Mass Spectrometry in endocrinology, beyond Steroids

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IFCC Satellite Meeting on Mass Spectrometry

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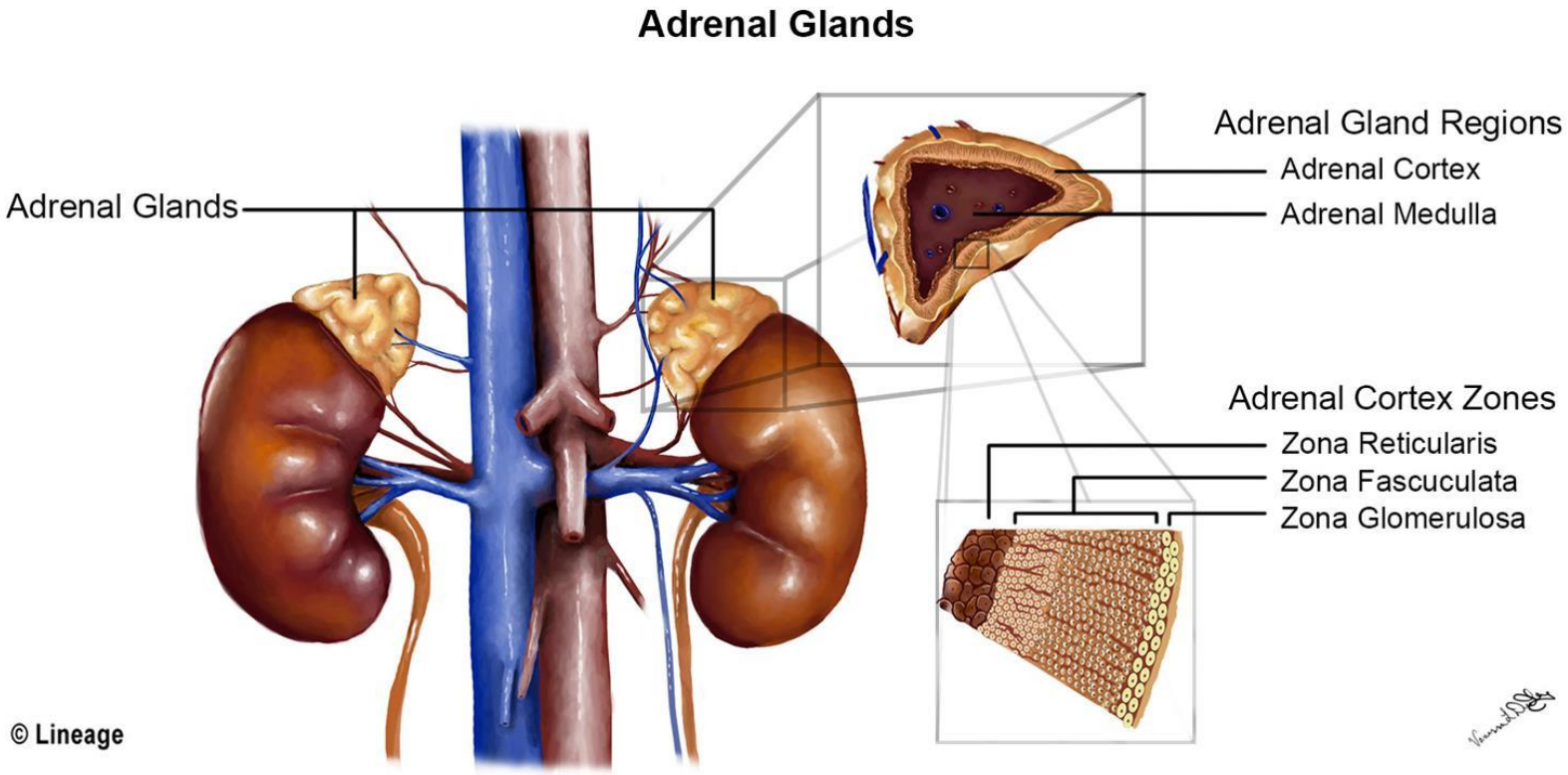
I declare no conflicts of interest

Steroid hormones

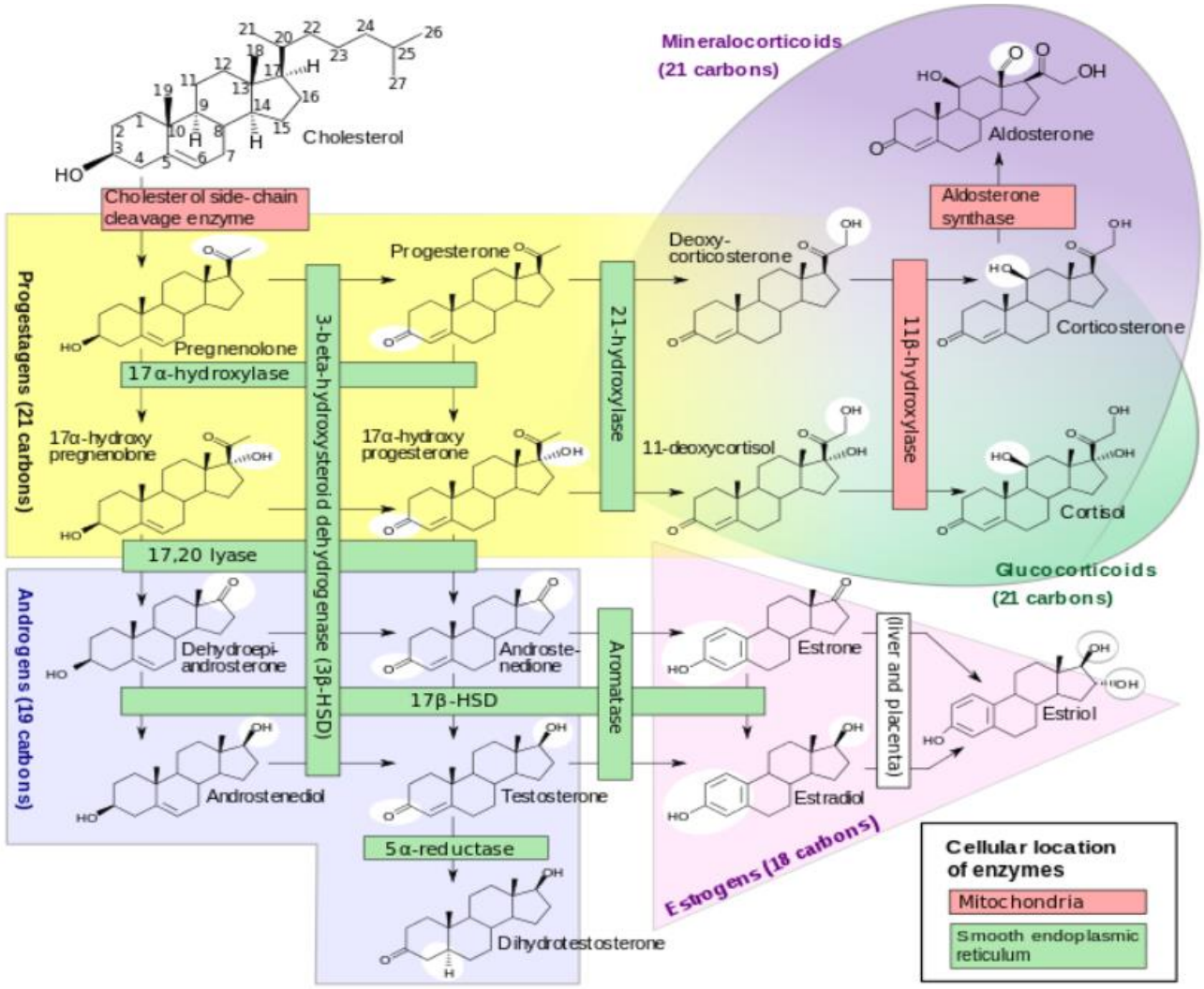
- Clinical chemistry lab→ many compounds (hormones…)
- Rosalyn Yalow and Solomon Berson: 1977-Nobel PRICE for the 1st assay by RIA for inulin→ revolution
- First LC-MS/MS methods in routine clinical diagnostics from the 70s
- LC-MS/MS is now routinely used in clinical practice

