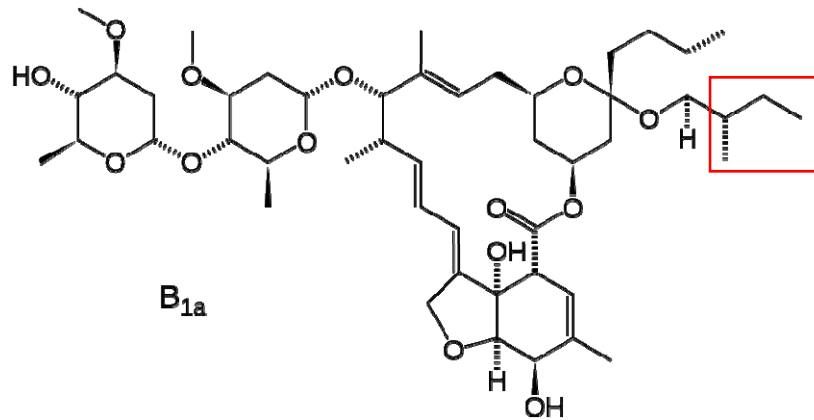




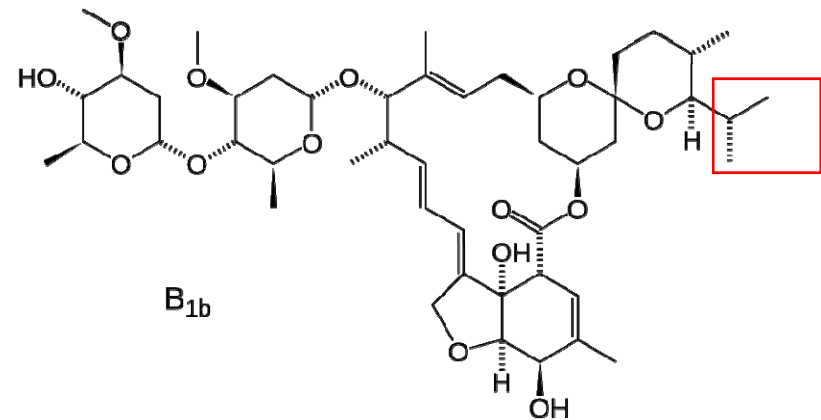
# Ivermectin Metabolites

Phornpimon Tiphara, PhD

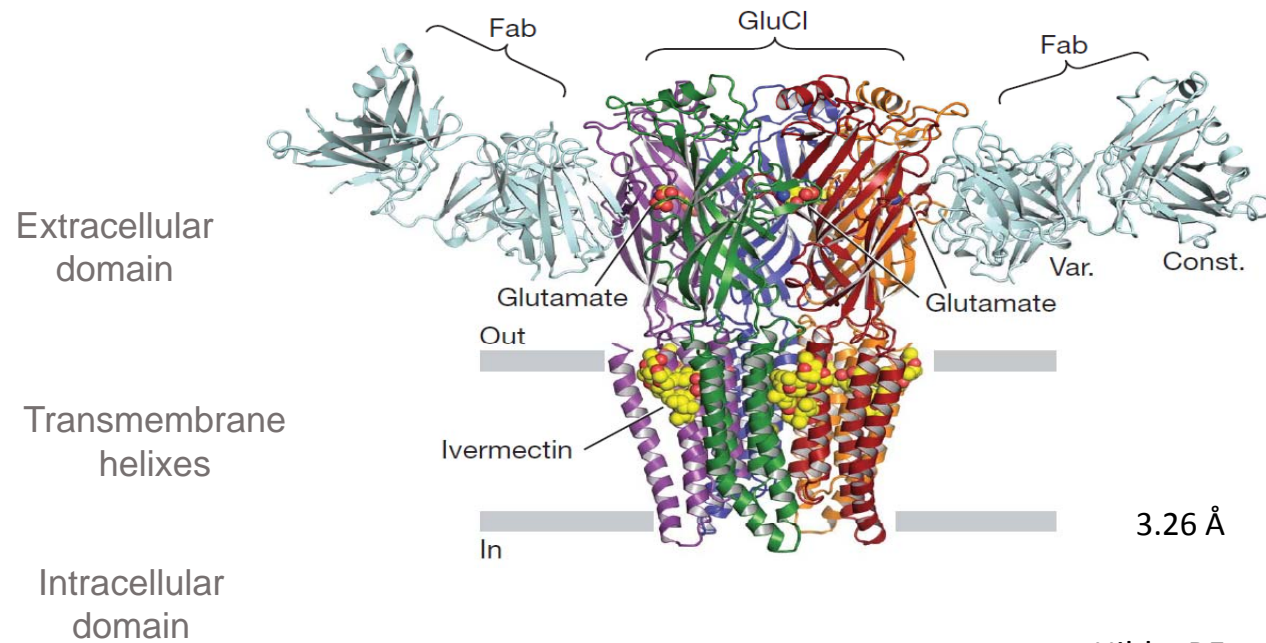
## B1a (>90%)



## B1b (<10%)

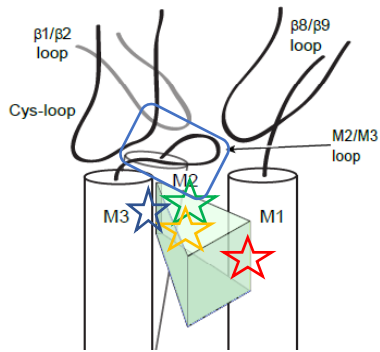
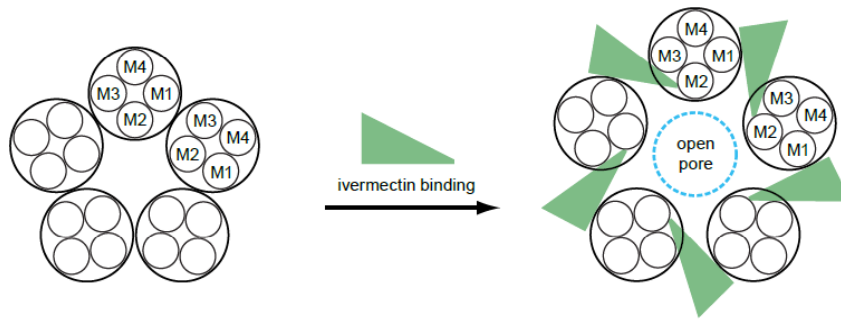


