TRANSFORMING PROMISING IDEAS INTO COMMERCIAL REALITY

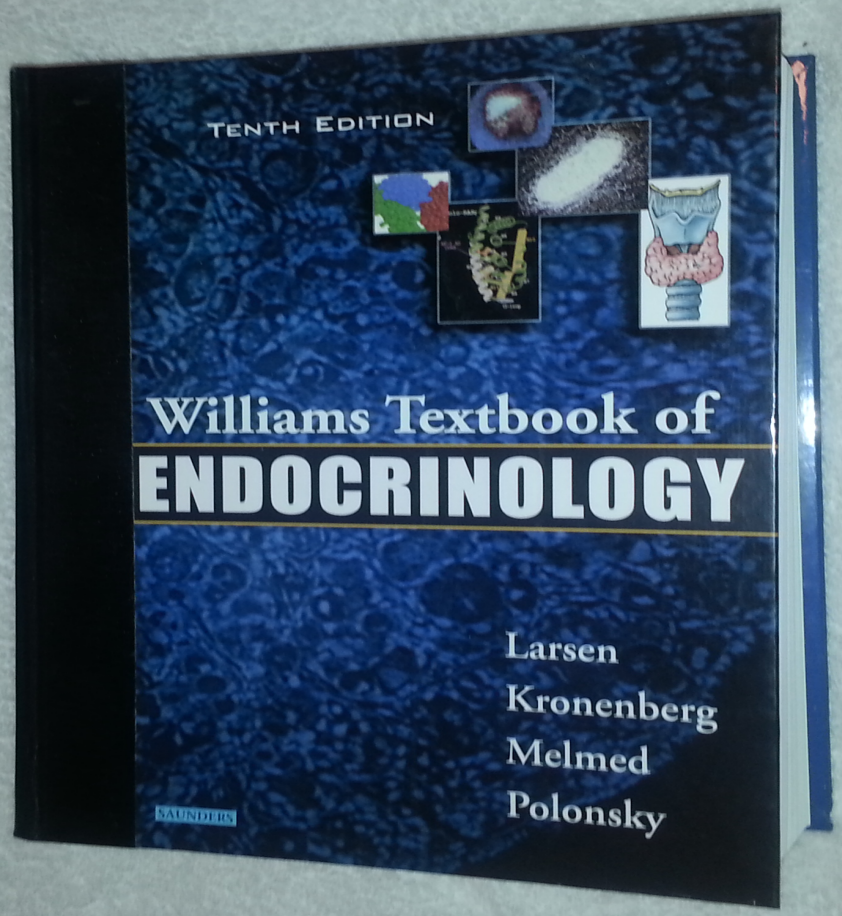
Using Supercritical Fluid Chromatography coupled with Tandem Mass Spectrometry to Provide Easier Solutions to Old Problems and New Solutions to Previously Unsolved Problems

By Jim Settlage

Senior Scientist, Princeton Lab

inVentiv Health clinical

Textbook od Endocrinology



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$$\text{mmol/L} = \frac{\text{mg/dL} \times 10}{\text{atomic or molecular weight}} \quad \text{or} \quad \text{mg/dL} = \frac{\text{mmol/L} \times \text{atomic or molecular weight}}{10}$$

Conversion of mEq/L to mmol/L is made by dividing mEq/L by the valence of the molecule. For the convenience of the reader, factors for converting conventional to SI units are included (see Young DS. Implementation of SI units for clinical laboratory data. Ann Intern Med 106:114–128, 1987). For the Units of Radiation, 1 bequerel (Bq) = 2.7×10^{-11} curies (Ci), 37 mBq = 1 mCi, 1 gray (Gy) = 100 rad, 1 sievert (Sv) = 100 rem.

Laboratory Parameter	SI	Conventional (C)	Conversion Factor (CF) CF × C = SI
Acetoacetate, plasma	<100 μmol/L	<1 mg/dL	97.95
Adrenal Steroids, plasma			
Aldosterone, supine, saline suppression	<240 pmol/L	<8.5 ng/dL	27.74
Aldosterone, upright, normal diet	140–560 pmol/L	5–20 ng/dL	27.74
Cortisol			
8 AM	140–690 nmol/L	5–25 μg/dL	27.59
4 PM	80–330 nmol/L	3–12 μg/dL	27.59
Overnight dexamethasone suppression	<140 nmol/L	<5 μg/dL	27.59
Dehydroepiandrosterone (DHEA)	7–31 nmol/L	2–9 μg/dL	3.467
Dehydroepiandrosterone Sulfate (DHEAS)	1.3–6.8 μmol/L	500–2500 ng/ml	0.002714
11-Deoxycortisol	<30 nmol/L	<1 μg/dL	28.86
17-Hydroxyprogesterone			
Women, follicular phase	0.6–3 nmol/L	0.2–1 μg/L	3.026
Women, lutesl phase	1.5–10.6 nmol/L	0.5–3.5 μg/L	3.026
Men	1.8–9 nmol/L	0.6–3 μg/L	3.026
Adrenal Steroids, urine			
Aldosterone	14–53 nmol/d	5–19 μg/d	2.774
Cortisol, free	55–276 nmol/d	20–100 μg/d	2.759
17-Hydroxycorticosteroids	5.4–27.6 μmol/d	2–10 mg/d	2.759
17-Ketosteroids			
Men	25–88 μmol/d	7–25 mg/d	3.467
Women	14–53 μmol/d	4–16 mg/d	3.467
Ammonia, as NH_3, plasma	6–47 μmol/L	10–80 μg/dL	0.5872
Angiotensin II, plasma	10–60 ng/L	10–60 pg/mL	—
Arginine Vasopressin (AVP), plasma			
Random fluid intake	0.0–2.8 pmol/L	1–3 pg/ml	9.1
Dehydration 18–24 h	5.5–13 pmol/L	4–14 pg/ml	9.1
Calciferols (as Cholecalciferol, Vitamin D_3) plasma			
1,25-Dihydroxycholecalciferol [1,25(OH)$_2$D]	36–144 pmol/L	15–60 pg/mL	2.400
25-Hydroxycholecalciferol (25-OH-D)	20–100 nmol/L	8–40 ng/mL	2.496
Calcitonin, plasma			
Normal	<19 ng/L	<19 pg/mL	—
Medullary cancer	>100 ng/L	>100 pg/mL	—
Calcium			
Ionized serum	1–1.4 mmol/L	4–5.6 mg/dL	0.2495
Total serum	2.2–2.6 mmol/L	9–10.5 mg/dL	0.2595
Catecholamines, urine			
Free Catecholamines	<590 nmol/d	<100 μg/d	5.911
Epinephrine	<273 nmol/d	<50 μg/d	5.458
Metanephrines	<7 μmol/d	<1.3 ng/d	5.485
Norepinephrine	89–473 nmol/d	15–89 μg/d	5.910
Vanillylmandelic Acid (VMA)	<40 μmol/d	<8 mg/d	5.046
Chloride, serum	98–106 μmol/L	98–106 mEq/L	—
Cholesterol, total plasma			
Desirable	<5.20 mmol/L	<200 mg/dL	0.02586
Borderline	5.20–6.18 mmol/L	200–239 mg/dL	0.02586
Undesirable	≥6.21 mmol/L	≥240 mg/dL	0.02586
Cholesterol, High-Density Lipoprotein (HDL Cholesterol), plasma			
Desirable	>1.55 mmol/L	>69 mg/dL	0.02586
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Desirable	<3.36 mmol/L	<130 mg/dL	0.02586
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Undesirable	≥4.14 mmol/L	≥160 mg/dL	0.02586
Corticotropin (ACTH) plasma, 8 AM	2–11 pmol/L	9–52 pg/mL	0.2202
Fatty Acids, Free (nonesterified) (FFA), plasma	0.4–0.7 mmol/L	10.6–18 mg/dL	0.03780
Gastrin, plasma	<120 ng/L	<120 pg/mL	—
Glucagon, plasma	50–100 ng/L	50–100 pg/mL	—

