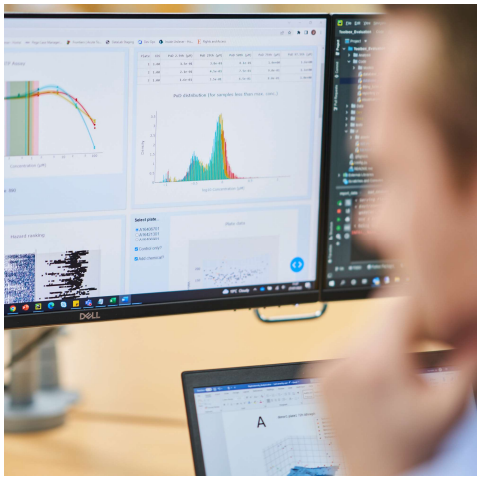
The detoxification of aldehydes by skin enzymes using a model system



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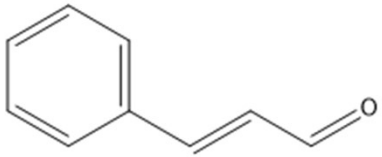
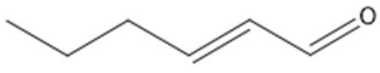
INTRODUCTION/BACKGROUND

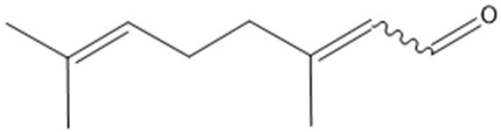
- Aldehydes such as cinnamaldehyde, citral and vanillin are organic compounds which can be strong smelling and are commonly used in fragrances.
- Aldehydes are electrophilic and have the ability to bind to proteins via Schiff base formation and cause skin allergy
- However, human skin has a natural ability to detoxify foreign compounds and understanding the biology helps refine risk assessment.



