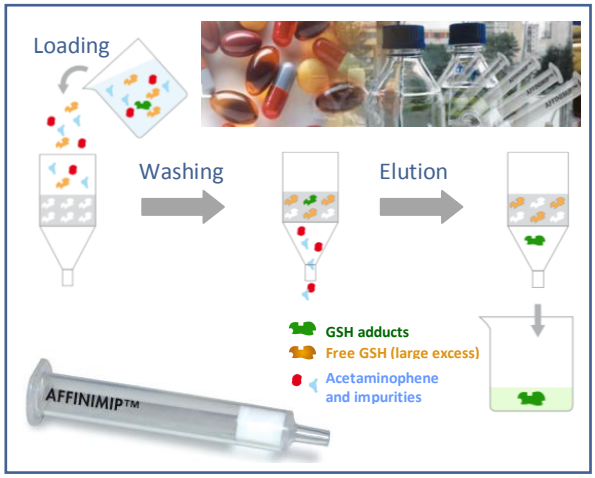
Sample Enrichment & Purification of Glutathione Adducts using AFFINIMIP® GSH

Selective Solid Phase Extraction Based on Molecularly Imprinted Polymers



Introduction

During Drug Development, the potential for a given molecule to generate 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 the presence of hepatic microsomes or hepatocytes. In order to separate the excess of GSH from potential adducts formed, a Molecularly Imprinted Polymer (MIP) specific to free GSH has been developed, named AFFINIMIP®GSH. This polymer has been used to develop a new and selective solid phase extraction method.

AFFINIMIP®GSH allows the separation between GSH, GSH adduct(s) and the drug candidate. This separation also results in an enrichment of GSH adduct. The drug candidates (Acetaminophen or Clozapine for example) are eliminated during the loading and the washing steps while GSH adduct(s) are extracted in the elution step with a high recovery (>80%). This collection fraction can be subject to classical analytical methods to detect GSH adducts with a better sensitivity than usual (an enrichment of adduct >4 or more could be easily obtained). AFFINIMIP® GSH permits a specific retention of free GSH which is completely removed.

Experimental conditions

Materials

All reagents and chemicals were ACS grade quality or better. Glutathione, acetaminophen and clozapine were obtained from Sigma Aldrich (Fluka). Microsomes were purchased from BD Biosciences.

Incubation of the drug candidates

An incubation of 1h at 37°C with microsomes (1mg/mL) in a buffer solution (0.1M pH=7.4) containing free GSH and Drugs (acetaminophen or clozapine or other potential drugs) is made in order to obtain potential adducts. Then, a dilution is realized with acetonitrile (ACN) containing 1.25% acetic acid to obtain the loading solution with 85/15 acetonitrile (1.25% acetic acid) / incubation solution. This solution was used as the loading solution.

Solid phase extraction (SPE) protocol

The SPE procedure used 3mL AFFINIMIP® GSH Cartridges. The details of each step are as follows:

- Condition the SPE cartridge with 10mL of ACN
- Load 6.6mL of the loading solution at 0.2mL/min
- Wash the cartridge with 5mL of 90/10 ACN (containing 1% of acetic acid)/ water (v/v)
- Elute GSH adduct(s) with 3mL of 70/30 ACN/Water (v/v)

The eluted solution is evaporated to dryness under nitrogen with a mini-vap evaporator at room temperature or with a Speed Vac device. The residue is dissolved in 250µL of mobile phase for further analysis or even lower volume 150µL.

Analysis

HPLC was performed on a ThermoFinnigan Surveyor System with a Thermo Hypersil gold column (50mm x 2.1mm). Separation was carried out using a mobile phase of deionized water-0.1% formic acid/ACN (gradient) at a flow rate of 0.2mL/min. The detection system was a Surveyor Plus Detector and a Surveyor MSQ Plus (ESI+, SIM). The injection volume was 20µL.

