MassHunter Study Manager: A Software Tool to Automate LC/MS Analysis for Drug Metabolism and Pharmacokinetics Studies

Application Note

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Abstract

MassHunter Study Manager is a software tool that automates LC/MS analyses for drug metabolism and pharmacokinetics (DMPK) studies, particularly for multiple sample sets and studies queued up for analysis on a single instrument. This tool enables LC/MS assays to be fully automated from compound MRM optimization to LC/MS method creation, data acquisition, data processing, and report generation. This workflow is successfully illustrated with *in vitro* metabolic stability assays of 38 pharmaceutical compounds using MassHunter Study Manager software interfaced to an Agilent 6460 Triple Quadrupole LC/MS System.[1] This software tool greatly streamlines the LC/MS workflow and increases analytical productivity.

Introduction

LC/MS/MS has proven to be the technique of choice for rapid quantitation of drugs and their metabolites in biological samples to support DMPK studies. However, in early stage drug discovery, where thousands of new chemical entities (NCEs) may be screened for ADME assessment, MRM optimization of a large set of compounds and subsequent data processing often becomes a bottleneck. Because of this, further streamlining and automation of the LC/MS analysis is crucial for improving productivity. To meet this need, the MassHunter Study Manager software tool automates all LC/MS analysis steps from compound MRM optimization, method creation, and data acquisition through to quantitation. This tool greatly reduces labor-intensive manual steps and increases productivity. In this work, an example of using MassHunter Study Manager for *in vitro* metabolic stability studies is shown for a set of pharmaceutical compounds.

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Results and Discussion

LC/MS workflow automation

MassHunter Study Manager is essentially a scheduler that queues sample sets and schedules the tasks needed to complete the LC/MS analysis automatically. Figure 1 shows a complete LC/MS workflow for *in vitro* screening assays and illustrates how MassHunter Study Manager automates the sample analysis.

To begin, the user creates a Microsoft Excel file with two worksheets: the first contains the compound information, including name, formula, and plate position; the second contains information on each sample, such as name, plate location, sample group, and which sample is to be used as the reference file for building the quantitation method. In this work, time zero samples were used in each case. Figure 2 shows an example of the two worksheets.

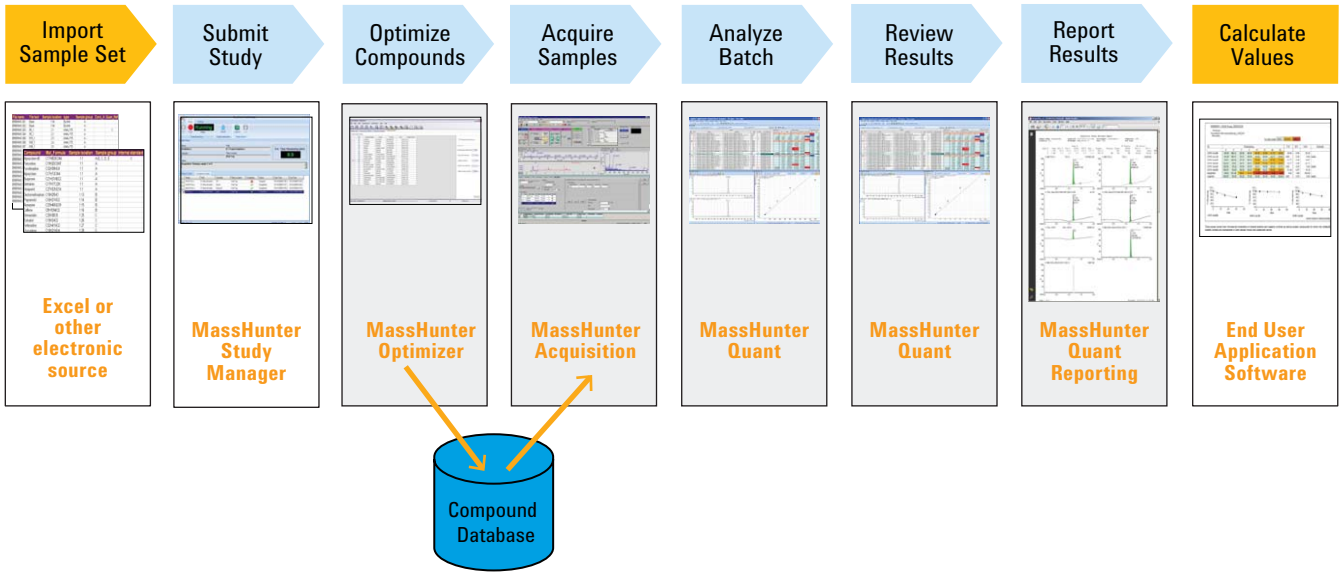

Figure 1. LC/MS Workflow for drug discovery screening assays. Gray panels indicate steps which are automated by using MassHunter Study Manager.

