Electrochemical simulation: a powerful tool for the elucidation and study of drug metabolism reactions.

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Electrochemical simulation: a powerful tool for the elucidation and study of drug metabolism reactions.

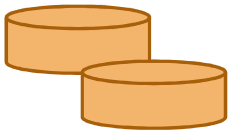
Conventional approaches in drug metabolism studies.

Electrochemistry/mass spectrometry for metabolite generation and identification.

The selected results of own studies.

Keywords:
antitumor compounds,
liver cell microsomes,
cytochrome P450,
oxidative drug metabolism,
electrochemical simulation
mass spectrometry

Degradation pathway of xenobiotics



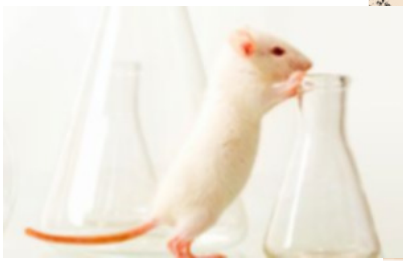
Xenobiotics (nonpolar, lipophilic)

Phase-I metabolism (activation): oxidation reactions (often catalysed by enzymes of the cytochrome P450 superfamily), reduction and hydrolysis reactions (intermediate metabolites – more polar, less lipophilic)

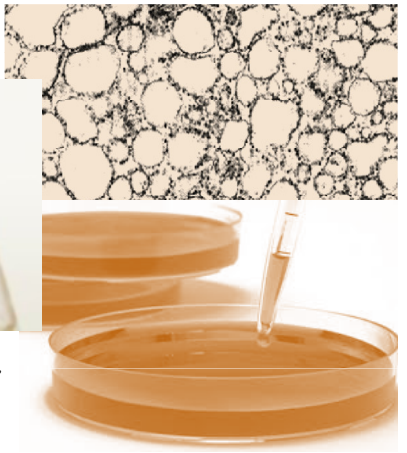
Phase-II metabolism (detoxification): enzyme-catalysed conjugation reactions with small polar endogenous compounds like tripeptide glutathione, glucuronic acid, sulfate, or glycine (metabolites – polar, water-soluble, easily excreted from the human body in urine or bile)

Conventional approaches in drug metabolism studies

In vivo and *in vitro* methods, using **laboratory animals, isolated liver cells (hepatocytes) or microsomes, containing active enzymes**, i.a. the cytochrome P450 system.



laboratory animals



liver cell microsomes

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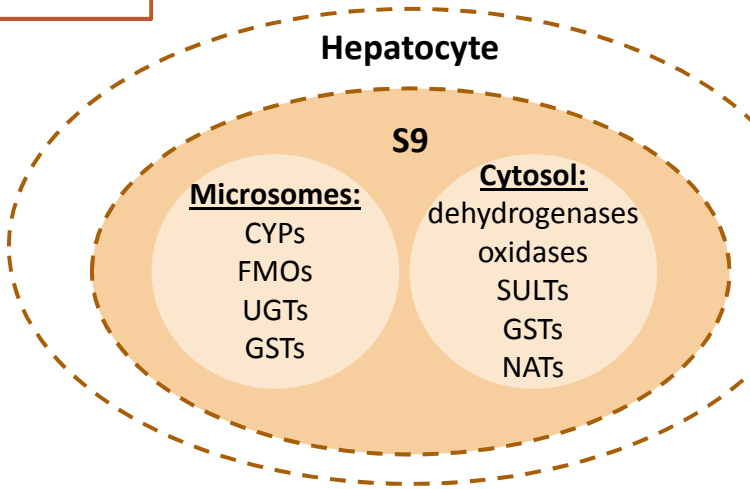

Distribution of drug metabolising enzymes in liver
CYP, cytochrome P450 monooxygenase; FMO, flavin-depe monooxygenase; UGT, UDP-glucuronosyl transferase; glutathione transferase; SULT, sulfotransf NAT, N-acetyltransferase.

- Laborious and time consuming.
- Metabolites may form adducts with the cell matrix, hence isolation and identification of reactive metabolites is hampered.
- Variations in the expression of cytochrome P450 isoforms in each organism have to be taken into account.

Electrochemistry/mass spectrometry (EC/MS)

IS – a powerful platform to simulate various tion and reduction processes.

ectrochemical cell for the simulation of the oxidative and ductive phase-I metabolism.

gh potential for screening of single target compounds in ase-II metabolomics studies.

st method for direct identification of reactive etabolites because of the absence of biological matrices.

elivers a redox fingerprint of a (drug) molecule, close to etabolic profile, in a very short time.

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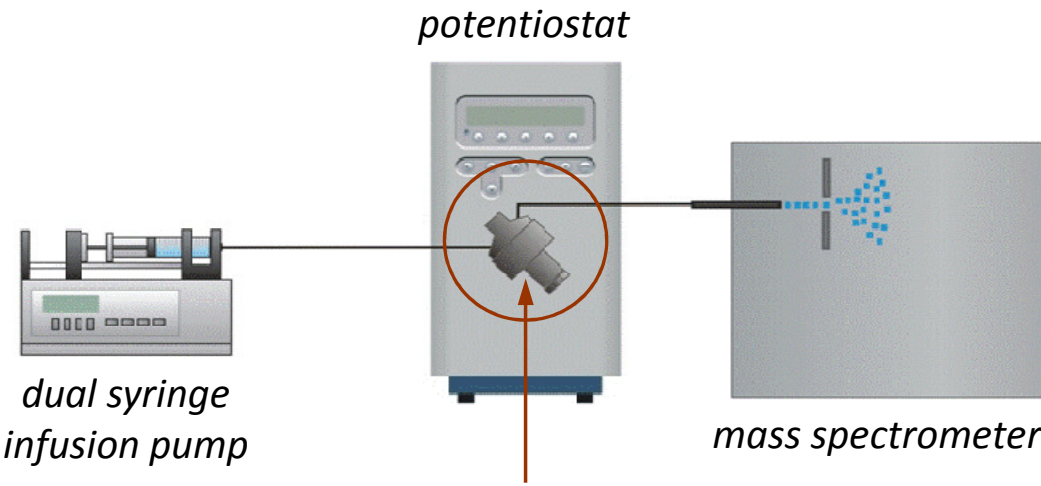
Instrumental set-up of EC system



