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In vitro **ADME**

Reactive Metabolite Assessment (Glutathione Trapping)

Background Information

Screening and structural characterization of reactive metabolites, as one of the major efforts to reduce attrition in drug development, has increasingly become an integral part of the ADMET-guided lead optimization process in drug discovery[2].

[2]Yan Z, Maher N, Torres R, Caldwell GW and Huebert N (2005) Rapid Commun Mass Spectrom 19(22); 3322-3330

- Reactive metabolite formation is thought to be one of the primary causes of idiosyncratic adverse drug reactions, often associated with drug-induced skin, liver and hematopoietic toxicities.
- Reactive metabolites, formed via drug metabolism in the body, are electrophilic species which can bind covalently to macromolecules such as proteins and DNA, affecting their function and potentially leading to toxicity.
- To minimise the risk of later stage failure - which is of considerable financial burden to the Pharmaceutical Industry - screening for reactive metabolite formation at an early stage in lead optimisation is now common practice[1].
- Chemical trapping agents, such as reduced glutathione (GSH), can form stable adducts with many reactive species. Trapping agents, incubated with liver microsomes, are now routinely used in the identification of reactive metabolites.
- Cyprotex now offer glutathione trapping studies using human liver microsomes and Q-TOF high resolution accurate mass instrumentation.
- By using high resolution accurate mass spectrometry, it improves detection of the conjugates and allows superior structural characterisation. The process utilises MS^E data acquisition, mass detect filtering and post acquisition data mining.

Protocol

Assay Design
Test article incubated with human liver microsomes and glutathione in the presence and absence of NADPH

Test Article Concentration
50 µM

Microsomal Concentration
1 mg/mL

Glutathione Concentration
1 mM

Quality Controls
Minus NADPH (negative control)
Ticlopidine (positive control)

Test Article Requirements
100 µL of 10 mM DMSO solution or equivalent amount of solid compound

Analysis Method
High resolution accurate mass Q-TOF

Data Delivery
Summary report including:

LC-MS chromatograms of the parent and reactive metabolites, along with spectra with and without fragmentation

Table including mass, name of proposed metabolite and formula, *m/z* found, mass error, retention time, absolute area and area percentage

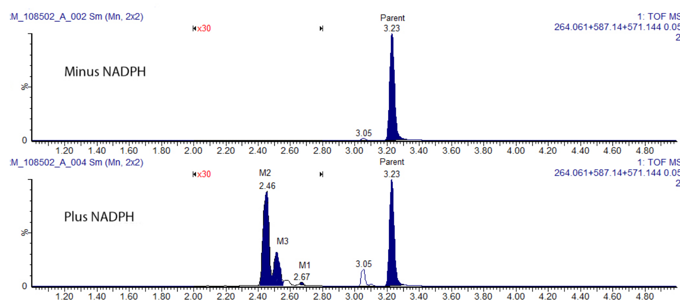
Structural elucidation (optional)

Comprehensive report (optional)

To find out more contact **enquiries@cyprotex.com**

Figure 1

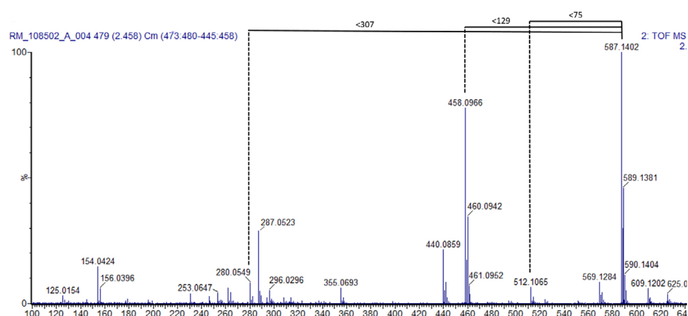
Representative XIC chromatogram of ticlopidine following incubation with human liver microsomes and glutathione in the absence and presence of NADPH.



In the presence of NADPH, reactive metabolite formation is evident. M1 represents a GSH adduct + reduction; M2 and M3 represent GSH adducts + hydration.

Figure 2

MSMS spectrum for ticlopidine following incubation with human liver microsomes, glutathione and NADPH.



The spectrum illustrates confirmation of reactive metabolite M2 through neutral losses for GSH and hydration.

Table 1

Table illustrating representative data for ticlopidine following incubation with human liver microsomes and glutathione in the presence of NADPH.

Name	Formula	Mass Difference	*m/z* found	Mass error (ppm)	Identifier	RT (min)	Neutral Loss
Parent	$C_{14}H_{14}ClNS$	-	264.0618	1.7	-	3.23	-
GSH + reduction	$C_{24}H_{31}ClN_4O_6S_2$	+307.0830	571.1443	-1.5	M1	2.67	307
GSH + hydration	$C_{24}H_{31}ClN_4O_7S_2$	+323.0790	587.1404	0.6	M2	2.45	75,129, 307
GSH + hydration	$C_{24}H_{31}ClN_4O_7S_2$	+323.0785	587.1398	-0.5	M3	2.52	129, 307

References

[1] Evans DC *et al*., (2004) *Chem Res Toxicol* **17(1)**; 3-16

[2] Yan Z *et al*., (2005) *Rapid Commun Mass Spectrum* **19(22)**; 3322-3330