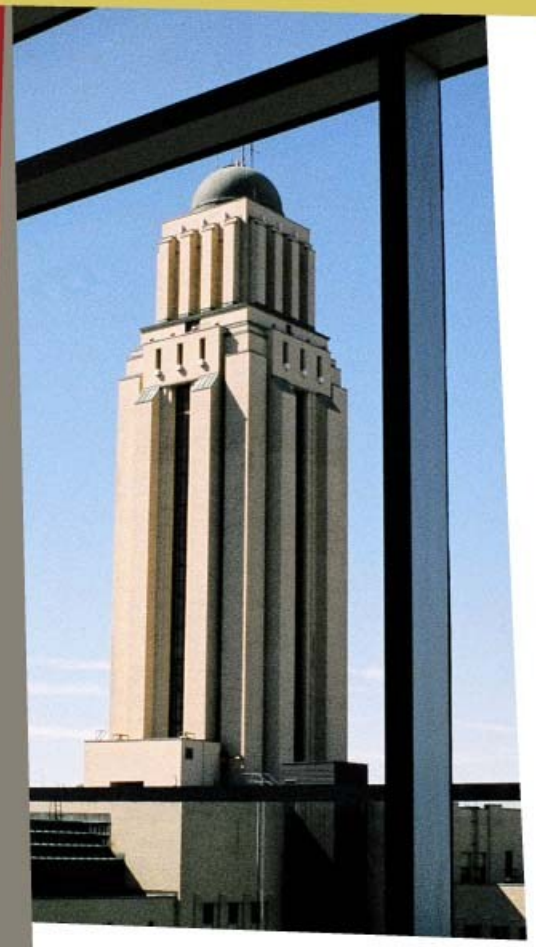
Université de Montréal



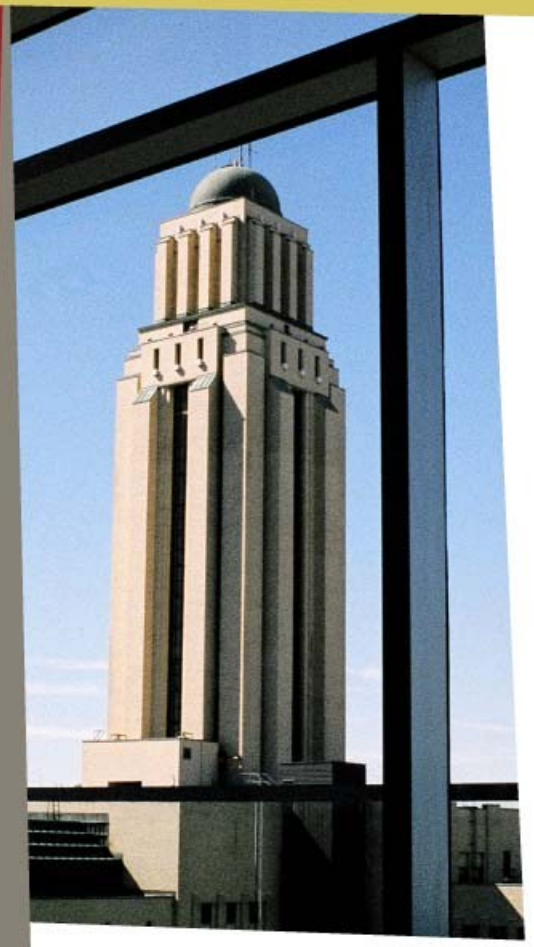
Application d'une nouvelle méthode SBSE modifiée pour l'analyse d'hormones dans l'eau par désorption thermique à diode laser avec APCI-MS/MS.

Sébastien Sauvé, Sung Vo Duy

Département de chimie

sebastien.sauve@umontreal.ca

Université de Montréal



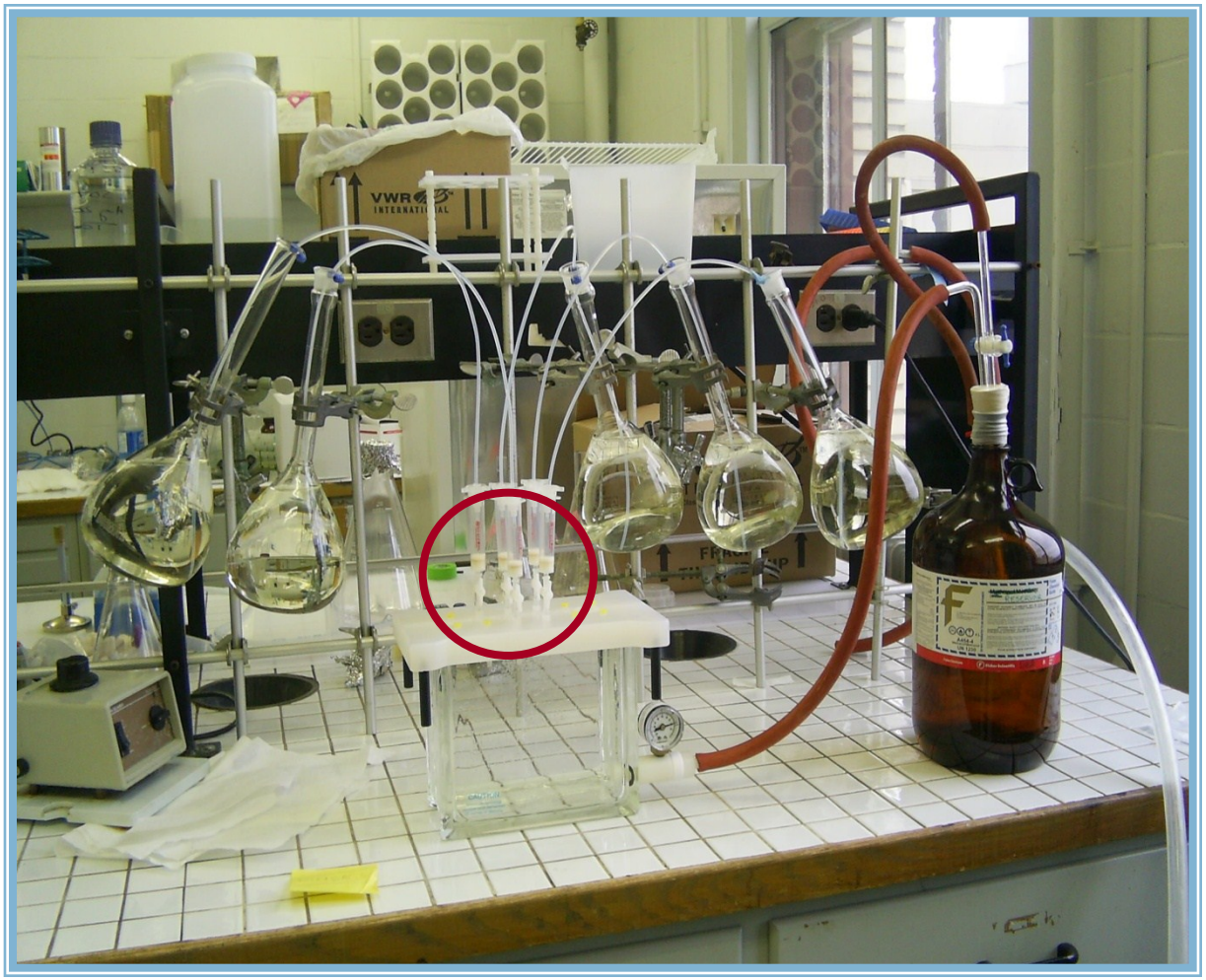
- 1re Au Québec avec ses écoles affiliées (HEC Montréal et Polytechnique Montréal)
- 2e Au Canada, 62 864 étudiants, 2600 professeurs
- 4e Au Canada en recherche, 526 millions de $ / an, 1re au Québec
- 84e Au monde, selon le classement du *Times Higher Education*