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Results

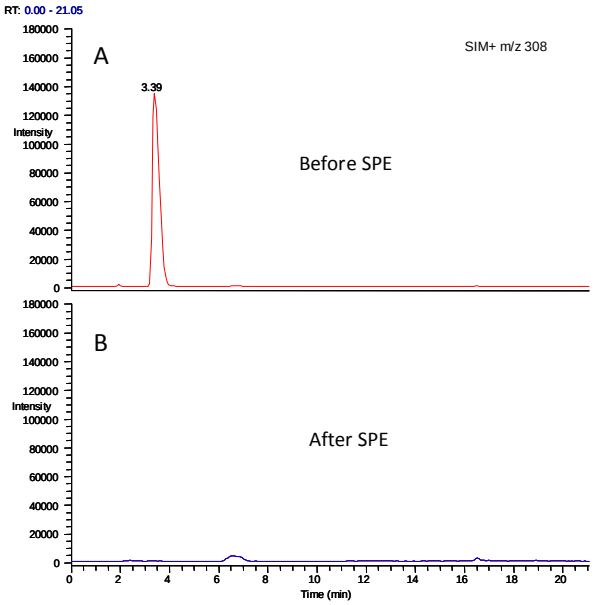

Figure1. Selected Ion Monitoring of **GSH** (m/z 308) after clozapine incubation with human liver microsomes in the presence of glutathione. A: Injection of the loading solution before purification by AFFINIMIP® GSH. B: Injection of the elution fraction after purification by AFFINIMIP® GSH.

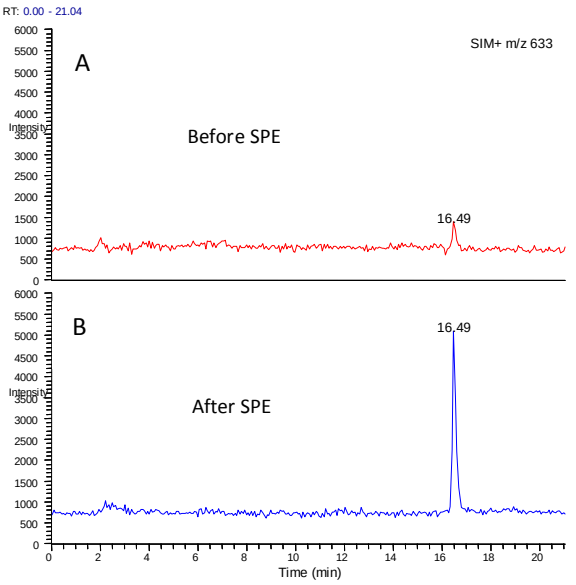

Figure3. Selected Ion Monitoring of **Clozapine** adduct (m/z 633) after clozapine incubation with human liver microsomes in the presence of glutathione. A: Injection of the loading solution before purification by AFFINIMIP® GSH. B: Injection of the elution fraction after purification by AFFINIMIP® GSH.

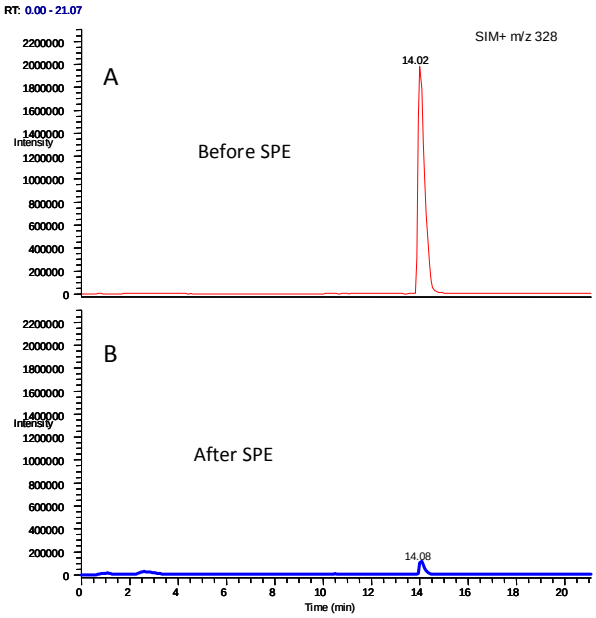

Figure2. Selected Ion Monitoring of **clozapine** (m/z 328) after clozapine incubation with human liver microsomes in the presence of glutathione. A: Injection of the loading solution before purification by AFFINIMIP® GSH. B: Injection of the elution fraction after purification by AFFINIMIP® GSH.