List of Metabolic stability compounds (group 1-8)

Compound Name	Formula	ISTD	Location	Comp Group
Nefazodone	$C_{25}H_{32}ClN_5O_2$		P1-B1	1
Nimodipine	$C_{21}H_{26}N_2O_7$		P1-B4	2
Nicardipine	$C_{26}H_{29}N_3O_6$		P1-B7	3
Midazolam	$C_{18}H_{13}ClFN_3$		P1-B10	4
Propafenone	$C_{21}H_{27}NO_3$		P2-B1	5
Terfenadine	$C_{32}H_{41}NO_2$		P2-B4	6
Buspirone	$C_{21}H_{31}N_5O_2$		P2-B7	7
Ticlopidine	$C_{14}H_{14}ClNS$		P2-B10	8
Bupivicain (IS)	$C_{18}H_{28}N_2O$	TRUE	P1-B2	1,2,3,4,5,6,7,8

Compound group1-8 for met stability (inject 6 uL)

Compound name	Sample Position	Sample Group	Quant Flag	Type	File Name
Blank	P1-A1	1		Blank	Blank_01
Blank	P1-A2	1		Blank	Blank_02
Blank	P1-A3	1		Blank	Blank_03
Blank	P1-A4	1		Blank	Blank_04
Blank	P1-A5	1		Blank	Blank_05
Nefazodone	P1-G1	1		Sample	Nefazodone_60 min_1
Nefazodone	P1-G2	1		Sample	Nefazodone_60 min_2
Nefazodone	P1-G3	1		Sample	Nefazodone_60 min_3
Nefazodone	P1-F1	1		Sample	Nefazodone_30 min_1
Nefazodone	P1-F2	1		Sample	Nefazodone_30 min_2
Nefazodone	P1-F3	1		Sample	Nefazodone_30 min_3
Nefazodone	P1-E1	1		Sample	Nefazodone_20 min_1
Nefazodone	P1-E2	1		Sample	Nefazodone_20 min_2
Nefazodone	P1-E3	1		Sample	Nefazodone_20 min_3
Nefazodone	P1-D1	1		Sample	Nefazodone_10 min_1
Nefazodone	P1-D2	1		Sample	Nefazodone_10 min_2
Nefazodone	P1-D3	1		Sample	Nefazodone_10 min_3
Nefazodone	P1-C1	1		Sample	Nefazodone_5 min_1
Nefazodone	P1-C2	1		Sample	Nefazodone_5 min_2
Nefazodone	P1-C3	1		Sample	Nefazodone_5 min_3
Nefazodone	P1-B1	1	TRUE	Sample	Nefazodone_0 min_1
Nefazodone	P1-B2	1		Sample	Nefazodone_0 min_2
Nefazodone	P1-B3	1		Sample	Nefazodone_0 min_3
Blank	P1-H1	2		Blank	Blank_13
Blank	P1-H2	2		Blank	Blank_14
Blank	P1-H3	2		Blank	Blank_15

Figure 2. Excel file with test compounds and sample information for a new study.

Map file generation

To optimize the acquisition method, run the samples, and process the data correctly, a one-time procedure is required to map the Microsoft Excel file information to the information needed for Agilent MassHunter Study Manager. This is performed using the Map File Generator software tool. The user provides a typical Excel file which the tool reads; Map File Generator then displays the column names used in the worksheets. Next, the user selects the appropriate **Map Column** from a drop-down list as indicated in Figure 3.