Steroid hormones



Source: https://step1.medbullets.com/endocrine/109001/adrenal-cortex-and-medulla

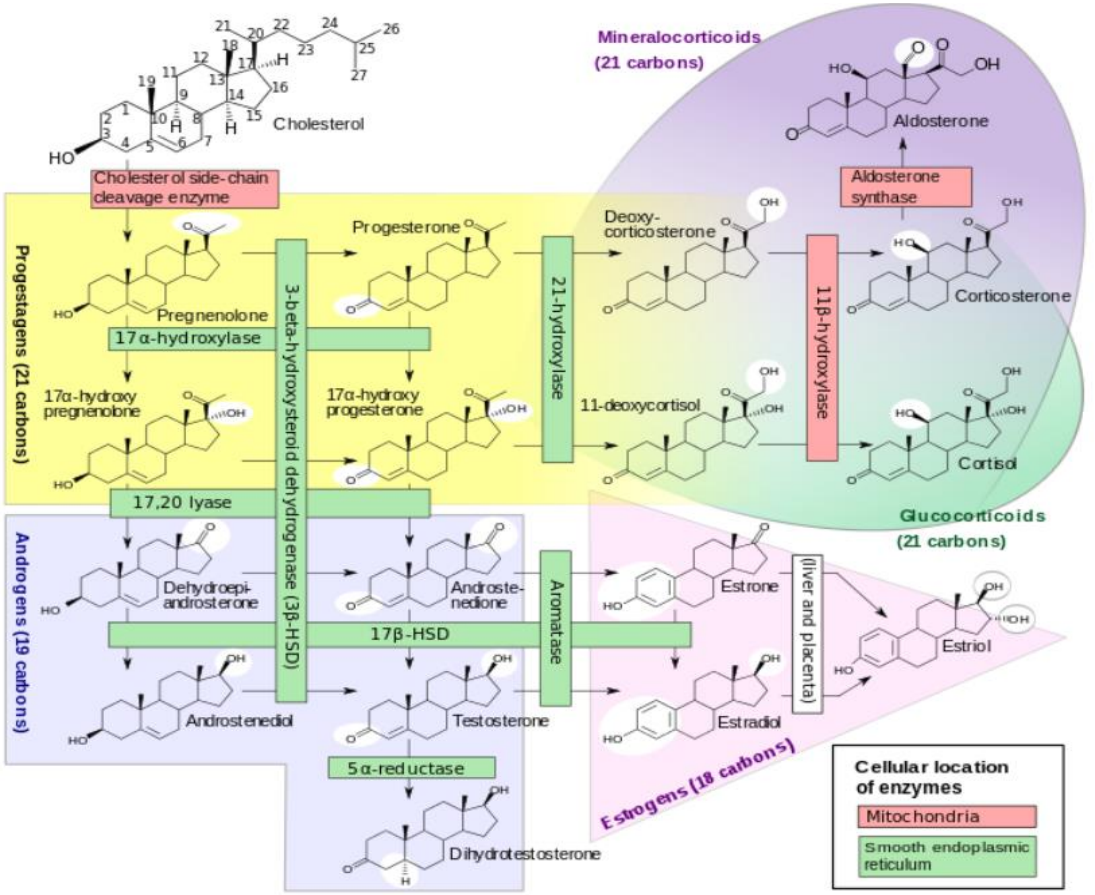
Steroid hormones



Steroid hormones

Crucial role : control of

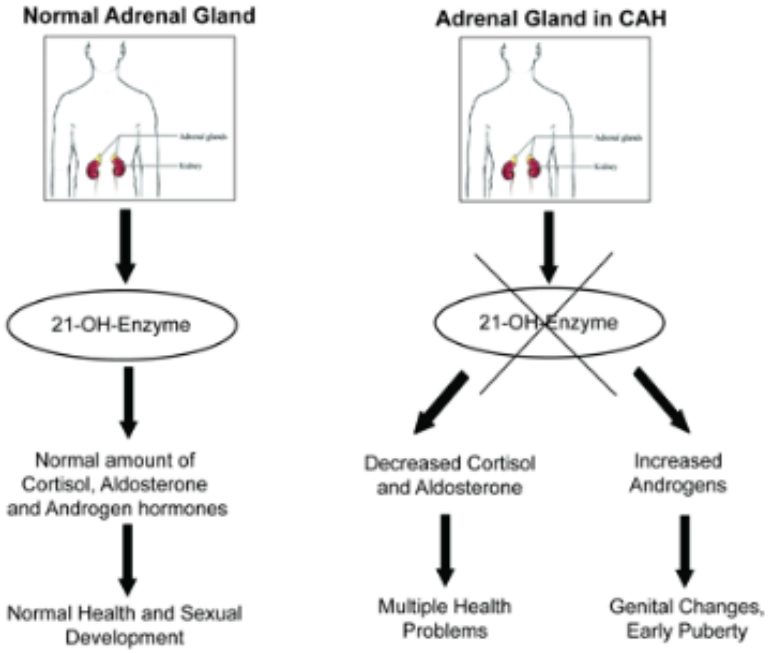
- metabolism
- immune functions
- inflammation



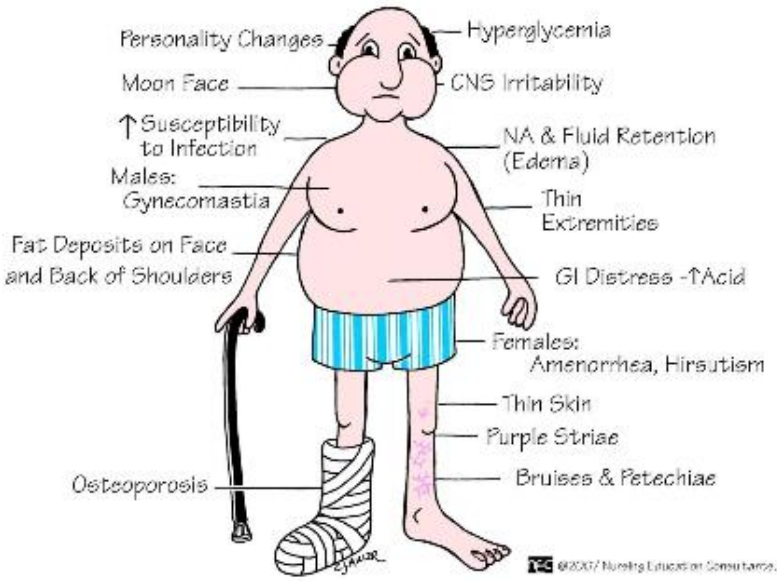
Steroid hormones

Modifications in steroid profiling reflect disease status and help research into a wide number of health disorders.

Congenital Adrenal Hyperplasia (CAH)



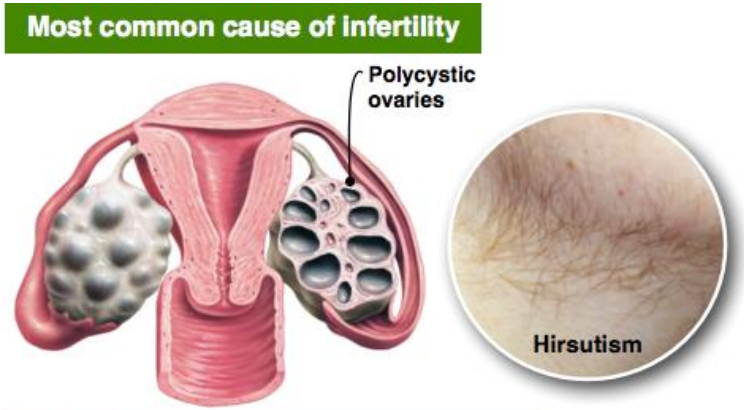
Cushing's syndrome



Steroid hormones

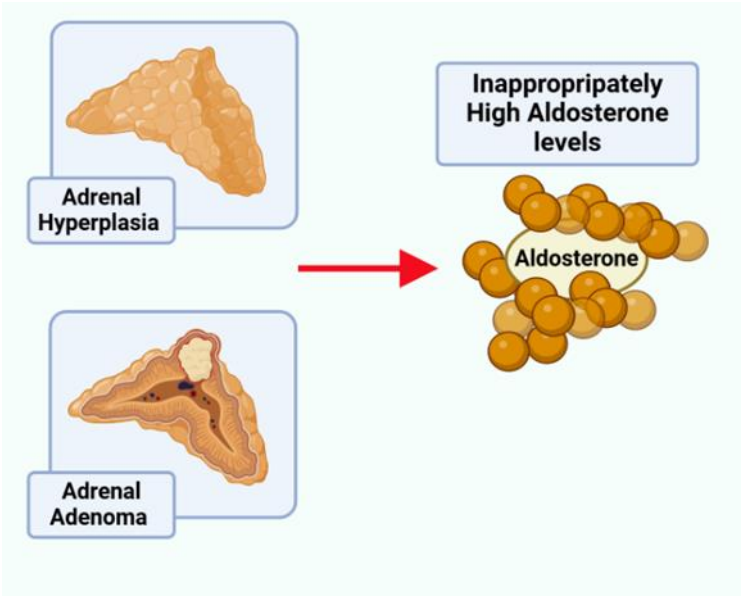
Modifications in steroid profiling reflect disease status and help research into a wide number of health disorders.