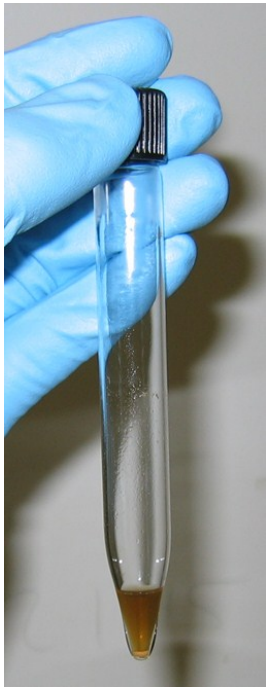
Médicaments dans l'environnement

Produits pharmaceutiques:

- Agents anti-infectieux et antiseptiques
- Antidépresseurs et antipsychotiques
- Agents cytostatiques et autres agents de chimiothérapie
- Régulateurs lipidiques et régulateurs d'hypertension
- Hormones (naturelles et de synthèse)
- Produits d'hygiène personnelle (e.g. caféine, détergents, cosmétiques) (PCP, *personal care products*)

SPE: Enrichissement





Université de Montréal

LC-MS/MS

Détection:
Spectrométrie de masse en tandem

(mode de suivi ciblé - *selected reaction monitoring SRM*)

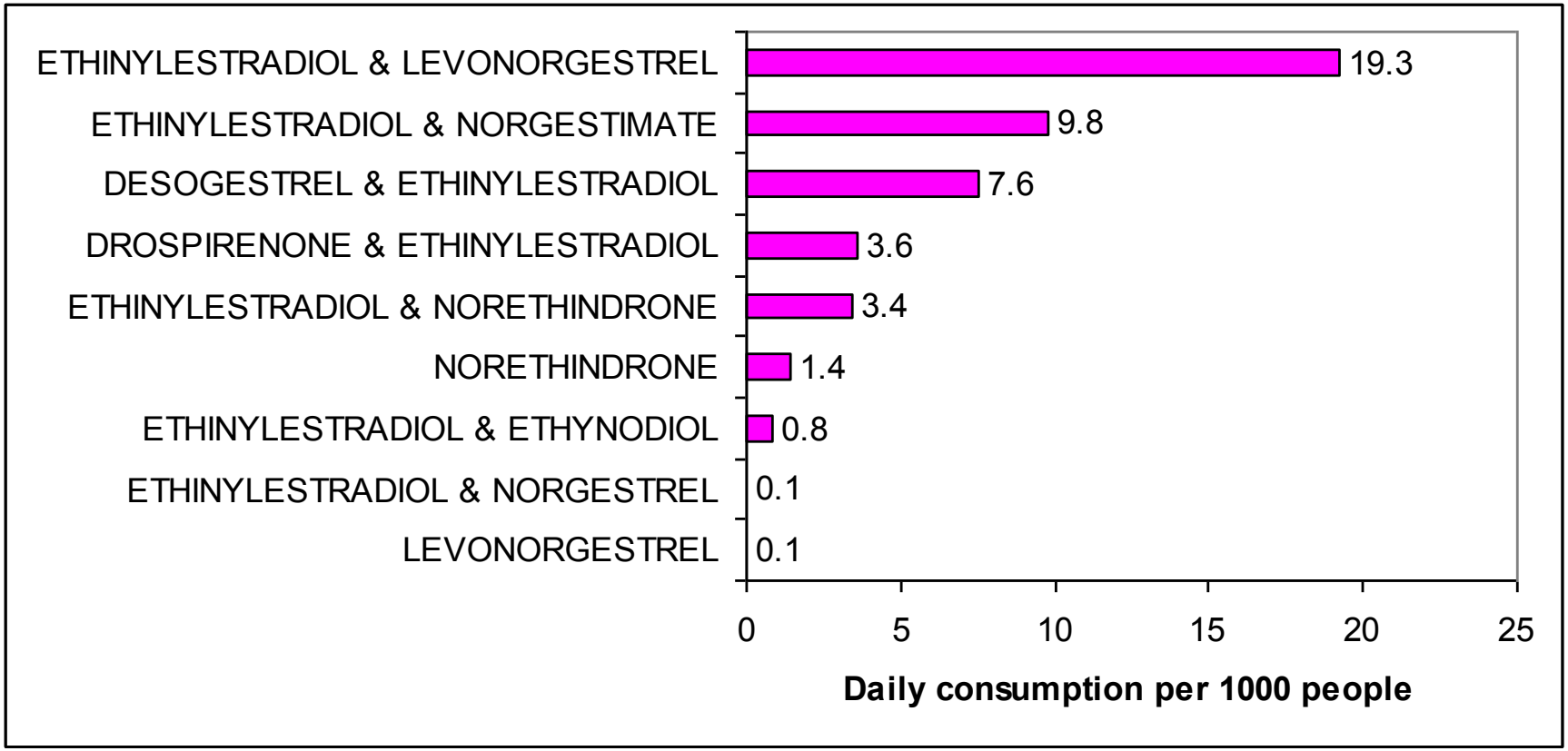
ThermoElectron TSQ Quantum EQuan MAX System

Université de Montréal

Hormones stéroïdiennes féminines

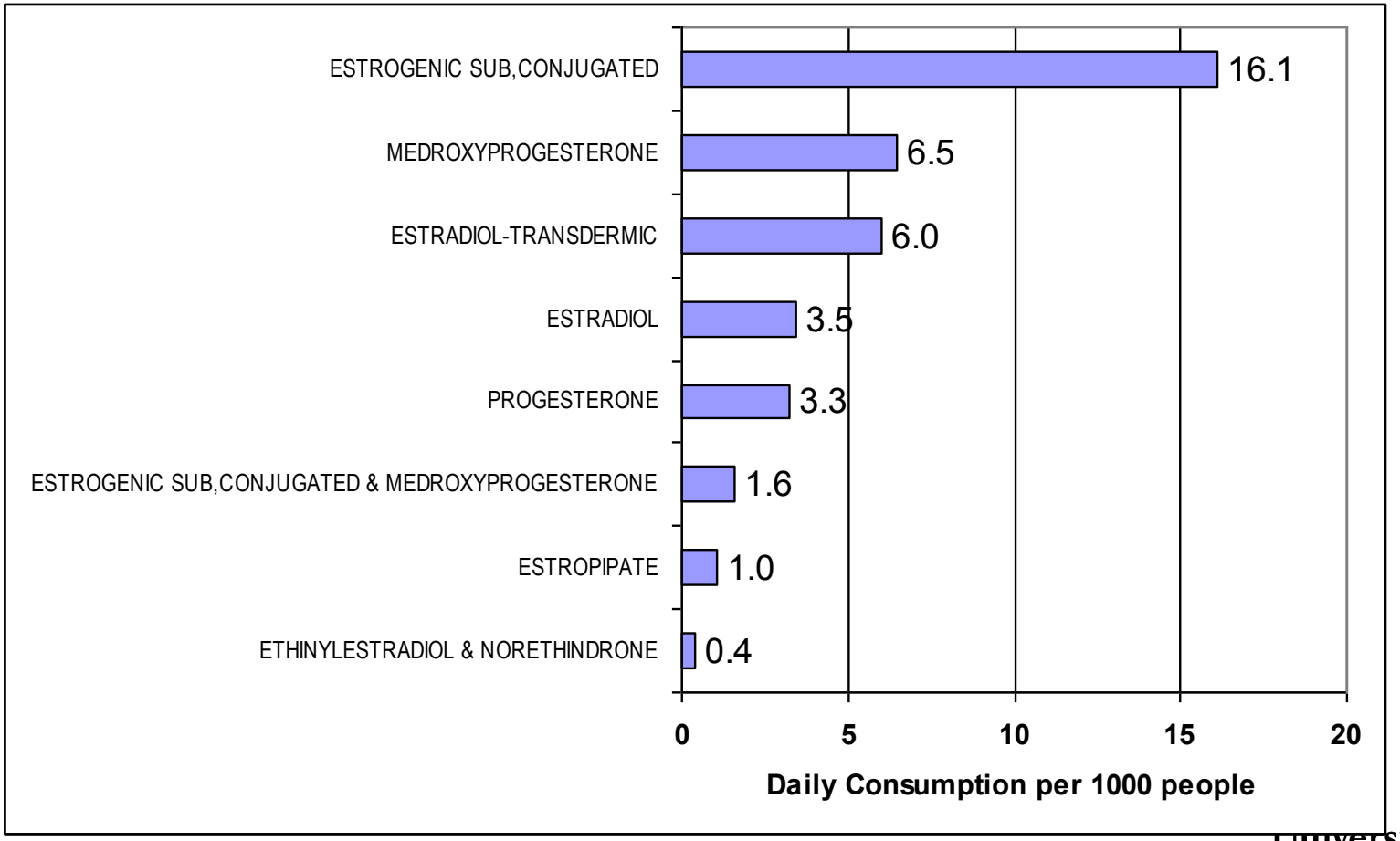
- Hormones naturelles
- Anovulants (contraception)
- Thérapie hormonale de remplacement (ménopause)

