Application Note
Drug Metabolism

Electrochemical Reactions upfront MS – EC/MS

Proteomics & Protein Chemistry
S-S bond reduction
HDX
Peptide bond cleavage
Na+, K+ removal
Drug-protein binding

Lipidomics & Fatty Acids
Cholesterol
Oxysterol
FAME Biodiesel

Drug Metabolism
Mimicking CYP 450
Phase I & II
Biotransformation

Synthesis (mg)
Metabolites & Degradants

Pharmaceutical Stability
Purposeful degradation
API testing
Antioxidants

Environmental
Degradation & persistence
Transformation products
Surface & drinking water

Food & Beverages
Oxidative stability
Antioxidants

Forensic Toxicology
Designer drugs
Illicit drugs

Healthcare & Cosmetics
Skin sensitizers

Genomics
DNA Damage
Adduct formation
Nucleic acid oxidation

Electrochemical Simulation of Phase I Metabolism of 3 Novel Cardiovascular Drugs

- **On-line EC-MS for rapid generation and identification of phase I metabolites**
- **Most metabolites found in human urine can be mimicked by EC**
- **"in-electro" a cost-effective alternative to in-vivo and in-vitro drug metabolism**

Introduction

In this study electrochemistry (EC) coupled with electrospray ionization mass spectrometry (ESI-MS) was used to investigate the metabolic fate of three novel cardiovascular drugs: Rivaroxaban (RIV), Aliskiren (ALS), and Prasugrel (PRS). Mimicry ("simulation") of the oxidative phase I metabolism was achieved in a simple amperometric thin-layer cell equipped with a boron-doped diamond (Magic Diamond™) working electrode. Structures of the electrochemically generated metabolites were elucidated from MS/MS experiments. The results obtained by EC-MS were compared with in-vivo studies by analyzing urine samples from patients after administration of the cardiovascular drug.

This application note is by courtesy of Dr. Małgorzata Szultka-Młyńska, Department of Environmental Chemistry and Bioanalytics, Nicolaus Copernicus University, 87-100 Toruń, Poland

Instrumental setup and experimental conditions

For online EC-MS experiments, the ROXY™ Potentiostat equipped with the ReactorCell™ (Antec Scientific, Boston, USA) and a boron-doped diamond working electrode was used. ALS, RIV, and PRS solutions (100 µM) were prepared in 10mM ammonium formate and adjusted to pH 7.4 with ammonia and acetonitrile (50:50, v/v). The solution was pumped through the electrochemical cell under continuous flow (8 µL/min) delivered by a syringe pump. The effluent of the cell was introduced into an Agilent 6410 quadrupole electrospray ionization-mass spectrometry (Agilent, Germany), where the positive ionization mode (ESI+) was applied. The schematics of the instrumental setup is shown in Figure 1.

Each mass voltammogram was recorded at least three times to ensure reproducibility of the measurements. The mass spectra of the drugs at different applied values of voltage were recorded to observe oxidation processes in real time. Post-processing of the data was performed with the Agilent MassHunter software.

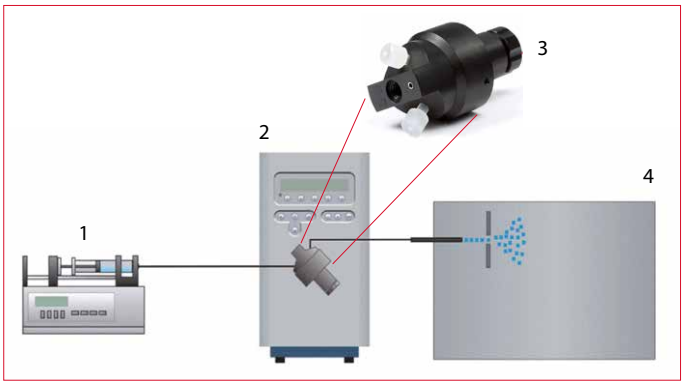


Figure 1: Schematics instrumental setup for online EC-MS: Syringe pump (1), ROXY Potentiostat (2), ReactorCell (zoom)(3), Mass Spectrometer (4).

Results

In Figure 2 the effect of the used electrode type is depicted for Rivaroxaban (RIV). The MS spectra clearly indicate that only the use of boron-doped diamond (Magic Diamond = MD) as electrode material results in oxidation of the drug compound. Platinum (Pt) as well as Glassy Carbon (GC) do not work. Same result was found for Aliskiren (ALS), and Prasugrel (PRS).