Caveat

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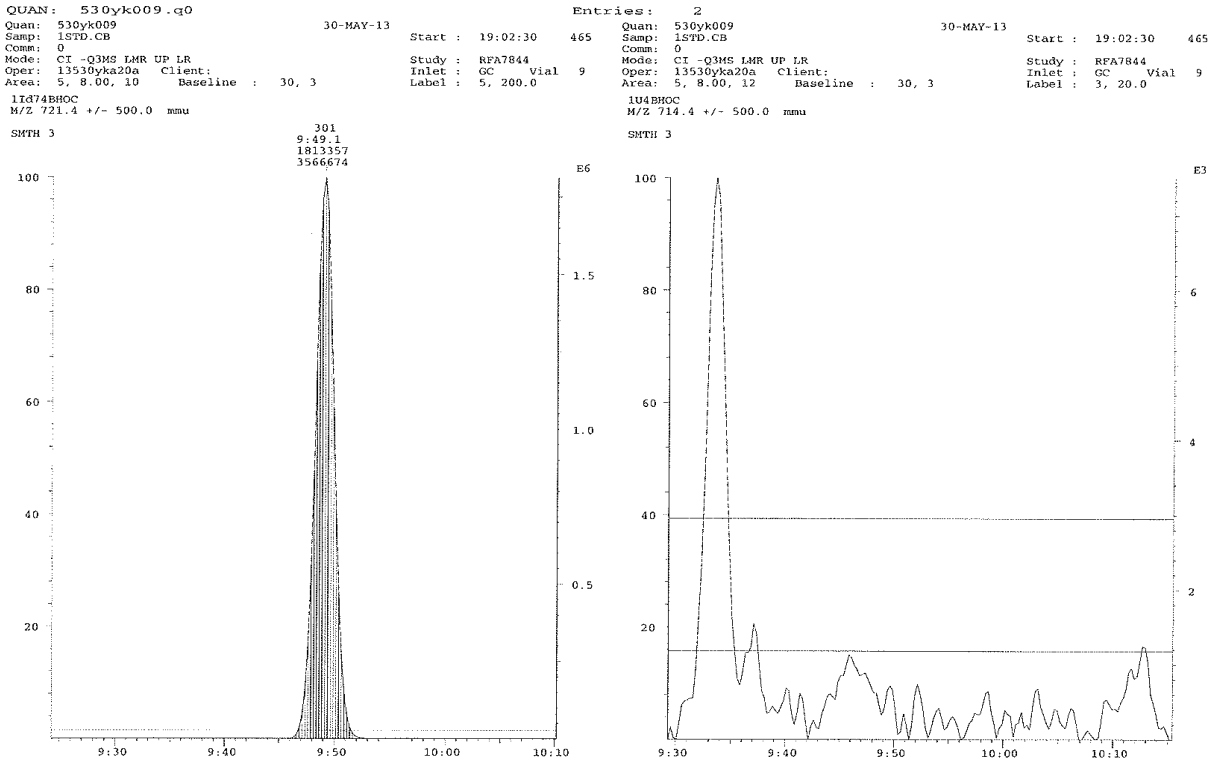
Why do we still do GC-MS/MS?

- “LC-MS/MS can do anything that GC-MS/MS can do.”

Why do we believe that?

- We can’t deny that GC provides an order of magnitude more chromatographic resolving power.
- But the selectivity provided by mass selective detection and MS/MS is remarkable.
- We usually don’t need much chromatography.
- And in bioanalysis when we are quantifying xenobiotics we know when we face a chromatographic challenge.

Typical Matrix Blank



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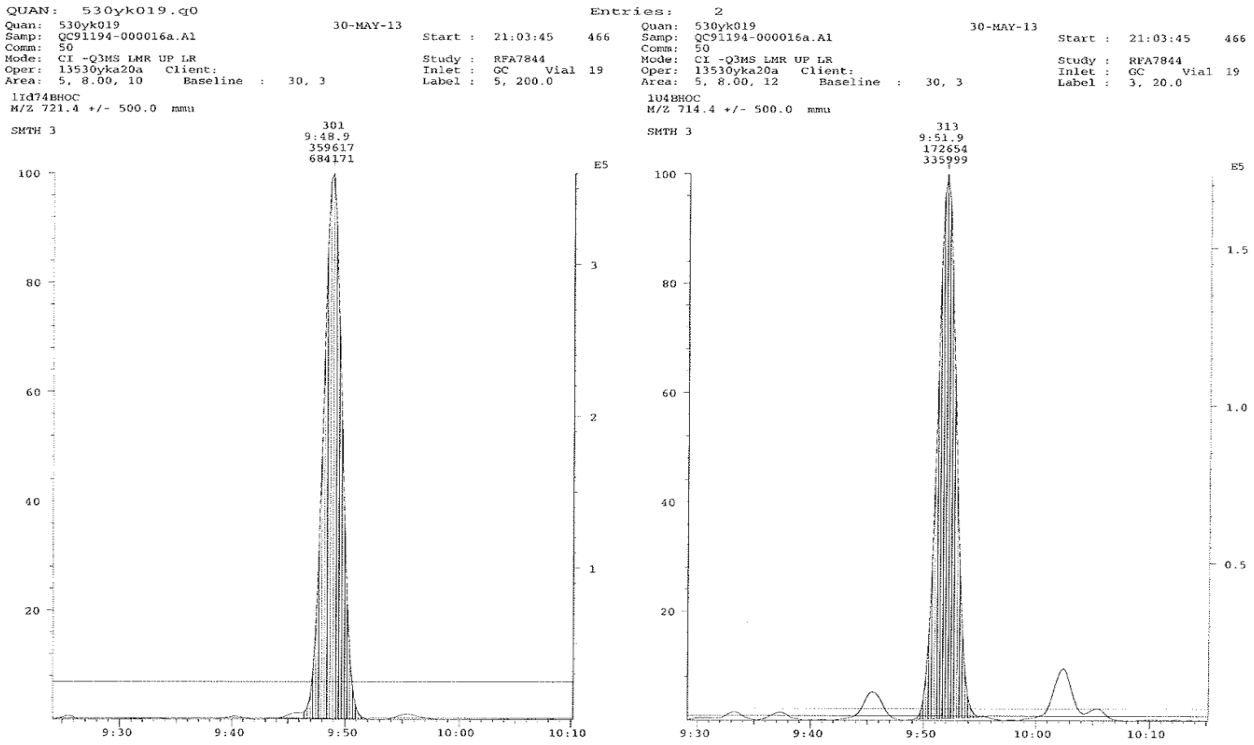
Matrix with Drug



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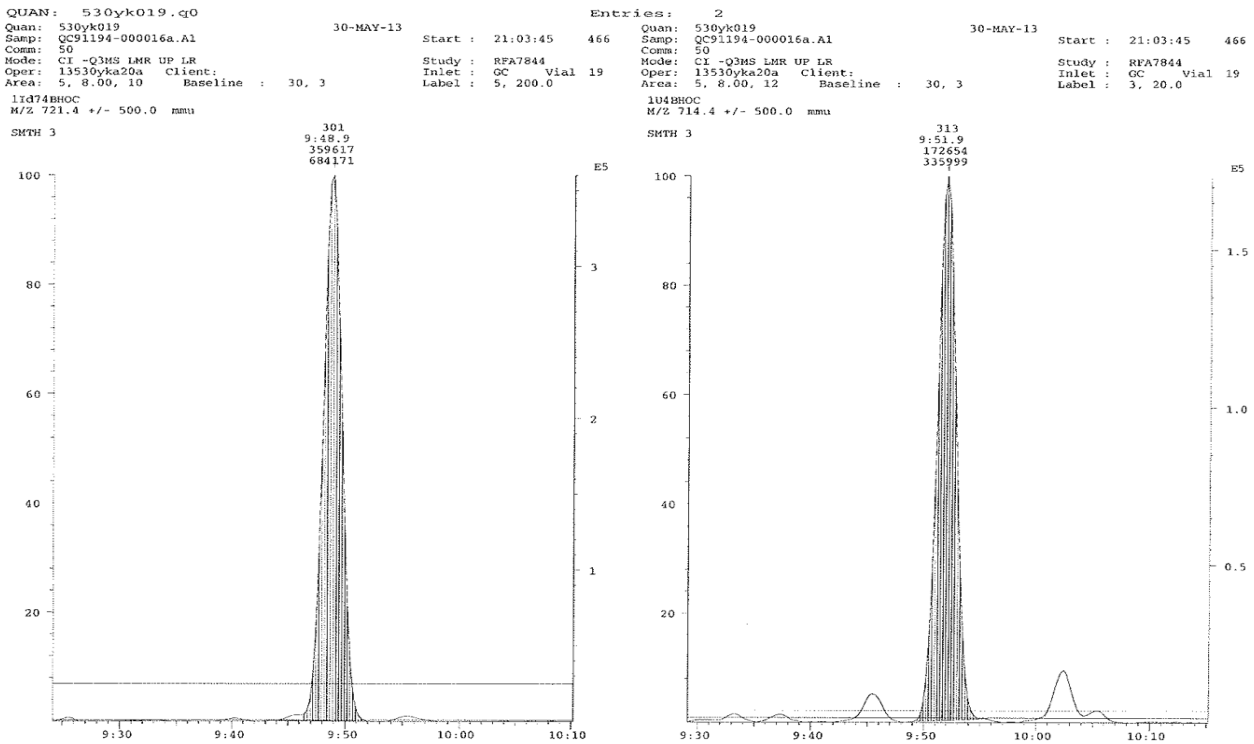
When quantifying endogenous compounds the challenges are significantly different.

- There usually is no available matrix blank.

