

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).

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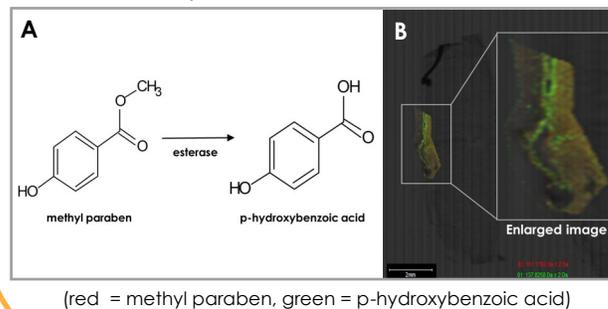
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Results cont.:

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.



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.