- high speed
- simplicity
- ease of automation
- low-cost instrumentation

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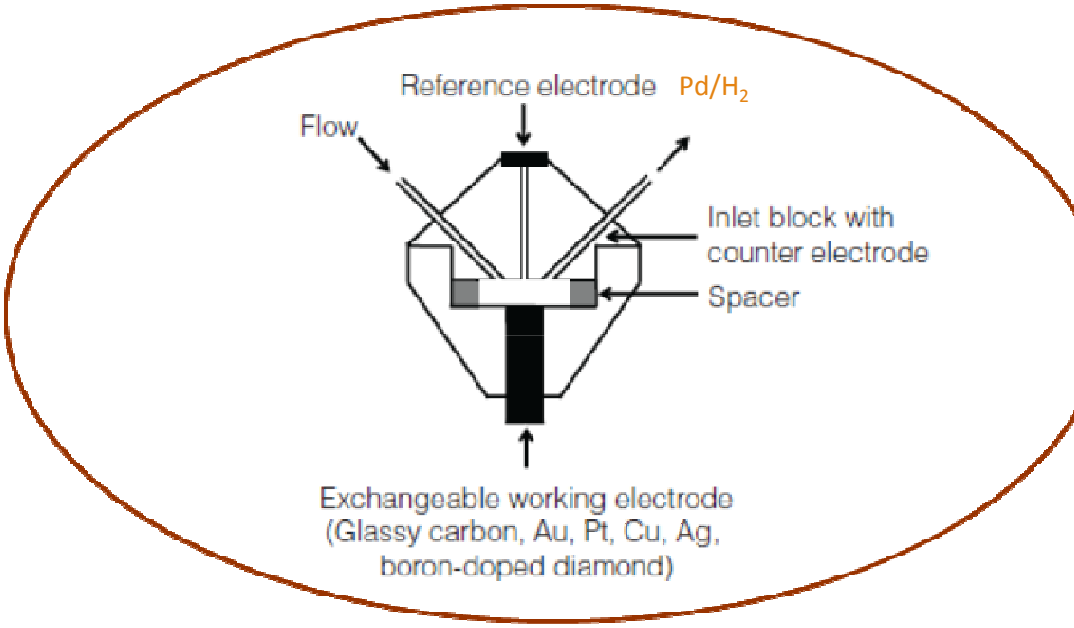
Simulation in an amperometric thin-layer cell



- low adsorption on the electrode surface
- easily exchangeable working electrode

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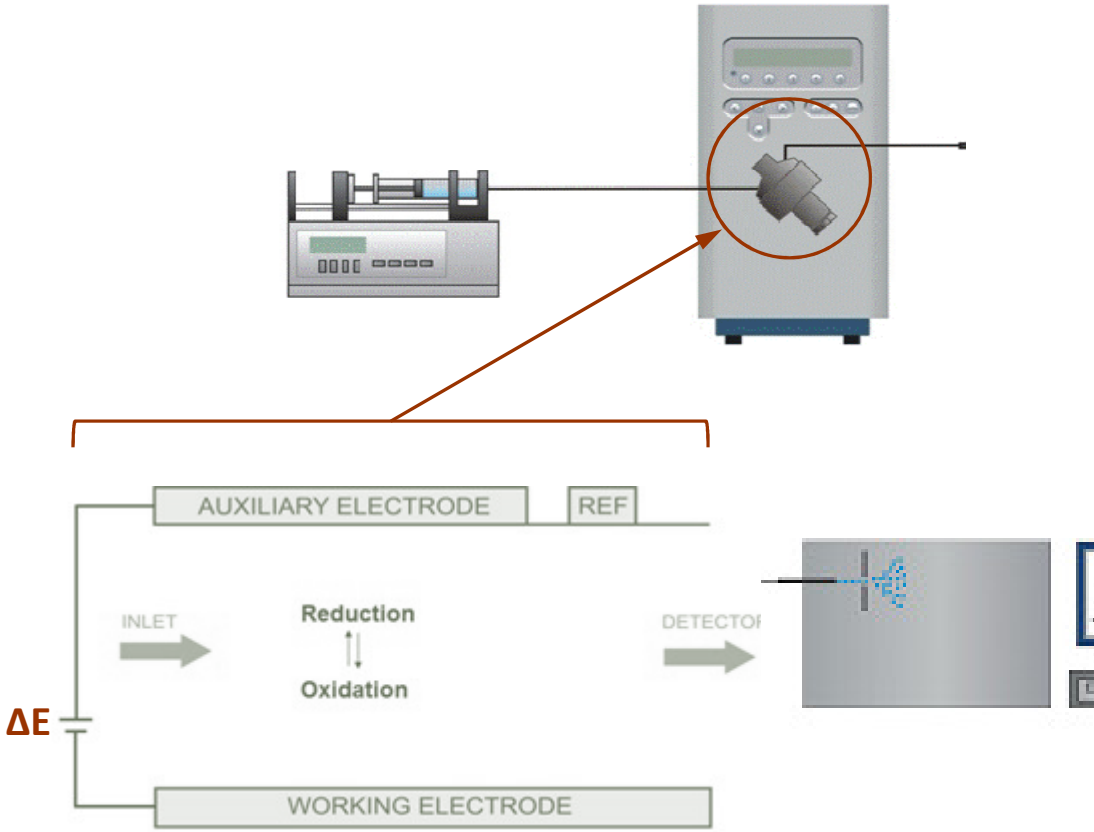
Electrochemical reaction



