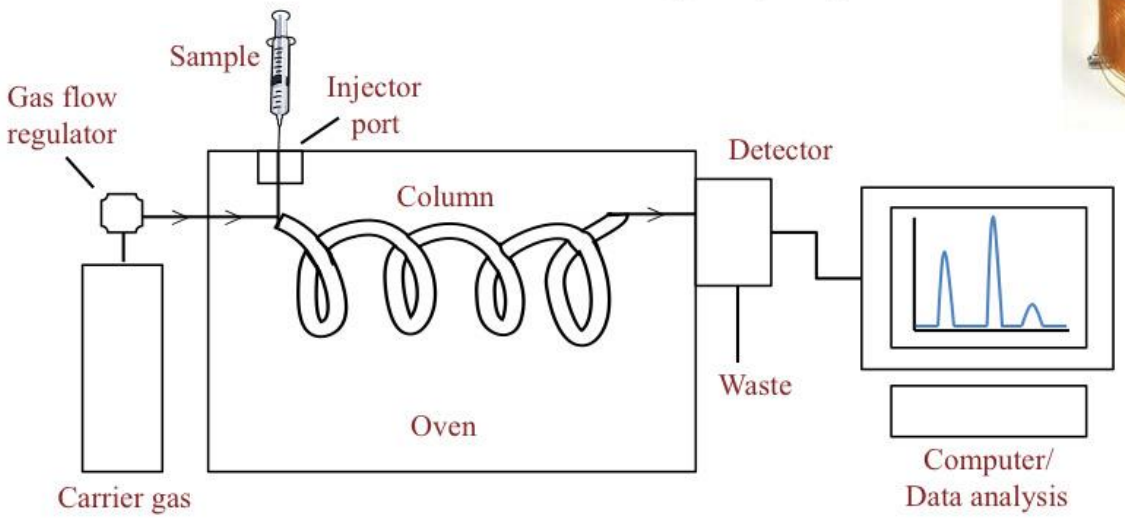
Kromatográfia és tömegspektrometria

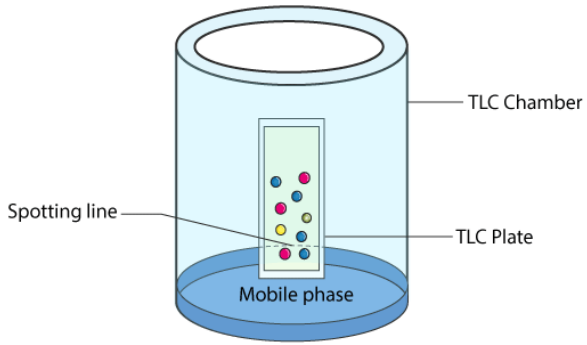
Dr. Karvaly Gellért Balázs
Semmelweis Egyetem Laboratóriumi Medicina Intézet
Tömegspektrometriai és Elválasztástechnikai Laboratórium

Gas Chromatography

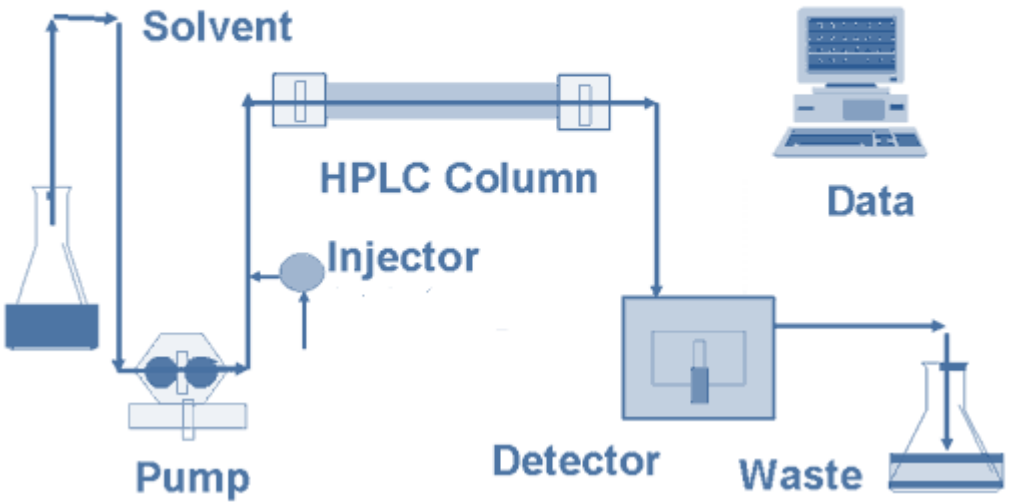


THIN LAYER CHROMATOGRAPHY

BYJU'S The Learning App



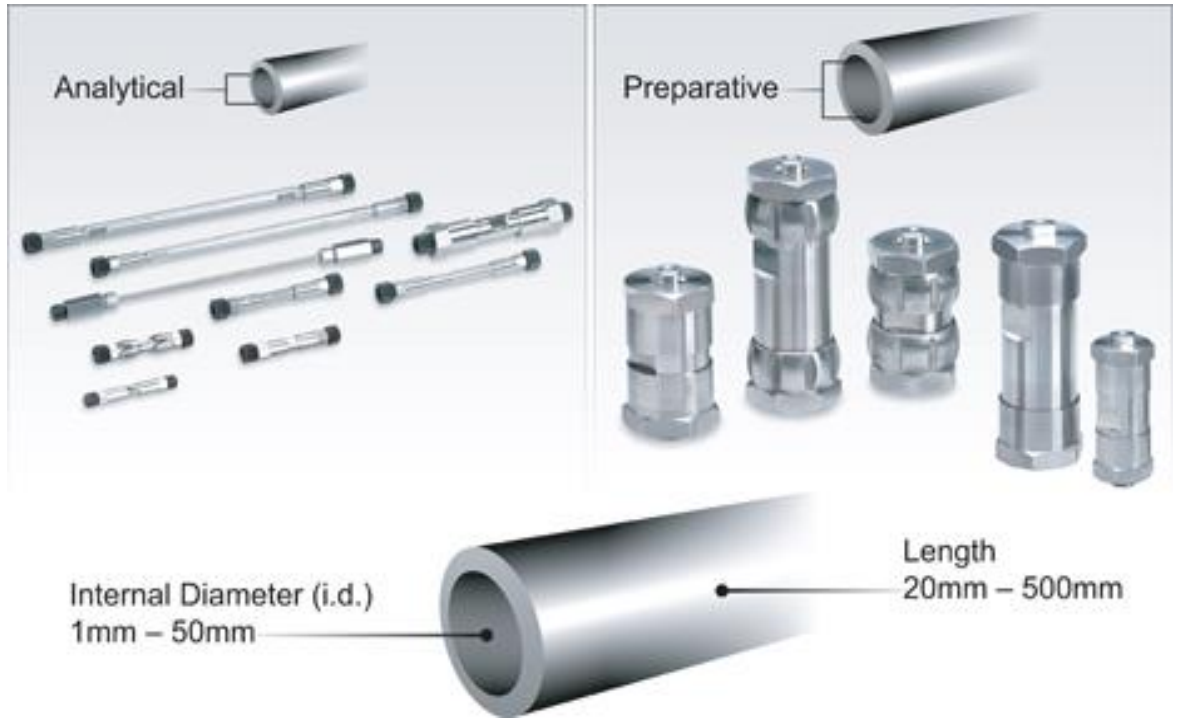
HPLC System

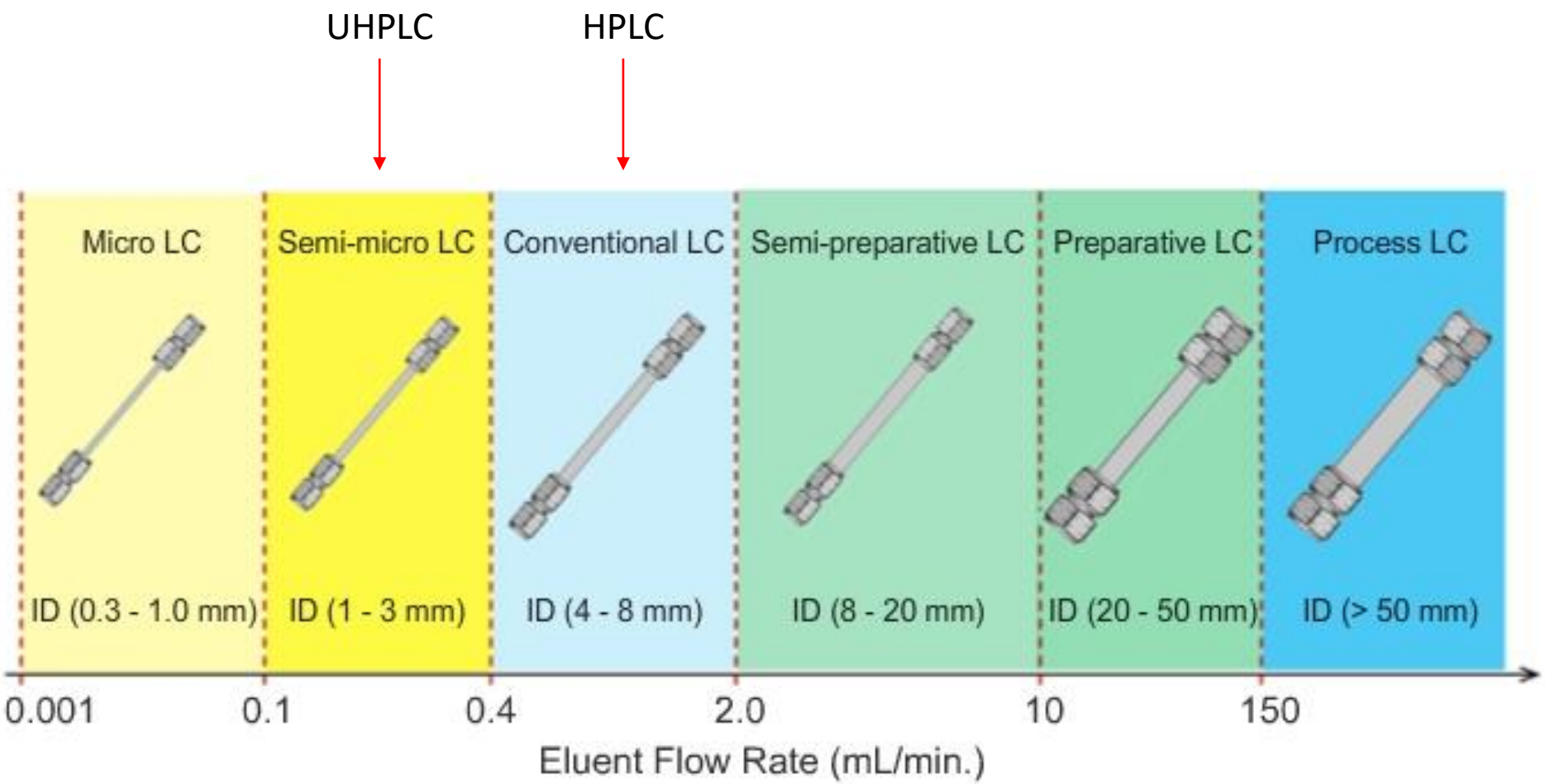


HPLC dimensions

www.waters.com

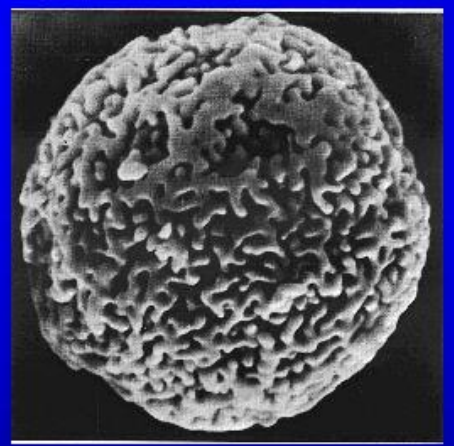
column ID	analytical	semi-prep	preparative	process
1-8 mm	X			
10-40 mm		X		
50-100 mm			X	
>100 mm				X
particle size (µm):	0.1-10	5-15	15-100	100+



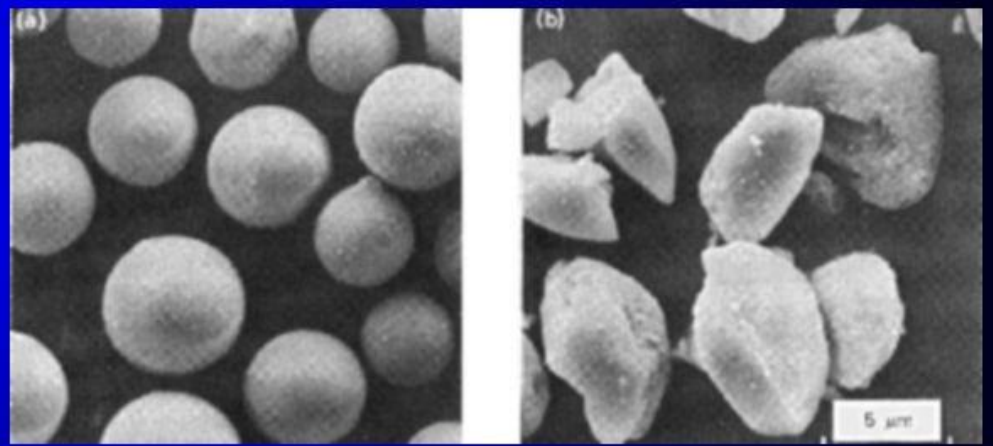

UHPLC
HPLC
Micro LC
Semi-micro LC
Conventional LC
Semi-preparative LC
Preparative LC
Process LC
ID (0.3 - 1.0 mm)
ID (1 - 3 mm)
ID (4 - 8 mm)
ID (8 - 20 mm)
ID (20 - 50 mm)
ID (> 50 mm)
0.001
0.1
0.4
2.0
10
150
Eluent Flow Rate (mL/min.)

The Most Popular Particle: Silica

- Different morphology for different applications:

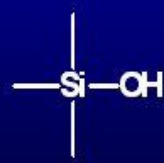


Macroporous spherical silica particle. [K.K.Unger, Porous silica, Elsevier, 1979]

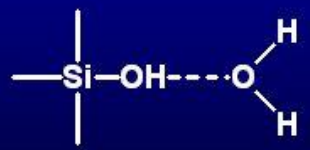


Electron microphotograph of spherical and irregular silica particles. [W.R.Melander, C.Horvath, Reversed-Phase Chromatography, in HPLC Advances and Perspectives, V2, Academic Press, 1980]

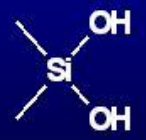
- Different chemistry:



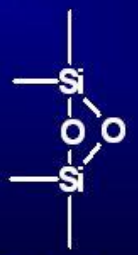
Free Silanol



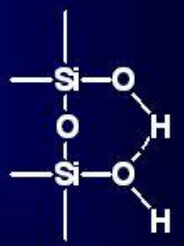
Adsorbed Water



Geminal Silanol



Dehydrated Oxide Siloxane



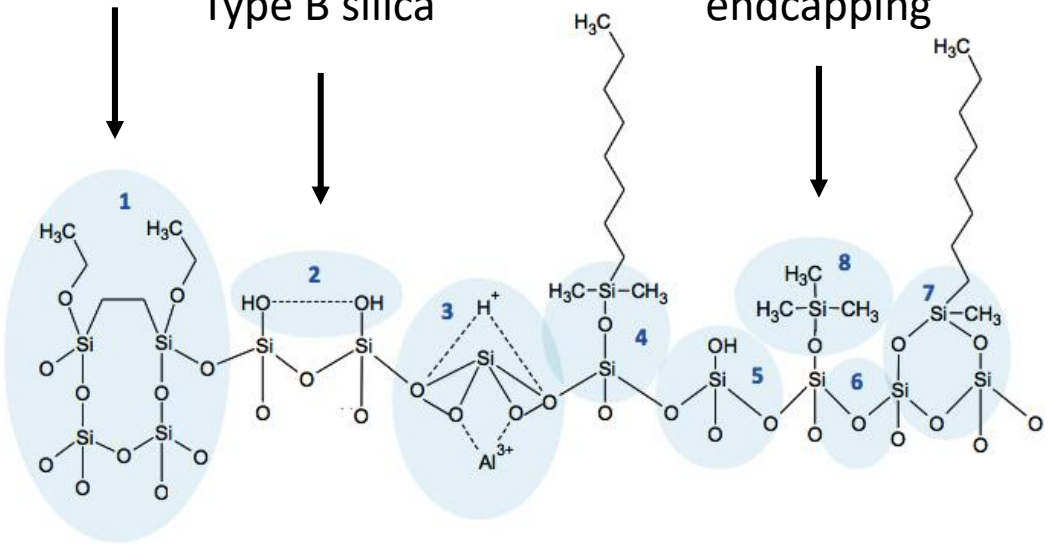
Bound and Reactive Silanols

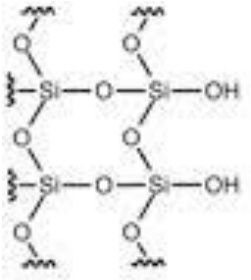
Silica-based stationary phases

hybrid silica

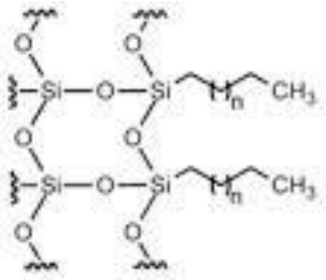
Type B silica

endcapping

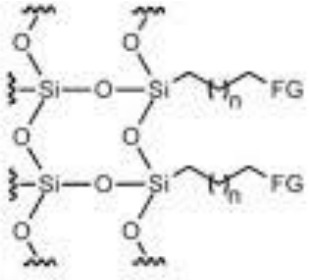




Normal phase silica



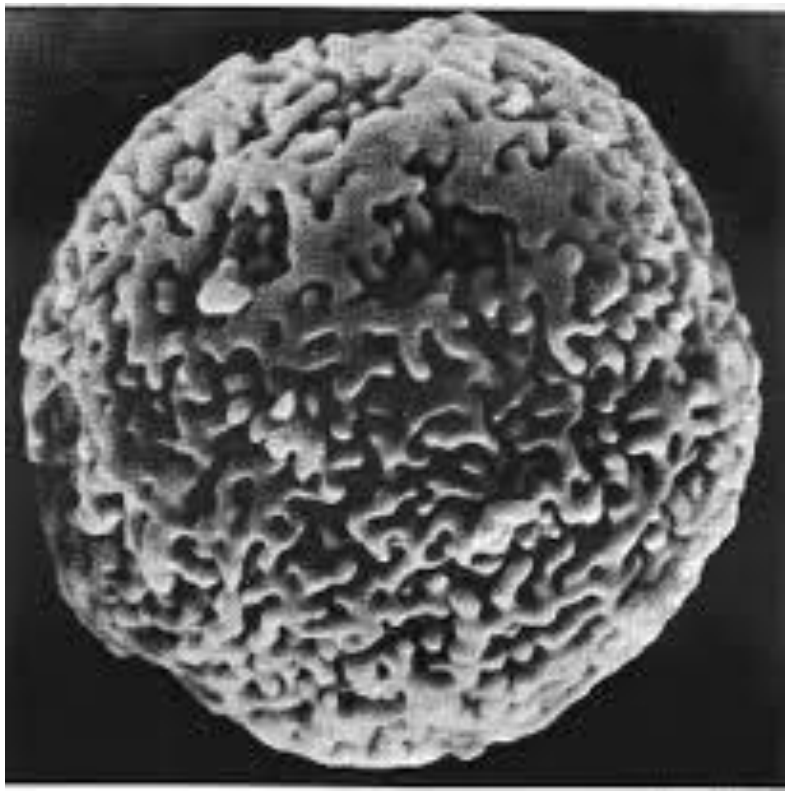
Reversed phase silica



Functionalized silica

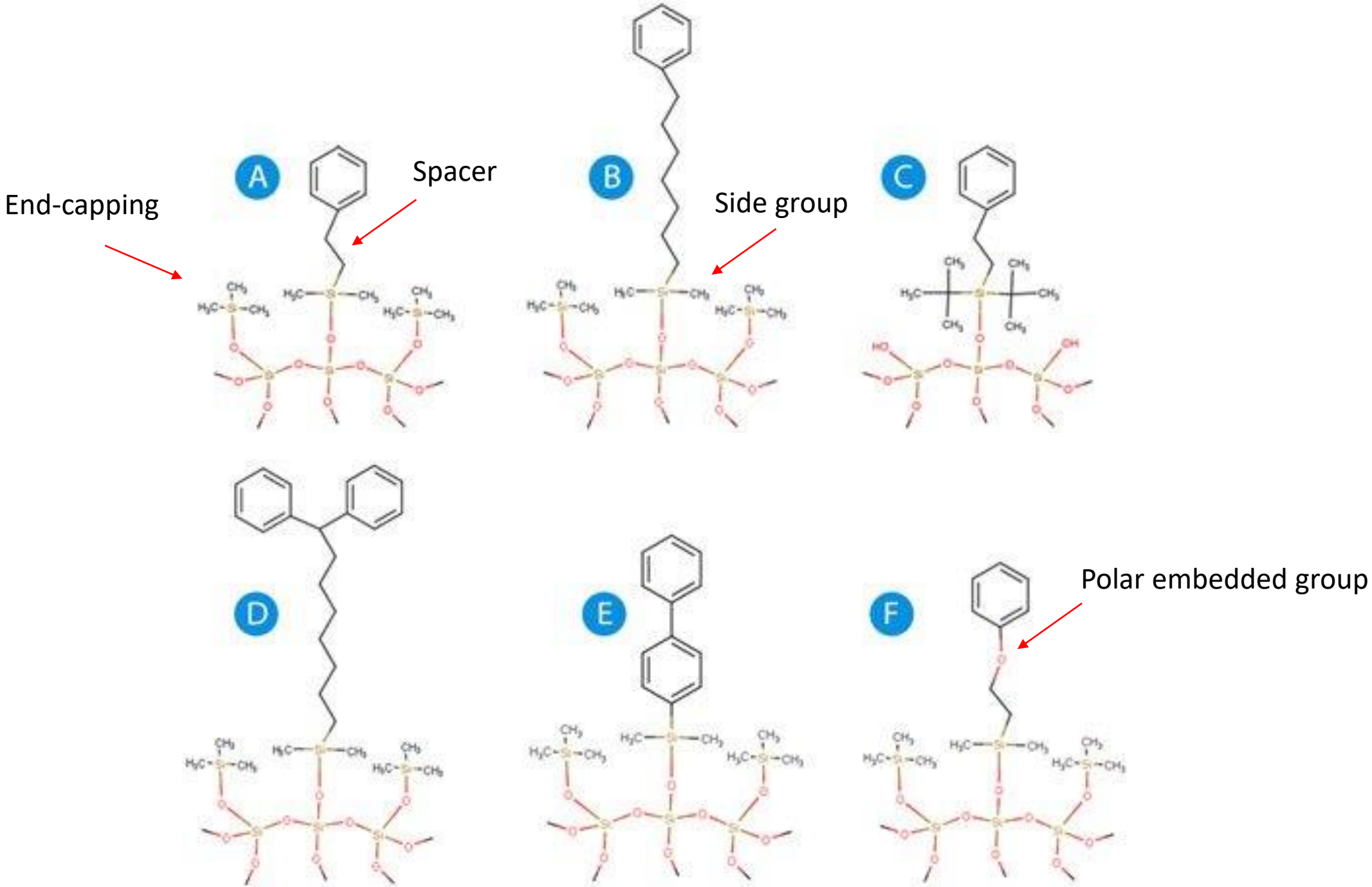
FG = $-NH_2$
$-CN$

d_p = 1-10 µm



density of –OH groups: 8-9 µmol/m^2

surface coverage of bonded phase: 40-50% of silanol groups


End-capping
A
Spacer
B
Side group
C
D
E
F
Polar embedded group

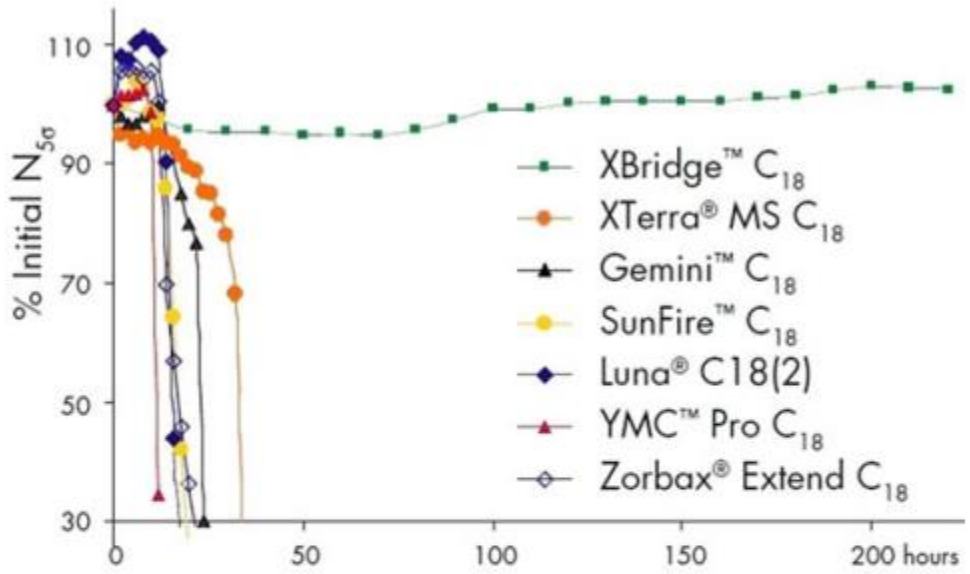


Figure 1. Stationary phase stability: efficiency loss as a function of time of purge with an aggressive mobile phase (analyte: acenaphthene; mobile phase: triethylamine, pH 10; 50 mM at 50°C). Figure reprinted with permission from reference (16).

phase as determined by gradient elution chromatography using acetonitrile/ammonium carbonate (pH 10, 10 mM) at 40°C for 8,000 min (Figure 3) (21). Phenomenex evaluated its hybrid sta-

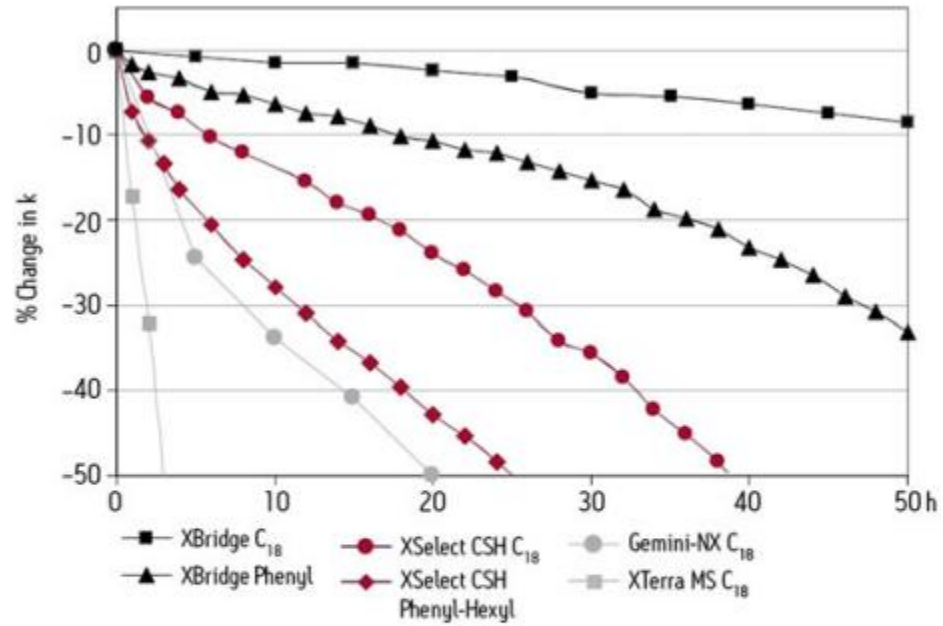
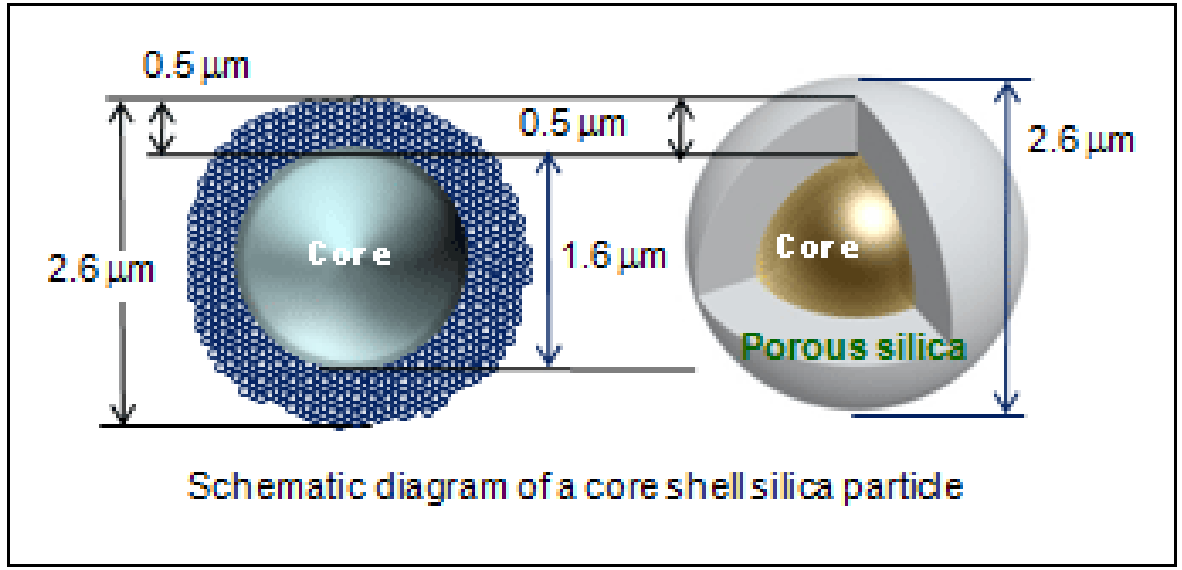
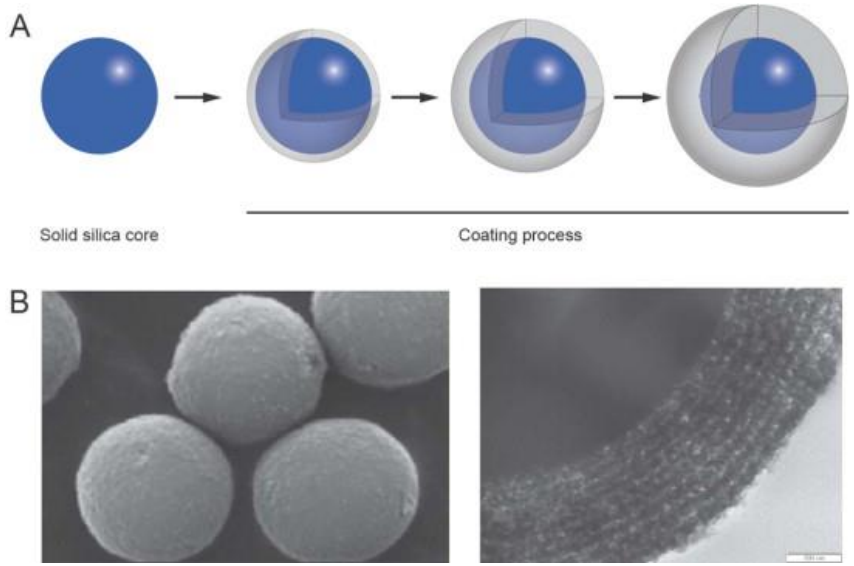


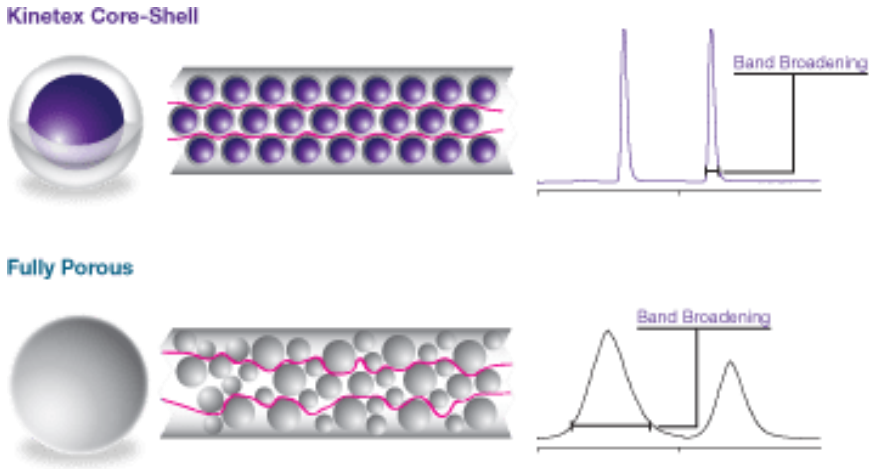
Figure 2. Results of accelerated base stability testing for six stationary phases, showing percent change of retention factor (*k*) for decanophenone versus exposure time (h) to aqueous sodium hydroxide (20 mM; pH 12.3) at 50°C. The stationary phases were purged at 0.85 mL/min for 1.8 h, washed for 10 min at 0.43 mL/min. Mobile phase: acetonitrile/water, 50:50 v/v. Columns: XTerra MS C18 (50 × 3 mm), Gemini C18 and Xbridge C18 (50 × 4.6); all other columns 30 × 3 mm. Figure reprinted with permission from reference (17).

Borges EM, Volmer DA. J Chrom Sci 2015;53:1107-22.



Schematic diagram of a core shell silica particle



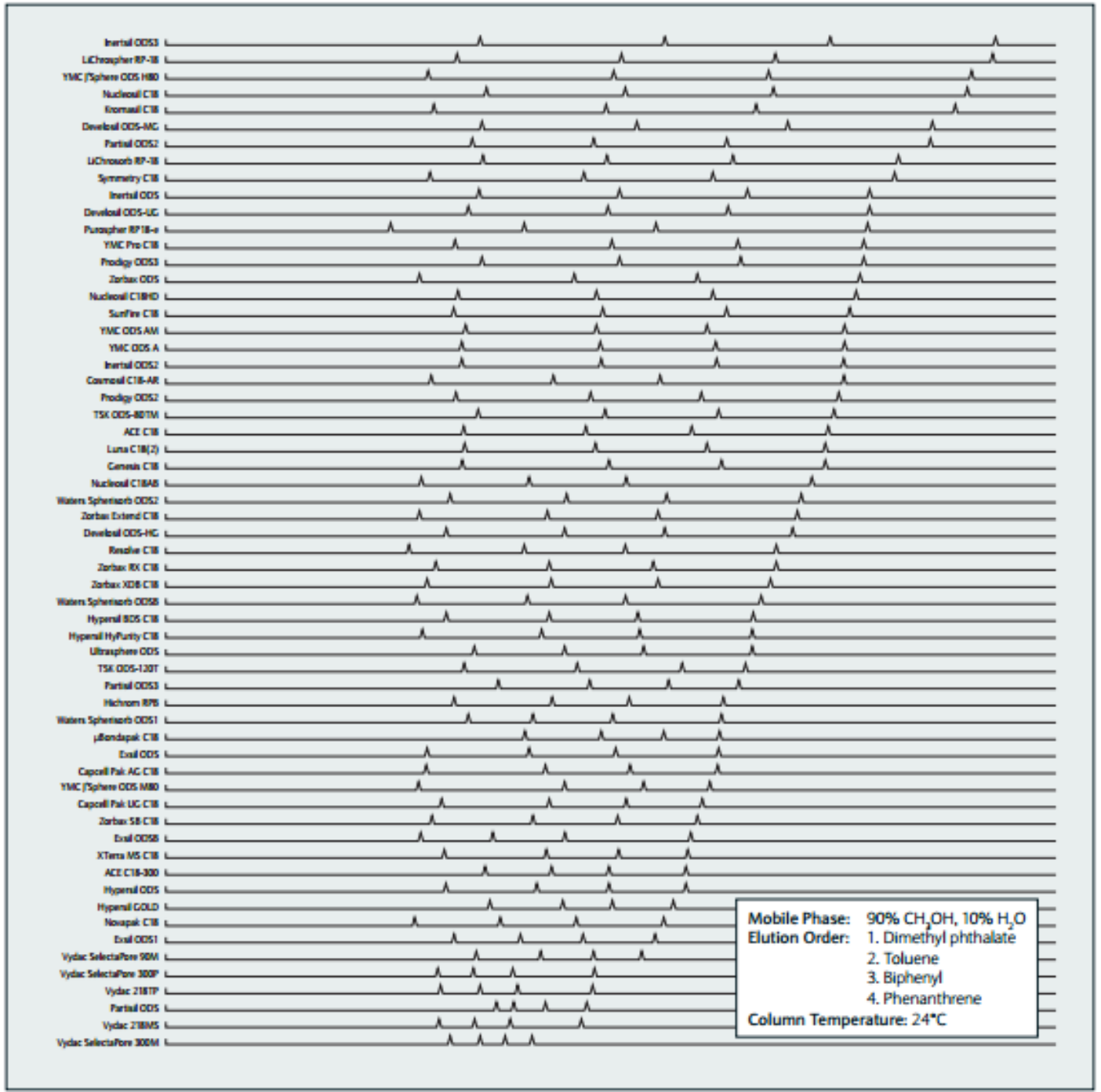


Fully Porous		Kinetex Core-Shell	Average Efficiency Gain with Kinetex*
5 µm	vs	5 µm	90 % Higher
3 µm	vs	2.6 µm	85 % Higher
1.7 µm	vs	1.7 µm	20 % Higher
1.7 µm	vs	1.3 µm	50 % Higher

* May not be representative of all separations.

