

# Reactive Metabolite Detection Study -Cysteine Trapping-

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From Drug Development Solutions  
Center



- Drug-induced liver injury (DILI) is caused by various mechanisms and it is difficult to predict it accurately in one type of assay
- Conducting various experiments and making a comprehensive judgment leads to accurate DILI risk evaluation

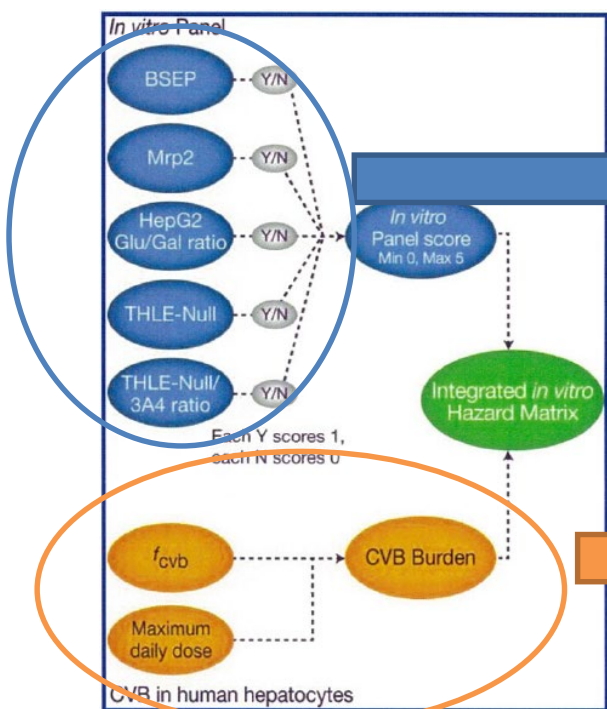


Figure 1. Overview of assays and their interrelationship.

Table 3. Selectivity and Specificity for the *in Vitro* Panel<sup>a</sup>

	Severe and Marked concern	Low concern	
2 or more signals	13	1	PPV (13/14) = 93%
1 or less signals	14	8	NPV (8/22) = 36%
	sensitivity (13/27) = 48%		specificity (8/9) = 89%
			correct = 58%

Table 5. Selectivity and Specificity for the Integrated *in Vitro* Hazard Matrix

	Marked and Severe concern	Low concern	
integrated <i>in vitro</i> hazard detected	27	2	PPV (27/29) = 93%
integrated <i>in vitro</i> hazard not detected	0	7	NPV (7/7) = 100%
	sensitivity (27/27) = 100%		specificity (7/9) = 78%
			correct = 94%

Add Covalent Binding (Reactive metabolite detection)



- Drug-induced liver injury (DILI) is caused by various mechanisms and it is difficult to predict it accurately in one type of assay
- Conducting various experiments and making a comprehensive judgment leads to accurate DILI risk evaluation

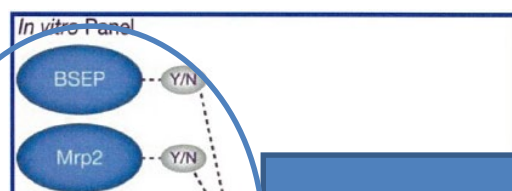


Table 3. Selectivity and Specificity for the *in Vitro* Panel<sup>a</sup>

	Severe and Marked concern	Low concern	
2 or more signals	13	1	PPV (13/14) = 93%
1 or less	1	8	NPV (8/23)

**Reactive Metabolite detection study is very important factor for DILI prediction**

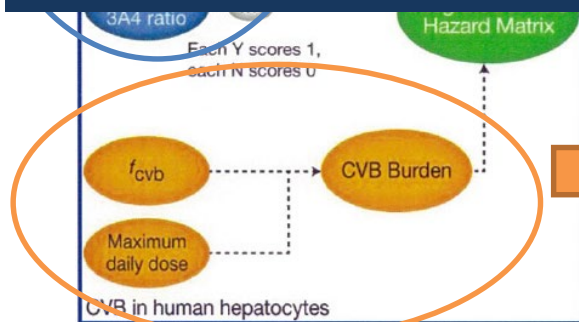


Figure 1. Overview of assays and their interrelationship.

**Add Covalent Binding (Reactive metabolite detection)**

Hazard Matrix

	Marked and Severe concern	Low concern	
integrated <i>in vitro</i> hazard detected	27	2	PPV (27/29) = 93%
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sensitivity (27/27) = 100%		specificity (7/9) = 78%	correct = 94%

Chemical Research in Toxicology  
Volume 25, Issue 8, 20 August 2012



To evaluate reactive metabolites...