Electrochemical conversion and optimization

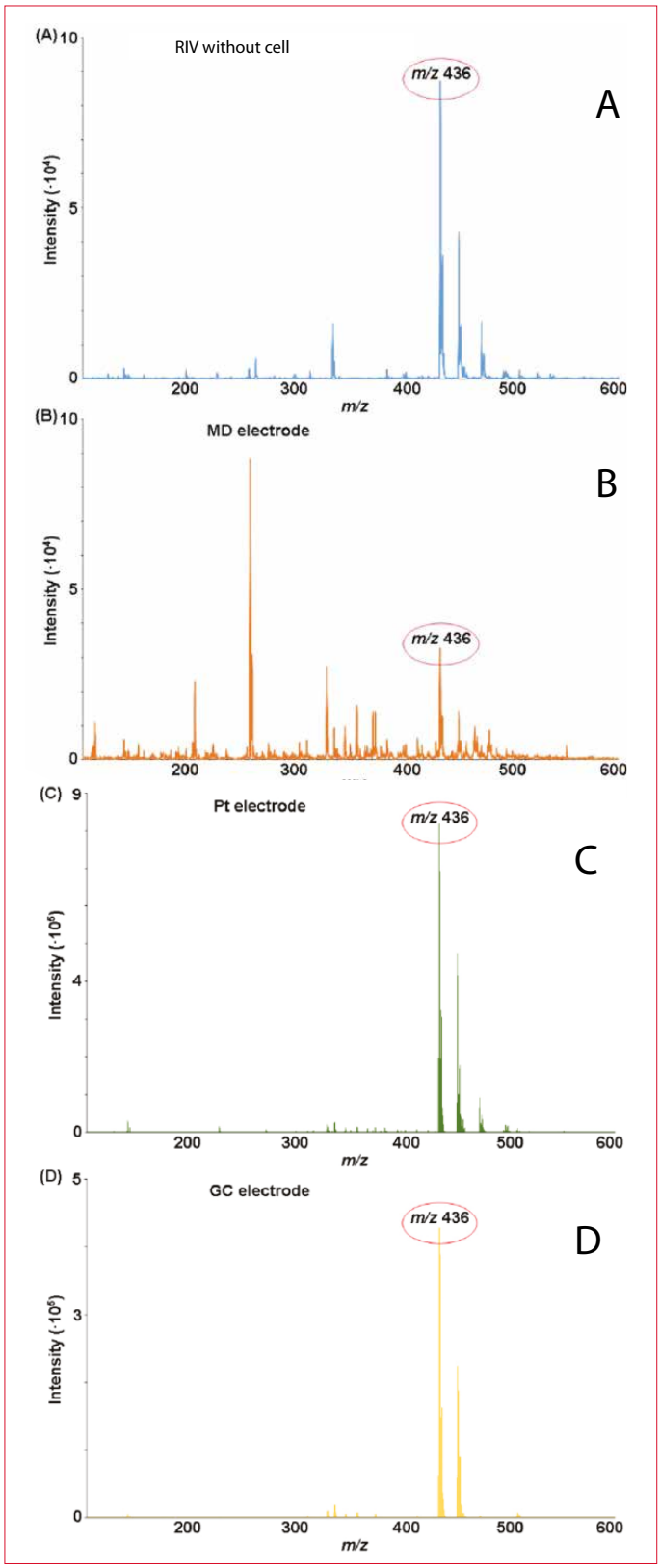


Figure 2: Effect of the working electrode on electrochemical conversion of Rivaroxaban (RIV). MS spectra for: RIV without cell (m/z 436) (A), MD electrode (B), Pt Electrode (C) and GC electrode (D).