Comparison Guide to C18 reversed phase HPLC columns. 4th ed. MacMod Analytical, 2008.
http://www.mac-mod.com/pdf/technical-report/036-ColumnComparisonGuide.pdf

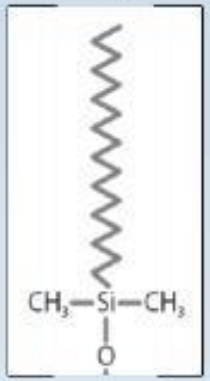
C18 Phases Compared According to Relative Hydrophobicity



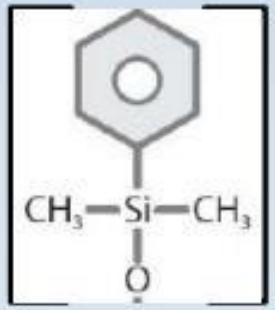
Common Reversed Phase Stationary Phase Ligands

Non Polar — Polar

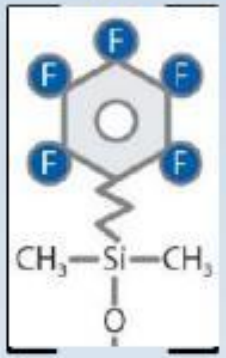
normal phase separations



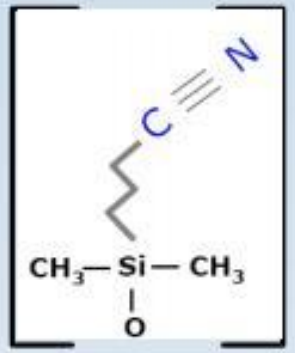
Alkyl



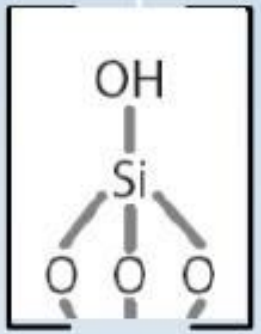
Phenyl



Fluoro

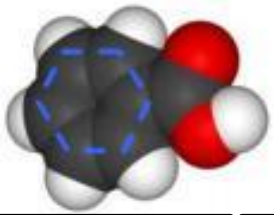


Cyano



Silica

Dispersive — **Pi-Pi Interactions** — **Electrostatic / Dipole** — **H Bonding**



reversed phase separations

RESTEK Chromatography Products
www.restek.com

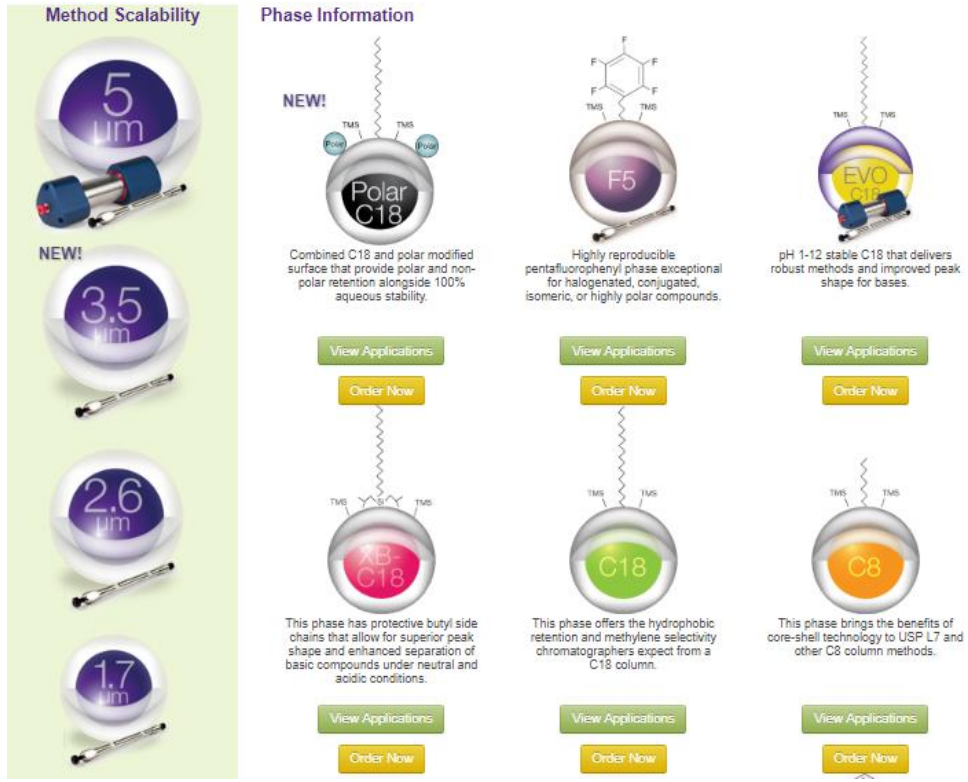
Choosing the mobile phase

- **The choice of mobile phase(s) depends on a number of factors:**
  - Type of separation (reversed phase/HILIC/normal phase/ion chromatography)
  - Type of detector
  - Chemical properties of the analyte: polarity, pKa, presence of aromatic rings and heteroatoms, steric properties

- **Basic types of HPLC separations:**
  - Isocratic: no change in mobile phase
    - No equilibration time
    - Lower solvent consumption
    - Fewer sources of error
    - Lower risk of losing reproducibility
  - Gradient: programmed changes in mobile phase compositions
    - Shorter runs
    - Longer column lifetime
    - Requires fast re-equilibration of detector → not used with electrochemical detection

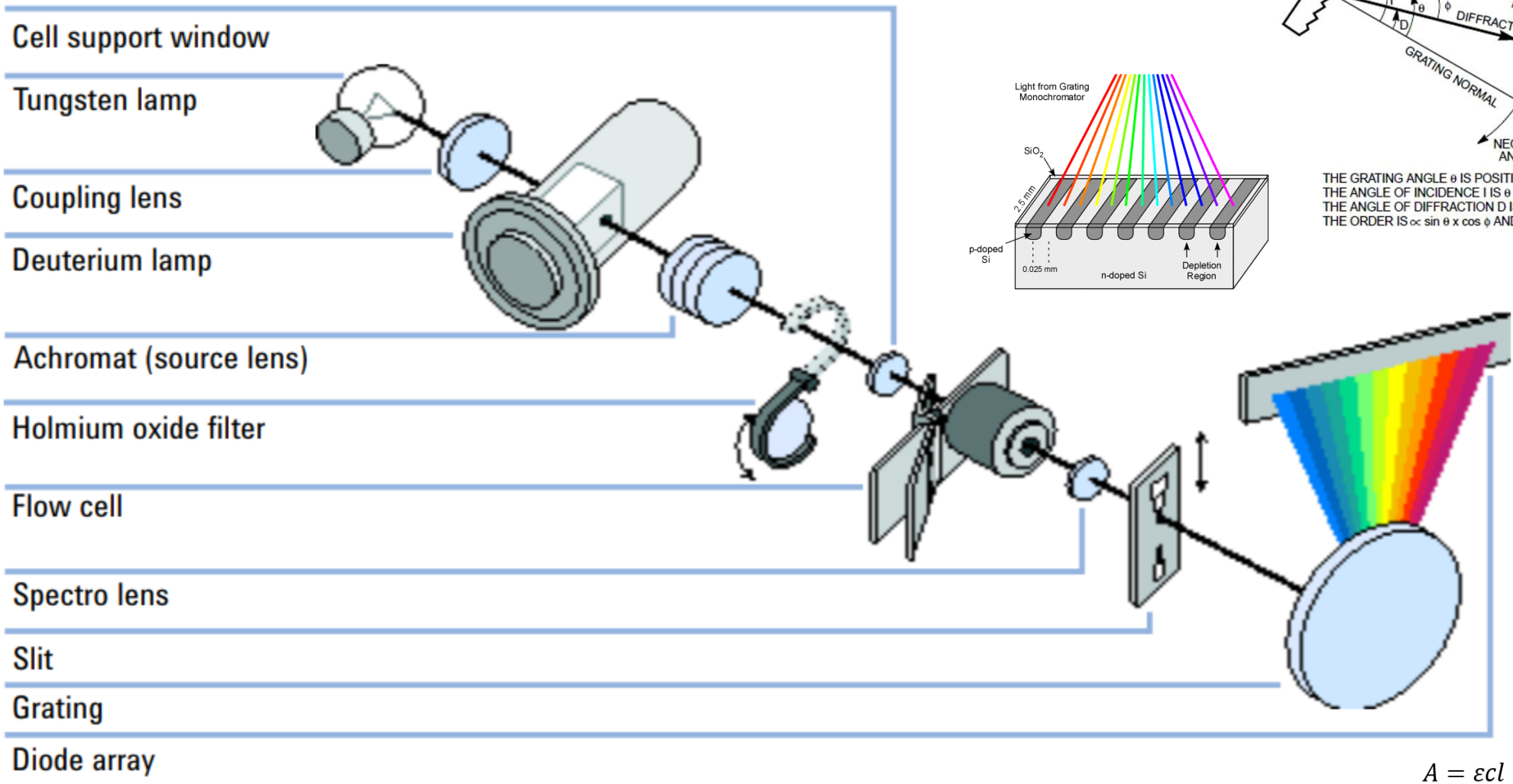
What can the chromatographer do to enhance selectivity, specificity and sensitivity?

- select the instrument configuration
- select the stationary phase
- select the mobile phases
- select the separation settings
  - analytical column dimensions
  - column space temperature
  - mobile phase gradient program
  - mobile phase flow rate
- select the detection settings



Classification of detectors

- Bulk-property detectors: refractive index
- Analyte specific detectors: absorbance, fluorescence, electrochemical, conductivity, radioactivity
- Mobile phase modification detectors: evaporative light scattering, corona discharge
- Hyphenated techniques: mass spectrometry, infrared spectroscopy, nuclear magnetic resonance spectroscopy



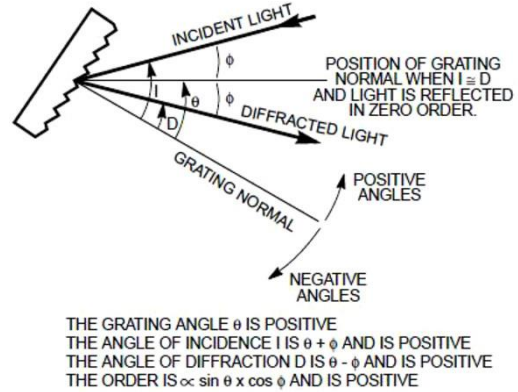
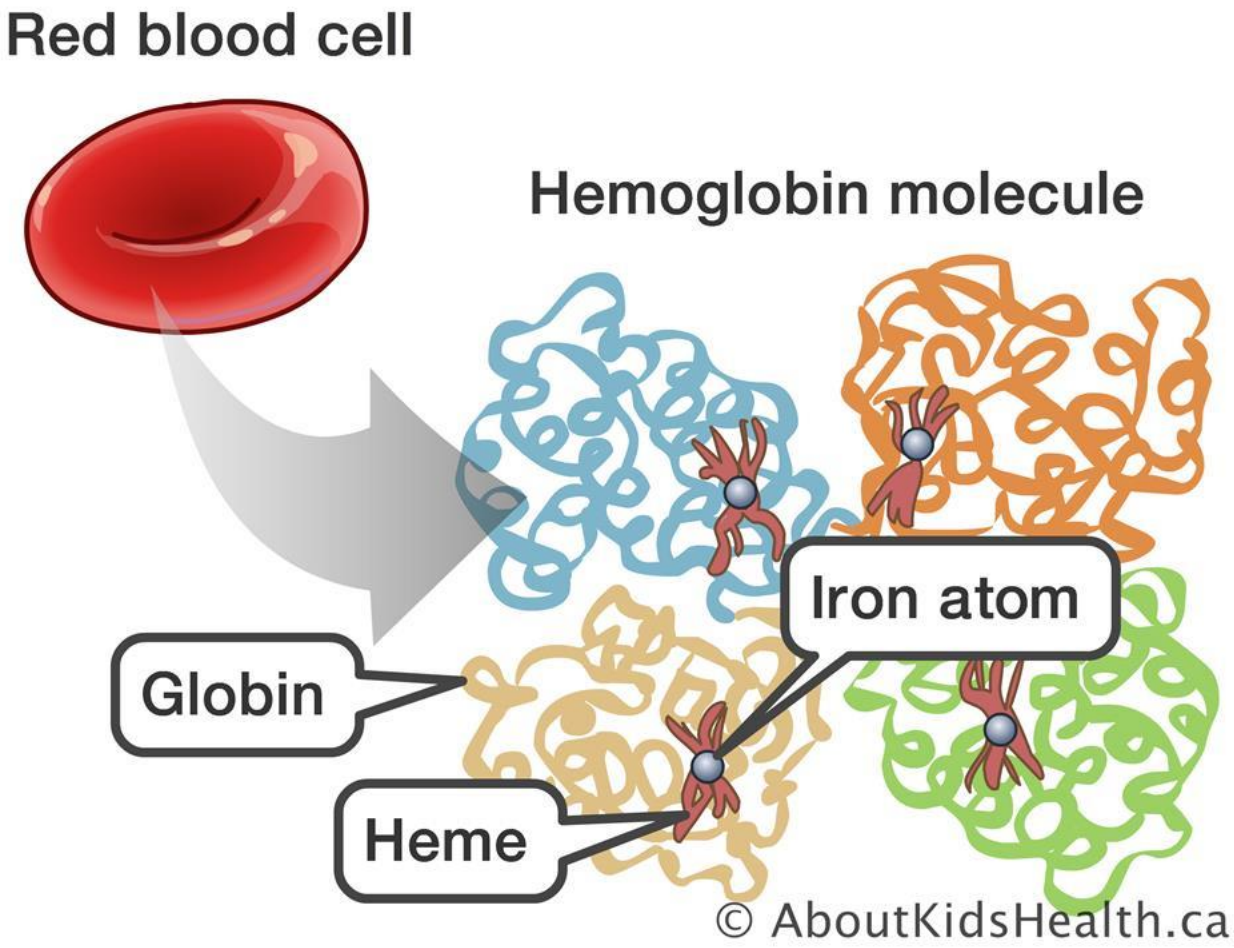


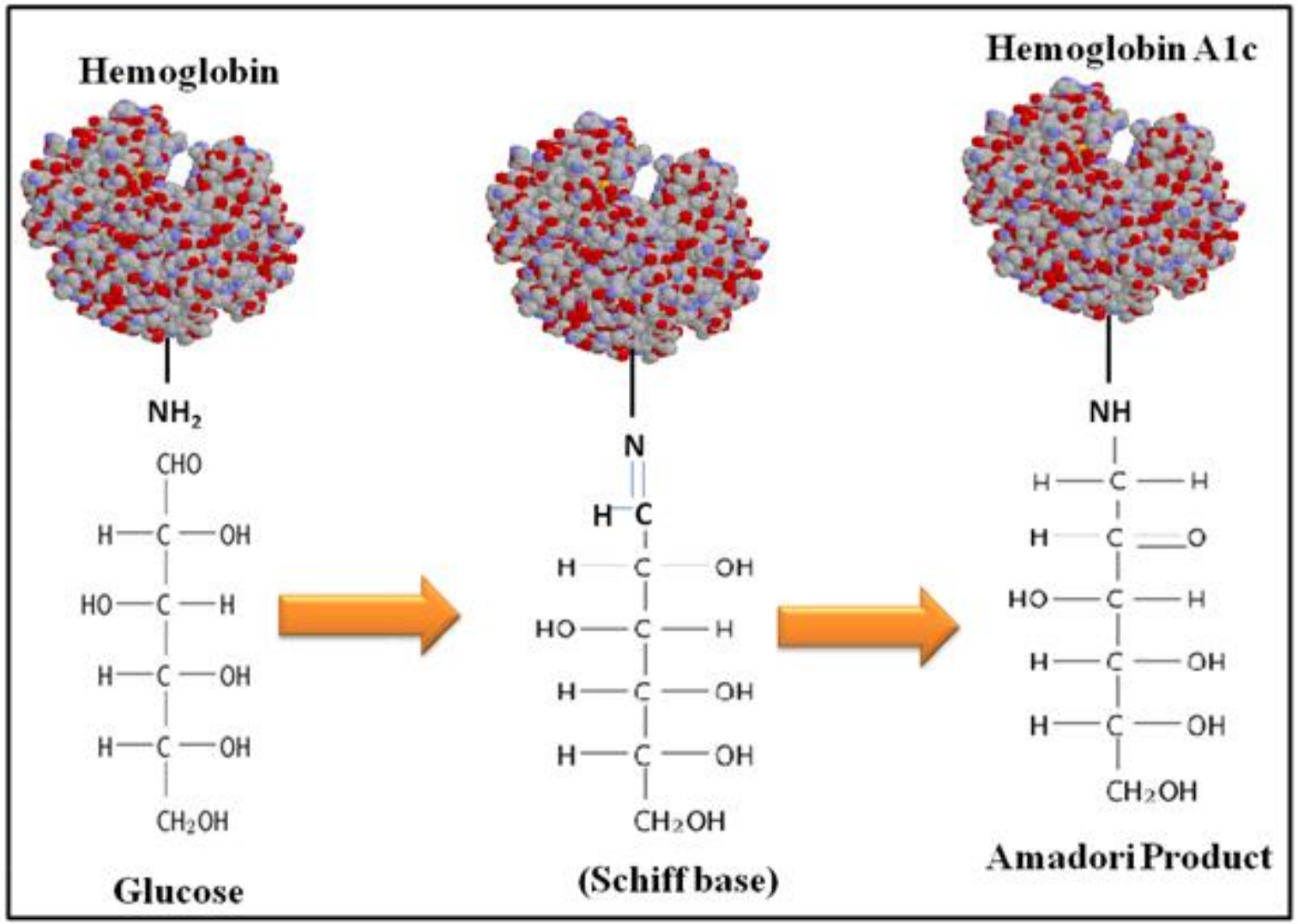
Figure 3 Optical System of the Detector

$$A = \varepsilon c l$$

A	Absorbance	
ε	Molar absorption coefficient	$M^{-1}cm^{-1}$
c	Molar concentration	M
l	optical path length	cm



- Makes up 96% of the dried content of RBC's
- HbA (>95%): α (α_1 and α_2) and β chains ($\alpha_2\beta_2$)
- A2 (1.5-3.5%): $\alpha_2\delta_2$
- F: $\alpha_2\gamma_2$
- Several pathological variants exist


Hemoglobin
NH2
CHO
H—C—OH
HO—C—H
H—C—OH
H—C—OH
CH2OH
Glucose
N
H—C
H—C—OH
HO—C—H
H—C—OH
H—C—OH
CH2OH
(Schiff base)
Hemoglobin A1c
NH
H—C—H
H—C—O
HO—C—H
H—C—OH
H—C—OH
CH2OH
Amadori Product

HbA1c	mmol/mol	%
Normal	Below 42 mmol/mol	Below 6.0%
Prediabetes	42 to 47 mmol/mol	6.0% to 6.4%
Diabetes	48 mmol/mol or over	6.5% or over

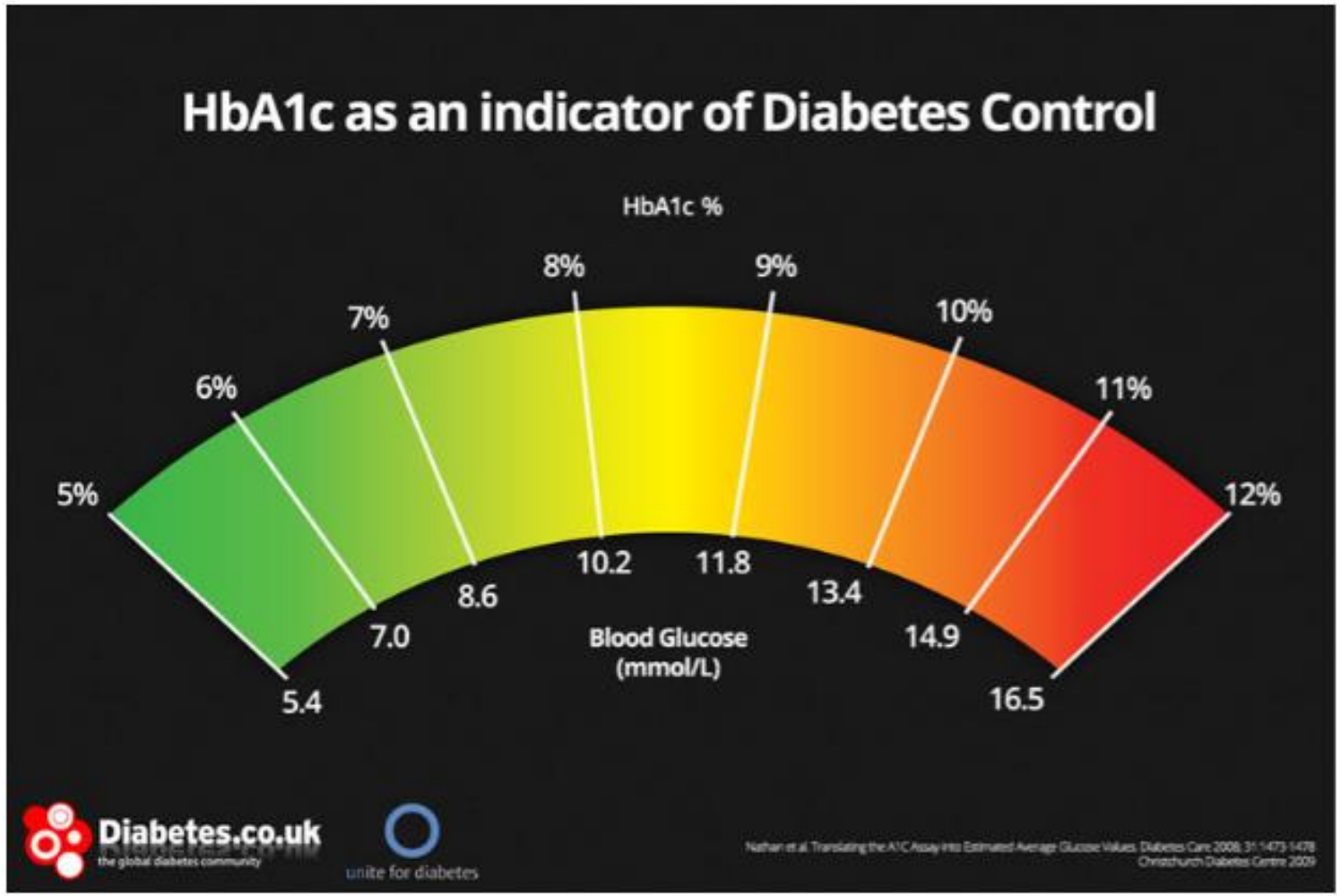


Table 4 The relationships between the hemoglobin A_{1c} units as % values to the mmol/mol values are presented at different levels of hemoglobin A_{1c}

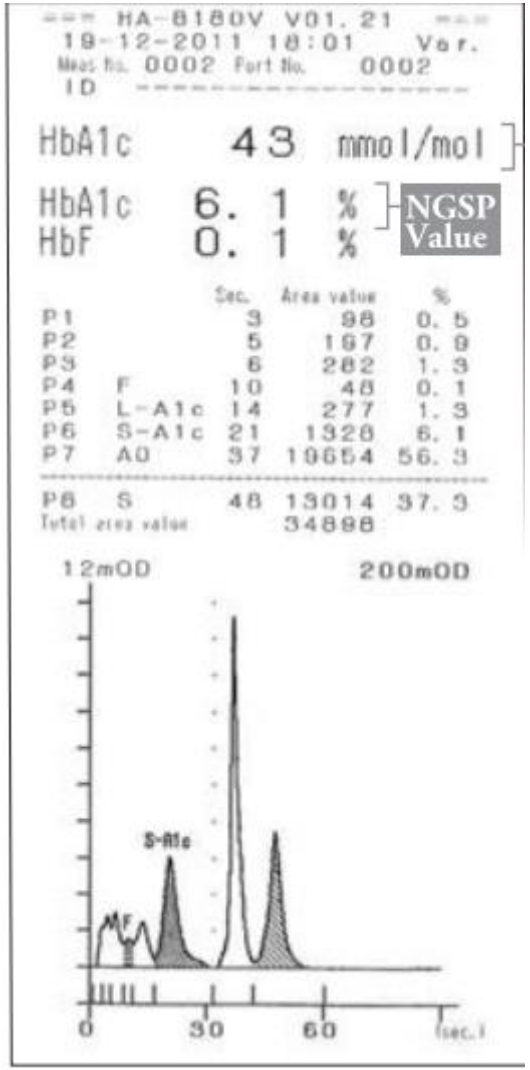
HbA_{1c}	HbA_{1c} (%)	HbA_{1c} (mmol/mol)
Reference limits	4.0-6.0	20-42
Diagnosis limit	6.5	48
Treatment limits, adults	7.0	53
children < 6 yr	7.5	69
children 6-12 yr	8.0	64
children 13-19 yr	8.5	58
Poor diabetic balance	9.0	75
Very poor diabetic balance	12.0	108

Applications of glycated haemoglobin measurements

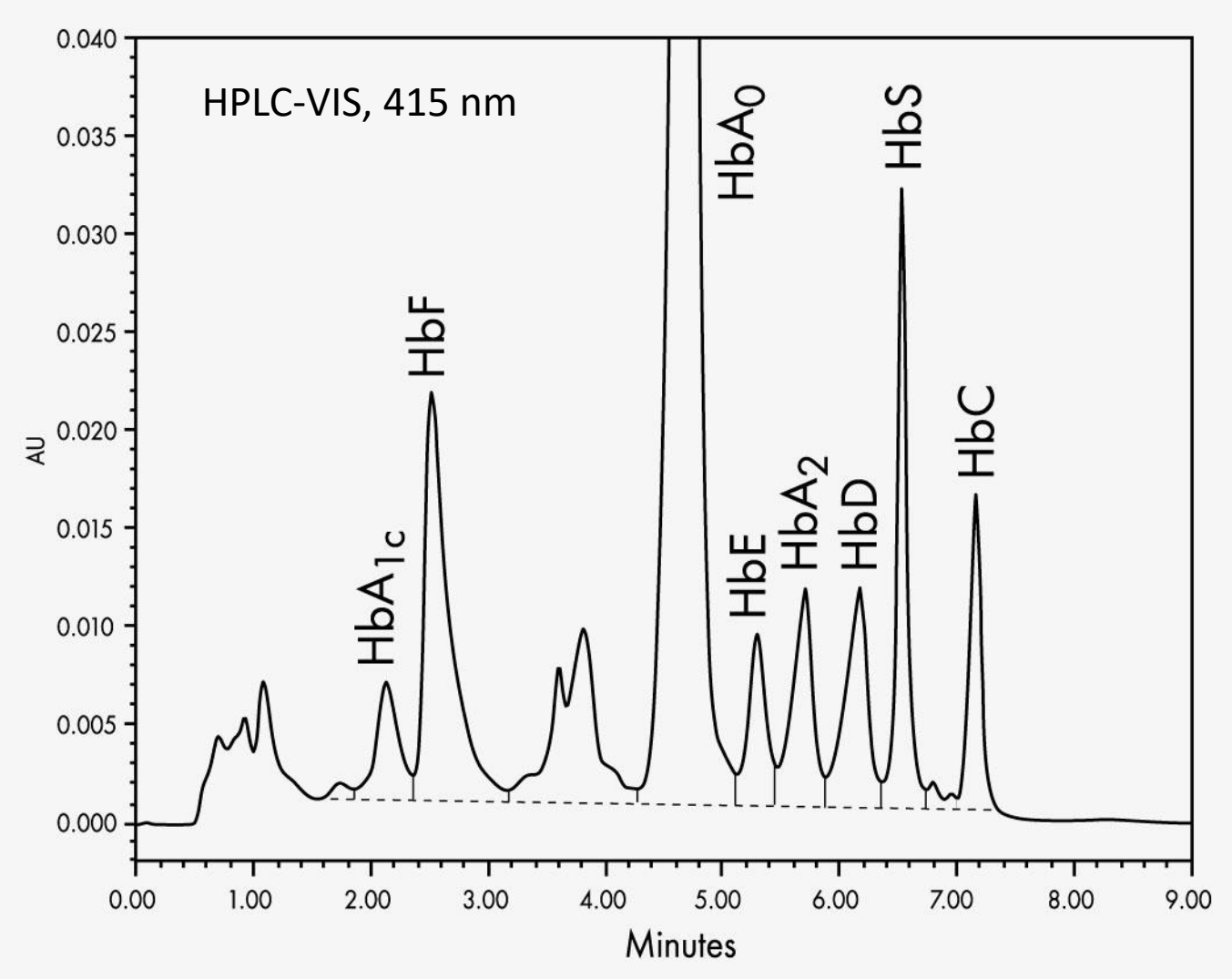
- Diagnosis of diabetes
- Monitoring the progress of therapy
- Results correlate with average blood glucose levels in past 6-12 weeks

Lab technology for evaluating HbA1c

- HPLC-VIS (420/500 nm)
- Separation by cation exchange chromatography
- Sample: whole blood, EDTA-coated tube
- Fully automated
- Run time: 48-90 s



http://www.arkray.eu/english/products/laboratory/habalc/ha-8180.html



https://www.chromsystems.com/products/hemoglobin-testing/hemoglobin-variants-hplc-15330.html

Pharmacokinetic-pharmacodynamic indices of antibiotics

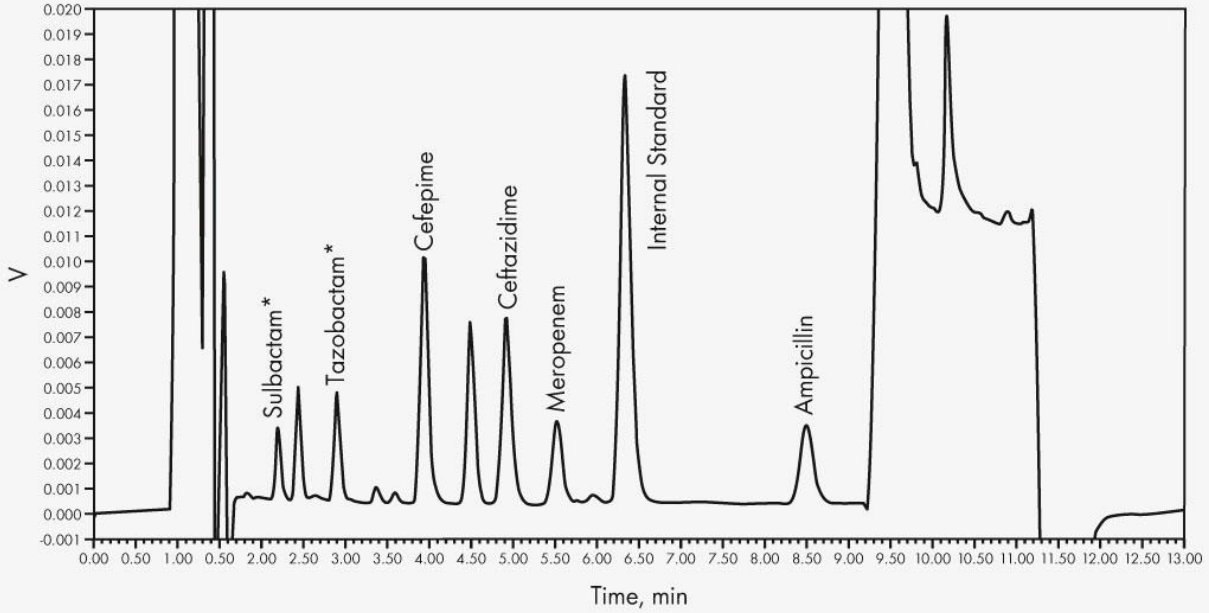
Pharmacodynamic index	$f\ T_{>MIC}$	C_{max}/MIC	AUC_{0-24}/MIC
Antimicrobials	β-Lactams Carbapenems Linezolid Erythromycin Clarithromycin Lincosamides	Aminoglycosides Metronidazole Fluoroquinolones Telithromycin Daptomycin	Fluoroquinolones Aminoglycosides Azithromycin Tetracyclines Glycopeptides Tigecycline Linezolid

Roberts JA et al. Brit J Clin Pharmacol 2011;73:27.