Biomarker “Blank”



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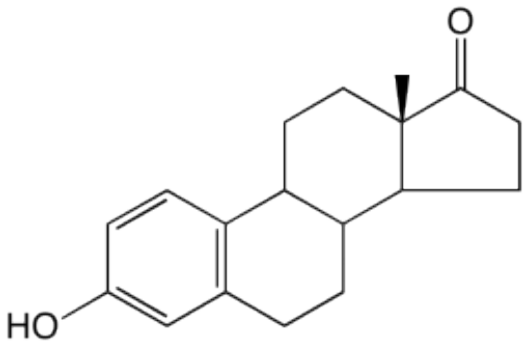
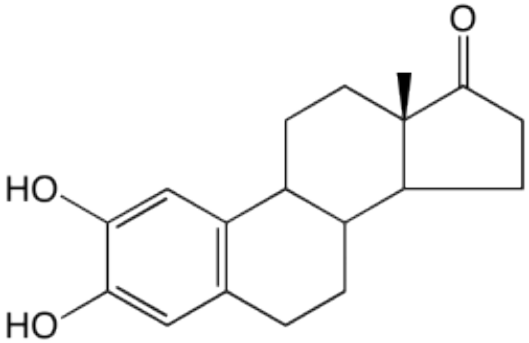
When quantifying endogenous compounds the challenges are significantly different.

- There usually is no available matrix blank.
- And for endogenous compounds there are often many very similar molecules.

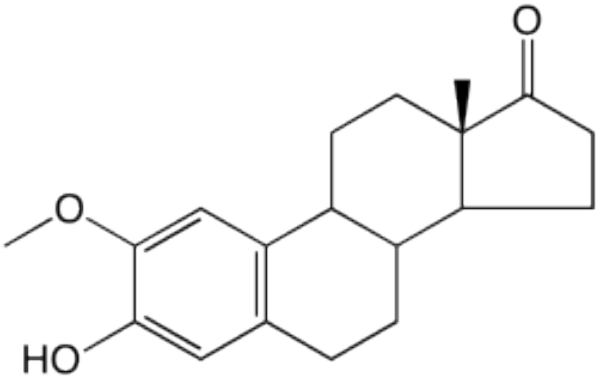
Estrone and Metabolites



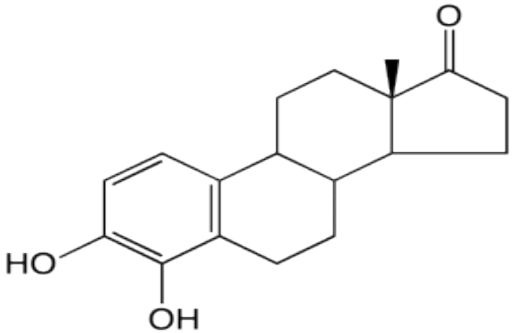
Estrone

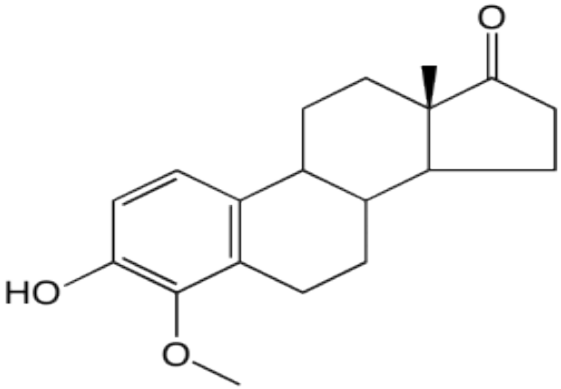


2- and 4-hydroxyestrone

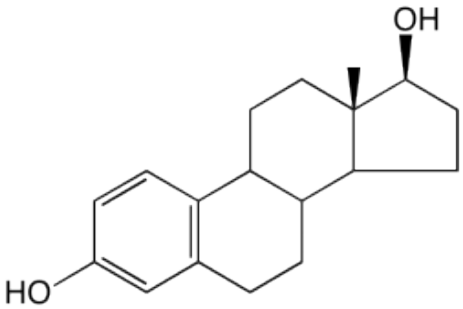


2- and 4-methoxyestrone





Estradiol and Metabolites



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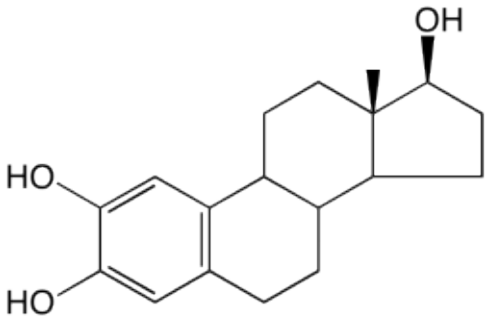
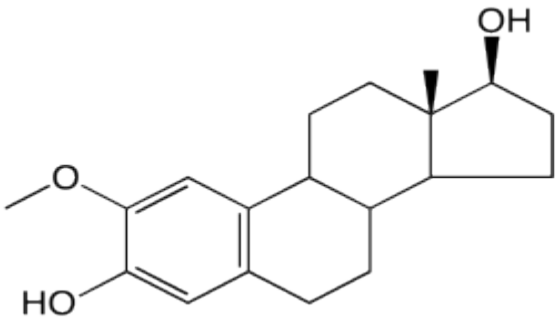
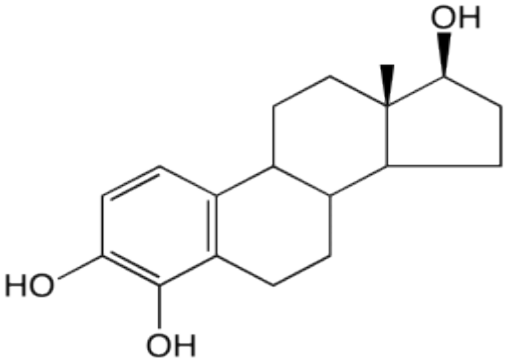
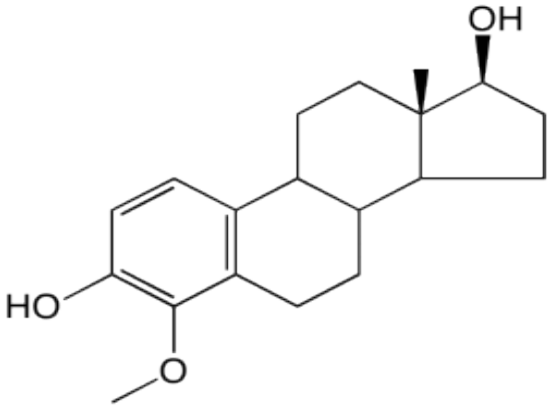