Polycystic Ovarian Syndrome



Clinical
- Hirsutism
- Menstrual irregularities
- Acanthosis nigricans
- Obesity
- Acne
- Insulin resistance and metabolic syndrome
- Sleep-disordered breathing
- Fatty liver disease

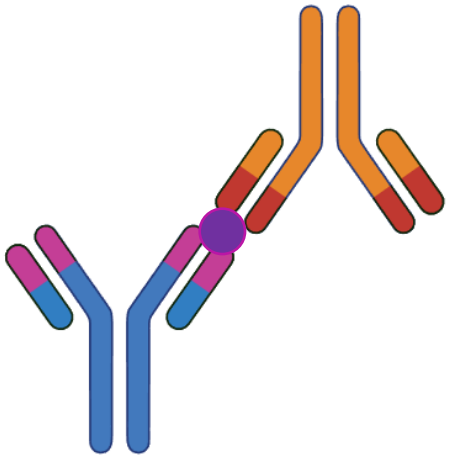
Hyperaldosteronism



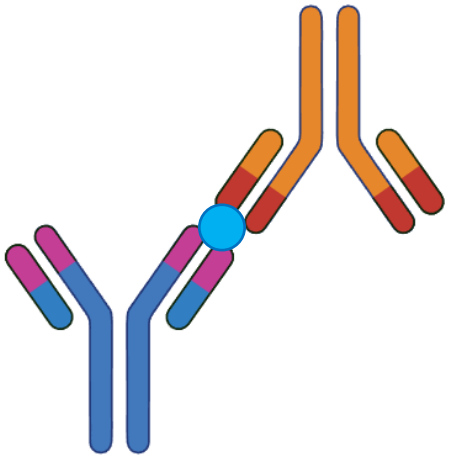
Steroid hormones

Quantification of steroids by immunoassays

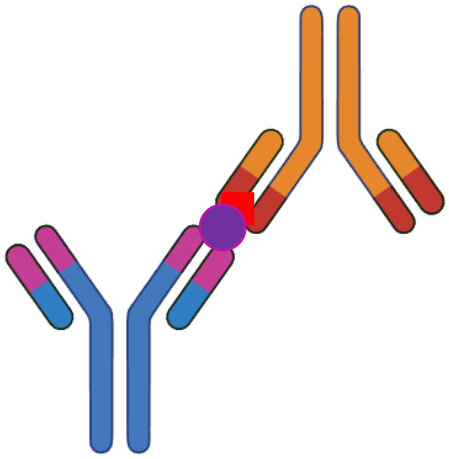
Specific interaction



Cross-reactivity



Interference



Clinical case

Mr K (28/12/75)

- Malaria attack ➡ RF grade V and hypertension (200/120 mmHg)
- GFR = 6 mL/min et Cr= 4.7 g/L
- Proteinuria = 3044 mg/L ➡ 700 mg/g Creatinuria
- Asthenia and headaches

Clinical case

Mr K (28/12/75)

- Hypertension (200/120 mmHg)

→ Aldosterone quantification

RIA > 1000 ng/L

Clinical case

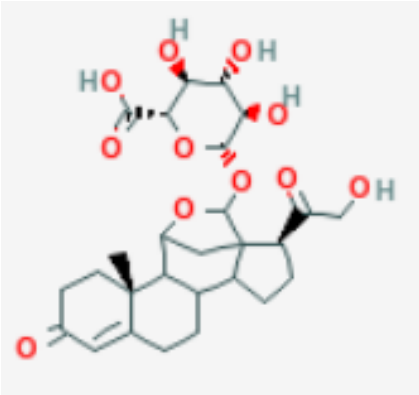
Mr K (28/12/75)

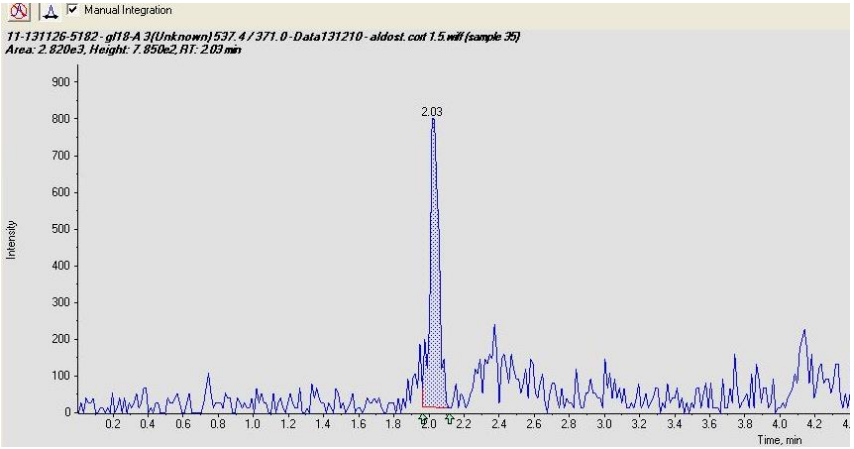
- Hypertension (200/120 mmHg)

→ Aldosterone quantification

RIA > 1000 ng/L vs LC-MS/MS = 66 ng/L (< 200 ng/L normal value)

➡ Presence of Aldosterone glucuronide

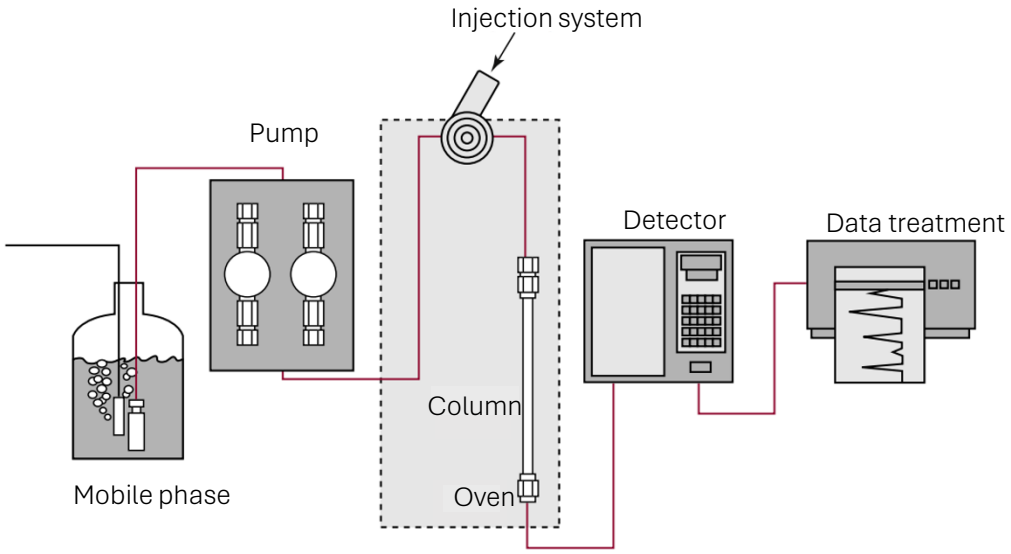


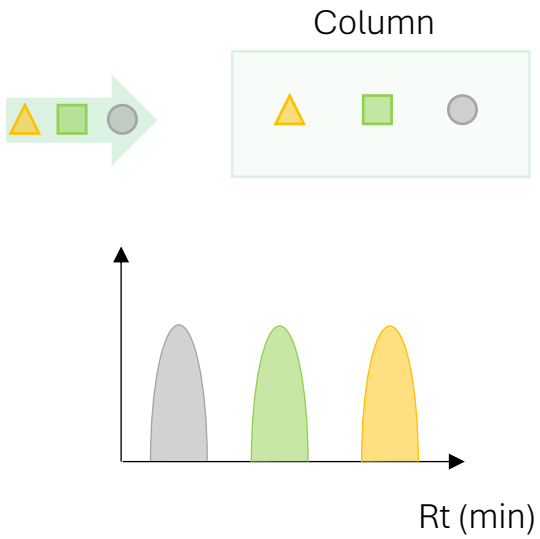


LC-MS/MS

1. Liquid chromatography

Separation technique of analyte based on the affinity for a stationary phase and a mobile phase.