Anovulants (contraceptifs)



Université de Montréal

Thérapie hormonale de remplacement (ménopause)



Université de Montréal

Proprerties of selected hormones

Hormones	Molecular mass (g/mol)	$LogK_{o/w}$ [a]	pK_a
E1	270.4	3.13	10.7
E2	272.4	4.01	10.7
E3	288.4	2.81	10.4
EE2	296.4	3.67	10.4
LEVO	312.4	3.48	NA
MPROG	344.5	3.50	NA
NOR	298.4	2.97	NA
PROG	314.5	3.87	---

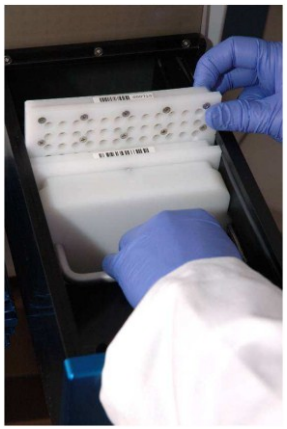
Université de Montréal

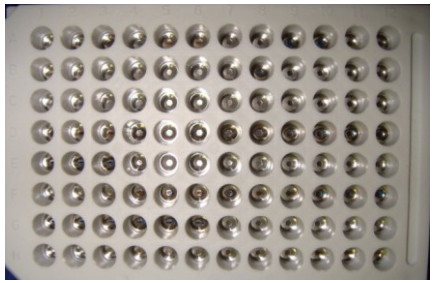
LDTD-APCI-MS/MS Analysis

→ **15 sec/sample** instead of 15-20 min with LC-MSMS

- ✓ technique combining thermal desorption (laser) with APCI
- ✓ deposition of a small sample volume (1-5 µL) using a 96-well plate; 2 min drying of solvent
- ✓ neutral analytes will desorb/volatilize the through the heat generated by laser
- ✓ APCI corona needle will ionise the analytes (ionisation mode +/-)
- ✓ vector gaz (air) will tranfser the analytes from the well to the MS

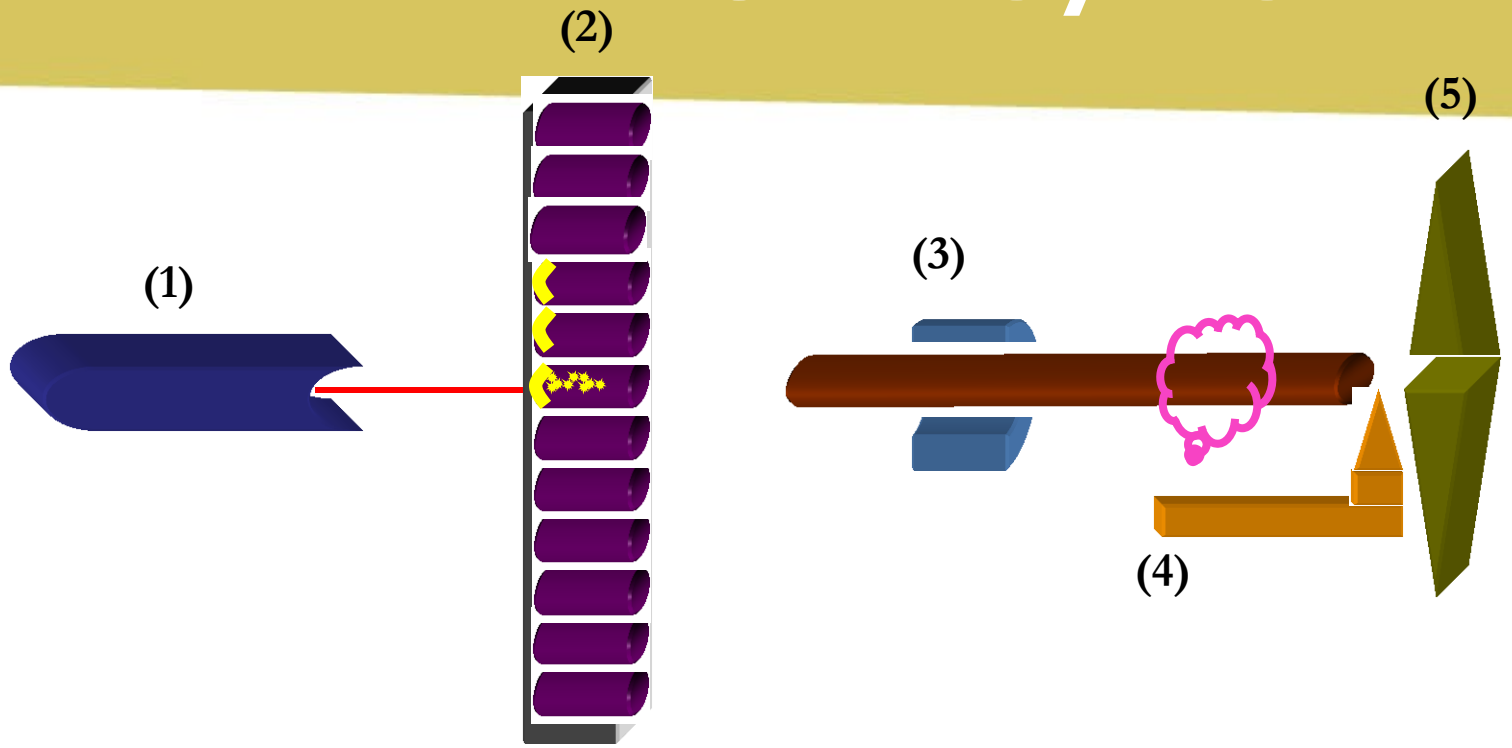
Échantillonneur →
960 échantillons





Université de Montréal

LDTD APCI-MS/MS



(1) Infrared laser (980 nm, 20W)

(2) LazWell Plate (96 wells): analyte desorption (1-10 µL spotted)

(3) Transfer tube transporting the neutrally desorded analytes to the APCI region

(4) Corona needle discharge region (APCI)

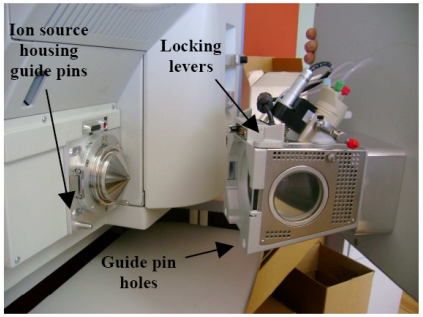
(5) MS inlet

Université de Montréal

Laser diode thermal desorption (LDTD)

