Mode.** Haloperidol parent in the 5 minute time point with excellent accuracy and resolution is shown on the left with a near perfect match of isotope pattern (inset). Product ion spectrum of hydroxy midazolam in the 5 minute time point acquired with IDA is shown on the right. Excellent mass accuracy and 30,000 resolution allow easy assignment of elemental composition, even to the product ions.

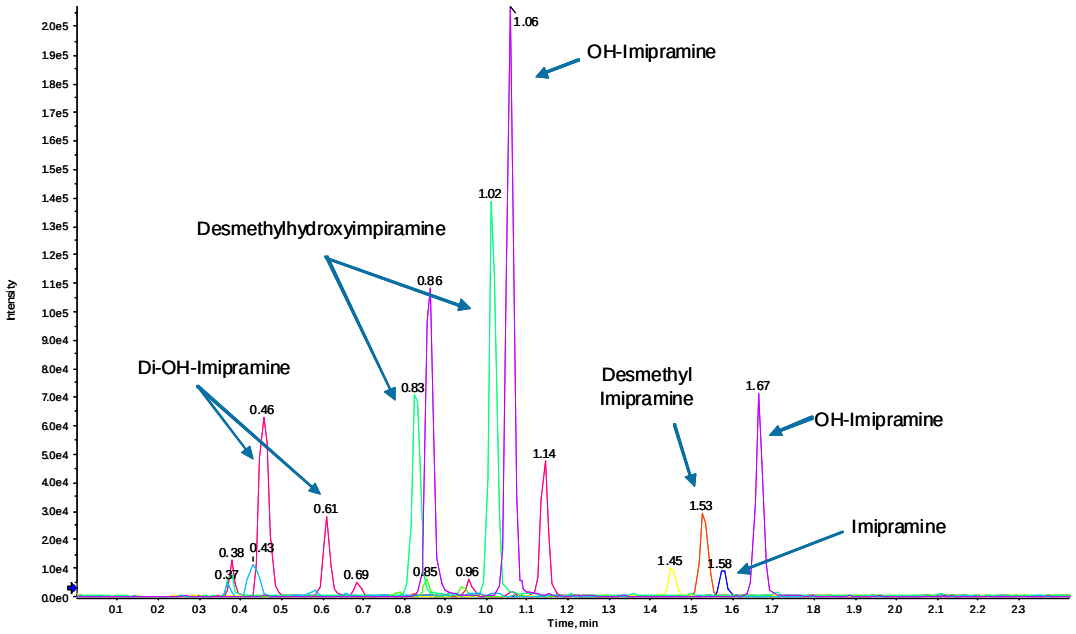


**Figure 6. Broad Coverage for Phase I Metabolites.** Overlay of accurate mass XIC's for imipramine incubation at 5 minutes. A wide range of imipramine Phase I metabolites were easily detected using a completely generic TOF MS IDA method in under 2.5 minutes.

## Conclusions

- The AB SCIEX TripleTOF™ 5600 is the first accurate mass instrument to offer quantitative performance comparable to a high performance triple quadrupole.
- The combination of speed, sensitivity, mass accuracy, and resolution enables routine quant / qual analysis. For metabolic stability we have shown the ability to perform parent quantification, metabolite detection, and obtain accurate mass MS/MS information all in a single run.
- MultiQuant™ and MetabolitePilot™ Software are powerful tools for efficient analysis of quantitative and qualitative data.
- With proven front end technology and high performance, the AB SCIEX TripleTOF™ 5600 system fits well with existing high throughput sample preparation and fast chromatography.

## Acknowledgements

The authors would like to thank Dr. Christie Hunter and Tanya Gamble for their invaluable contributions and review.

**For Research Use Only. Not for use in diagnostic procedures.**



Publication number: P-0480110-01

**Headquarters**
353 Hatch Drive Foster City CA 94404 USA
Phone 650-638-5800
**www.absciex.com**

**International Sales**
For our office locations please call the division headquarters or refer to our website at
**www.absciex.com/offices**