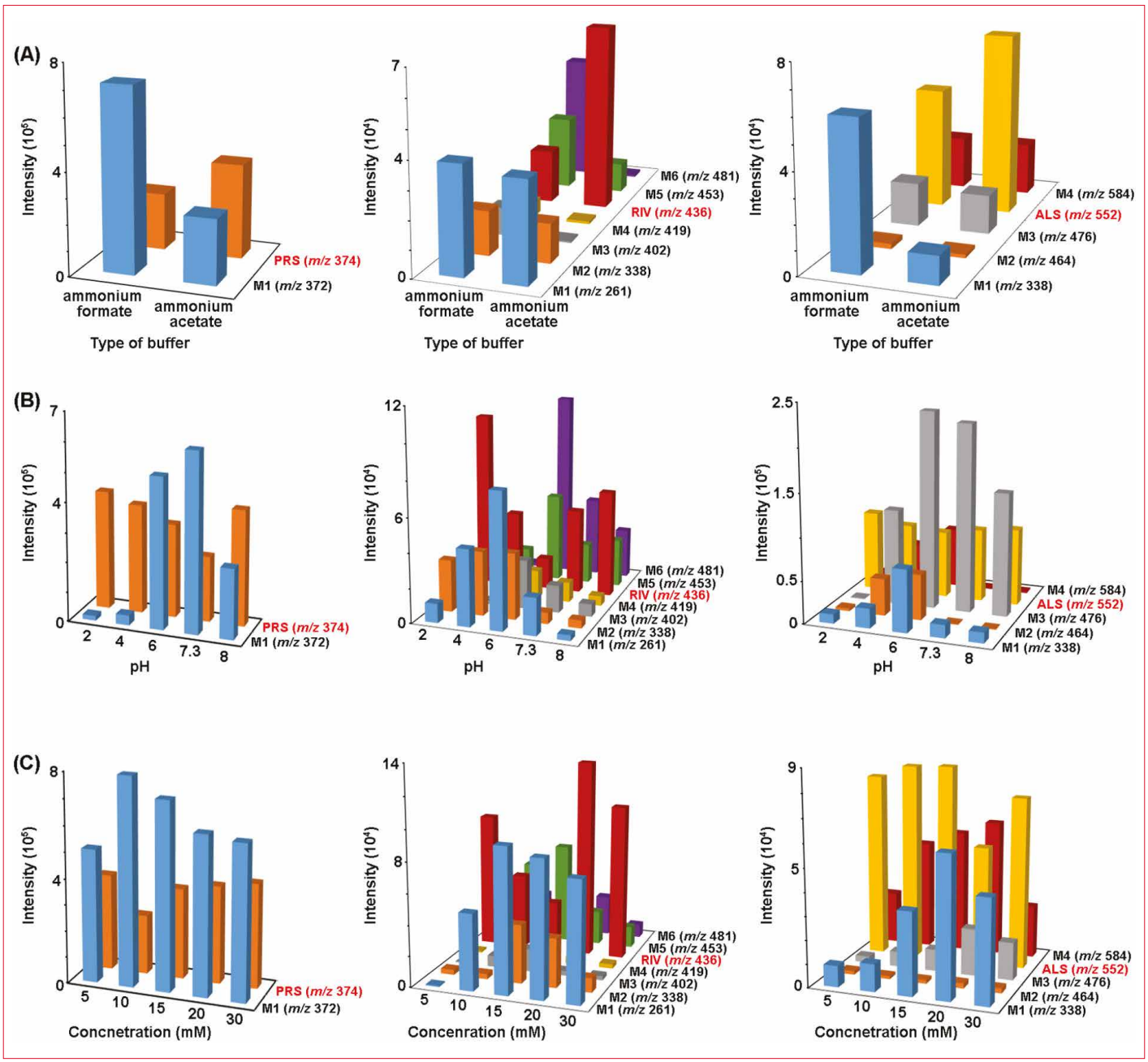


Figure 3: Effect of the mobile phase on the signal intensity of target compounds (PRS, RIV, ALS) and their electrochemically generated derivatives/metabolites (A) type of buffer, (B) pH of ammonium formate, and (C) concentration of ammonium formate. Optimal conditions: 20mM ammonium formate adjusted to pH 7.4

In Figure 3 the results from the optimization experiments are graphically presented. For all 3 cardiovascular drugs the use of 20mM ammonium formate buffer adjusted to pH 7.4 in combination with a boron-doped diamond working electrode resulted in highest conversion into the metabolites.

Electrochemical oxidation and identification of metabolites

The electrochemical oxidation behavior of the 3 drugs was studied by using the on-line EC/ECI-MS setup. A mass voltammogram was recorded using a continuous voltage ramp from 0 to 3000 mV. The mass voltammogram for RIV is depicted in Figure 4. For ALS and PRS, see reference [1].