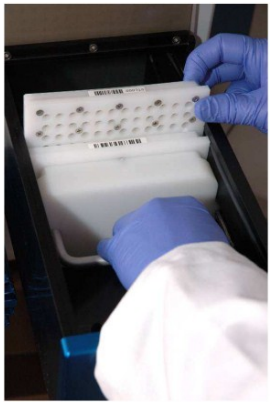
LDTD

Installation



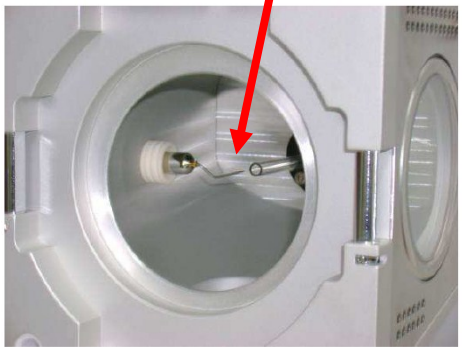
Auto-sampler

960 samples

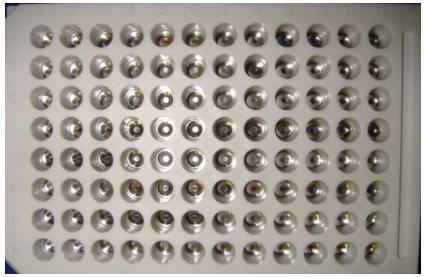


IR Laser

(980 nm, 20 W)



Corona needle position (APCI)

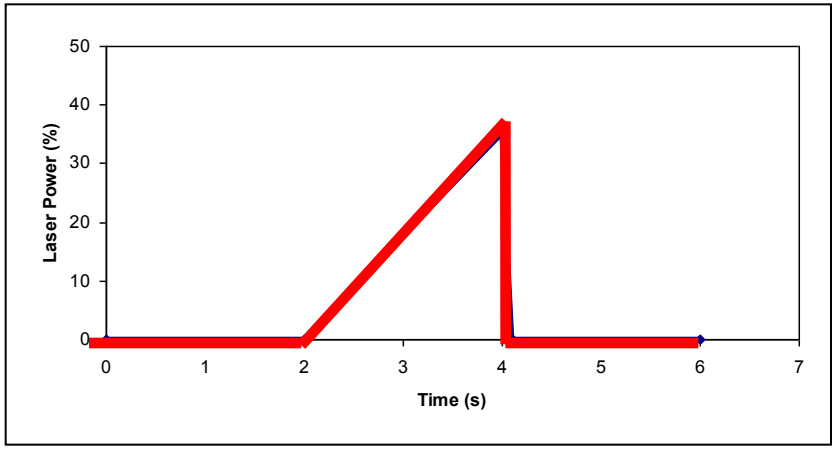


Can ramp up to 3000°C/sec.
Laser power is defined in %
Normally ~100-150°C

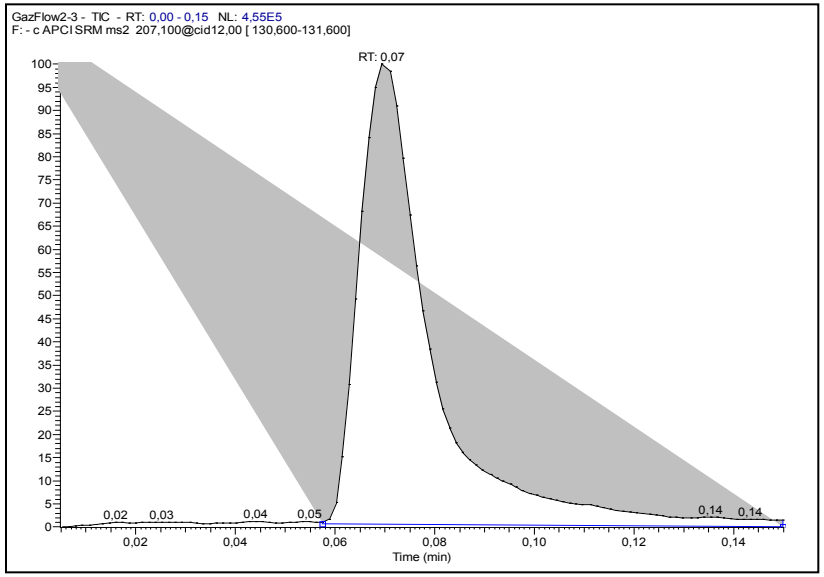
Université de Montréal

LDTD parameter optimisation

Laser Power: 35%
Gas Flow: 3 L/min
Deposition volume: 2 µL
Laser pattern duration: 6 s



Laser pattern



LDTD peak shape

Université de Montréal

LDTD Optimization

- No need to optimize liquid chromatography - it has been completely eliminated!

- Optimization for MS (precursor) and MS/MS (SRM transitions) conditions in NI and PI mode.

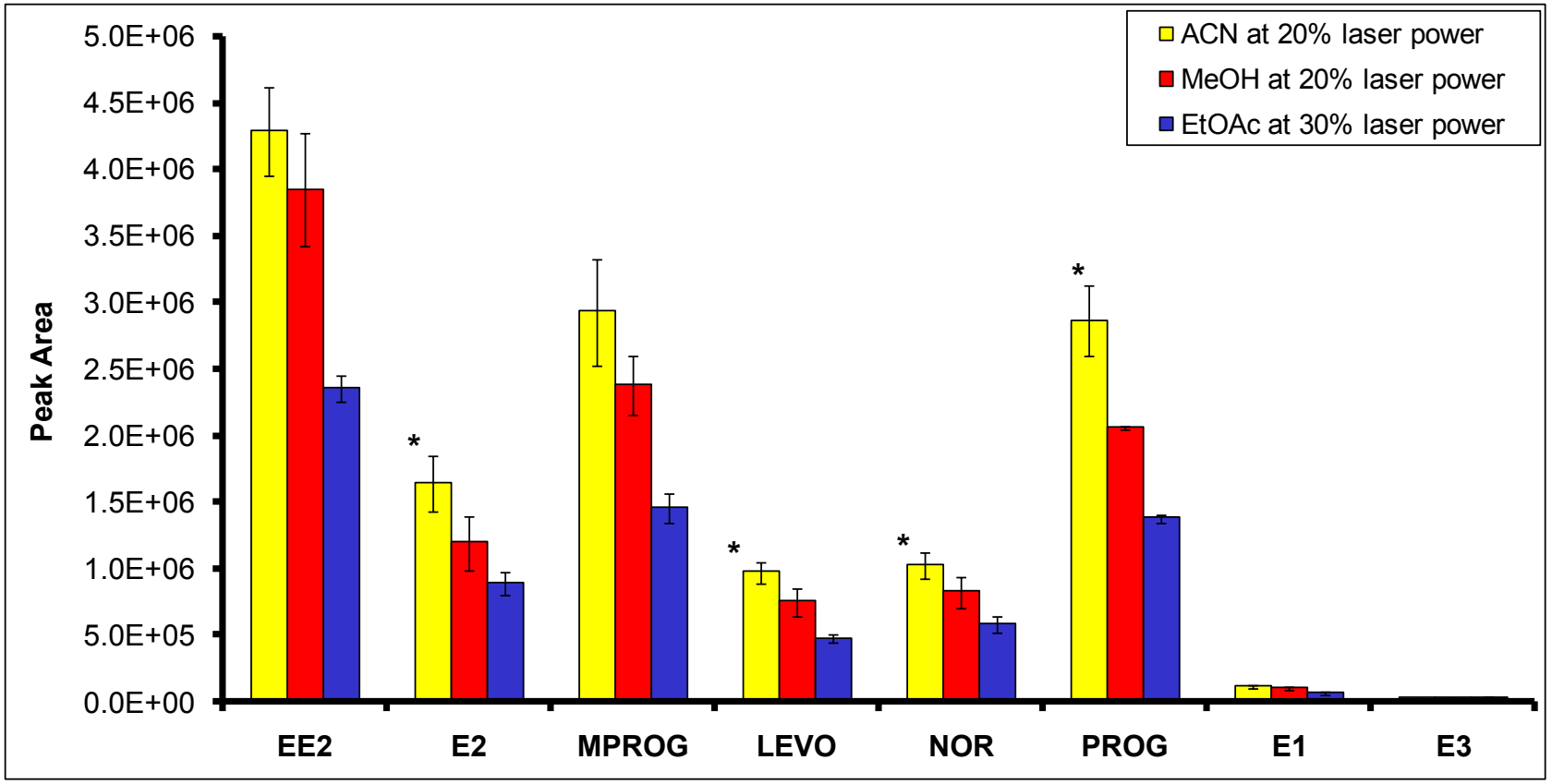
  → A minimum of 2 SRM transitions were selected + their ion ratios