# Mode of action

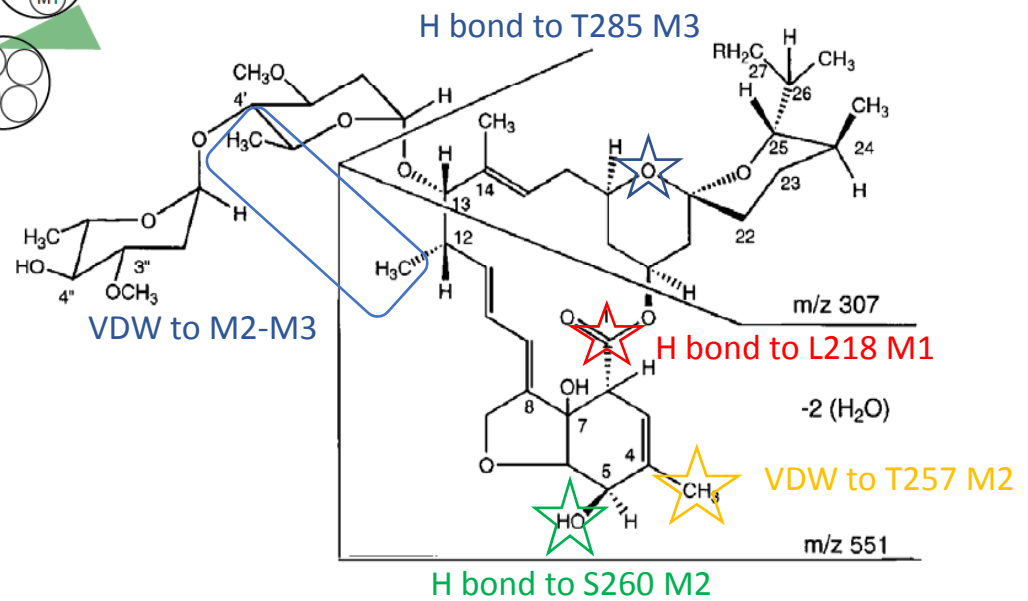


Hibbs RE *et al.*, Nature, 2011

# Binding site



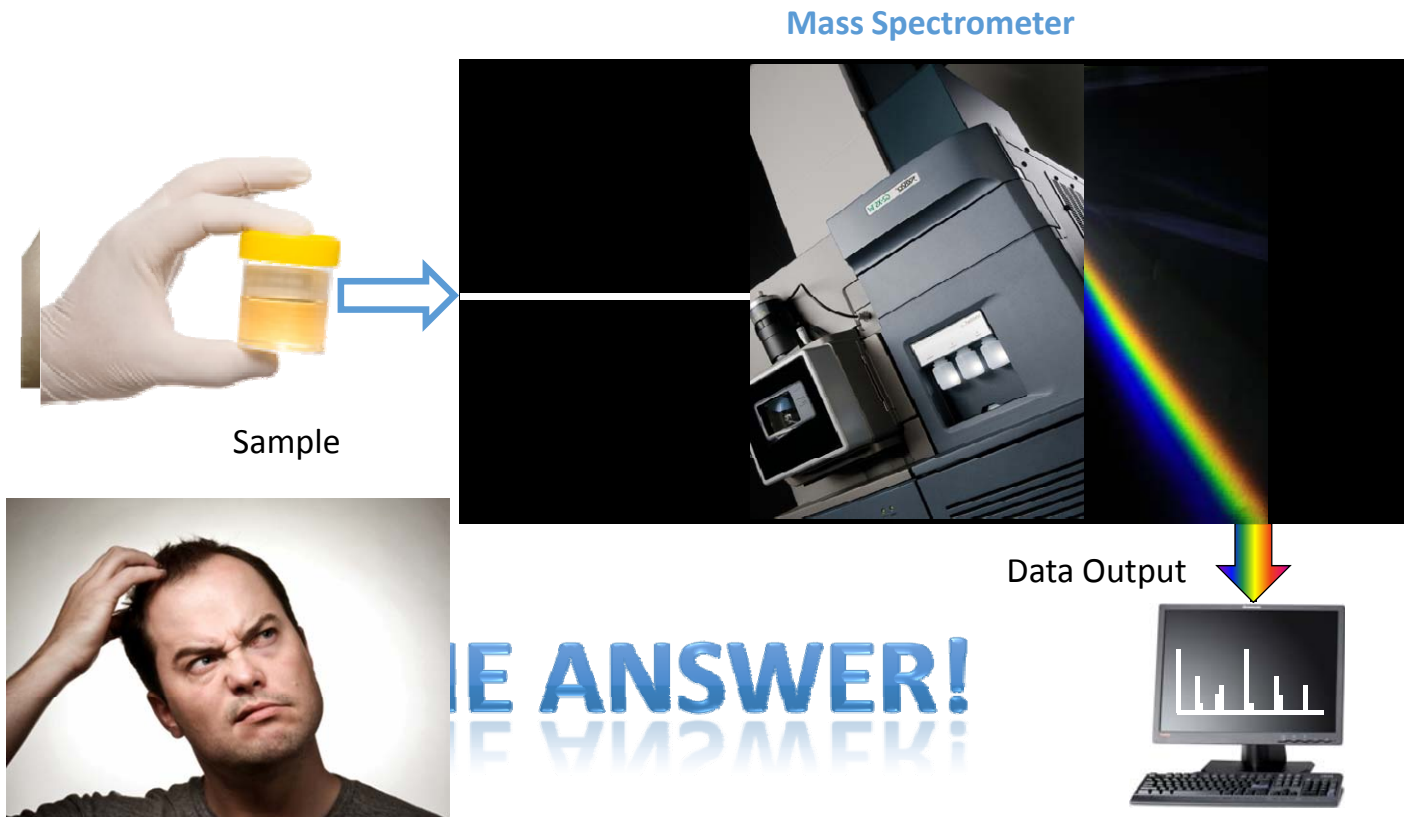
Hibbs RE *et al.*, Nature, 2011



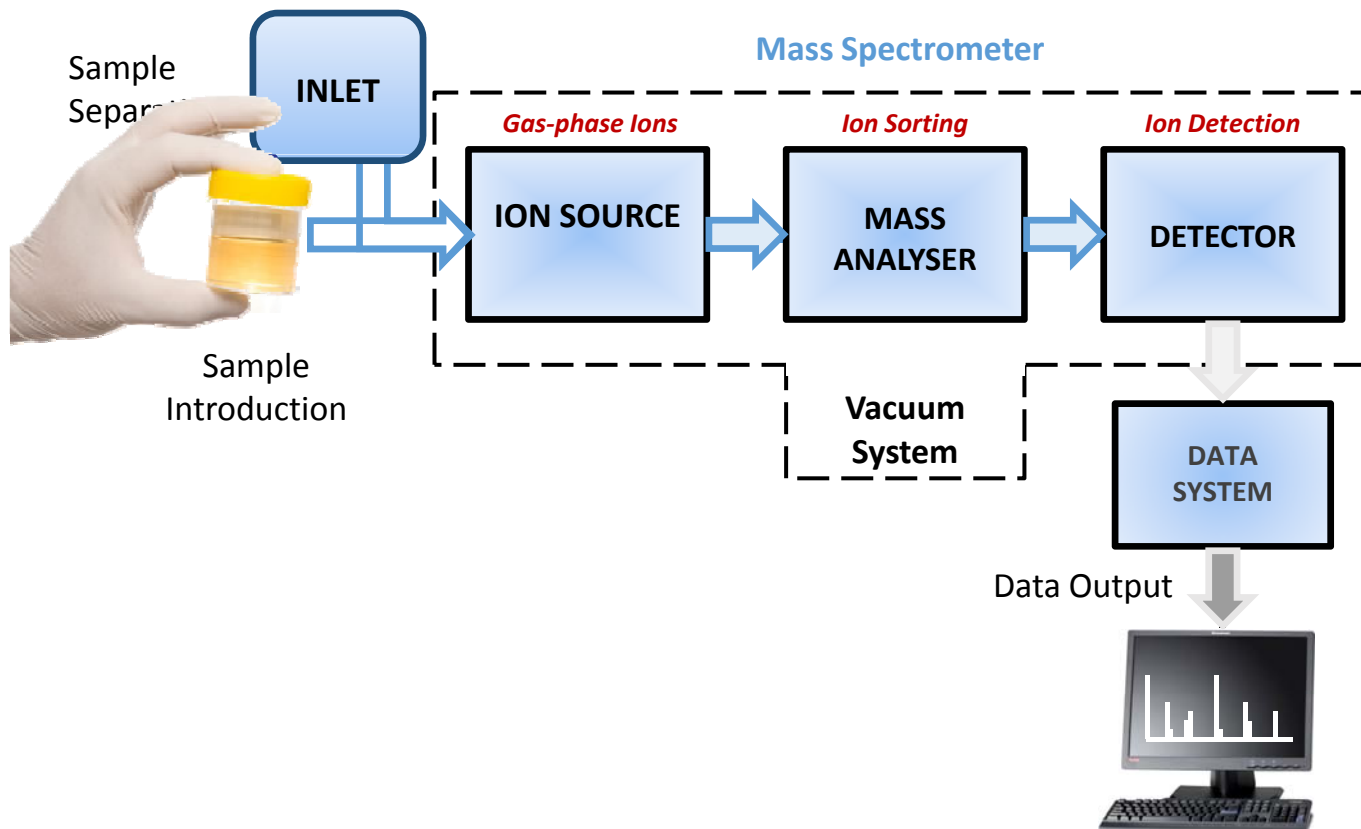
# Metabolite Identification by UPLC-MS/MS

What is Mass Spectrometry?

# Mass Spectrometry Flow Path - Fantasy

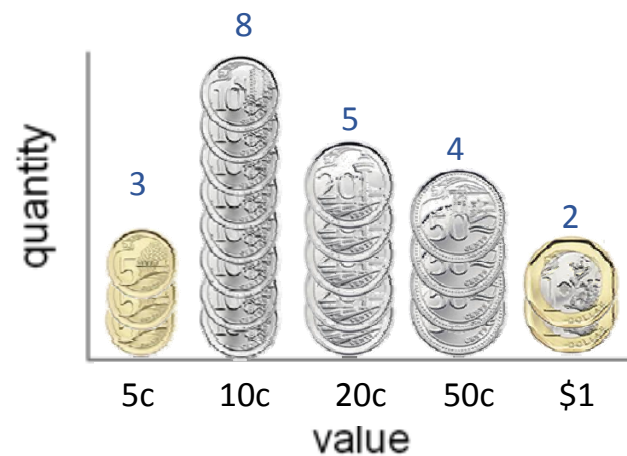


# Mass Spectrometry Flow Path - Reality



# Fundamental Concepts: Mass Analysis

- Sorting and Counting
  - Pocket change (mixture of coins)
  - 5c, 10c, 20c, 50c, \$1
  - Sort change by value or size
  - Concept of visual interpretation
- Mixture of molecules
- Molecules of different size, weight
- Separation by mass ( $m/z$ )
- Spectrum



What MS can tell us:

***What is there, and how much.***

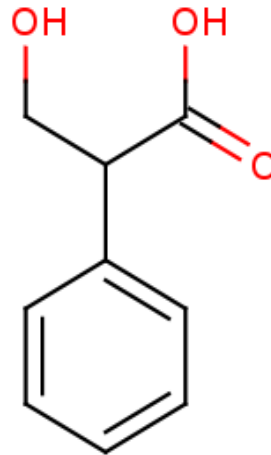
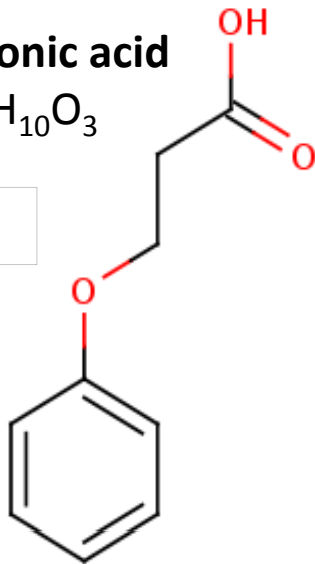


## Specificity : when mass resolution fails

**3-Phenoxypropionic acid**

Formula:  $C_9H_{10}O_3$

166.0630



**Tropic acid**

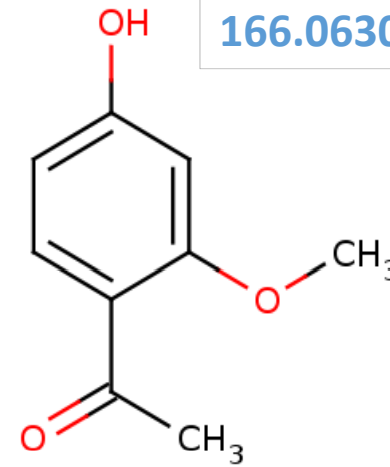
Formula:  $C_9H_{10}O_3$

166.0630

**Isopeonol**

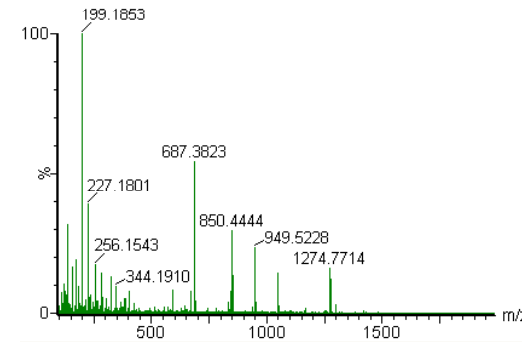
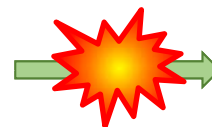
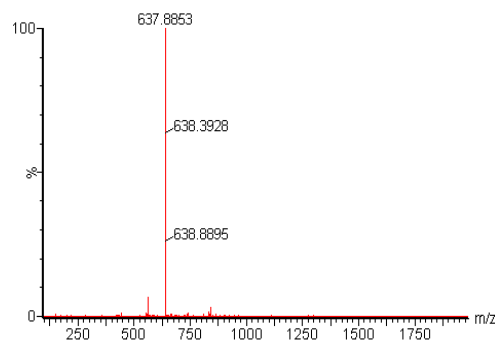
Formula:  $C_9H_{10}O_3$

166.0630

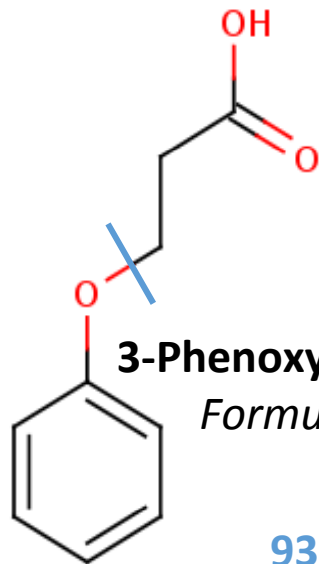


# Fundamental Concepts MS/MS

MS #1	Fragmentation Chamber	MS #2
Sorting molecules	Breaking molecules	Sorting Pieces



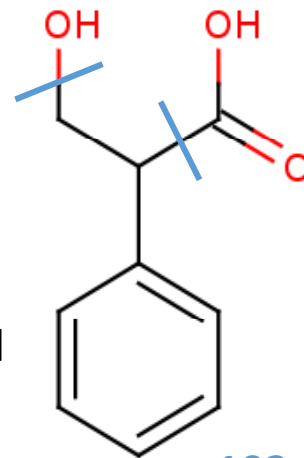
## Specificity : when mass resolution fails