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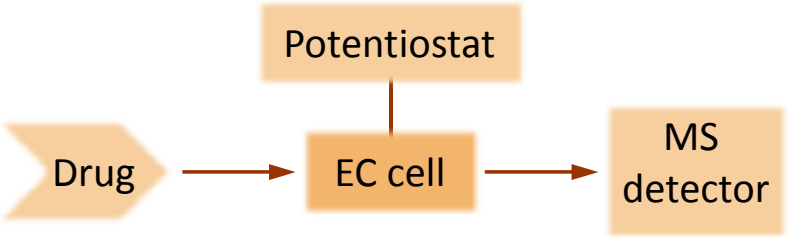
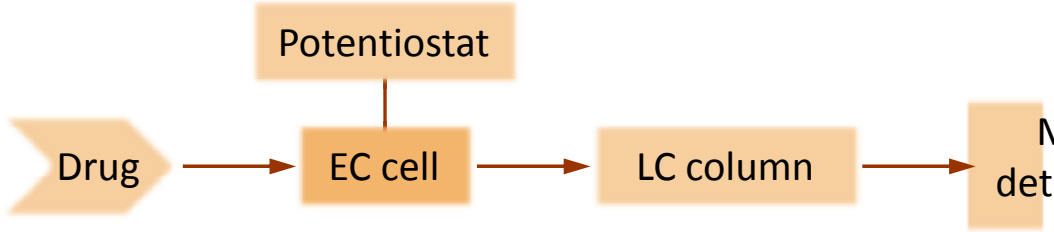
Set-ups of the most common approaches for the simulation of drug metabolism reactions

A **EC/MS system**



B **EC/LC/MS system**



Adapted from Baumann et al., Expert Opin Drug Metab Toxic

Electrochemistry/mass spectrometry (EC/MS)

MS – a powerful platform to simulate various tion and reduction processes.

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Typical phase I reactions and the possibility for the electrochemical simulation

Typical reactions catalysed by cytochrome P450	*Simulation by electroche*
Allylic and aliphatic hydroxylation	Succesfully mimicked potentials
Benzylic hydroxylation	Sucessfully mimicked, but low yields
Desalkylation of amines	Readily mimicked
Desalkylation of ethers and thioethers	Ethers: possible Thioethers: not mimicked
Hydroxylation of aromatics	Possible
Epoxide formation	Not mimicked
Oxidation of heteroatoms (*N, S*)	Sucessfully mimicked (but formation only in low yield
Alcohol and aldehyde oxidation	Not mimicked
Dehydrogenation	Readily mimicked

Adapted from Lohmann et al., LC-GC Europe, Ja

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RESEARCH OBJECTIVES

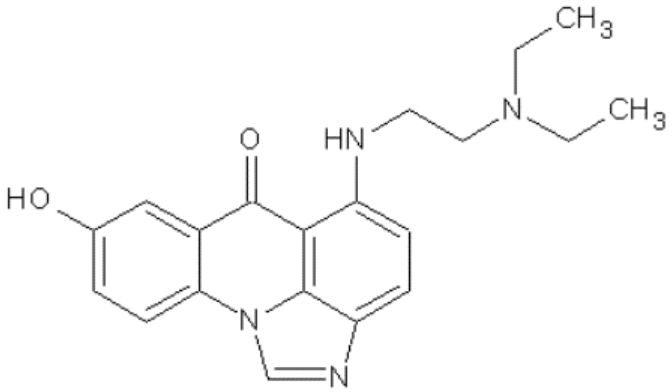
Financial support by the National Science Centre (Pola
(project SONATA No 2012/07/D/NZ7/03395)

- **Investigation phase-I metabolism of the model antitumor acridine derivatives, C-1311 and C-1748, under electrochemical conditions coupled to mass spectrometry (EC/MS).**

- **Comparison the results obtained by EC/MS with conventional *in vitro* microsomal approach.**

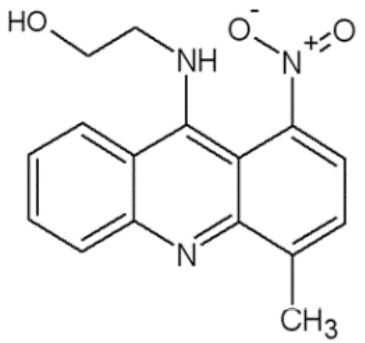
THE STUDIED COMPOUNDS

- different structures
- various metabolic pathways



5-diethyloaminoethyloamino-8-hydroxyimidazoacridinone
C-1311

- **inhibitor of both topoisomerases and certain receptor kinases**, including FMS-like tyrosine kinase FLT3 *(Chau et al., 2006; Skwarska et al., 2010)*
- **activity against advanced solid tumors** under phase I and II of clinical trials, also tested for the treatment of autoimmune diseases *(Capizzi et al., 2008; Isambert et al., 2010)*



9-(2'-hydroxyethyloamino)-4-methylo-1-nitroacridine
C-1748

- **strong cytotoxic activity against colon cancer cel lines** (HCT8 HT29) *(Augustin et al., 2010)* and high antitumor activity aga several prostate (LnCaP, JCA, PC3, TSU) and colon carcin xenografts (HCT8) in nude mice *(Chen et al., 2002; Tadi et al., 20*
- **low mutagenic potential and slight myelosuppressive proper** *(Narayanan et al., 2005)*

