# The ADME–Hub: Formalizing and Automating Information Flow in the Preparation and Measurement of Lead Optimization assays

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## Overview

A software system for early-ADME studies is presented that collects plate and assay information remotely and efficiently at the point-of-plate-preparation. The system uses a convenient barcoding system to transfer setup data to the instrumentation laboratory.

## Introduction

Performing empirical lead optimization in pharmaceutical discovery involves the experimental determination of metabolic fitness characteristics for a large set of compounds using a battery of assays involving mass spectrometry. Preparing plates of samples requires a researcher with expertise in assay preparation. This researcher (the "Plate Preparer") is often distinct from the LC/MS expert measuring the quantitative results of the assay with a mass spectrometer, and the work is often performed in a different laboratory space.

The Plate Preparer generally has access to all the data needed for setting up the experiment and tracking compounds. The LC/MS expert only needs to add the appropriate ion transition information and the instrumental setup to perform the measurement. In the most efficient system, information about the plate setup would blend seamlessly with the ion transition and instrument setup information, and accompany the sample data through the measurement review process.

Several information transfer solutions have been presented within the area of early ADME, including the use of Electronic Laboratory Notebooks for storage of plate information. However, the retrieval of this information often requires an importing process within the software which is neither automated nor tracked.

One well-documented solution involving transition information and barcoded plate setup is an impressive custom software system that relies on file input for plate setup, and is restricted to a single autosampler configuration (Luippold et al.)[1].
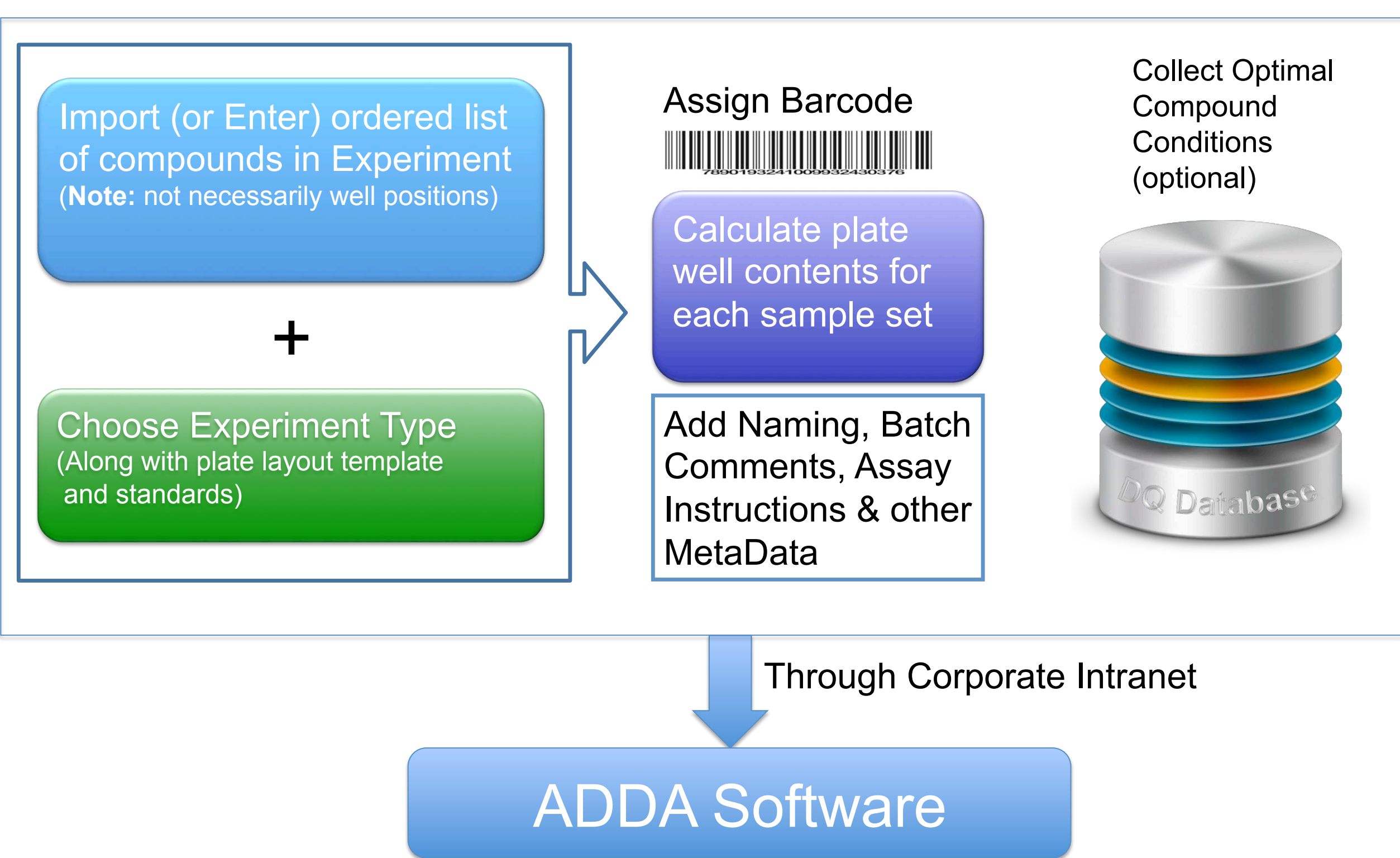
Figure 1: The ADME-Hub software allows the Plate Preparer to enter all compound-specific information necessary to run the batch of compounds, and link it to the plate's barcode.