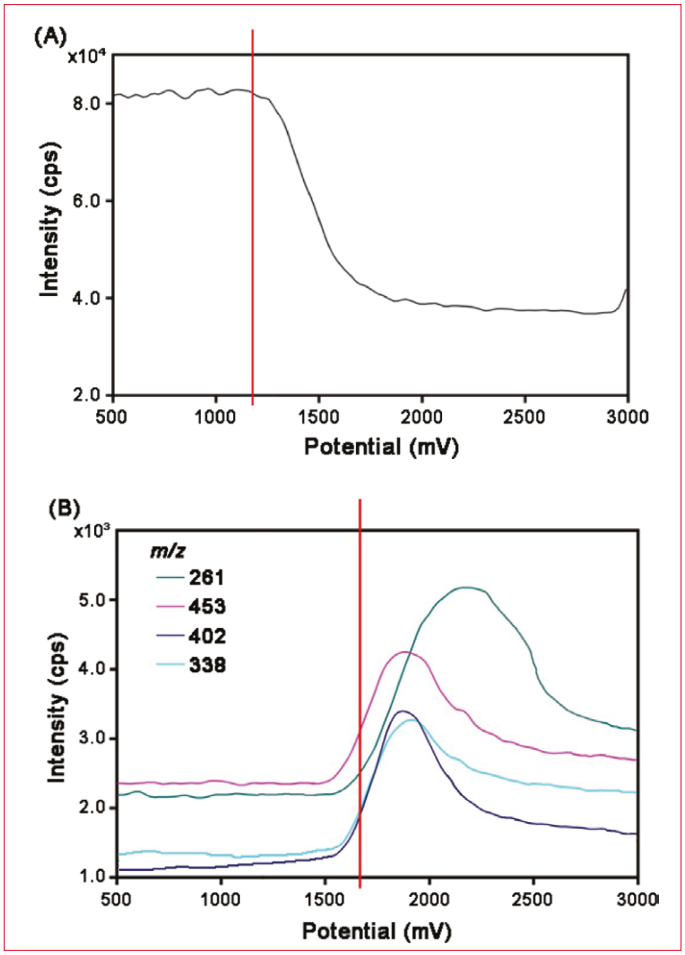


Figure 4: (A) Mass voltammogram of RIV. Oxidation of RIV starts at a potential of approximately 1200 mV, evident by the decline of the signal intensity. (B) Main oxidation product ions are formed at voltages > 1600 mV, with m/z 261, 338, 402 and 453. Working electrode: Magic Diamond (BDD); mobile phase: 20mM ammonium formate ((pH=7.4 ± 0.6):acetonitrile (50:50; v/v); flow rate of mobile phase: 8 µL/min. ROXY Potentiostat equipped with ReactorCell (Antec Scientific). MS: Agilent 6410 quadrupole ESI-MS (Agilent,Germany), in positive ionization mode (ESI+).

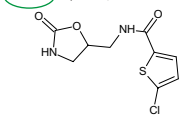
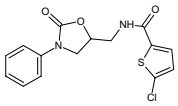
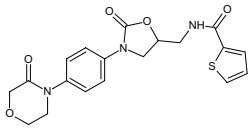
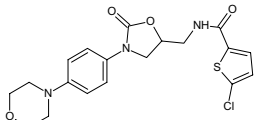
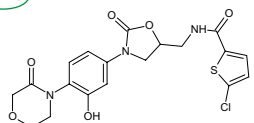
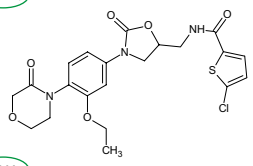
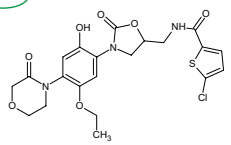
Product mass/proposed chemical structure	Intensity	Potential metabolic reaction	Proposed by
RIV $[M+H]^+ = 436$ *Electrode*: MD *Mobile phase: A:* 20 mM ammonium formate (pH = 6.0 ± 0.6); B: 50% acetonitrile *Flow rate:* 8 µL/min			
RIV-M1 m/z = 261 	Major	$C_{10}H_{10}NO$ loss	*On-line* EC/ESI-MS *O -line* EC UHPLC-ESI-MS
RIV-M2 	Minor	$C_4H_6NO_2$ loss	*On-line* EC/ESI-MS *O -line* EC
RIV-M3 	Medium	Cl loss	*On-line* EC/ESI-MS *O -line* EC
RIV-M4 	Minor	O loss	*On-line* EC/ESI-MS *O -line* EC
RIV-M5 	Major	OH gain	*On-line* EC/ESI-MS *O -line* EC UHPLC-ESI-MS
RIV-M6 	Minor	OC_2H_5 gain	*On-line* EC/ESI-MS UHPLC-ESI-MS
RIV-M7 	Minor	OH gain + OC_2H_5 gain	*On-line* EC/ESI-MS UHPLC-ESI-MS