2- and 4-hydroxyestradiol

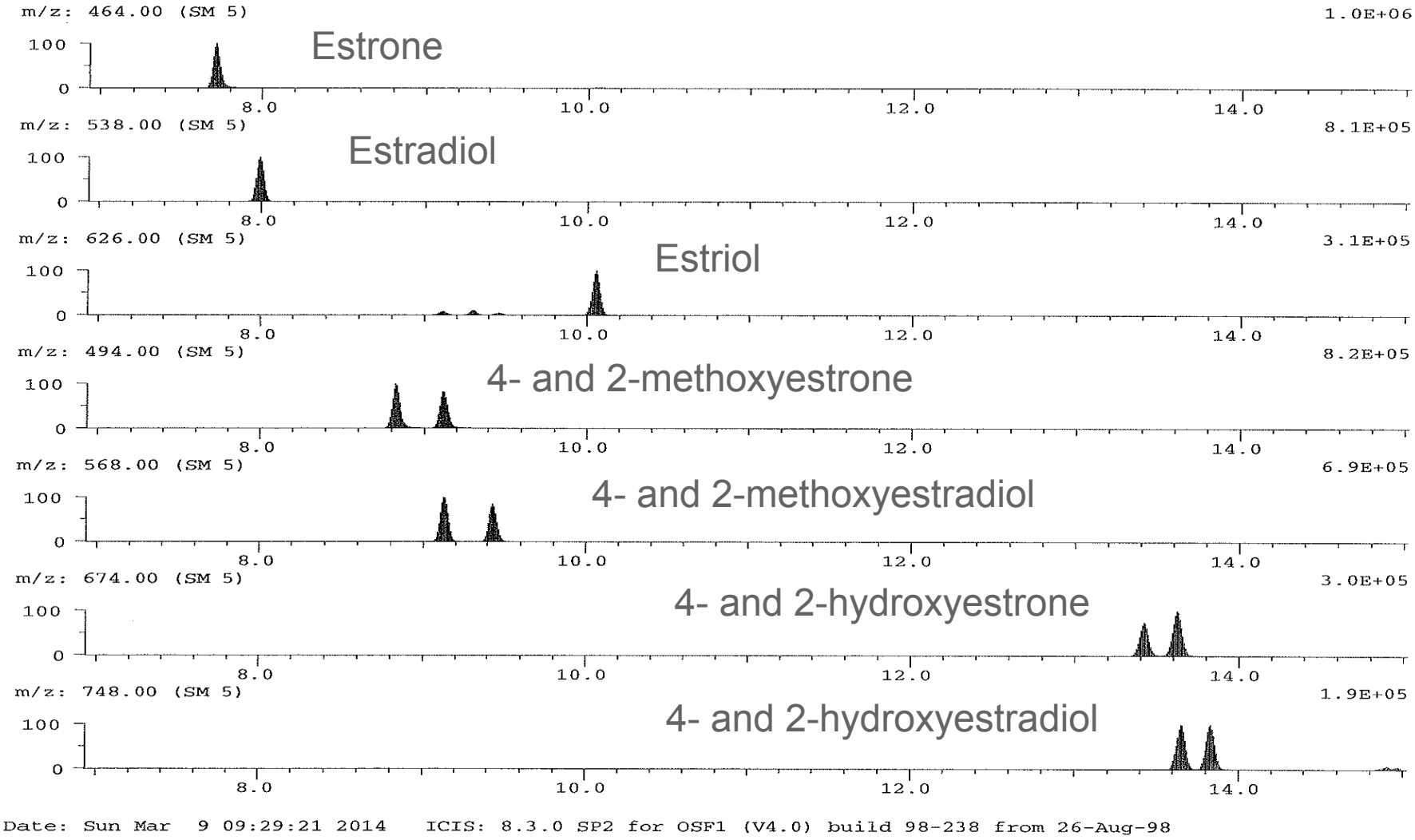


2- and 4-methoxyestradiol

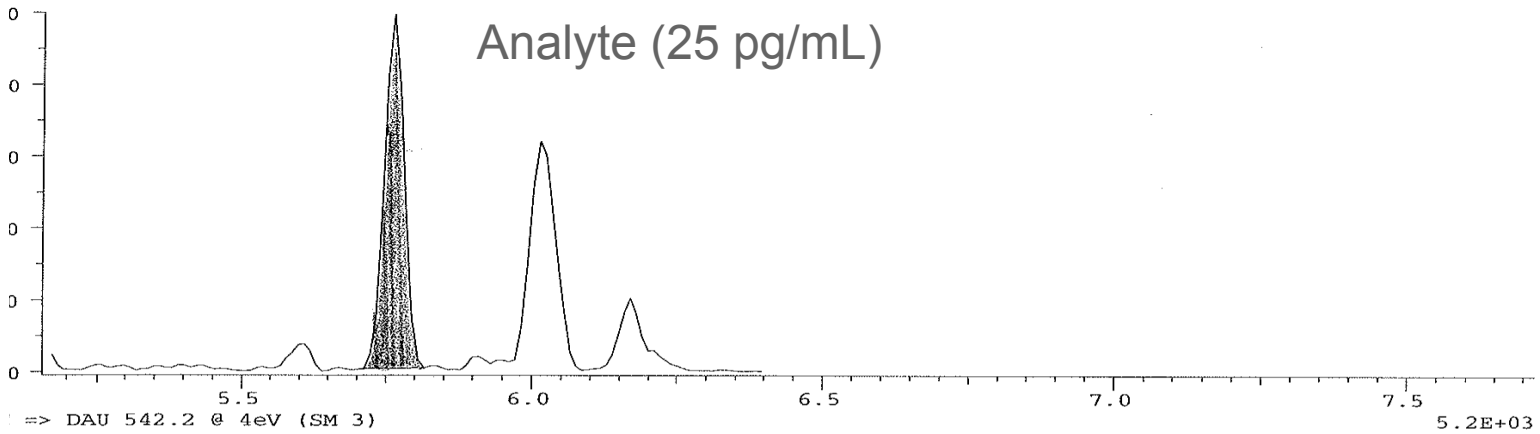


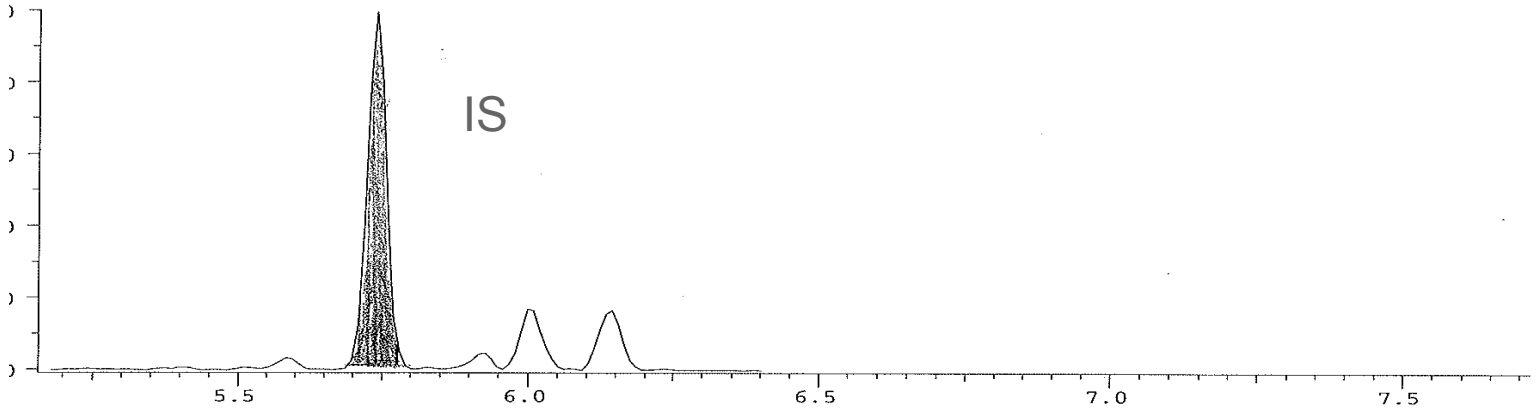


11 Estrogens

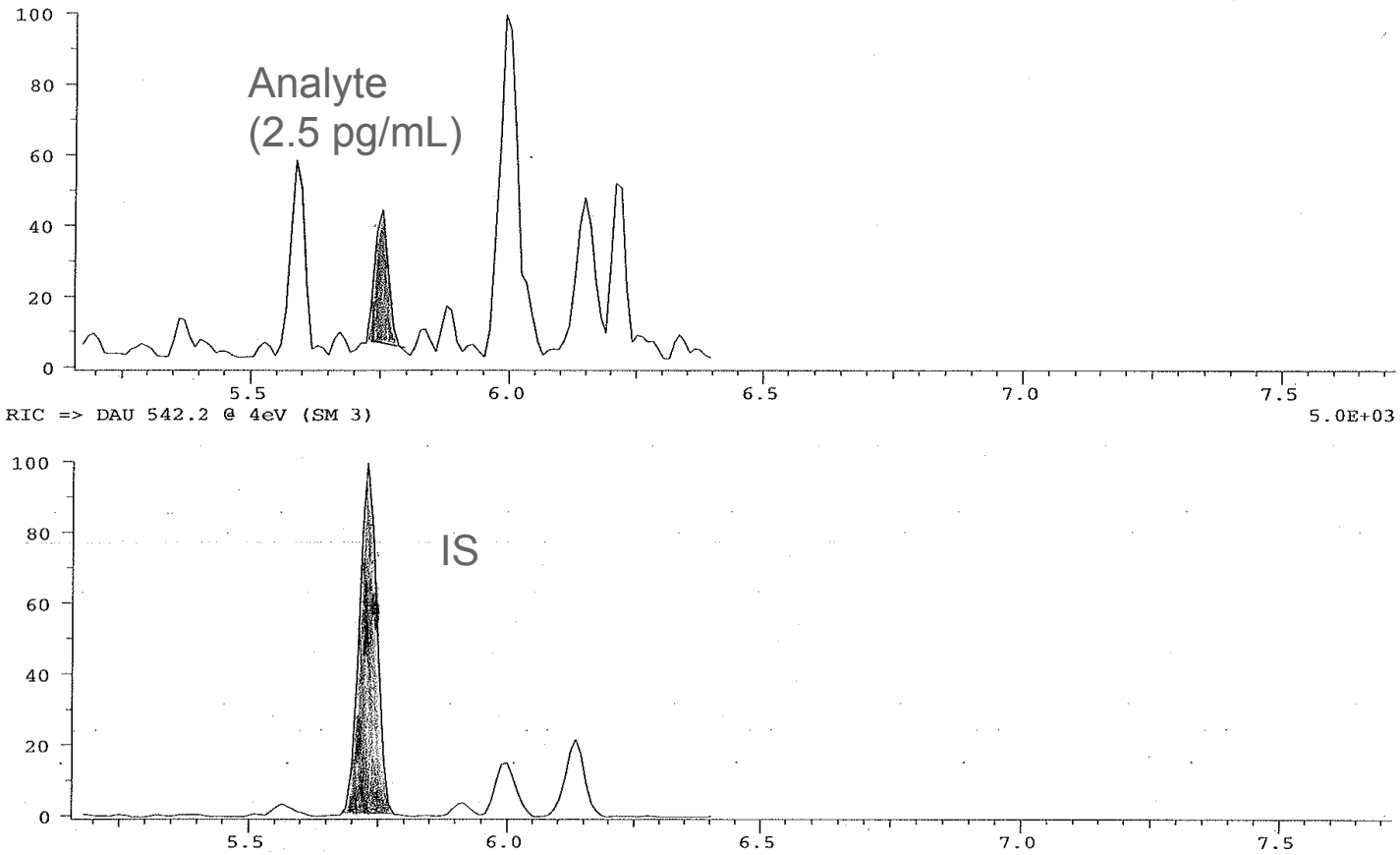


Chromatographic needs at 25 pg/mL





Chromatographic needs at 2.5 pg/mL



It’s Not What We Can See…….

