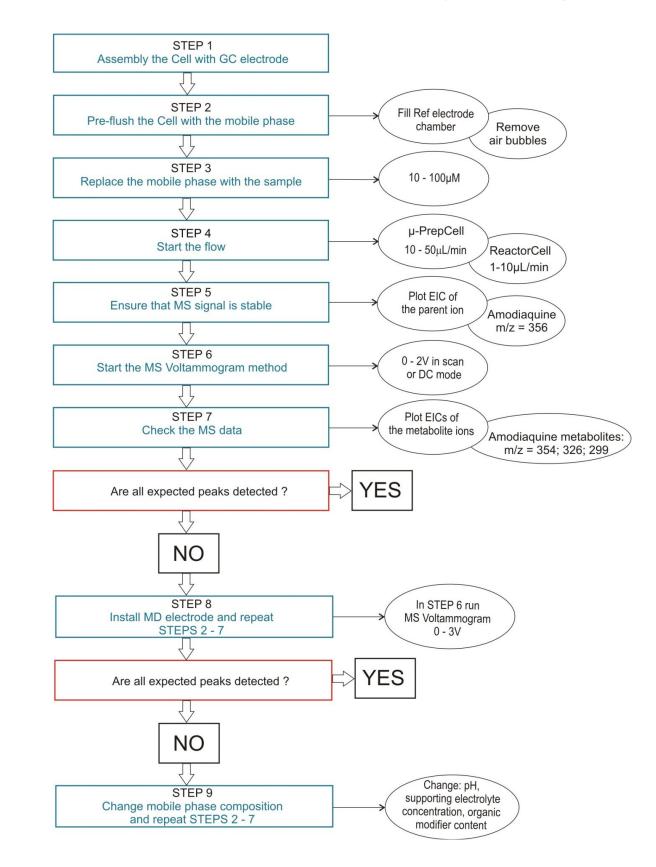
## Antec®

## ROXY™ EC system DEMO guide





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## ROXY<sup>™</sup> EC system DEMO guide

This DEMO Guide card covers the most important aspects about the performance of the ROXY EC system DEMO. It is recommended to read the  $\mu$ -PrepCell or ReactorCell user manual before performing the experiments. <u>Note:</u> this guide does not replace the  $\mu$ -PrepCell user manual (p/n 204.0010) or ReactorCell user manual (p/n 210.7014).

**Step 1 – Assembling the cell with GC electrode**: GC electrode and mount the cell as described in the corresponding manuals.  $\mu$ -PrepCell: Do not forget to insert the metal spacers (at least thickness of 150  $\mu$ m) and O-ring. The WE electrode contact pin should be screwed in completely (be aware of locking mechanism) to assure proper contact. It is recommended to check if none of the electrodes are short-circuited using a voltmeter.

**Step 2 – Pre-flushing the Cell with the mobile phase**: For the analysis of Amodiaquine the recommended mobile phase is 20mM ammonium formate (pH 7.4 adjusted with ammonium hydroxide) in 50% acetonitrile. Prime the cell with mobile phase and ensure that the reference electrode reservoir is filled and air bubble-free.

**Step 3 – Replace the mobile phase with the sample**: For system check/DEMO the suggested test compound is 10µM Amodiaquine in 20mM ammonium formate (pH 7.4 adjusted with ammonium hydroxide) in 50% acetonitrile.

**Step 4 – Optimal flow rate**: The recommended flow rate for analysis of Amodiaquine in the  $\mu$ -PrepCell is 50  $\mu$ L/min. For ReactorCell the recommended flow rate is 10  $\mu$ L/min.

**Step 5 – Check the MS signal:** It is recommended to plot EIC trace of the parent ion of Amodiaquine at m/z of 356 to ensure that MS signal is stable and optimize it if necessary.

**Step 6 – Acquiring of the MS Voltammogram**: To record of MS Voltammogram select the DC or scan mode. MS Voltammogram will give you a good indication of the optimal potential required for the formation of specific metabolites. Verify that the potential determined with a MS Voltammogram is indeed the optimal potential by executing a direct measurement at that specific potential before starting your metabolite synthesis. In the ROXY<sup>TM</sup> potentiostat the DC and Scan mode are available for efficient metabolite synthesis. The <u>DC mode</u> is based on applying a static (single) potential during the whole conversion process. Note that the synthesis of different metabolites of one compound may require operation at different potential settings. In the <u>Scan mode</u> stabile oxidation conditions are obtained by continuous scanning between two preset potentials values (E1 and E2) with a certain scan rate (unit: mV/s). More details about using the DC and Scan mode can be found in the User manual.

**Step 7 – Checking the results**: Check the registered MS data and plot EIC traces of Amodiaquine metabolites at m/z of 299; 326 and 354. For other compound trace the EICs of the relevant peaks. It can be helpful to compare the spectra with conditions when no potential is applied.

**Step 8 – Exchange the electrode:** Samples can show electrode-dependent oxidation patterns and it is recommended to test samples with both the MD and GC electrode. The MD electrode is more inert (less absorption) and has a wider working potential range for electrochemical oxidation in aqueous solution.

**Step 9 – Change the mobile phase composition:** It can be necessary to adjust the mobile phase composition to optimize conversion. The ReactorCell or  $\mu$ -PrepCell require the use of an electrolyte at concentrations of 10 mM or <u>higher</u> (e.g. ammonium formate, ammonium acetate) in the mobile phase. The supporting electrolyte will assure the stable working conditions and conversion. Alternatively, formic acid at concentration 0.1 - 1 % can be used as supporting electrolyte. In many cases pH of 7.4 can be used, but change pH to lower value, especially when MD electrode is used could increase the yield in metabolite formation.

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