Enzymatic transformations of C-1311 and C-1748

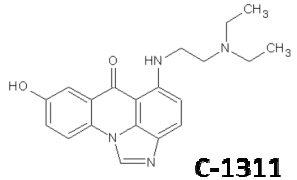
***Table**: Gradient profile utilized for all HPLC analyses.*

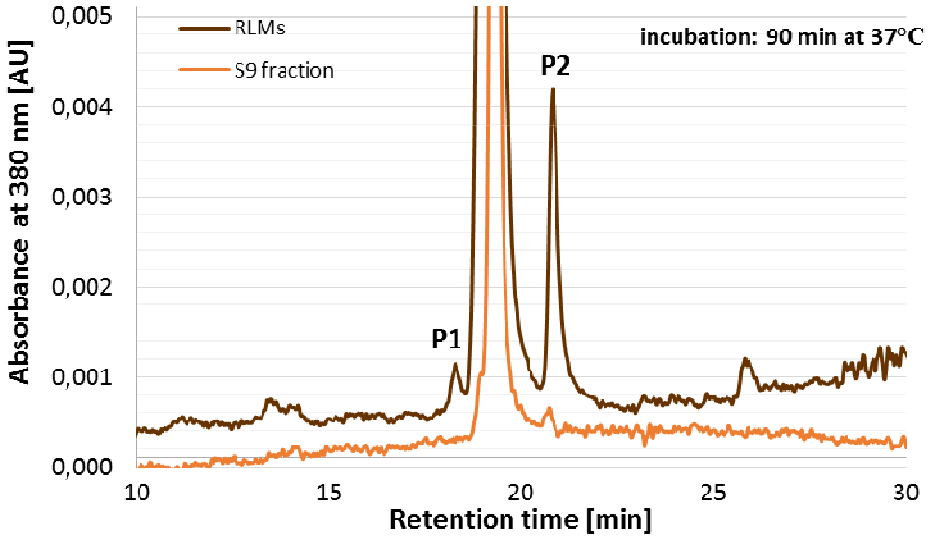
Time [min]	Flow [mL/min]	Mobile phase [%]		Curve
		A	B	
-	1	85	15	-
25	1	20	80	6
28	1	0	100	6
30	1	85	15	6

***A:** 50 mM **aqueous amm** **formate buffer** (pH 3.4)*
methanol for HPLC
***B:** **Methanol for HPLC** + 5*

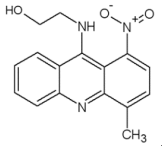
▪ RP-HPLC-DAD/ESI-MS analysis

A





B



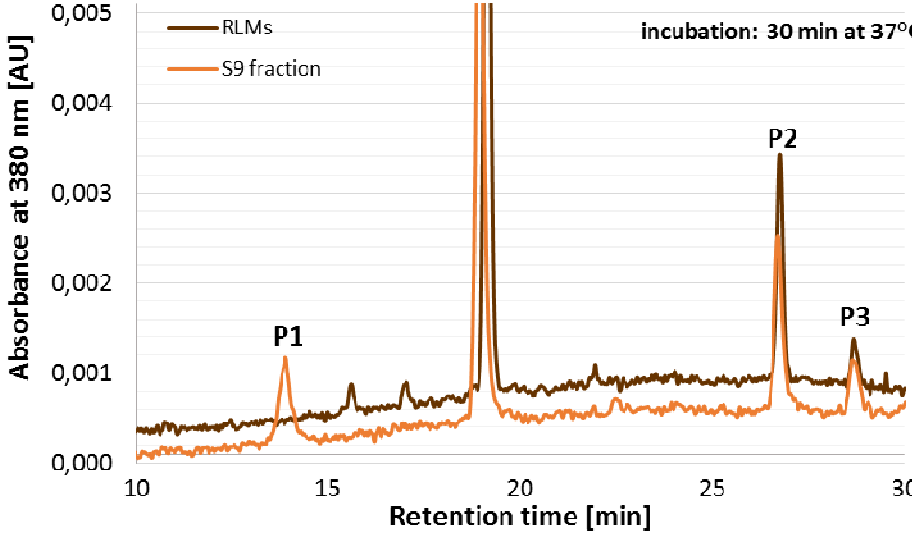
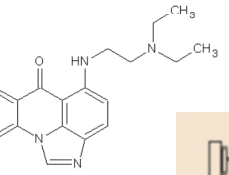
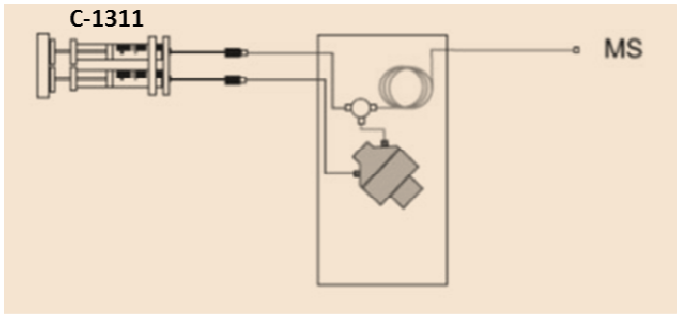


***Figure:** Linear chromatograms of the reaction mictures containing 0.05 mM (**A**) **C-1311** (**B**) **C-1748**, 1 mM NADPH and 2 mg/mL microsomal (S9) fraction of liver cells in sodium phosphate buffer (pH7.4).*

Electrochemical simulation of C-1311 metabolism

Parameter	Electrosynthesis conditions
type of working electrode	glassy carbon (GC)
EC potential range	+/- 2000 mV (10 mV steps)
flow rate	10 µL/min
Reaction medium	H_2O + MeOH (1:1) + 0.1% v/v HCOOH