- It’s not the peaks that we can see that we need to worry about.
- Inaccuracy will result from any peak with the same mass that co-elutes with our analyte.
- The chance of this happening increases at lower concentration.

Chromatographic Resolution

- **For endogenous compounds there is no blank.**
- **We can't demonstrate specificity.**
- **Endogenous compounds share similarities in structure with numerous other compounds.**
- **Mass selective detection and MS/MS may be ineffective.**
- **We don't know how much chromatographic resolution we need.**
- **The lower we go in concentration the greater the challenge to achieve specificity.**
- **For successful evaluation of biomarkers specificity is critically important, impossible to prove and difficult to achieve.**

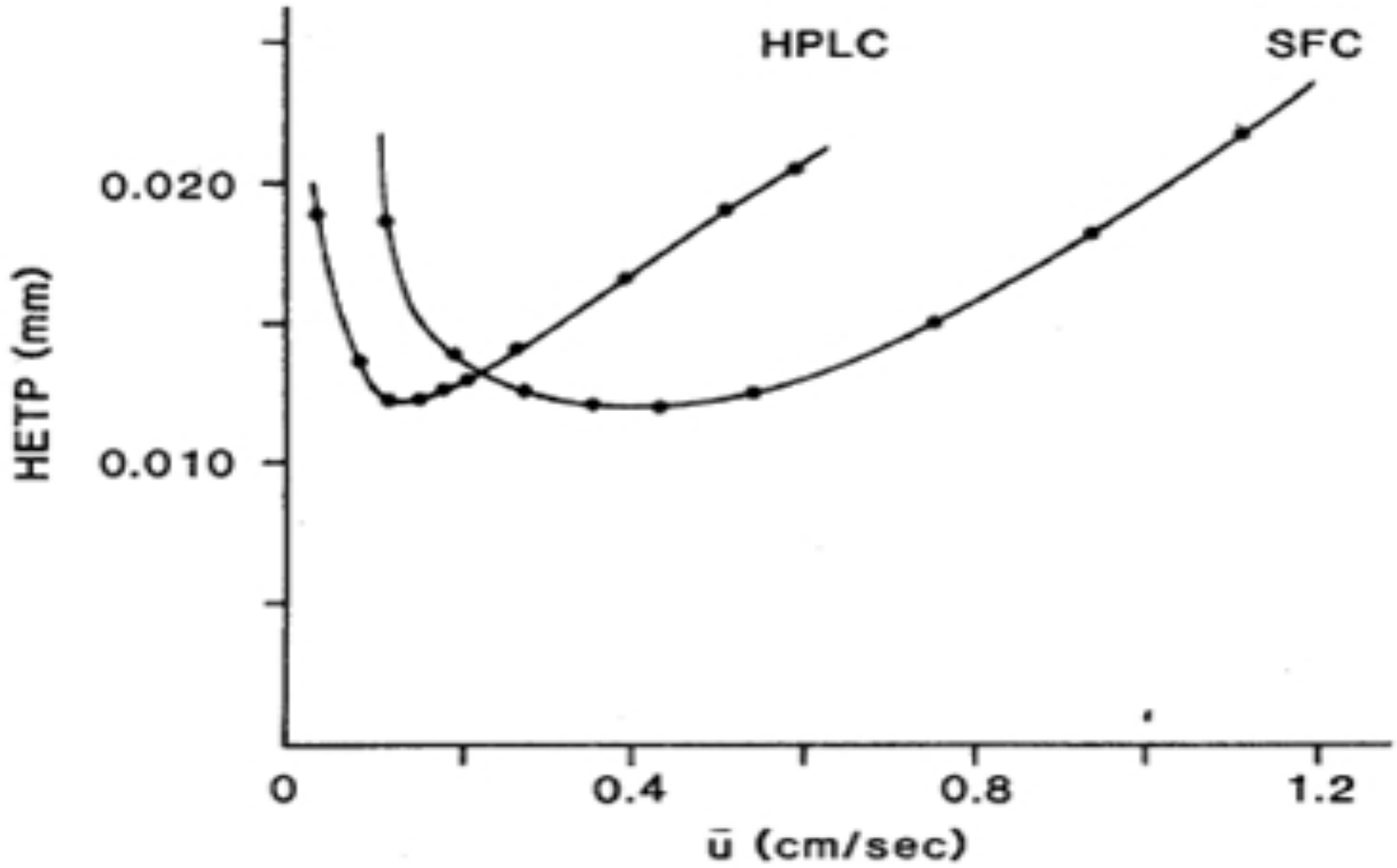
What do we really want?

- **A chromatographic system that will chromatograph a broad range of molecules**
- **With 10^5 theoretical plates**
- **Has a large number of parameters that can be manipulated**
- **Provides sensitivity down to 1 pg/mL**
- **That will provide fast analysis**
- **And is environmentally friendly**
- **And cheap**

Properties of SFC[2]

	Density ($g\ cm^{-3}$)	Diffusion ($cm^2\ s^{-1}$)	Viscosity ($g\ cm^{-1}\ s^{-1}$)
Gas	10^{-3}	10^{-1}	10^{-4}
Supercritical fluid	$10^{-1} - 1$ *Liquid-like*	$10^{-4} - 10^{-3}$ *Liquid-like*	$10^{-4} - 10^{-3}$ *Gas-like*
Liquid	1	$< 10^{-5}$	10^{-2}

Van Deemter Plots[3]



Properties of SFC^2

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Supercritical Fluid Chromatography

.....”the low viscosity of supercritical fluids results in lower pressure drops along the column, thus up to ten columns can be assembled, serially, to afford up to 200,000 theoretical plates.”[2]

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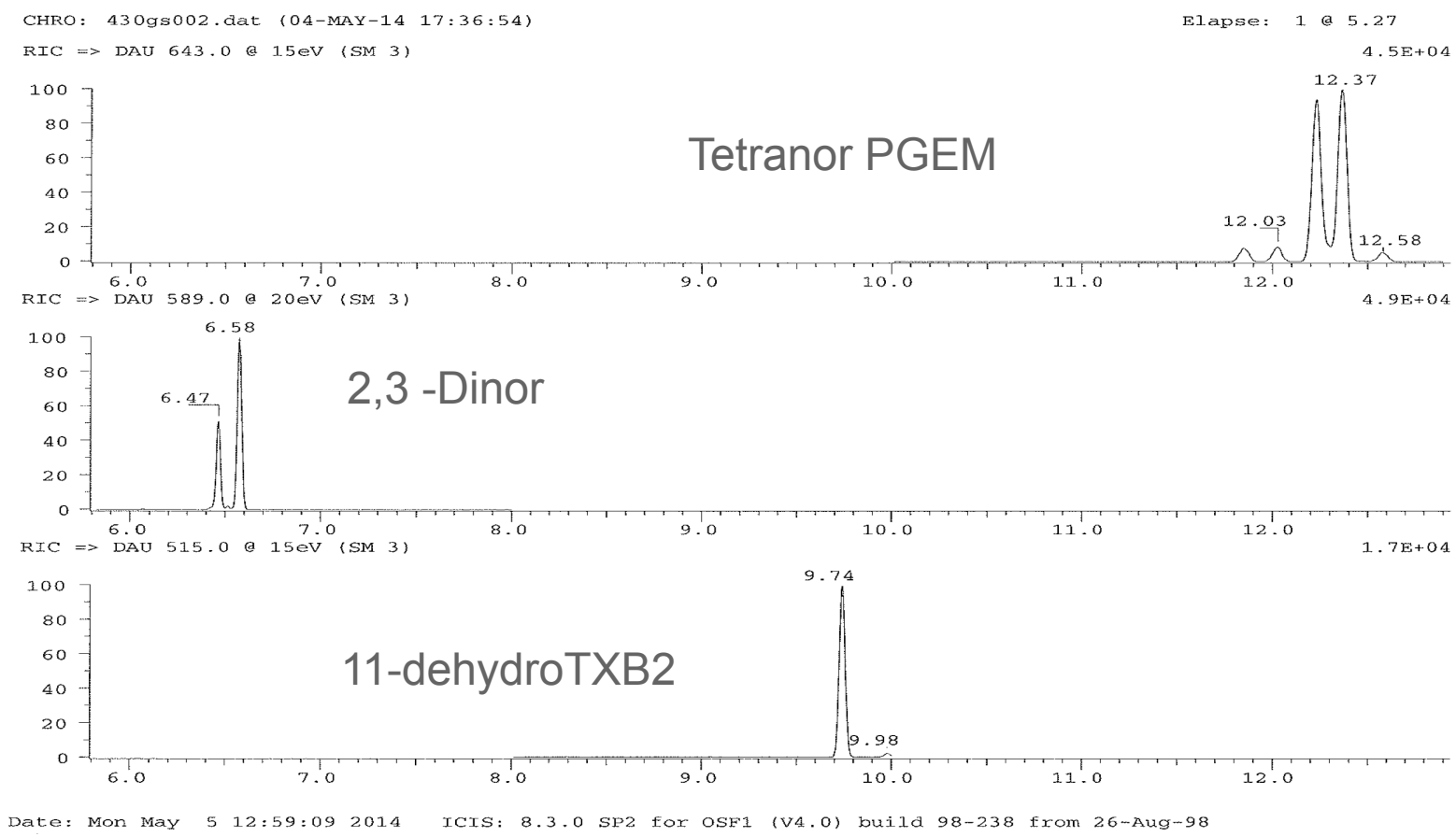
Ionization