- Parameters of the LDTD-APCI source are optimized for signal intensity :

  - ✓ solvent choice for analyte deposition in the well cavities
  - ✓ laser power (%)
  - ✓ carrier gas flow rate (L/min)
  - ✓ mass deposition (deposition volume in µL) into plate well
  - ✓ laser pattern

LDTD Optimization

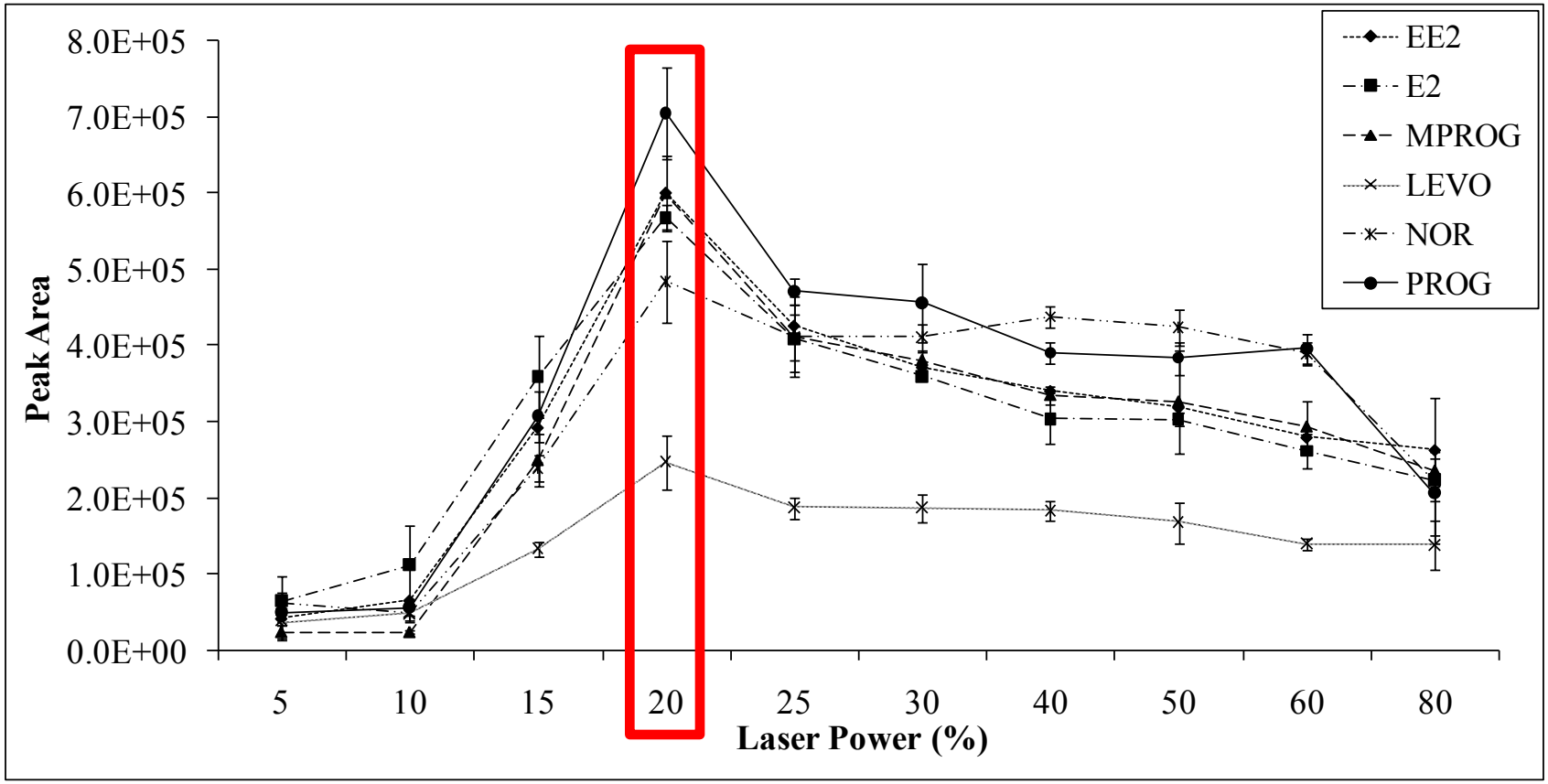
1) Solvant choice for deposition: example for hormones in NI and PI mode