**3-Phenoxypropionic acid**

Formula:  $C_9H_{10}O_3$

93.0331



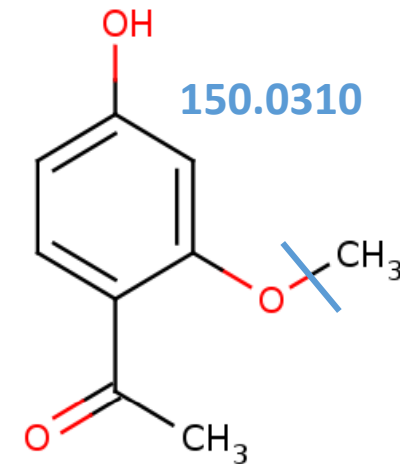
**Tropic acid**

Formula:  $C_9H_{10}O_3$

103.0550

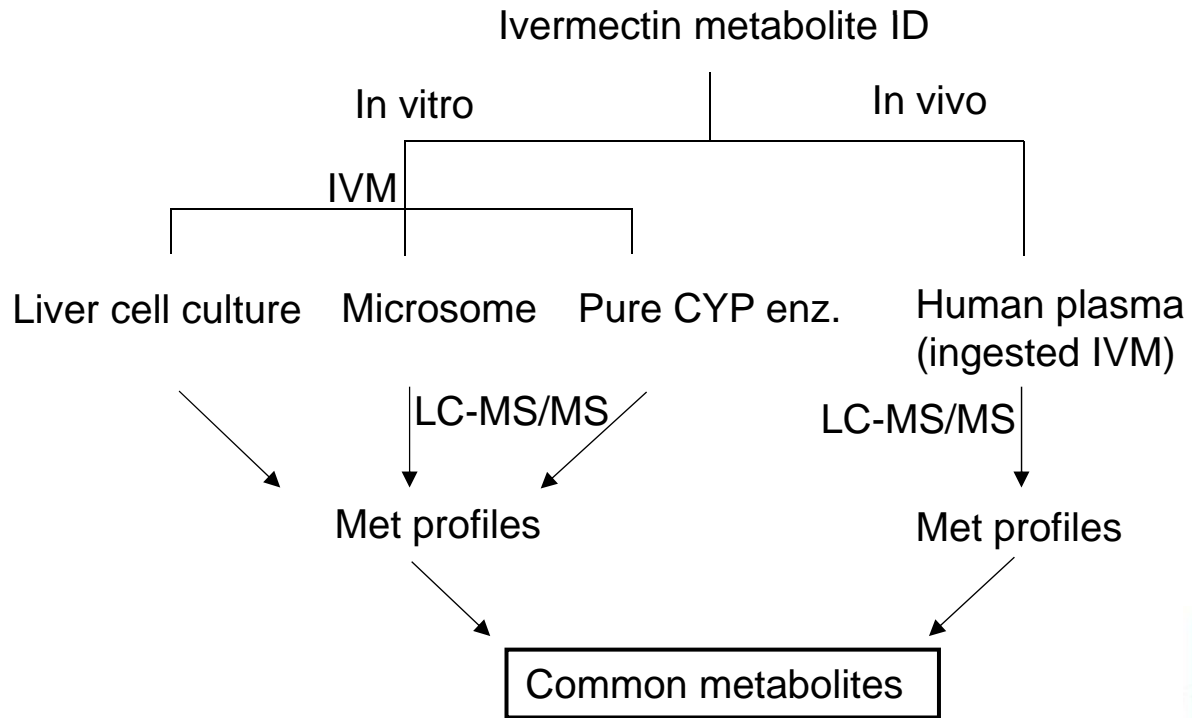
**Isopeanol**

Formula:  $C_9H_{10}O_3$

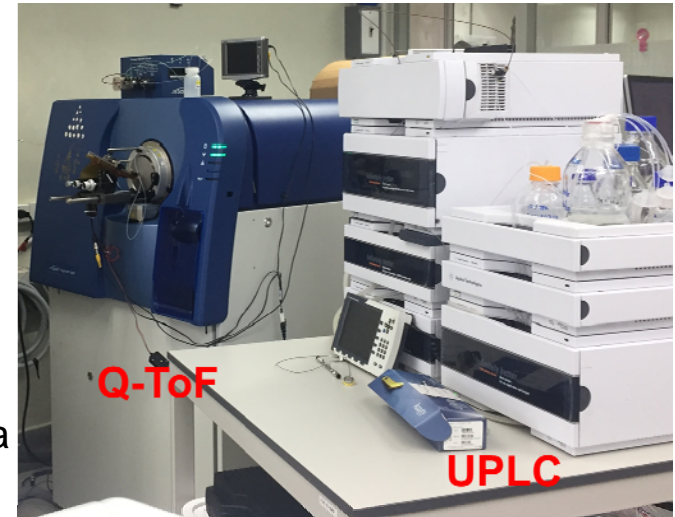