- Sensitivity requires efficient ionization.
- The bar is set very high by the efficiency of electron capture in GC-MS.
- There are two ways to approach that level of ionization that are available to both LC and SFC.
  - Dissociative ionization

Prostaglandins by GC



Prostaglandins by SFC

Example Chromatogram for Methoxime / PFB derivative

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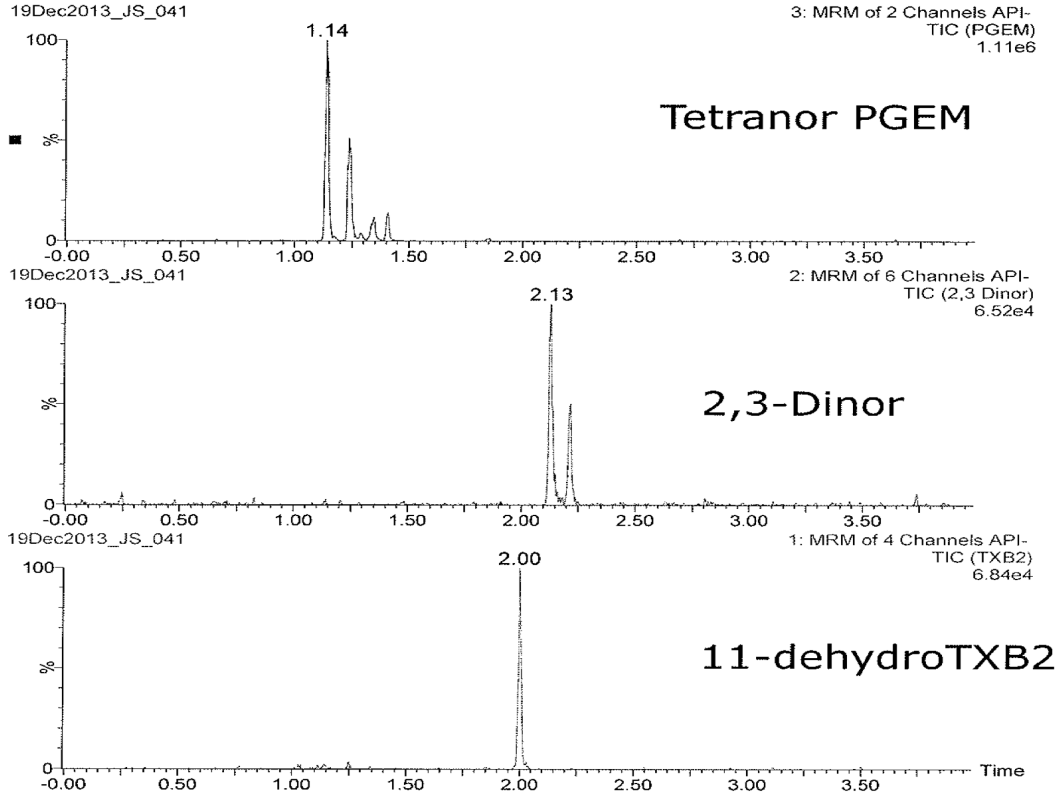
THE SCIENCE OF WHAT'S POSSIBLE.®



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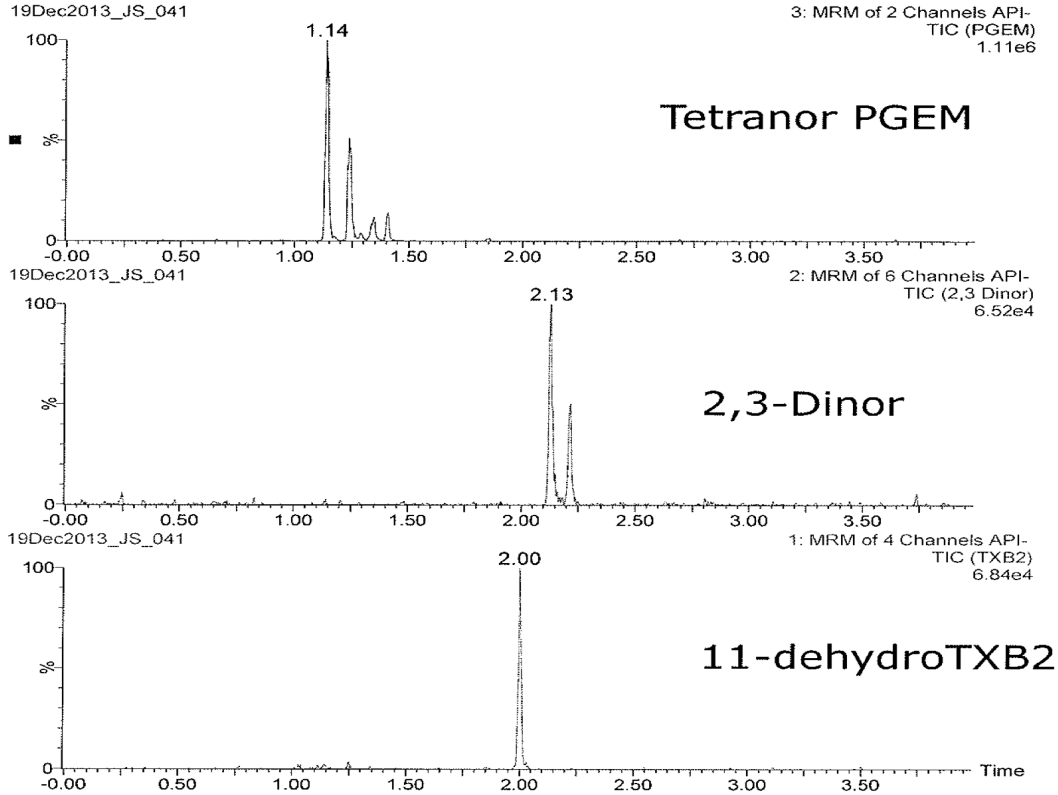