Université de Montréal

LDTD Optimization

2) Laser power (%): **example for hormones in PI mode**

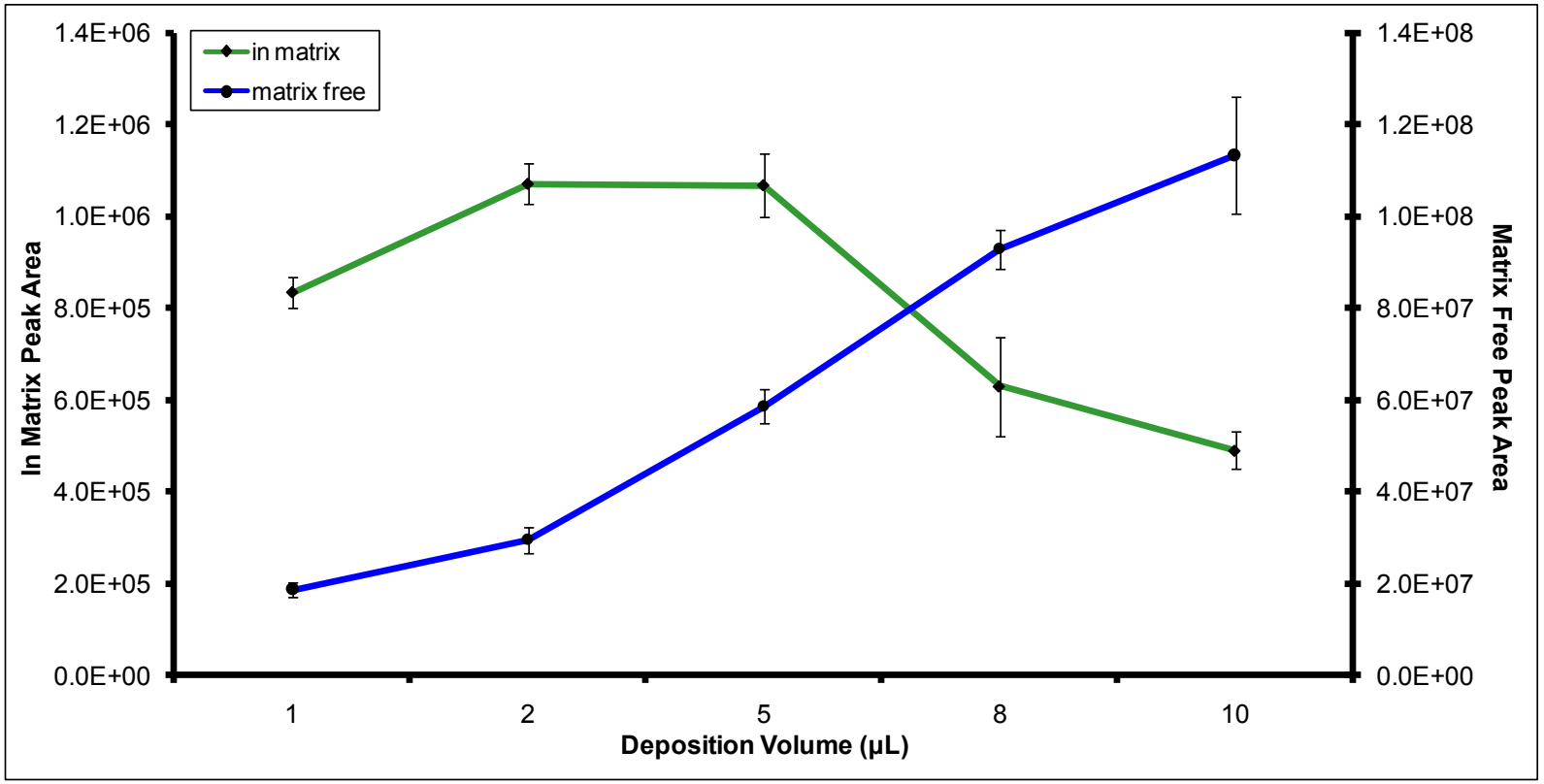


- Optimal laser power is 20%.

Université de Montréal

LDTD Optimization

4) Deposition volume (µL): example for PROG in PI mode

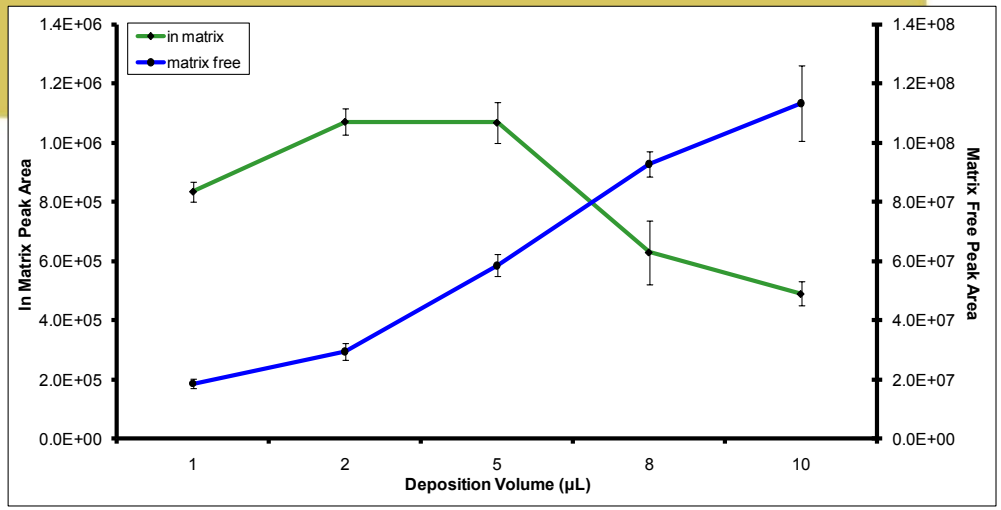


- A clean-up step in sample preparation or a sample dilution will allow for higher S/N ratios, improving the overall sensitivity and reducing the LD of the method.

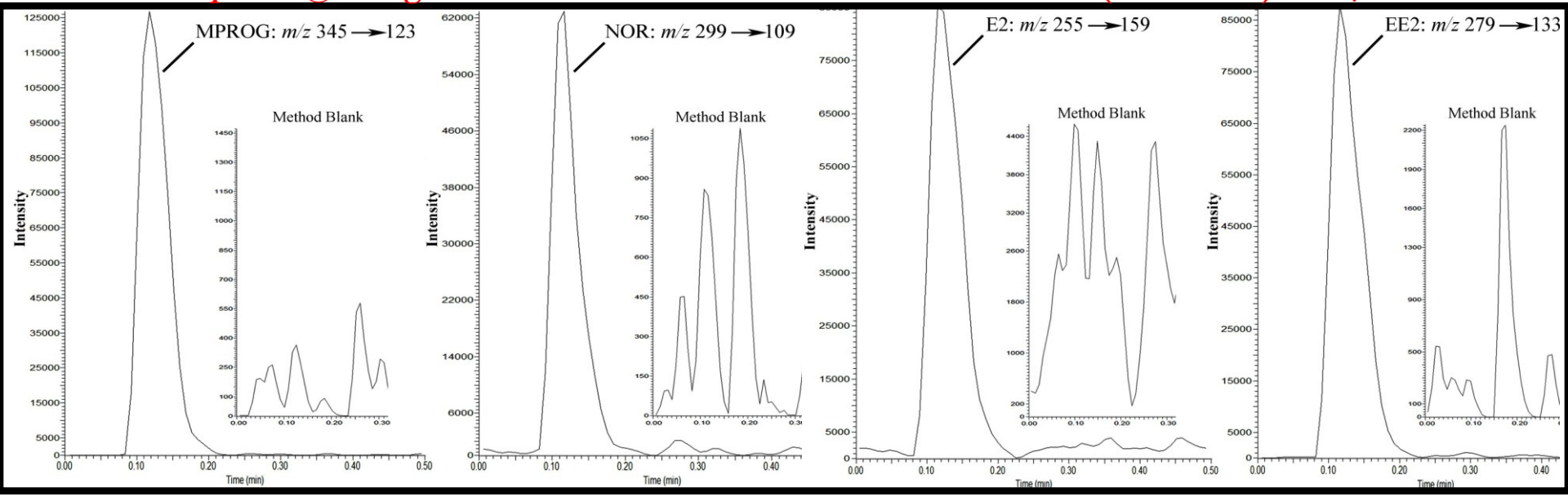
Université de Montréal

LDTD-MS/MS

- ✓ Complex matrices lower the intensity
- ✓ Sometimes need an important wash procedure for LDTD-MS/MS
- ✓ No separation (no chromatography)



Wastewater spiked @ 30 ng/L of hormones → manual SPE with 500 mL (CF = 2000) → 4 µL into well



Référence: Fayad B.P., *Anal .Chem.*, 2010 (82), 639.

Results for Feminizing Hormones

Compound	Ionisation Mode	Matrix-free				In matrix		
		Linearity range (µg/L)	Sensitivity	R^2	ILD (µg/L)	Sensitivity	R^2	MDL (ng/L)
E1	NI	9 - 684	0.0421	0.9999	9	0.0129	0.9984	30
E2	NI	18 - 588	0.0084	0.9998	10	0.0030	0.9972	36
	PI	18 - 588	0.0384	0.9995	5	0.0035	0.9997	13
E3	NI	80 - 749	0.0038	0.9986	24	0.0016	0.9977	30
EE2	NI	39 - 915	0.0062	0.9993	17	0.0019	0.9946	42
	PI	57 - 915	0.0178	0.9990	8	0.0024	0.9988	14
LEVO	PI	47 - 780	0.0128	0.9990	11	0.0025	0.9965	20
MPROG	PI	35 - 677	0.0630	0.9990	6	0.0098	0.9964	30
NOR	PI	41 - 634	0.0144	0.9989	9	0.0031	0.9950	25
PROG	PI	21 - 670	0.0320	0.9995	6	0.0080	0.9971	18

