

# Automation for Metabolic Stability Assay

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## Abstract:

Metabolic stability using liver microsomes represents a well-established *in vitro* model to estimate hepatic clearance, a major determinant factor of *in vivo* bioavailability. A drastically increased number of new chemical entities are generated by combinatorial chemistry, demanding high throughput methods for metabolic stability screening. Our laboratory has developed and validated the automation of a 96-well format metabolic stability assay

## Introduction:

The clinical development of new drugs has often been discontinued because of unfavourable pharmacokinetic properties other than lack of efficacy, animal toxicity, commercial or other reasons. ADME properties are now analyzed earlier in the process of drug development. Hepatic clearance is a major determinant of *in vivo* bioavailability.

The metabolic stability determination using liver microsomes represents a well-established *in vitro* model to estimate hepatic clearance. This model has been widely utilized to obtain early prediction of hepatic clearance for compounds known to possess desired biological activity, accelerating the identification of lead compounds. Currently, a drastically increased number of new chemical entities are generated by combinatorial chemistry, demanding high throughput methods for metabolic stability screening.

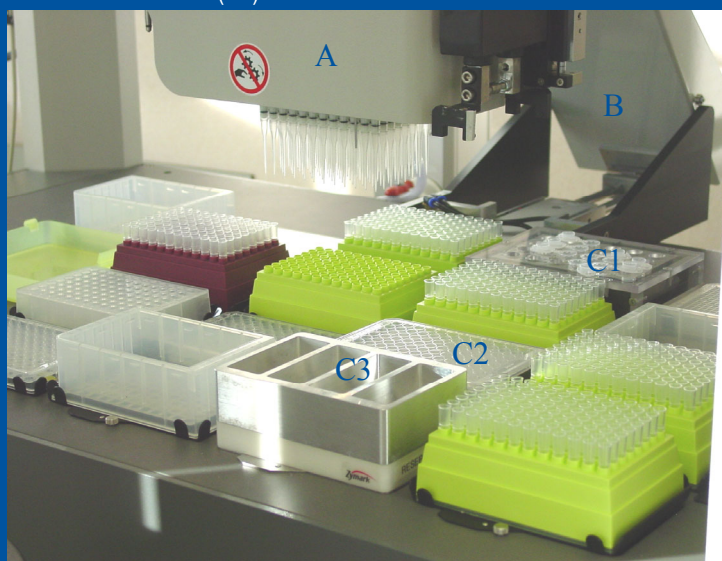
We here describe a metabolic stability assay in 96-well microplates on a Caliper LifeSciences Staccato automation platform combined with liquid chromatography/tandem mass spectrometry (LC-MS/MS) for the analysis of samples.

Picture 1 - Detail of the metabolic stability layout on Sciclone ALH:

A: 96-pipette Head - Sciclone ALH;

B: Tips ejection system;

C: thermostat locations for Eppendorf tubes (C1), 96-well-plate (C2) or reservoir (C3)



## Methods:

Compounds (1  $\mu$ M) were incubated with reaction mixture (160  $\mu$ l) consisting of 0.5 mg/ml of human liver microsomal proteins and NADPH-generating system (1 mM NADP, 5 mM glucose 6-phosphate, 1 U/ml glucose 6-phosphate dehydrogenase, and 5 mM MgCl<sub>2</sub>) in 100 mM Tris buffer (pH 7.4). Reactions were initiated by adding NADPH-generating system. After incubation at 37 °C for 10 and 30 min, reaction was stopped by collecting 40  $\mu$ l of the reaction mixture and mixing it with 30  $\mu$ l of acetonitrile in a 384-well microplate. After stirring, the 384-well microplate was centrifuged for 20 min (4000 g). The disappearance of parent drug was determined using LC-MS/MS method with Turbo Ionspray source and the metabolic stability on human liver microsomes was assessed by the percentage of remaining product at 10 and 30 min. Experiments were performed on a Caliper LifeSciences Staccato station.

## Materials:

Caliper LifeSciences Staccato system included :

- Liquid Handler: CaliperLS Sciclone ALH 500 equipped with a High Volume 96 tip Head, Z8 and Gripper
- Incubator: Kendro CO<sub>2</sub> Cytomat 6000
- Plate handler and Stacker: CaliperLS Twister II
- Scheduler: Clara software

Detection: Quattro Micro spectrometer (Waters)

Biological material: human liver microsomal proteins were purchased from BD Gentest™ (Becton Dickinson, Le Pont de Claix, France).

## Results:

Compound	Time (min)	N	Plate 1				Plate 2				Plate 3				Plate 4				InterPlate			
			Mean (nM)	SD	CV%	%*	Mean (nM)	SD	CV%	%*	Mean (nM)	SD	CV%	%*	Mean (nM)	SD	CV%	%*	Mean (nM)	SD	CV%	%*
LF1	0	10	920	43	4.7	100	808	48	5.9	100	859	12	1.4	100	626	35	5.6	100	811	129	16	100
	10	10	221	14	6.6	24	119	2.6	2.2	15	191	13	6.6	22	123	2.6	2.1	20	169	50	29	21
	30	10	7	4.3	63	0.7	2	0.6	27	0.3	5	0.5	9.7	0.5	2	0.0	3.2	0.3	4	2.6	66	0.5
LF2	0	10	1077	96	8.9	100	1006	30	3.0	100	1011	42	4.1	100	888	110	12	100	1005	83	8.2	100
	10	10	1144	80	7.0	106	1034	21	2.0	103	1126	2.2	0.2	111	950	32	3.3	107	1078	89	8.2	107
	30	10	704	63	9.0	65	608	52	8.6	60	691	35	5.1	68	544	50	9.2	61	637	75	12	63

\* : Percentage of remaining product

Table 1 –Reproducibility and repeatability studies for metabolic stability determination of 2 compounds on human liver microsomes.

## Discussion:

Reproducibility and repeatability results for automated metabolic stability assays are acceptable CV% ( $\leq 15\%$  up until 80 % of metabolisation).

Metabolic stability tests can be performed on a complete automated platform thus reducing technician resources and enabling higher throughput. Nevertheless, these data were obtained with soluble compounds. Further optimizations are ongoing on less soluble compounds in order to obtain satisfactory reproducibility. Preliminary experiments show encouraging results (data not shown).

In a near future, this metabolic stability assay, providing hepatic clearance estimation, will be coupled to metabolite identification for investigation of liability sites to improve drug design.