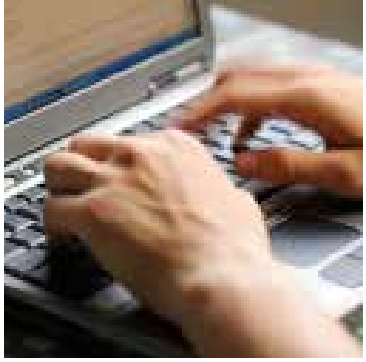
Application Note
Neuroscience



ALEXYS Analyzer for Highest Sensitivity in Neurotransmitter Analysis

Monoamines and Metabolites
Noradrenaline
Dopamine
Serotonin
5-hydroxyindole acetic-acid (5-HIAA)
3,4-dihydroxyphenylacetic-acid (DOPAC)
homovanillic acid (HVA)

OPA derivatized amines and amino acids
GABA and Glutamate
Histamine (LNAAs)
4-aminobutyrate (GABA)
Glutamate (Glu)
LNAAs

Choline and Acetylcholine
Choline (Ch)
Acetylcholine (ACh)

Markers for oxidative stress
3-nitro-L-Tyrosine
8-OH-DPAT

Glutathione and other thiols

ROXY™ EC System - Events Programming & Settings

- **Dialogue™ software for ROXY EC system**
- **Automated Recording of Mass Voltammograms**
- **Simulating Cytochrome P450 Oxidation using EC in combination with MS**
- **Phase I and II Oxidative Metabolism**

Introduction

The ROXY™ EC system is delivered with Dialogue™ software for ROXY (p/n 210.9005). The software package contains a set of event table files (*.evt) for the automated recording of mass voltammograms and for use in phase I and phase II metabolomics studies. The set of pre-programmed event tables files can be used as a starting point for your own research and can be easily modified to suit your specific needs. This appendix provides detailed background information about the supplied event table files and relevant Dialogue settings

Dialogue™ settings

The Dialogue software control both the ROXY EC System and the mass spectrometer (start data-acquisition), the latter by means of an analog trigger (contact closure, RELAY 1). The RS232 settings of both the DECADE II and syringe pump are configured in the Dialogue settings menu (figure 1).

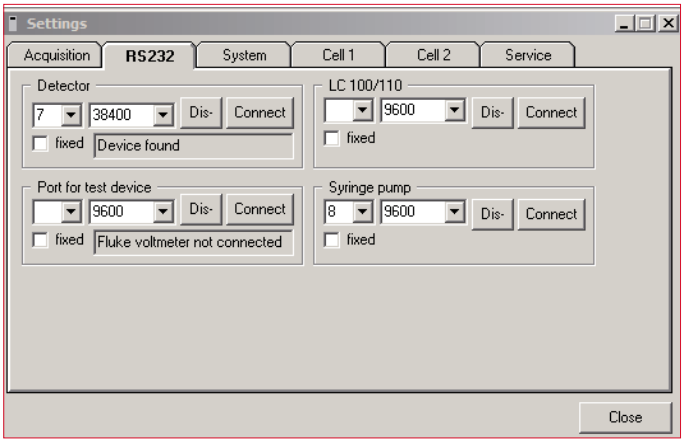


Figure 1: Dialogue settings window.

For all ReactorCells and devices (syringe infusion pump) separate settings tabs are available in the Dialogue window for parameteric control. In figure 3 the devices settings tab is shown as an example. In this window all relevant syringe pump settings can be configured such as syringe volume, syring diameter, flow rate and flow direction (infuse/withdraw). By means of the start/stop buttons the pump can be activated with the configured settings.

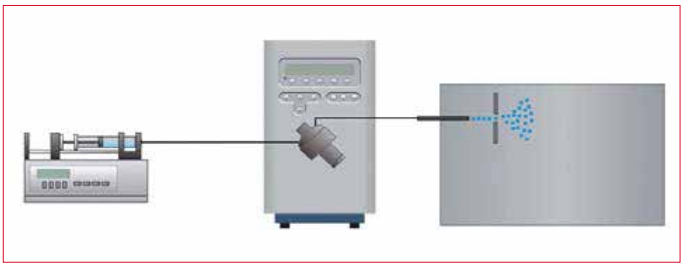


Figure 2: ROXY™ EC System including ReactorCell™ and dual syringe infusion pump.