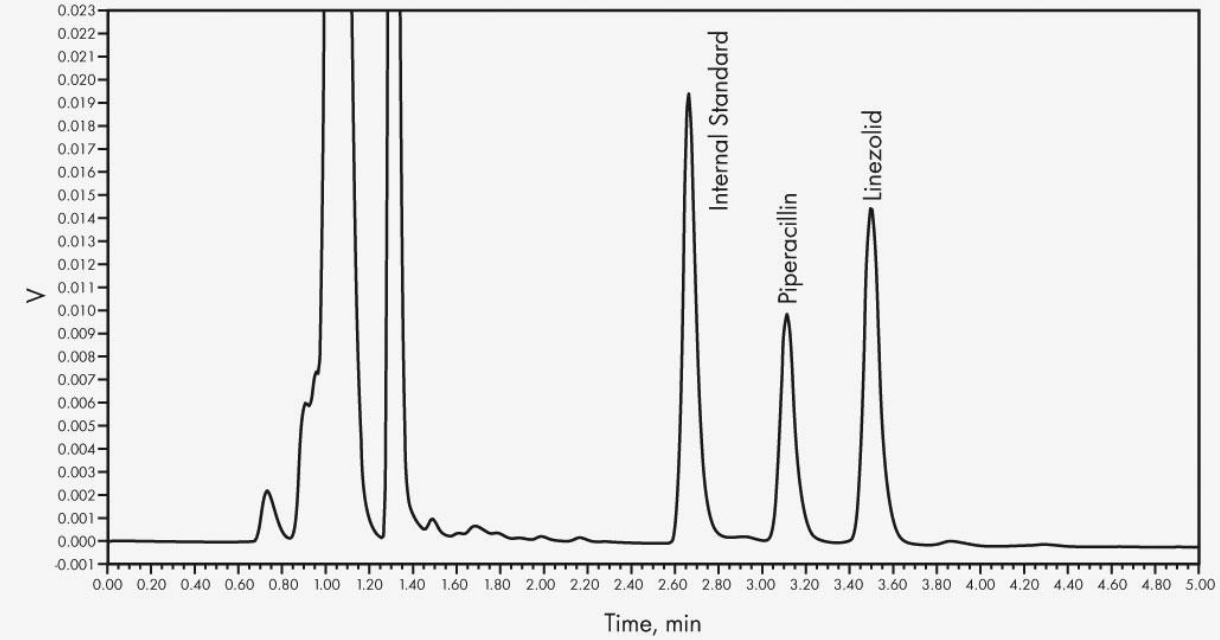
Assaying 6 antibiotics and 2 β-lactamase inhibitors in serum using reversed-phase HPLC with UV detection

- Analytes: ampicillin, cefepime, ceftazidime, linezolid, meropenem, piperacillin, sulbactam, tazobactam
- Preanalytical considerations:
  - Analytes are unstable
  - Serum should be separated, 100 µL mixed with 20 µL solution of preservative
  - Store sample frozen
- HPLC conditions:
  - 2 groups of analytes
    - Group 1: gradient run (13 min)
      - Detection at 210 nm: ampicillin, sulbactam, tazobactam
      - Detection at 290 nm: cefepime, ceftazidime, meropenem
    - Group 2: isocratic run (5 min)
      - Detection at 255 nm: linezolid, piperacillin
  - Column temperature: 22 °C

https://www.chromsystems.com/antibiotics-in-serum-plasma-61000.html

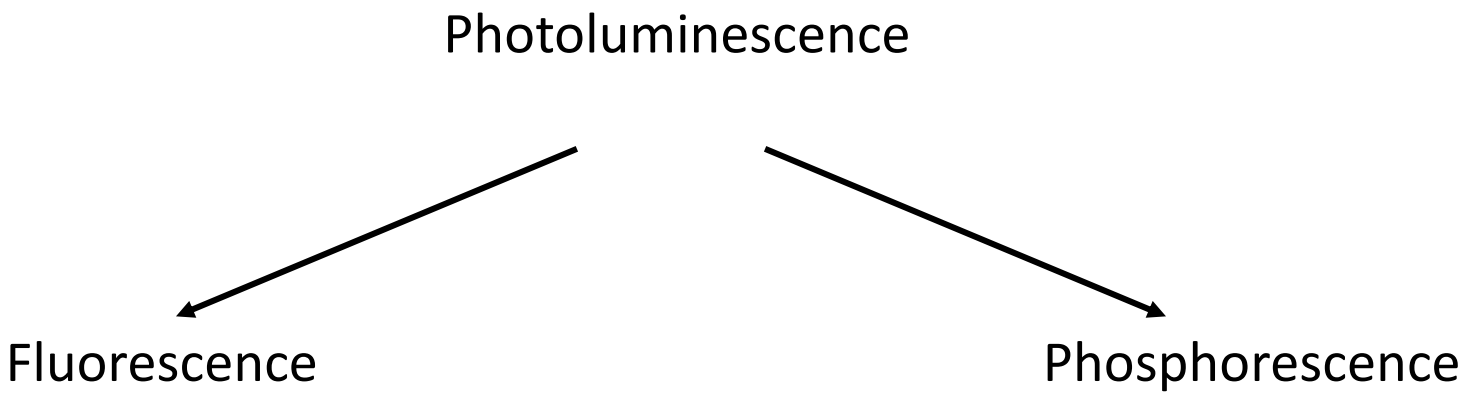


Group 1
Gradient run
210/290 nm

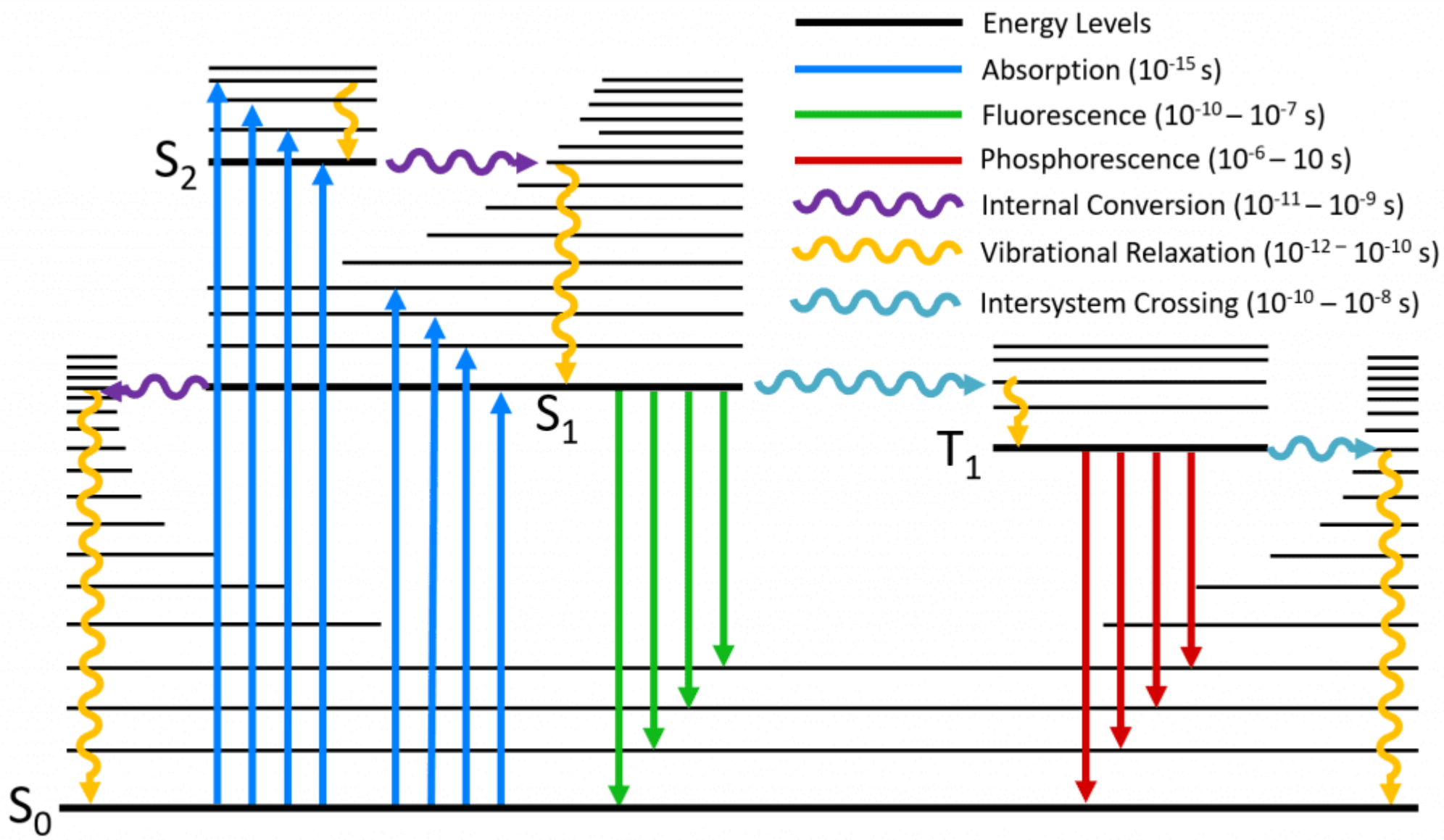


Group 2
Isocratic run
255 nm

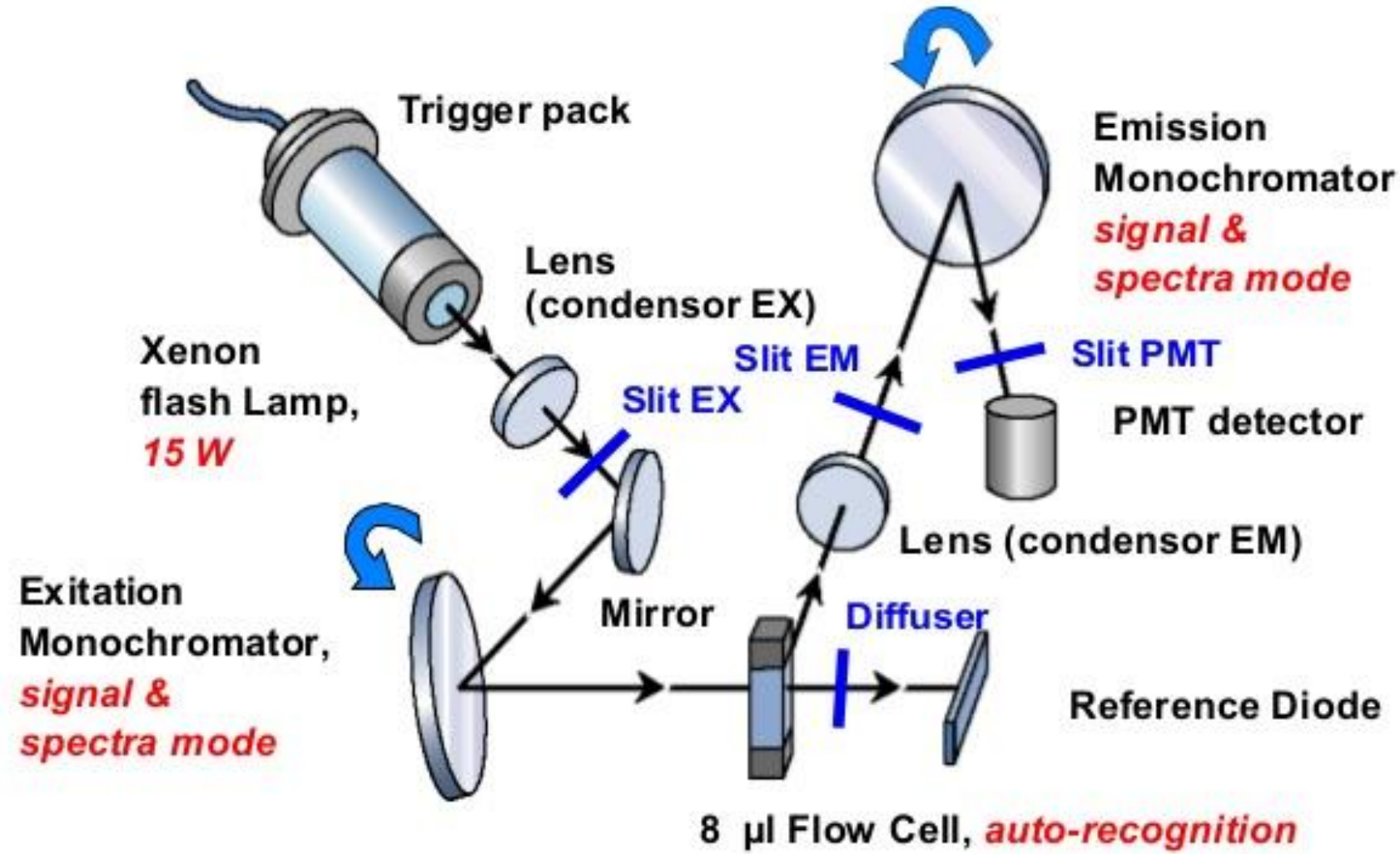
Fluorescence detection


Photoluminescence
Fluorescence
Phosphorescence

Jablonski diagram



Fluorescence Detection



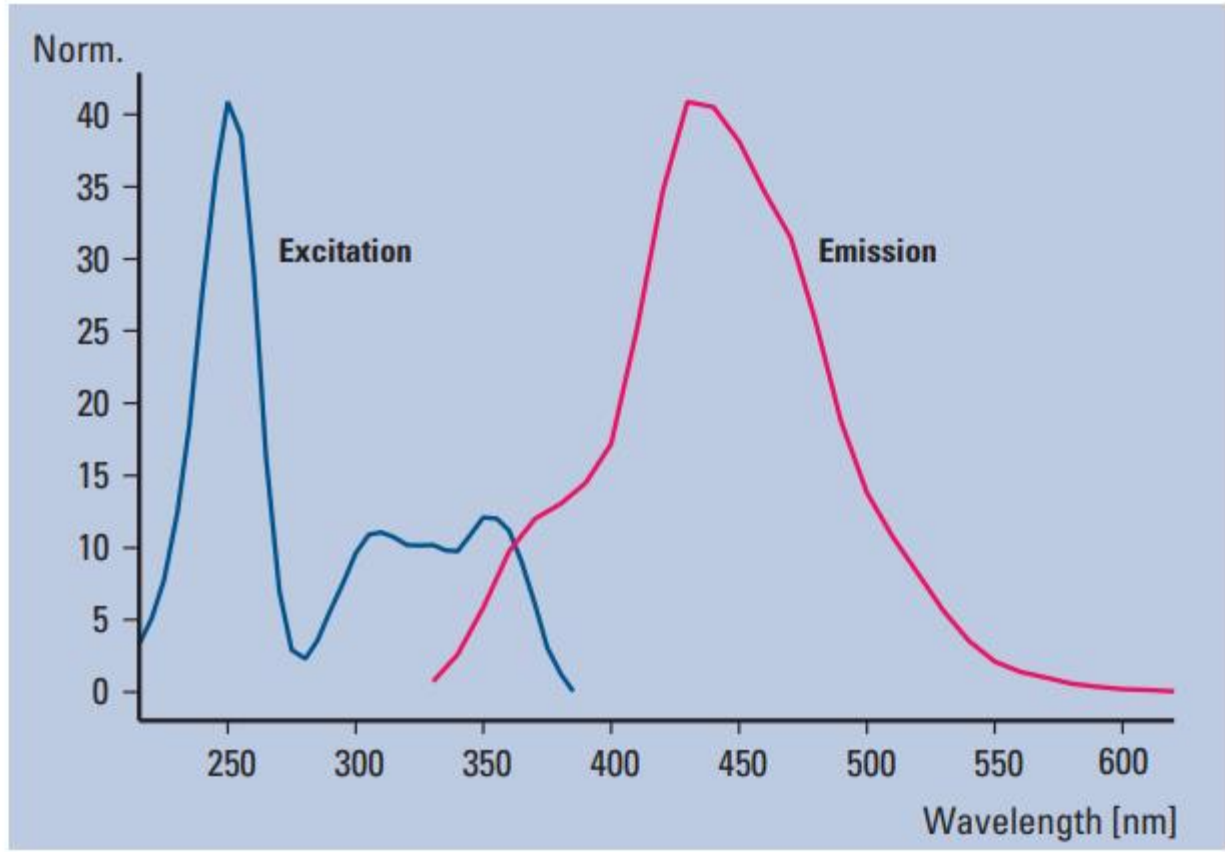
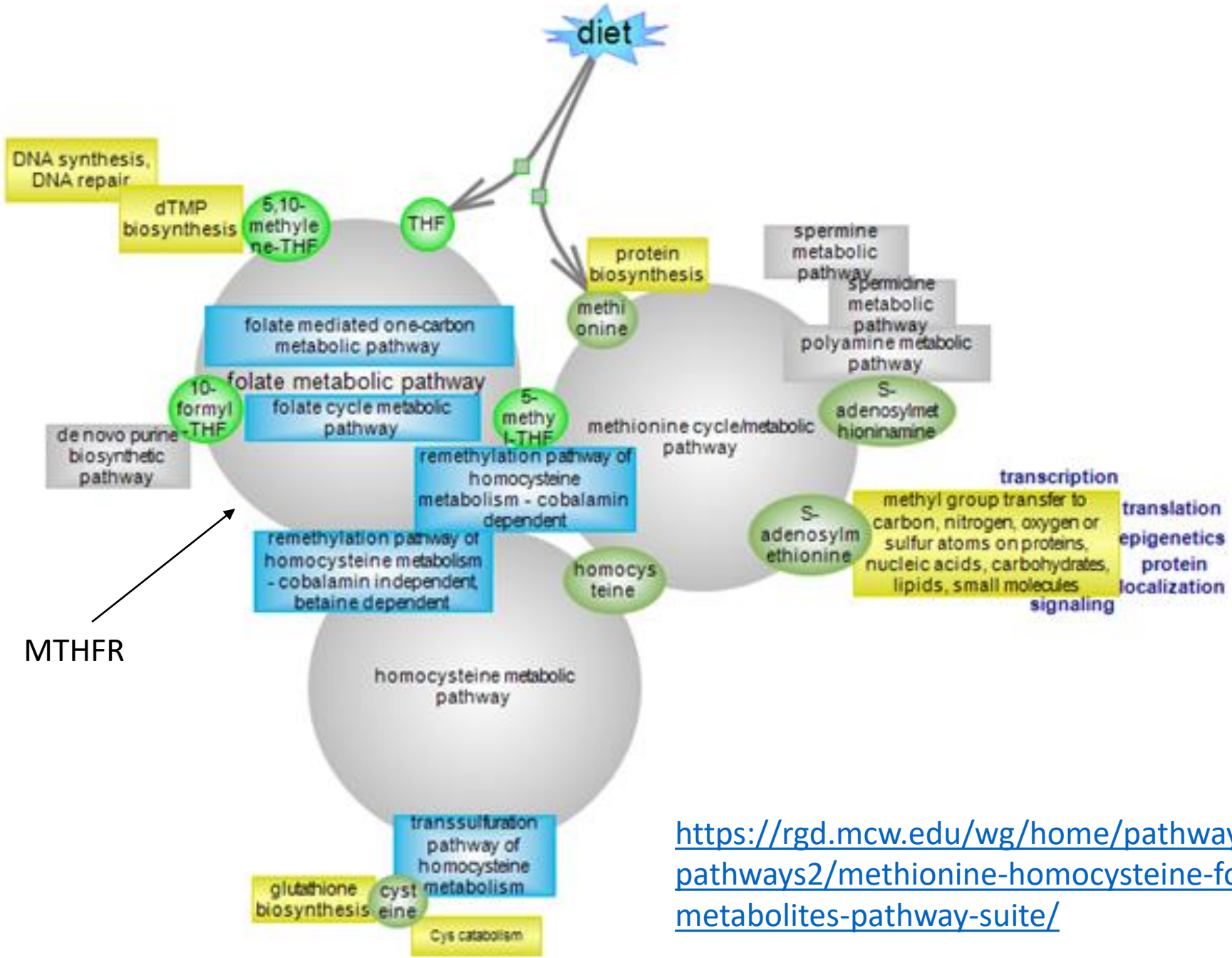


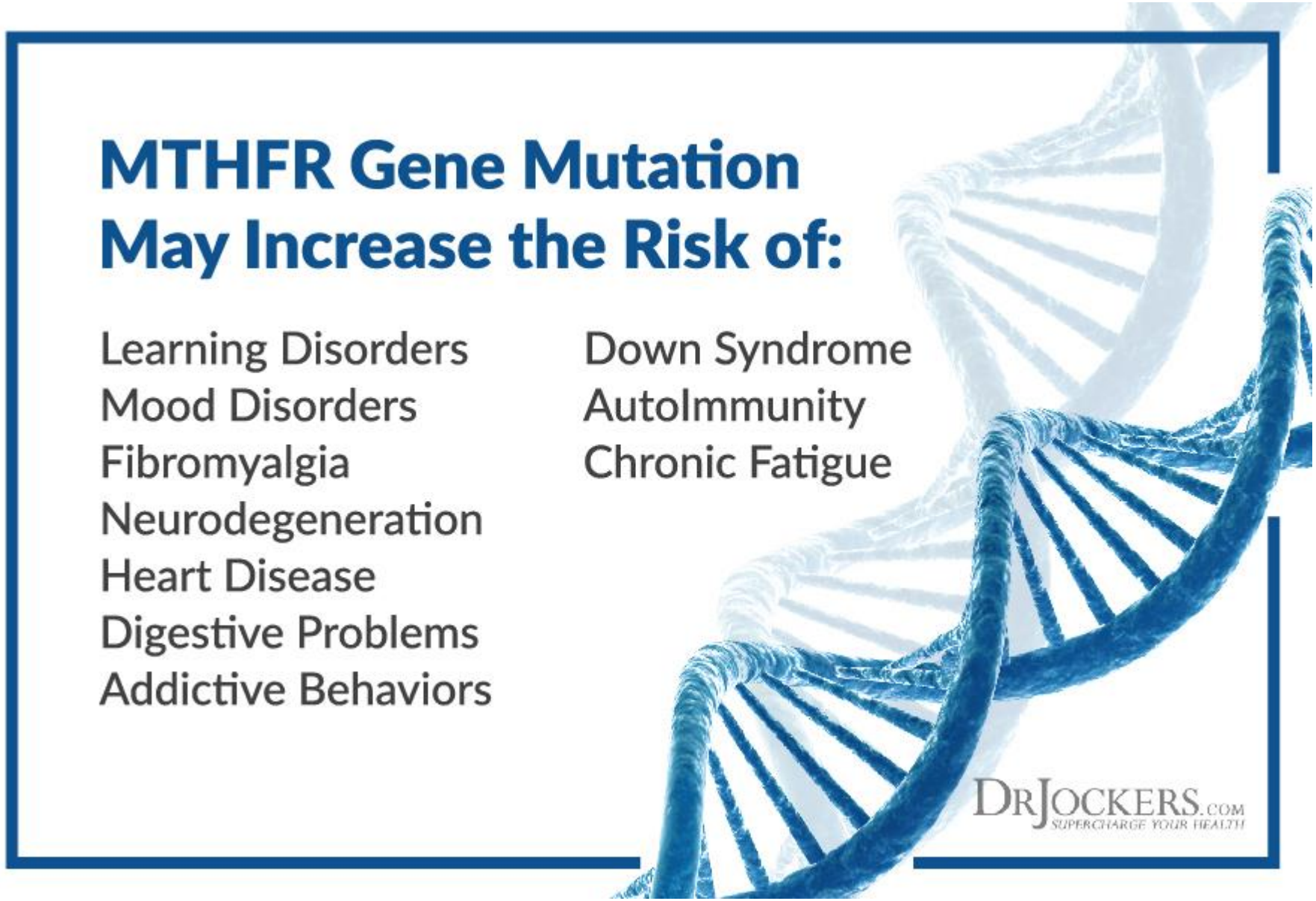
Figure 5
Excitation and emission spectra of quinidine
Excitation spectrum with emission at 440 nm, emission spectrum with excitation at 250 nm of 1 µg/ml quinidine.
Detector settings: step size 5 nm, PMT 12, Response time 4 s.

Clinical laboratory parameters frequently assayed using HPLC with fluorescence detection

Homocysteine



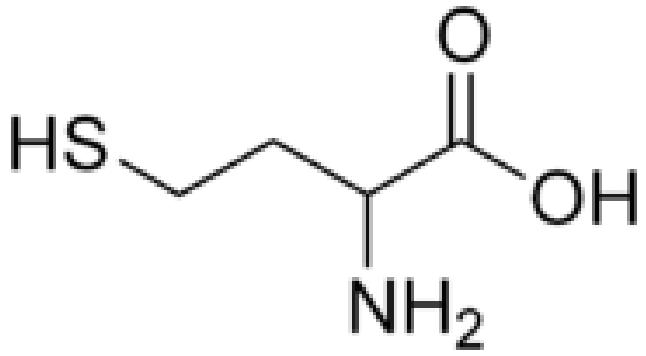
https://rgd.mcw.edu/wg/home/pathway2/molecular-pathways2/methionine-homocysteine-folate-and-related-metabolites-pathway-suite/



http://myhealthmaven.com/mthfr-gene-mutation-impact-health/

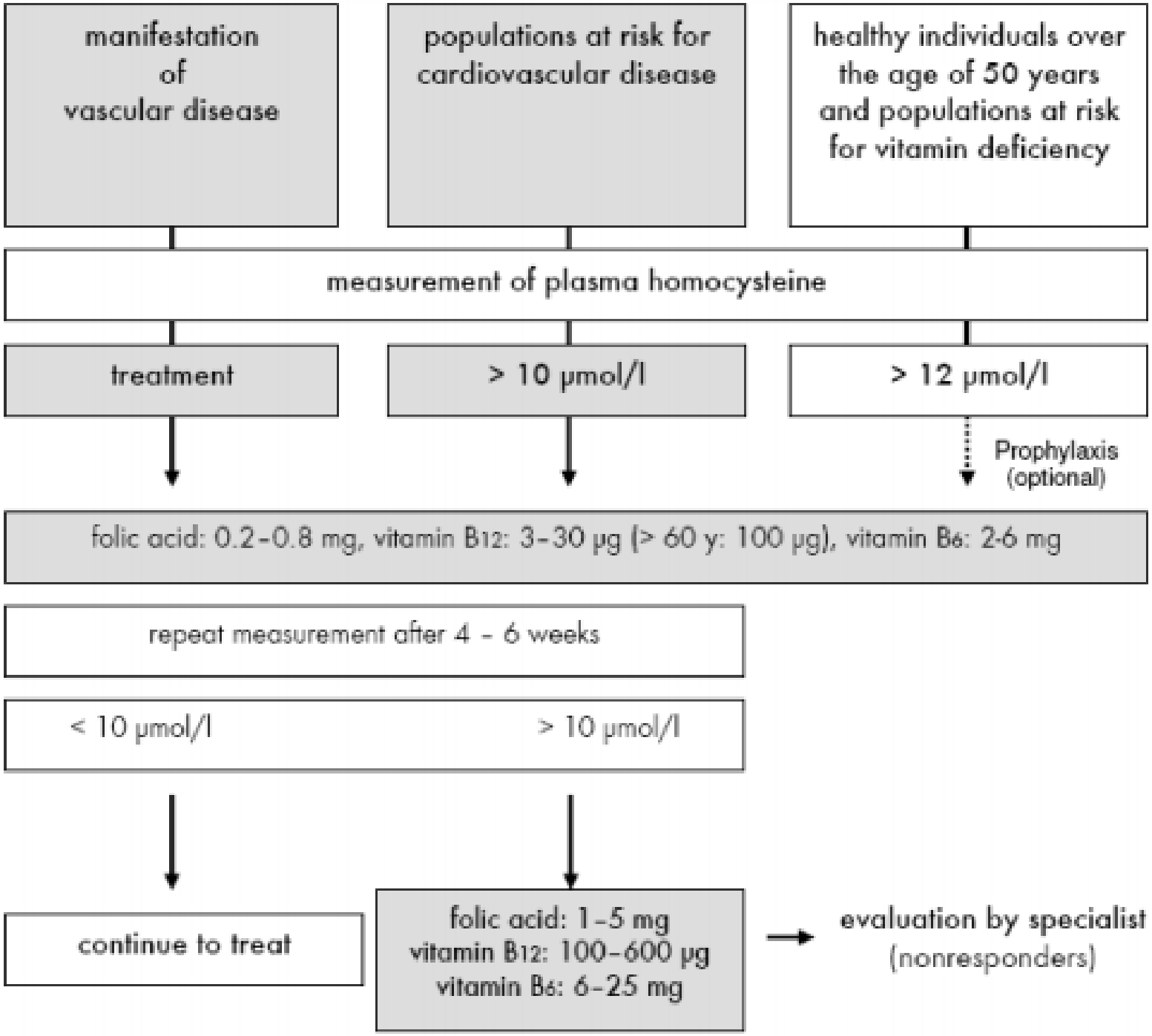
Homocysteine: clinical lab details

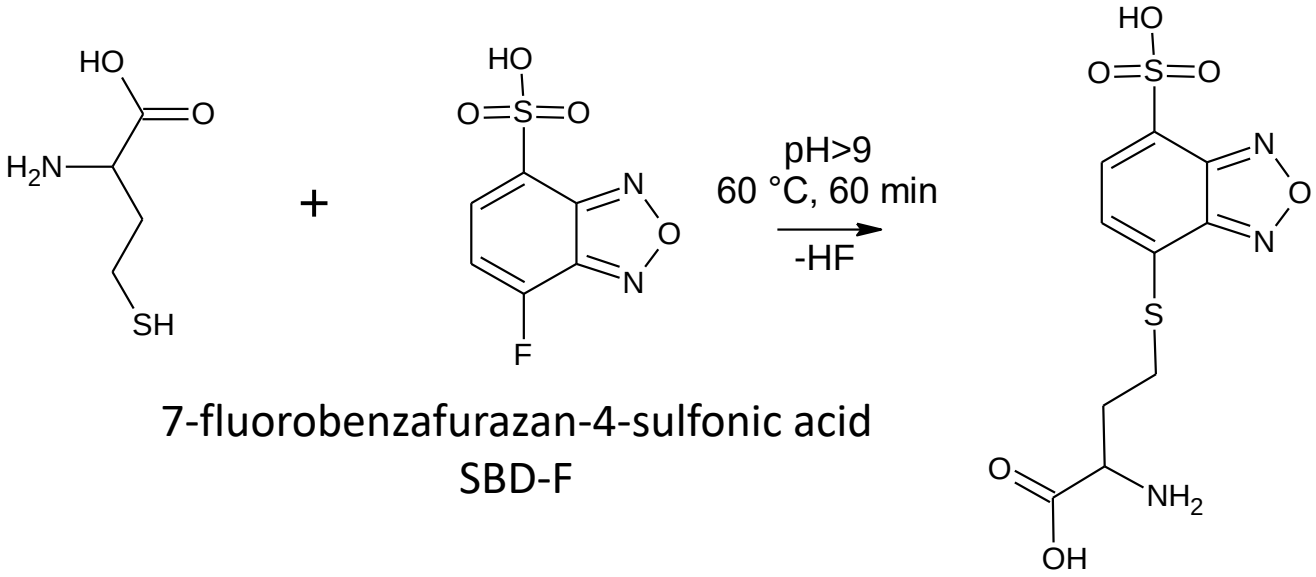
- specimen: plasma (EDTA, heparinized)
- specimen stability: ambient, 1 h. Refrigerated: 1 week. Frozen: 3 months
- 0-30 y: 4.6-8.1 µmol/l
- 30-59 y: males – 6.3-11.2 µmol/l, females – 4.0-7.9 µmol/l
- >59 y: 5.8-11.9 µmol/l



Pathology of homocysteine

- plasma and urine levels elevated in homocystinuria
- plasma levels elevated in Vitamin B-12 deficiency, vitamin B-6 deficiency, folic acid deficiency
- plasma levels may be elevated in hypothyroidism, impaired kidney function, SLE
- drugs can increase homocysteine levels: methotrexate, carbamazepine


manifestation of vascular disease
populations at risk for cardiovascular disease
healthy individuals over the age of 50 years and populations at risk for vitamin deficiency
measurement of plasma homocysteine
treatment
> 10 µmol/l
> 12 µmol/l
Prophylaxis (optional)
folic acid: 0.2–0.8 mg, vitamin B12: 3–30 µg (> 60 y: 100 µg), vitamin B6: 2-6 mg
repeat measurement after 4 – 6 weeks
< 10 µmol/l
> 10 µmol/l
continue to treat
folic acid: 1–5 mg
vitamin B12: 100–600 µg
vitamin B6: 6–25 mg
evaluation by specialist
(nonresponders)

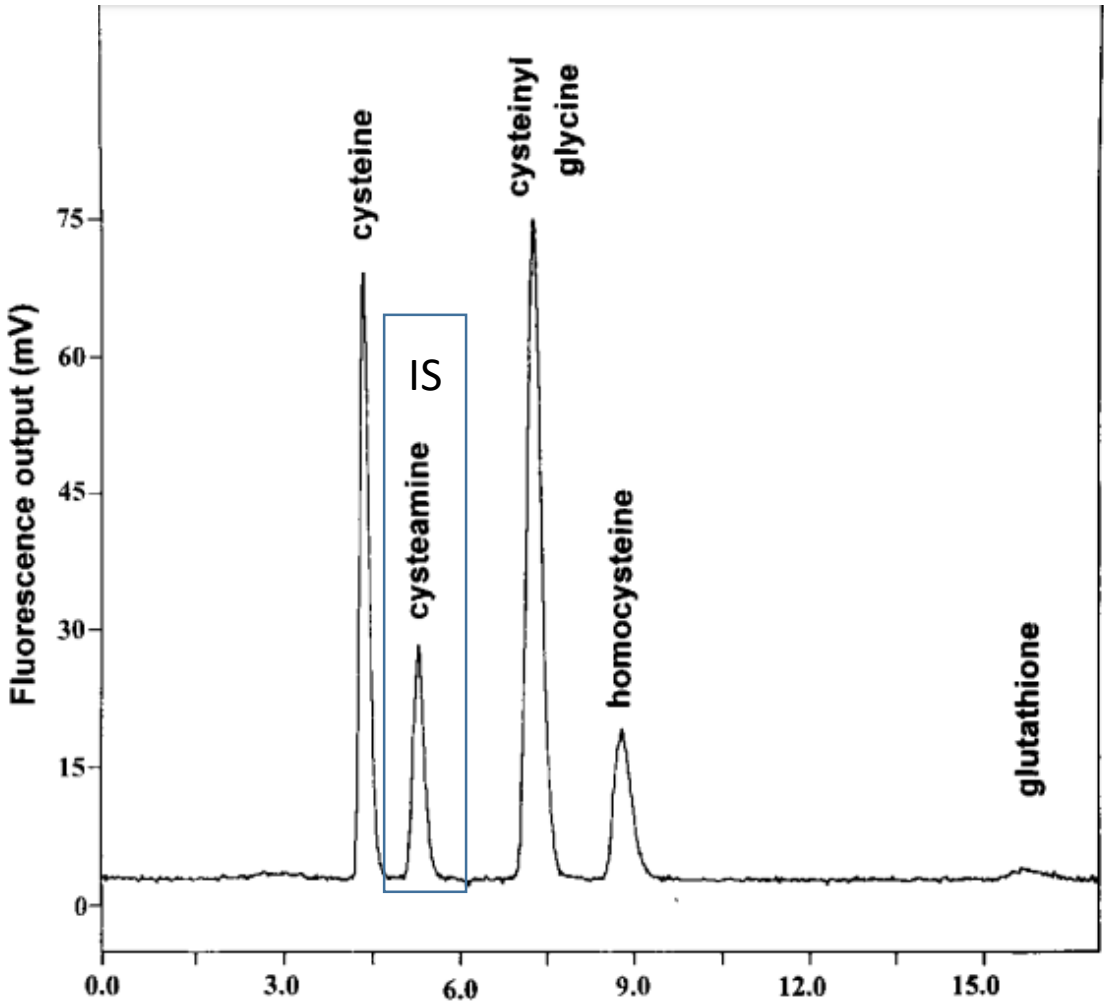


7-fluorobenzafurazan-4-sulfonic acid
SBD-F

Sample volume: 150 µL

1. Liberation: 100 mL/L tri-n-butylphosphine in DMF 4 °C/30 min
2. Deproteinization: 150 µL TCA solution (0.1 g/mL)
3. Derivatization

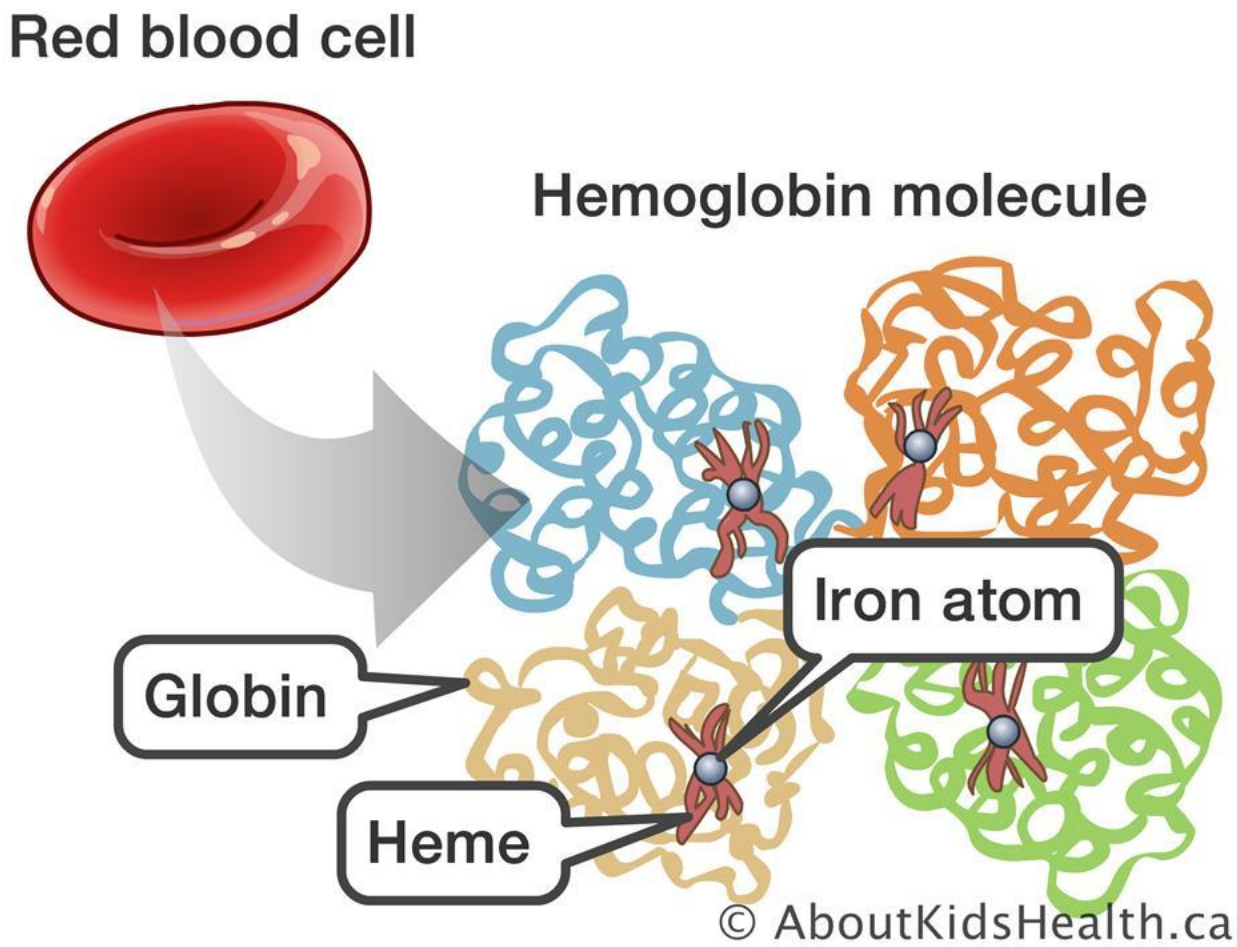
SP: C18 250x4x6 mm, 5 µm (ambient)
MP: 0.1 M KH_2PO4 (pH=2.0):acetonitrile 96:4
FR: 0.8 mL/min
EX: 385 nm
EM: 515 nm

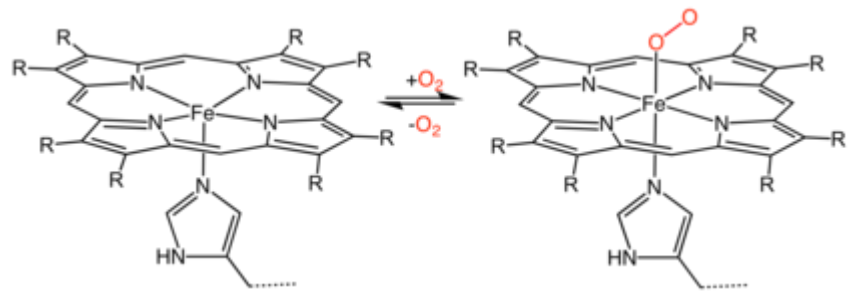


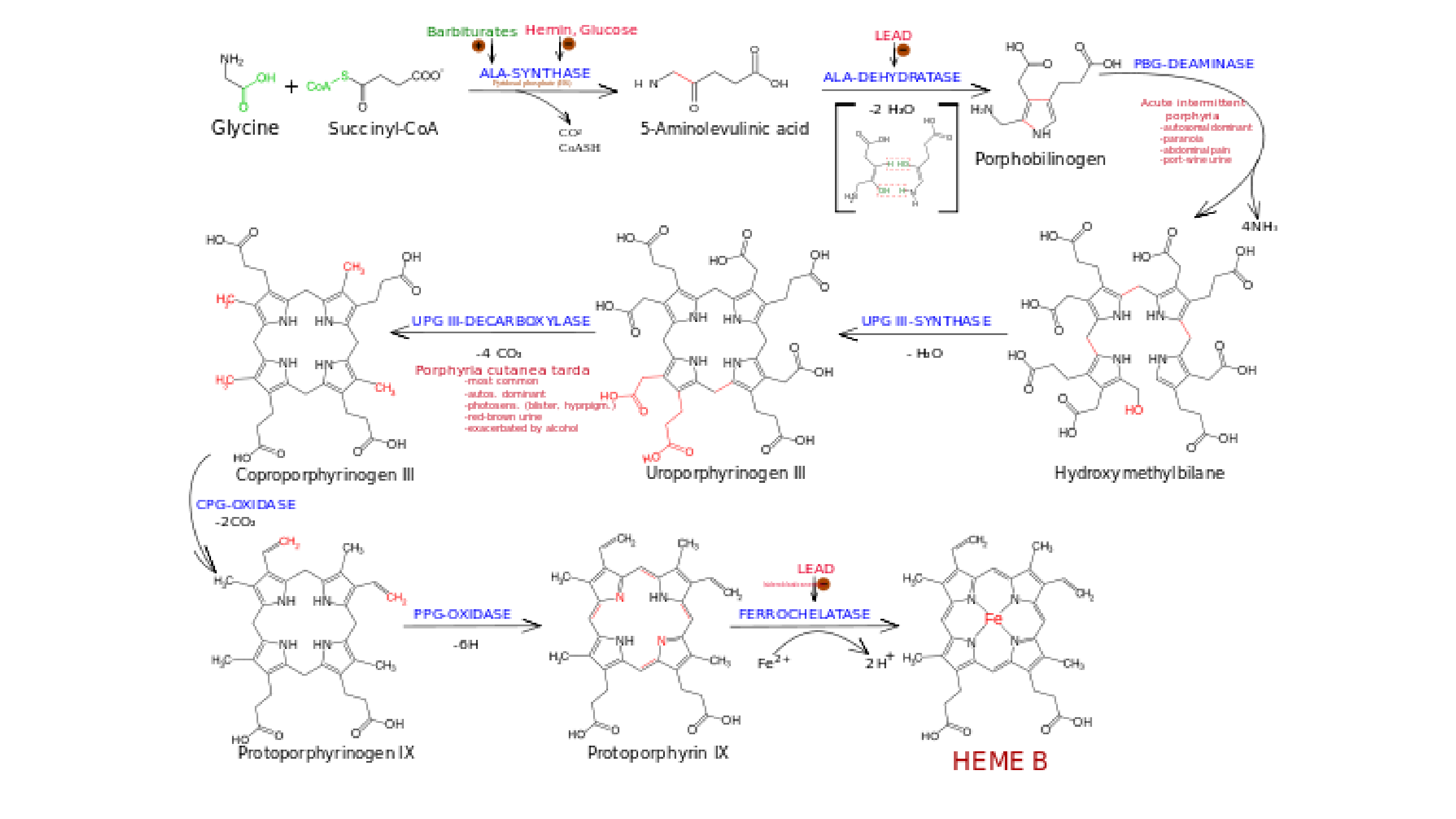
Kuo K et al. Clin Chem 1997;43:1653.