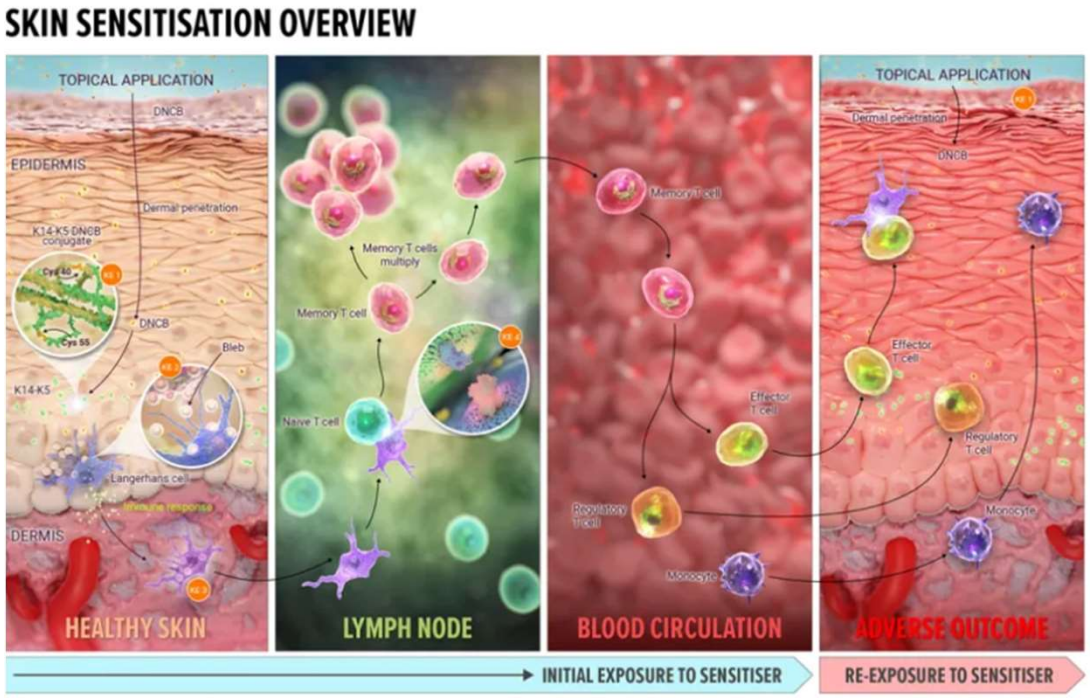
Cinnamaldehyde



Trans-2-hexenal

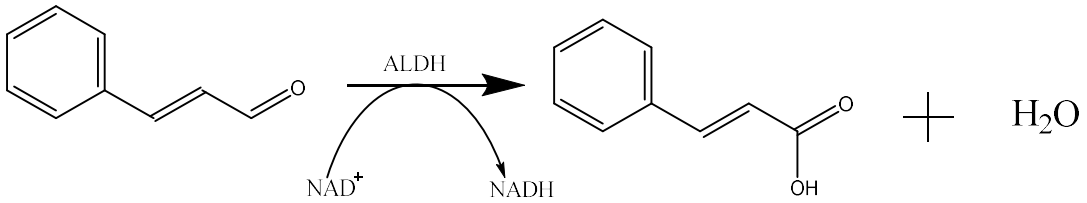


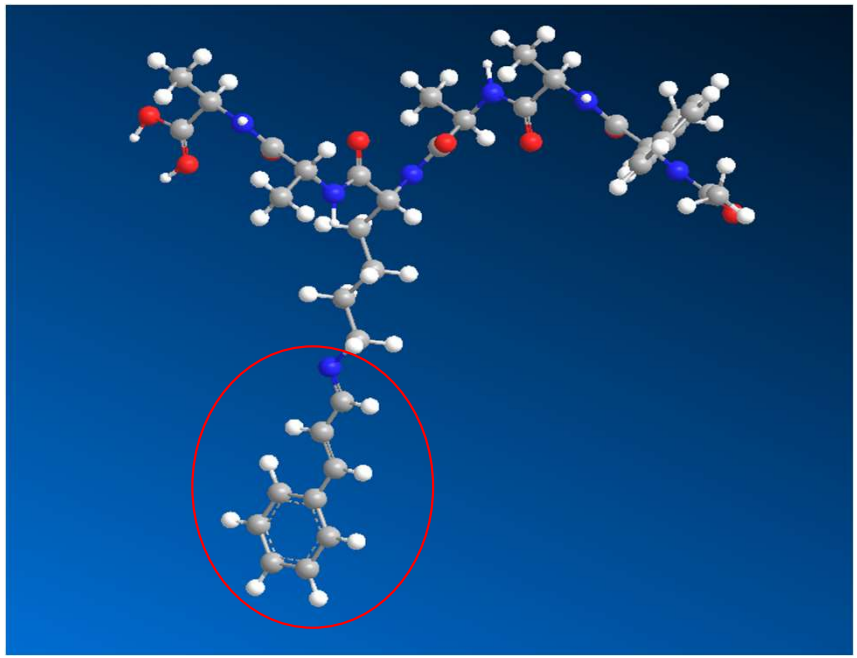
Citral



INTRODUCTION/BACKGROUND

- Enzymes such as ALDH and AO readily metabolise these aldehydes into less toxic carboxylic acids which can safely be excreted.
- Aldehydes react with proteins and enzyme concomitantly
- Peptide reactivity assays can be used to routinely measure protein reactivity to chemicals
- Adding the detoxification pathway of aldehydes to the associated mathematic model used for risk assessment a better understand of the risk can be found.