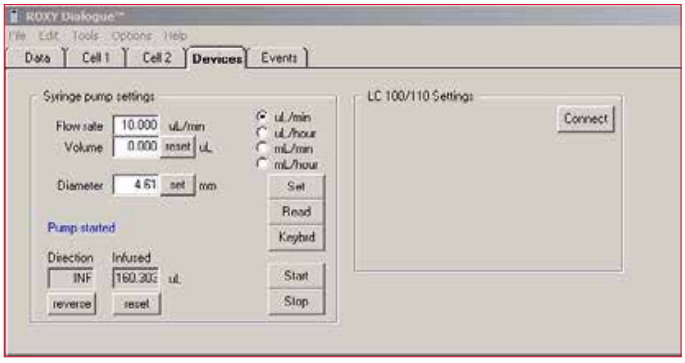


Figure 3: Dialogue syringe pump settings window.

The 'events' tab in Dialogue (figure 4) offers the possibility to compose an event table for the execution of an automated sequence of parametric control events. It is used for the automated recording of for instance a mass voltammogram with the ROXY EC system (see next paragraph). The event table consists of a series of data lines in which settings of the ROXY potentiostat or infusing pump can be changed with 0.01 min (0.6 s) time resolution.

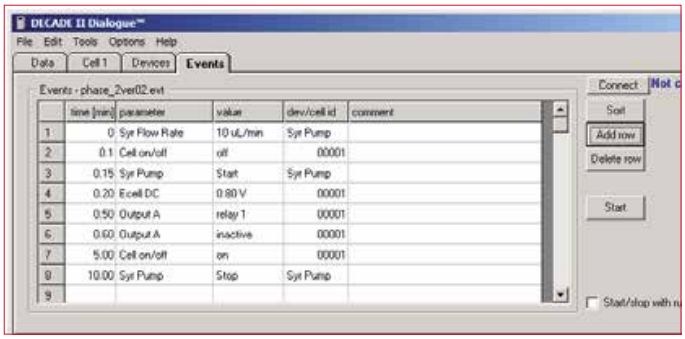


Figure 4: Screen shot of events table in Dialogue.

Mass voltammogram

A mass voltammogram visualizes the ion abundance versus m/z as a function of applied potential to the ReactorCell in a 3D plot.

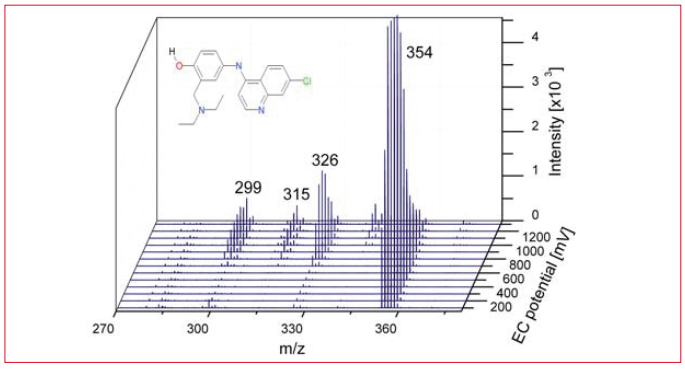


Figure 5: Example of a mass voltammogram of Amodiaquine. Ion abundance versus m/z as a function of EC potential.

Table 1

Dialogue event program for recording of a mass voltammogram. File name: voltammogram_standard_rev02.evt				
Step	**Time (min)**	**Parameter**	**Value**	**Device/ID***
01	0.00	Syr Flow Rate	10µL/min	Syr Pump
02	0.05	Syr Pump	Start	Syr Pump
03	0.45	Cell on/off	on	00001
04	0.50	Ecell DC	0.10V	00001
05	1.00	Ecell DC	0.20V	00001
06	1.50	Output A	relay 1	00001
07	1.55	Ecell DC	0.30V	00001
08	1.60	Output A	inactive	00001
09	2.00	Output A	relay 1	00001
10	2.05	Ecell DC	0.40V	00001
11	2.10	Output A	inactive	00001
12	2.50	Output A	relay 1	00001
13	2.55	Ecell tDC	0.50V	00001
14	2.60	Output A	inactive	00001
15	3.00	Output A	relay 1	00001
16	3.05	Ecell tDC	0.60V	00001
17	3.10	Output A	inactive	00001
18	3.50	Output A	relay 1	00001
19	3.55	Ecell tDC	0.70V	00001
20	3.60	Output A	inactive	00001
21	4.00	Output A	relay 1	00001
22	4.05	Ecell tDC	0.80V	00001
23	4.10	Output A	inactive	00001
24	4.50	Output A	relay 1	00001
25	4.55	Ecell tDC	0.90V	00001
26	4.60	Output A	inactive	00001
27	5.00	Output A	relay 1	00001
28	5.05	Ecell tDC	1.00V	00001
29	5.10	Output A	inactive	00001
30	5.50	Output A	relay 1	00001
31	5.55	Ecell tDC	1.10V	00001
32	5.60	Output A	inactive	00001
33	6.00	Output A	relay 1	00001
34	6.05	Ecell tDC	1.20V	00001
35	6.10	Output A	inactive	00001
36	6.50	Output A	relay 1	00001
37	6.60	Output A	inactive	00001
38	7.00	Output A	relay 1	00001
39	7.10	Output A	inactive	00001
40	7.50	Output A	relay 1	00001
41	7.60	Output A	inactive	00001
42	8.00	Syr Pump	Stop	Syr Pump
43	8.10	Cell on/off	off	00001
44	8.20	Ecell tDC	0.10V	00001

