Electrochemically-Modulated Preconcentration and Cleanup On-Line with ES-MS

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The use of electrochemically-modulated preconcentration and cleanup combined on-line with electrospray mass spectrometry (EMPC/ES-MS) in the analysis of organics is demonstrated. This sample handling approach is similar to the electrochemical technique termed adsorptive stripping analysis [1] in that retention of the targeted analytes at the working electrode of an electrochemical flow cell involves a non-electrolytic adsorption process that is controlled through the proper combination of the solvent system, the working electrode material, and the applied potential. The studies described here concentrate specifically on the analysis of the pharmaceutically important drug tamoxifen and a metabolite 4-hydroxytamoxifen using a glassy carbon working electrode within a thin-layer, flow-by electrode cell for preconcentration [2]. Electrode treatment, solvent systems, and applied potentials necessary to accumulate and strip tamoxifen and 4-hydroxytamoxifen were investigated and optimized. Detection limits (DLs) using a 5.0 min preconcentration period with selected reaction monitoring for tamoxifen (m/z 372 → 72) were bracketed as 0.010 nM < DL< 0.025 nM. These detection levels are at least 10 times better than those levels reported for pristine solutions using potentiometric adsorptive stripping analysis [3]. The ability to simultaneously detect low nanomolar levels of both tamoxifen and 4-hydroxytamoxifen in pristine solution and 1/10 diluted urine was also demonstrated.

All experiments were performed on either a PE SCIEX API165 single quadrupole or PE SCIEX API365 triple quadrupole mass spectrometer using a "TurboIonSpray" source. The thin-layer electrochemical cell used in this work has been described in detail elsewhere [2]. *Analytes:* tamoxifen; SIM *m/z* 372, SRM *m/z* 372→72 and 4-hydroxytamoxifen; SIM *m/z* 388, SRM *m/z* 388→72. Retention of these species is via adsorptive accumulation at the GC electrode of the cell involving hydrophobic, π-π, and electrostatic interactions *Solvents:* The analytes have limited solubility in H_2O. A solvent comprised of 50% CH_3OH/50% H_2O preserves hydrophobic electrode interaction, and provides rapid elution and adequate ES-MS signal. *Electrolytes:* 5.0 mM NH_4OAc/0.75%(v/v) HOAc (pH ≈ 4) provided the necessary conductivity, minimal signal suppression, and protonated the tertiary amine of each analyte facilitating detection.

Figure 1 shows, for an optimized experiment, the plots of mass spectrometer response for the protonated molecule of tamoxifen (*m/z* 372) recorded in SIM mode during replicate experiments using 25 nM tamoxifen deposited from and stripped into a solution composed of H_2O/CH_3OH (1/1) containing 5.0 mM NH_4OAc and 0.75% (v/v) HOAc. The plot in (a) is an expansion of the first replicate in (b) illustrating the timing sequence of the experiment and the various figures of merit used to define the performance of the system. Estimated recovery was ≈ 38%. The plot in (b) is the *m/z* 372 signals recorded for three replicate experiments demonstrating the rate of sample throughput (~10 min/sample) and the reproducibility (2.6% RSD) of the stripping peak heights for 2.5 min deposition and 2.5 min washout times. Note that only ~ 150 µL of sample was consumed per analysis.

The plots in Figure 2 show the blank subtracted relative stripping peak heights for tamoxifen (●) and 4-hydroxytamoxifen (▼) in 1/10 diluted urine at concentrations of 5, 10 and 20 nM acquired in MRM mode (deposit 3.0 min at -1.4 V, wash 3.25 min at -1.25 V, strip = 0 V). The solid line curves were obtained using least squares fits of the data to eq 4 below. Error bars represent ± 1 std dev. for 4 replicate analyses. Note that the nonlinearity exhibited by the EMPC/ES-MS stripping peak analytical curves in Figure 2 is precisely that expected! The MS stripping peak ion current plotted, i_{MS} is related to the quantity of analyte on and liberated from the electrode surface during the stripping process, *N*, by eq 1. The proportionality constant

FIGURE 1
EC/ES-MS SIM Detection of 25 nM Tamoxifen
50/50 (v/v) H_2O/CH_3OH, 5.0 mM NH_4OAc/0.75% v/v HOAc