Clinical laboratory parameters frequently assayed using HPLC with fluorescence detection

Porphyrins


Red blood cell
Hemoglobin molecule
Iron atom
Globin
Heme
© AboutKidsHealth.ca


+O2
-O2
Fe
HN


Barbiturates
Hemin, Glucose
ALA-SYNTHASE
Glycine
Succinyl-CoA
CO2
CoASH
5-Aminolevulinic acid
LEAD
ALA-DEHYDRATASE
-2 H2O
Porphobilinogen
PBG-DEAMINASE
Acute intermittent porphyria
-autosomal dominant
-paranoia
-abdominal pain
-port-wine urine
4NH3
UPG III-DECARBOXYLASE
-4 CO2
Porphyria cutanea tarda
-most common
-autos. dominant
-photosens. (blister, hyperpigm.)
-red-brown urine
-exacerbated by alcohol
UPG III-SYNTHASE
- H2O
Coproporphyrinogen III
Uroporphyrinogen III
Hydroxymethylbilane
CPG-OXIDASE
-2CO2
PPG-OXIDASE
-6H
Protoporphyrinogen IX
Protoporphyrin IX
LEAD
FERROCHELATASE
Fe2+
2 H+
HEME B

Types of porphyrias

Acute porphyrias	Chronic porphyrias
Aminolevulinic acid dehydratase deficiency (ALAD)	X-linked dominant protoporphyria (XLPDD)
Acute intermittent porphyria (AIP)	Congenital erythropoietic porphyria (CEP)
Hereditary coproporphyria (HCP)	Porphyria cutanea tarda (PCT)
Porphyria variegata (VP)	Erythropoietic protoporphyria (EPP)

Signs and symptoms of acute porphyria may include:

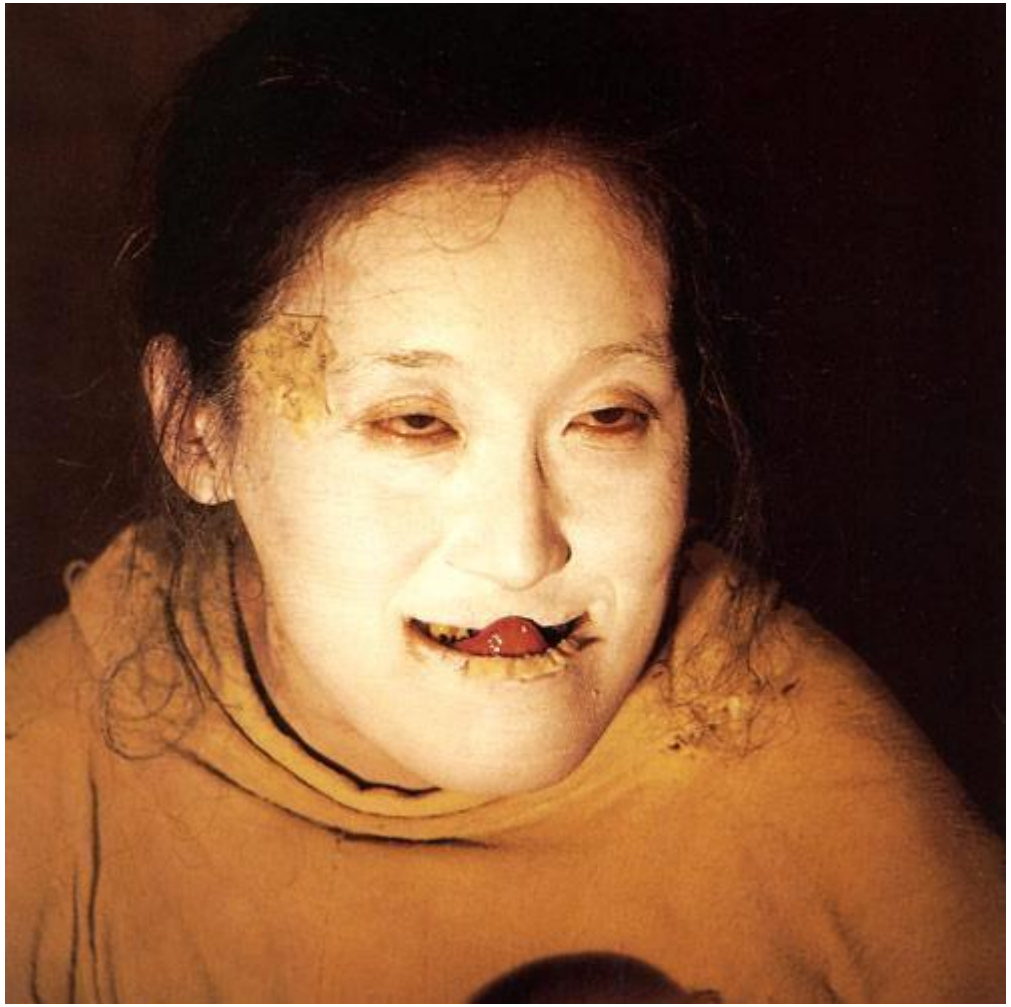
- Severe abdominal pain
- Pain in your chest, legs or back
- Constipation or diarrhea
- Nausea and vomiting
- Muscle pain, tingling, numbness, weakness or paralysis
- Red or brown urine
- Mental changes, such as anxiety, confusion, hallucinations, disorientation or paranoia
- Breathing problems
- Urination problems
- Rapid or irregular heartbeats you can feel (palpitations)
- High blood pressure
- Seizures

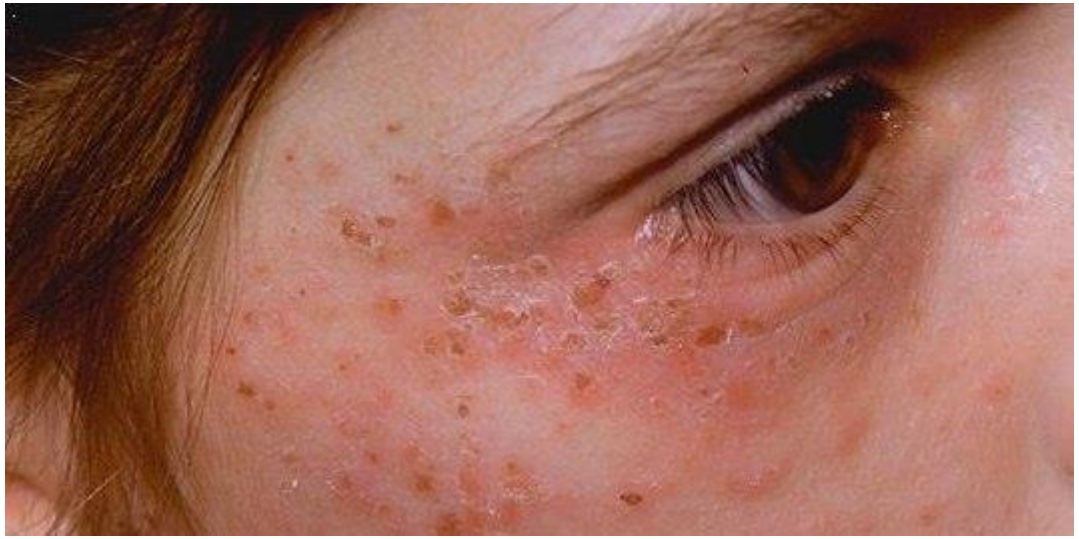
Cutaneous porphyrias

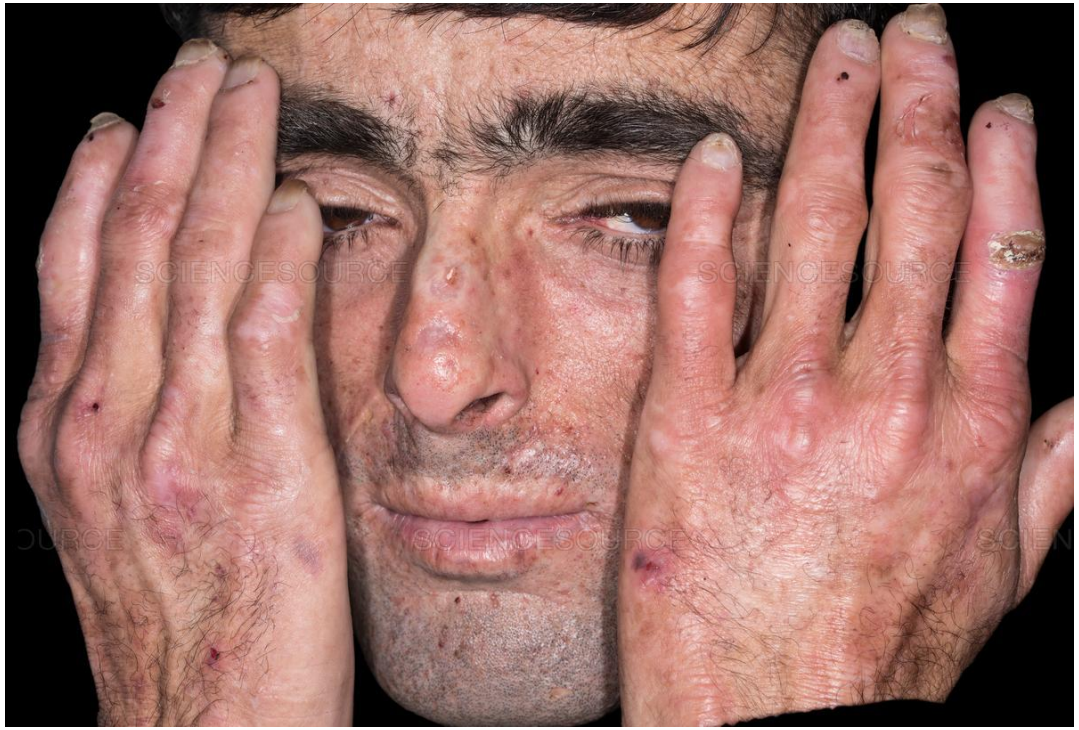
Cutaneous porphyrias include forms of the disease that cause skin symptoms as a result of sensitivity to sunlight, but these forms don't usually affect your nervous system. Porphyria cutanea tarda (PCT) is the most common type of all the porphyrias.

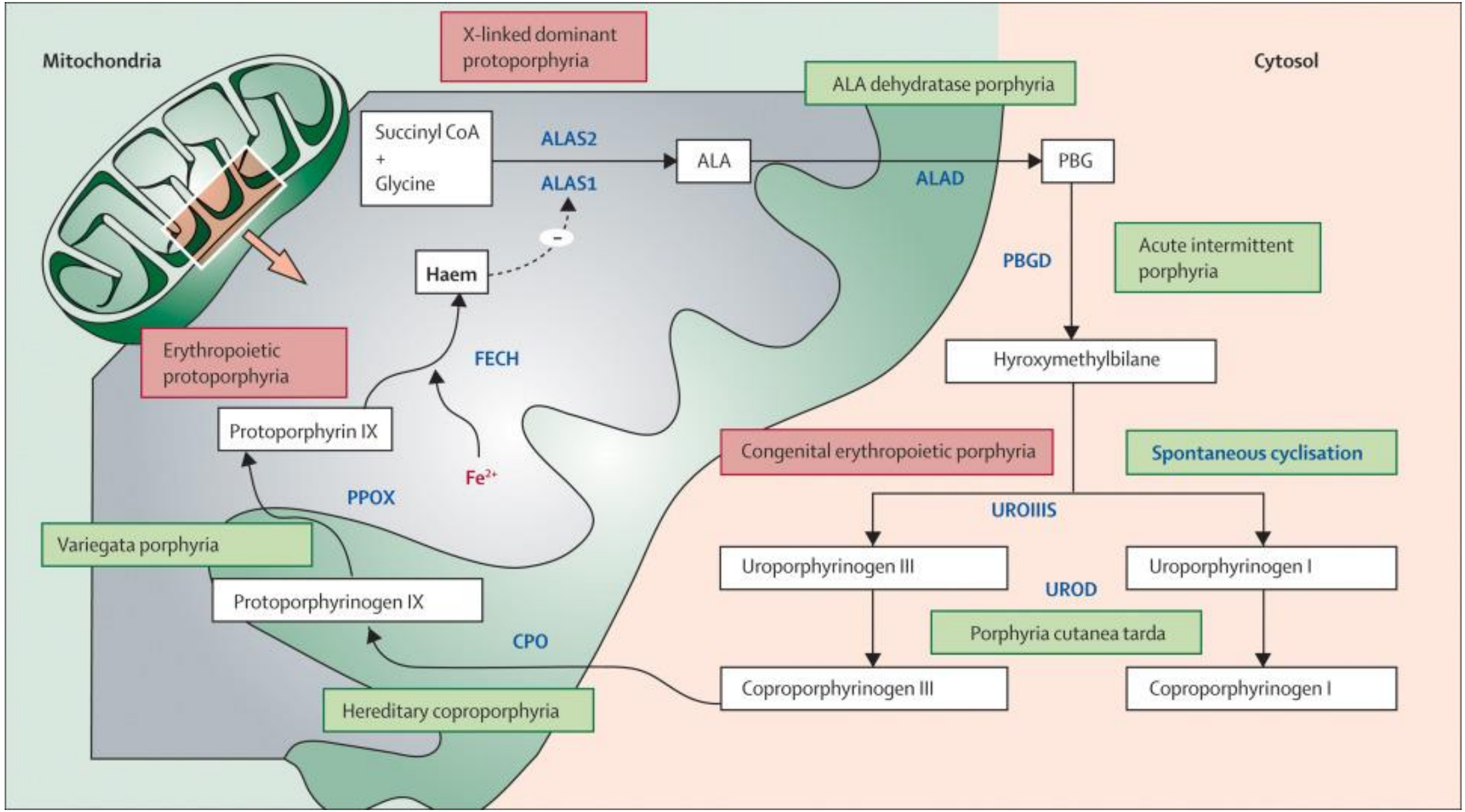
As a result of sun exposure, you may experience:

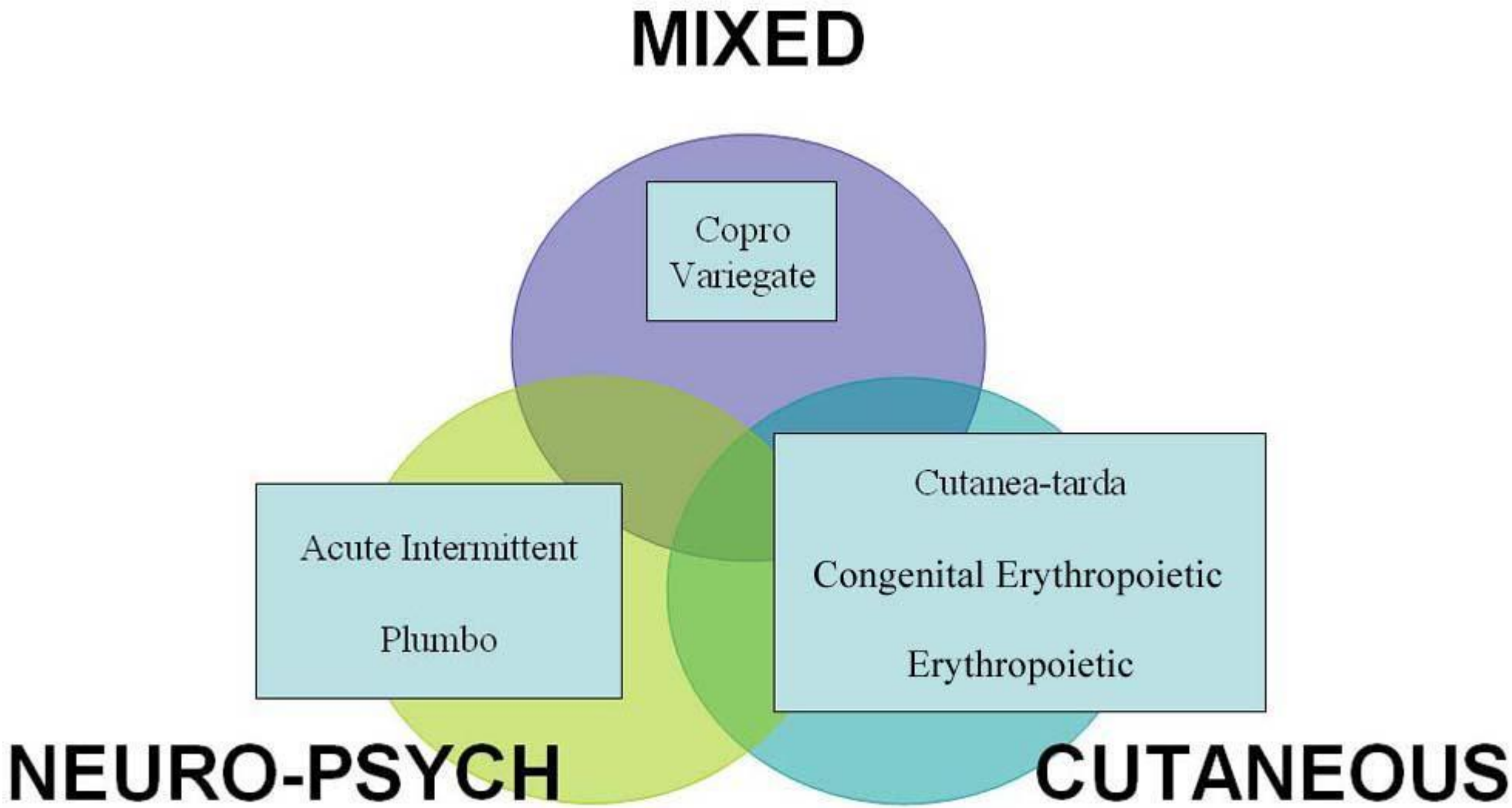
- Sensitivity to the sun and sometimes artificial light, causing burning pain
- Sudden painful skin redness (erythema) and swelling (edema
- Blisters on exposed skin, usually the hands, arms and face
- Fragile thin skin with changes in skin color (pigment)
- Itching
- Excessive hair growth in affected areas
- Red or brown urine








Mitochondria
Cytosol
X-linked dominant protoporphyria
ALA dehydratase porphyria
Succinyl CoA + Glycine
ALAS2
ALAS1
ALA
ALAD
PBG
Haem
PBGD
Acute intermittent porphyria
Erythropoietic protoporphyria
FECH
Hyroxymethylbilane
Protoporphyrin IX
Fe2+
Congenital erythropoietic porphyria
Spontaneous cyclisation
PPOX
UROIIIS
Variegata porphyria
Uroporphyrinogen III
Uroporphyrinogen I
Protoporphyrinogen IX
UROD
Porphyria cutanea tarda
CPO
Coproporphyrinogen III
Coproporphyrinogen I
Hereditary coproporphyria



https://emedicine.medscape.com/article/1389981-overview

EX: 400 nm (Soret-band)
EM: 620 nm

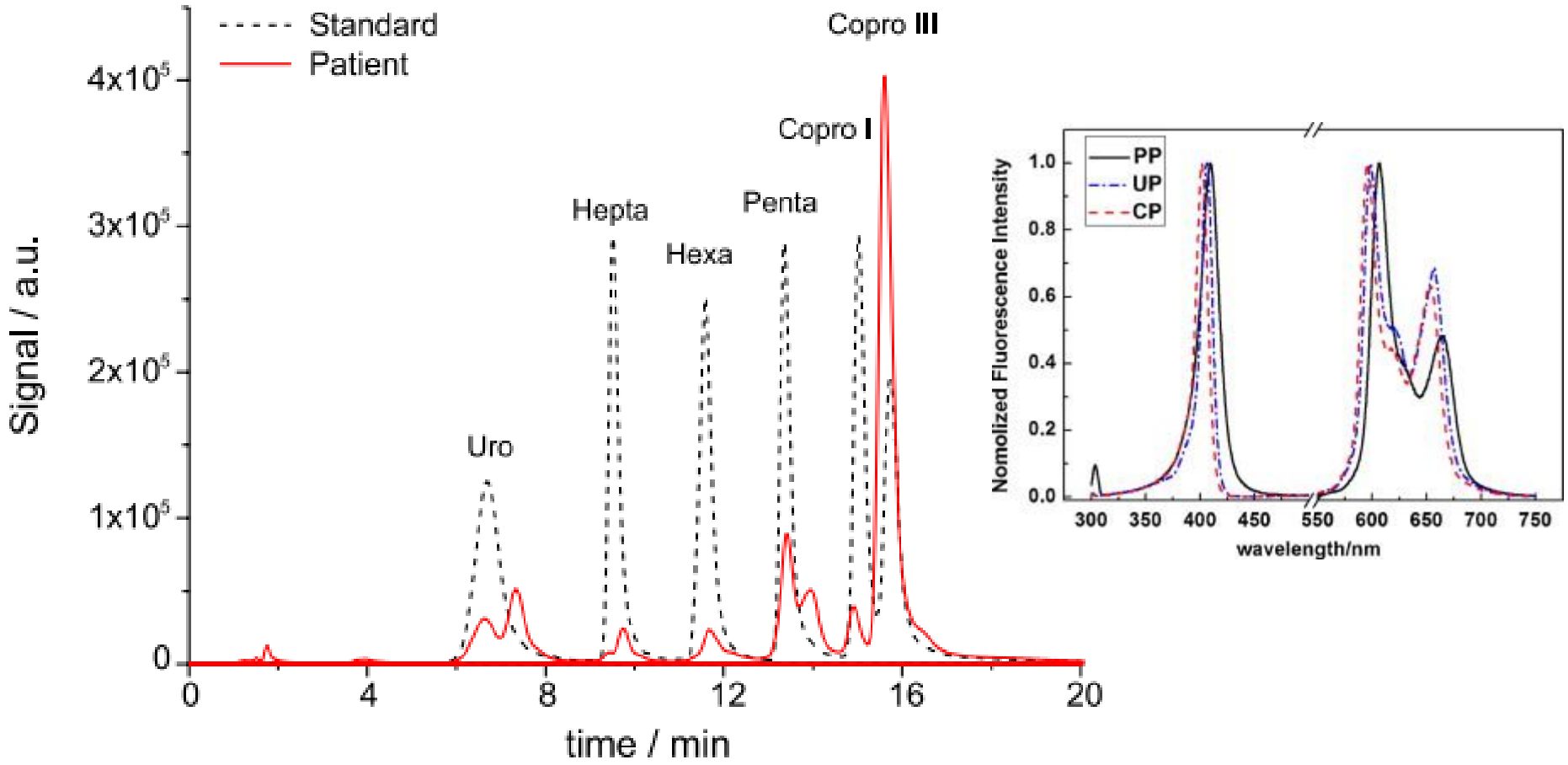
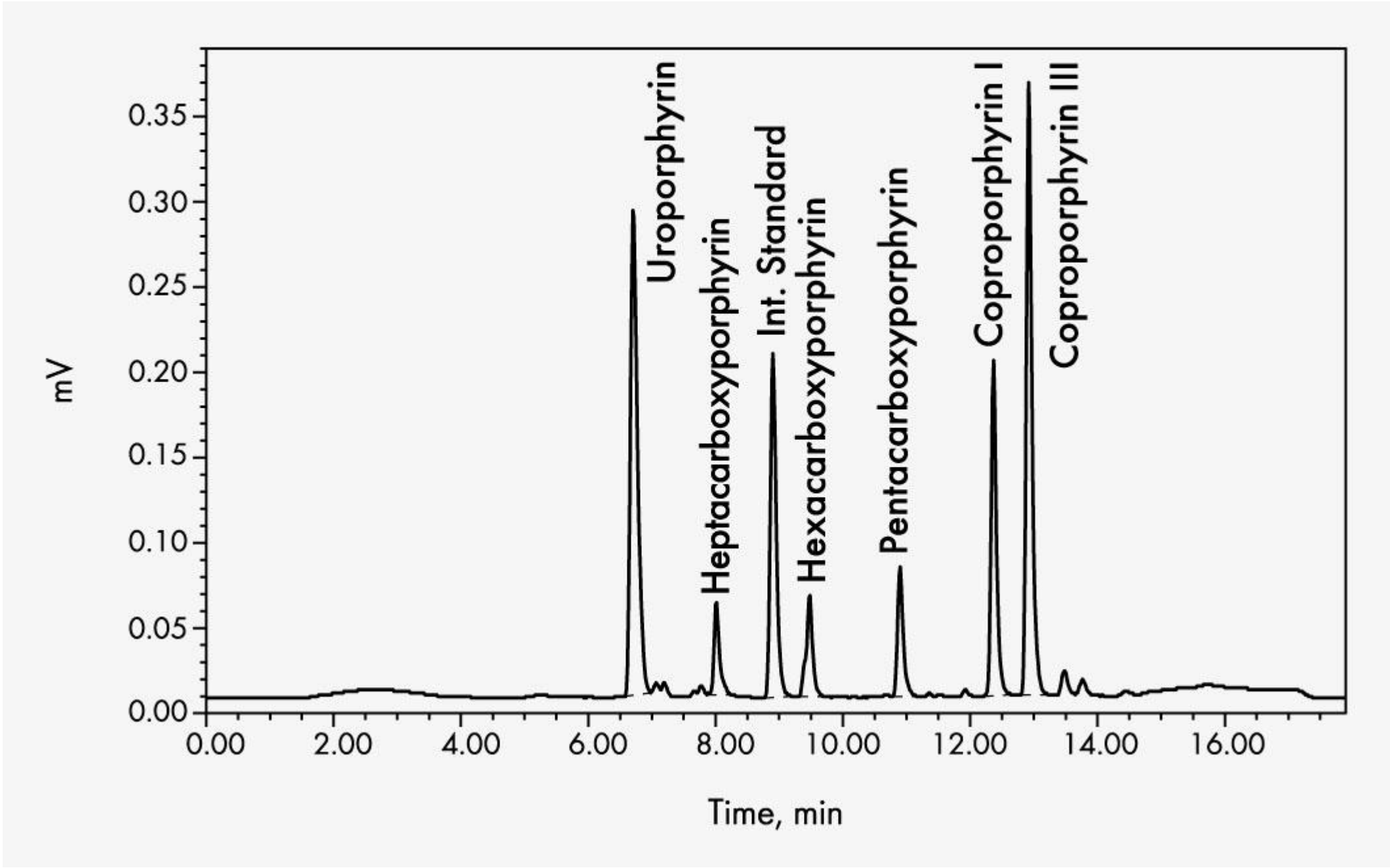


Figure 2. Comparative HPLC/fluorescence traces obtained for a standard mixture of porphyrins (dashed line) and the urine of a porphyric patient (solid line). Eluent A: 1.0 mol L^{-1} ammonium acetate buffer containing 10% acetonitrile at pH 5.7. Eluent B: methanol:acetonitrile (9:1, v/v). Patient 2 was clinically diagnosed as an acute intermittent porphyria carrier. Porphyrin concentration was 500 nmol L^{-1}.

Cypriani MGSC et al. J Braz Chem Soc 2014;25:1918.


Uroporphyrin
Heptacarboxyporphyrin
Int. Standard
Hexacarboxyporphyrin
Pentacarboxyporphyrin
Coproporphyrin I
Coproporphyrin III
mV
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
Time, min

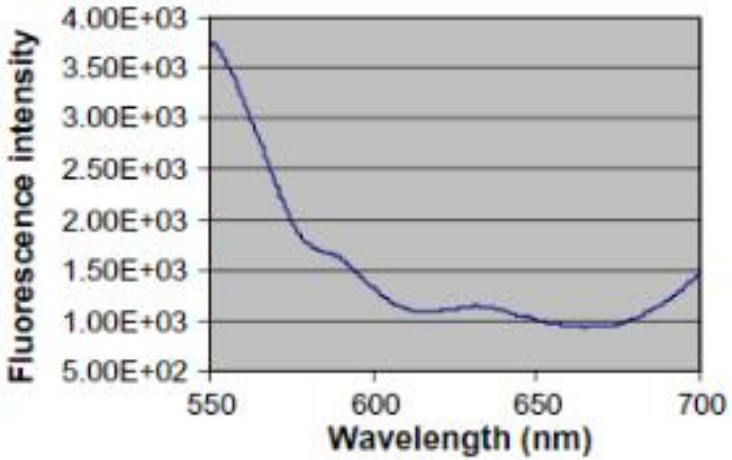


Figure 3 Florescence emission spectrum of normal plasma: no porphyrin like emission is observed. (The nonzero baseline is due to scattered exciting light).

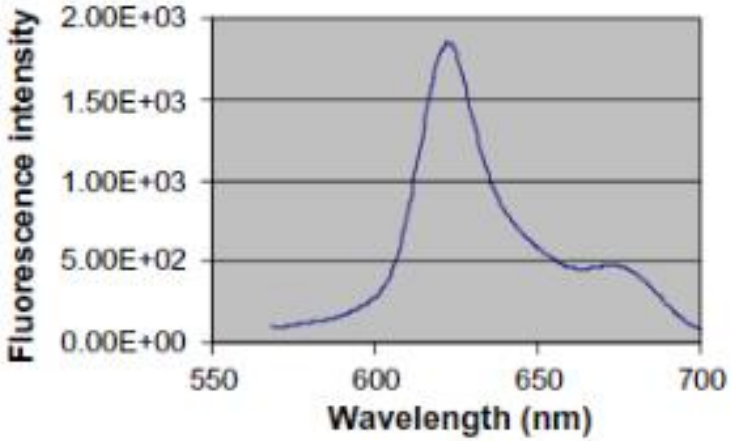


Figure 4 Florescence emission spectrum of plasma of VP patient: excitation, 400 nm and 625 indicates fluorescence emission maximum of 625 nm.

Fateen E et al. Egypt J Med Hum Genet 2011;12:49.

Table 2. Second-line Biochemical Testing for Acute Porphyrias: Laboratory Findings to Differentiate Between AIP, HCP and VP.

Acute Porphyria	HMBS activity in RBCs	Urine PBG	Urine ALA	Urine porphyrins	Fecal porphyrins	Plasma porphyrins
AIP	Decreased in ~90% of cases	elevated	elevated	Markedly increased; mostly uroporphyrin	Normal or slightly increased	Normal or slightly increased
HCP	normal	elevated	elevated	Markedly increased; mostly coproporphyrin	Markedly increased; mostly coproporphyrin III	Normal or slightly increased
VP	normal	elevated	elevated	Markedly increased; mostly coproporphyrin III	Markedly increased; mostly protoporphyrin	Markedly increased; Fluorescence peak (at neutral pH) at ~626nm
ADP	normal	normal	elevated	Markedly increased; mostly coproporphyrin III	Normal or slightly increased	Normal or slightly increased

J Clin Pathol. 2014 Jan;67(1):60-5. doi: 10.1136/jclinpath-2012-201367. Epub 2013 Aug 1.

Urinary excretion of porphyrins, porphobilinogen and δ-aminolaevulinic acid following an attack of acute intermittent porphyria.

Marsden JT[1], Rees DC.

Author information

Abstract

BACKGROUND AND OBJECTIVES: The porphyrias are a group of rare, mainly inherited, diseases caused by a deficiency of one of the enzymes of the haem biosynthesis pathway. The biochemical hallmark of an acute attack is an increase in urine porphobilinogen (PBG), together with an increase in urinary excretion of δ-aminolaevulinic acid (ALA) and total urine porphyrins (TUP). In patients with acute intermittent porphyria (AIP) the concentrations of the porphyrin precursors are thought to remain elevated for many years following an acute attack, although this has not been well documented.

METHODS: We measured urine ALA, PBG and TUP excretion in 20 patients with AIP following an attack of acute porphyria over a time period of 3 months to 23 years after their last documented acute attack.

RESULTS: We showed that urinary concentrations of all metabolites remain elevated for many years. The urinary half life of TUP was 5.3 years, ALA 7.7 years and PBG 10.6 years. Even after 20 years, PBG concentrations remained elevated above the normal range.