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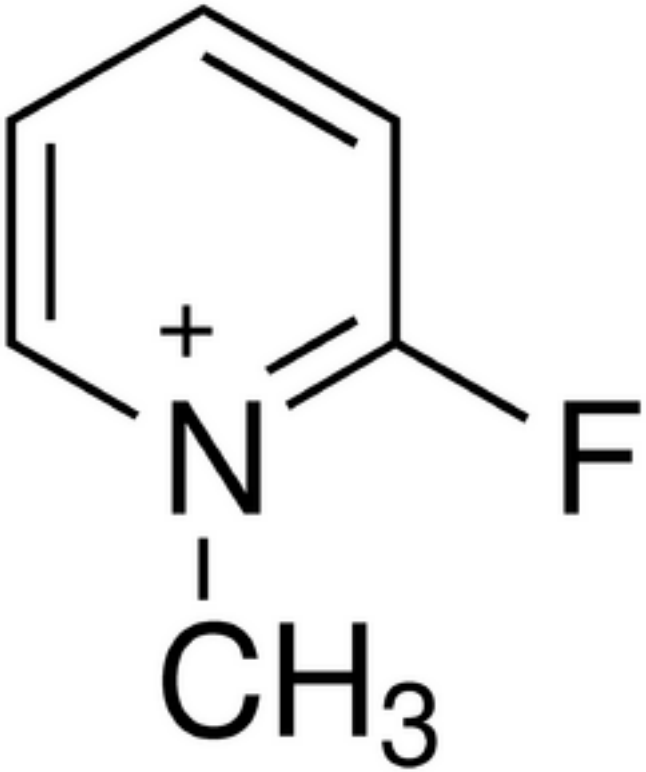
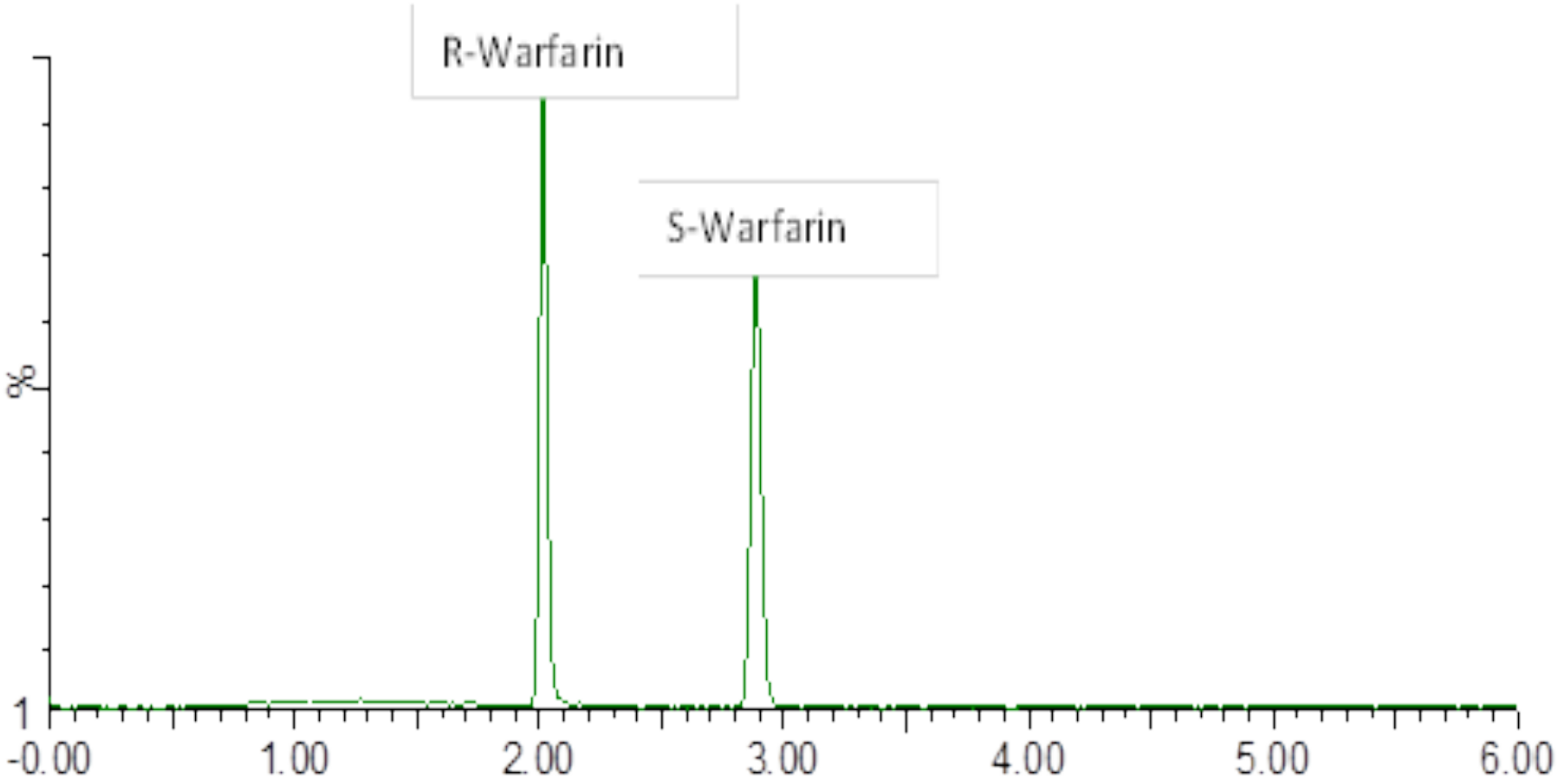
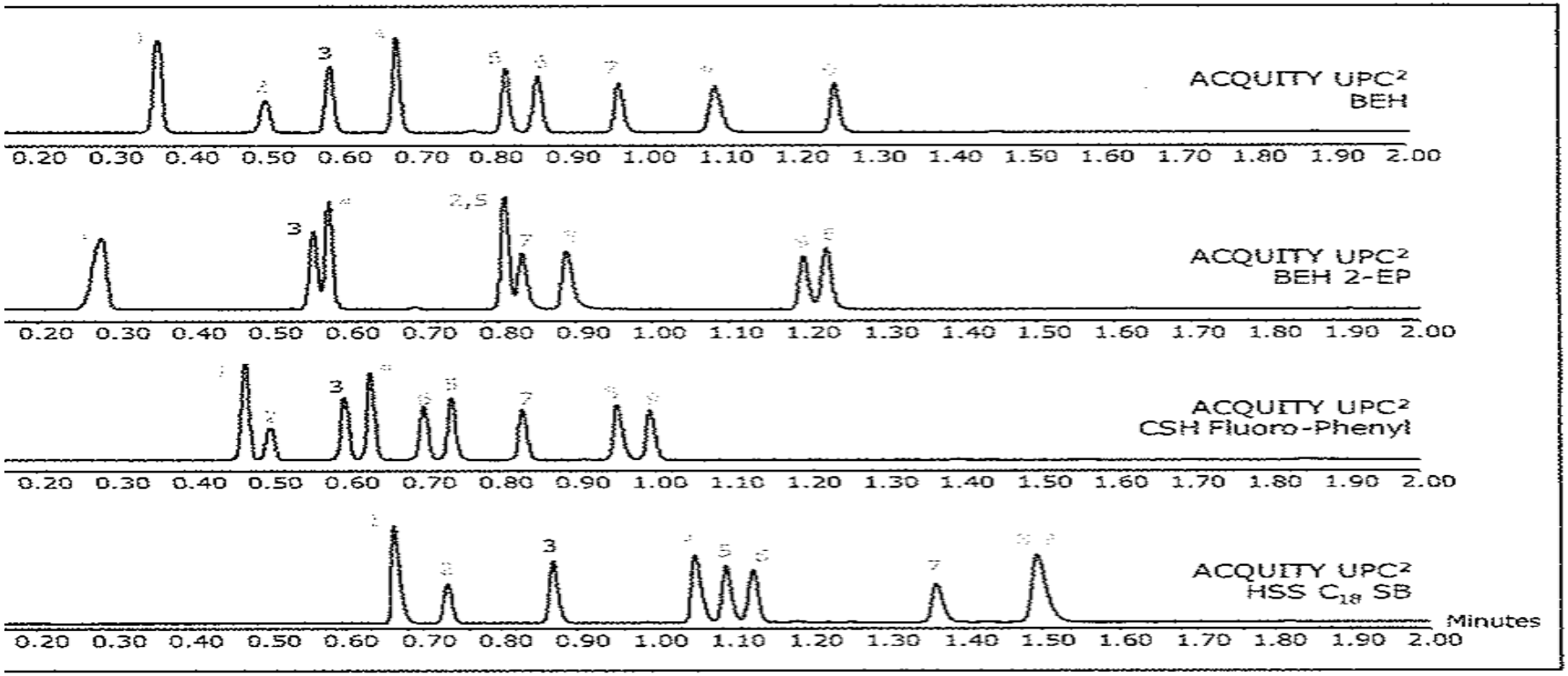
2-fluoro-1-methylpyridinium



Separation of Enantiomers



Nine Endogenous Steroids by SFC <1.5 min



rations (UV) of steroid standards on ACQUITY UPC² columns including: (a) BEH, (b) BEH 2-EP, (c) CSH™ (d) HSS C_{18} SB. All columns were 3.0 x 50 mm, 1.7-µm configurations except for the HSS C_{18} SB which is a e. Steroid compounds are the following: (1) androstenedione, (2) estrone, (3) 17α-OHP [17α-hydroxyprogesterone,) 11-deoxycortisol, (6) estradiol, (7) corticosterone, (8) aldosterone, and (9) cortisol. Colored peak assignments with similar molecular weights and m/z fragments

