

Metabolic Stability in Microsomes

- Phase I metabolism, CYP450 mediated
- Phase II metabolism, UGT mediated

Purpose

The assessment of metabolic stability of new chemical entities is essential in the lead optimization process because it provides information on the rate to which a molecule is metabolized by hepatic enzymes. We provide information of compound stability towards hepatic Phase I metabolism (CYP450 mediated), Phase II metabolism (by glucuronic acid conjugation), or a combined approach of Phase I and II. By using liver microsomes of different species (e.g. rat, human) potential species related differences in the rate of metabolism can be detected.

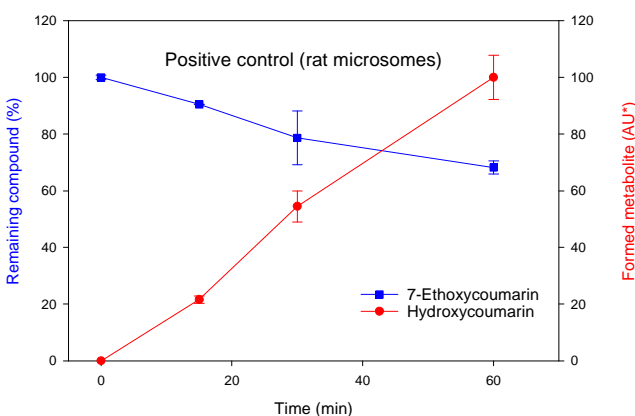
Assay protocol

Incubation solutions are prepared containing a microsomal suspension in phosphate buffer. For testing phase I metabolic stability, a NADP-regenerating system is used, for phase II (glucuronidation) UDPGA is applied, for the combined phase I and II approach both cofactors systems are employed. The reaction is initiated by the addition of test compound after pre-incubation at 37°C. After e.g. 0, 15, 30 and 60 minutes, the reaction is stopped by addition of ethyl acetate or acetonitrile (sample extraction or precipitation respectively). The corresponding loss of parent compound is determined by LC-MS/MS.

Data analysis

The amount of test compound in the samples is expressed in percentage of remaining compound compared to time point zero (100%).

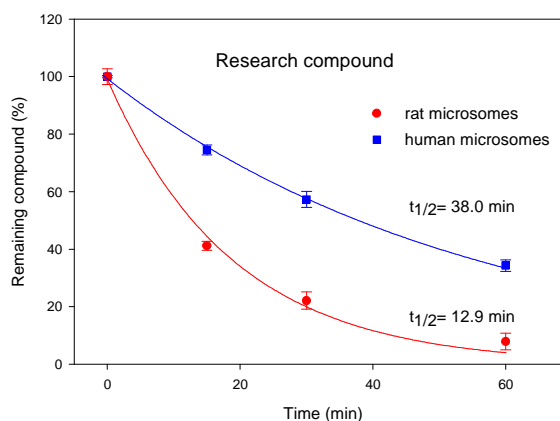
These percentages are plotted against the corresponding time points. The half-life of each compound is determined using the regression curve associated to this representation.



*Arbitrary units

Model validation

Validation is performed by running two controls groups in parallel to the assay: positive controls using 7-ethoxycoumarin (for phase I, metabolized to hydroxycoumarin) and 7-hydroxycoumarin (glucuronidated) for phase II metabolic stability as reference compounds to prove the quality of the microsomal enzymatic activity. Negative controls, using boiled microsomes without regenerating system are employed to ensure that the potential apparent loss of parent compound in the assay incubation is due to metabolism.



Please don't hesitate to contact us for a customized quotation

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