Adduct of cinnamaldehyde on lysine in a model peptide

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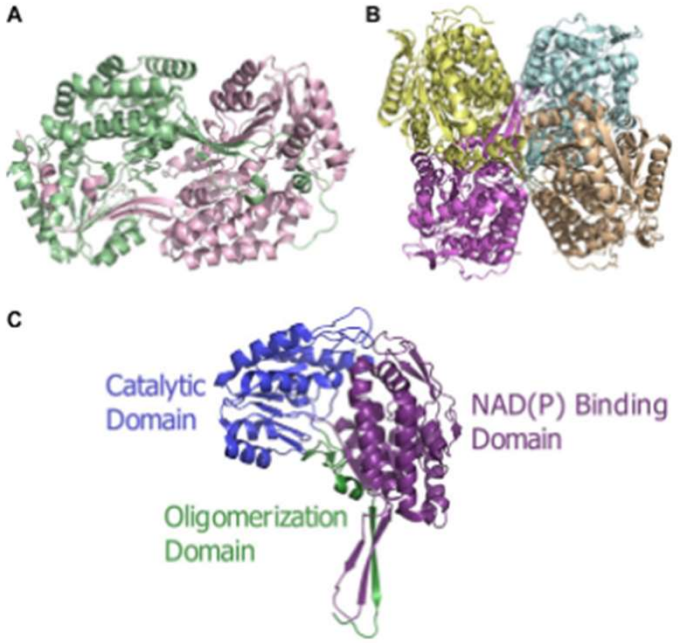
METHOD DEVELOPMENT OF AN ALDH ASSAY

- Goal is to develop a standardised assay to determine ALDH activity towards a series of aldehydes.
- The original assay design used isolated ALDH from Baker's yeast.
- The next best alternative is to use isolated human ALDH. However, this was unavailable to obtain so human pooled skin cytosol was used.
- Assay used human skin cytosol with the addition of NAD as a cofactor.

Figure 1



A. Structure of human ALDH3A1
B. Structure of human mitochondrial ALDH
C. Three conserved domains on an ALDH monomer

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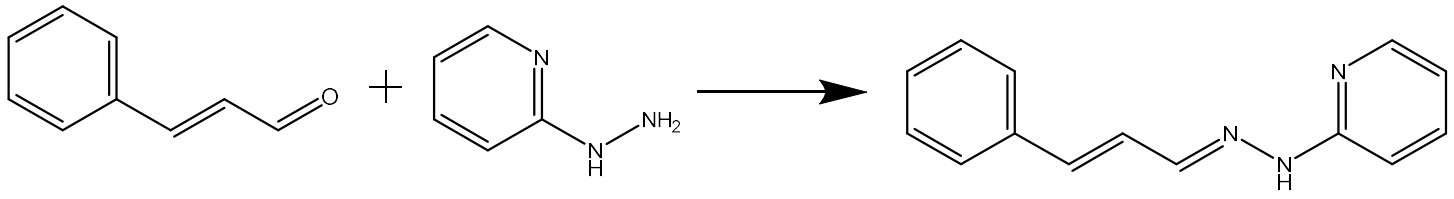
METHOD DEVELOPMENT OF AN ALDH ASSAY

- Model aldehydes selected to cover wide range of structures.
- Availability of matching acid considered
- Reaction mix was equal volumes of cytosol solution, NAD cofactor and test chemical with final volume being > 99% PBS.
- Reaction was terminated with 3x total volume 2-hydrazinopydridine in 0.1 % formic acid acetonitrile.
- Choice of derivatising agent based upon:
  - Availability
  - Fast derivatisation at standard conditions

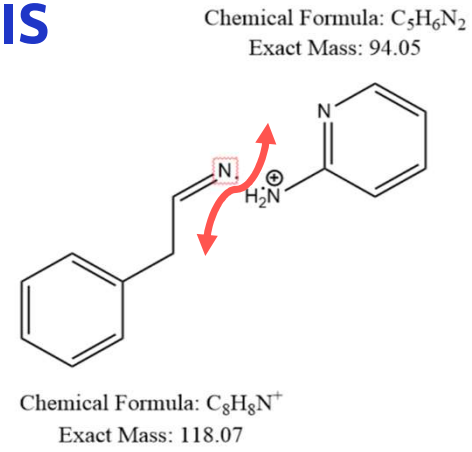
Chemical	Molecular weight / 134.18 g mol^{-1}	CAS	Proposed product
Trans-2-hexenal	98.14	6728-26-3	Trans-2-hexenoic acid
Phenylacetaldehyde	120.15	122-78-1	Phenylacetic acid
Cinnamaldehyde	132.16	104-55-2	Cinnamic acid
2-phenylpropanaldehyde	134.18	93-53-8	2-phenylpropionic acid
Vanillin	152.15	121-33-5	Vanillic acid
Isovanillin	152.15	621-59-0	Isovanillic acid
Citral	152.23	5392-40-5	Geranic acid