150.0310

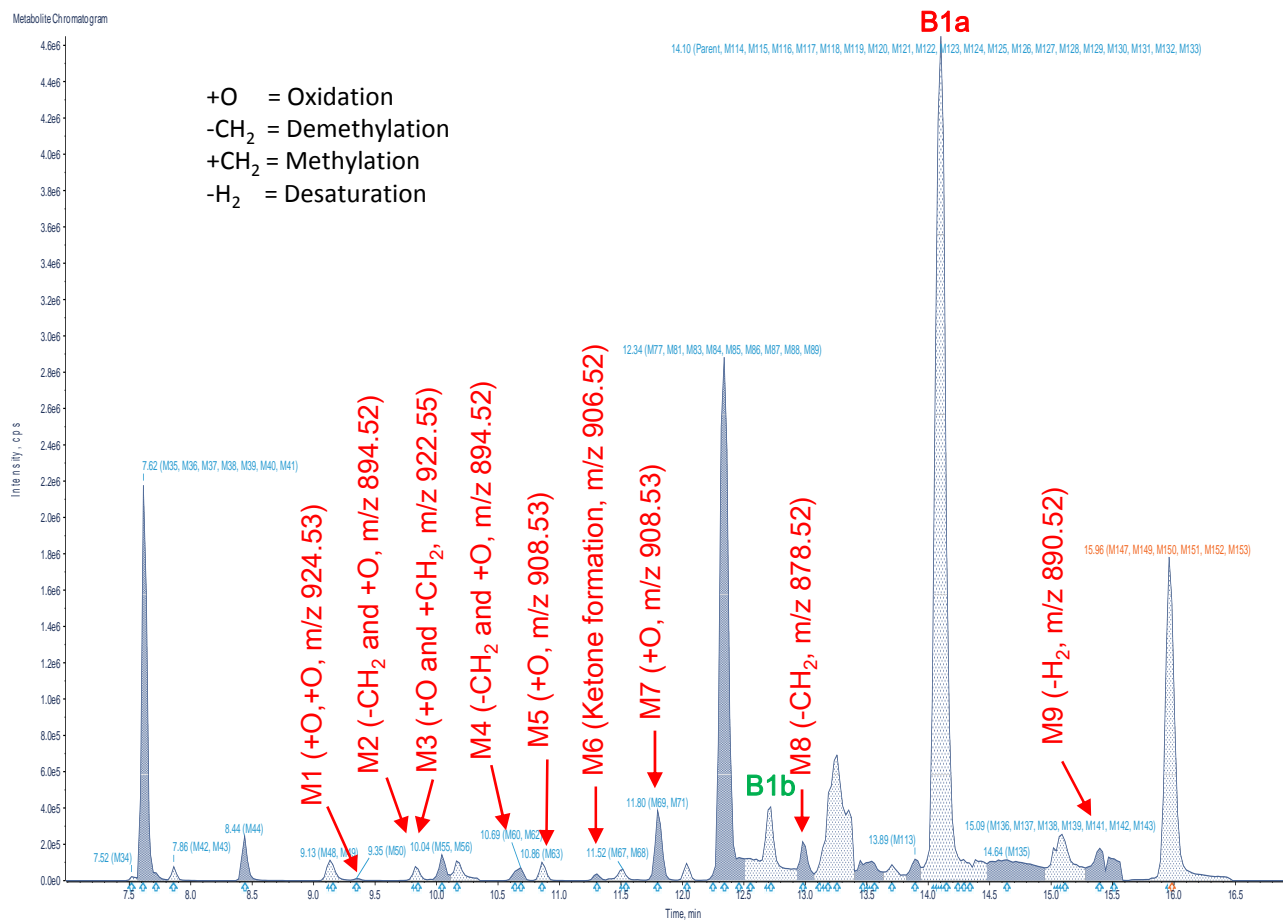
# Experimental



- Confirm structure by NMR
- Develop LCMS quantitation assay
- Pharmacokinetic properties



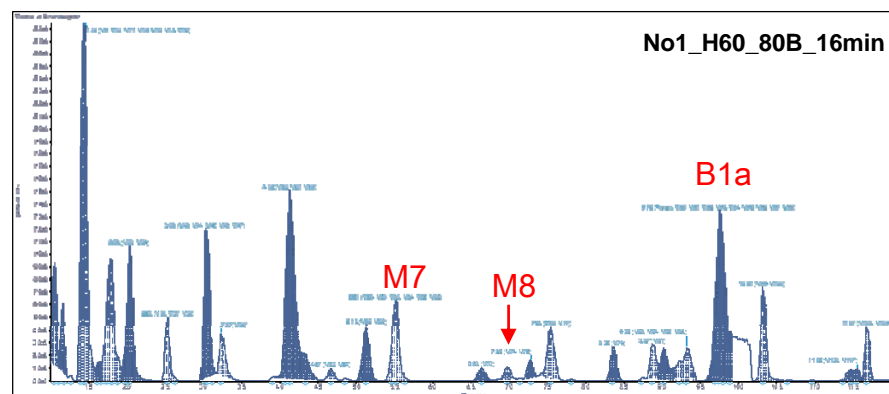
# IVM metabolite - Microsome result (10 $\mu$ M IVM, 60 min)