- Most accurate method is Covalent Binding study, but this study needs radio-labeled test article
- Therefore it is difficult to conduct it in early stage development
- Generally, in early stage, trapping study is conducted to evaluate reactive metabolites instead of Covalent Binding study  
Most major trapping study is Glutathione (GSH) trapping

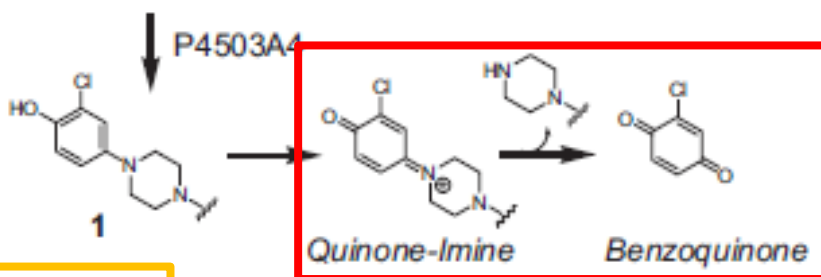
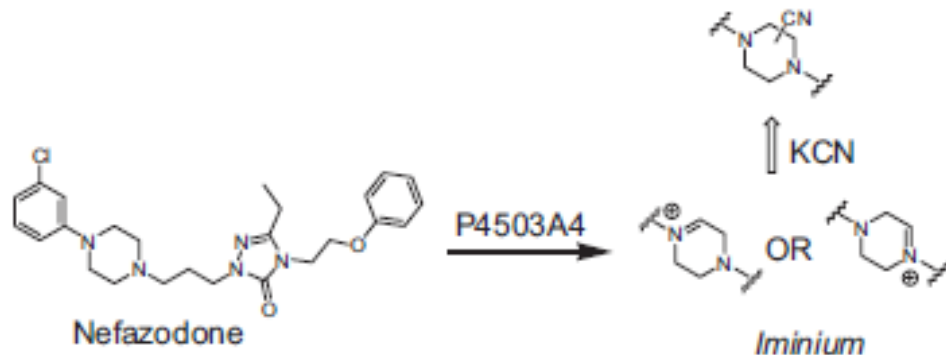


This time, we has started to offer *Cysteine (Cys) trapping study* as an alternative study of GSH trapping

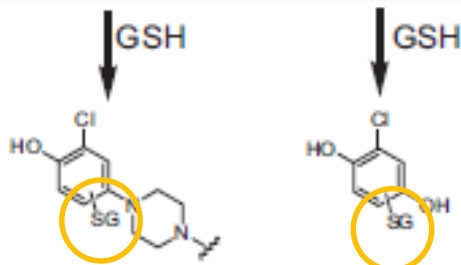
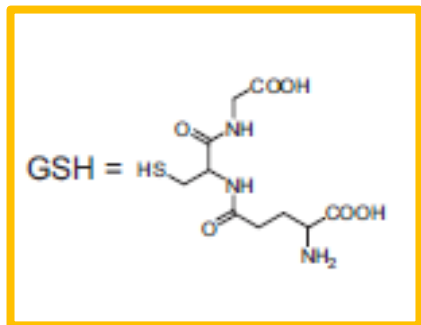
✓ higher quantitativity ✓ higher throughput  
✓ lower cost



# Metabolic pathway of Nefazodone



Reactive Metabolites not detectable



GSH adduct  
Detoxification, stabilization and detectable

DMD 2008;36(6):1016-29



## Trapping Studies

	GSH trapping			Cys trapping	
Trapping reagent	$^3\text{H}$ - or $^{35}\text{S}$ -GSH	Dansyl GSH	$^3\text{H}$ - or $^{35}\text{S}$ -GSH + Stable isotope	$^{35}\text{S}$ -Cys	$^{35}\text{S}$ -Cys
Detection	HPLC-RAD	HPLC-Fluorecent	LC-MS/MS HPLC-RAD	HPLC-RAD	Liquid Scintillation Counter
Quantitativity	**	*	***	**	***
Throughput	**	***	*	**	***
Cost	High	Low	High	Low	Low

**Our Method!**





## Cys Trapping

### Reagents

Enzyme: 50-donor human liver microsomes (Sekisui XenoTech)

Cofactors: NADPH and UDPGA

Trapping reagent: Radio-labeled cysteine ( $^{35}\text{S}$ )

### Assay procedure

- Mix reagents and incubate at  $37^{\circ}\text{C}$  for 60 min
- Stop the reaction and separate the cys-adduct and non-adduct by **solid phase extract plate**
- Measure the radioactivity of adduct fraction by **liquid scintillation counter**





## Study Design

- 1 positive control (e.g. Nefazodone) and 1 solvent control
- 1 concentration (100  $\mu\text{M}$ )
- 30 compounds can be evaluated per plate