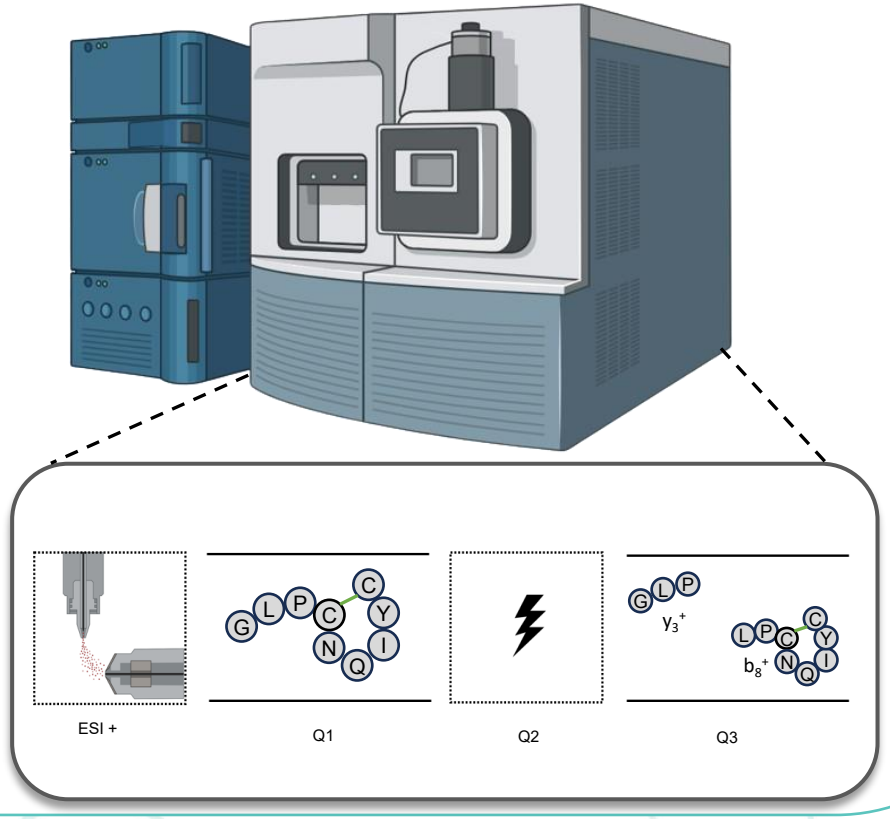
LC-MS/MS

2. Mass spectrometry

Analytical technique that ionizes chemical species contained in a sample and analyzes the produced ions in the gas phase.

Mass spectrometer

- Ion source (ionization of sample)
- Analyzer (*m/z* separation in time or in space)
- Detector (ion detection and generation of the mass spectrum)



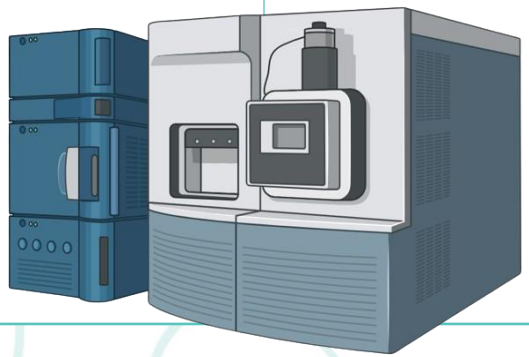
LC-MS/MS

Advantages

- High sensivity
- Specificity
- Reproducibility
- Ability to perform simultaneous analysis
- No variation LOT to LOT

Disadvantages

- Price
- Method development
- Technical aspect
- Noise and heat generation
- Nitrogen generator and compressed air or linked supply
- Review of peaks one by one
- Annual maintenance contract



IA versus LC-MS/MS

Criteria	*Immunoassays (IA/RIA)*	*Mass Spectrometry (MS)*
Principle	Uses antibodies to detect specific steroids	Separates and detects molecules based on mass-to-charge ratio
Sensitivity	Good	Excellent
Specificity	**Moderate to low** (cross-reactivity is common)	**High** (distinguishes structurally similar compounds)
Interferences	Frequent (e.g., heterophilic antibodies, rheumatoid factor)	Rare (if sample prep is adequate)
Precision / Accuracy	Variable; depends on antibody quality	Very high
Multiplexing capability	Limited (typically one analyte per assay)	Yes (can measure multiple steroids simultaneously)
Cost & Accessibility	Lower cost; widely used in routine labs	Higher cost; increasingly available
Typical errors	**Underestimation or overestimation** due to interferences	Fewer errors; requires technical expertise

Lab-developed test
or
Ready-to-use kit?

Ready-to-use kit

15 steroids separated in 2 panels

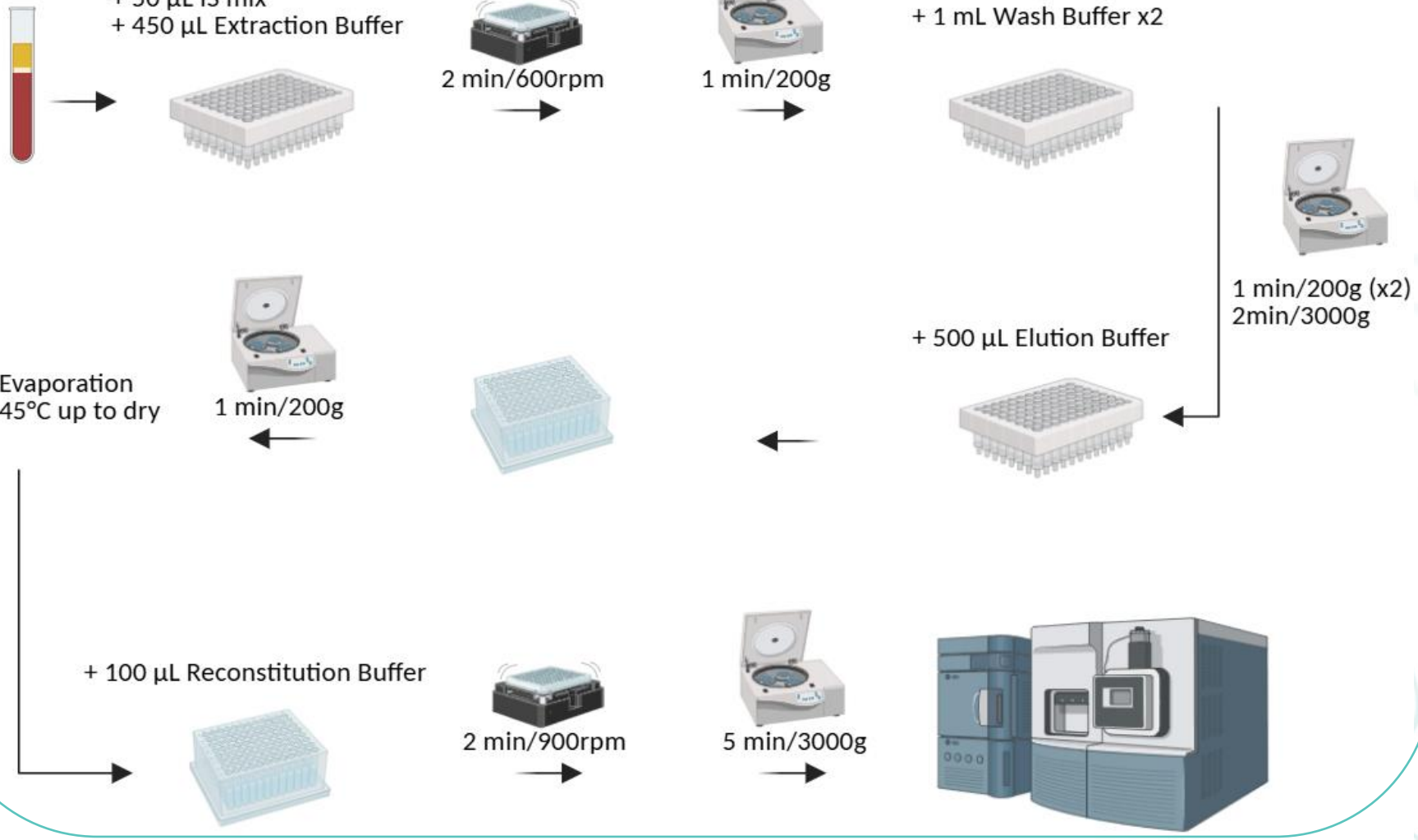
Panel 1

- Aldosterone
- Cortisol
- Cortisone
- Corticosterone
- 11-deoxycortisol (S)
- 21-desoxycortisol