Negative control

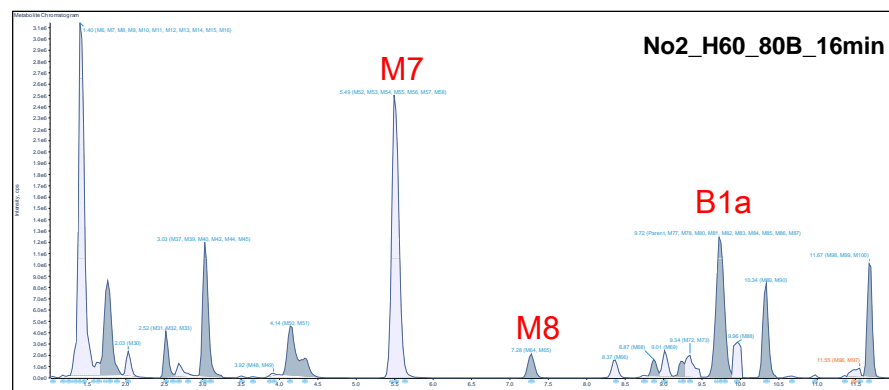
- 0 min
- No NADPH
- No IVM

# IVM metabolite – Human plasma extract

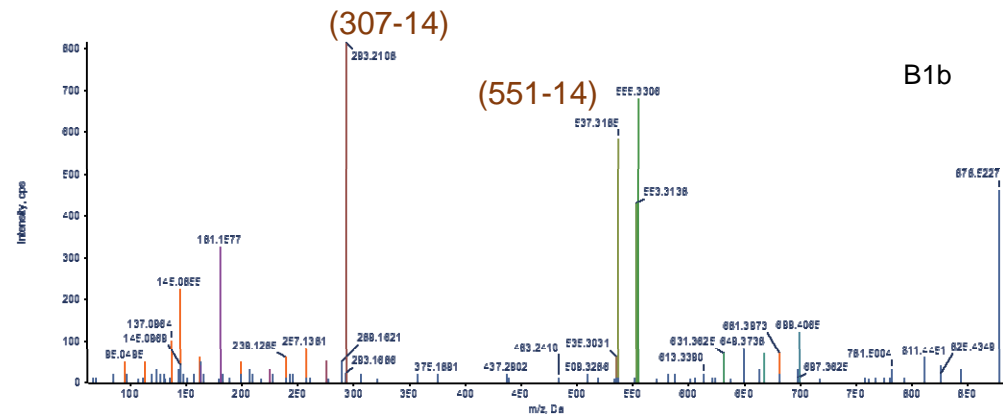
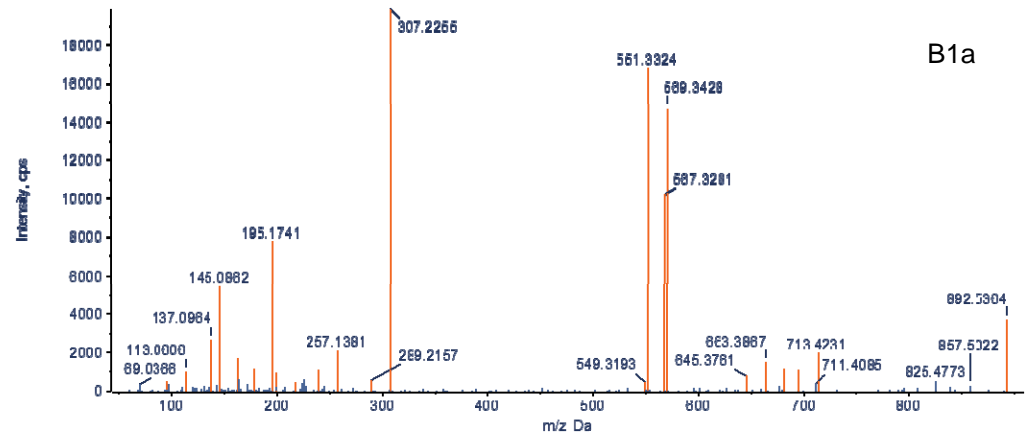
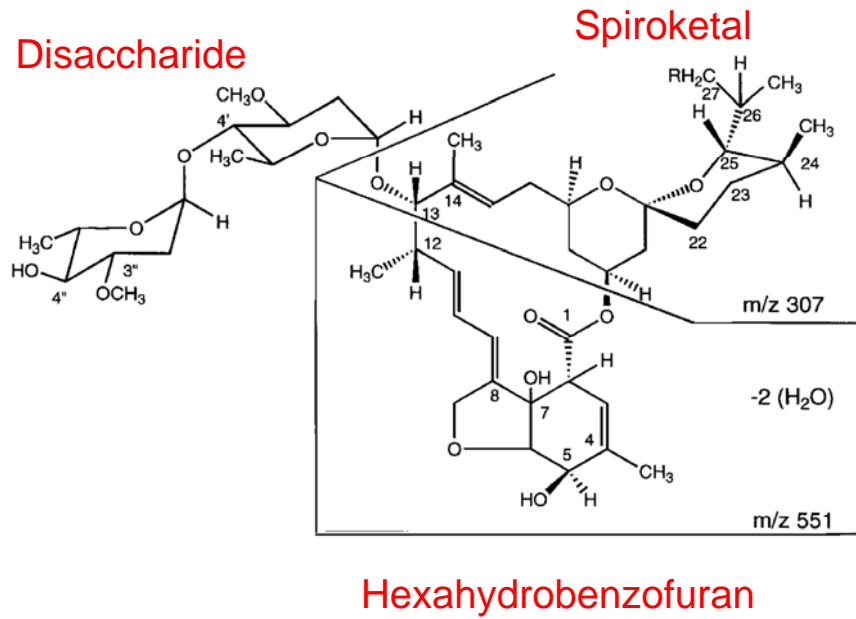


M7 (+O, m/z 908.53)

M8 (-CH<sub>2</sub>, m/z 878.52)