## End point

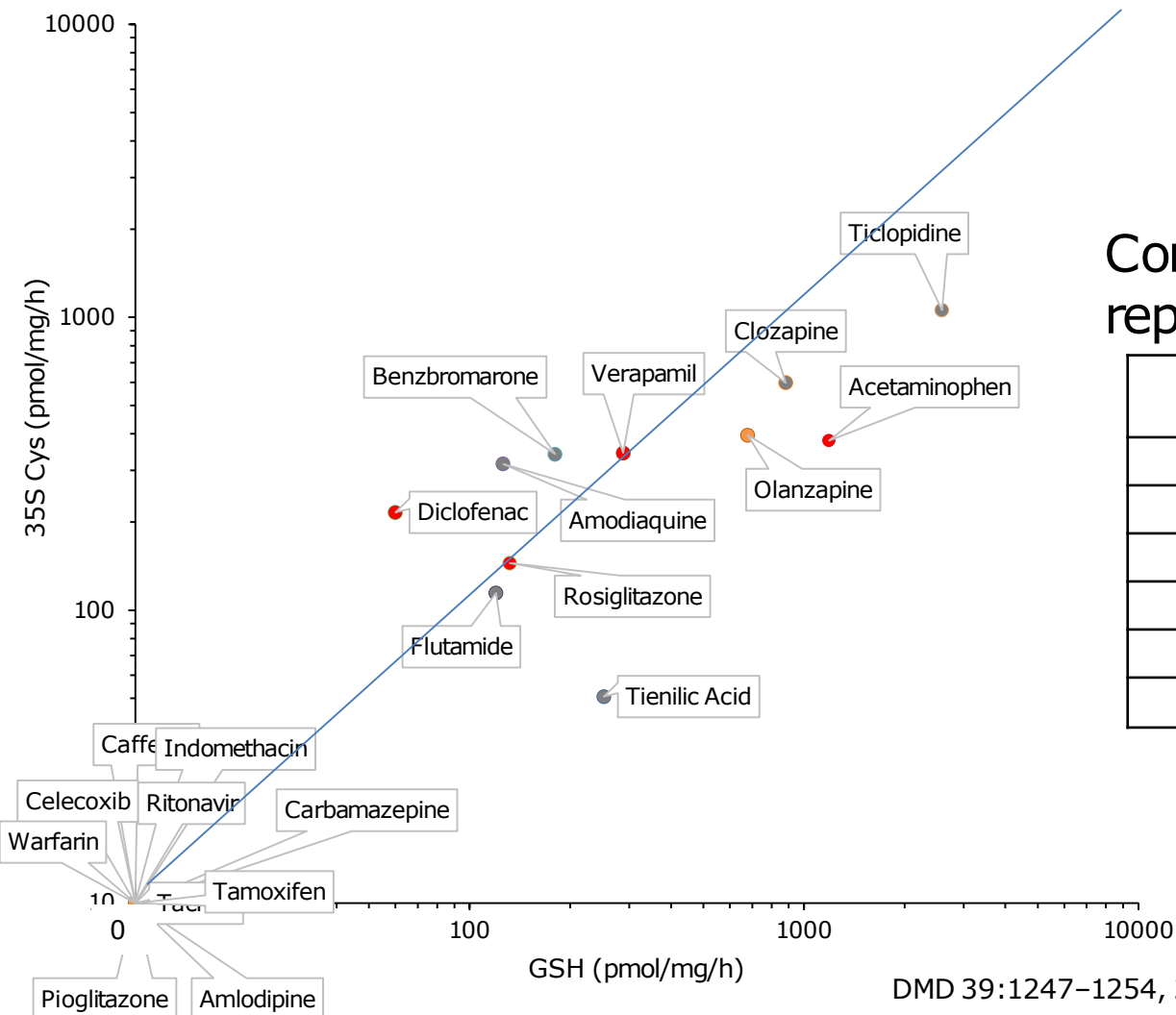
Formation rate of reactive metabolite (pmol/h/mg protein)







## Correlation with GSH trapping



## Comparison with previously reported data of Cys trapping

Compounds (pmol/h/mg protein)	SMD data	Previously reported
Carbamazepine	18.3	<100
Clozapine	944	429
Diclofenac	329	257
Nefazodone	2631	2333
Rimonabant	0	<100
Troglitazone	358	265

J. Toxicol Sci. 2019;44(3):201-211.

DMD 39:1247-1254, 2011



# *Special Offer*

## By 20th March, 2022

We are offering special price for this study!

<https://www.xenotech.com/about/events/65-off-new-cysteine-trapping-service-for-a-limited-time/>

To get more detail information about this offer, please use

**Contact Us** form on XenoTech's website

<https://www.xenotech.com/>





# Thank you for watching!

