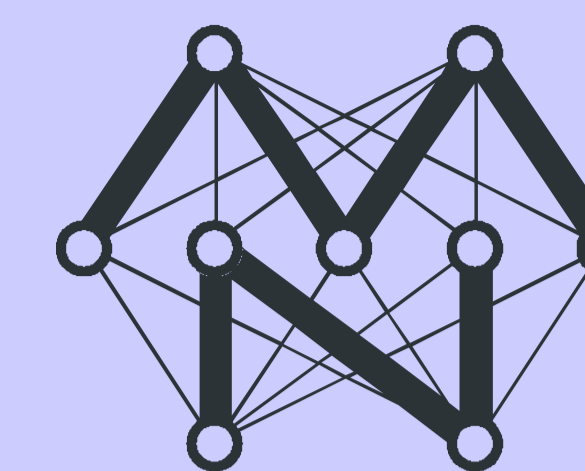


OLIMPIC - Overcoming current Limitations In Metabolism Prediction of Industrial Chemicals

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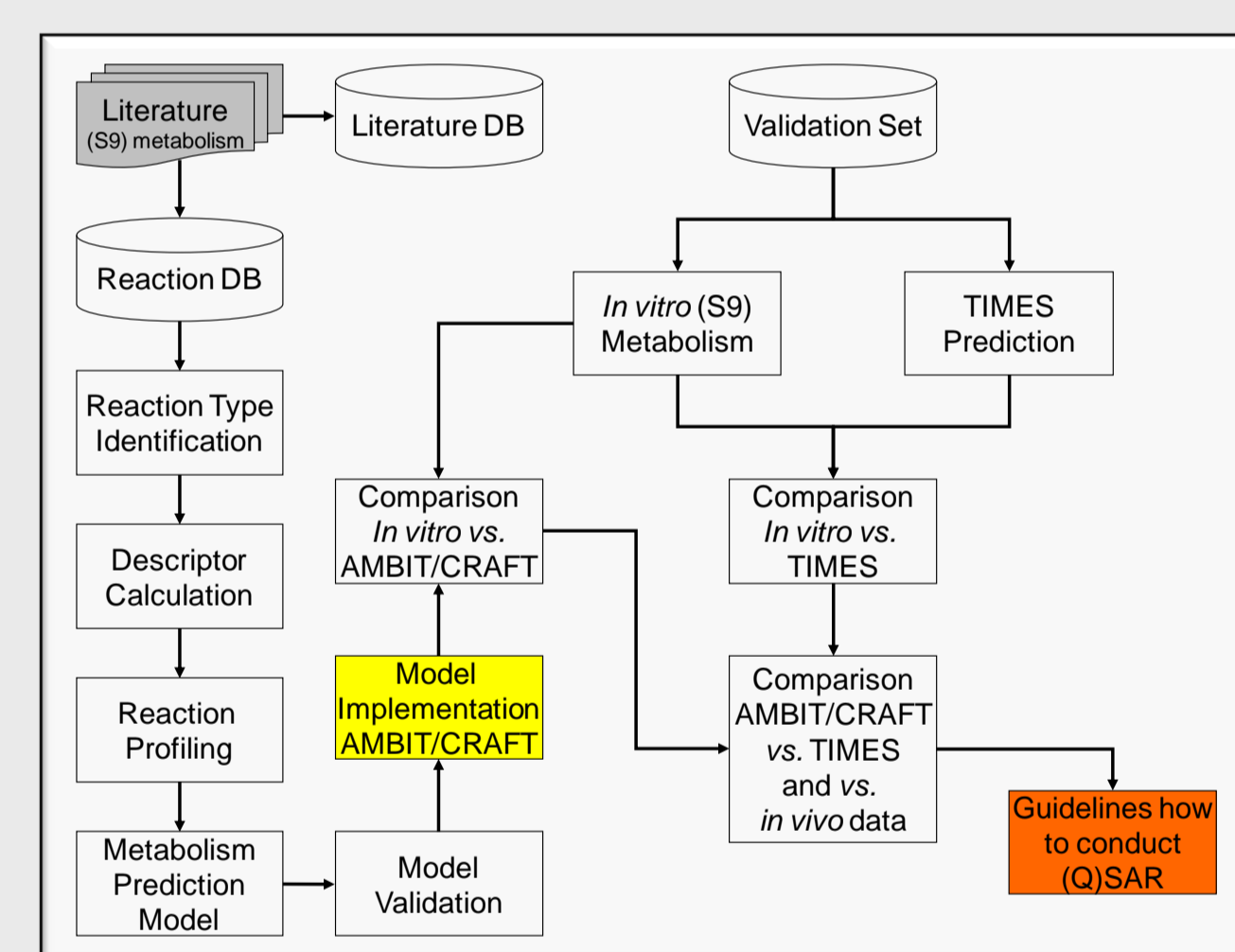
Introduction