The system described here gains efficiency by combining a pre-setup software module (the "ADME-Hub") to collect chemical, positioning, and assay information within the plate with an automated setup module (the ADDA Software). The ADME-Hub provides an efficient interface to lay out plates, taking advantage of known experimental configurations and common additions (e.g. Calibration Standards) to provide an efficient plate setup environment. Importing (from Excel or LIMS) is also available. In addition, it allows for batch comments and instructions based on the type of assay chosen (see Figure 1).

The autosampler (ADDA) software uses barcode information to access the ADME-Hub output, and correctly sets the mass spectrometry parameters for each analysis, using barcode pairs to dynamically inform the software where each plate has been positioned for analysis. A plate can thus be placed in any open plate position on the deck.

## Methods

### Transport Study
The transport assay set contained the compound **Quinidine**, arranged across 7 plates. In sequence:
Blank 1, Blank 2, Blank 3,  A-B (1), Blank 4, A-B (2), Rec (1), Rec (2), Time Zero (1), Time Zero (2).

**Details:** RRCK cells were seeded into transwell plates and capped. Plates were placed in a shaking incubator overnight at 37°C. Quinidine was dosed to transwell plates at 2µM, plates were incubated for 90 minutes at 37°C. At 90 minutes, 300µL sample from plates 3 and 5 (A->B) was diluted with 300µL deionized water and 120 µL acetonitrile containing internal standard. Recovery and T0 plates consisted of 20 µL sample, 130µL buffer, 150µL deionized water and 120µL acetonitrile containing internal standard. Sample plates were sealed and mixed prior to analysis.

### Metabolic Stability
The metabolic stability study for **Verapamil** was conducted with pooled (n=50 donors) human liver microsomes (HLM). Comments captured in Figure 2 give preparation details and method directions.

