Application Note | Drug Metabolism

Using Labskin to characterise xenobiotic metabolising enzymes

Objective:

To compare xenobiotic metabolism distribution and levels between Labskin and *ex vivo* human skin to determine whether Labskin could be used as a prediction tool for xenobiotic metabolism in human skin through proteomic and substrate based mass spectrometry imaging (SB-MSI) analysis.

Method:

- *Ex vivo* skin was obtained from the Human Tissue Bank (university of Bradford).
- Proteomics: Samples were homogenised, treated with detergent and debris removed. The crude fraction was centrifuged and the cytosolic fraction collected and digested ready for analysis using label-free quantification and peptide identification.
- SB-MSI: Samples were treated with SB-MSI probes (e.g. methyl paraben) on the surface for 48 hours. Samples were snapped frozen, sectioned (12 µm), coated with MALDI matrix ready for analysis.

Results:

Proteomic analysis found consistent expression of several xenobiotic metabolising enzymes (XME) expressed between Labskin and *ex vivo* skin. The XME profile between Labskin and *ex vivo* skin were agreeable (Table 1).

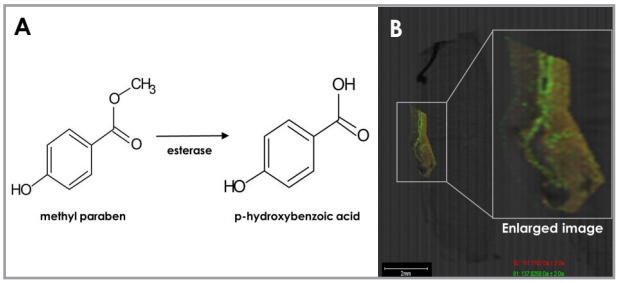
SB-MSI found esterase activity for the metabolism of methyl paraben into p-hydroxybenzoic acid (Figure 1).

Results cont.:

Table 1 - Expression of soluble phase I & II XMEs in Labskin and *ex vivo* human skin. The variation in colour highlights the similarities in XME expression between Labskin and *ex vivo* skin (fmol/µg, ND = not detected).

Phase	Protein Name	Labskin 05	Labskin 06	Human Skin 07	Human Skin 52	Human Skin 56	Human Skin 57	Human Skin 58	Human Skin 60
I	Aldo-keto reductase 1A1	ND	2.1	1.3	ND	ND	3.8	5	ND
	Aldehyde dehydrogenase 1A1	ND	2.4	9.2	12.9	ND	19.8	6.3	18.9
	Carboxylesterase 1	ND	0.3	15.3	17.3	ND	6.3	2.6	19
	Esterase D	2.6	4.5	14.7	14.1	7.7	19.5	14.4	22.2
II	Glutathione S-transferase P1-1	56.3	22.7	50.8	54.5	65.3	150.2	137.7	129.1

Figure 2 - (A) Diagram showing metabolism reaction of SB-MSI probe methyl paraben with esterase to form p-hydroxybenzoic acid. (B) MSI image of Labskin treated with methyl paraben highlighting esterase activity .



(red = methyl paraben, green = p-hydroxybenzoic acid)

Summary:

Label-free proteomic analysis highlighted similar distribution of XMEs in Labskin and *ex vivo* human skin. The location of esterase activity was identified using a XME probe (methyl paraben) in Labskin.

Contact us

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