** ID is the sensor board / cell identifier. This is a five digit code identifying to which sensor board the programmed event will apply. 00001 activates board / cell 1, 00010 activates board / cell 2 and 00011 activates both board / cell 1 and 2, etc.*

In table 1 all steps of the Dialogue event program are listed for the acquisition of a mass voltammogram in the voltage range of 100 – 1200 mV with 100 mV steps (File: voltammogram_standard .evt).

The commands of the mass voltammogram event program are explained in table 2.

Table 2

Commands used in events table	
Parameter	**Description**
Syr Flow Rate	Setting the flow rate of the syringe pump
Syr Pump	Start/Stop of the syringe pump
Cell on/off	Turn on/off of the ReactorCell
Ecell DC	Applying voltage on the ReactorCell
Output A (relay1)	Starting run on the MS
Output A (inactive)	Switching relay off for next trigger signal

The first MS measurement is recorded with the ReactorCell off, subsequently the ReactorCell potential is ramped from 100 – 1200 mV with incremental steps of 100 mV every 0.5 minute. For each cell potential mass spectra are recorded for 0.45 minute and saved in separate data files (13 files in total).

The first acquisition of a mass spectrum start after 1.5 minute delay and will contain data for the ReactorCell off. The delay is calculated based on the following parameters (delay time > Vd/F):

- The dead volume Vd between ReactorCell and the ion source of the mass spectrometer. For p/n 180.0219 "ROXY outlet assembly 2, MS" this volume is 12.7 µL (1 meter red PEEK tubing ID 127µm, see reference 1)
- The flow rate F of the syringe infusion pump, in this case is 10 µL/min.

A slightly longer delay time is taken than based on the calculation to compensate for the tolerance in tubing inner diameter (affects volume) and dilution in the tubing (Poiseuille flow profile).

The start of the mass spectrometer run is triggered by means of RELAY 1. The run duration is set to 0.45 minutes in the mass spectrometer data-acquisition software. The steps 43 and 44 prepare the system for next measurement; in step 43 Reactor-Cell is turned off and in step 44 proper EC potential is applied (0.1V). The total time of the method is 8.2 min. The operation principle is shown graphically in fig 6.

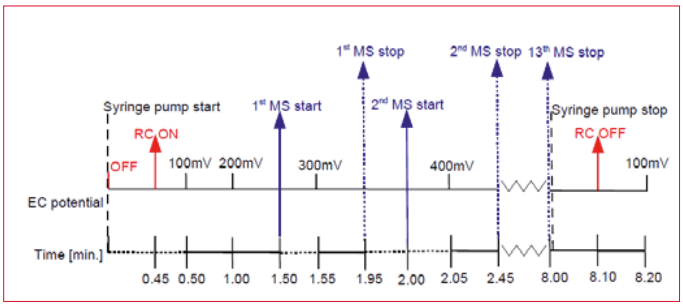


Figure 6: Operation principle of mass voltammogram acquisition. RC – ReactorCell; MS – mass spectrometer. Details see text.

In table 3 an event table is shown for a fast EC scan of target compounds. Voltage range: 100 – 1200 mV, with 100 mV steps (File name: voltammogram_fast_rev02.evt). The run time of the event program is 3.6 minutes and all mass spectra are acquired in one file. This fast measurement can be useful to make a quick estimation of the optimal working electrode potential for phase I and II metabolism.

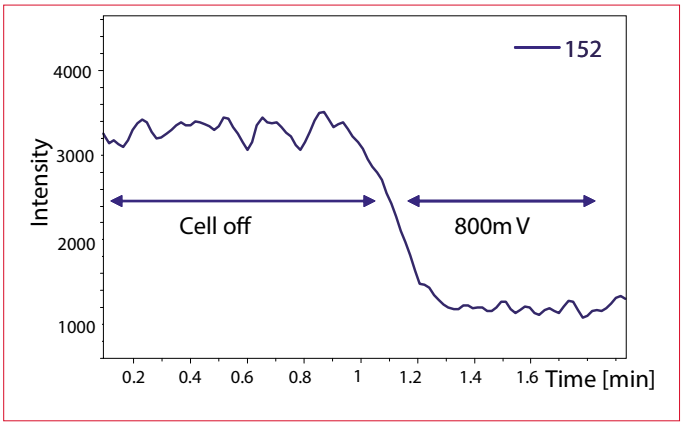


Figure 7 Extracted ion chromatogram of Acetaminophen.