### Transferable Information
Each plate was barcoded with a unique barcode. Using the ADME-Hub software, compound-specific information was entered into the system (Compound names, Internal Standards, and well positions). In addition, assay-type-specific information was included such as the plate preparation protocol used, and instructions on how to handle the plate.
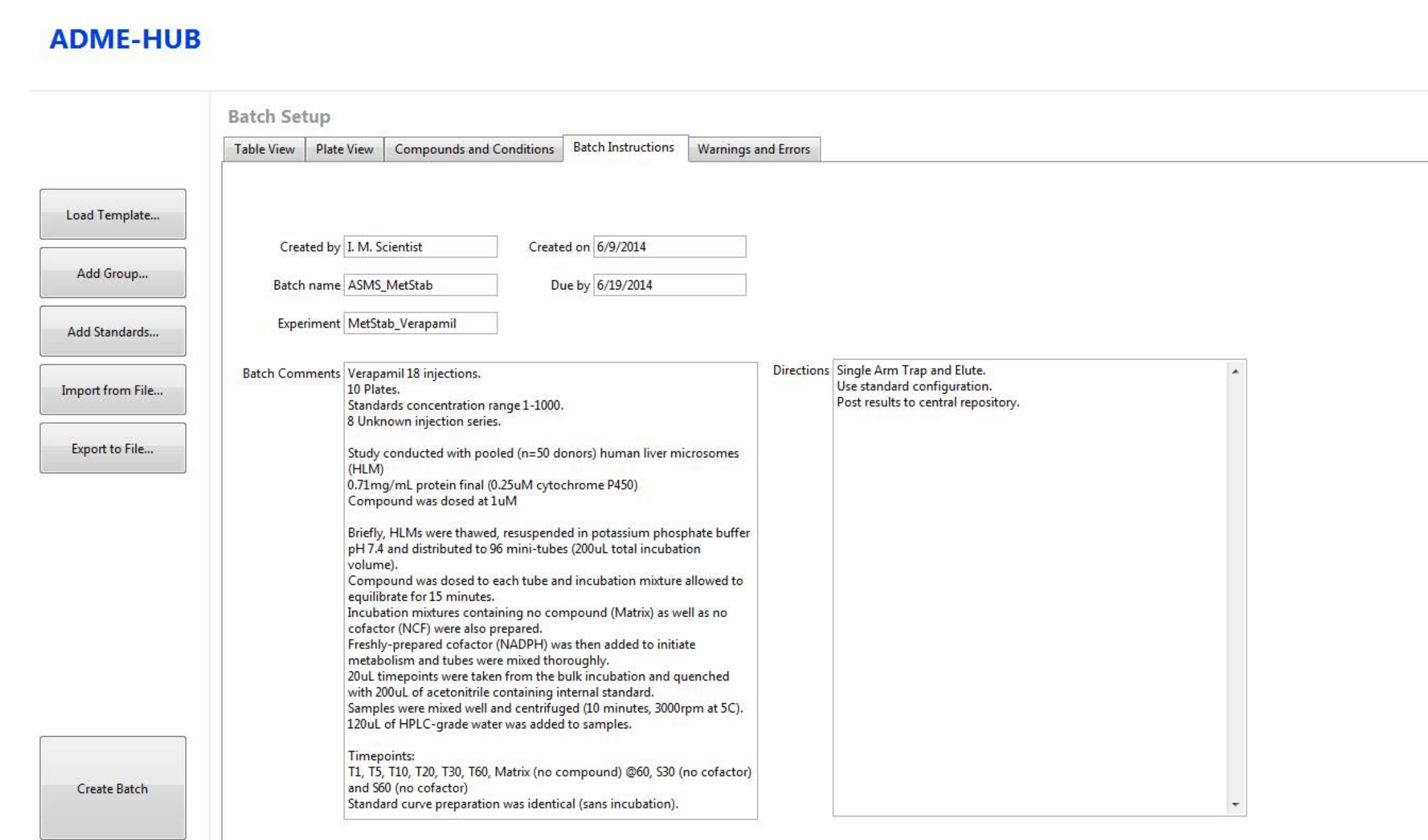
Figure 2: Comments and instructions are entered or imported into the ADME-HUB software on the Batch Instructions tab. The metabolic stability experiment plate preparation procedure is shown in this example.

Figure 3: The ADME-Hub software allows the Plate Preparer to enter all compound-specific information necessary to run the batch of compounds. In this figure, the table view for the verapamil metabolic stability experiment is shown.

The plates were submitted to the mass spectrometry lab with their barcodes for identification.

Barcodes were also placed on the bottom of the tray at each plate position on the autosampler. On setup, the barcode of the plate and the barcode of the plate position were both scanned for all plates sequentially. A handheld barcoding device was used for all barcode identification.

The plates were run and the results compiled. An Apricot Designs Dual Arm (ADDA) autosampler, Shimadzu LC-20 pumping system, and an ABSCIEX 4000 QTRAP were used to perform the measurement. The ADDA™ software was used to analyze the results.