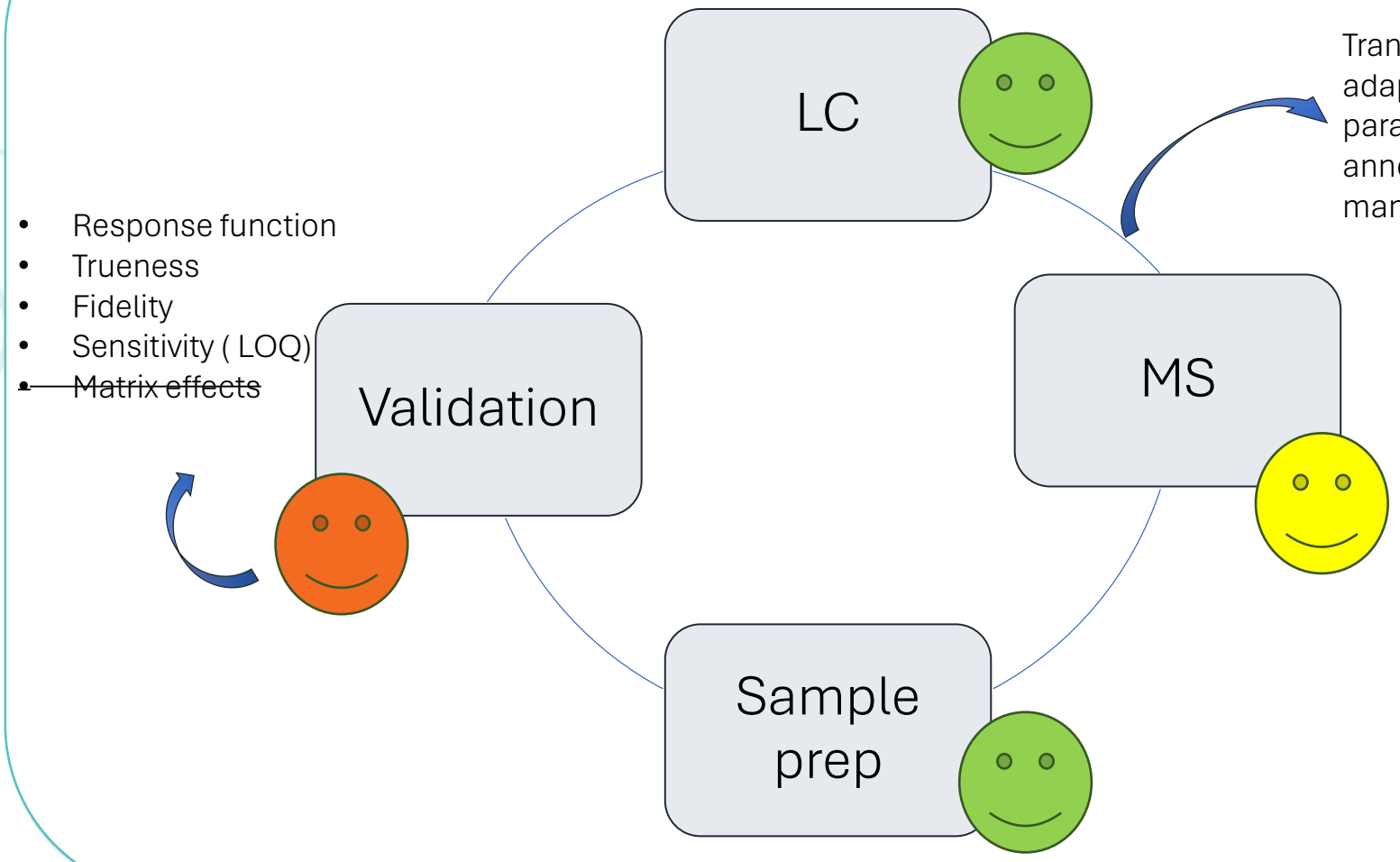
Panel 2

- Androstenedione
- Dehydroepiandrosterone
- Dehydroepiandrosterone sulfate
- 11-desoxycorticosterone
- Estradiol (E2)
- 17 α-hydroxyprogesterone
- Progesterone
- Testosterone
- Dihydrotestosterone

LC-MS/MS : Sample preparation



Ready-to-use kit



Analytical evaluation

- A calibration curve: 6 points➔ responses determined by calculating the integrated peak area ratio between endogenous steroids to deuterated steroids.
- Precision (intra-assay and inter-assay) and an accuracy profile were established➔ n=3 in triplicate, 3 days.
- Trueness was calculated with the expected values of the internal control ➔ n=3 in triplicate, 3 days.
- The limit of quantification (LOQ) was calculated with the lowest concentration that we tested.
- Correlation with other devices.

Analytical evaluation Results

Substance		Precision						Desirable specification EFLM
		Intra-assay			Inter-assay			
		Level 1	Level 2	Level 3	Level 1	Level 2	Level 3	
aldosterone	Concentration (µg/L)	0,10	0,25	1,03	0,10	0,25	1,03	
	CV(%)	**2,6**	**3,8**	**2,3**	**2,6**	**4,5**	**3,9**	18,30%
corticosterone	Concentration (µg/L)	0,83	3,99	30,30	0,83	3,99	30,30	
	CV(%)	**2,8**	**6,4**	**2,7**	**3,3**	**11,1**	**9,0**	15%
cortisol	Concentration (nmol/L)	71,2	170	494	71,2	170	494	
	CV(%)	**1,4**	**3,1**	**2,8**	**2,8**	**3,6**	**3,6**	8,15%
cortisone	Concentration (µg/L)	2,06	12,3	29,3	2,06	12,3	29,3	
	CV(%)	**3,3**	**4,2**	**6,1**	**9,6**	**11,3**	**6,3**	15%
11-deoxycortisol	Concentration (µg/L)	0,26	1,38	9,14	0,26	1,38	9,14	
	CV(%)	**3,7**	**5,5**	**2,8**	**3,9**	**5,8**	**4,1**	10,70%
Androstenedione	Concentration (µg/L)	0,28	1,16	9,3	0,28	1,16	9,3	
	CV(%)	**10**	**1,8**	**3,1**	**10,5**	**5,9**	**3,8**	15%
DHEA	Concentration (µg/L)	1,99	11,6	38,5	1,99	11,6	38,5	
	CV(%)	**1,8**	**1,3**	**1,5**	**4,7**	**3**	**2,35**	15%
DHEAS	Concentration (µmol/L)	0,69	4,116	13,43	0,69	4,116	13,43	
	CV(%)	**2,7**	**2,6**	**2,2**	**4,8**	**2,6**	**2,2**	3,00%
Dihydrotestosterone	Concentration (µg/L)	0,08	0,37	1,06	0,08	0,37	1,06	
	CV(%)	**13,5**	**2,1**	**2,5**	**22,2**	**2,5**	**2,8**	5,80%
Estradiol	Concentration (ng/L)	80	390	2460	80	390	2460	
	CV(%)	**2,0**	**1,3**	**1,8**	**8,0**	**4,0**	**2,6**	7,50%
17-Hydroxyprogesterone	Concentration (µg/L)	0,31	1,53	9,01	0,31	1,53	9,01	
	CV(%)	**5,0**	**1,4**	**1,9**	**8,3**	**4,2**	**3,2**	14,15%
Progesterone	Concentration (µg/L)	0,32	3,26	15,6	0,32	3,26	15,6	
	CV(%)	**3,1**	**1,3**	**1,8**	**8,8**	**3,7**	**2,3**	9%
Testosterone	Concentration (nmol/L)	0,69	5,24	27,24	0,69	5,24	27,24	
	CV(%)	**3,3**	**1,2**	**1,9**	**5,7**	**4,2**	**3,4**	6,25%