CONCLUSIONS: Our study highlights the difficulties of using urinary analysis for diagnosing recurrent attacks, and also raises important questions about the pathophysiology of the condition.

KEYWORDS: Inherited Pathology; Laboratory Tests; Metabolism

PMID: 23908454 DOI: 10.1136/jclinpath-2012-201367

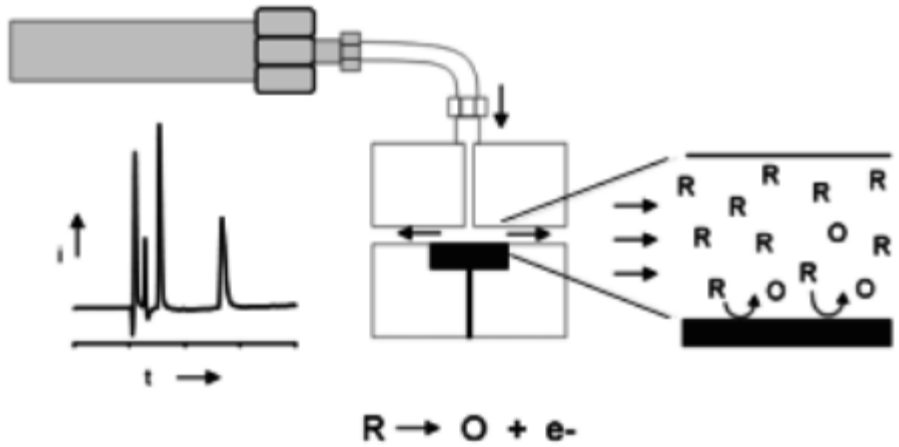
[Indexed for MEDLINE]

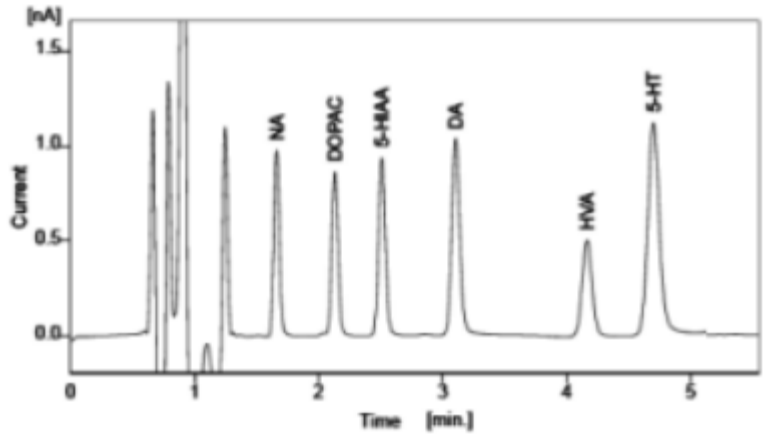
How to approach porphyrin tests

- Urine: dilute and shoot
- Faeces: extract with organic solvent, concentrate+dilute with aqueous buffer
- Blood: separate red blood cells and add water for hemolysis

Electrochemical detection

Amperometric, coulometric and voltammetric detection modes





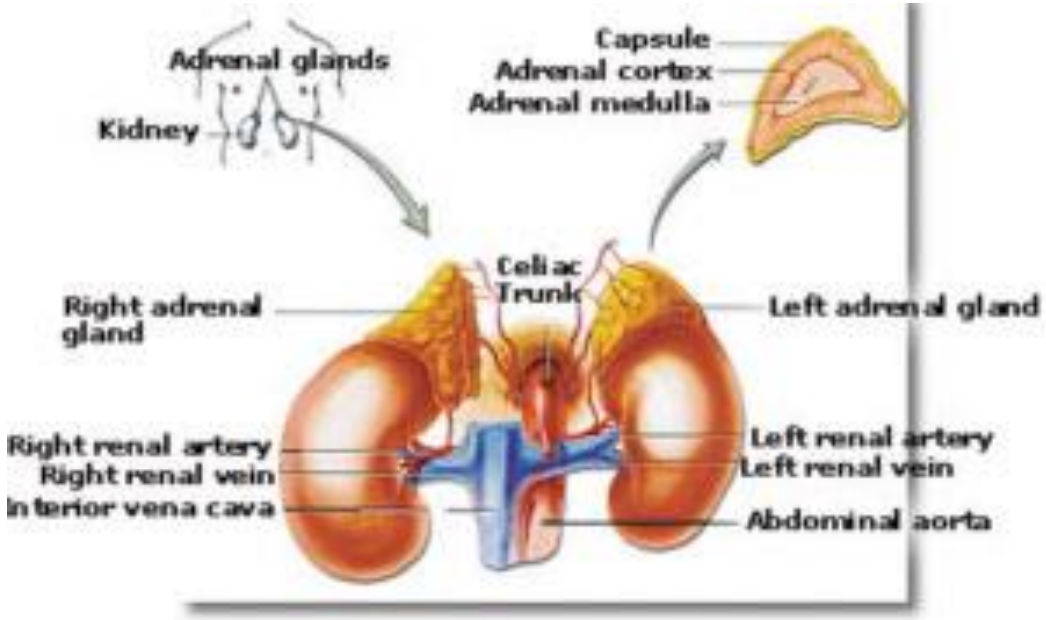
A sample is introduced in HPLC and separated on the chromatographic column. The column is connected to an ECD cell, an electrochemical sensor where a reaction takes place at an electrode. Electrochemically active substances that elute from the column undergo an electrochemical reaction, electrons are transferred resulting in an electrical current.

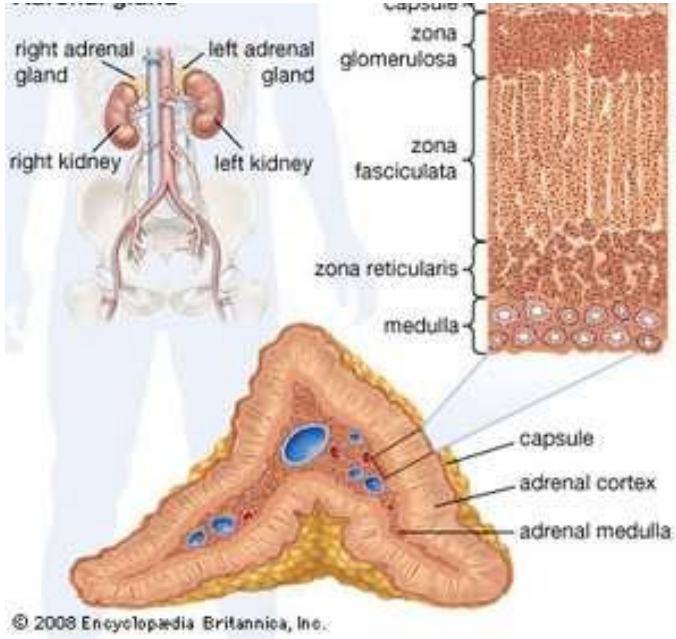
https://www2.chemistry.msu.edu/courses/cem837/Electrochemical%20Detection.pdf

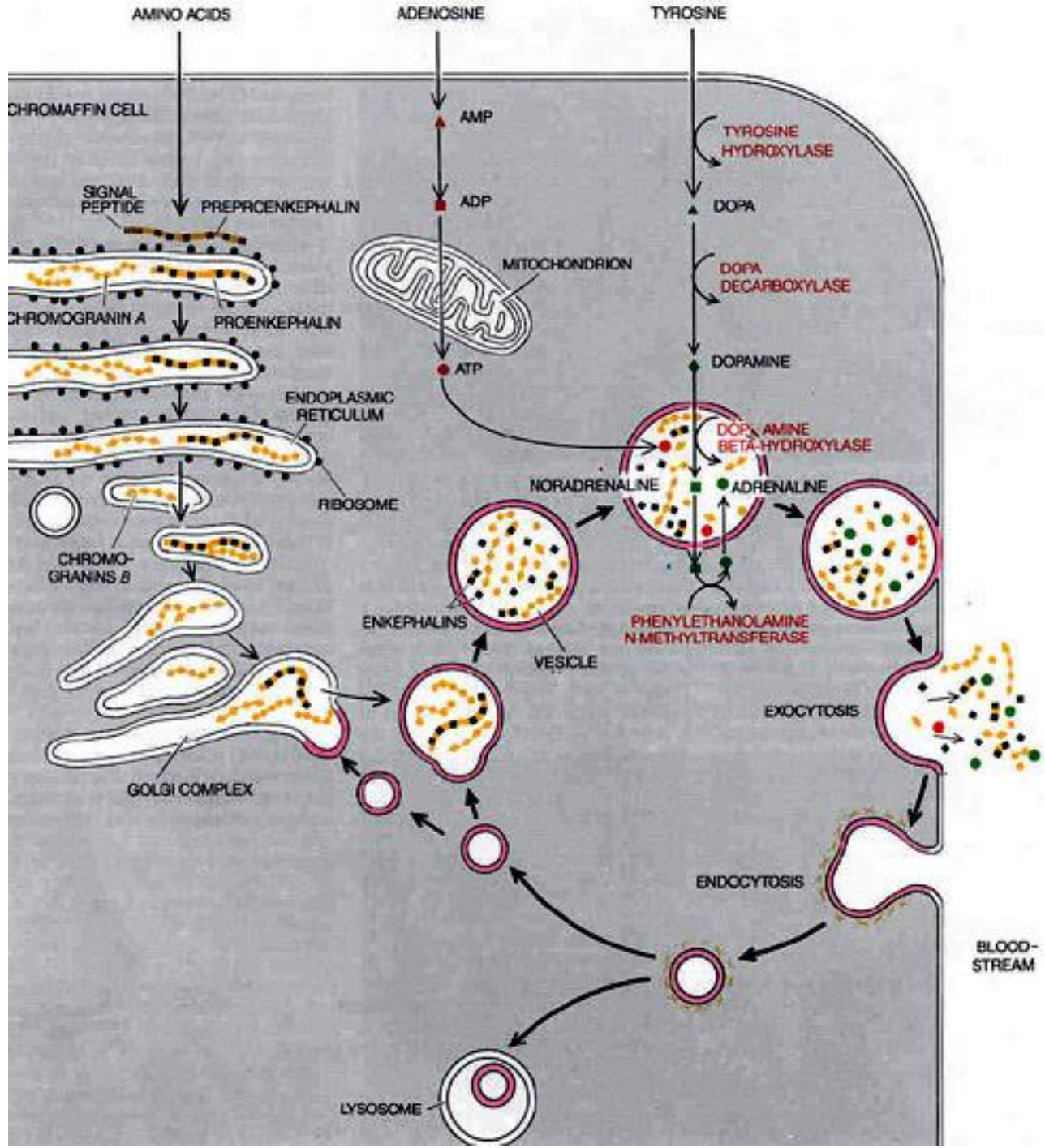
Pros and cons of using HPLC-ECD systems

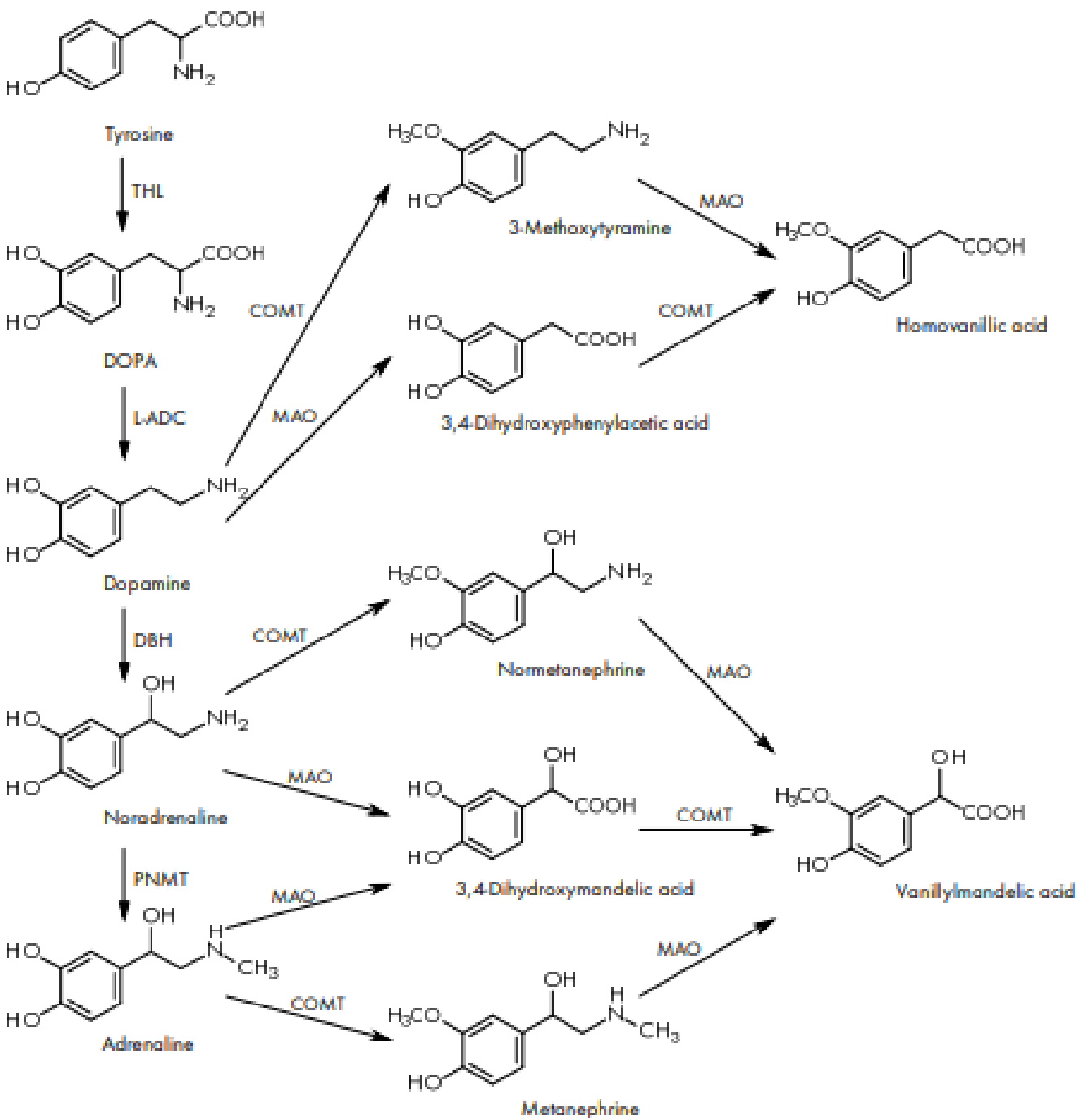
- **Advantages:**
  - High selectivity
  - MS-level sensitivity
  - Electrochemical cell is enduring and maintainable by user

- **Disadvantages:**
  - Only isocratic methods can be used efficiently
  - No opportunity to obtain structural information on analytes
  - Sensitive to chemical contaminations, requires user maintenance


Adrenal glands
Kidney
Capsule
Adrenal cortex
Adrenal medulla
Right adrenal gland
Left adrenal gland
Right renal artery
Right renal vein
Left renal artery
Left renal vein
Abdominal aorta

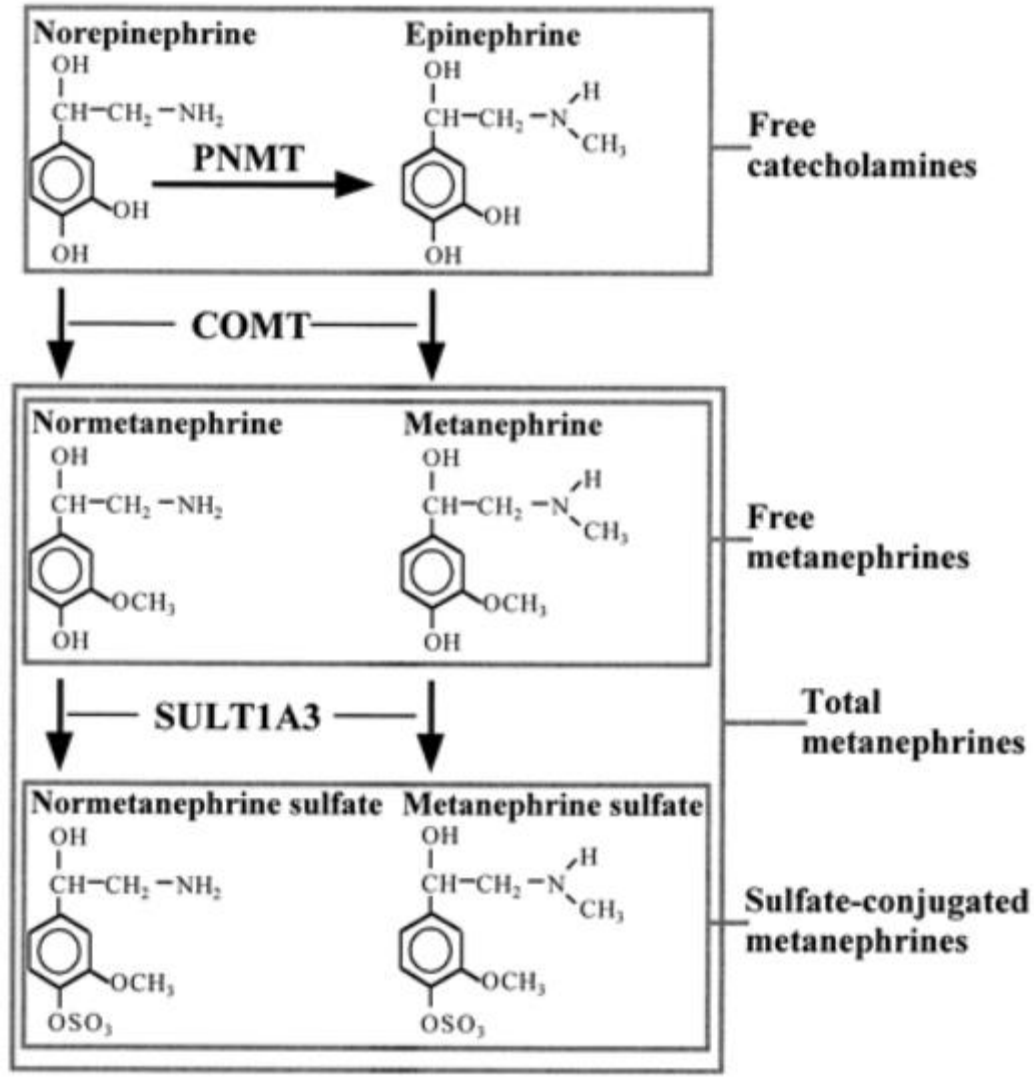

right adrenal gland
left adrenal gland
right kidney
left kidney
zona glomerulosa
zona fasciculata
zona reticularis
medulla
capsule
adrenal cortex
adrenal medulla
© 2008 Encyclopædia Britannica, Inc.


AMINO ACIDS
ADENOSINE
TYROSINE
CHROMAFFIN CELL
AMP
ADP
ATP
TYROSINE HYDROXYLASE
DOPA
DOPA DECARBOXYLASE
DOPAMINE
SIGNAL PEPTIDE
PREPROENKEPHALIN
PROENKEPHALIN
CHROMOGRANIN A
MITOCHONDRION
ENDOPLASMIC RETICULUM
RIBOSOME
DOPAMINE BETA-HYDROXYLASE
NORADRENALINE
ADRENALINE
CHROMO-GRANINS B
ENKEPHALINS
VESICLE
PHENYLETHANOLAMINE N-METHYLTRANSFERASE
EXOCYTOSIS
GOLGI COMPLEX
ENDOCYTOSIS
BLOOD-STREAM
LYSOSOME


Tyrosine
THL
DOPA
LADC
Dopamine
DBH
Noradrenaline
PNMT
Adrenaline
COMT
MAO
3-Methoxytyramine
3,4-Dihydroxyphenylacetic acid
Homovanillic acid
Normetanephrine
3,4-Dihydroxymandelic acid
Vanillylmandelic acid
Metanephrine

Chromaffin cell tumors

- account for about 0.2-0.6% of hypertension cases
- genetic disposition or sporadic
- 10-17% of tumors are malignant
- phaeochromocytoma (80-85%), paraganglioma (15-20%)
- neuroblastoma: neural crest origin, most common malignancy at 0-1 y


Norepinephrine
Epinephrine
PNMT
Free catecholamines
COMT
Normetanephrine
Metanephrine
Free metanephrines
SULT1A3
Total metanephrines
Normetanephrine sulfate
Metanephrine sulfate
Sulfate-conjugated metanephrines

Diagnostic workup of catecholamine producing chromaffin cell tumors

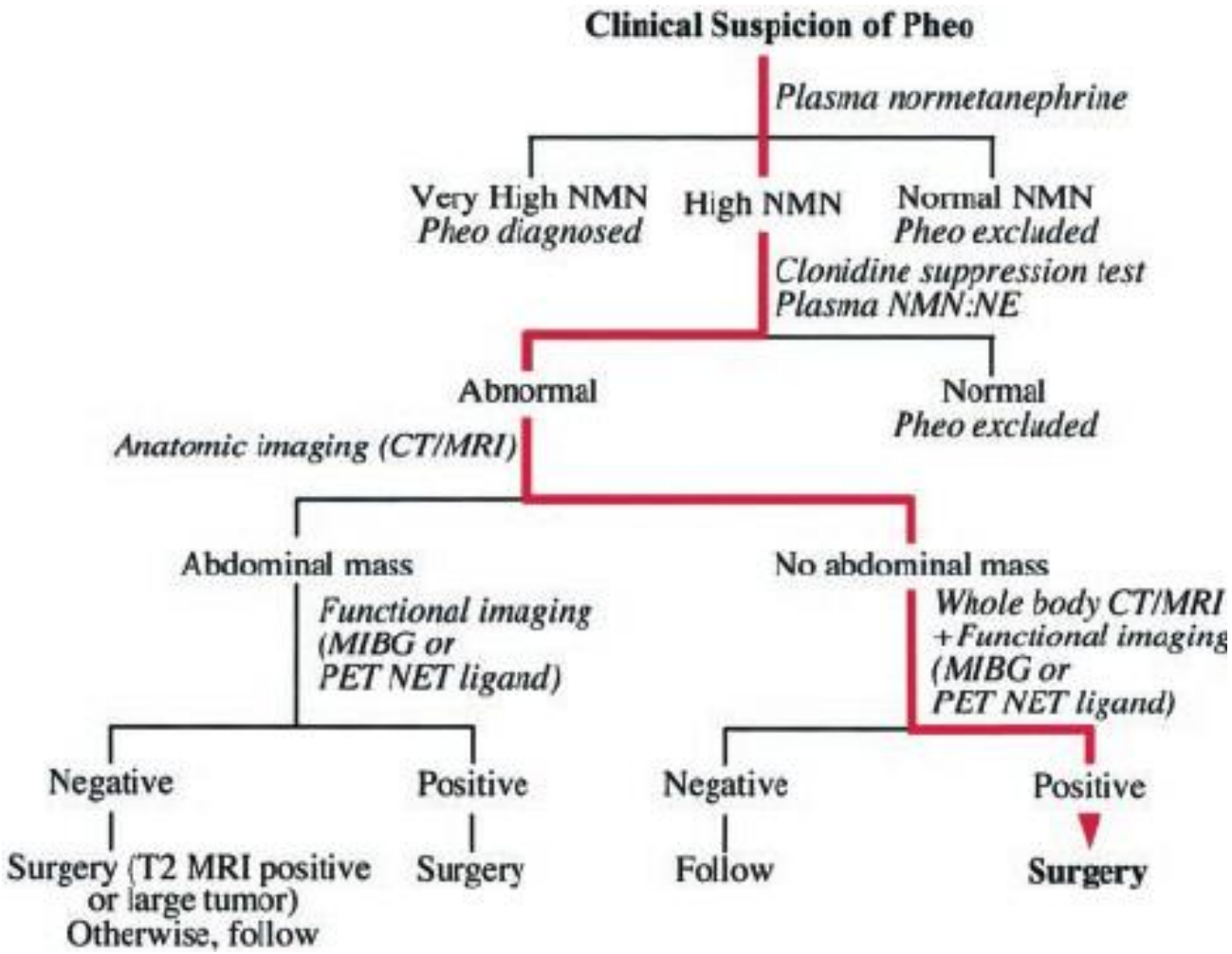


Table 1. Plasma concentrations (medians and ranges) and urinary outputs of catecholamine *O*-methylated metabolites in the reference population and patients with and without PPGLs.

Test panel	Reference population[a]	No PPGL	PPGL
Plasma free metabolites	n = 586[a]	n = 1820	n = 236
Normetanephrine, pg/mL	62 (18–201)	67 (11–365)	642 (45–25 444)
Metanephrine, pg/mL	29 (5–89)	30 (0.2–145)	120 (5–6889)
Methoxytyramine, pg/mL	4.9 (1.4–17.6)	4.8 (0.4–36.7)	14.1 (0.6–11 444)
Urine free metabolites	n = 580[a]	n = 1756[b]	n = 226[b]
Normetanephrine, μg/day	21 (4–100)	22 (1–170)	229 (9–3478)
Metanephrine, μg/day	18 (2–61)	16 (0.2–172)	64 (1–3547)
Methoxytyramine, μg/day	33 (4–136)	34 (2–212)	50 (8–3202)
Urine deconjugated metabolites	n = 581[a]	n = 1757[b]	n = 226[b]
Normetanephrine, μg/day	189 (41–803)	212 (26–2678)	1239 (172–21 850)
Metanephrine, μg/day	105 (17–446)	108 (1–991)	419 (9–14 946)
Methoxytyramine, μg/day	188 (52–2185)	197 (20–2990)	323 (58–13 031)

[a] Inclusion of the reference population in the table is to provide a comparison with patients without PPGLs. Specified ranges do not indicate the reference intervals that were used, which are supplied in the online Data Supplement. Among the 590 subjects of the reference population, measurements of plasma concentrations and urinary outputs of metabolites were not possible in up to 10 patients.

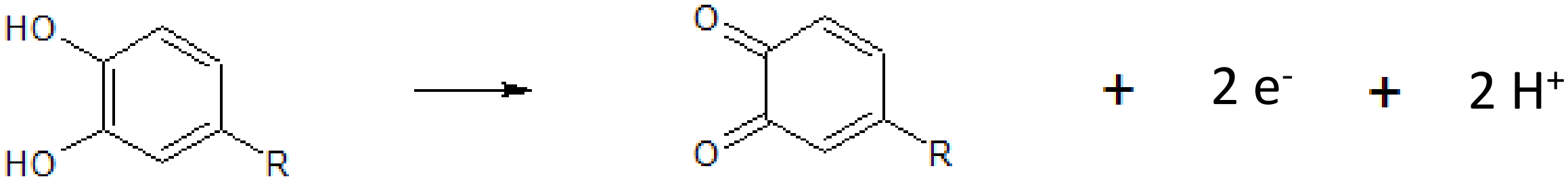
[b] Urinary measurements were not possible in up to 64 of the 1820 patients without PPGLs and 10 patients with PPGLs. To convert pg/mL to pmol/L and μg/day to nmol/day, divide values for normetanephrine, metanephrine, and methoxytyramine by 0.1832, 0.1972, and 0.1672, respectively.

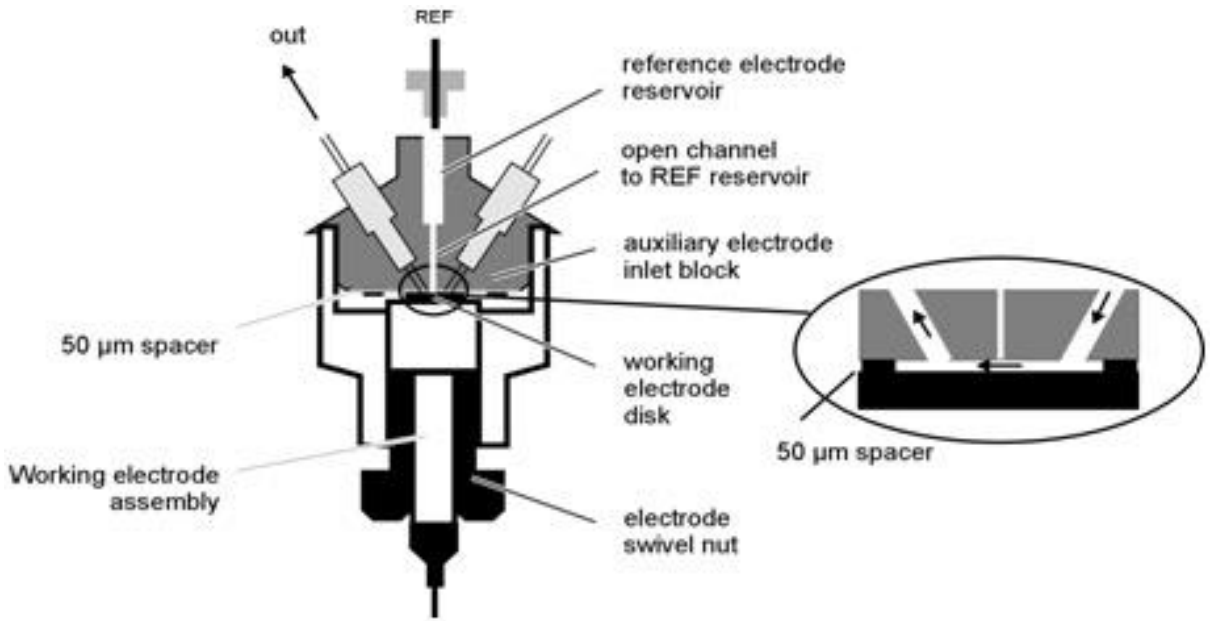
Eisenhofer G et al. Clin Chem 2018;64:1646.

The prerequisite of conducting efficient biochemical tests is a precise preanalytical workflow

- **Plasma tests**:
  - correct collection tube
  - seated vs supine sampling
  - sampling environment (patient stress)
  - specimen storage and transport

- **Urine tests:**
  - collected vs random urine
  - pH control
  - specimen storage and transport


HO
HO
R
O
O
R
+ 2 e⁻ + 2 H⁺


out
REF
reference electrode reservoir
open channel to REF reservoir
auxiliary electrode inlet block
50 µm spacer
working electrode disk
50 µm spacer
Working electrode assembly
electrode swivel nut

SUMMARY OF RECOMMENDATIONS

1.0 Biochemical Testing for Diagnosis of Pheochromocytoma and Paraganglioma (PPGL)

1.1 We recommend that initial biochemical testing for PPGLs should include measurements of plasma free metanephrines or urinary fractionated metanephrines. (**1**|⊕⊕⊕⊕)

1.2 We suggest using liquid chromatography with mass spectrometric or electrochemical detection methods rather than other laboratory methods to establish a biochemical diagnosis of PPGL. (**2**|⊕⊕◯◯)

1.3 For measurements of plasma metanephrines, we suggest drawing blood with the patient in the supine position and use of reference intervals established in the same position. (**2**|⊕⊕◯◯)

1.4 We recommend that all patients with positive test results should receive appropriate follow-up according to the extent of increased values and clinical presentation. (**1**|⊕⊕◯◯)

Weak recommendation

Low quality evidence

Lenders JWM et al. J Clin Endocrin Metab 2014;99:1915.