➢Fayad P, Prévost M, Sauvé S. 2010. Laser Diode Thermal Desorption-Atmospheric Pressure Chemical Ionization Tandem Mass Spectrometry Analysis of Selected Steroid Hormones in Wastewater: Method Optimization and Application. *Analytical Chemistry* 82:635-645

Université de Montréal

Disadvantages of LDTD versus LC

- ✓ Very polar analytes (conjugated drug metabolites) or higher mass analytes (e.g. proteins easily ionized with ESI) can be tough to ionize by APCI.
- ✓ Limits of detection are not as good – may sometime need SPE

- **Solutions to this and other difficulties:**

1) No chromatographic separation (no column is used to separate same masses in time) → Could de resolved by using two ionization modes (PI and NI)

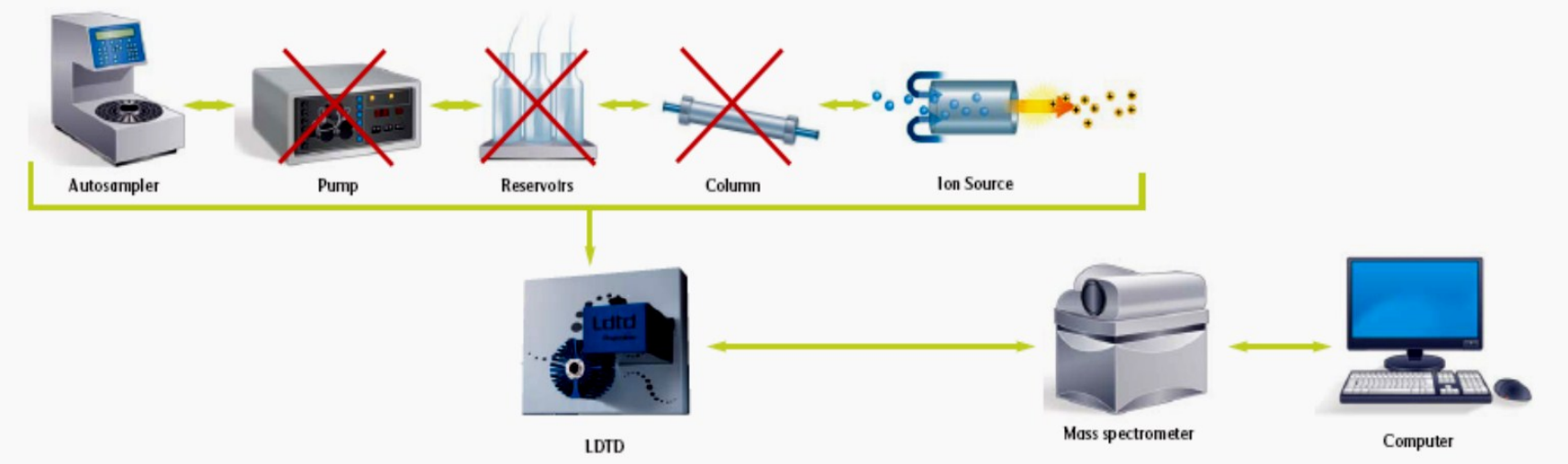
2) Complex matrices can reduce signal intensity due to competitive interfering compounds in the APCI region → Must utilize a proper washing step in sample preparation or try sample dilution

3) Polar or higher mass analytes usually more suitable for ESI type analyses → Different solvants can be used to improve signal response and use derivatization

Université de Montréal

Advantages of the LDTD versus LC:

- ✓ Analysis is done in 15 sec opposed to several minutes in LC-MS/MS
- ✓ Small sample volume (1-10 µL) compared to 1+ mL for *on-line* SPE LC-MS/MS
- ✓ Fewer sources of contamination (tubing, column, injection loop or port, divert valve)
- ✓ Lower backround noise resulting from solvant use
- ✓ Lower operation costs (solvants, columns, tubing, syringes, pumps)



Source: www.ldtd-ionsource.com/fra/default.asp

Université de Montréal

LDTD

- Ultrafast alternative for some compounds
- Some limited separation capabilities
- Not very sensitive for ultratrace analysis in water
- Seems ideal for solids where sample preparation is required

Utiliser le SBSE en éluant pour analyses avec LDTD + CD

Reagents	Stir bar 1	Stir bar 2	Stir bar 3	Stir bar 4
β-CD	0 mg	0 mg	90 mg	90 mg
Dimethylsulfoxide	0 µL	0 µL	0 µL	100 µL
OH-PDMS	900 mg	900 mg	900 mg	900 mg
PTMS	180 mg	180 mg	180 mg	180 mg
TEOS	0mg	90 mg	90 mg	90 mg
TMSPMA	180 mg	180 mg	0 mg	0 mg
GPTMOS			180 mg	180 mg
PMHS			90 mg	90 mg
Dichloromethane			1350 µL	1350 µL
TFA (95% in water)	225 µL	225 µL	225 µL	225 µL

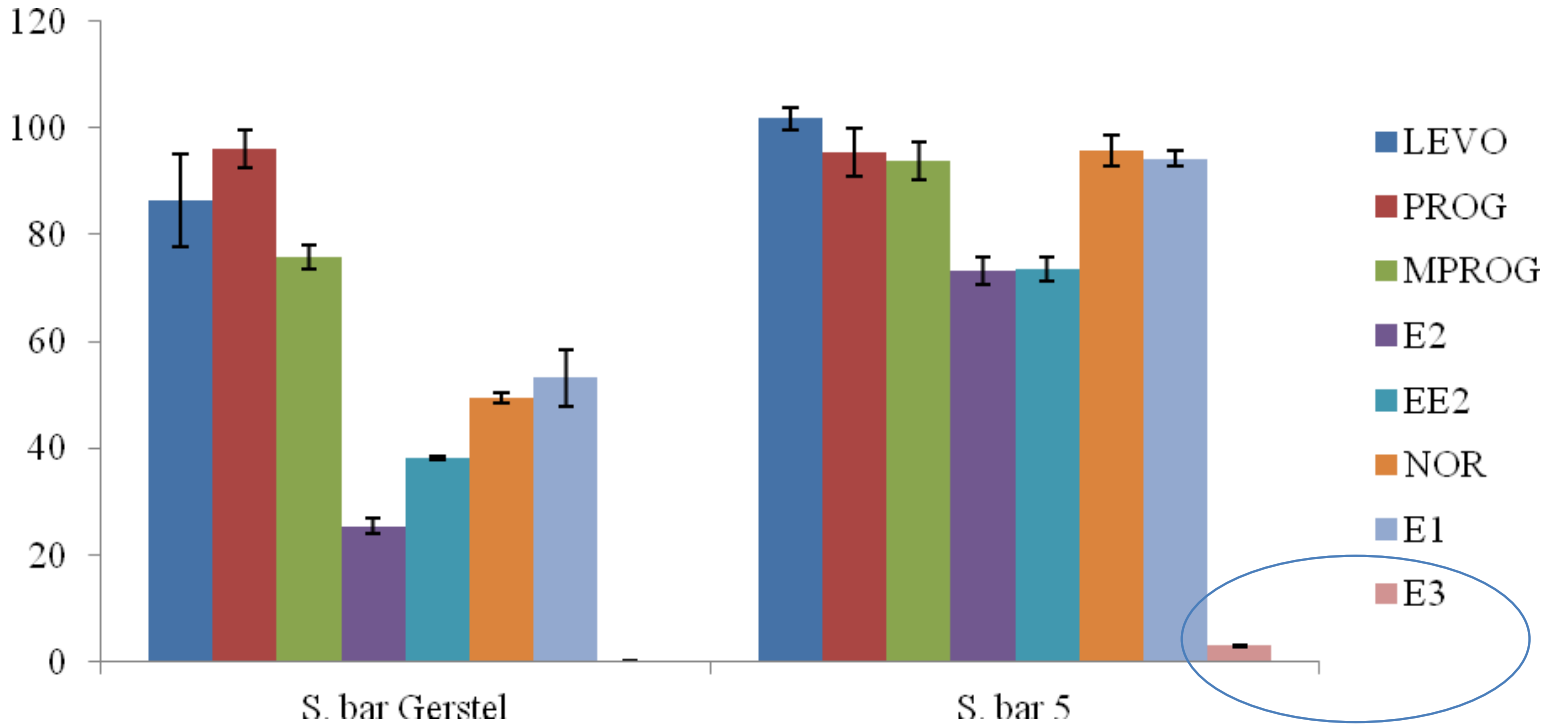
Université de Montréal

Standard SBSE vs. « house blend » SBSE + cyclodextrin

- Optimised for:
  - Extraction (+ added NaCl)
  - Desorption period
  - Desorption volume
  - Best recoveries