Analytical evaluation Results

Substance		Precision						Desirable specification EFLM
		Intra-assay			Inter-assay			
		Level 1	Level 2	Level 3	Level 1	Level 2	Level 3	
aldosterone	Concentration (µg/L)	0,10	0,25	1,03	0,10	0,25	1,03	
	CV(%)	**2,6**	**3,8**	**2,3**	**2,6**	**4,5**	**3,9**	18,30%
corticosterone	Concentration (µg/L)	0,83	3,99	30,30	0,83	3,99	30,30	
	CV(%)	**2,8**	**6,4**	**2,7**	**3,3**	**11,1**	**9,0**	15%
cortisol	Concentration (nmol/L)	71,2	170	494	71,2	170	494	
	CV(%)	**1,4**	**3,1**	**2,8**	**2,8**	**3,6**	**3,6**	8,15%
cortisone	Concentration (µg/L)	2,06	12,3	29,3	2,06	12,3	29,3	
	CV(%)	**3,3**	**4,2**	**6,1**	**9,6**	**11,3**	**6,3**	15%
11-deoxycortisol	Concentration (µg/L)	0,26	1,38	9,14	0,26	1,38	9,14	
	CV(%)	**3,7**	**5,5**	**2,8**	**3,9**	**5,8**	**4,1**	10,70%
Androstenedione	Concentration (µg/L)	0,28	1,16	9,3	0,28	1,16	9,3	
	CV(%)	**10**	**1,8**	**3,1**	**10,5**	**5,9**	**3,8**	15%
DHEA	Concentration (µg/L)	1,99	11,6	38,5	1,99	11,6	38,5	
	CV(%)	**1,8**	**1,3**	**1,5**	**4,7**	**3**	**2,35**	15%
DHEAS	Concentration (µmol/L)	0,69			0,69	4,116	13,43	
	CV(%)	**2,7**			**4,8**	**2,6**	**2,2**	3,00%
Dihydrotestosterone	Concentration (µg/L)	0,08			0,08	0,37	1,06	
	CV(%)	**13,5**			**22,2**	**2,5**	**2,8**	5,80%
Estradiol	Concentration (ng/L)	80			80	390	2460	
	CV(%)	**2,0**	**1,3**	**1,8**	**8,0**	**4,0**	**2,6**	7,50%
17-Hydroxyprogesterone	Concentration (µg/L)	0,31						
	CV(%)	**5,0**						14,15%
Progesterone	Concentration (µg/L)	0,32						
	CV(%)	**3,1**						9%
Testosterone	Concentration (nmol/L)	0,69						
	CV(%)	**3,3**	**1,2**	**1,9**	**5,7**	**4,2**	**3,4**	6,25%

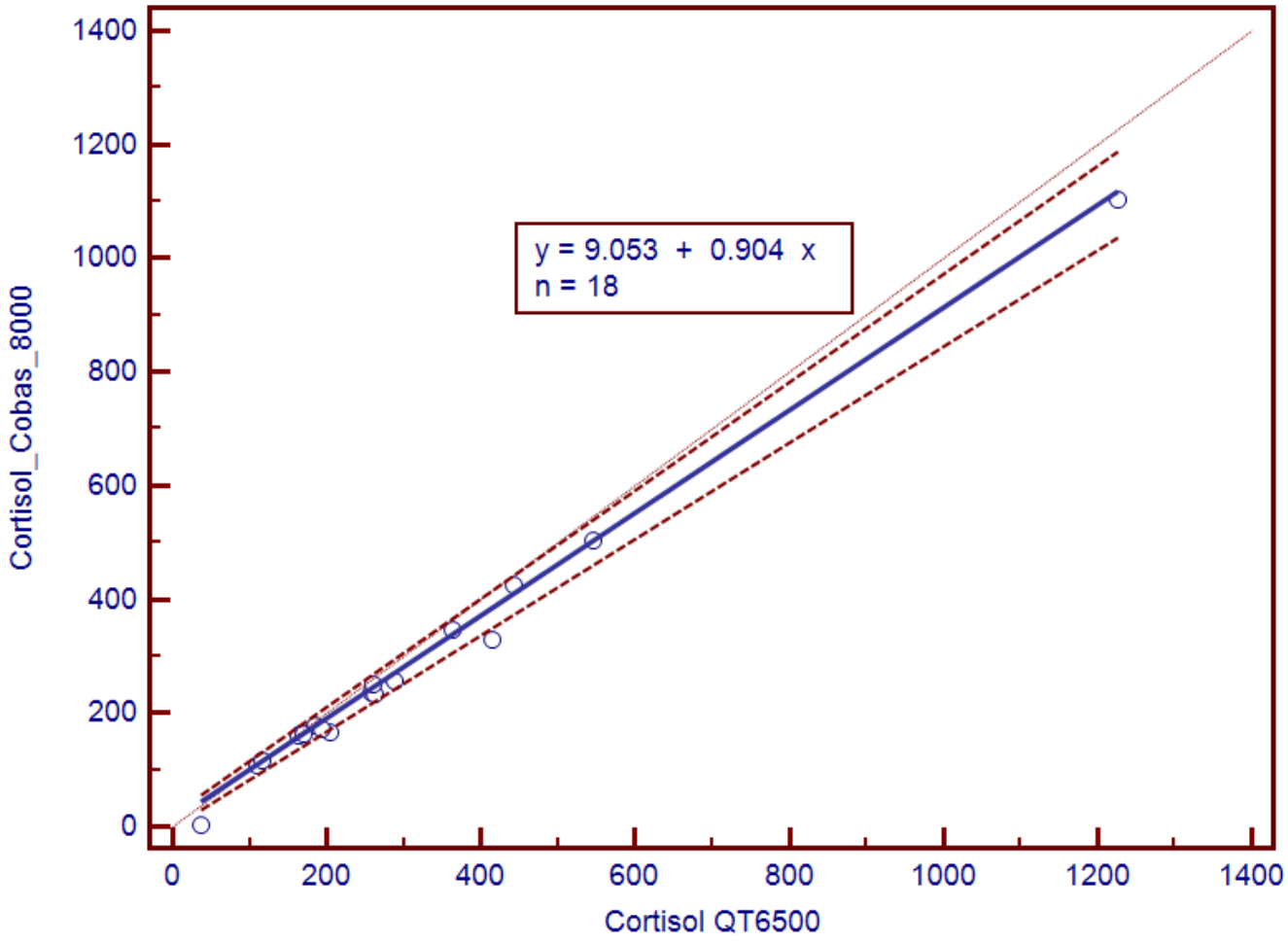
Cal 1 : 40 ng/L

Normal values : <6 ng/L (boys <9yo)

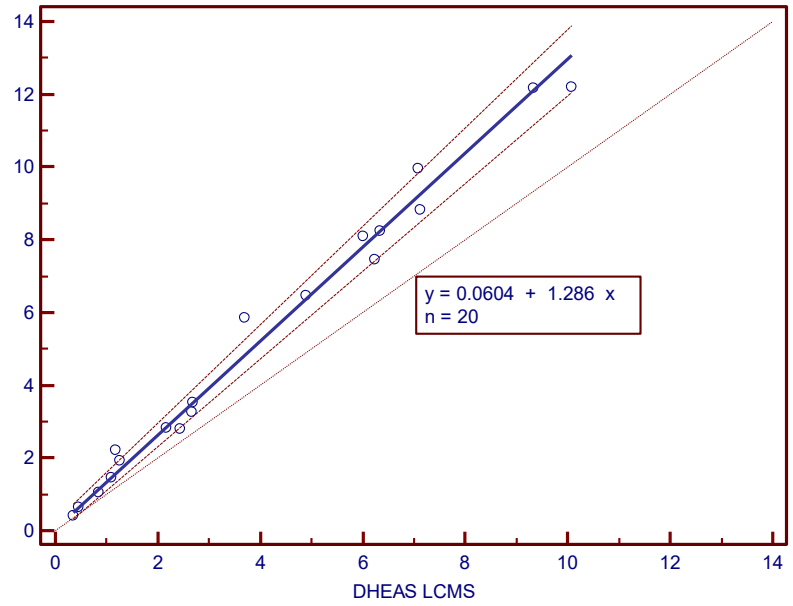
Analytical evaluation Results

Substance	Recovery(%) n=3. 3 days			Desirable biais (EFLM)
	Level 1	Level 2	Level 3	
aldosterone	0.09	0.23	0.90	
	-2.8	**0.8**	**2.1**	12.6%
corticosterone	0.85	4.18	28.80	
	-1.6	**-2**	**0.4**	**15%**
cortisol	68.7	163	469	
	-0.5	**-0.2**	**1.3**	12.8%
cortisone	2.08	12.4	30	
	-0.4	**-1.93**	**-0.13**	**15%**
11-deoxycortisol	0.31	1.49	9.27	
	-4	**-2**	**0.8**	**15%**
21-deoxycortisol	0.098	0.403	2.53	
	-2.4	**2.1**	**3.1**	**15%**
Androstenedione	0.278	1.09	8.95	
	-1.3	**1.5**	**2.3**	**15%**
DHEA	1.92	12.1	38.6	
	1.8	**2.1**	**2.6**	**15%**
DHEAS	0.735	4.185	12.999	
	-3.5	**-6.9**	**0.8**	5.5%
Dihydrotestosterone	83	392	1130	
	-1.1	**1.9**	**0.04**	9.7%
Estradiol	80	406	2560	
	0.8	**1.1**	**2.2**	5.0%
17-Hydroxyprogesterone	0.273	1.47	8.7	
	3	**2.8**	**2.4**	12.0%
Progesterone	0.299	3.05	15	
	-2.5	**1.6**	**1.6**	**11.0%**
Testosterone	0.721	5.27	27.9	
	0.4	**-1**	**-1.1**	13.0%