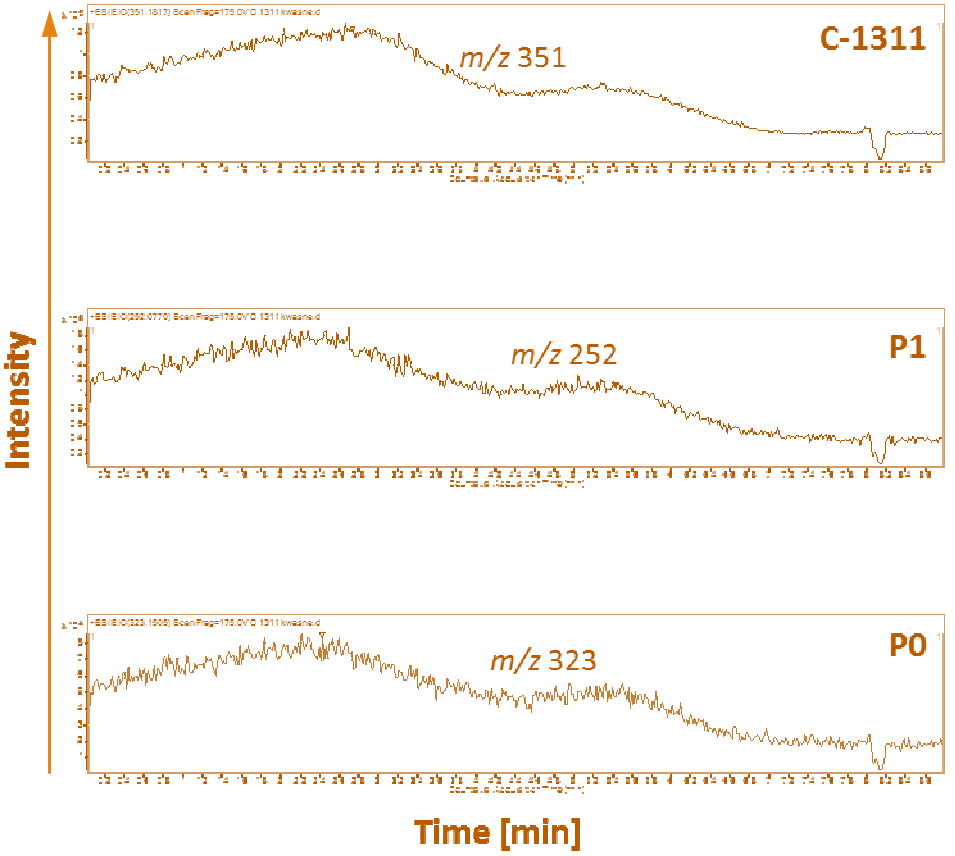


Figure: *C-1311 abundance vs. electrosynthesis time.*

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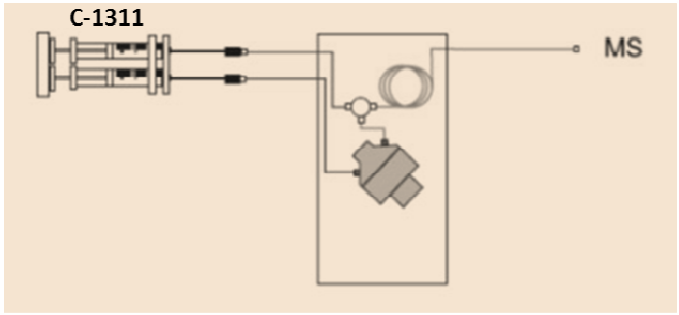