Nine steroids by SFC

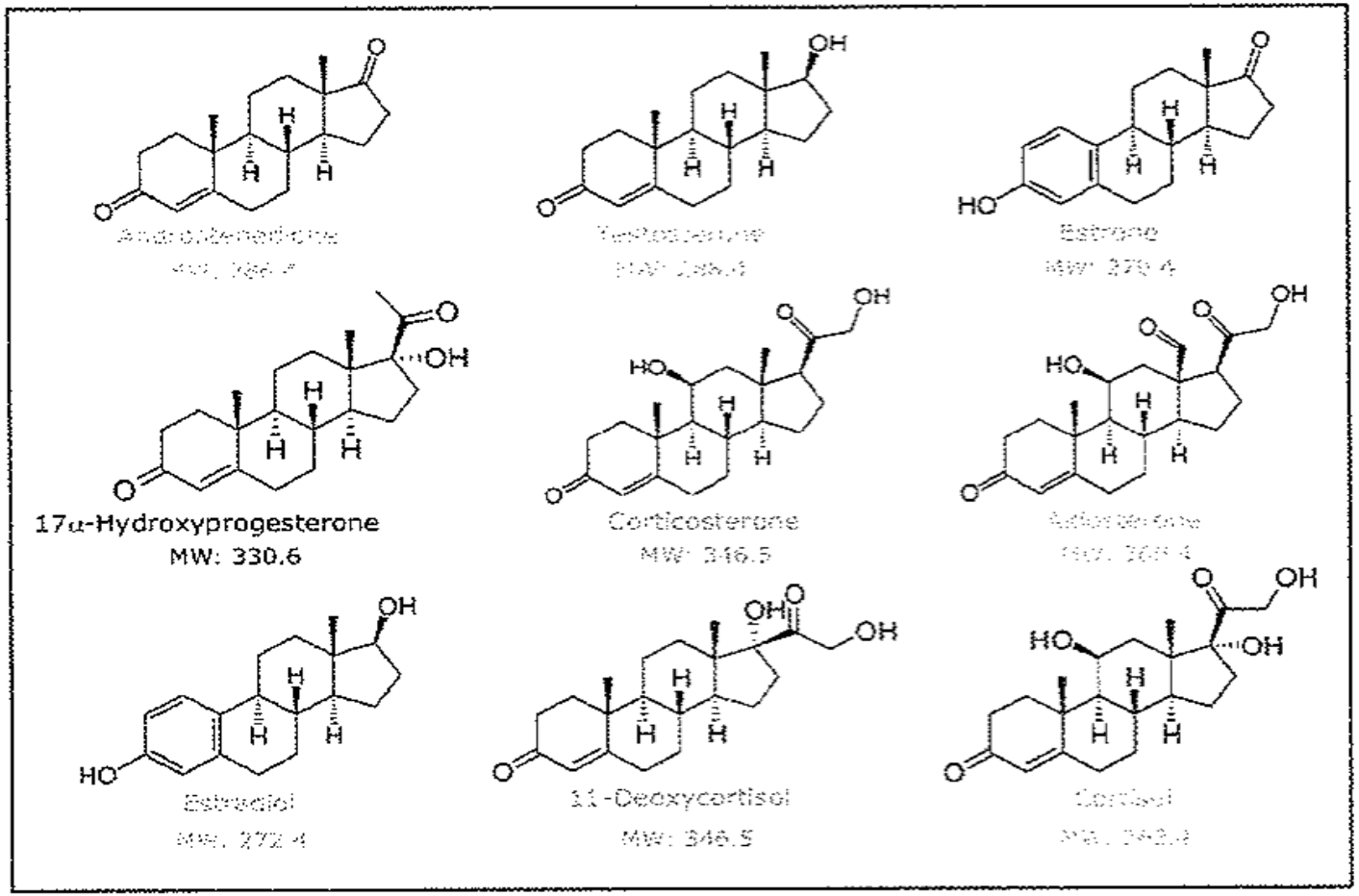
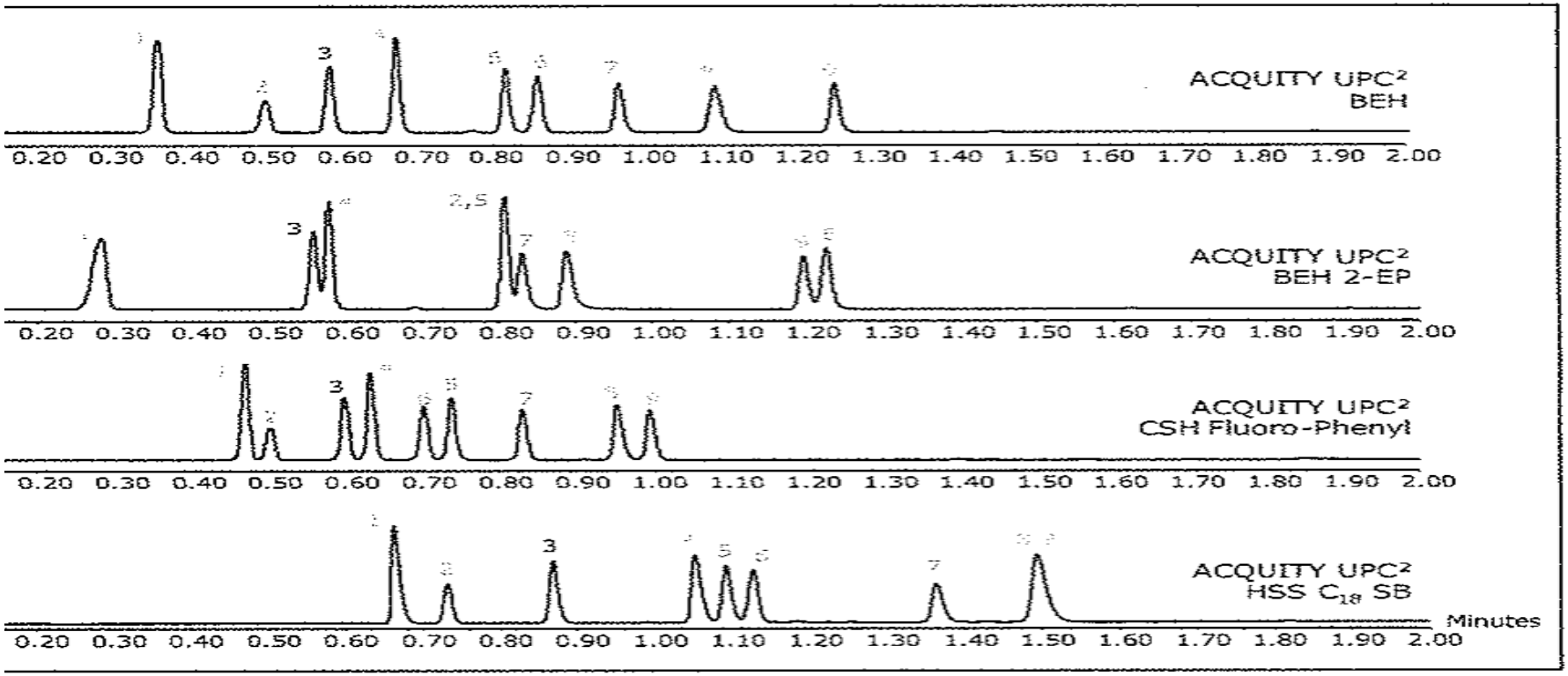


Figure 1. Structures of steroids evaluated. Compounds with the same color font indicate compounds with similar molecular weights that generate similar MS fragments.

Nine Endogenous Steroids by SFC <1.5 min



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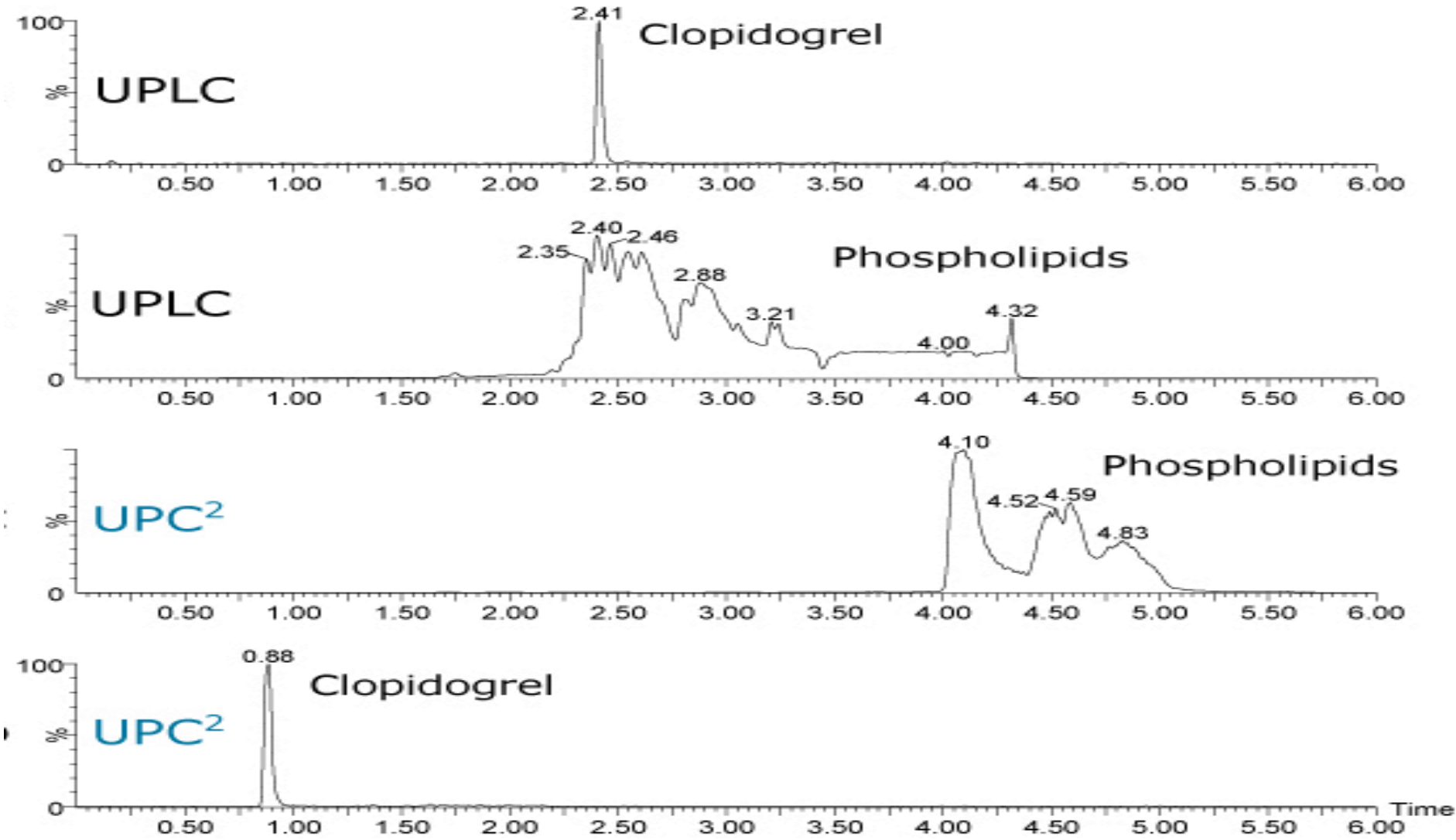
Orthoganality!



Thank you for your attention.

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