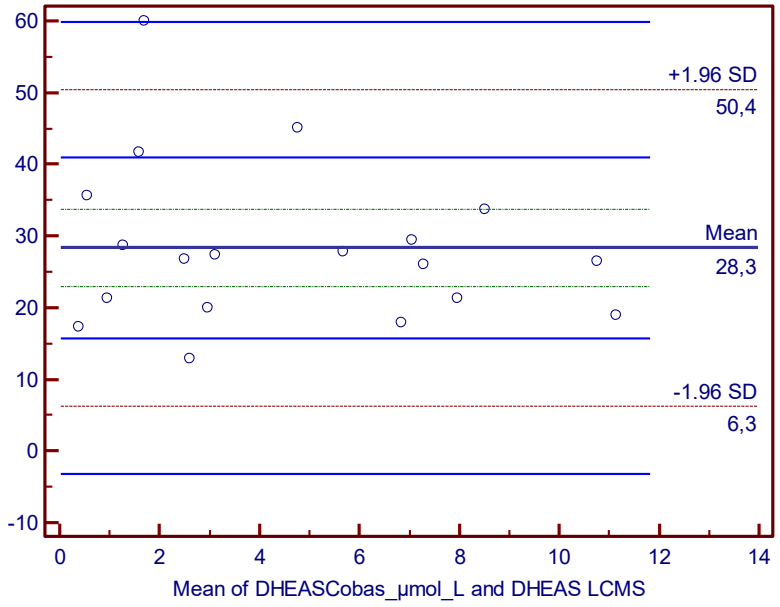
Correlation



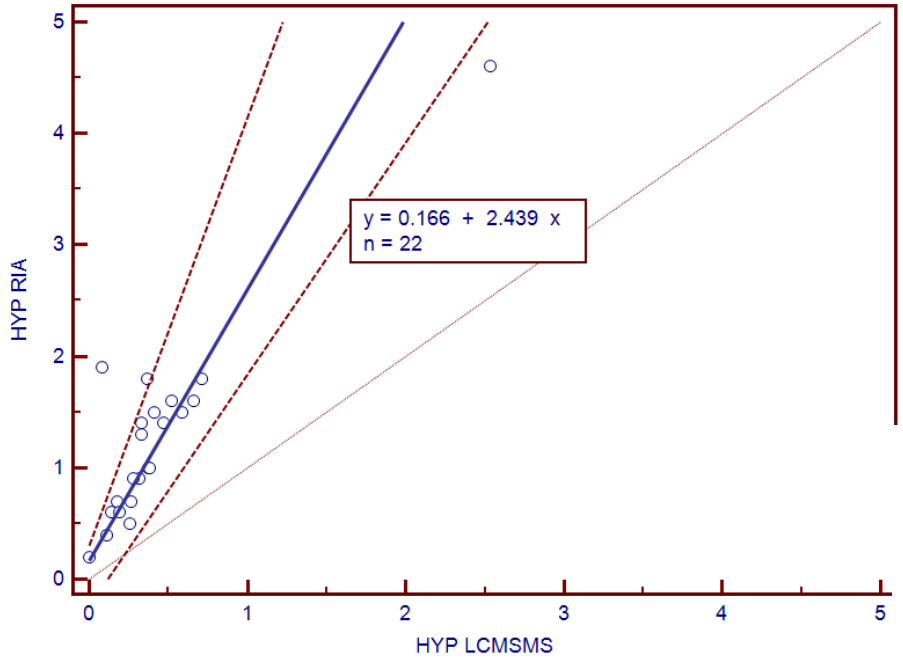
Correlation



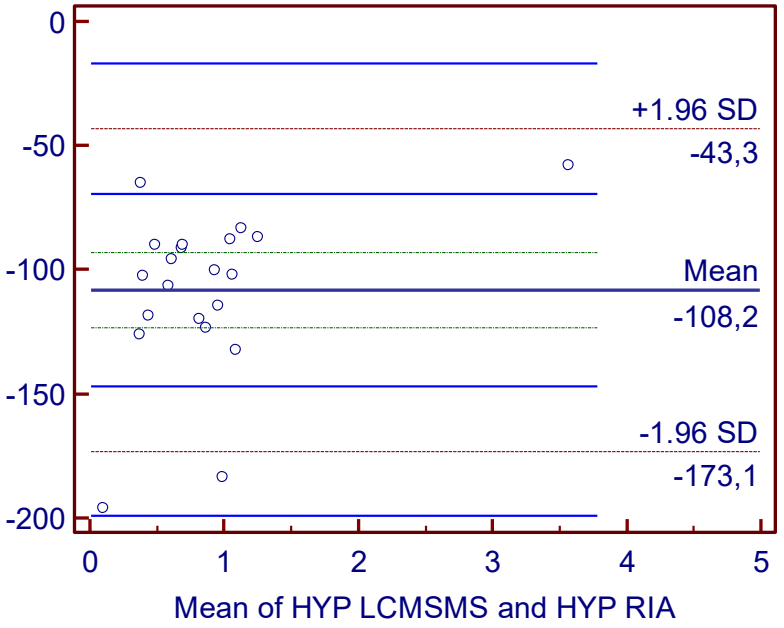
y = 0,0474608 + 1,291257 x	
Systematic differences	
Intercept A	0,04746
95% CI	-0,09749 to 0,2392
Proportional differences	
Slope B	1,2913
95% CI	1,2053 to 1,3534
Random differences	
Residual Standard Deviation (RSD)	0,2884
± 1.96 RSD Interval	-0,5652 to 0,5652
Linear model validity	
Cusum test for linearity	No significant deviation from linearity (P=0,66)



Correlation



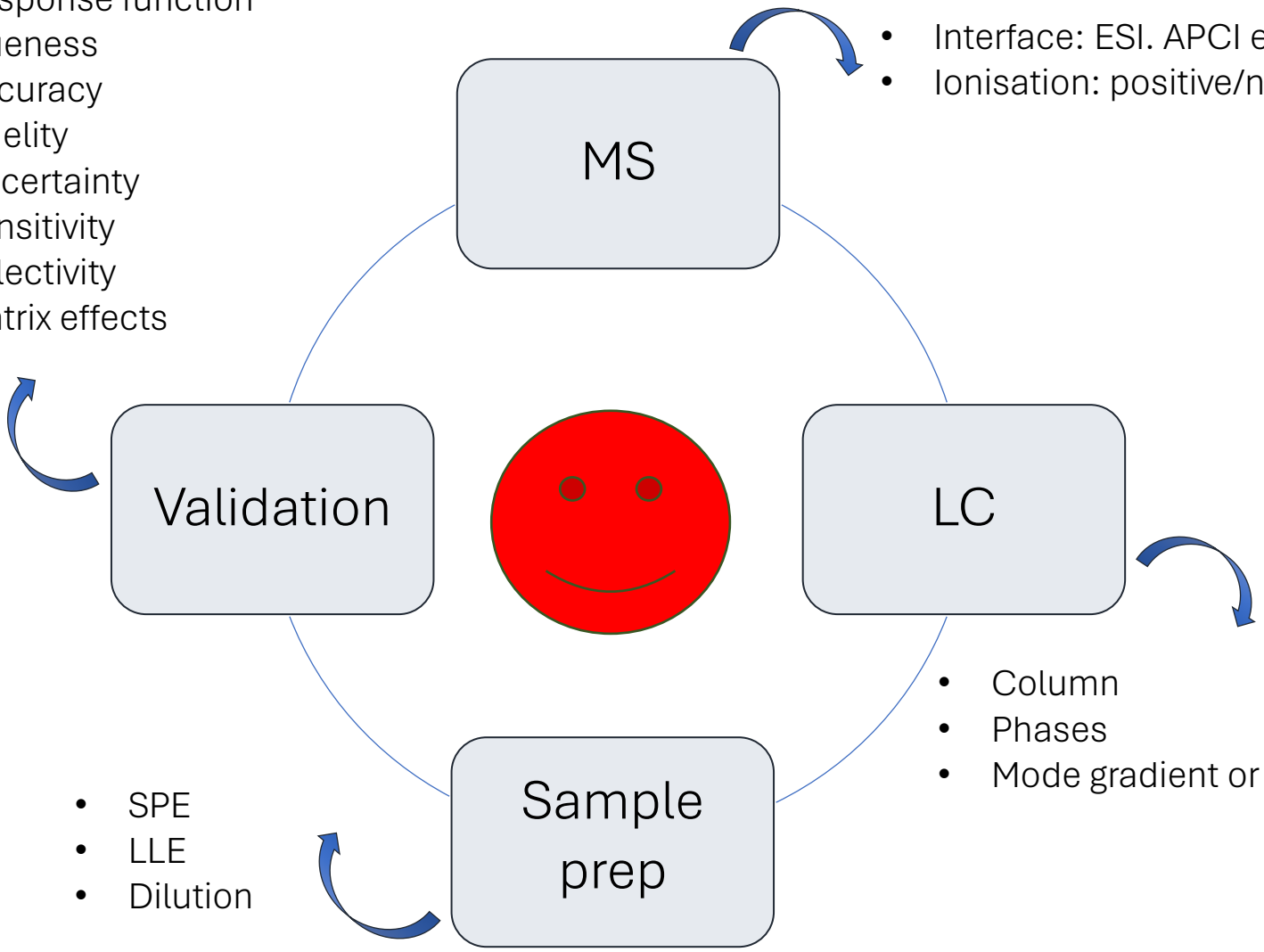
y = 0,195092 + 2,454080 x	
Systematic differences	
Intercept A	0,1951
95% CI	-0,3231 to 0,3274
Proportional differences	
Slope B	2,4541
95% CI	2,0045 to 3,8462
Random differences	
Residual Standard Deviation (RSD)	0,2235
± 1.96 RSD Interval	-0,4380 to 0,4380
Linear model validity	
Cusum test for linearity	No significant deviation from linearity