Catecholamines:
epinephrine, norepinephrine, dopamine

urine: free

- extraction using cation exchange
- WE: 550 mV
- analysis time: <5 min

plasma: free

- extraction using aluminum oxide
- WE: 550 mV
- SP: ambient
- analysis time: <20 min

Metanephrines:
metanephrine, normetanephrine, 3-methoxytyramine

urine: free or total

- hydrolysis of conjugated fraction (cc acid, 95 °C, 30 min)
- extraction using cation exchange
- WE: 750 mV
- analysis time: <10 min

plasma: free

- extraction using cation exchange
- SP: ambient
- analysis time: <5 min

End products:
vanilmandelic acid, homovanillic acid

urine: free

- extraction using anion exchange
- WE: 700-850 mV
- SP: ambient
- analysis time: <20 min

Electrochemical detection: amperometric
Working electrode: glassy carbon
Reference electrode: Ag/AgCl

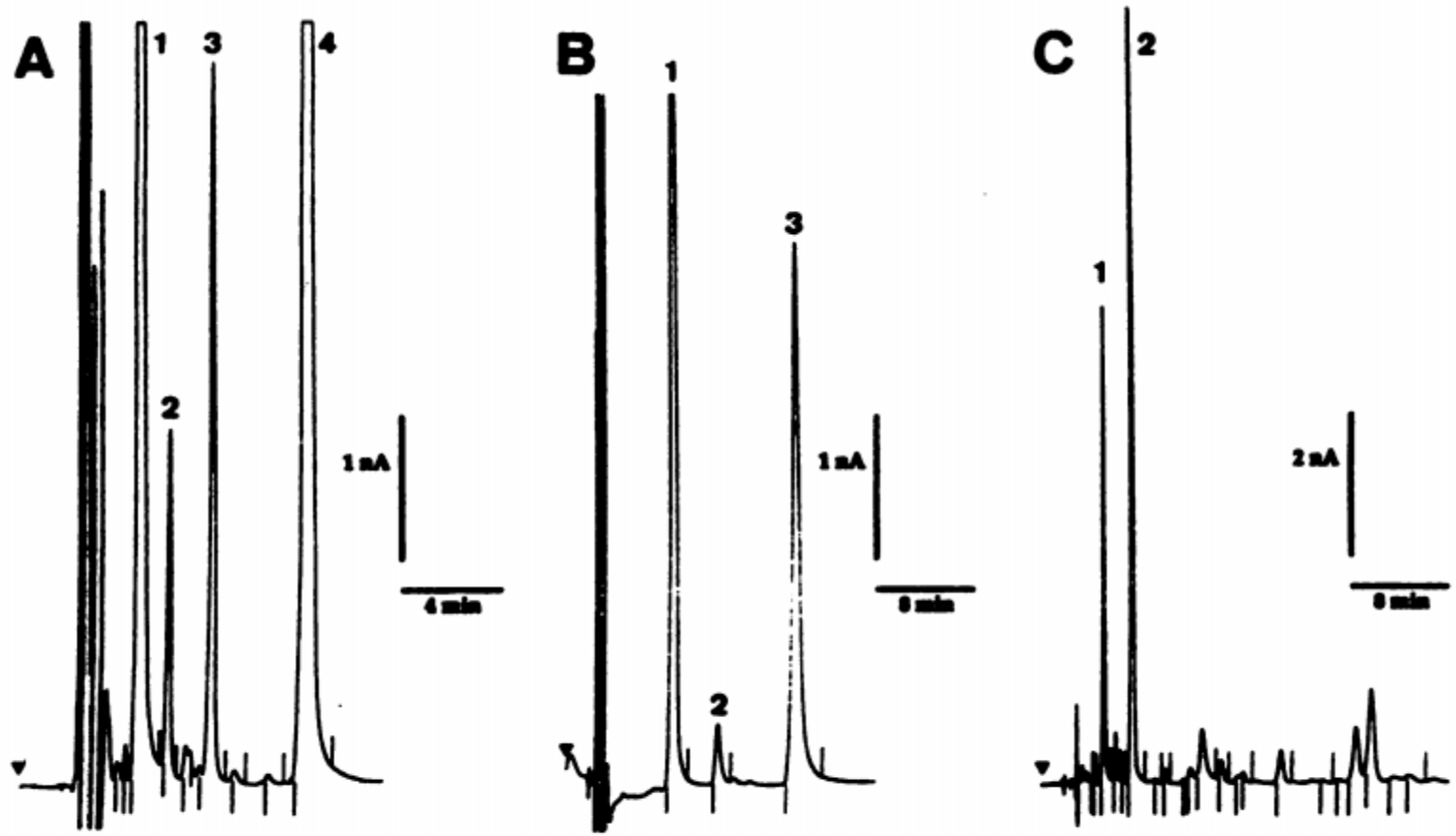
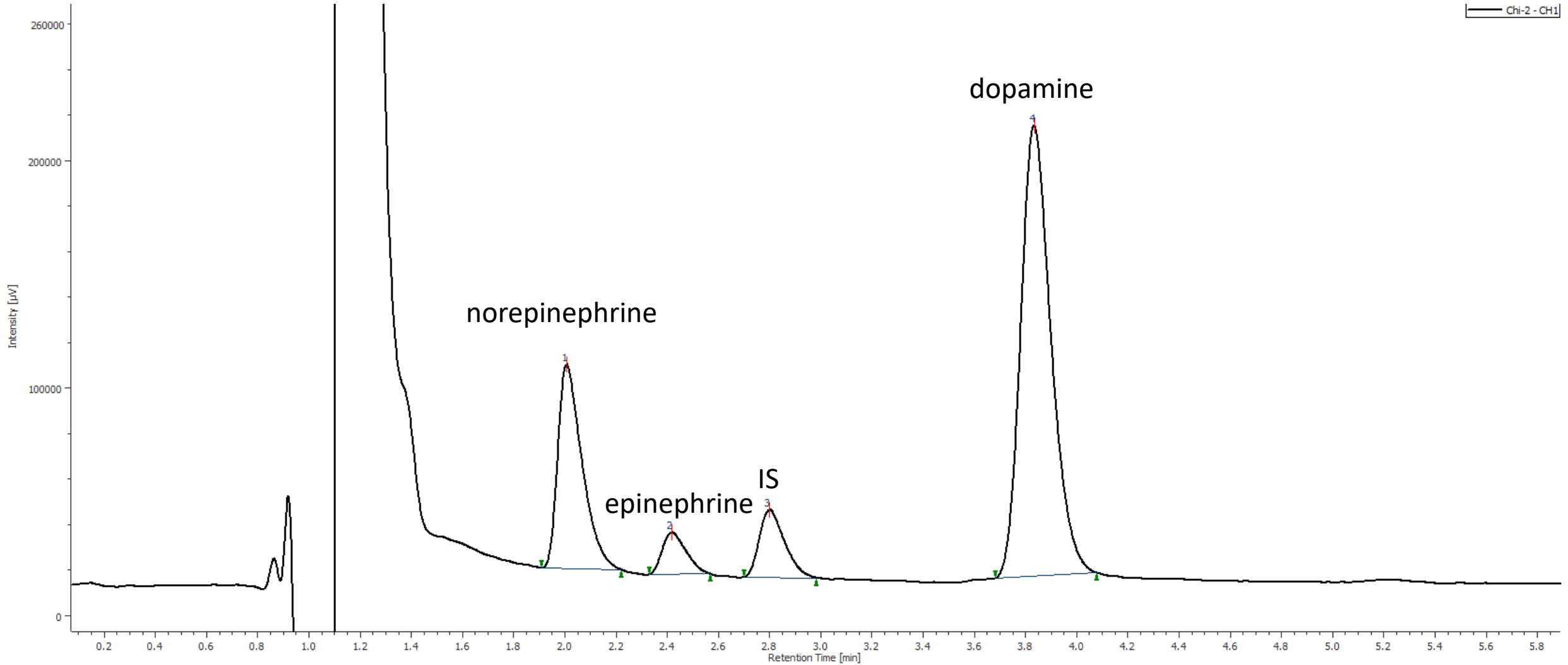
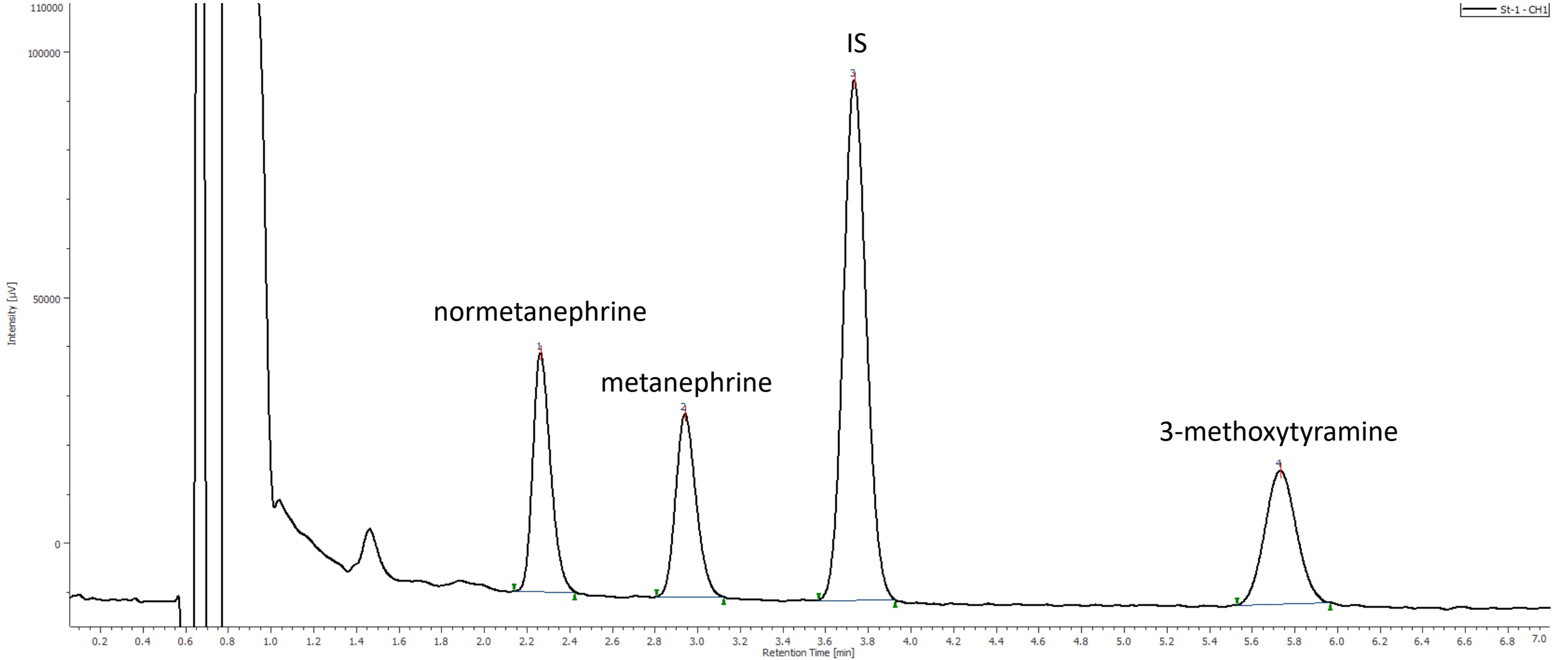
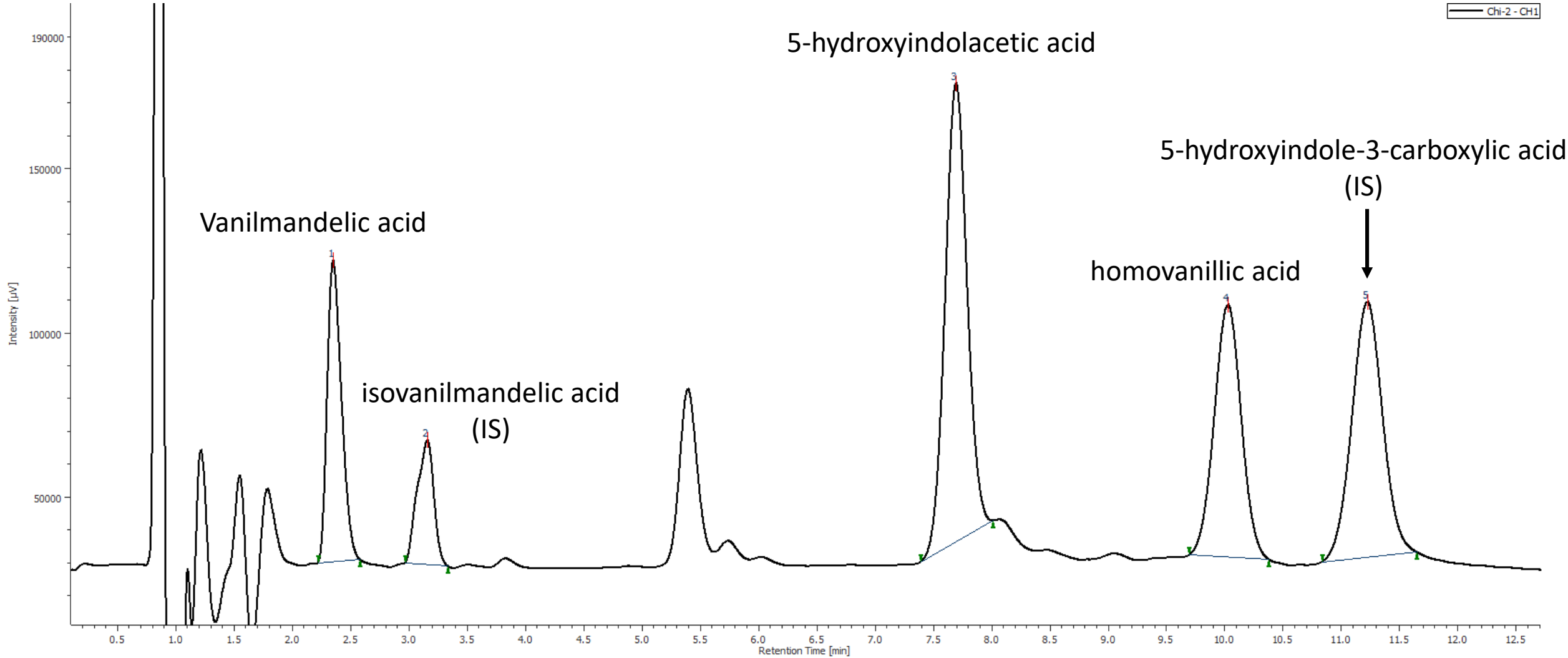


Fig. 1. Chromatograms of urinary catecholamines (A), metanephrines (B), and VMA (C) from a patient with a predominantly NE-secreting pheochromocytoma (no. 8).

A: (*1*) norepinephrine, 5.1 μmol/L; (*2*) epinephrine, 0.13 μmol/L; (*3*) dihydroxybenzylamine (internal standard); (*4*) dopamine. *B*: (*1*) normetanephrine, 19 μmol/L; (*2*) metanephrine, 1.0 μmol/L; (*3*) MHBA (internal standard). *C*: (*1*) VMA, 49 μmol/L; (*2*) iso-VMA (internal standard). The 24-h urinary volume was 1.65 L.


Chi-2 - CH1
dopamine
norepinephrine
epinephrine
IS
Intensity [µV]
Retention Time [min]
260000
200000
100000
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
3.0
3.2
3.4
3.6
3.8
4.0
4.2
4.4
4.6
4.8
5.0
5.2
5.4
5.6
5.8

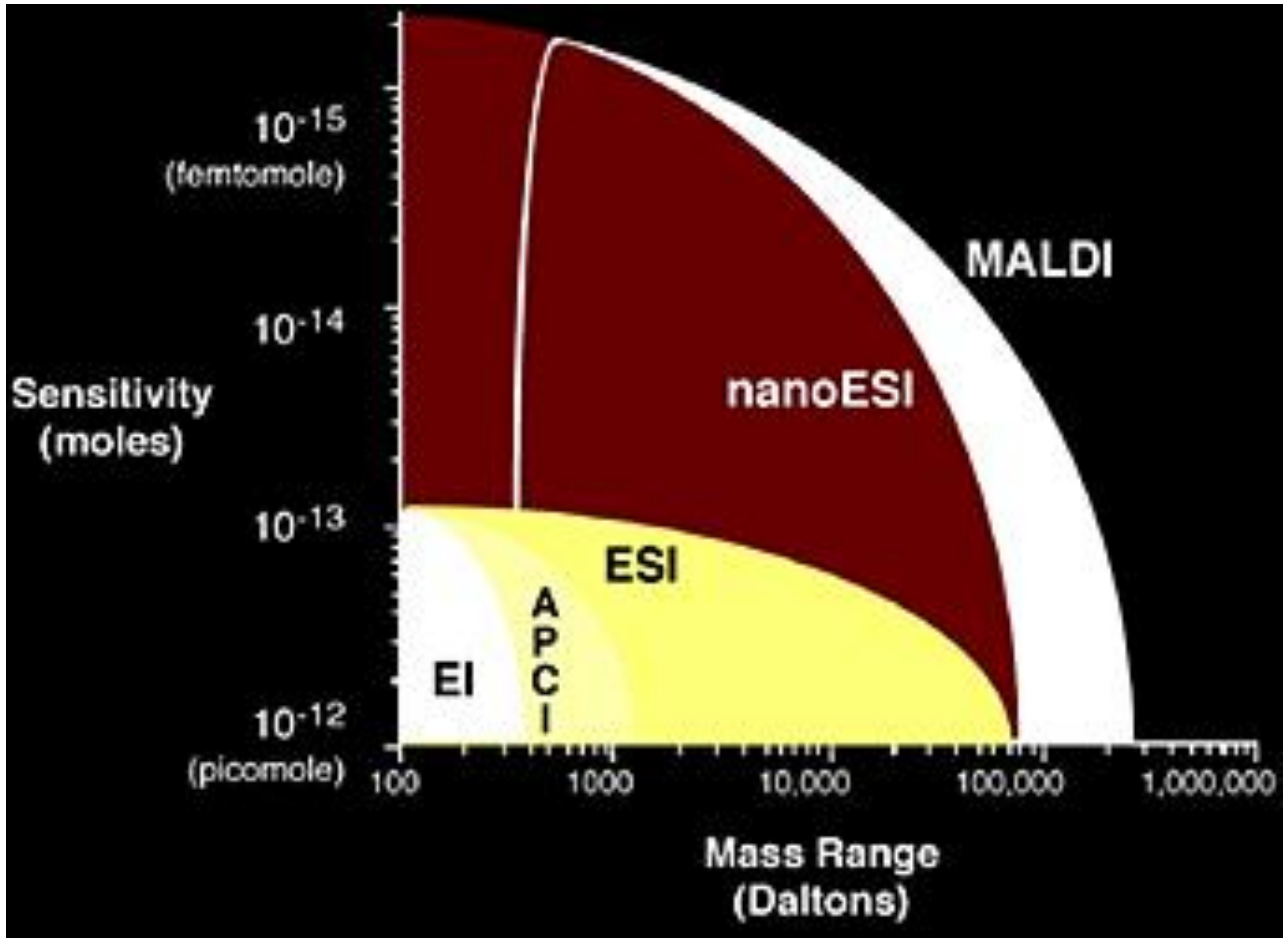

St-1 - CH1
110000
100000
50000
0
Intensity [µV]
normetanephrine
metanephrine
IS
3-methoxytyramine
1
2
3
4
0.2 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8 2.0 2.2 2.4 2.6 2.8 3.0 3.2 3.4 3.6 3.8 4.0 4.2 4.4 4.6 4.8 5.0 5.2 5.4 5.6 5.8 6.0 6.2 6.4 6.6 6.8 7.0
Retention Time [min]


Chi-2 - CH1
5-hydroxyindolacetic acid
5-hydroxyindole-3-carboxylic acid
(IS)
Vanilmandelic acid
homovanillic acid
isovanilmandelic acid
(IS)
Intensity [µV]
Retention Time [min]

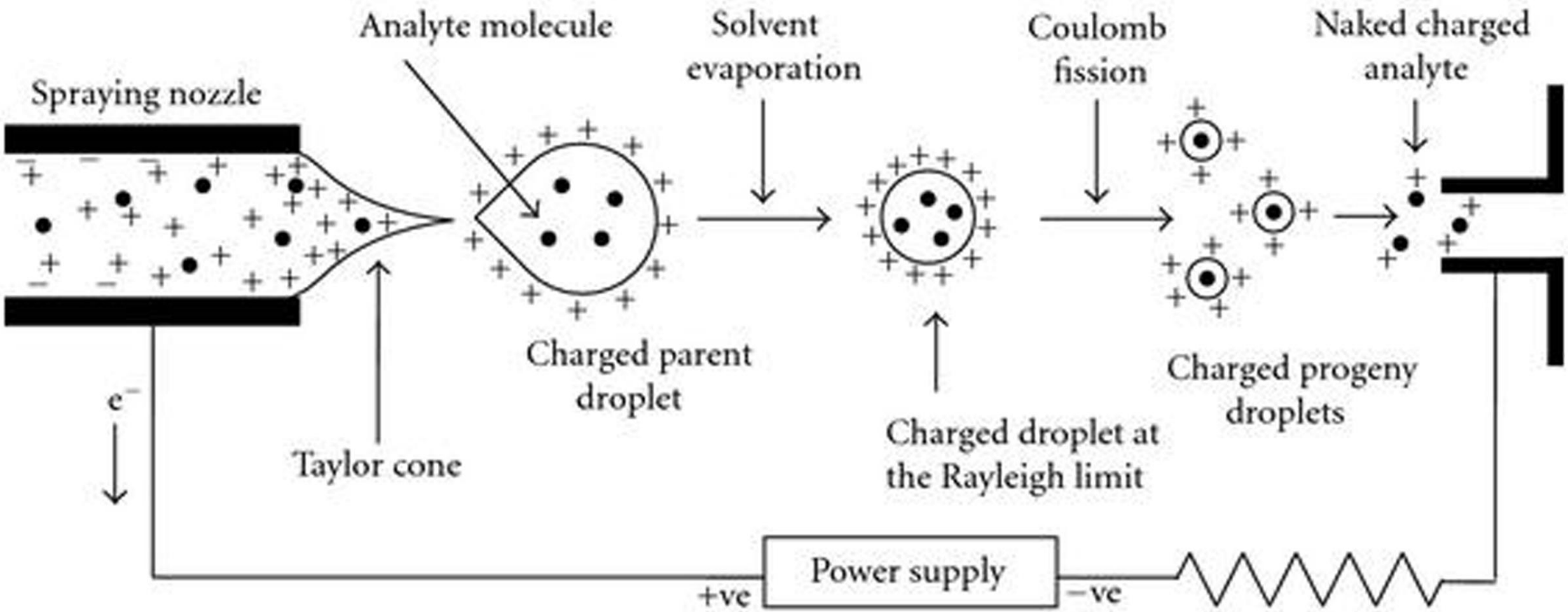
Introduction to mass spectrometry

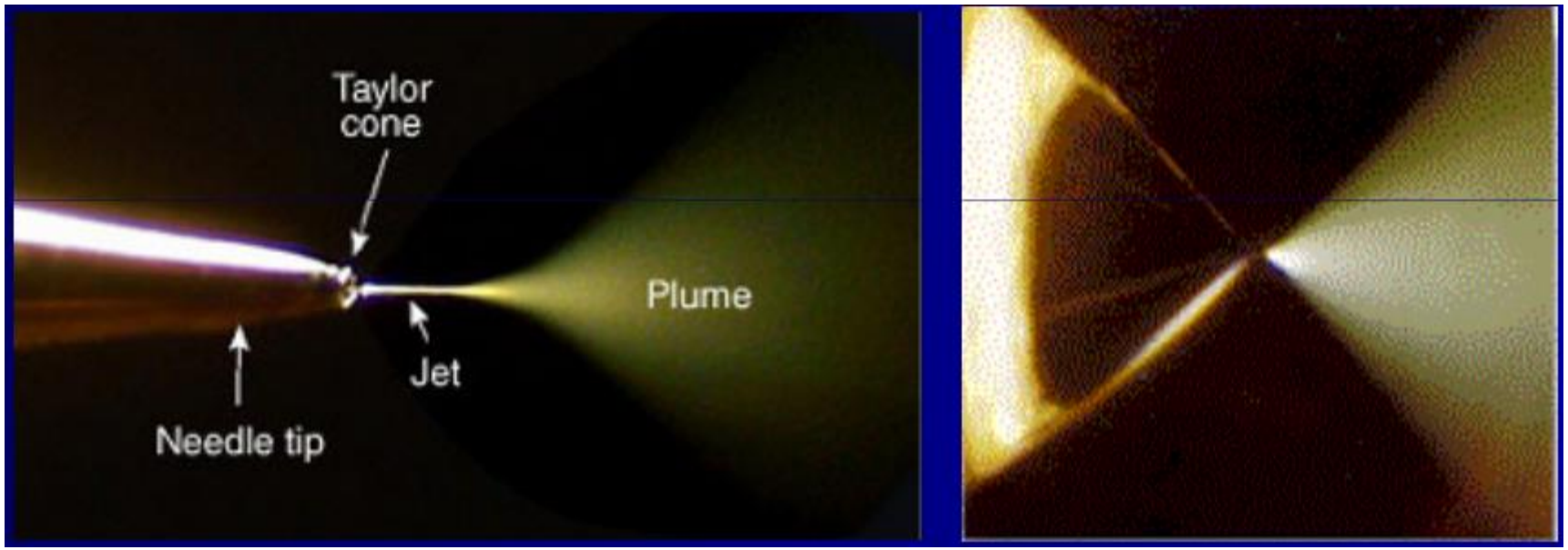
Ion source	Mass analyzers
Electrospray ionization (ESI)	*Quadrupole*
Atmospheric pressure chemical ionization (APCI)	Ion trap
Atmospheric pressure photoionization (APPI)	Linear ion trap
Electron ionization	*Quadrupole-ion trap*
Chemical ionization	*Time of flight*
Nanospray ionization	Double focussing magnetic sector
Spark ionization	Radiofrequency mass analyzer
Thermospray ionization	Fourier transformation mass analyzer
Field ionization	Magnetic sector
Field desorption	Ion cyclotron resonance mass analyzer
Fast atom bombardment	Reversed geometry mass analyzer
Multiphoton ionization	Hybrid
Plasma desorption	Electronic sector
Laser desorption (MALDI, SELDI, infrared)	Orbitrap
Corona discharge ionization	

Mass ranges and sensitivities attainable using various ionization techniques

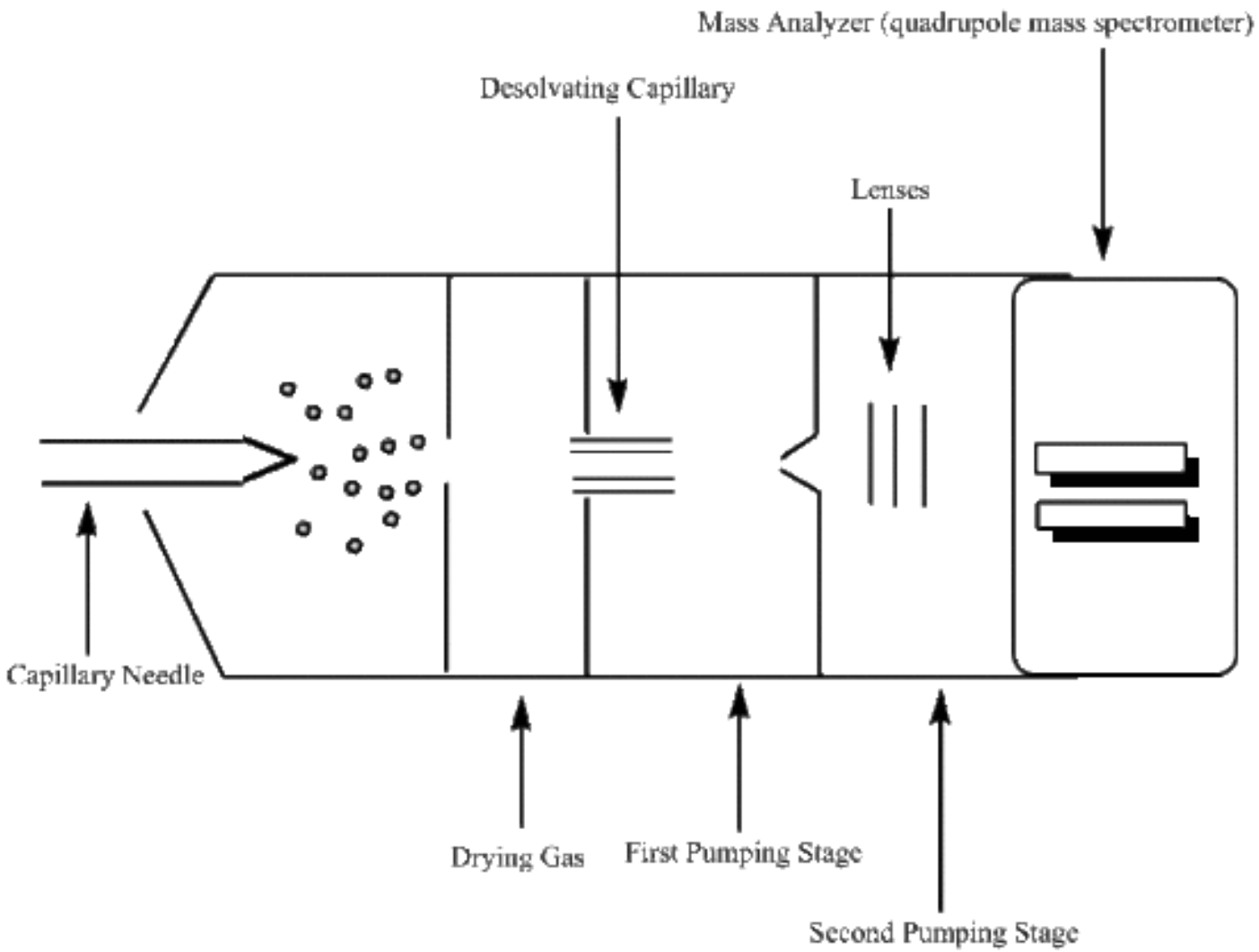


Electrospray ionization




Taylor
cone
Plume
Jet
Needle tip

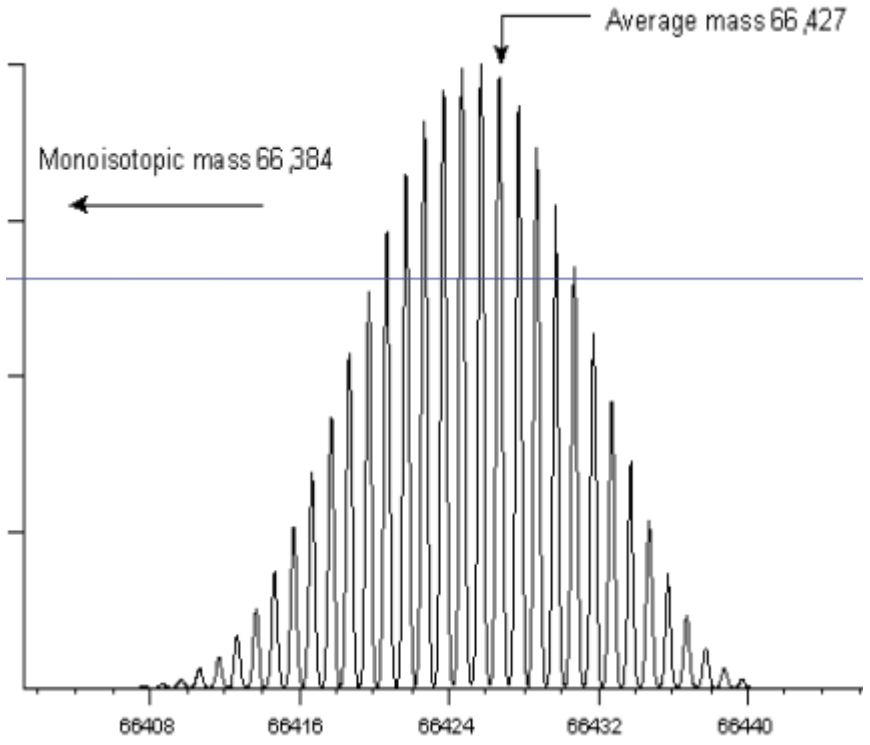
Simplified overview of the electrospray unit

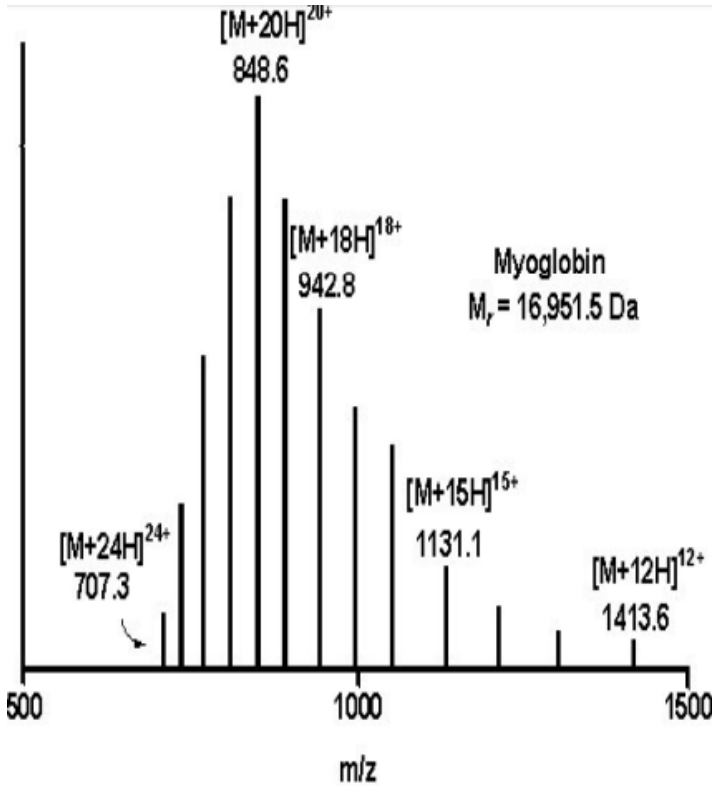


Formation of adducts during electrospray ionization

Observed	Explanation	Mass
$[M+H]^+$	Protonation	M+1
$[M-H]^-$	Deprotonation	M-1
$[M-H-nH_2O]^-$	Deprotonation and loss of H_2O	M-1-(nx18)
$[M+Cl]^-$	Ion attachment	M+35 (37)
$[M-2H+Na]^-$	M + Na adduct	M+21
$[M-H-CO_2]^-$	Carbon dioxide loss	M+45
$[M+H+CH_3CN]^+$	In presence of CH_3CN	M+42
$[M+H+CH_3CN+nH_2O]^+$	Water-acetonitrile cluster	M+42+(nx18)

Macromolecules have peak envelopes





3% methanol

3% methanol

3% methanol

3% methanol

50% methanol

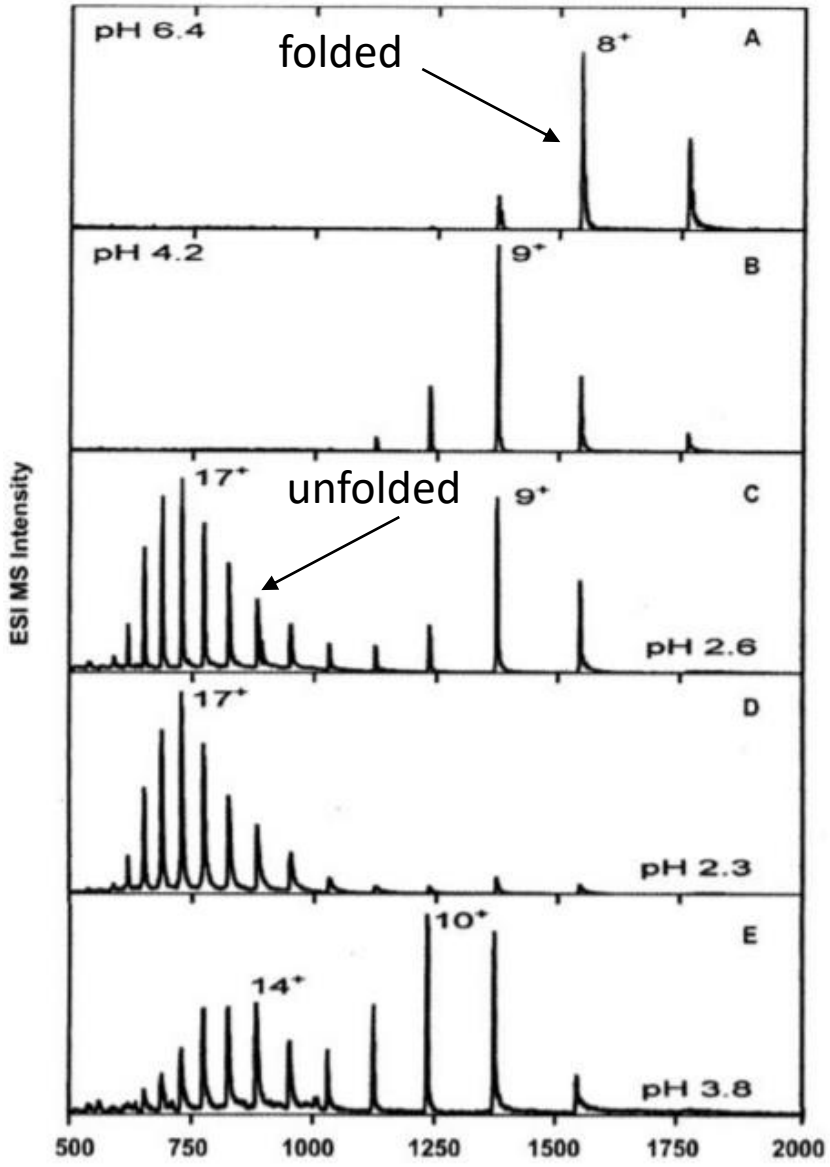


FIG. 3. **ESI mass spectra of cytochrome *c* in water containing 3% methanol and 0.5 mM ammonium acetate.** *A*, pH 6.4; *B*, pH 4.2; *C*, pH 2.6; *D*, pH 2.3; and *E*, in 50% methanol and 0.5 mM ammonium acetate at pH 3.8. (Reprinted with permission from Ref. 21.)

Native folded conformation, charge centered on +8 charge state

Native folded conformation, charge centered on +9 charge state

Yan X et al. Mol Cell Proteom 3 (2004), 10.