Data can be presented as extracted ion chromatogram (figure 7) of desired m/z ratio value, in case of acetaminophen m/z of 152 should be traced.

Table 3

Dialogue event program for a 'fast' mass voltammogram.
File name: voltammogram_fast_rev02.evt

Step	Time (min)	Parameter	Value	Device/ID*
01	0.00	Syr Flow Rate	10µL/min	Syr Pump
02	0.05	Syr Pump	Start	Syr Pump
03	0.10	Cell on/off	off	00001
04	0.15	Output A	relay 1	00001
05	0.20	Output A	inactive	00001
06	0.50	Cell on/off	on	00001
07	0.75	Ecell DC	0.20	00001
08	1.00	Ecell DC	0.30	00001
09	1.25	Ecell DC	0.40	00001
10	1.50	Ecell DC	0.50	00001
11	1.75	Ecell DC	0.60	00001
12	2.00	Ecell DC	0.70	00001
13	2.25	Ecell DC	0.80	00001
14	2.50	Ecell DC	0.90	00001
15	2.75	Ecell DC	1.00	00001
16	3.00	Ecell DC	1.10	00001
17	3.25	Ecell DC	1.20	00001
18	3.50	Syr Pump	Stop	Syr Pump
19	3.55	Cell on/off	off	00001
20	3.60	Ecell DC	0.10	00001

The steps 19 and 20 prepare the system for next measurement; in step 19 ReactorCell is turned off and in step 20 the initial EC potential is applied (0.1V).

Oxidative Metabolism, Phase II

In phase II oxidative metabolomics studies the adduct formation of reactive metabolite with reagents like glutathione are analyzed. In table IV an example event program for the acquisition of a phase II experiment at an applied potential of 800 mV is listed. The program run time is 15.10 minutes in total. The acquisition of mass spectrum start after 5.0 minute delay and will contain data for ReactorCell off.

Table 4

Dialogue event program for phase II metabolism
File name: phase2_rev03.evt

Step	Time (min)	Parameter	Value	Device/ID*
01	0.00	Syr Flow Rate	10 µL/min	Syr Pump
02	0.10	Cell on/off	off	00001
03	0.15	Syr Pump	Start	Syr Pump
04	0.20	Ecell DC	0.80 V	00001
05	5.00	Output A	relay 1	00001
06	5.20	Output A	inactive	00001
07	5.30	Cell on/off	on	00001
08	15.00	Syr Pump	Stop	Syr Pump
09	15.10	Cell on/off	off	00001

The first 5 minutes the mass spectra are acquired with the ReactorCell turned OFF. Subsequently, for 5 minutes the mass spectra are collected with the ReactorCell at an applied potential of 800mV. All mass spectra are stored in one file.

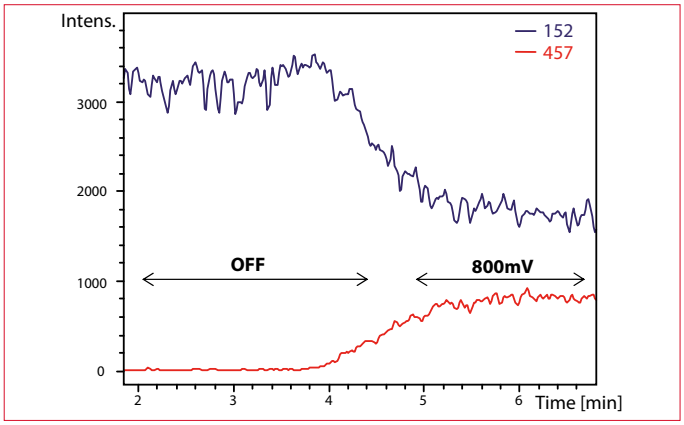


Figure 8 Example of phase II metabolism experiment. The blue line represents extracted ion chromatogram (EIC) of acetaminophen, and the red line the EIC of the NAPQI-GSH adduct (acetaminophen metabolite conjugated with glutathione).