Université de Montréal

Standard SBSE vs. « house blend » SBSE + cyclodextrin



Extraction recoveries (%) of 8 hormones in pure water with Gerstel stir bar and stir bar 5 (modified Gerstel stir bar with in-house coating)

Université de Montréal

Propriétés des hormones testées

Hormones	Molecular mass (g/mol)	$LogK_{o/w}$ [a]	pK_a
E1	270.4	3.13	10.7
E2	272.4	4.01	10.7
E3	288.4	2.81	10.4
EE2	296.4	3.67	10.4
LEVO	312.4	3.48	NA
MPROG	344.5	3.50	NA
NOR	298.4	2.97	NA
PROG	314.5	3.87	---

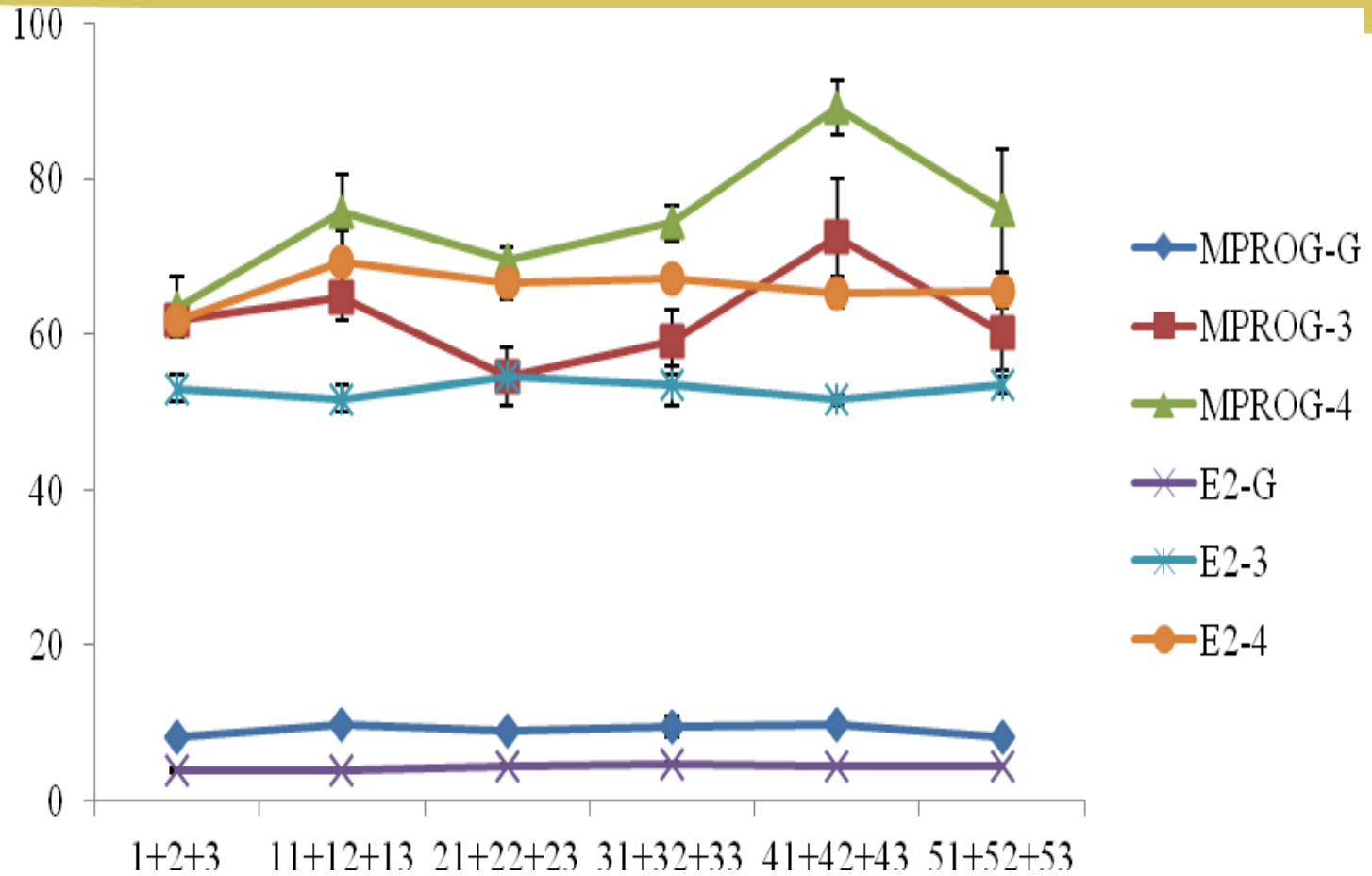
Université de Montréal

Standard SBSE vs. « house blend » SBSE + cyclodextrin

- Tested:
  - Repeatability (performances of multiples home-made bars made using the same protocol)
  - Stability (repeated extractions of same bar)
  - Using real matrices (tap and wastewater)

Université de Montréal

Recouvrements successifs pour SBSE modifié avec ajout de cyclodextrine



Lifetime of three stir bars from batch C for 53 extractions for two hormones (each point is a mean of three consecutive extractions)

Talanta 101 (2012) 337–345

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Using a novel sol–gel stir bar sorptive extraction method for the analysis of steroid hormones in water by laser diode thermal desorption/atmospheric chemical ionization tandem mass spectrometry

S. Vo Duy [a], P.B. Fayad [a], B. Barbeau [b], M. Prévost [b], S. Sauvé [a,*]

[a] Department of Chemistry, Université de Montréal, Montreal, Quebec, Canada
[b] Department of Civil, Geological and Mining Engineering, École Polytechnique de Montréal, Montreal, Quebec, Canada

ARTICLE INFO

Available online 28 September 2012

Keywords:
Stir bar sorptive extraction
Polydimethylsiloxane/phenyltrimethylsiloxane/β-cyclodextrin
Sol–gel

ABSTRACT

A new coating material was used for a stir bar sorptive extraction (SBSE) method coupled to a high throughput sample analysis technique. This allowed for a simple procedure for fast determinations of eight steroid hormones (estriol, estradiol, ethynylestradiol, estrone, progesterone, medroxyprogesterone, levonorgestrel, northindrone) in water. Sample pre-treatment was performed using an in-house SBSE method based on a polydimethylsiloxane/phenyltrimethylsiloxane/β-cyclodextrin sol–gel material. The analytes were desorbed by liquid extraction prior to their analysis by laser diode thermal

Université de Montréal

Questions ou pdf?

sebastien.sauve@umontreal.ca

Thermo SCIENTIFIC

Phytronix Technologies

Fonds de recherche sur la nature et les technologies Québec

CRSNG NSERC

CHAIRE INDUSTRIELLE CRSNG EN EAU POTABLE

INNOVATION.CA

CANADA FOUNDATION FOR INNOVATION | FONDATION CANADIENNE POUR L'INNOVATION

Université de Montréal