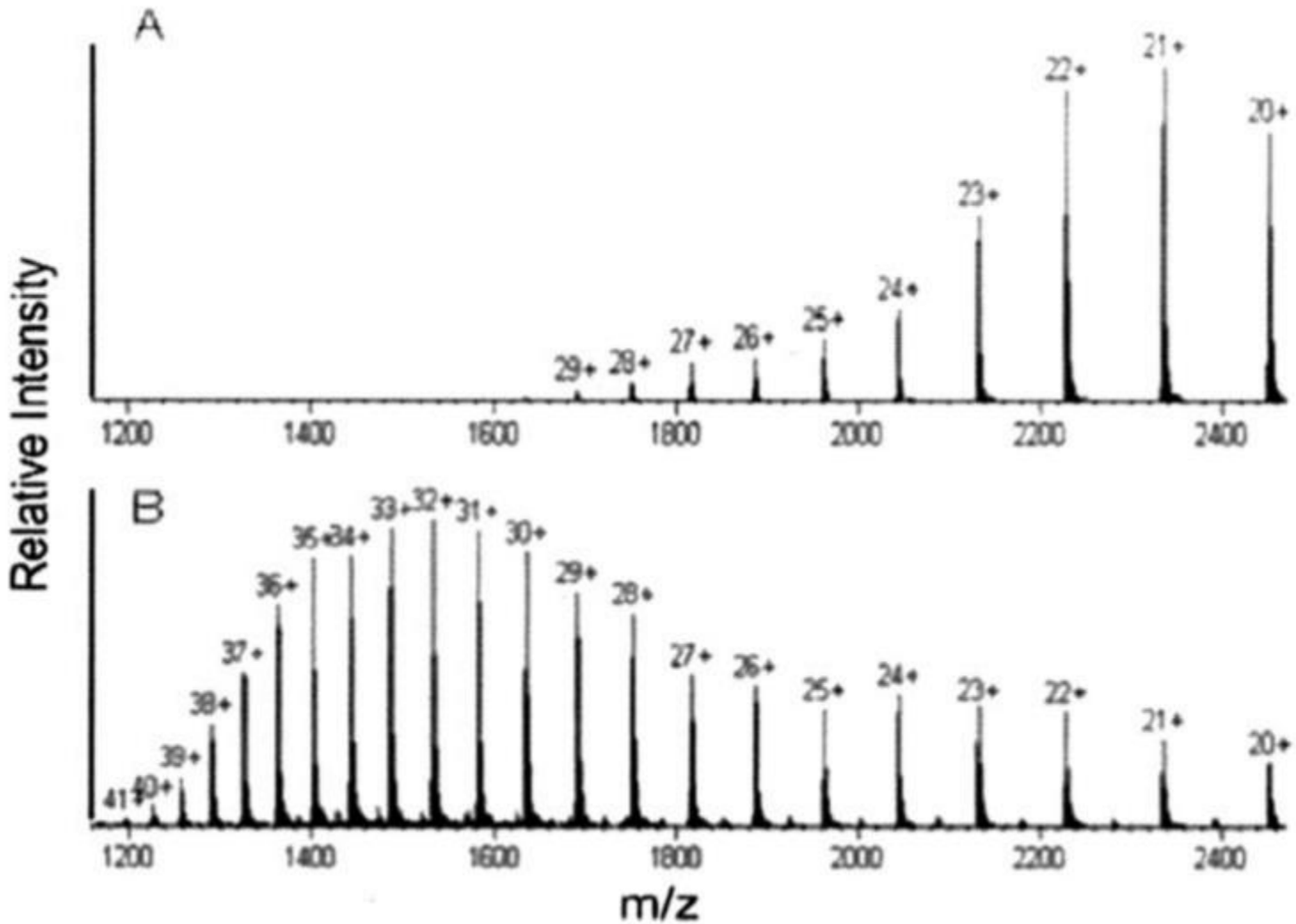
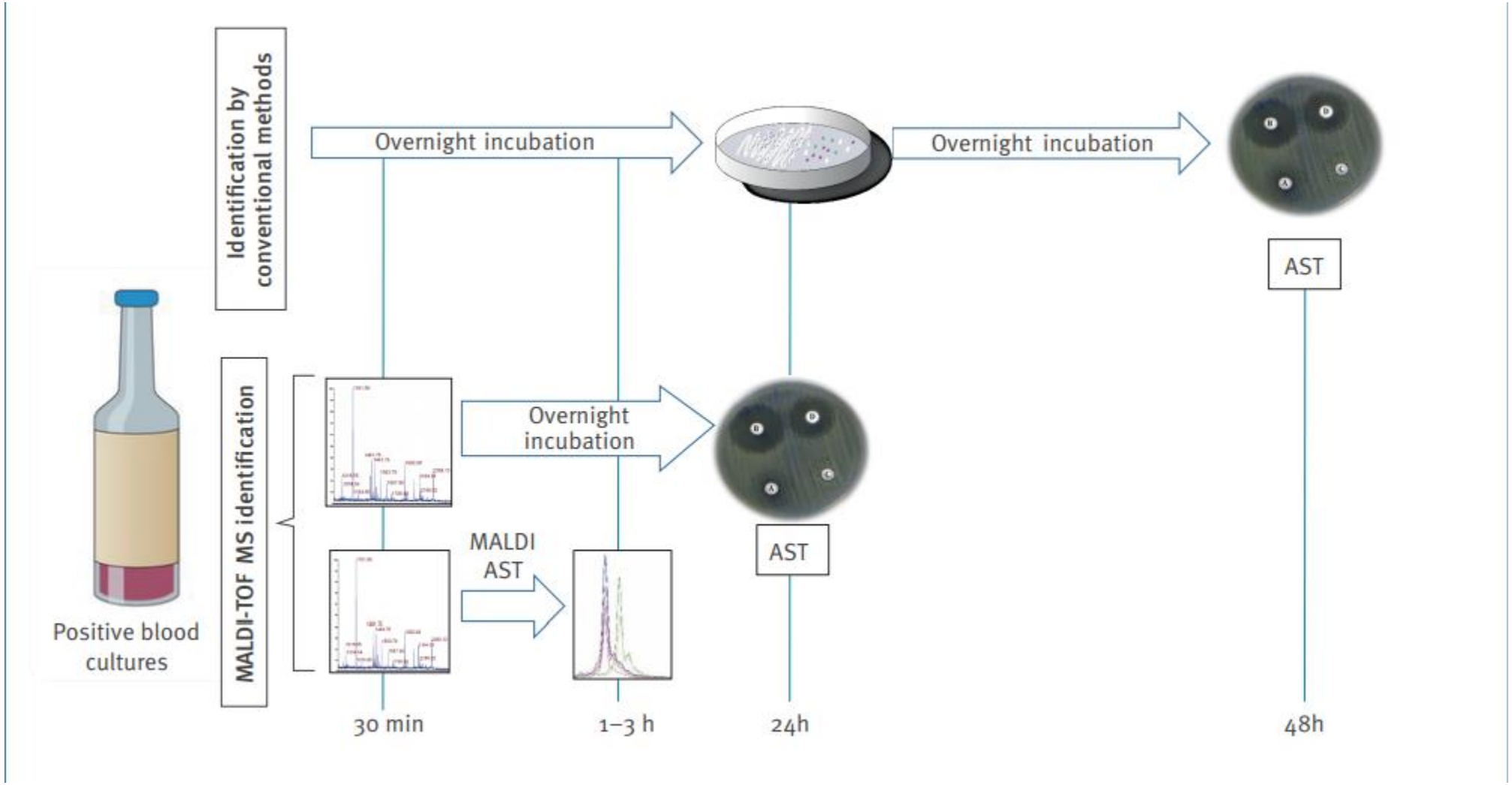


FIG. 4. **Positive ion ESI mass spectra of rhM-CSFβ sprayed from acetonitrile/H_2O containing 0.1% trifluoroacetic acid solution through a column.** *A*, Native state; and *B*, selectively reduced and blocked sulfhydryls C_{157}–C_{159}.

Yan X et al. Mol Cell Proteom 3 (2004), 10.

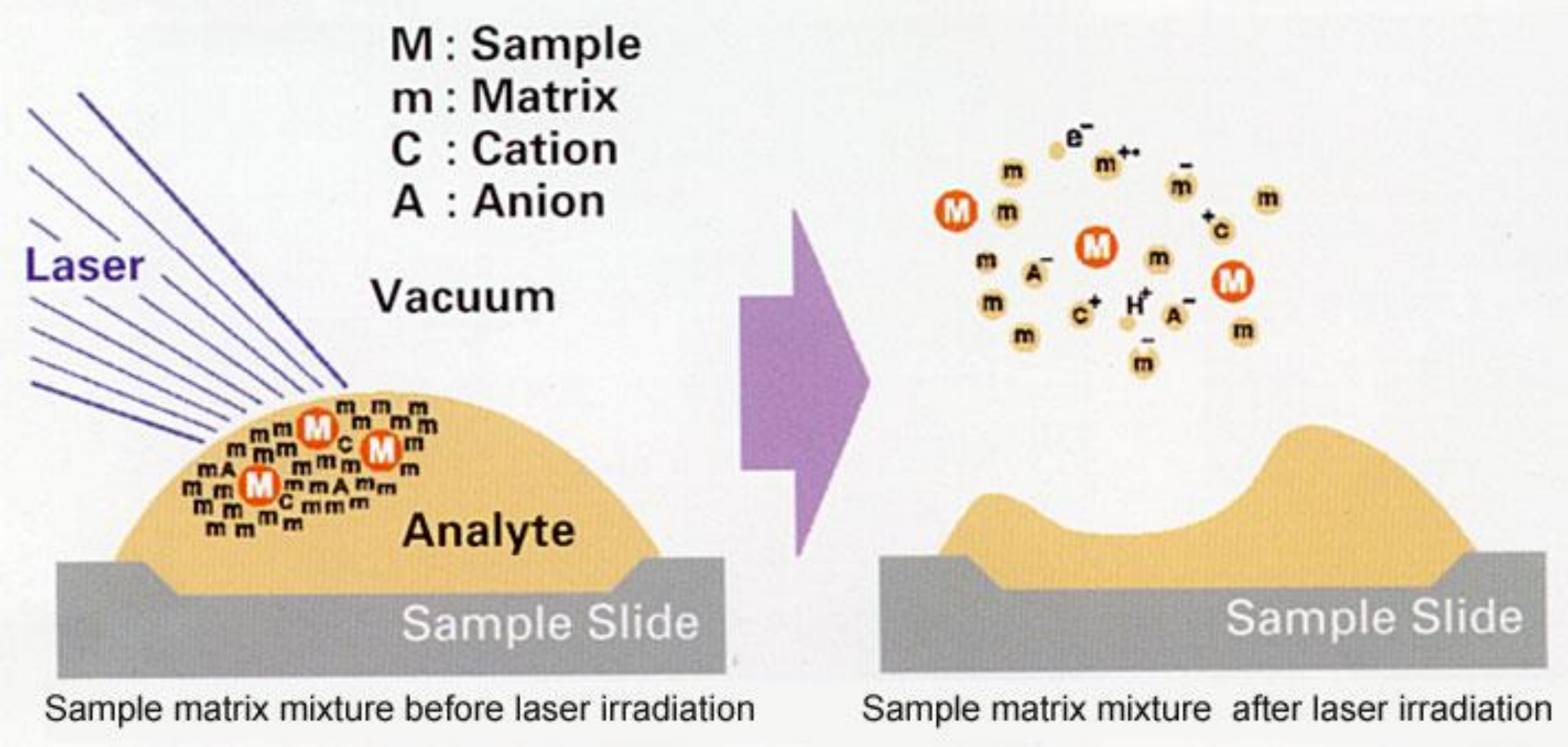
Matrix-assisted laser desorption ionization

MALDI-TOF has revolutionized the identification of microorganisms in clinical and public health laboratories



Rodríguez-Sánchez B et al. Euro Surveill 2019;24:pii=1800193.

Matrix-assisted laser desorption ionization

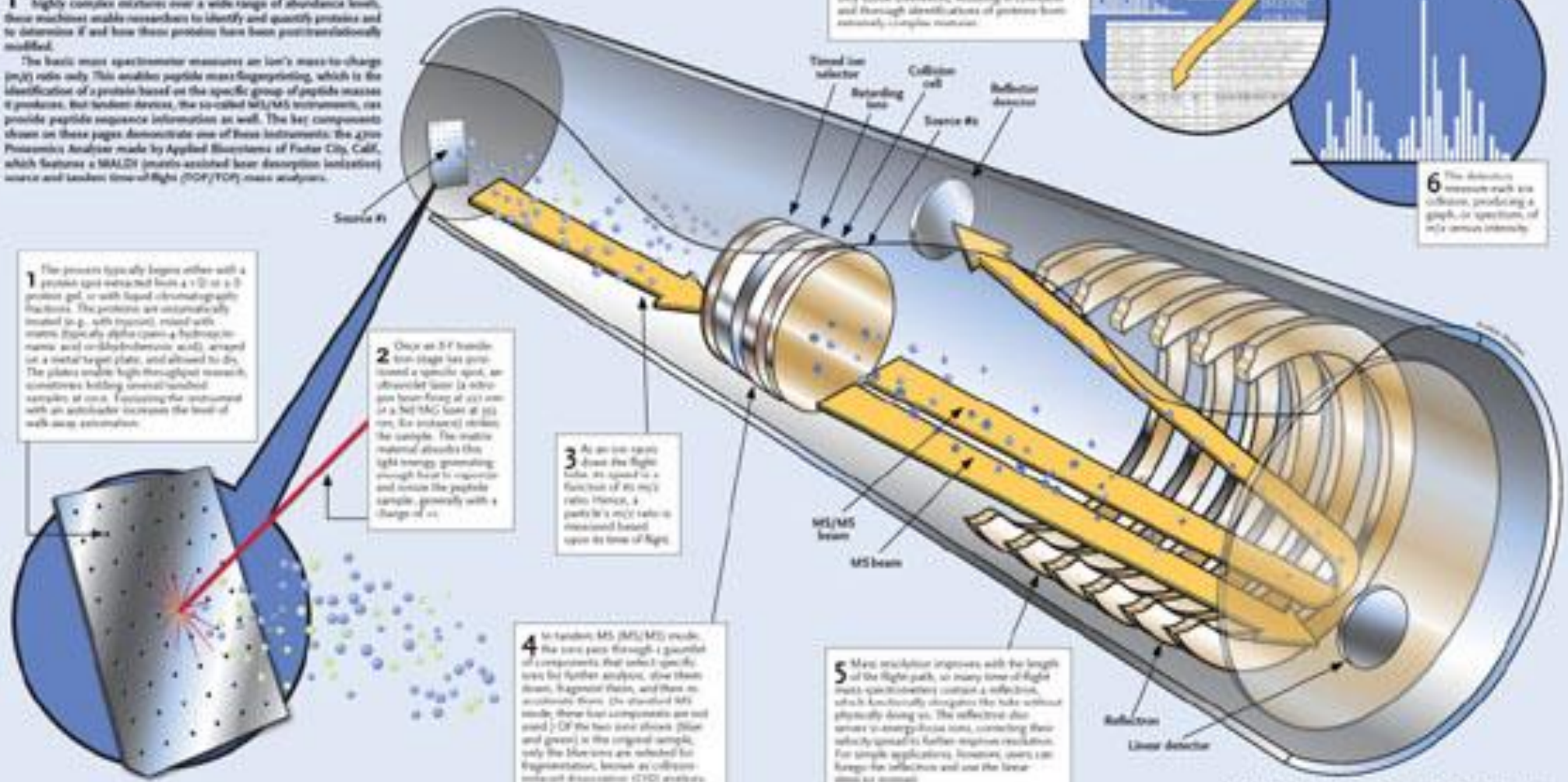


MALDI-TOF/TOF Mass Spectrometer

By Jeffrey M. Perkel

Perhaps no tool has been as instrumental to the proteomics revolution as the mass spectrometer. With the ability to deconvolute highly complex mixtures over a wide range of abundance levels, these machines enable researchers to identify and quantify proteins and to determine if and how those proteins have been post-translationally modified.

The basic mass spectrometer measures an ion's mass-to-charge (*m*/*z*) ratio only. This enables peptide mass fingerprinting, which is the identification of a protein based on the specific group of peptide masses it produces. But tandem devices, the so-called MS/MS instruments, can provide peptide sequence information as well. The key components shown on these pages demonstrate one of those instruments: the 4700 Proteomics Analyzer made by Applied Biosystems of Foster City, Calif., which features a MALDI (matrix-assisted laser desorption ionization) source and tandem time-of-flight (TOF/TOF) mass analyzers.



1 The process typically begins either with a protein spot extracted from a 1-D or a 2-D protein gel, or with liquid chromatography fractions. The proteins are enzymatically treated (e.g., with trypsin), mixed with matrix (typically alpha-cyano-4-hydroxycinnamic acid or dihydroxybenzoic acid), arrayed on a metal target plate, and allowed to dry. The plates enable high-throughput research, sometimes holding several hundred samples at once. Equipping the instrument with an autoloader increases the level of walk-away automation.

2 Once an X-Y translation stage has positioned a specific spot, an ultraviolet laser (a nitrogen laser firing at 337 nm or a Nd:YAG laser at 355 nm, for instance) strikes the sample. The matrix material absorbs this light energy, generating enough heat to vaporize and ionize the peptide sample, generally with a charge of +1.

3 As an ion races down the flight tube, its speed is a function of its *m*/*z* ratio. Hence, a particle's *m*/*z* ratio is measured based upon its time of flight.

4 In tandem MS (MS/MS) mode, the ions pass through a gauntlet of components that select specific ions for further analysis, slow them down, fragment them, and then accelerate them. (In standard MS mode, these four components are not used.) Of the two ions shown (blue and green) in the original sample, only the blue ions are selected for fragmentation, known as collision-induced dissociation (CID) analysis.

5 Mass resolution improves with the length of the flight path, so many time-of-flight mass spectrometers contain a reflectron, which functionally elongates the tube without physically doing so. The reflectron also serves to energy-focus ions, correcting their velocity spread to further improve resolution. For simple applications, however, users can forego the reflectron and use the linear detector instead.

6 The detector(s) measure each ion collision, producing a graph—or spectrum—of *m*/*z* versus intensity.

7 Comparing the resulting constellation of *m*/*z* values against a database identifies the peptide, and by extension, the protein from which it derives. Tandem MS/MS analysis of fragmented peptides produces an actual sequence, providing a more accurate protein ID. Today's mass spectrometers have high enough resolution and mass accuracy to distinguish peptides bearing only subtle differences, resulting in confident and thorough identifications of proteins from extremely complex mixtures.

Jeffrey M. Perkel can be contacted at jperkel@the-scientist.com.

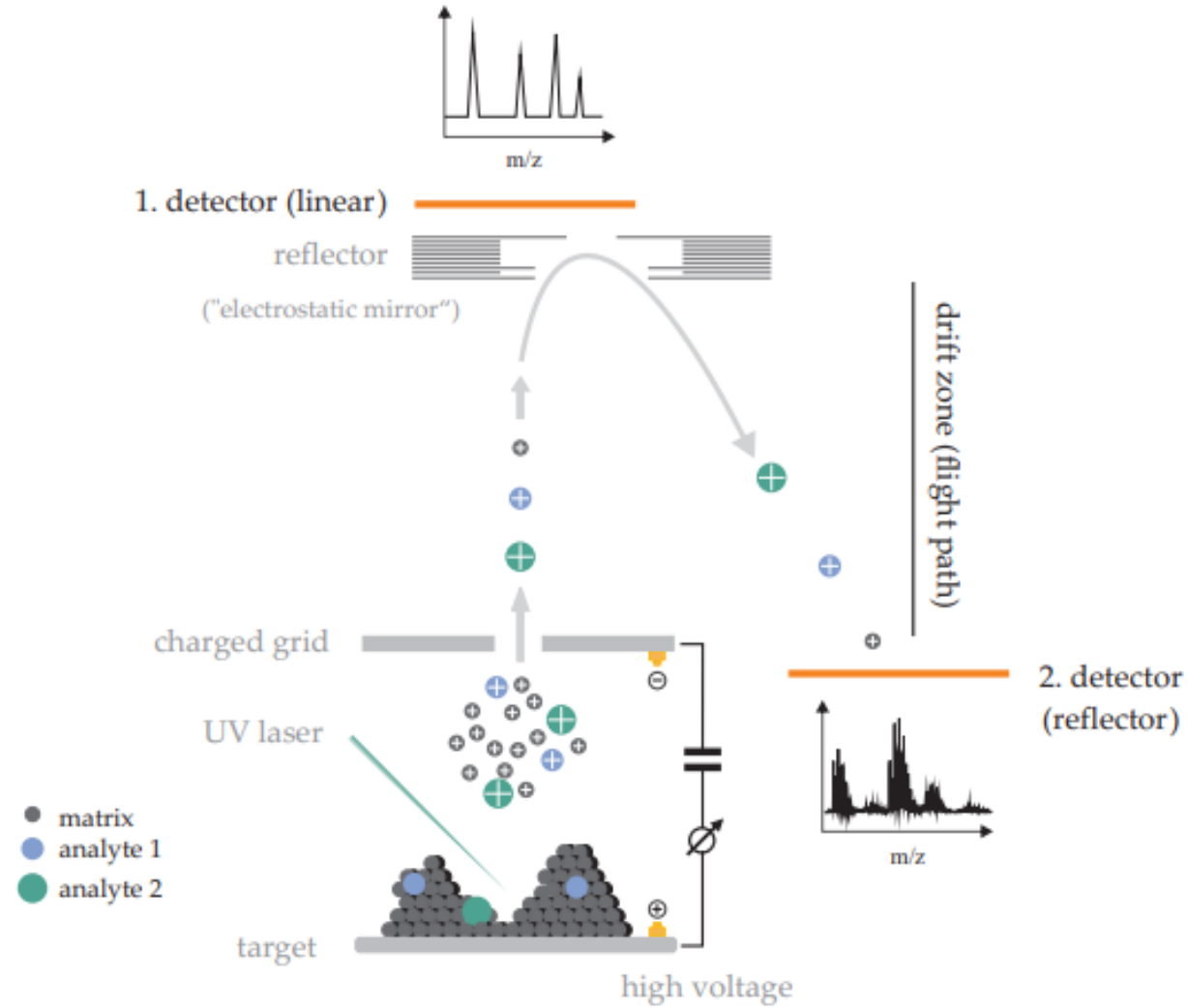


Figure 1. Simplified schema of the positive ionization matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) process occurring in the mass spectrometer (for details see text and Reference [18]). The influence of the detection using either the linear or the reflector mode is emphasized in the figure.

Leopold J et al. Biomolecules 2018;8:173.

Types of MALDI matrices

- classical organic matrices
  - most widely used:
  - proteins - α-cyano-4-hydroxycinnamic acid (CHCA), sinapinic acid (SA)
  - carbohydrates - 2,5-dihydroxybenzoic acid (DHB),
  - lipids – CHCA, DHB
- liquid crystalline matrices
- inorganic matrices
  - graphite

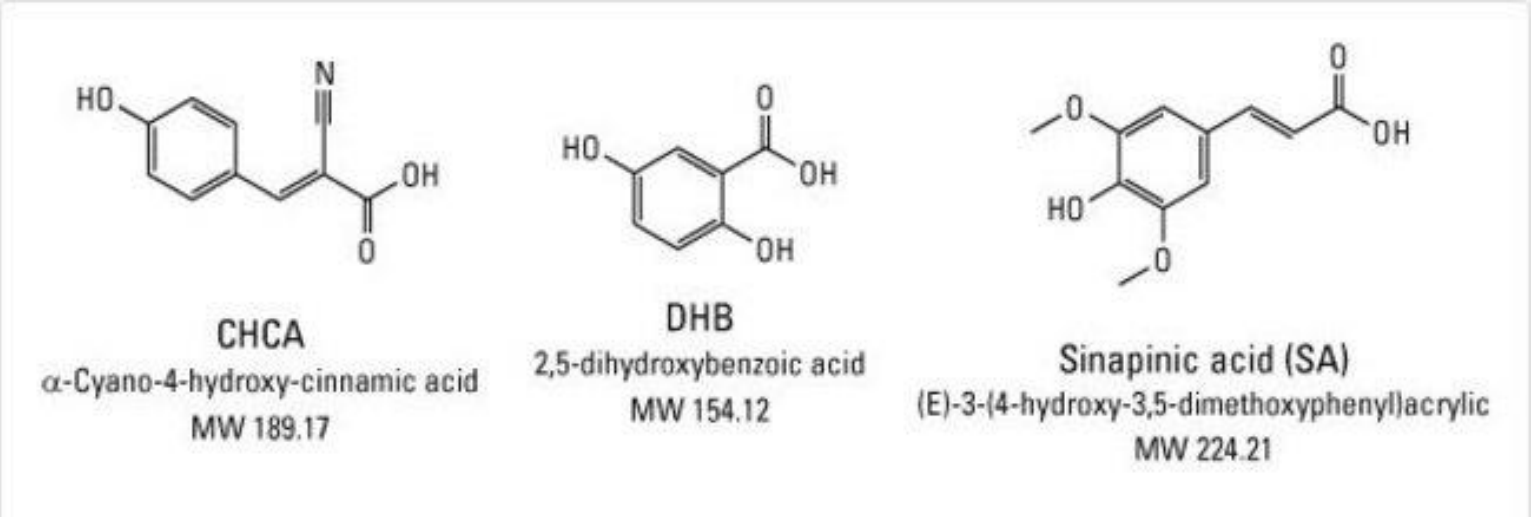
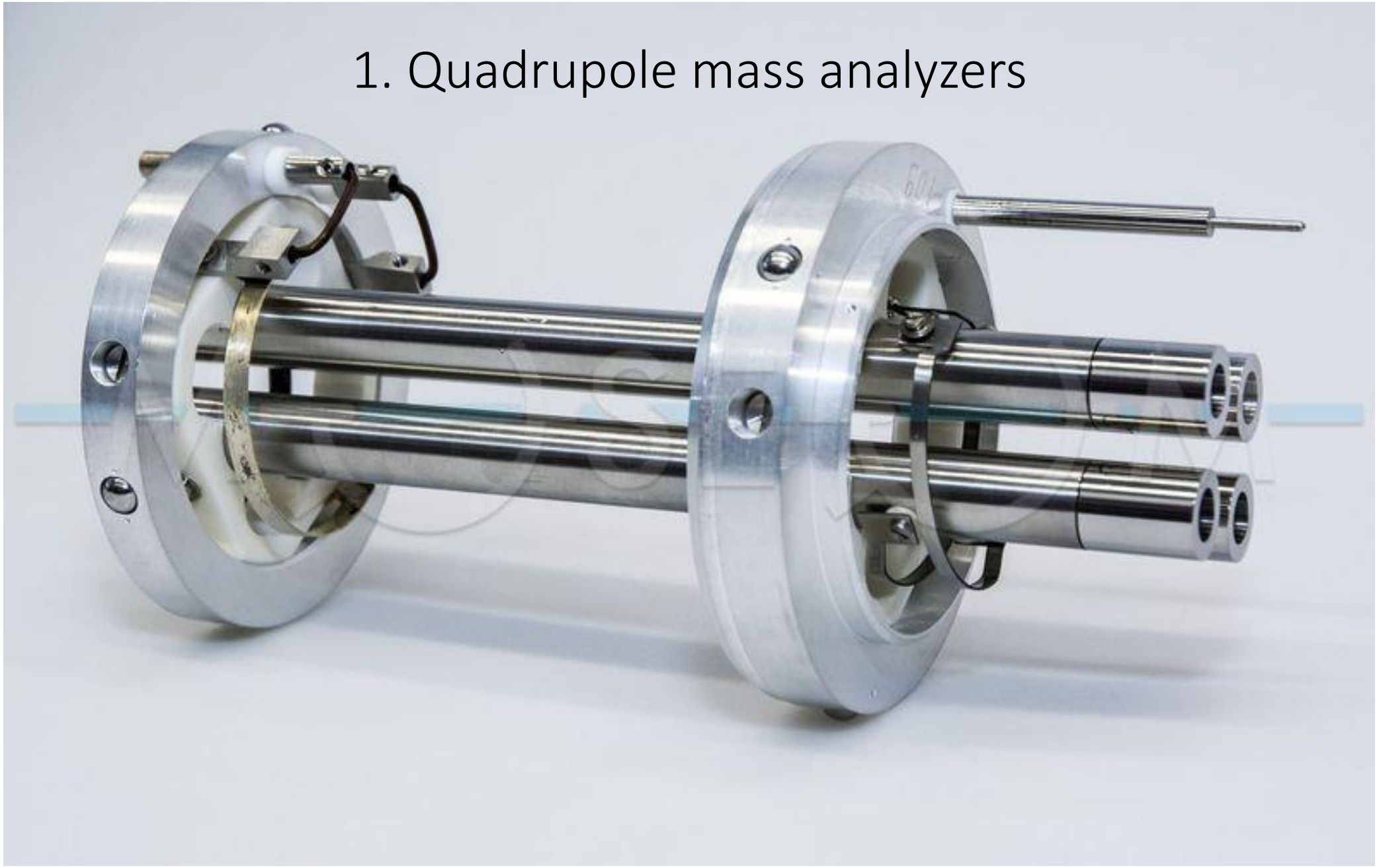


TABLE 2 List of common matrices used for UV-MALDI methods

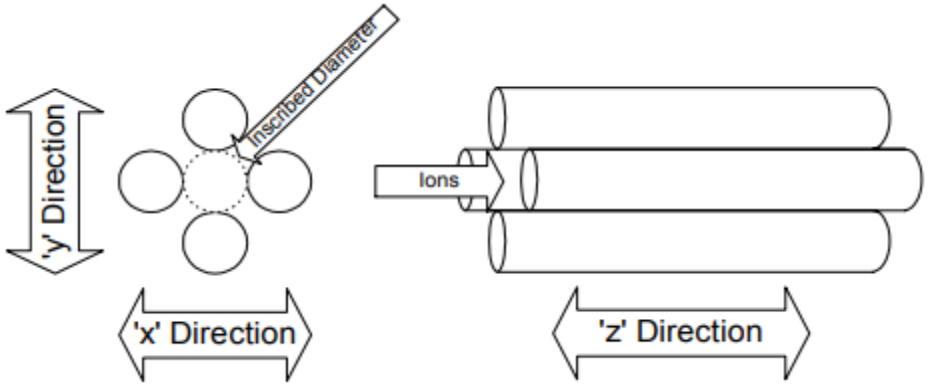
Chromophore matrix(es)[a]	Sample type(s) analyzed
PA, HPA, 3-aminopicolinic acid	Oligonucleotides, DNA, and biopolymers
DHB	Oligosaccharides
CCA	Peptides and triacylglycerol
SA	Proteins
HABA	Peptides, proteins, glycoproteins
MBT	Peptides, proteins, synthetic polymers
DHAP	Glycopeptides, phosphopeptides
THAP	Oligonucleotides

[a] PA, picolinic acid; HPA, 3-hydroxypicolinic acid; SA, 3,5-dimethoxy-4-hydroxycinnamic acid; HABA, 2-(−4-hydroxyphenylazo)benzoic acid; MBT, 2-mercaptobenzothiazole; DHAP, 2,6-dihyroxyacetophenone; THAP, 2,4,6-trihydroxyacetophenone.

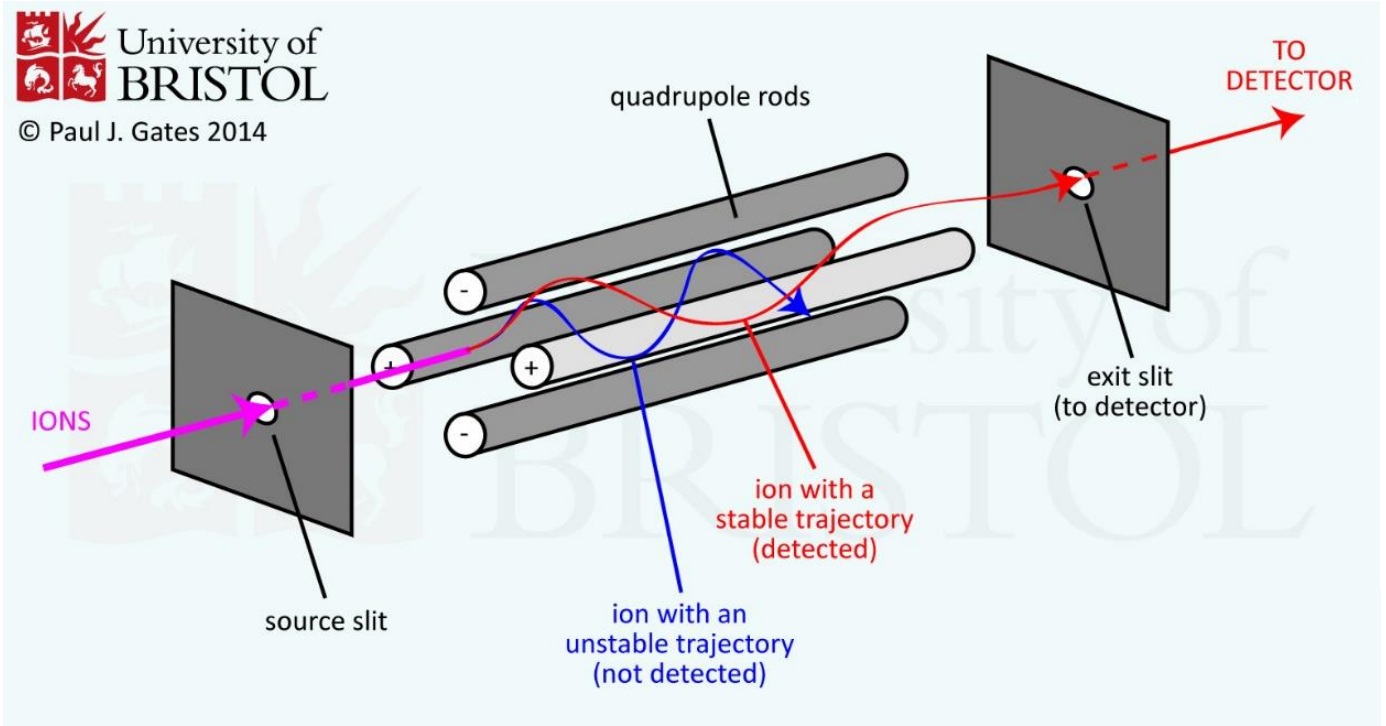
1. Quadrupole mass analyzers

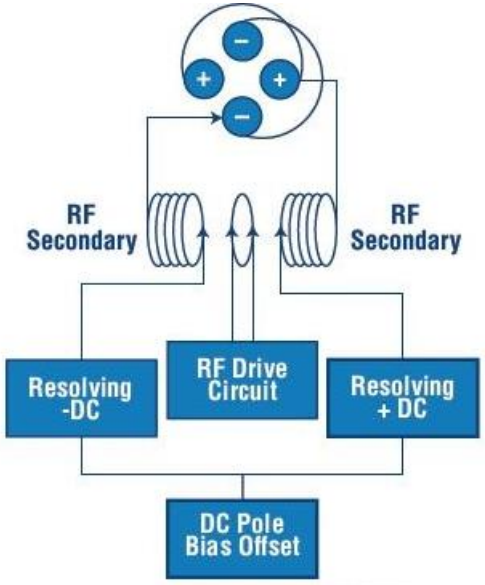


Operating principle

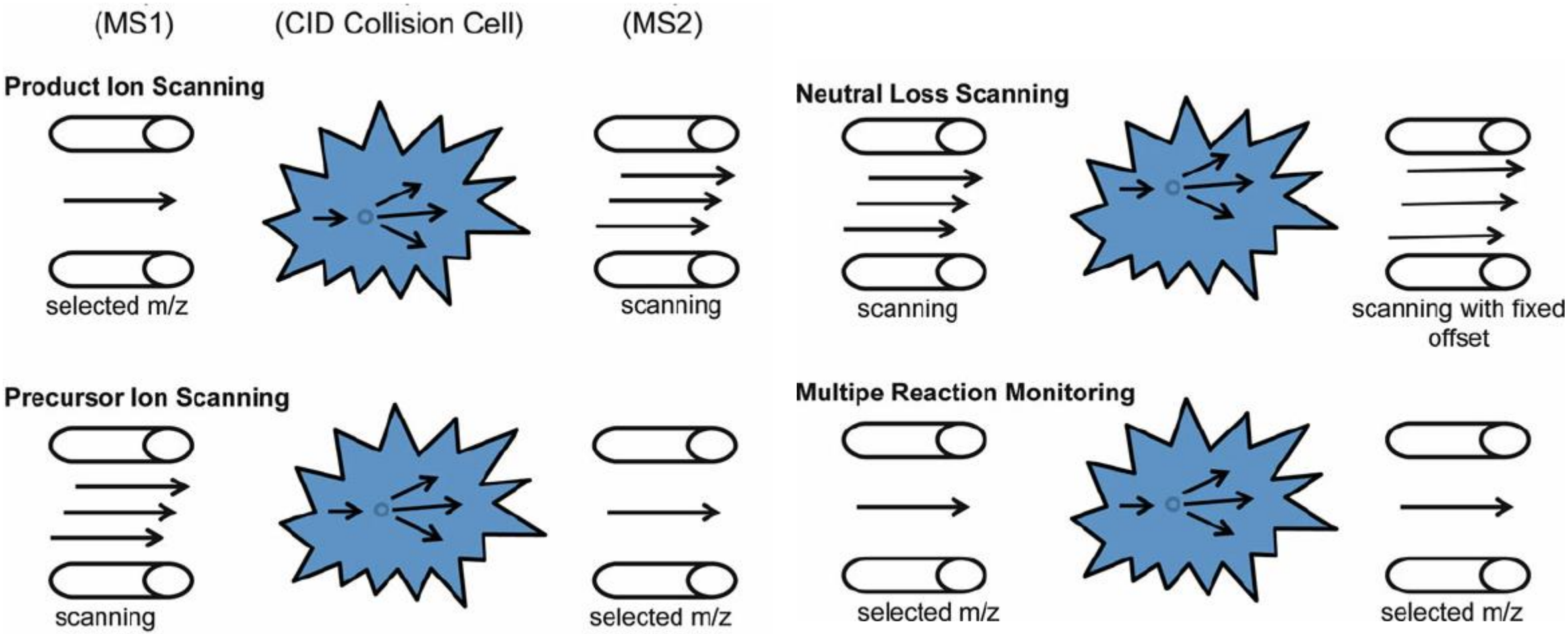


- Applied voltages:
  - fixed frequency oscillating RF voltage to generate harmonic ion trajectories
  - DC voltage difference between poles which defines mass resolution
  - DC offset voltage: defines axial ion energy





Types of tandem-in-space MS^2 experiments



Screening neonates for inborn metabolic diseases

Samples are taken 48-72 h after birth

https://www.youtube.com/watch?v=vxshWngJ114

Targeted inborn disorders-I

- **Disorders of amino acid metabolism**
  - Phenylketonuria (Phenylalanine acculumates, 1:17 000)
  - Maple syrup disease (leucine, isoleucine and valine breakdown impaired, ketoacids accumulate, 1:150 000))
  - Tyrosinaemia types I and II (tyrosine accumulates, type I: 1:100 000)
  - Citrullinaemia I (urea cycle affected, ammonia accumulates, 1:57 000)
  - Arginosuccinate aciduria (urea cycle affected, ammonia accumulates, 1:70 000)
  - Homocystinuria (methionine breakdown impaired, homocysteine and methionine accumulate in plasma, 1:100 000)
- **Fatty acid oxidation disorders**
  - Short-chain acyl-CoA dehydrogenase deficiency (SCAD)
  - Middle-chain acyl-CoA dehydrogenase deficiency (MCAD)
  - Long-chain hydroxyacyl-CoA dehydrogenase deficiency (LCHAD)
  - Very long-chain acyl-CoA dehydrogenase deficiency (VLCAD)
  - Multiplex acyl-CoA dehydrogenase deficiency (MADD)
  - Carnitine-palmitoyl transferase deficiency (CPT-I, CPT-II)
  - Carnitine transport disorder (CT)