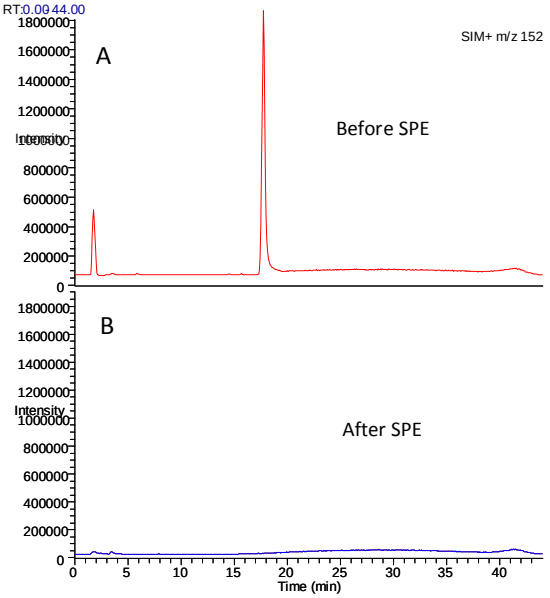

Figure4. Selected Ion Monitoring of **acetaminophen** (m/z 152) after acetaminophen incubation with human liver microsomes in the presence of glutathione. A: Injection of the loading solution before purification by AFFINIMIP® GSH. B: Injection of the elution fraction after purification by AFFINIMIP® GSH.

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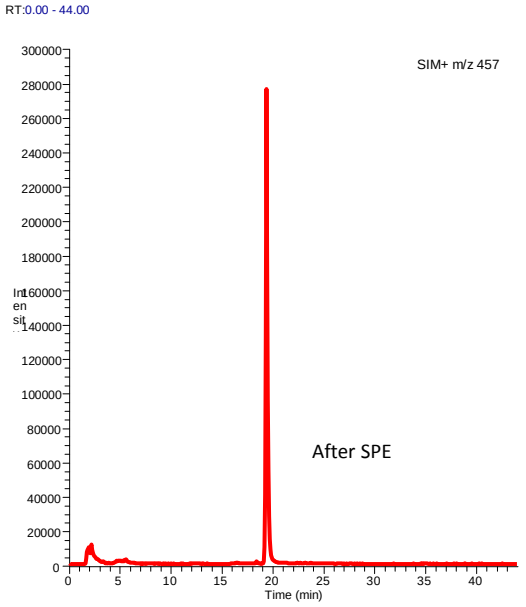

Figure5. Selected Ion Monitoring of **Acetaminophen adduct** (m/z 457) after acetaminophen incubation with human liver microsomes in the presence of glutathione. Injection of the elution fraction after purification by AFFINIMIP® GSH.

Conclusion

The analysis of the elution solution after purification on AFFINIMIP®GSH shows that the free GSH present in a large excess is completely removed (figure 1). The same goes for the drug candidate where less than 3% is in the elution fraction (figure 2 or 4). Furthermore, we observe that we obtain a better sensitivity for adduct detection after treatment with AFFINIMIP®GSH (figure 3 or 5). For clozapine adduct, figure 3 shows an amplification of the adduct peak after SPE (by 5) due to a combination between enrichment (by 4) and the removal of ion suppression after purification. Indeed, AFFINIMIP®GSH permits to delete the effect of ion suppression when mass spectrometry is used for detection. After treatment, the sample is purified and the GSH adduct is clearly observed. GSH adduct(s) were extracted in the elution step with a high recovery (>80%, obtained with S-lactoylglutathione).

To sum up, the sample issued from in vitro microsomal incubations containing an excess of free GSH, unreacted drugs and some GSH adduct(s) can be efficiently loaded on AFFINIMIP® GSH cartridges for solid-phase extraction. A selective elution of potential GSH adducts has been obtained, while free GSH in excess is irreversibly retained on the MIP cartridge and the unreacted drug, such as clozapine and acetaminophen, is eliminated during the washing steps. This solid-phase extraction procedure allows a high enrichment of potential adducts, increasing the sensitivity for their further detection. The analysis is reliable by reducing common and problematic ion suppression phenomena.

References

New method for selective extraction of GSH adducts using molecularly imprinted polymers. S. Bayoudh*, C. Perollier, D. Derrien, P. Baumy, J. Le Gourrierec, T. Arnauld MipTec, 14.-16. October, 2008, Basel – Poster

Negative Ion Tandem Mass Spectrometry for the Detection of Glutathione Conjugates, Chem Res Toxicol. 18, 630 (2005).

Related products

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Founded in 2004, POLYINTELL develops, manufactures and markets innovative products based on intelligent polymers for sample preparation, selective extraction, purification and detection of specific target analytes.

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