## Results

For both the transport and the metabolic stability study in sequence, the ADDA software was placed into the barcode-reading mode (see Figure 4). Barcodes for the plate and the plate position on the autosampler deck were taken in pairs. During barcode reading, the software display updated to reflect the samples on the deck, giving the user timely feedback as to which samples were contained in each plate (see Figure 4). No keyboard inputs were necessary during plate positioning, allowing the user to perform the barcode reading with one hand and place the plates on the deck with the other.
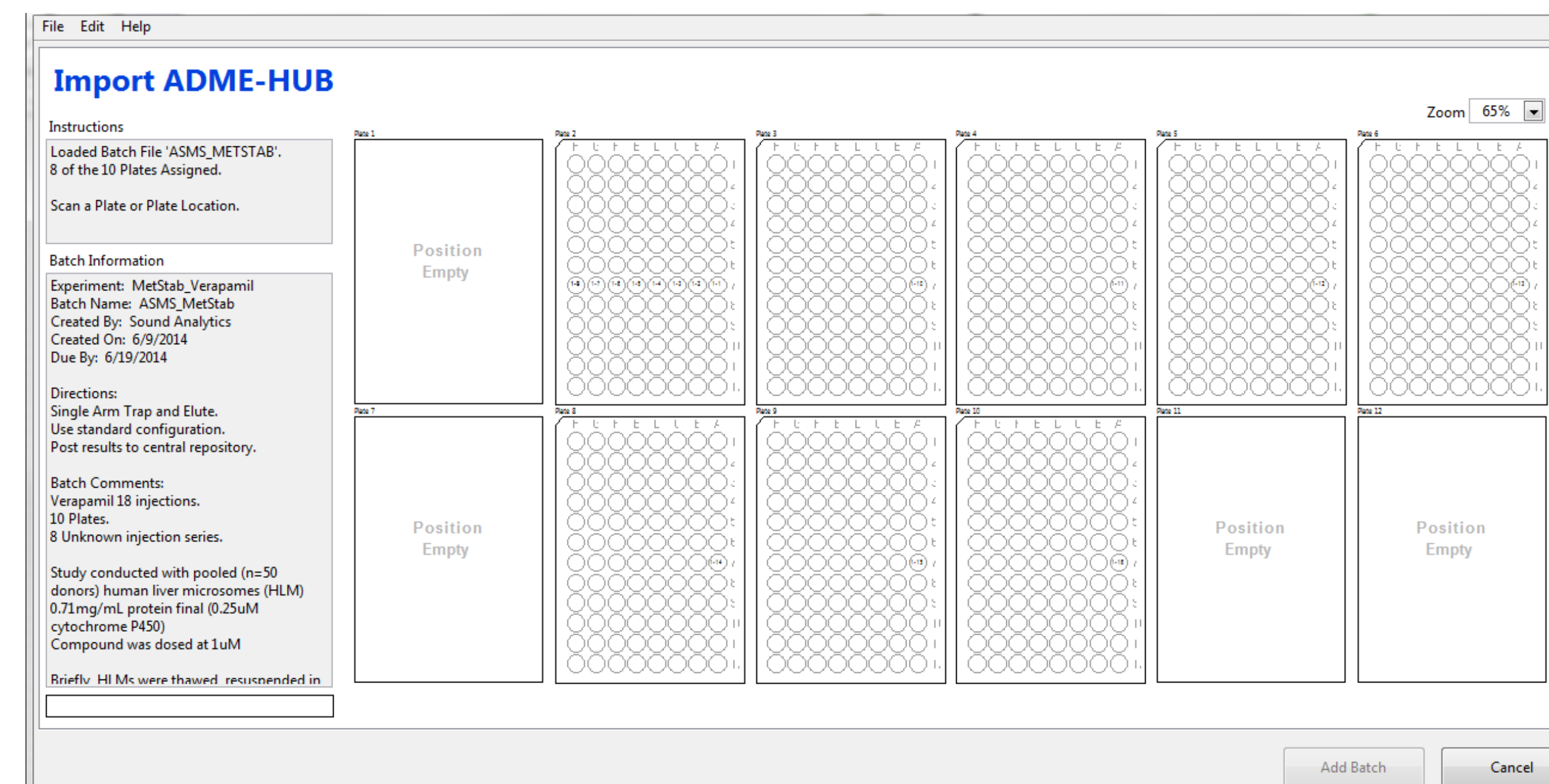
Figure 4: ADDA Software barcode entry screen with 8 of the 10 plates in the batch mapped. A plate and a rack position are scanned in series, causing the software to import the compound map (set up in the ADME-HUB) to the correct plate position on the autosampler.

For the transport study, the order in which the barcodes were read (plate-then-position, or position-then-plate) was switched around for different plates, and the plates were placed on the rack in a different order than specified in the ADME-Hub software. However, the plates were processed by the autosampler in the order given in the ADME-Hub, demonstrating the independence of the measurement process from the plate placement process.

The injection port and the analysis mode used (trap and elute) were set in the ADDA™ software.

Results were calculated for the metabolic stability study, as shown in Figure 5. The regression for the standard curve had R = 0.998.

## Conclusions

The execution of this procedure has demonstrated that plate preparation information can be collected remotely and passed to the instrument setup software by way of a database and a barcode. This procedure provides a method to increase the efficiency of operation by minimizing the downtime of the mass spectrometer, while at the same time minimizing manual data transcription errors.

With this setup, it is possible for the mass spectrometry scientist to gather all necessary knowledge of the contents of the plate from the barcode. The barcode refers the software to remote information collected with the ADME-Hub software. The demonstration of this behavior is novel for the instrumentation configured.