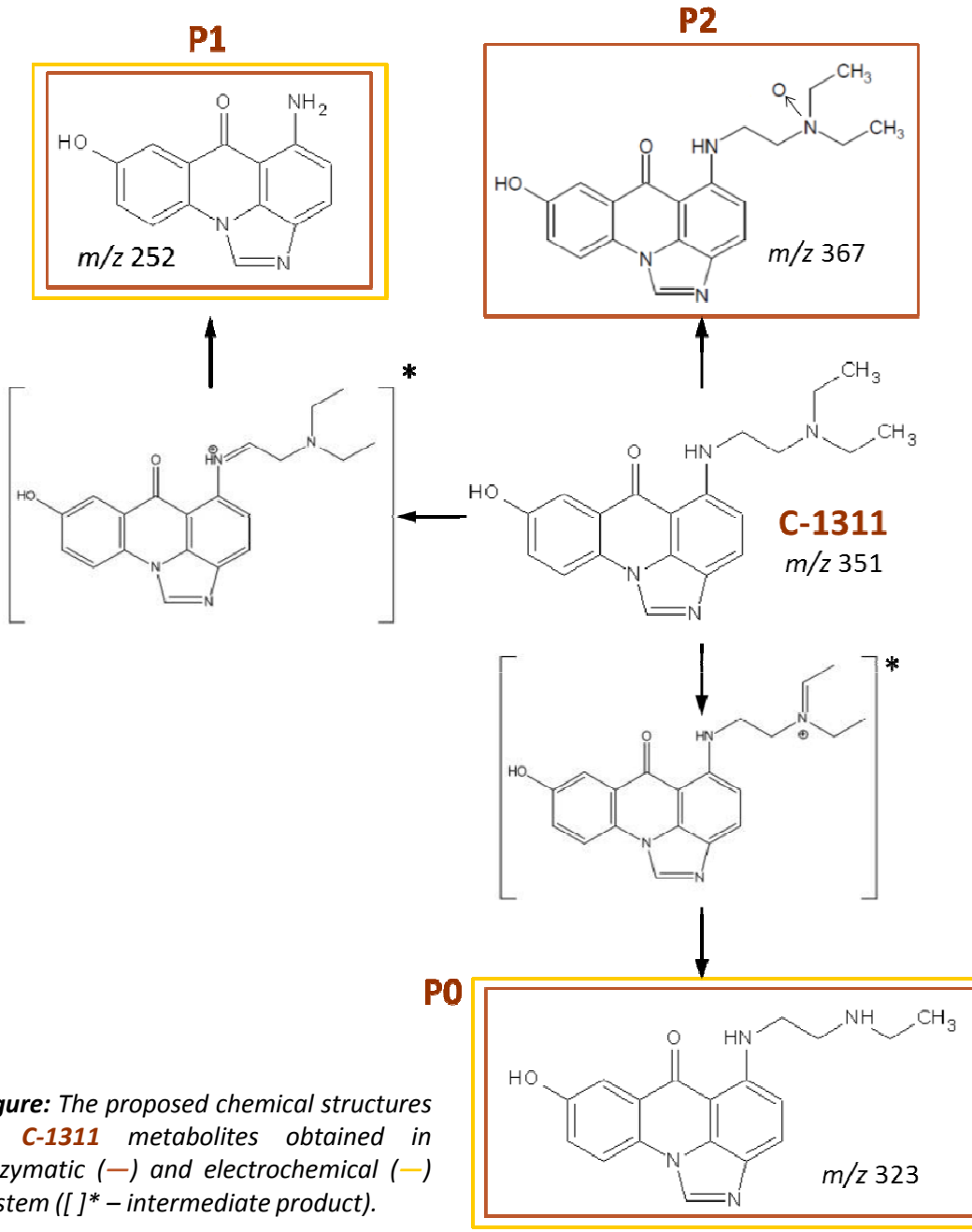
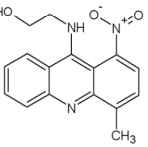
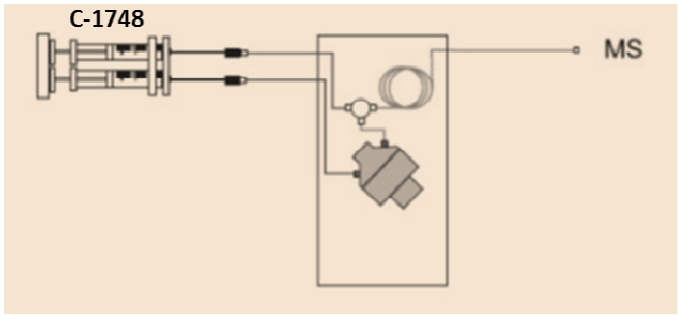


Figure: *The proposed chemical structures of* ***C-1311*** *metabolites obtained in enzymatic (—) and electrochemical (—) system ([ ]* – intermediate product).*

Electrochemical simulation of C-1748 metabolism

Parameter	Electrosynthesis conditions
type of working electrode	glassy carbon (GC)
EC potential range	+/- 2000 mV (10 mV steps)
flow rate	10 µL/min
Reaction medium	NH_4C00H (pH 7.7) + 25% v/v ACN