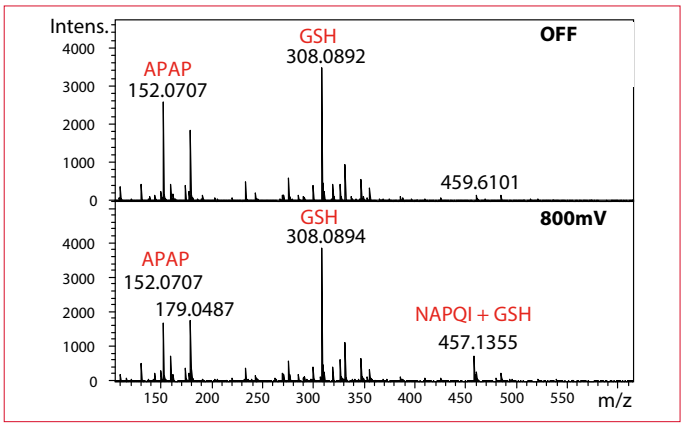


Figure 9 Mass spectra acquired for ReactorCell OFF (t = 3 min after start MS acquisition) and EC=800mV (t=7 min), respectively. The mass spectra are extracted from the experiment showed in figure 8.

Additional programs

Dialogue contains two additional event tables. The first event table, voltammogram_standard_one_file_rev01.evt, is based on the program for the acquisition of a mass voltammogram in the voltage range of 100 – 1200 mV with 100 mV steps (See Table 1). But in the event table listed in table 5 all data will be saved in one MS file.

This fast measurement can be useful to make a quick estimation of the optimal working electrode potential for phase I and II metabolism. The EIC of desired m/z ratio can be plotted versus potential. This method is optimized for use with Glassy Carbon electrode, but it can be easily adjusted for Magic Diamond when a higher voltage range is required.

Table 5

Dialogue event program for recording of a mass voltammograms with data stored in one MS file.
File name: voltammogram_standard_one_file_rev01.evt

Step	Time (min)	Parameter	Value	Device/ID*
01	Syr Pump	Start	Syr Pump	Syr Pump
02	0.10	Cell on/off	off	00001
03	0.15	Ecell DC	0.10 V	00001
04	0.45	Cell on/off	on	00001
05	0.5	Ecell DC	0.10	00001
06	1	Ecell DC	0.20	00001
07	1.5	Output A	relay 1	00001
08	1.55	Ecell DC	0.30	00001
09	1.6	Output A	inactive	00001
10	2.05	Ecell DC	0.40	00001
11	2.55	Ecell DC	0.50	00001
12	3.05	Ecell DC	0.60	00001
13	3.55	Ecell DC	0.70	00001
14	4.05	Ecell DC	0.80	00001
15	4.55	Ecell DC	0.90	00001
16	5.05	Ecell DC	1.00	00001
17	5.55	Ecell DC	1.10	00001
18	6.05	Ecell DC	1.20	00001
19	8.00	Cell on/off	off	00001
20	8.10	Ecell DC	0,10	00001
21	8.30	Syr Pump	Stop	Syr Pump

Conclusion

Dialogue software offers flexibility in programming the ROXY Potentiostat and external devices for automated operation. It is a convenient tool for programming mass voltammograms, and triggering the MS data acquisition.

The event table, phase1_OFF_ON_1potential_rev01.evt (Table 6), can be used when a simple 'OFF- ON' experiment is required. At first the spectra with ReactorCell OFF will be acquired and subsequently the ReactorCell will be switched ON using the potential specified in the event table. This potential will be applied till the end of the measurement. The program is similar to the method named phase2rev03.evt but in this case a shorter delay is programmed (no mixing coil taken into account). This method is universal and can be adjusted with respect to potential and analysis time.

Table 6

Dialogue event program for a control measurement with ReactorCell OFF and ON at constant potential.
File name: phase1_OFF_ON_1potential_rev01.evt

Step	Time (min)	Parameter	Value	Device/ID*
01	0.00	Cell on/off	off	00001
02	0.10	Syr Flow Rate	10 uL/min	Syr Pump
03	0.15	Syr Pump	Start	Syr Pump
04	0.20	Ecell DC	0.80 V	00001
05	0.40	Cell on/off	on	00001
06	0.50	Output A	relay 1	00001
07	0.60	Output A	inactive	00001
08	10.00	Syr Pump	Stop	Syr Pump
09	10.10	Cell on/off	off	00001

References

1. Antec, *"Schematic installation drawing of LC connections in ROXY EC system"*, part number 180.7161A

Figure 10: ROXY™ EC System.

Ordering number

210.0070	ROXY™ EC System

For research purpose only. The information shown in this communication is solely to demonstrate the applicability of the ROXY system. The actual performance may be affected by factors beyond Antec's control. Specifications mentioned in this application note are subject to change without further notice.

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