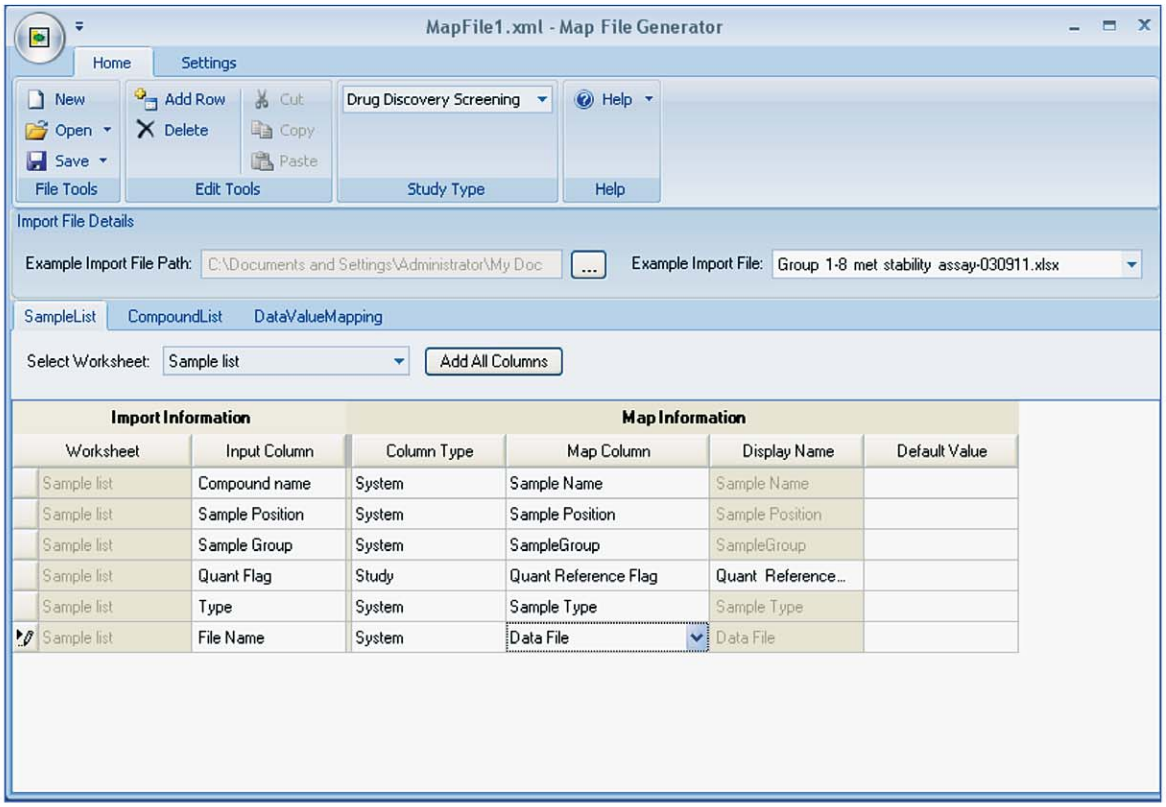

Figure 3. Map File Generator being used for the sample list.

Submitting studies

The user begins by submitting a study with the Study Manager (Figure 4). Next, the user selects one of the four Study Creator options: **Bioanalysis**, **Drug Discovery Screening**, **Optimizer Automation**, and **Worklist Only**. For *in vitro* ADME screening, choose either **Optimizer Automation** or **Drug Discovery Screening**. Optimizer Automation is used to determine MRM conditions for a set of compounds which will be screened later. Drug Discovery Screening is used to integrate compound optimization and analysis in a continuous workflow for each compound set. In either case, the next step is to provide the name of the Excel file.