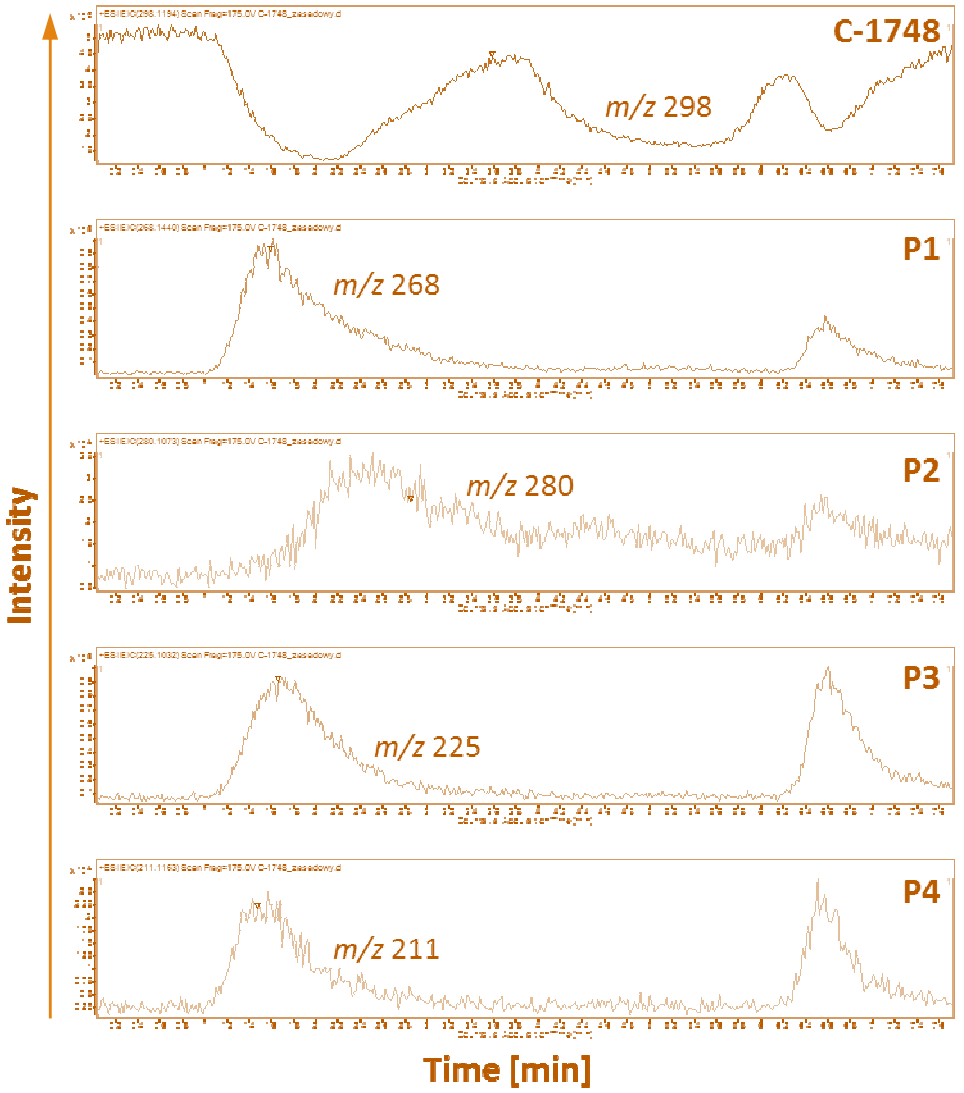


Figure: *C-1748 abundance vs. electrosynthesis time.*

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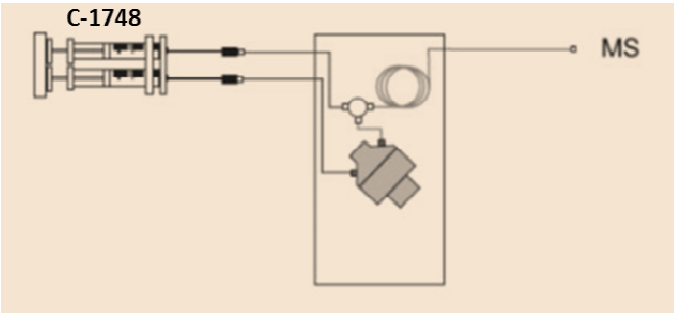


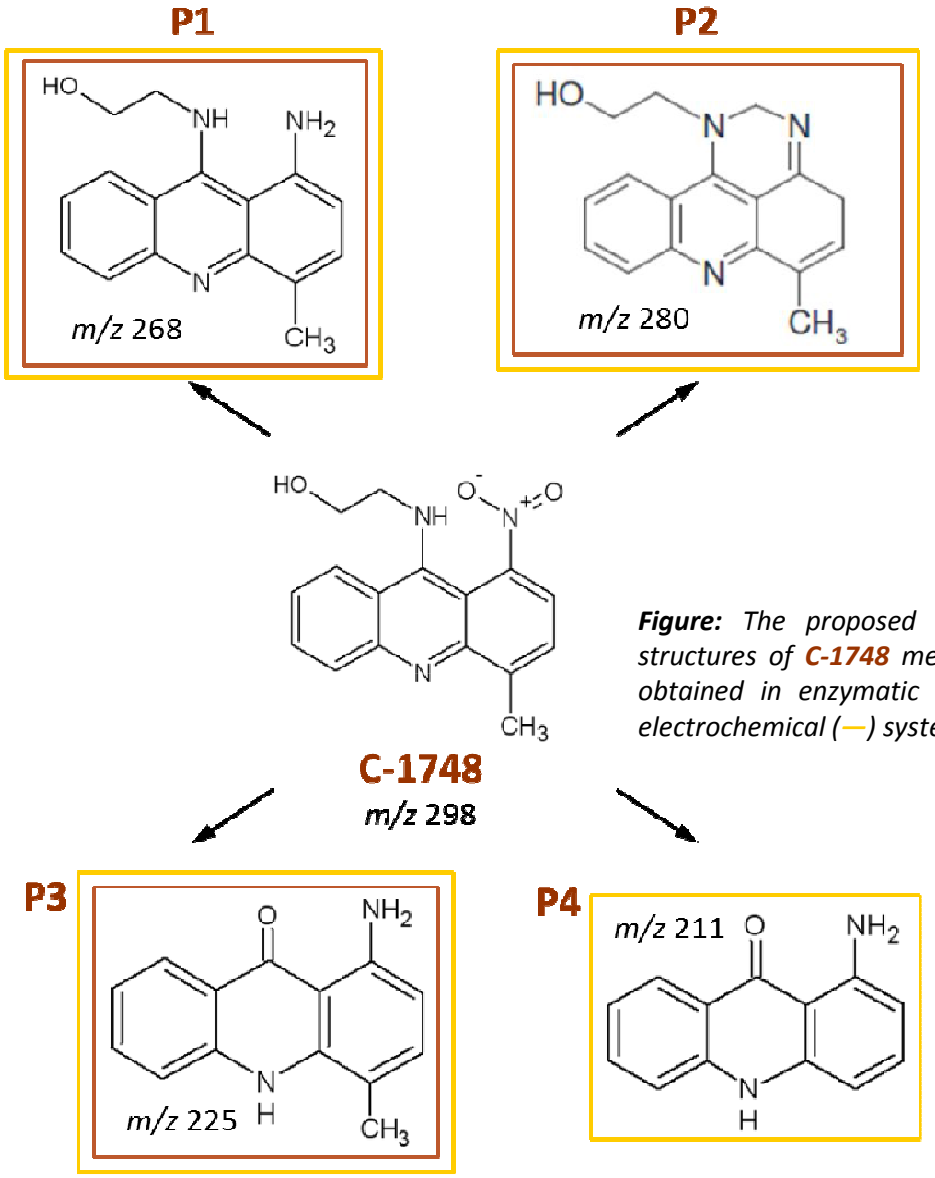


Figure: *The proposed ch structures of* ***C-1748*** *meta obtained in enzymatic (– electrochemical (—) system*

SUMMARY & CONCLUSIONS

Electrochemical system is very well-suited for t simulation of the oxidative and reductive metaboli of antitumor acridine derivatives.