Strategic combinations and tiered application of alternative testing methods to replace or minimize the use of animal models in risk assessment are attracting much attention. The aim of the CEFIC-LRI funded OLIMPIC project is to provide improvements in the prediction of metabolism of industrial chemicals in mammals. The holistic investigation will consider *in vivo*, *in vitro* and *in silico* data in order to identify discrepancies between the different methods and to enhance the predictive power of *in silico* models for metabolism.

In this poster we will report on the compilation of a data set on the metabolism of chemicals in rats, the measurements of S9 *in vitro* metabolism data for additional 30 compounds, the evaluation of the software package TIMES, and the chemoinformatics reactivity modeling. The metabolic degradation of the food dye curcumin (E100) will be exemplarily shown.

Workflow

In the figure below the sequence of the individual work packages is assembled to a workflow.



Data Set

The training data set based on literature data on rat S9 metabolism (data set 1) comprises 358 reactions. A second data set covering metabolism by rat microsomes was compiled. This data set on metabolism by rat microsomes (data set 2) comprises 3390 reactions.

The data on rat S9 metabolism is quite limited. Therefore, the chemoinformatics investigation is focused on data set 2 extending the scope of the chemoinformatics modeling from rat S9 metabolism to rat microsomes.

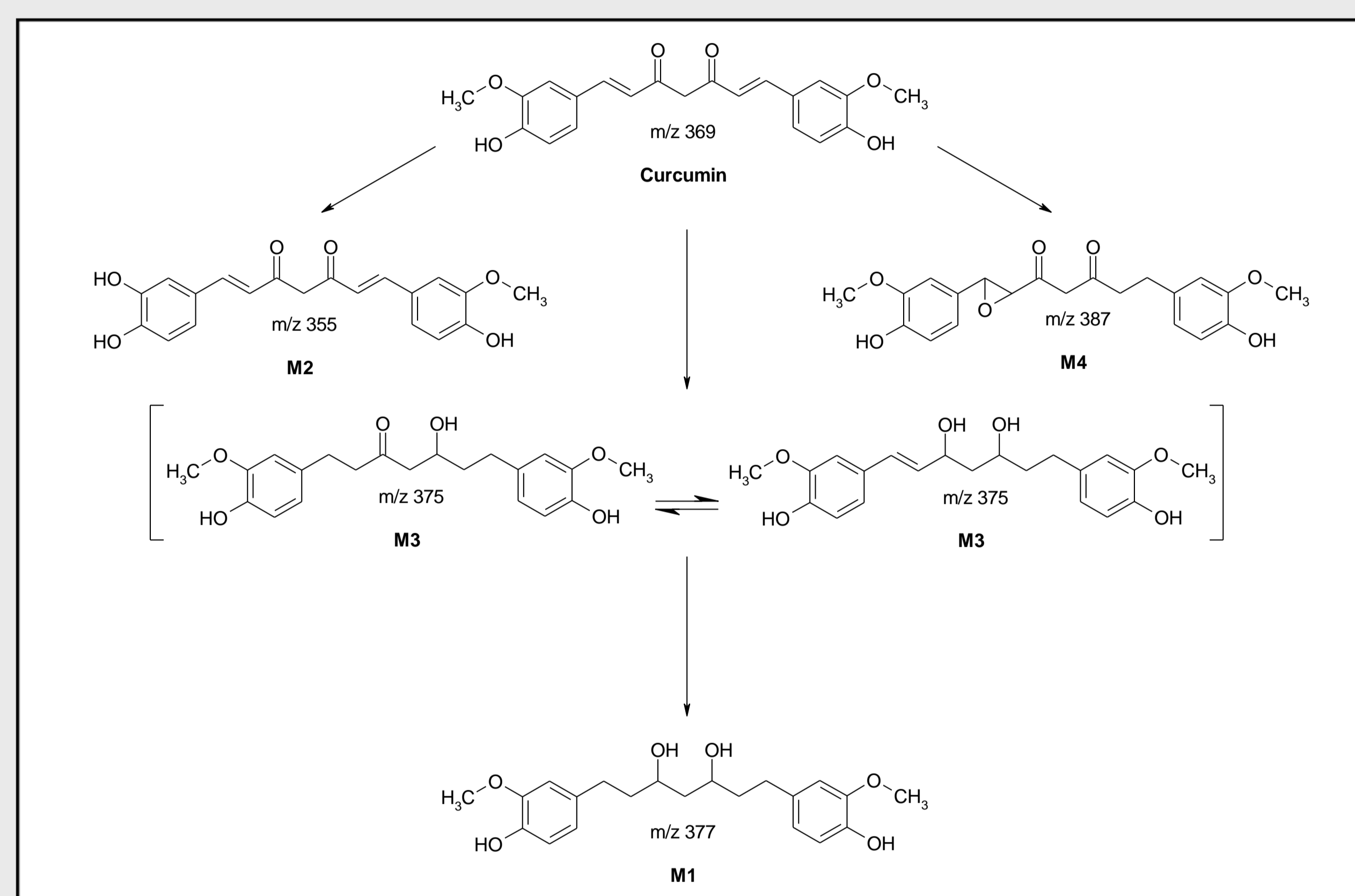
	Data set 1 (rat S9)	Data set 2 (rat microsomes)
Unique compounds	414	3924
Unique reactions	358	3390
Unique substrate	77	1043
Unique product	340	3018