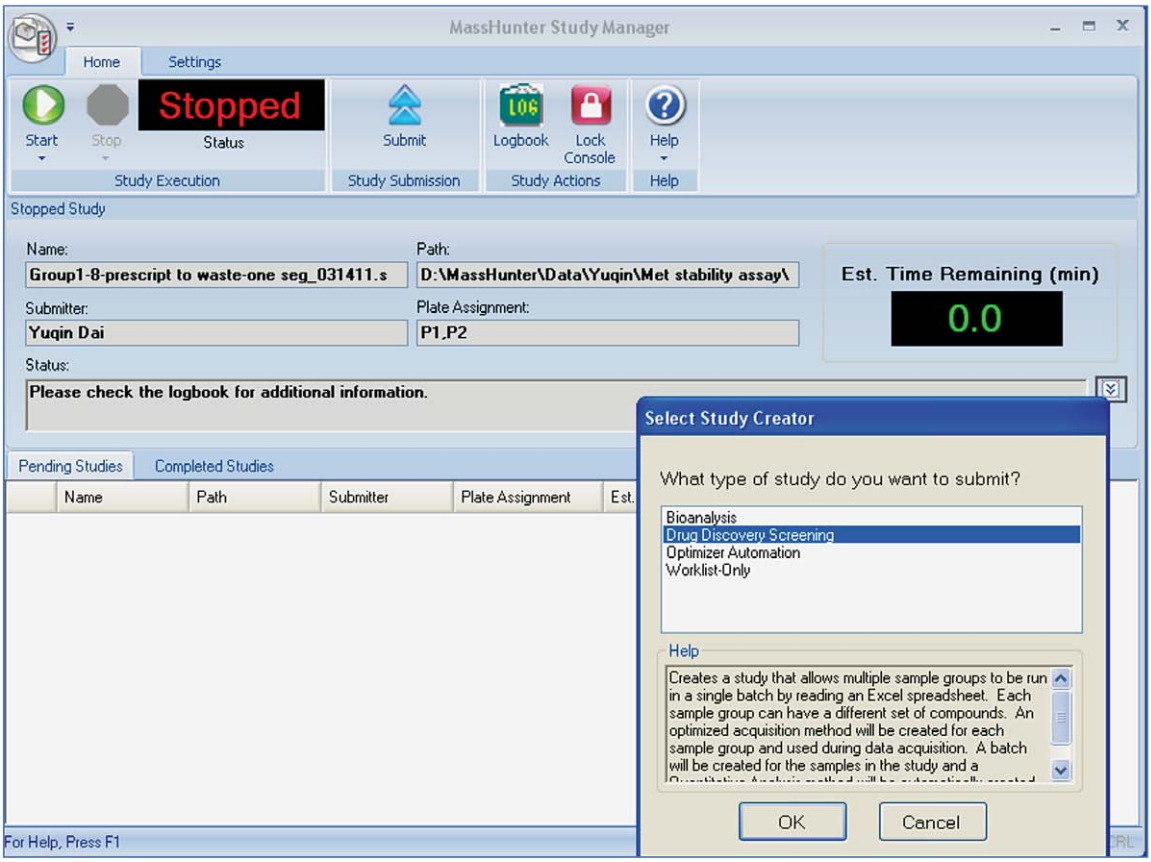

Figure 4. MassHunter Study Manager and submission process.

Running studies

When the analysis of the submitted study commences, Study Manager schedules and completes the following tasks:

1. Determines which compounds have never been optimized
2. Optimizes those compounds and updates the database
3. Creates an acquisition method for each sample set
4. Acquires data for all samples in the study
5. Builds quant method and determines areas and concentrations for all samples
6. Generates an Excel output file to determine ADME properties

Figure 5 shows a study in progress.