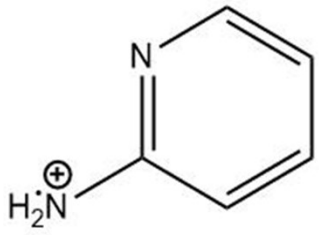
Reaction scheme for the derivatisation of cinnamaldehyde

MASS SPECTROMETRY ANALYSIS

- Product ion is very sensitive on LC-MS/MS
  - m/z 94 common ion regardless of aldehyde
- Analysis was done using LC-MS/MS in ESI+



Predicted mass spec splitting



Product ion used for quantification of derivatised aldehydes

- Standards of each derivatised aldehyde and equivalent acid used to quantify concentration.

Chemical	m/z parent	m/z daughter 1	m/z daughter 2	Run time / min	Mobile phase A	Mobile phase B
Cinnamaldehyde-Der	224.1	94.0	120.0	2.5	0.01 % formic acid (aq)	Acetonitrile
Cinnamic acid	147.0	77.0	103.0	2.5	10 mM TBA, 15 mM AcOH, 5% MeOH (aq)	Acetonitrile
2-phenylpropanaldehyde-Der	226.1	94.9	132.0	2.5	0.01 % formic acid (aq)	Acetonitrile
2-phenylpropionic acid	151.0	82.9	128.0	2.5	10 mM TBA, 15 mM AcOH, 5% MeOH (aq)	Acetonitrile

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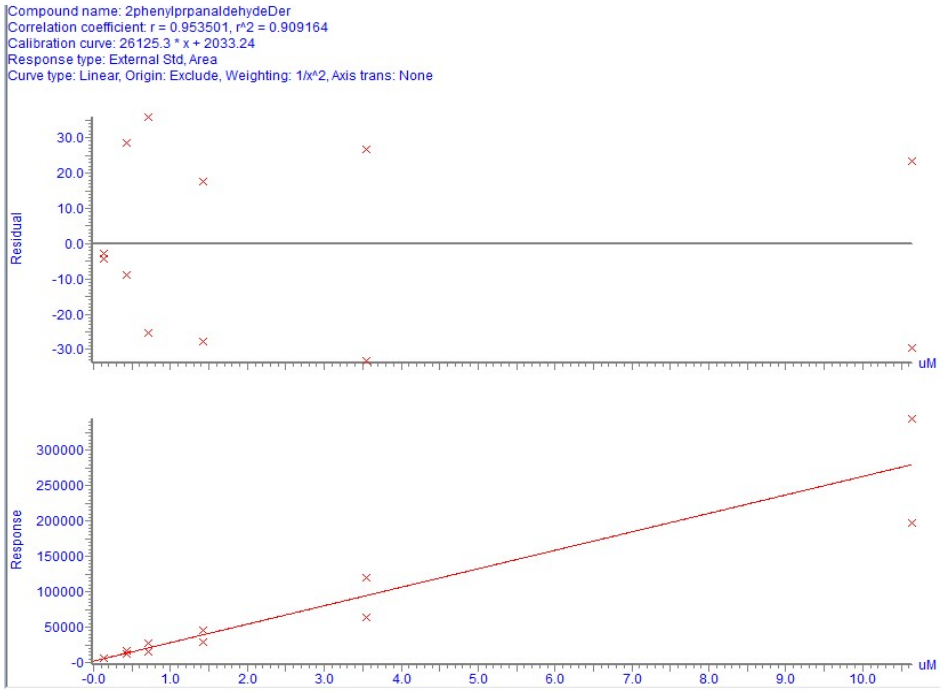
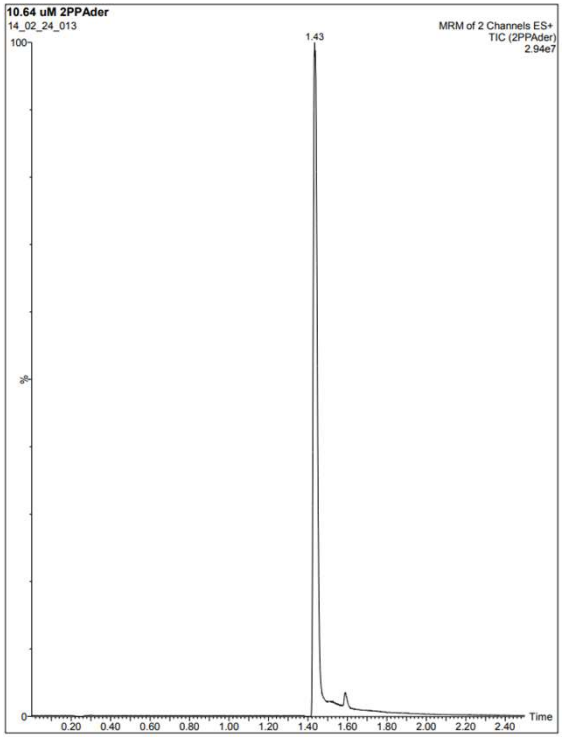
2-PHENYLPROPANALDEHYDE EXAMPLE

