Supplementary Information for

A Microfluidic Technique for Quantification of Steroids in Core Needle Biopsies

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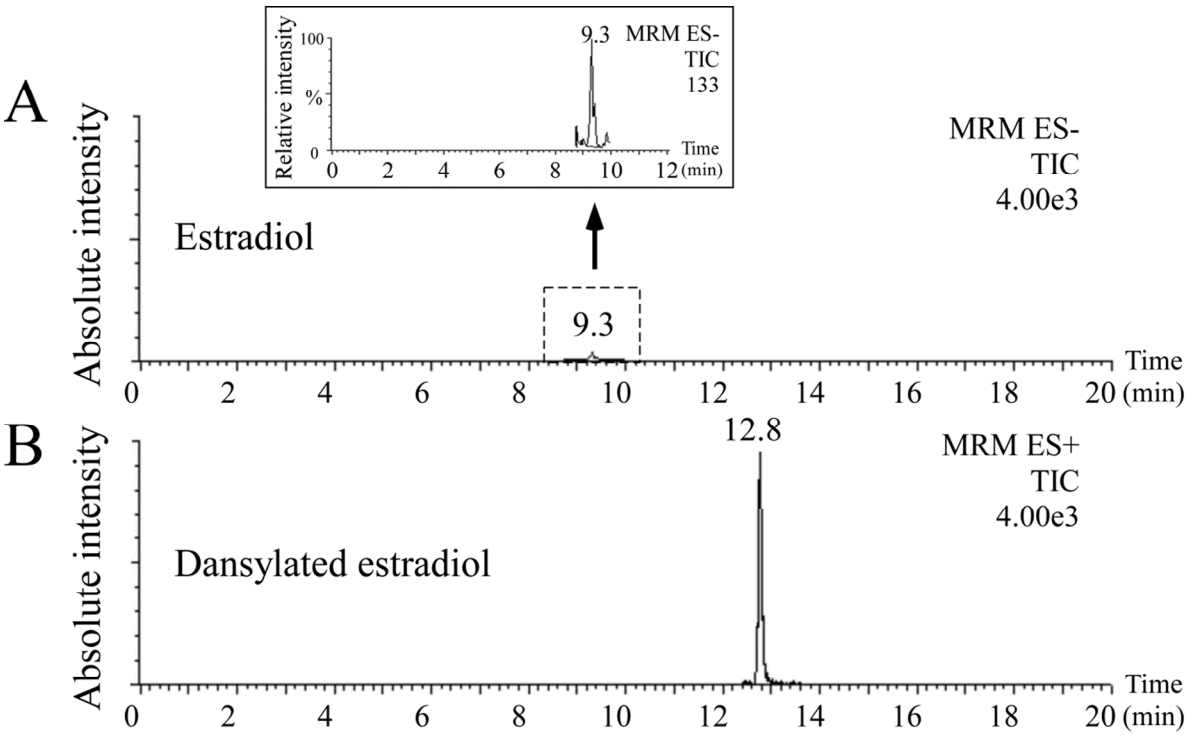

Figure S1. Sensitivity improvement for measurement of estradiol by derivatization with dansyl chloride. (The LC-MS/MS conditions were optimized for the detection of estradiol with or without derivatization, respectively.) (A) Multiple reaction monitoring (MRM) chromatogram of the 271/145 MRM transition for estradiol (20 pg/mL) in negative ion mode (the inset is a magnified reproduction of the peak). (B) MRM chromatogram of the 506/171 MRM transition for dansylated estradiol (20 pg/mL) in positive ion mode.