We expect that future work will center around the ability to quickly set up diverse plate formats within the ADME-Hub, diversifying the instrument/software set from which the ADME-Hub can import and export samples.
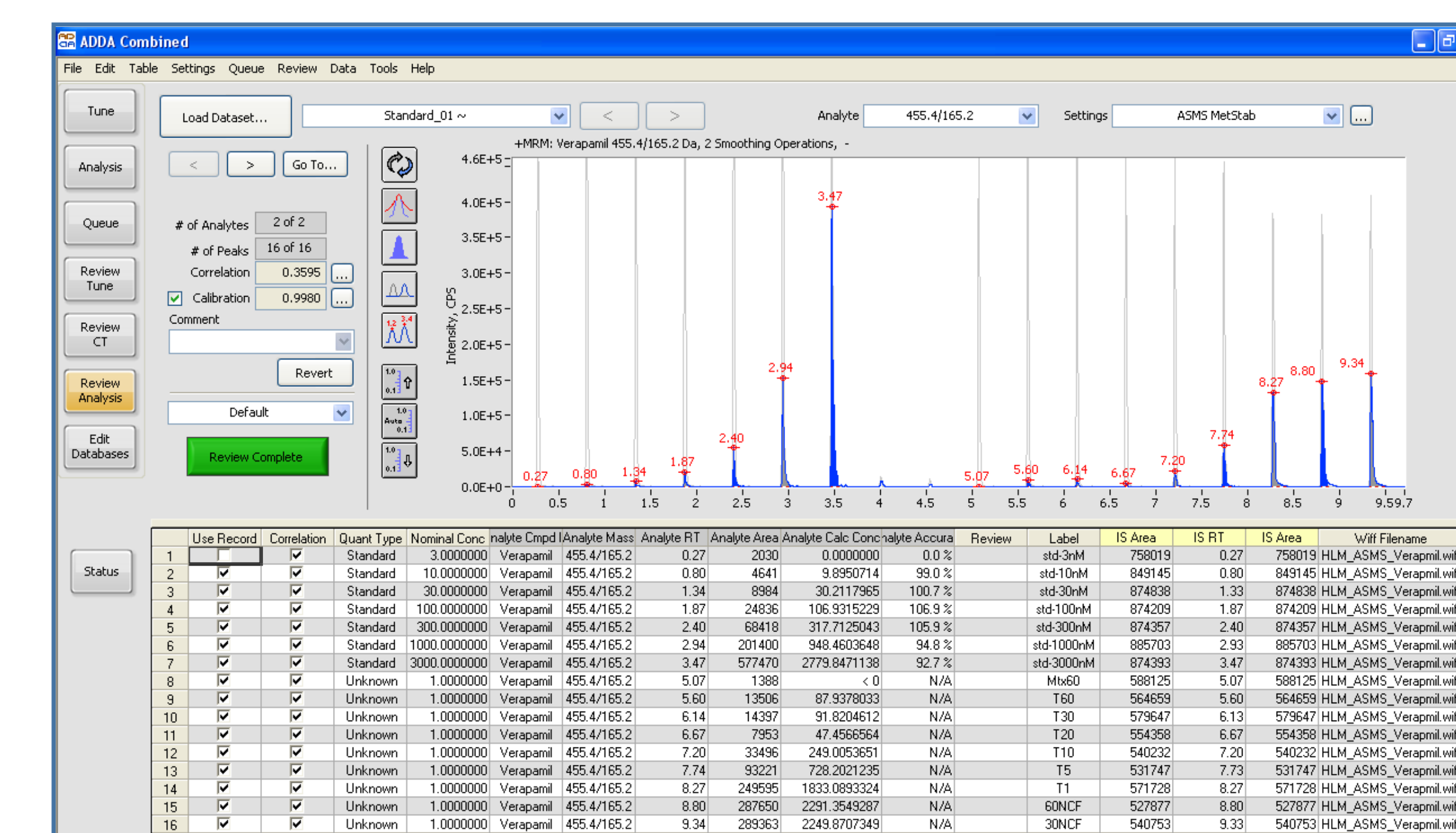
Figure 5: Standards and metabolic stability raw data from a multiply-injected run for Verapamil. The first 7 peaks are the standards, followed by 2 blanks, and the metabolic stability data.

Reference [1] Luippold, A. H., Arnhold, T., Jörg, W., Krüger, B., & Süssmuth, R. D. (2011). Application of a rapid and integrated analysis system (RIAS) as a high-throughput processing tool for in vitro ADME samples by liquid chromatography/tandem mass spectrometry. Journal of biomolecular screening: the official journal of the Society for Biomolecular Screening, 16(3), 370–377.