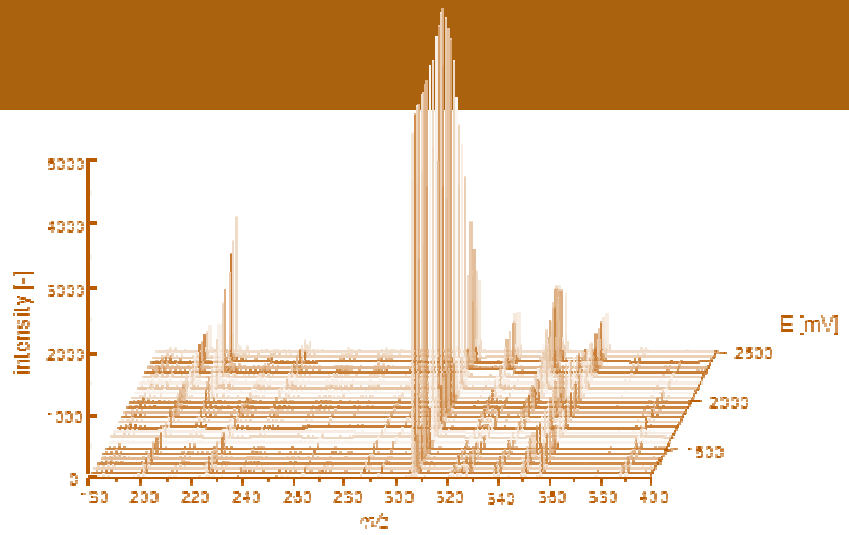
Electrochemical conversion of C-1311 and C-1748 into phase-I metabolites was successfully achieved.

- Two main metabolic products of C-1311 (side chain degradation products) and three products of C-1748 were detected both in the conventional enzymatic approach and in the electrochemical simulation.

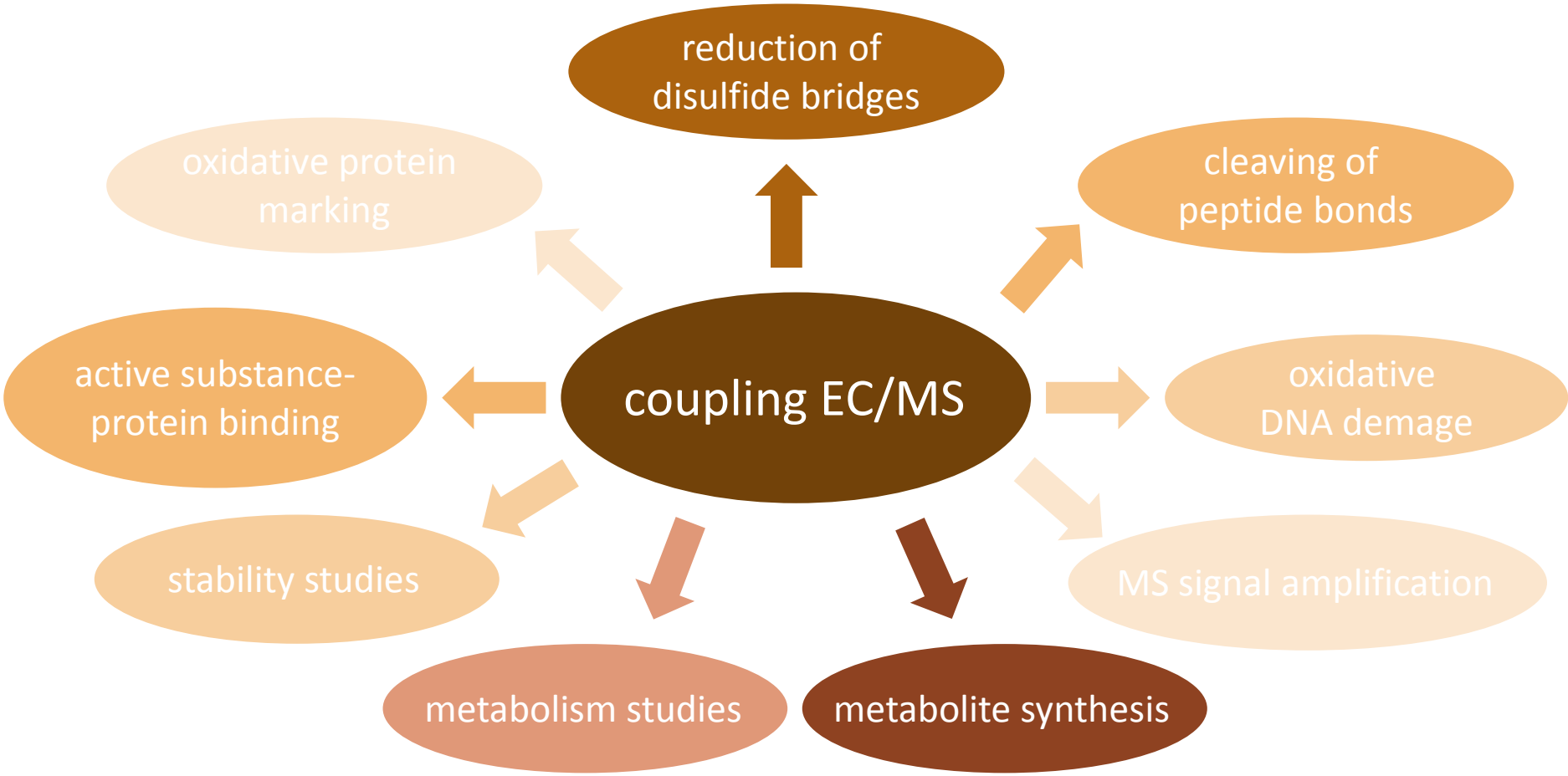
An additional product of C-1748 (1-aminoacridone) was found only in the electrochemical simulation.

SUMMARY & CONCLUSIONS

The purely instrumental approach, based on electrochemical conversion, is a feasible alternative to the conventional microsomal studies.



Application areas of electrochemistry/mass spectrometry



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Weronika Hewelt-Belka[2], MSc
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Thank you for your attention!