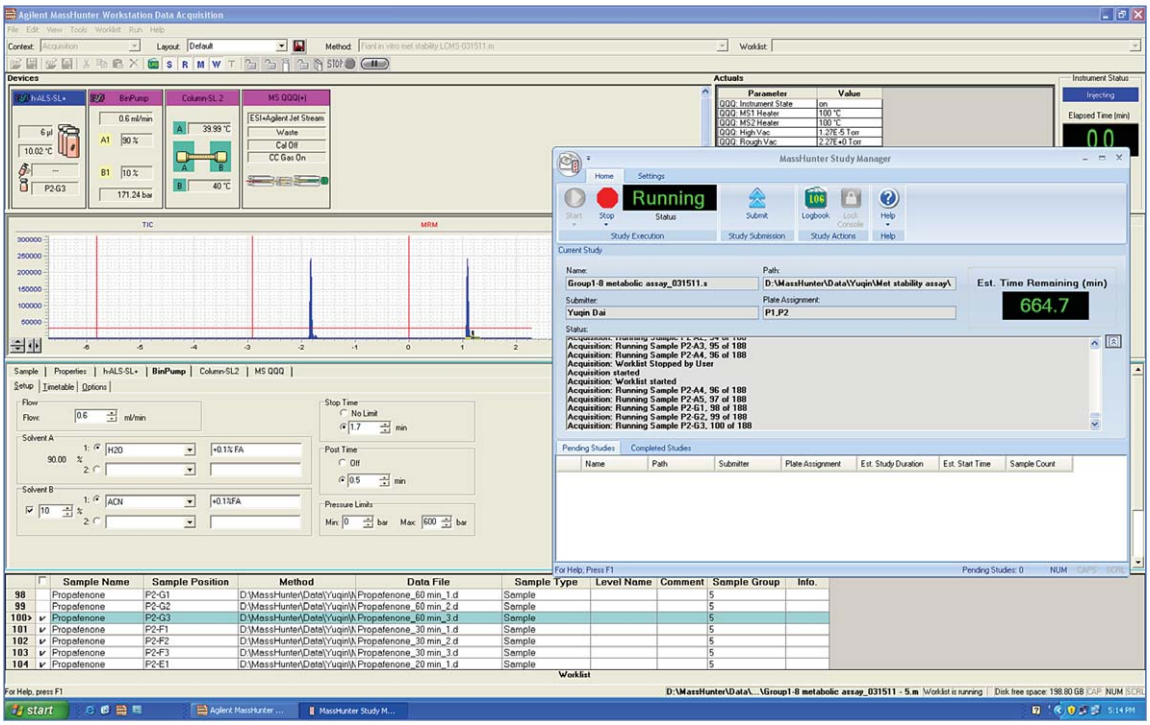

Figure 5. A screening study in progress.

Viewing results

The latest version of MassHunter Quantitative Analysis software was updated to accommodate Drug Discovery Screening, where a batch of samples or a study contains sets of samples, each set having one or more distinct compounds. This not only enables the building of a single method and displaying results for one sample set at a time, but also allows the data to be presented and verified more easily (Figure 6).

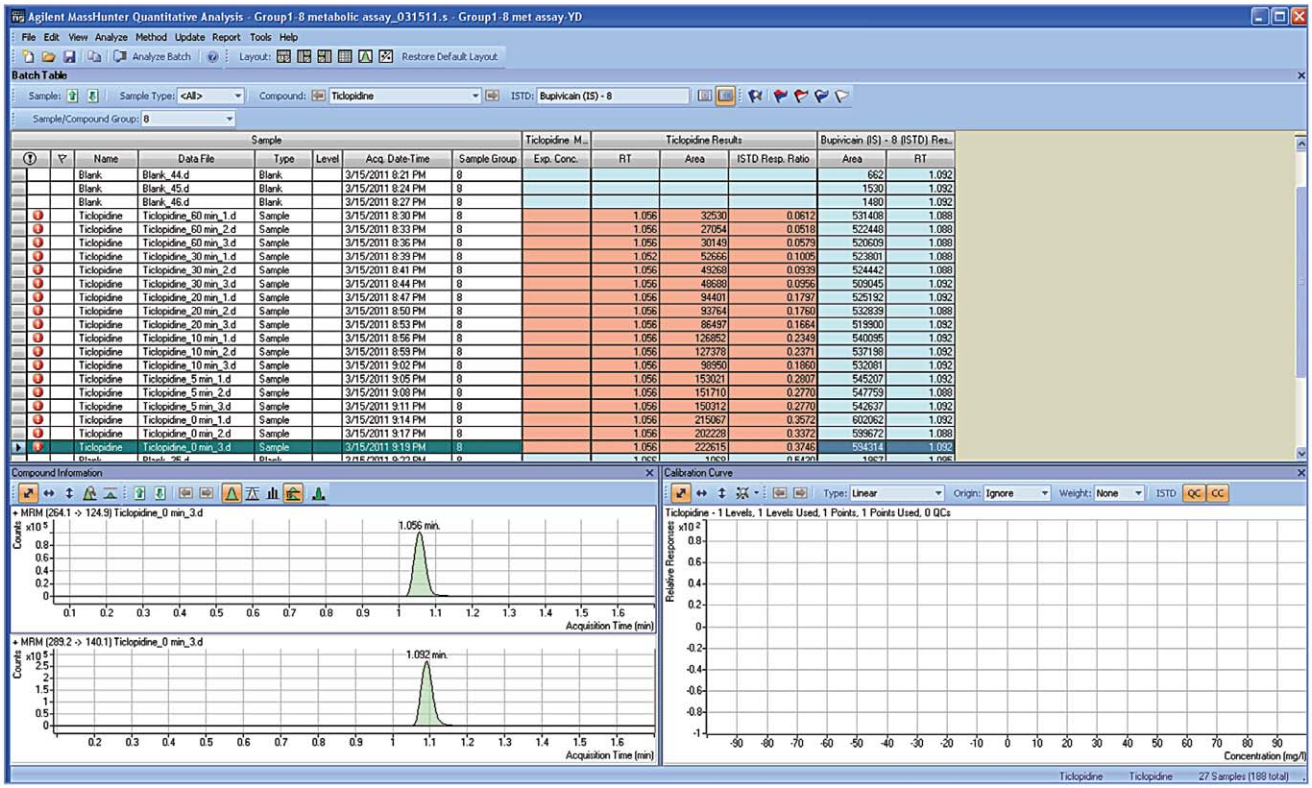

Figure 6. Quant results showing a single sample set.