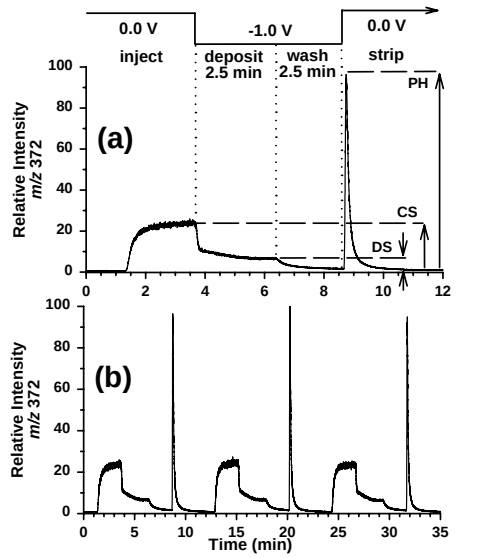
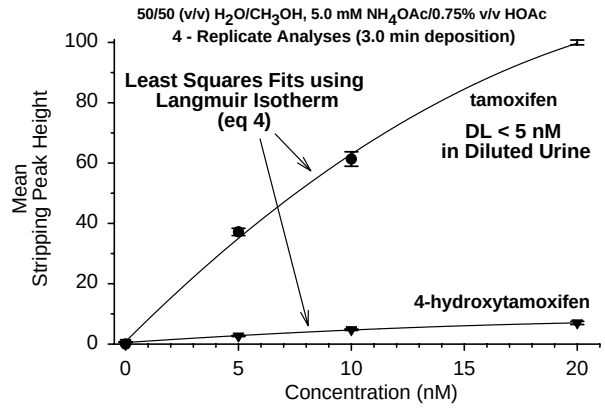


FIGURE 2
Simultaneous Detection of Tamoxifen and 4-Hydroxytamoxifen in 1/10 Diluted Urine Using MRM



K_{MS} incorporates mass spectral ionization efficiency, ion sampling effects, etc. Moreover, N is equal to the product of the effective electrode area A and the electrode surface density of adsorbed analyte Γ (eq 2). Because analyte accumulation is via adsorption, Γ, in turn, can be related to analyte solution concentration C_A by the Langmuir isotherm given by eq 3, which assumes monolayer coverage of the electrode surface by the analyte. B_A is the surface adsorption coefficient of the analyte and Γ_M the maximum surface density for the adsorbing analyte species. Substitution for Γ in eq 2 then yields eq 4, to which the data in Figure 2 are fitted.

$$i_{MS} = K_{MS} \bullet N \quad (1)$$

$$i_{MS} = K_{MS} \bullet N \bullet A \bullet \Gamma \quad (2)$$

$$\Gamma = \Gamma_M \bullet \{B_A \bullet C_A / (1 + B_A \bullet C_A)\} \quad (3)$$

$$i_{MS} = K_{MS} \bullet N \bullet A \bullet \Gamma_M \bullet \{B_A \bullet C_A / (1 + B_A \bullet C_A)\} \quad (4)$$

The results presented here show that EMPC/ES-MS is a viable means to perform on-line preconcentration and cleanup of organic analytes. Analyte retention/elution from the "stationary phase" is controlled by changing the electrode potential rather than via a change in mobile phase composition. Detection levels of less than 5 nM tamoxifen in 1/10 diluted urine were possible using a 3.0 min preconcentration time. Only about 20 µL of urine was required per analysis (100-150 µL total sample volume). Cursory experiments not discussed here demonstrated that the same protocols could be applied to the analysis of the other triphenylethylene-based drugs clomifen and toremifen as well as the phenothiazine-based drugs promethazine and chlorpromazine.

[1] Wang, J., In *Electroanalytical Chemistry*; Bard , A. J., Ed.; Marcel Dekker: New York, 1989; Vol 16, pp. 1-88. [2] Pretty, J. R; Van Berkel, G. J., *Rapid Commun. Mass Spectrom.* **1998**, *12*, 1644-1652. [3] Wang, J., et al., *Talanta* **1997**, *45*, 273-278.

J.R.P. and H. D. acknowledge support through an appointment to the Oak Ridge National Laboratory (ORNL) Postdoctoral Research Associates Program administered jointly by the Oak Ridge Institute for Science and Education and ORNL. Doug Goeringer (ORNL) is thanked for help with the Langmuir isotherm analysis. The ES-MS instrumentation used in the work was provided thorough a Cooperative Research and Development Agreement with PE SCIEX (CRADA No. ORNL96-0458). This research was sponsored by the National Cancer Institute under Interagency Agreements DOE 0485-F053-A1 and NCI Y1-CB-0016-01. ORNL is managed by Lockheed Martin Energy Research Corporation. Inc.