Determination of Metabolic Stability in Liver Microsomes

1. Objective:

The metabolic stability of a test compound, e.g. drug substance in liver microsomes of different species is determined in order to assess the potential of this compound to form undesired potentially toxic or pharmacologically inactive metabolites due to phase I metabolism or to accumulate in the body due to lacking or negligible metabolic degradation. The determination of the metabolic stability is therefore a measure to describe the metabolic fate.

The determination of the metabolic stability in liver microsomes summarizes all the possible reactions.

2. Introduction

Liver microsomes are subcellular fractions (mainly endoplasmatic reticulum) containing many drug-metabolizing enzymes, e.g. cytochrome P450s (CYPs), flavin-monooxygenases, carboxylesterases, and epoxide hydrolase. Therefore they are widely used as an *in vitro* model system in order to investigate the metabolic fate of xenobiotics.

The most prominent group of drug metabolizing enzymes is the super family of cytochrome P450s (CYPs) These haem-containing enzymes play a key role in the metabolism (mainly oxidation) of a variety of chemically diverse compounds including food compounds, pharmaceutical agents, carcinogens, and environmental pollutants.

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Human liver microsomes contain the following CYP isoenzymes involved in drug metabolism: CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4. Of these isoenzymes CYP3A4 plays a major role in metabolism of xenobiotics as it is the most abundant CYP in human liver (approx. 28 %) and it is involved in metabolism of more than 50 % of all pharmaceuticals applied in present-day medication.

3. Short summary: Metabolic Stability Procedure

- Preparation of buffers and stock solutions of test compound (usually in DMSO or water)
- Incubation of reaction mix including liver microsomes (desired species, usually human and/or rat), test compound, and NADPH for different time points, e.g. 10, 15, 30, and 60 minutes or single time points, e.g. 60 minutes.
- End of reactions by addition of stop reagent, sample preparation
- Determination of loss of parent compound (compared to zero time point control and/or no NADPH-control) using LC-MS or LC-MS/MS methods.
- Results: Metabolism (percentage of test compound metabolized after a certain time) and half-life time (in case of time course study).

4. Marker Reactions

The following marker reactions and marker substrates can be employed as quality criteria of the metabolic capability of the microsomes:

CYP	Marker Substrate	Marker Reaction
1A2	7-ethoxyresorufin	7-ethoxyresorufin O-deethylation
2A6	coumarin	coumarin 7 - hydroxylation
2B6	S-mephenytoin	S-mephenytoin N-demethylation
2C8	paclitaxel	paclitaxel 6a-hydroxylation
2C9	diclofenac	diclofenac 4'-hydroxylation
2C19	S-mephenytoin	S-mephenytoin 4'-hydroxylation
2D6	bufuralol	bufuralol 1'-hydroxylation
2E1	chlorzoxazone	chlorzoxazone 6-hydroxylation
3A4	testosterone	testosterone 6ß-hydroxylation