New Method for Selective Phase Extraction of GSH-adducts Using Molecularly Imprinted Polymers AFFINIMIP®GSH

POLYINTELL intelligent polymers
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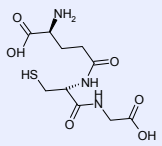
Introduction

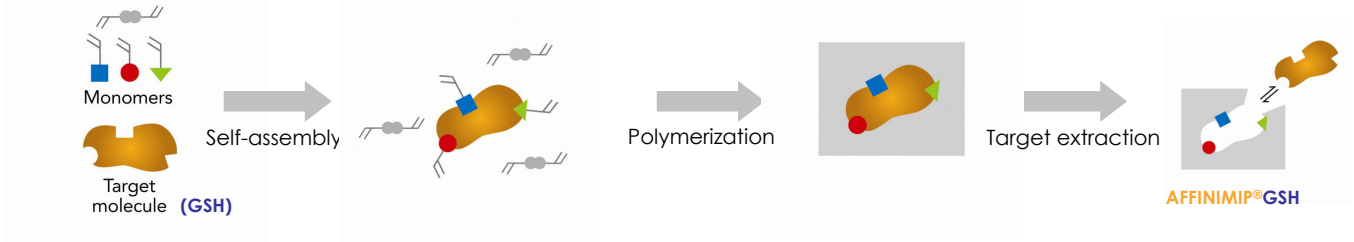
During Drug Development, the potential for a given molecule to give some reactive intermediates that can further bind to endogenous macromolecules is important to be checked. This potential of reactive intermediates formation is currently assessed with a reactive endogenous nucleophile such as Glutathione (GSH).
The reaction involves *in vitro* incubation with an excess of GSH in hepatic microsomes or hepatocytes. In order to separate the excess of GSH from potential adducts formed, an imprinted polymer specific for free GSH retention has been developed: AFFINIMIP®GSH.

Glutathione



AFFINIMIP synthesis

AFFINIMIP is a process to create a three-dimensional network that has a « memory » of the shape and functional group positions of the template molecule.



During the polymer synthesis, functional and cross-linking monomers are copolymerized in the presence of the target analyte (the imprint molecule). The functional monomers form a complex with the imprint molecule, and following copolymerization, their functional groups are maintained in position by the highly crosslinked polymeric structure. Subsequent removal of the imprint molecule reveals binding sites that are complementary in size and shape to the analyte.

Material & Methods

Reference solution

Microsomes (1 mg/mL) in Buffer solution 0.1M pH=7.4 containing free GSH and GSH adducts (tested ratio from 20/1 to 300/1)

Loading solution

Addition of acetonitrile (with 1% acetic acid) in order to obtain a solution with 85/15 acetonitrile(1% acetic acid) / reference solution, after microsomes precipitation and centrifugation.

AFFINIMIP®GSH cartridges

Sorbent mass: 200 mg

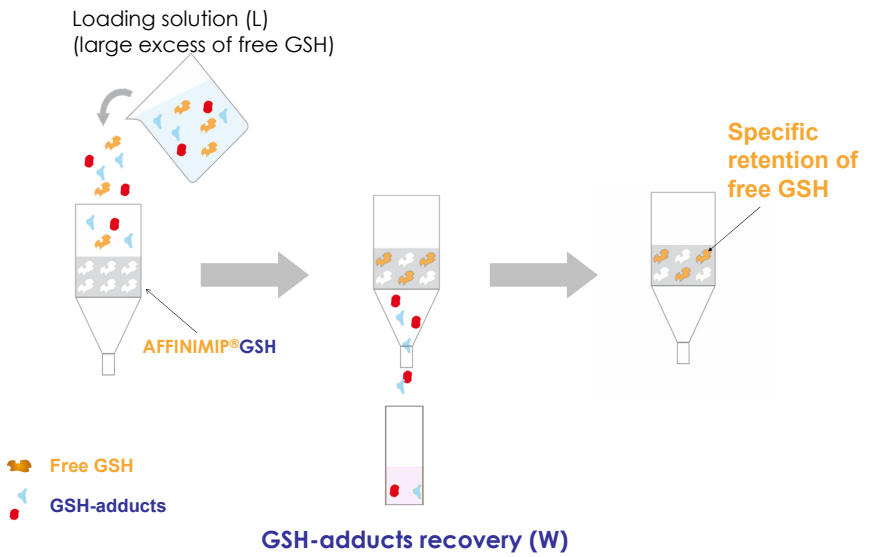
Extraction protocol

Step	Solvent
Loading (L)	85/15 acetonitrile(1% acetic acid) / reference solution
Washing steps **Recovery fractions of GSH-adducts (W)**	4 x 1mL 90/10 ACN /water 4 x 500µL 90/10 ACN /water

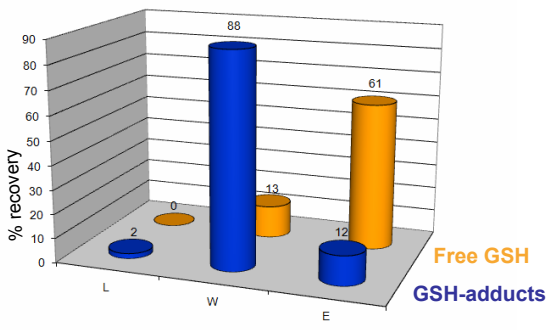
HPLC analysis

Hypersil Gold column C18 100 x 4,6 mm d.i. (5µm) - Mobile Phase : water (0.1% TFA), UV – MS detection

Application : Separation of free GSH from GSH-adducts from *in vitro* microsomal incubations



% Recovery of GSH-adducts and free GSH (LC UV-MS analysis)



90% recovery of GSH adducts with only 13 % of free GSH

Conclusion

The sample issued from *in vitro* microsomal incubations containing an excess of free GSH and some GSH-adduct(s) can be efficiently loaded on AFFINIMIP®GSH cartridges for solid-phase extraction. During washing steps, a selective elution of potential GSH-adducts could be obtained, while free GSH in excess is retained on the MIP cartridge. This solid-phase extraction procedure allows concentration of potential adducts, therefore increasing the sensitivity for their further detection.