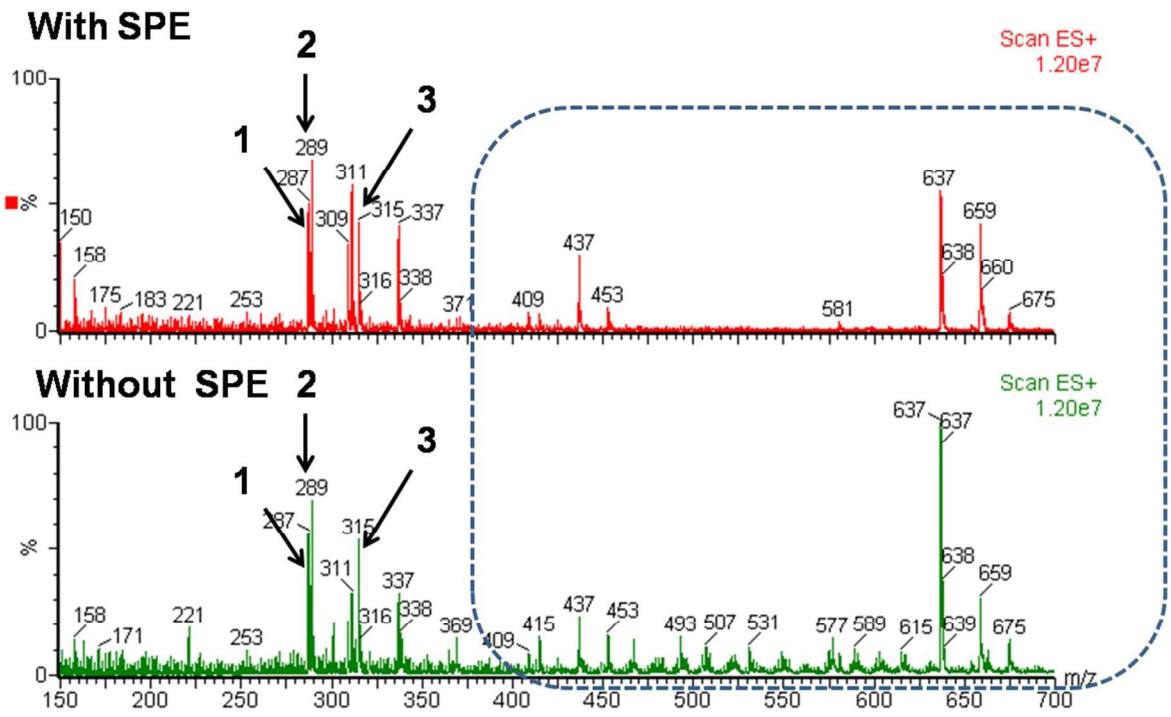

Figure S2.Sample cleanup. Rat tissue samples were spiked with 1 μg/mL AD, TS and PG. Representative direct infusion MS spectra of rat tissue samples extracted by DMF after (above, red) or before (below, green) an additional on-chip cleanup by solid phase extraction (SPE). Analyte peaks are labeled as 1 (AD), 2 (TS), and 3 (PG), and their peak heights changed only modestly after SPE cleanup. In contrast, the peak intensities of a series of unidentified interferants (dotted blue box, m/z 400-700) are significantly reduced after SPE cleanup.

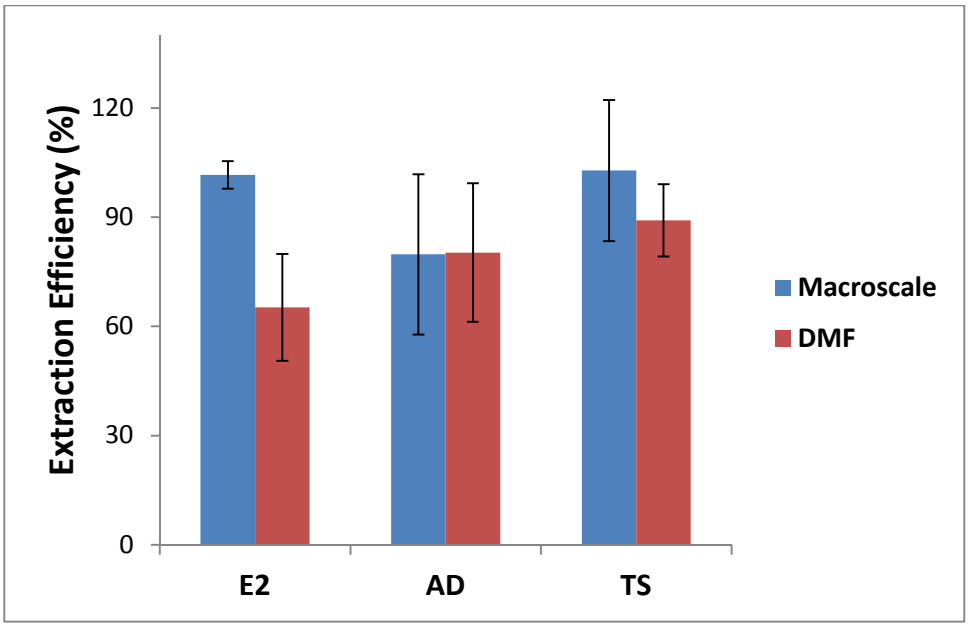

Figure S3. Extraction efficiency. The analyte recoveries for E2, AD, and TS in spiked rat tissue were determined using the DMF technique (red) and a conventional macro-scale method (blue). Each sample (~100 mg for macroscale and ~5 mg for DMF) was spiked with a mixture of exogenous E2, AD, and TS prior to extraction. The concentration and volume of the spiking solutions (2.5 µL/100 ng/mL standards for DMF and 20 µL/100 ng/mL standards for macro-scale) were selected such that perfect extraction would yield a 5 ng/mL concentration of each analyte in the final (extracted, reconstituted) solutions that were analyzed. Analyte concentrations in non-spiked tissue samples were measured and the average values were subtracted from those determined from spiked samples. These values were expressed as percent recoveries relative to spiked amounts. Error bars represent ± 1 S.D. for averages obtained from three independent tissue samples.

Table S1. HPLC-MS/MS with multiple reaction ion monitoring (MRM) conditions for eight deuterated and non-deuterated hormones.

Analyte	Retention time window (min)	MRM transition	Cone voltage (V)	Collision energy (eV)
AD	8.5-10.2	287/97	30	20
		287/107	30	20
ADd7	8.5-10.2	294/100	30	20
		294/113	30	20
TS	8.9-10.3	289/97	35	25
		289/109	35	25
TSd3	8.9-10.3	292/97	35	25
		292/109	35	25
PG	9.75-11.25	315/97	30	25
		315/109	30	25
PGd9	9.75-11.25	324/100	30	25
		324/113	30	25
Dansylated E2	12.2-13.6	506/171	50	35
Dansylated E2d3	12.2-13.6	509/171	50	35

Table S2. Calibration data for four steroid hormones.

Analyte	Slope	Intercept	R^2	Intraday CV (%)	Interday CV (%)	LOD (fmol)
DansylatedE2	0.0153	0.113	0.9997	1.3	1.6	3.6
AD	0.0156	0.086	0.9967	7.5	5.0	1.6
TS	0.0137	0.018	0.9996	4.2	7.5	5.8
PG	0.0305	0.116	0.9988	5.3	6.0	8.5