cyprotex AN EVOTEC COMPANY

In vitro **ADME**

Reactive Metabolite Assessment (Stable Label 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]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.
- 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 and post acquisition data mining.
- Cyprotex now offers the reactive metabolite screen with the addition of stable label glutathione, which, when incubated in a 1:1 ratio with unlabelled glutathione, produces an easily recognisable isotopic doublet with a difference of 3 amu. This additional diagnostic tool increases the robustness of identified conjugations.

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 (1:1 ratio of unlabelled to stable label)

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

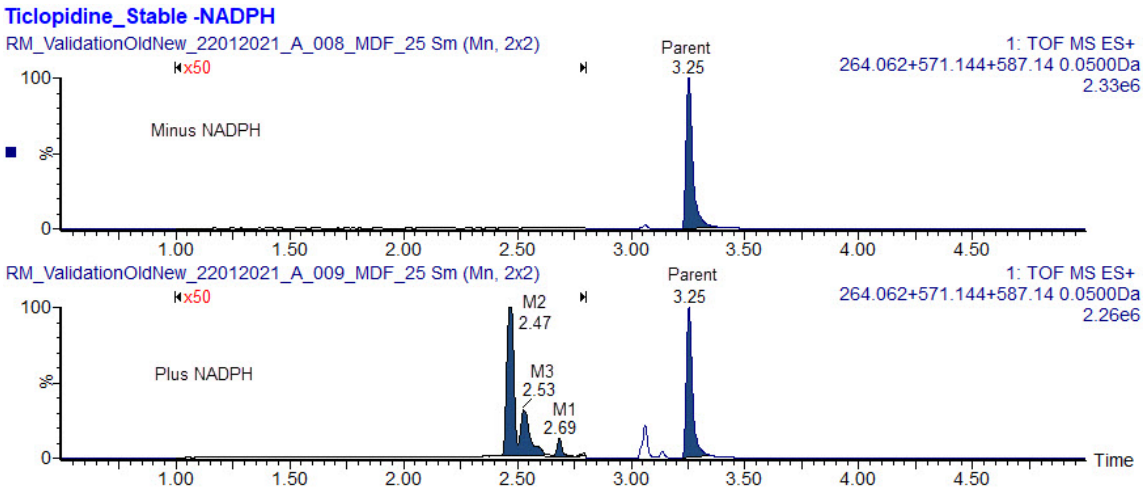
Table including mass, name of proposed metabolite and formula, *m/z* found, mass error, retention time, absolute area, and area percentage

Structural elucidation (optional)

Comprehensive report (optional)

Figure 1

Representative XIC chromatogram of ticlopidine following incubation with human liver microsomes and stabel and unlabelled 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

High energy MS^E spectrum for ticlopidine + hydration + GSH following incubation with human liver microsomes, stable label and unlabelled glutathione, and NADPH.

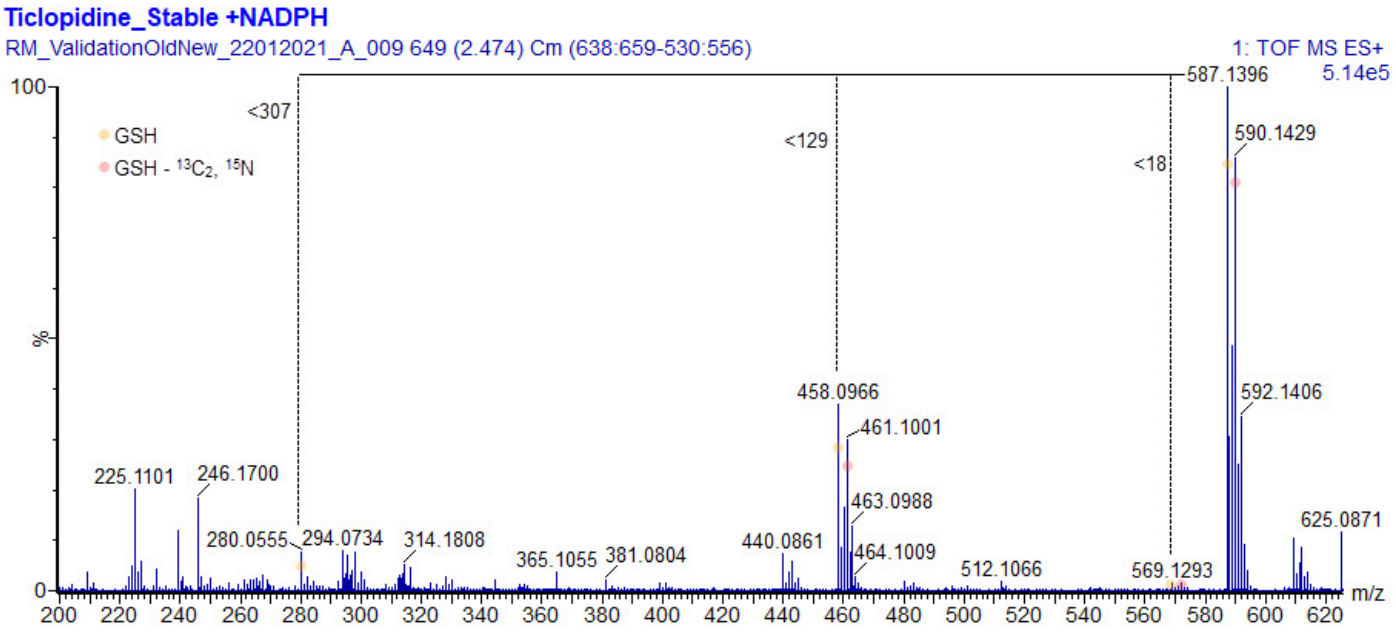


Table 1

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

Name	Formula	m/z Found	Mass Error (ppm)	Identifier	RT (min)	Neutral Loss	Stable Label Observed
Parent	$C_{14}H_{14}ClNS$	264.0616	1.0	Parent	3.26	-	-
GSH + reduction	$C_{24}H_{31}ClN_4O_6S_2$	571.1449	-0.4	M1	2.69	305, 307	Yes
GSH + hydration	$C_{24}H_{31}ClN_4O_7S_2$	587.1397	-0.6	M2	2.47	75, 129, 307	Yes
GSK + hydration	$C_{24}H_{31}ClN_4O_7S_2$	587.1393	-1.3	M3	2.53	307	Yes

References

[1] Evans DC *et al.* (2004) Drug–protein adducts: An industry perspective on minimizing the potential for drug bioactivation in drug discovery and development. *Chem Res Toxicol* **17(1)**; 3-16

[2] Yan Z *et al.* (2005) Rapid detection and characterization of minor reactive metabolites using stable-isotope trapping in combination with tandem mass spectrometry. *Rapid Commun Mass Spectrom* **19(22)**; 3322-3330