Table 1: Proposed structure of electrochemically generated Rivaroxaban (RIV) metabolites. Seven oxidation products with m/z 261, 338, 402, 419, 453, 481, and 498 were detected by EC–MS. The oxidation products encircled in green were later on confirmed as metabolites from in-vivo experiments. For structures of Aliskiren (ALS), and Prasugrel (PRS) metabolites, see ref. [1]

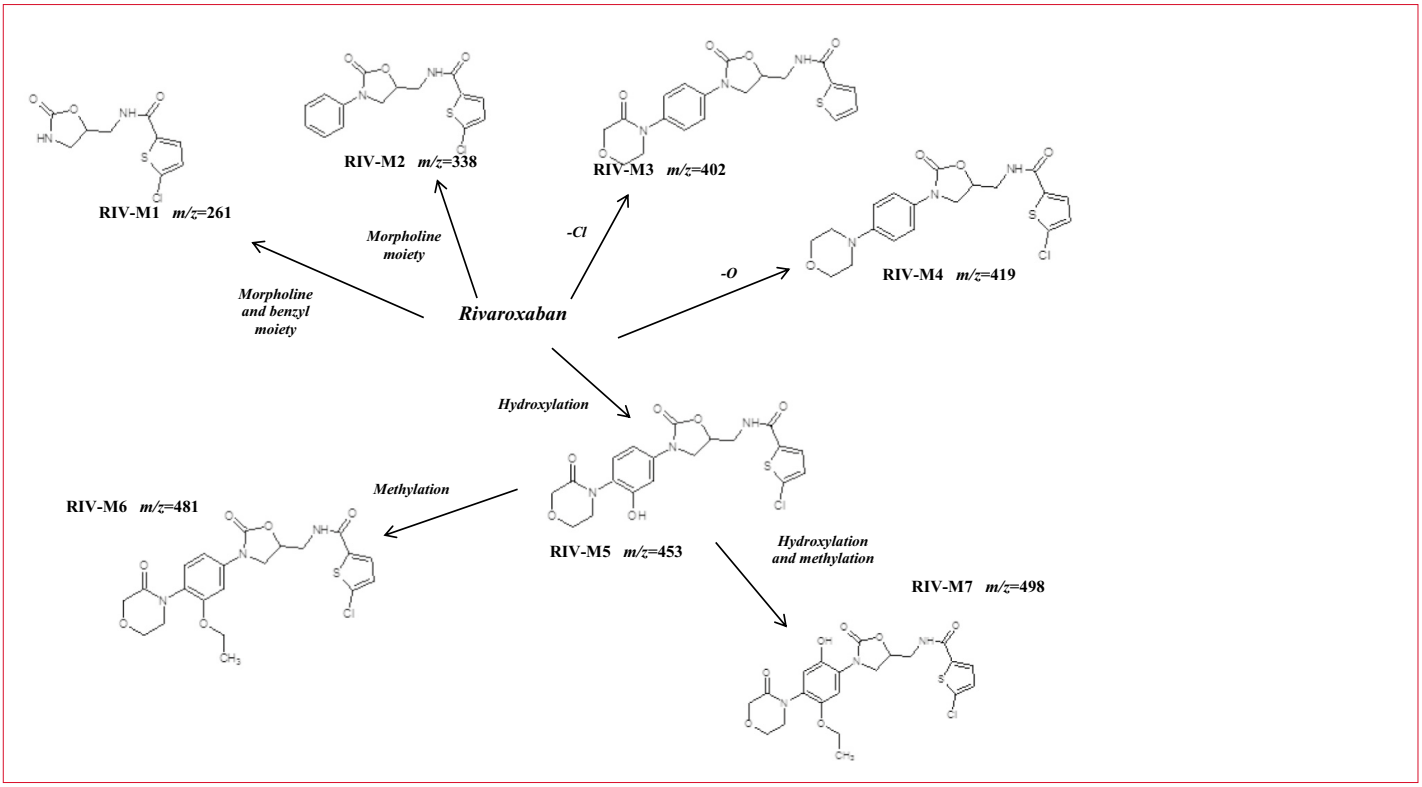


Figure 5: Proposed transformation pathway of Rivaroxaban (RIV)