Lab-developed method

LDT method

- Response function
- Trueness
- Accuracy
- Fidelity
- Uncertainty
- Sensitivity
- Selectivity
- Matrix effects
- ....

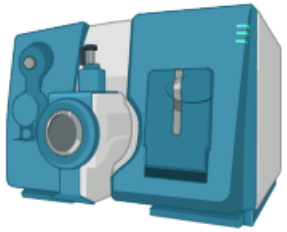


- Interface: ESI. APCI etc
- Ionisation: positive/negative

- Column
- Phases
- Mode gradient or not

- SPE
- LLE
- Dilution

LDT method



Precursor ions
Product ions
MRM parameters

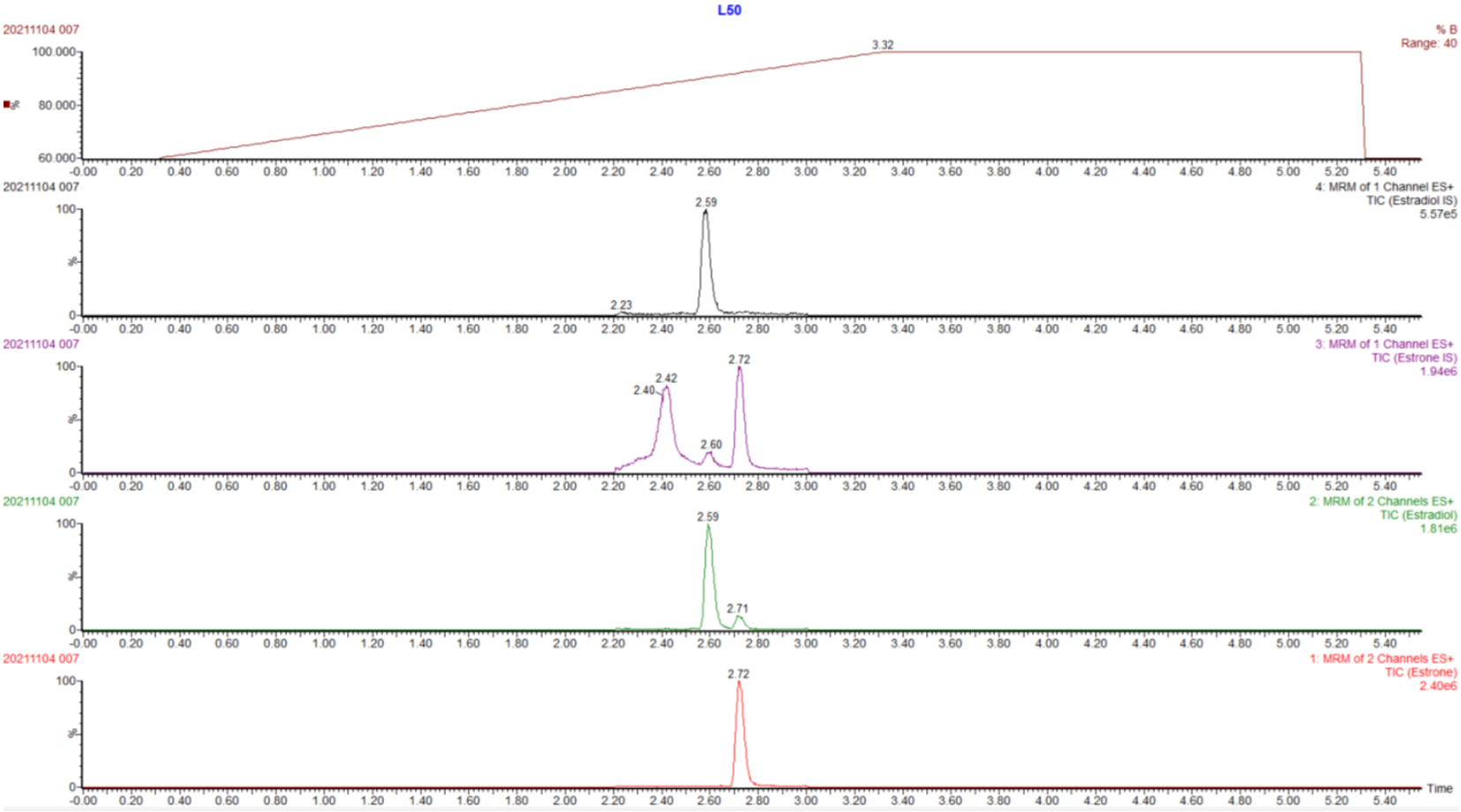
Phases
Gradient
Optimization of the separation

Extraction method development
SPE
LLE
...

Validation

Recovery	Accuracy
Matrix effect	Trueness
Linearity	Measurement
Carry over	uncertainty
LLOQ	...

Oestradiol and oestrone



Oestradiol and oestrone

- Precision (intra and inter-assay CV)

n= 8 (1-800 pg/mL) X 4 X 3 days

Concentration E1 (pg/mL)	Within-asssay CV (%)	Inter-assay CV (%)
1	15.6	21.7
2	8.8	17.3
4	3.2	3.2
20	2.9	4.1
40	4.1	4.1
200	2.6	6.1
400	4.6	4.6
800	1.9	3

The intra-assay CV did not exceed 4.6% and inter-assay CV 6.1% for E1

Concentration E2 (pg/mL)	Within-asssay CV (%)	Inter-assay CV (%)
1	9.2	13.2
2	6.4	19.1
4	6.9	7.6
20	1.7	3.6
40	4.5	5.4
200	3.1	4.2
400	3.5	4.6
800	1.9	1.9

The intra-assay CV did not exceed 6.9% and inter-assay CV 7.6% for E2 (EFLM= 7,5%)

- LOQ:
  E1= 3.3 pg/mL
  E2 = 3.7 pg/mL

Conclusion

- Experience is needed to develop an LDT method ➔ less for a kit
- +/- of the kits:
  - easiest BUT you don't know anything about the extraction plate, column, mobile phase…

    ➔ If there is a problem➔difficult to solve…
  - Price

Acknowledgments

My team: “Department of clinical chemistry in the University of Liege. Belgium”

CHU de Liège

CLINICAL CHEMISTRY ULiège

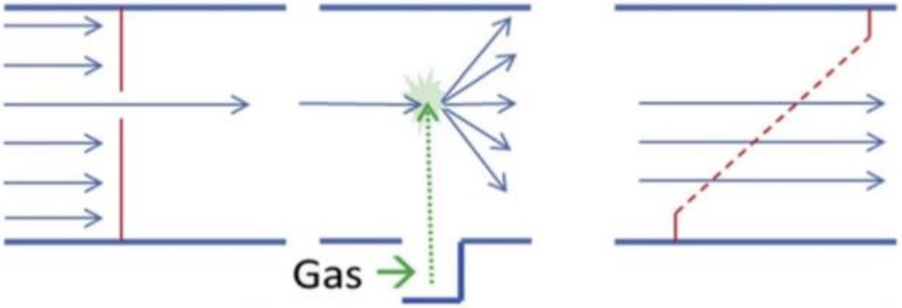
LIÈGE université

Unilab Lg.

3 modes

Full Scan
Q1 and Q3 scanned together;
no collision gas

Product Ion
Q1 set, Q3 scanned;
collision gas on



Selective reaction monitoring
Q1 and Q3 set;
collision gas on