- Calibration was run before and after analytes and an average was taken for quantification.

- Problem with mass spec drift over long run leads to two distinct calibrations with the way to fix it is to add an internal standard.



Calibration of derivatised 2-phenylpropanaldehyde



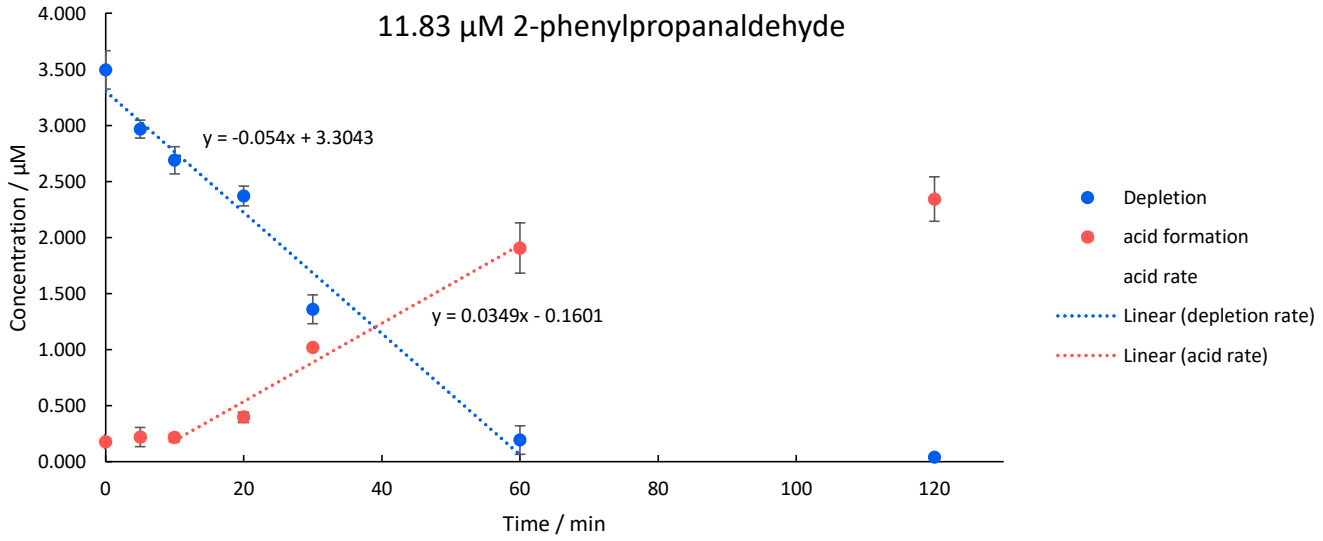
Chromatogram of 1 concentration of derivatised 2-phenylpropanaldehyde

2-PHENYLPROPANALDEHYDE EXAMPLE

- 30 µl of NAD (3.42 mM), 2-phenylpropanaldehyde (variable concentration), and cytosol (1.6 mg protein ml^{-1}) were added to a well and at each time point quenched with 270 µl 2-hydrazinopyridine (19.24 mM).