After reviewing the quantitation results, an Excel output file is produced using the Drug Discovery template, from which metabolic stability profile results can be readily calculated (Figure 7).

Conclusions

Agilent MassHunter Study Manager enables fully automated compound optimization, method building, data acquisition and processing of large numbers of compounds for ADME studies. The intuitive and easy-to-use Study Manager minimizes manual operator interaction for creating the study and processing the results, substantially increasing productivity and the efficient use of resources.

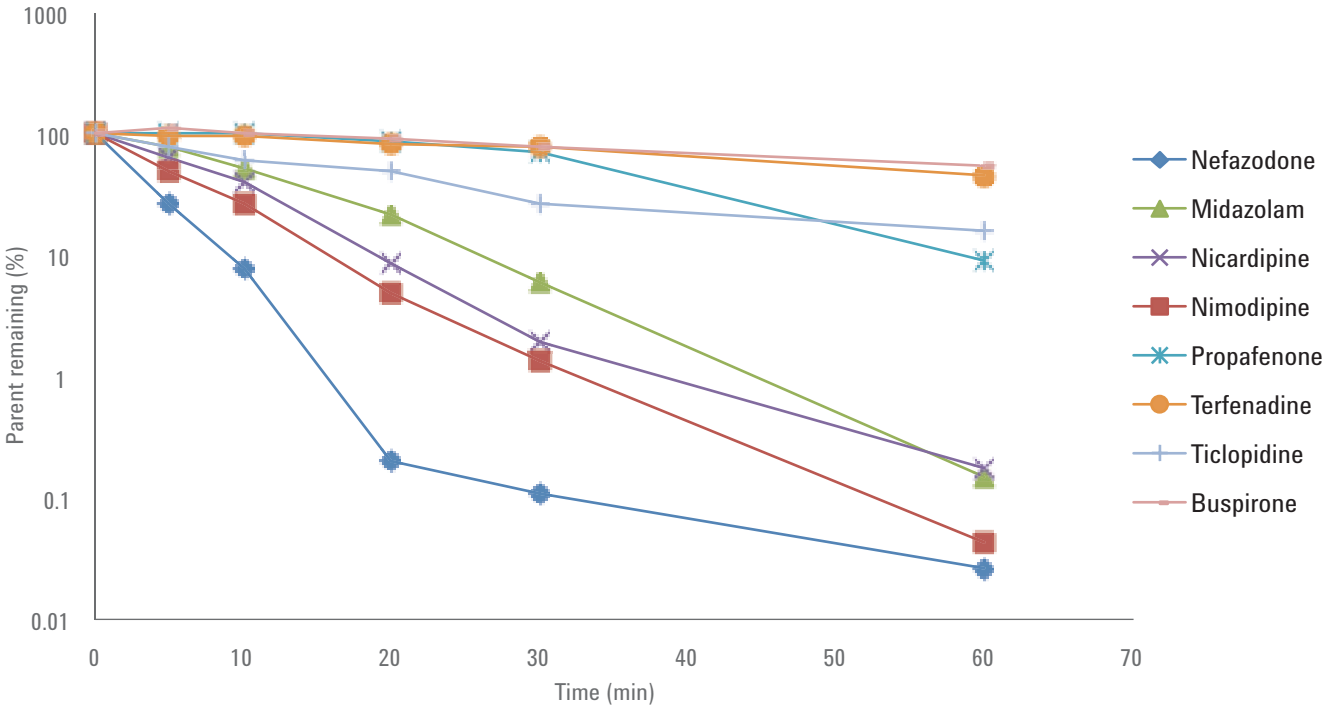

Figure 7. Metabolic stability profiles of eight pharmaceutical compounds in human liver microsomes.

References

1. Romm, M.V., *et al*. Ultrafast Analysis of Metabolic Stability Assays Using Rapid Fire-High Resolution Accurate Mass *Agilent Application Note,* **2011,** publication number 5990-8344EN.

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