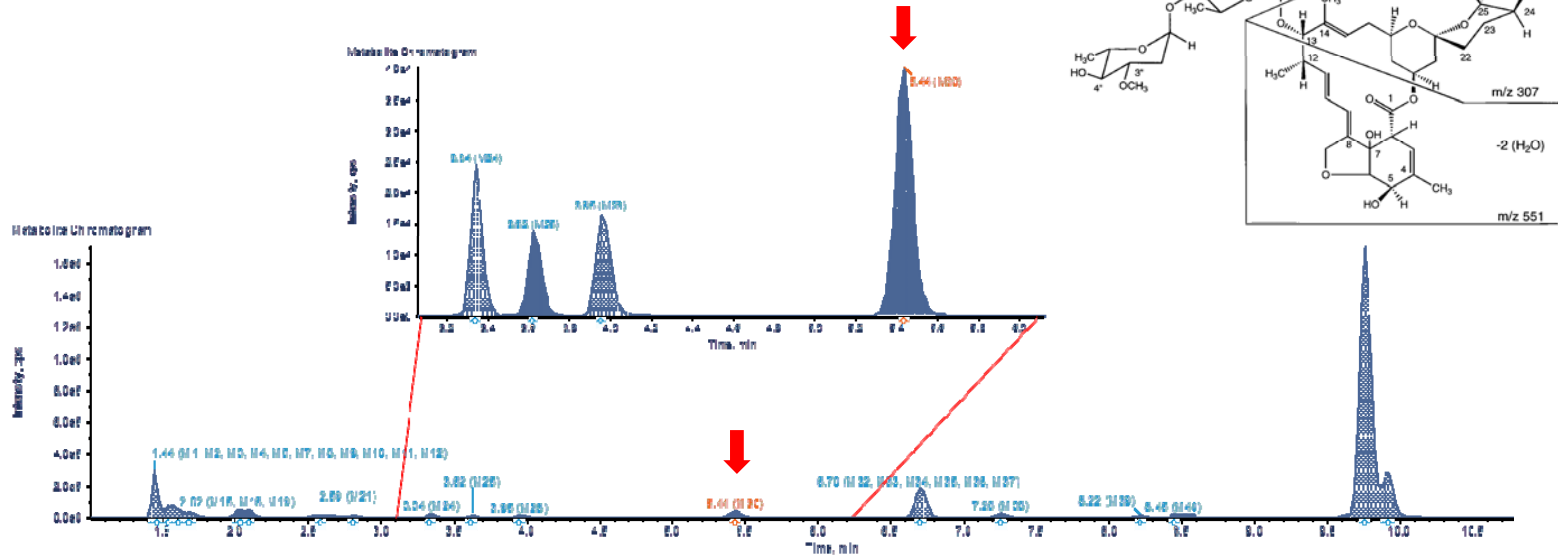


# MS/MS

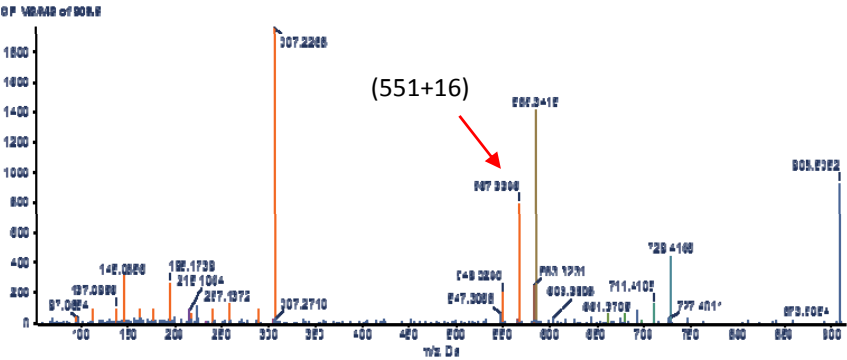
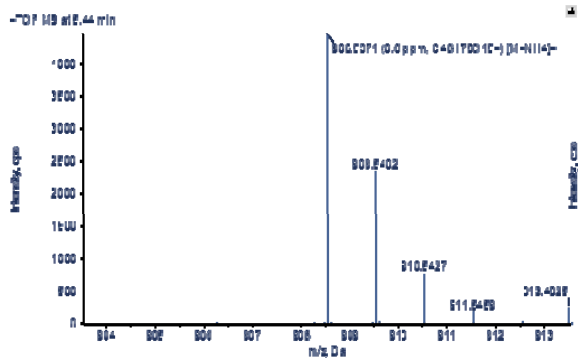


Zeng Z *et al.*, Xenobiotica, 1998

# M7 Oxidation metabolite (at benzofuran) m/z 908.53

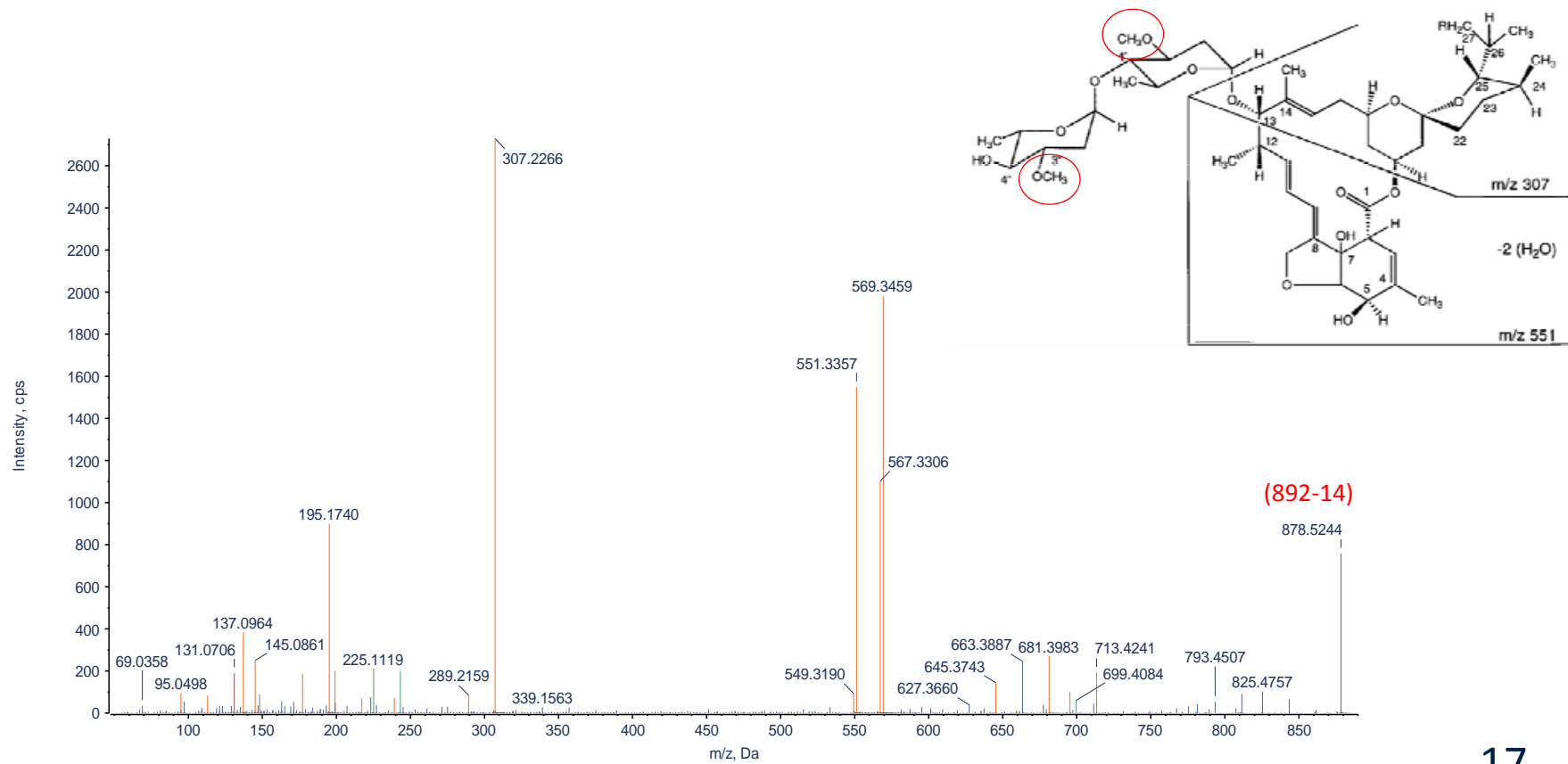


MS





# M8 Disaccharide demethylation m/z 878.52



# Conclusions

- An *in vitro* metabolism protocol in human pooled liver microsome was developed successfully
- Several ivermectin *in vitro* metabolites were identified using pooled human liver microsome
- Two of these metabolites were identified also in human plasma
  - Benzofuran oxidation
  - Disaccharide demethylation
- Further work is needed to characterise the metabolism pathway for each metabolite
- Further work is needed to confirm the structure of the therapeutically relevant metabolites



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Siribha Apinan  
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BILL & MELINDA  
GATES *foundation*



Thank you.



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