Experimental S9 Metabolism Measurements

Experimental setup

Metabolic clearance of curcumin (CAS: 000458-37-7) was performed by standard incubations in rat liver S9-fraction in a cofactor (NADPH) containing buffer system at 37 °C. After incubation proteins of incubates were precipitated by the addition of acetone and the supernatant was analyzed by LC/UV and LC/MS/MS.

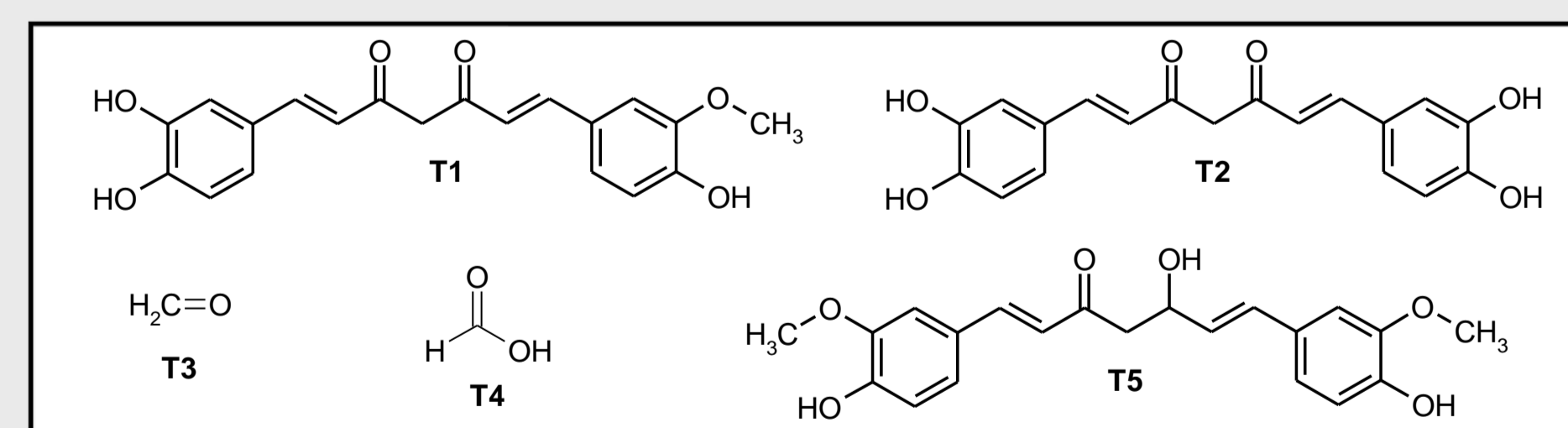
Metabolic pathway of curcumin in rat liver S9-fraction



Evaluation of TIMES

OASIS TIMES was used to generate *in silico* the metabolites of curcumin. Curcumin was in the applicability domain of TIMES. TIMES predicts five metabolites of curcumin.