Proposed transformation (metabolic) pathway

In Figure 5 the proposed metabolic pathway of RIV is shown. The electrochemical conversion of RIV includes the loss of 16 Da (O), 36 Da (Cl), 150 Da ($C_4H_6NO_2$, morpholine moiety), and 178 Da ($C_{10}H_{10}NO_2$, morpholine, and benzyl moiety). The main oxidation product, RIV-M5, is related to the hydroxylation reaction, with the formation of a hydroxyl metabolite and probably metabolism occurring at the morpholinone moiety. The formation of the electrochemical metabolite, RIV-M6, presumably involves methylation of the metabolite RIV-M5 and formation of the electrochemical metabolite, RIV-M7, presumably involves simultaneous hydroxylation and methylation of metabolite RIV-M5. For the proposed metabolic pathway of ALS and PRS, see reference [1].

Conclusion

In this study, EC–MS was applied as a purely analytical approach to evaluate the oxidation behavior of Rivaroxaban (RIV), Aliskiren (ALS), and Prasugrel (PRS). We found that:

On-line EC–MS allows to rapidly generate and identify the oxidation products of the three cardiovascular drugs.

Most metabolites of RIV, ALS and PRS found in-vivo (urine samples) could be generated electrochemically.

Electrochemistry has emerged as an interesting alternative to generate detect and predict the metabolic pathway in early drug development processes. It can rapidly mimic the main oxidative reactions (Phase 1) that occur in the human body, without biological interactions.

"in-electro" a real alternative to in-vivo and in-vitro drug metabolism.

Analysis of urine samples from patients

Urine samples obtained from patients treated with selected cardiovascular drugs were used for comparison with the proposed electrochemical simulation. After intake of RIV, ALS and PRS, the urine samples were analyzed by UHPLC–MS/MS and the presence of drug and metabolites were confirmed by MS spectra. In case of RIV the drug was detected in the urine samples at a concentration of 5000 ng/mL. The information gained by the analysis of the urine samples is summarized in Table 1. Four different metabolites of RIV were found in the urine (Fig. 6). Metabolite RIV-M1 was identified as the major metabolite. Metabolites RIV-M1 and RIV-M5 were found in almost equal amounts of 7 to 8% of the dose. Moreover, two other minor metabolites, RIV-M6 and RIV-M7, were observed in the urine samples. But, unchanged rivaroxaban was the major component in the urine. Approximately, 36% of the dose was excreted as unchanged drug in human urine.

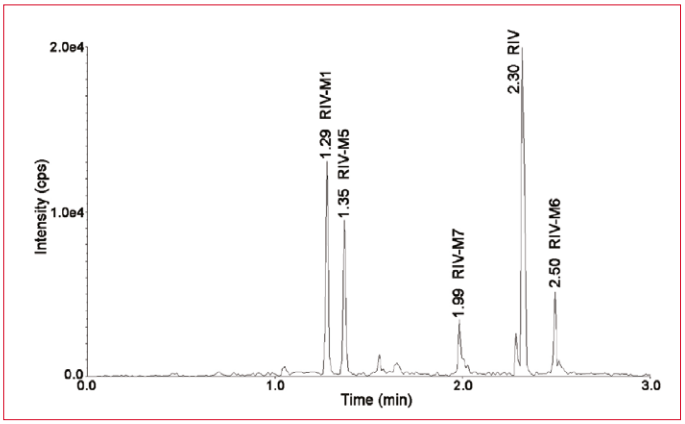


Figure 6: UHPLC-MS chromatogram of the extracted urine sample obtained from a patient after administration of RIV

Reference

1. Małgorzata Szultka-Młyńska; Journal of Chromatography B 1093–1094 (2018) 100–112

Figure 7: ROXY™ EC System consisting of ROXY Potentiostat, dual syringe pump and ReactorCell.

Ordering information

210.0070A	ROXY™ EC system, incl. dual syringe pump, ReactorCell, electrodes, LC connection kit for phase I and II reactions and Dialogue Elite software for system control. All parts included for described application.

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