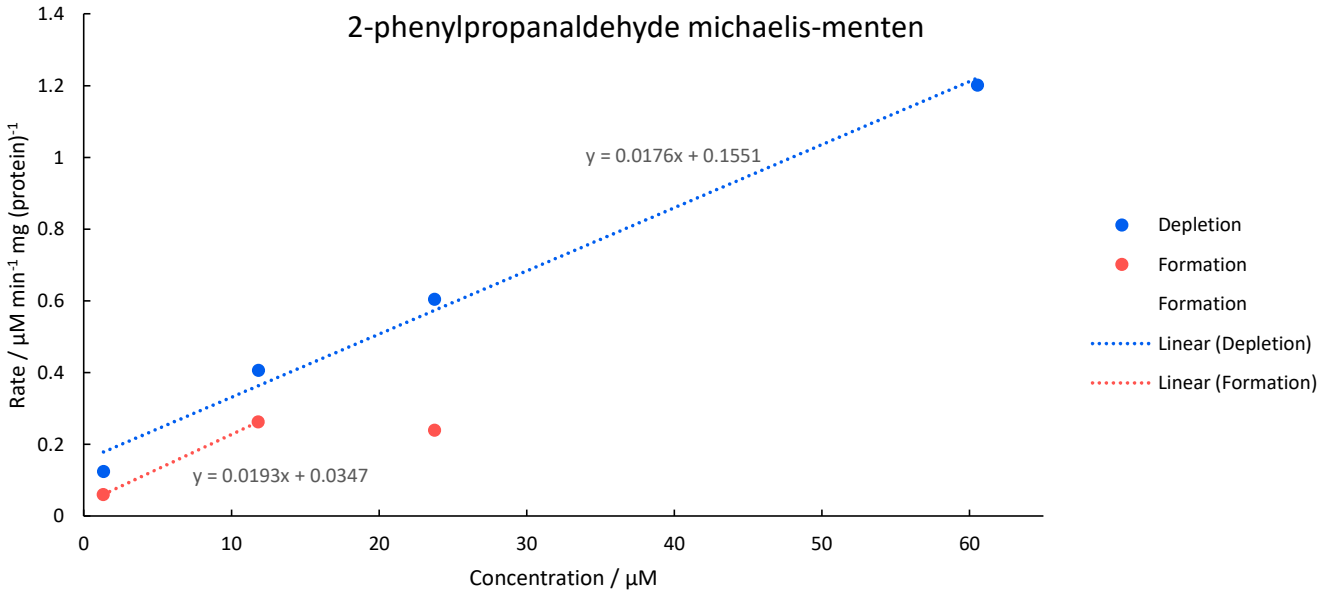
- Time points were 0, 5, 10, 20, 30, 60 and 120 minutes and each point was done in triplicate and an average was taken.

- Difference in depletion and formation due to protein binding



DATA INTERPRETATION

- Due to not reaching a clear V_{max} for depletion for most of the aldehydes, it is hard to find a clear estimation for Km
- However, since the acid formation reaches a clear V_{max}, Km can be determined. Km = 5.0 µM
- ALDH is present and active in skin cytosol



CONCLUSION

- We have demonstrated that ALDH is active in skin cytosol and can detoxify model aldehydes
- We have standardised the assay for skin cytosol (donor variability)
- Future step would be a standardisation of the assay using isolated enzyme
- Rate of clearance via ALDH might be integrated into a mathematical models which also consider peptide reactivity (such as DPRA/kDPRA) to understand the behaviour of aldehydes once they enter the skin.
- Could provide useful information for risk assessment

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Thank You

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