Scheme of curcumin metabolites predicted by TIMES



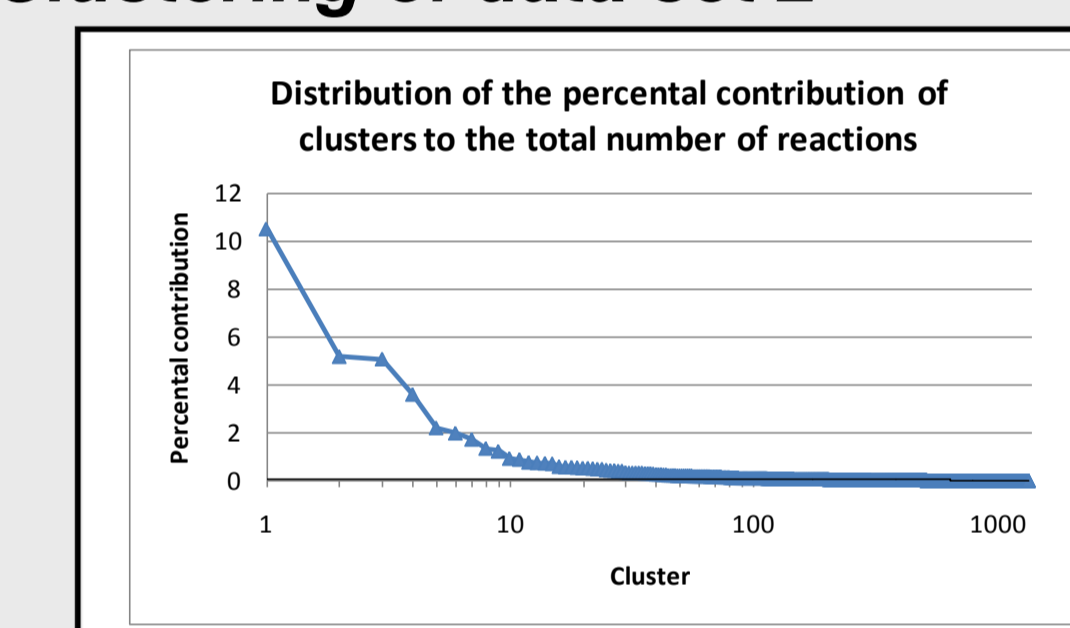
Although only the metabolite M2 (S9-fraction incubation), which is equal to T1 (TIMES evaluation) was detected *in vitro* and *in silico*, the main functionalization reactions were predicted correctly. The metabolite M2 resp. T1 was also detected *in vitro* by Tamvakopoulos et al.[1]

MOSES.Metabolism

Metabolite Prediction

- The prediction has to consider the chemo- and regioselectivity.
- The reactions are clustered by their reaction centers in order to identify the most relevant reaction types.
- Probabilities for each reaction type are derived from the reaction database by statistical analysis.
- For the metabolite prediction the reaction rules are applied to the query compounds. The metabolites are ranked by the probability of the applied reaction rule.

Clustering of data set 2



Depiction of the reaction centers of the top clusters

Cluster	Reaction center
1	
2	
3	
4	
5	
6	
7	
8	
9	

Metabolite Prediction with the Current Model

- The metabolite M2 resulting from the O-demethylation of a methoxy group attached to an aromatic ring is predicted as main metabolite with the highest likelihood.
- Glucuronidation or sulphation of the aromatic hydroxy group are predicted at the second rank. The S9-fraction does not catalyze phase II reactions.

Acknowledgements



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References

[1] Tamvakopoulos C, Sofianos ZD, Garbis SD, Pantazis P. Analysis of the *in vitro* metabolites of diferuloylmethane (curcumin) by liquid chromatography--tandem mass spectrometry on a hybrid quadrupole linear ion trap system: newly identified metabolites. *Eur. J. Drug Metab. Pharmacokinet.* **2007**, *32*, 51-57.

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