Targeted inborn disorders-II

- **Disorders of organic acid metabolism**
  - Beta-ketothiolase deficiency
  - Glutaric acidemia type 1
  - Isovaleric acidemia
  - Methylmalonic acidemia
  - Propionic acidemia
  - 3-hydroxy-3-methylglutaryl-CoA lyase deficiency
  - 3-methylcrotonyl-CoA carboxylase deficiency
  - Multiplex carboxylase deficiency

- **Endocrine and other disorders**
  - Hypothyreosis
  - Galactosemia
  - Biotinidase deficiency

The intracellular processing of fatty acids

Short-chain fatty acid: C2-C5

Middle-chain fatty acid: C6-C13

Long-chain fatty acid: C14-

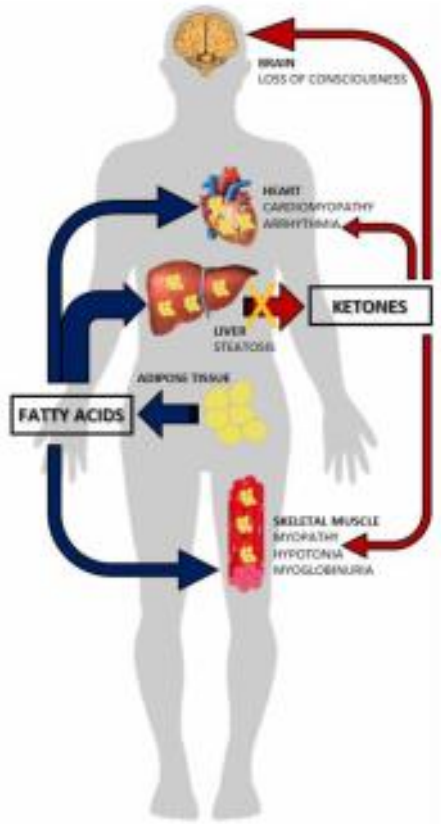
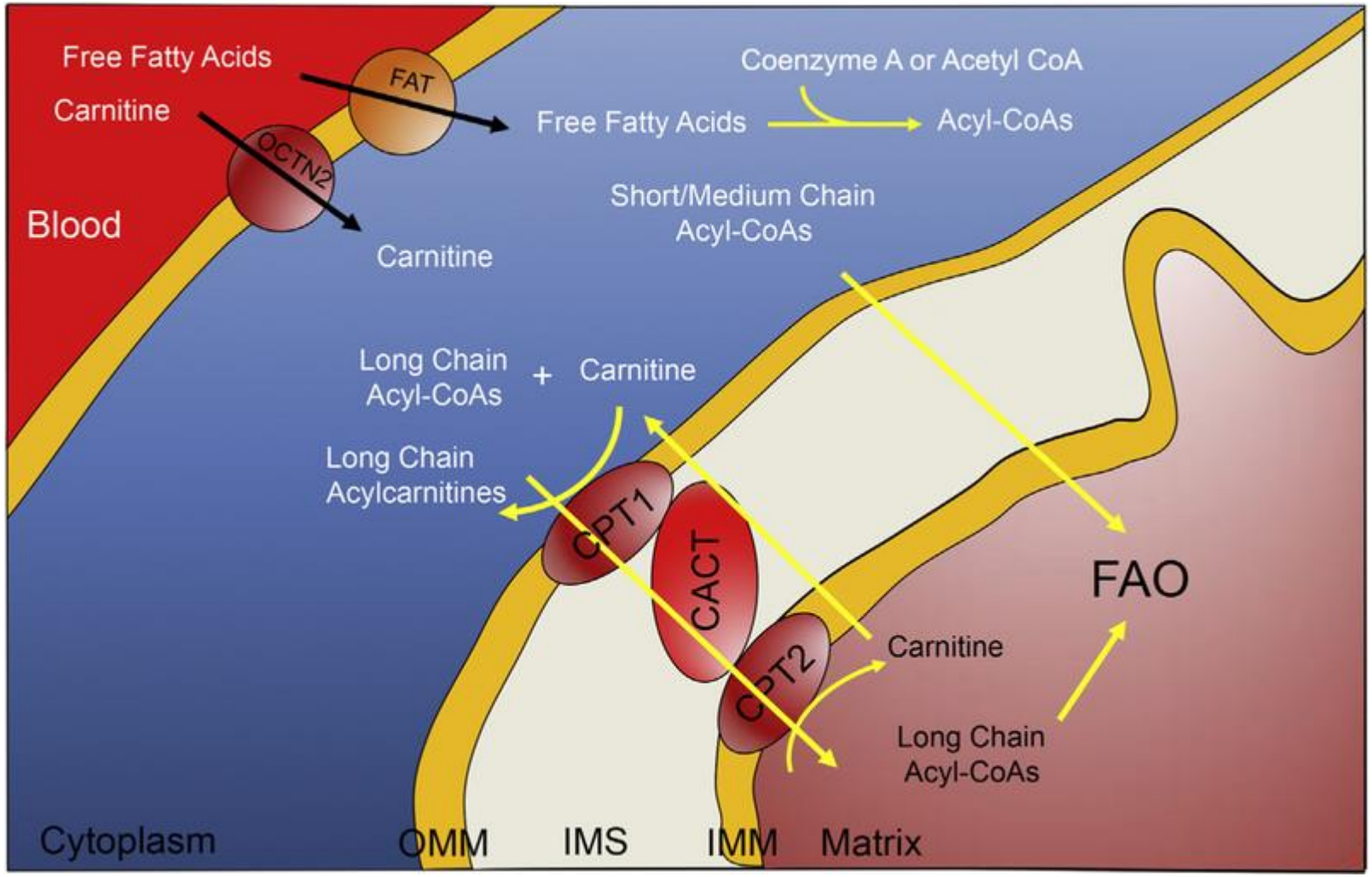
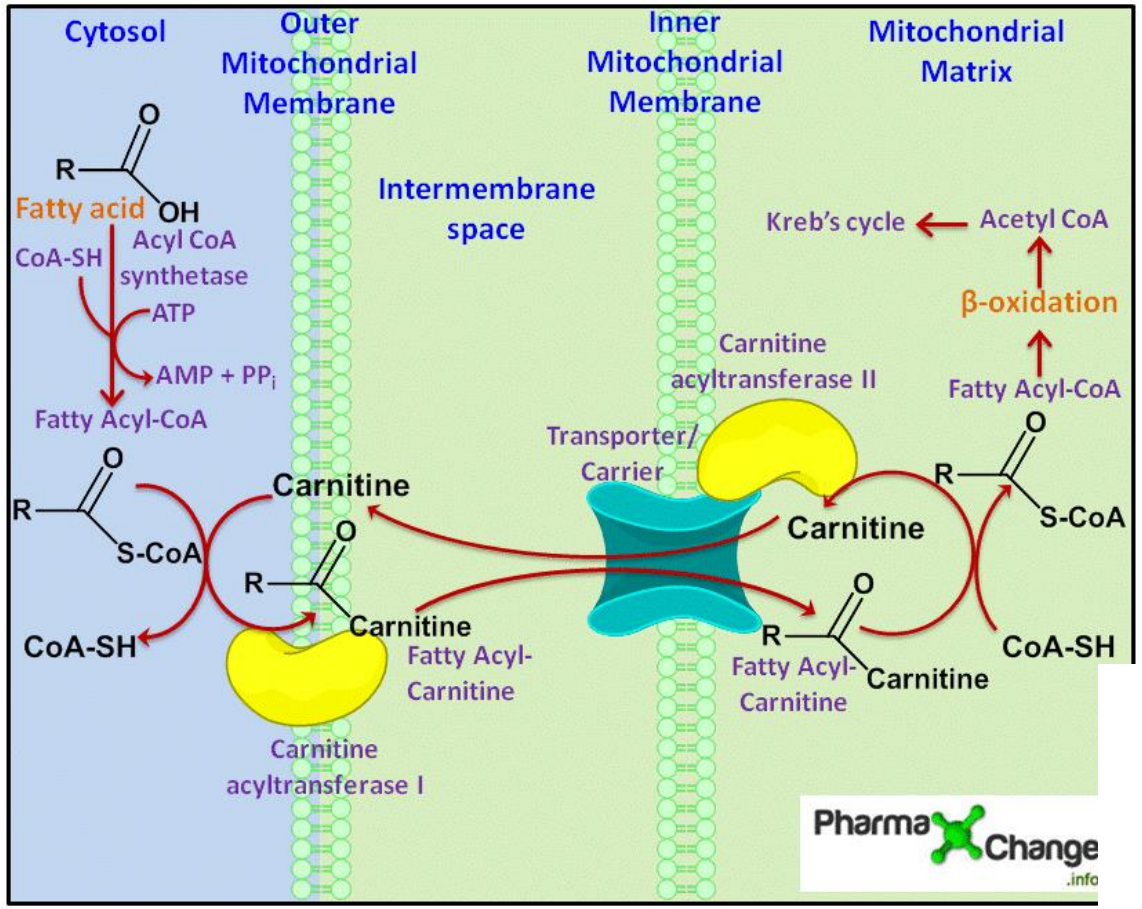


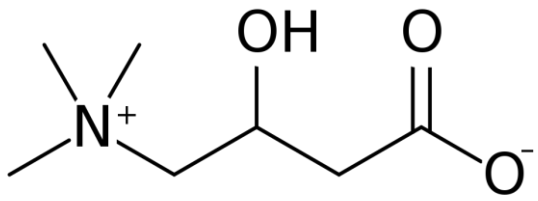
FIGURE 1. Fatty acids oxidation during fasting
During periods of fasting, fatty acids released from the adipose tissues are oxidized in the liver, skeletal muscle, and cardiac muscle for energy production. The brain does not directly utilize fatty acids, but oxidizes ketone bodies derived from β-oxidation of fatty acids in the liver. When fatty acid oxidation is defective, fats released from the adipose tissue cannot be oxidized, and accumulate in organs such as the skeletal and cardiac muscles, impairing their function. Moreover, the liver is unable to produce ketones bodies resulting in energy deficiency.

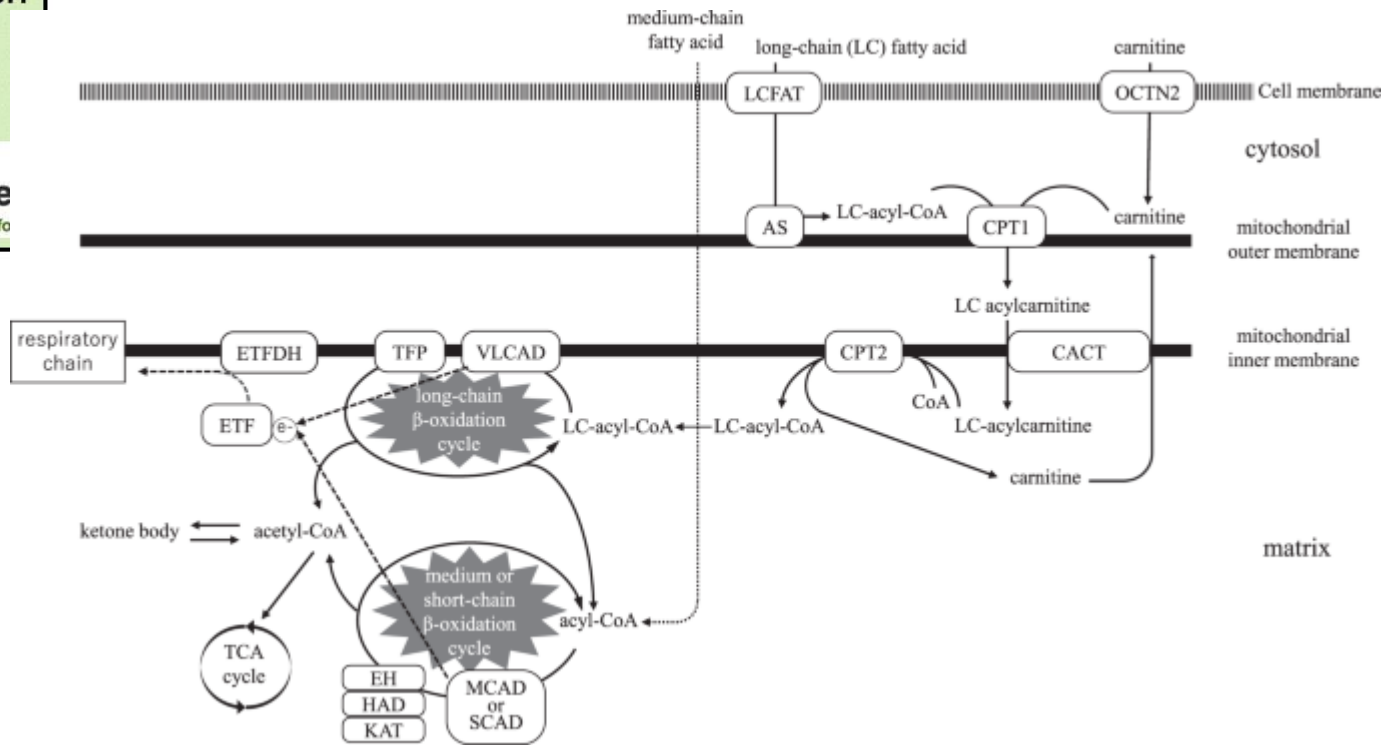
The intracellular processing of fatty acids



El-Gharbawy A et al. Pediatr Clin North Am 2018;65:317

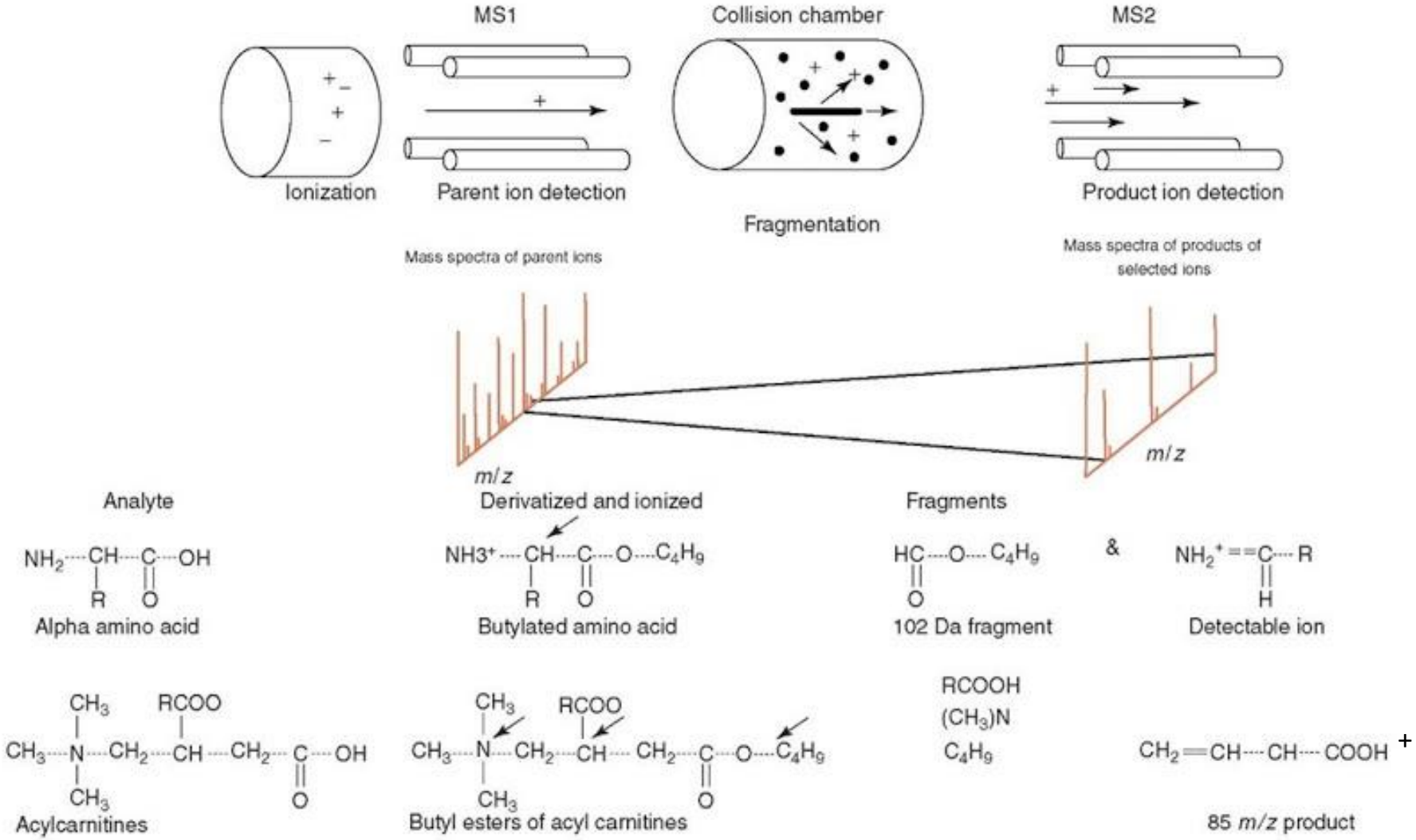

Cytosol
Outer Mitochondrial Membrane
Inner Mitochondrial Membrane
Mitochondrial Matrix
Intermembrane space
Fatty acid
CoA-SH
Acyl CoA synthetase
ATP
AMP + PPi
Fatty Acyl-CoA
Kreb's cycle
Acetyl CoA
β-oxidation
Fatty Acyl-CoA
Carnitine acyltransferase II
Transporter/ Carrier
Carnitine
Carnitine
Carnitine
Fatty Acyl-Carnitine
Fatty Acyl-Carnitine
Carnitine
CoA-SH
CoA-SH
Carnitine acyltransferase I
PharmaXChange.info


OH
O
N+
O-


medium-chain fatty acid
long-chain (LC) fatty acid
carnitine
LCFAT
OCTN2
Cell membrane
cytosol
AS
LC-acyl-CoA
CPT1
carnitine
mitochondrial outer membrane
LC acylcarnitine
respiratory chain
ETFDH
TFP
VLCAD
CPT2
CACT
mitochondrial inner membrane
ETF
e-
long-chain β-oxidation cycle
LC-acyl-CoA
LC-acyl-CoA
CoA
LC-acylcarnitine
carnitine
ketone body
acetyl-CoA
matrix
medium or short-chain β-oxidation cycle
acyl-CoA
TCA cycle
EH
HAD
KAT
MCAD or SCAD

IEMs Associated with Myopathy	Inheritance	Clinical Phenotype
Defects in energy metabolism		
Carnitine shuttle defects		
Primary systemic carnitine deficiency	AR	HCM, hypotonia, muscle weakness, fatigue
Carnitine palmitoyl transferase deficiency type 2 (CPT2) deficiency[b]	AR	Muscle weakness, rhabdomyolysis, exercise intolerance (isolated muscle phenotype), CM, hepatomegaly, hypoglycemia, seizures, cystic kidneys (severe infantile)
Carnitine acylcarnitine translocase (CACT) deficiency	AR	CM, arrhythmias, muscle damage, hepatomegaly, hypoglycemia
FAODs		
VLCAD deficiency[b]	AR	HCM, arrhythmias, sudden death, muscle weakness, exercise intolerance, recurrent rhabdomyolysis, hypoketotic hypoglycemia, "Reye-like" hepatic syndrome
LCHAD deficiency[b]	AR	Sudden death, "Reye-like" hepatic syndrome, hypoketotic hypoglycemia, myopathy, recurrent rhabdomyolysis, CM, retinopathy
TFP deficiency[b]	AR	Sudden death, "Reye-like" hepatic syndrome, hypoketotic hypoglycemia, CM, recurrent, rhabdomyolysis, peripheral neuropathy
MAD deficiency	AR	Muscle weakness, CM, hypoglycemia, hepatopathy, respiratory dysfunction, encephalopathy, acidosis
Mitochondrial respiratory chain defects		
Respiratory chain complexes I-V	AR	Myopathy, CM, hepatopathy, Leigh syndrome, epilepsy, developmental delay ± lactic acidosis
Coenzyme Q deficiency		Myopathy, proteinuria, ataxia, low tissue Coenzyme Q, corrected by Coenzyme Q supplementation

El-Gharbawy A et al. Pediatr Clin North Am 2018;65:317



https://onlinelibrary.wiley.com/doi/epdf/10.1002/rcm.6856

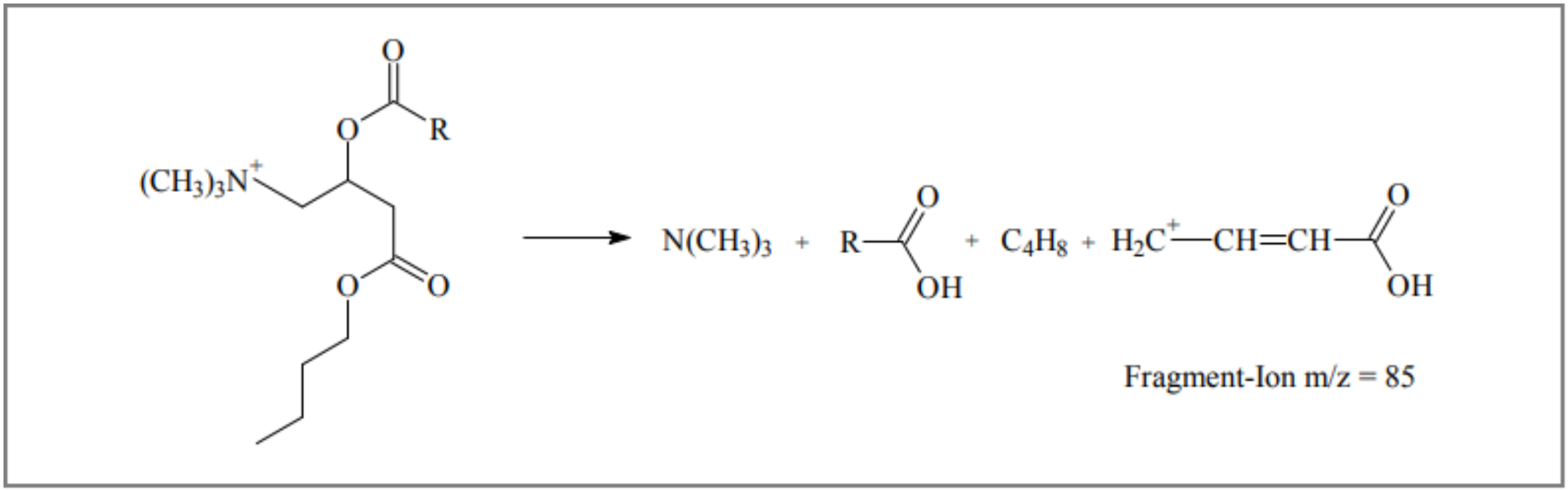


Abbildung 7: Fragmentierung der butylierten Acylcarnitine unter Abspaltung des charakteristischen Fragment-Ions m/z = 85
R = Alkylrest (z.B. R = CH_3 für Acetylcarnitin)

Assayed metabolites

- Amino acids with **neutral loss scan** m/z=102
  - Ala, Asp, Glu, Leu, Met, Phe, Tyr, Val

- Amino acids and succinylacetone (SUAC) using **multiple reaction monitoring**
  - Arg, Cit, Gly, Orn, Pro, SUAC

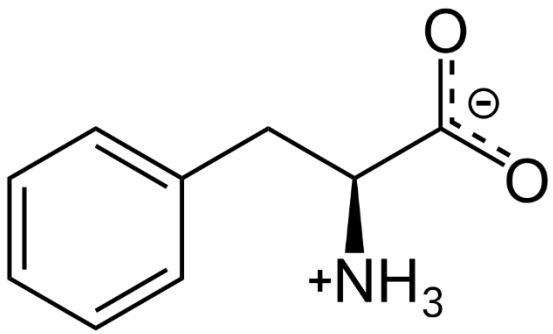
- Acylcarnitines and free carnitine using **precursor scan** of m/z=85
  - Carnitine, C2-carnitine, C3-carnitine, C4-carnitine, C5-carnitine, C6DC-carnitine, C6-carnitine, C8-carnitine, C10-carnitine, C12-carnitine, C14-carnitine, C16-carnitine, C17-carnitine

Sample preparation

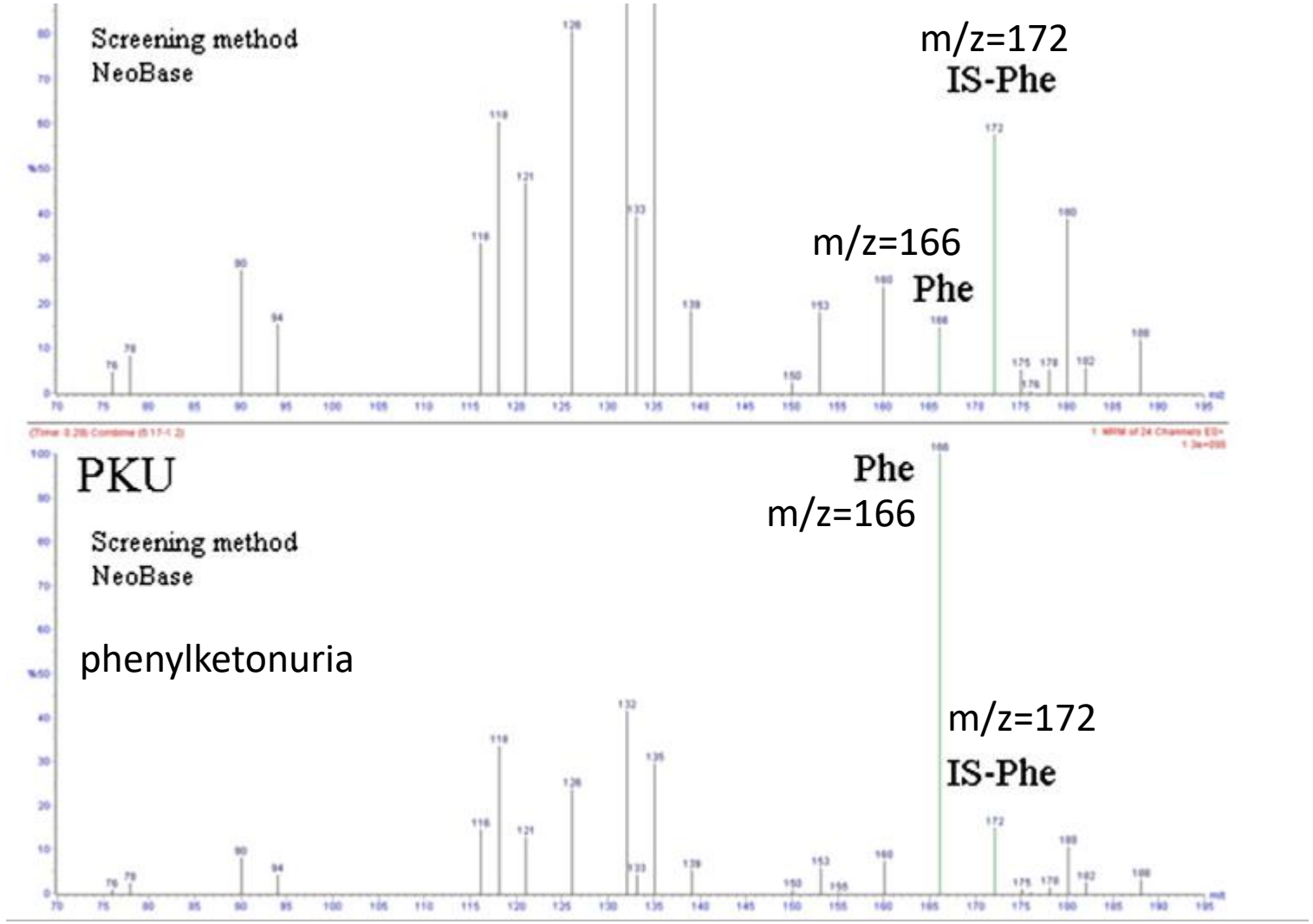
- Punch out 3.2 mm dried blood spots into a 96-well plate
- add 200 µL internal standard mixture, agitate for 20 min
- transfer supernatant, evaporate to dryness
- add solution of derivatizing reagent, incubate
- evaporate to dryness
- add reconstitution solution, agitate briefly
- inject aliquot into the LC-MS/MS system

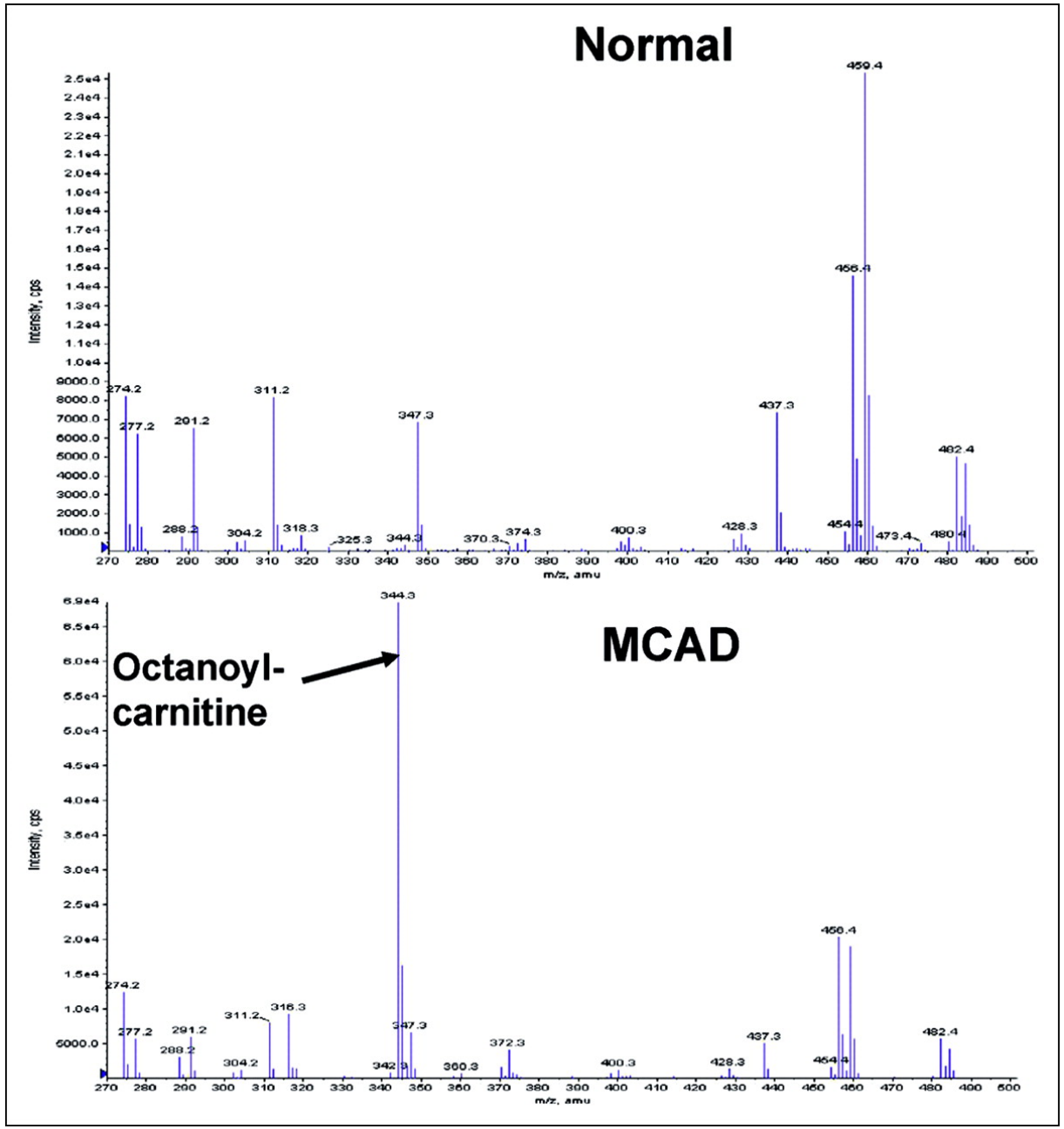
Analysis using LC-MS/MS

- HPLC: isocratic, flow-injection analysis is used (no analytical column)
- ionization: (+) ESI
- mass analyzer: triple quadrupole



$C_9H_{11}NO_2$
165.2





Cowan T NeoREviews 2005;6:e539.

Diagnostic applications of the analysis of serum steroid profiles using LC-MS/MS

- Screening, diagnosis and therapy monitoring:
  - Congenital adrenal hyperplasia
    - 21-hydroxylase deficiency (CYP21A2)
    - 11β-hydroxylase deficiency
    - 17α-hydroxylase/17-20 lyase deficiency (CYP17A)
    - 3β-hydroxysteroid dehydrogenase deficiency
    - Congenital lipoid adrenal hyperplasia
    - P450 oxydoreductase deficiency
  - Cushing syndrome
  - Primary/secondary adrenocortical insufficiency
  - hyperaldosteronism
  - adrenocortical tumors
  - other functional changes in the HPA axis
  - adverse effects of drug therapies
  - Polycystic ovary syndrome

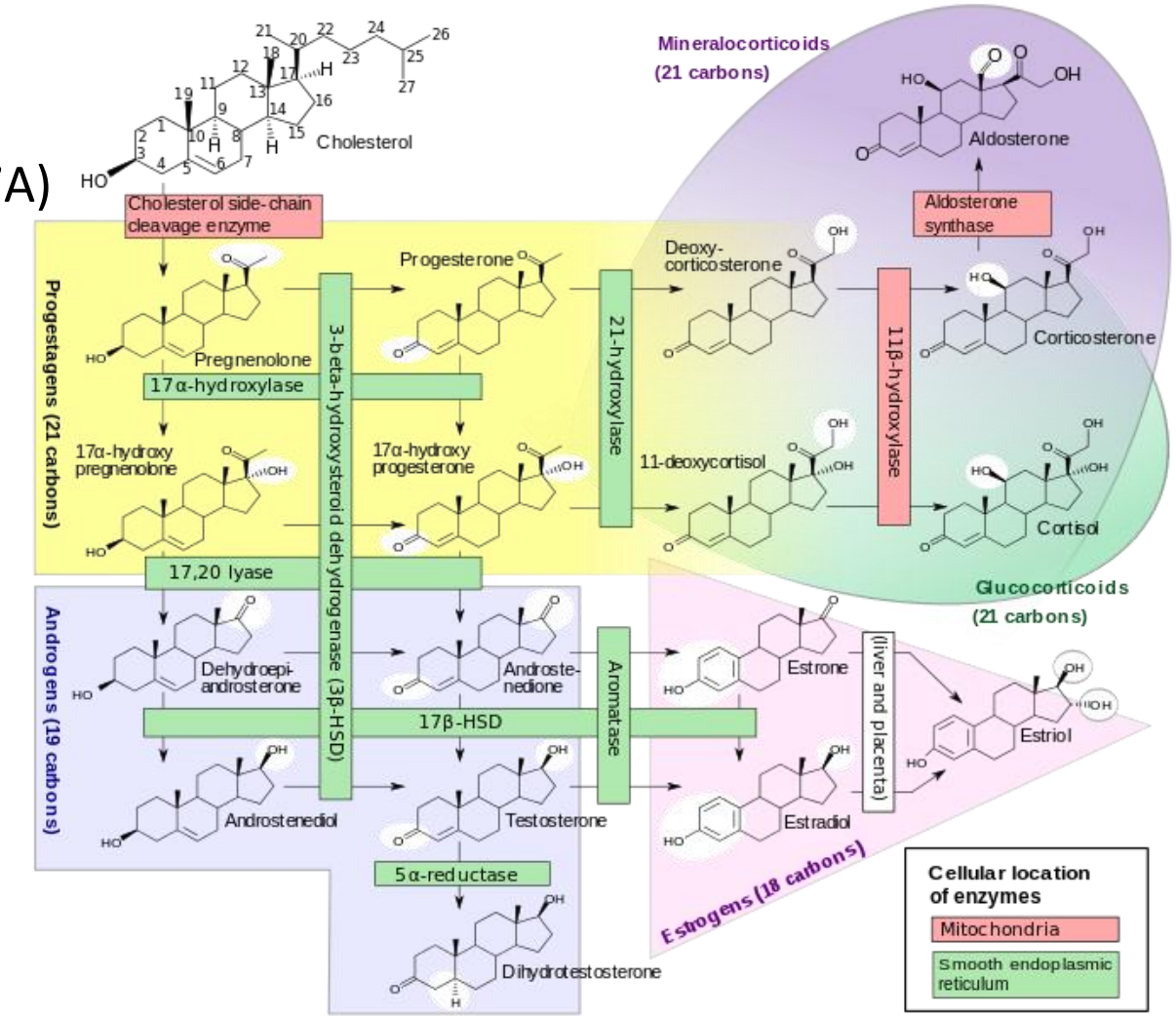
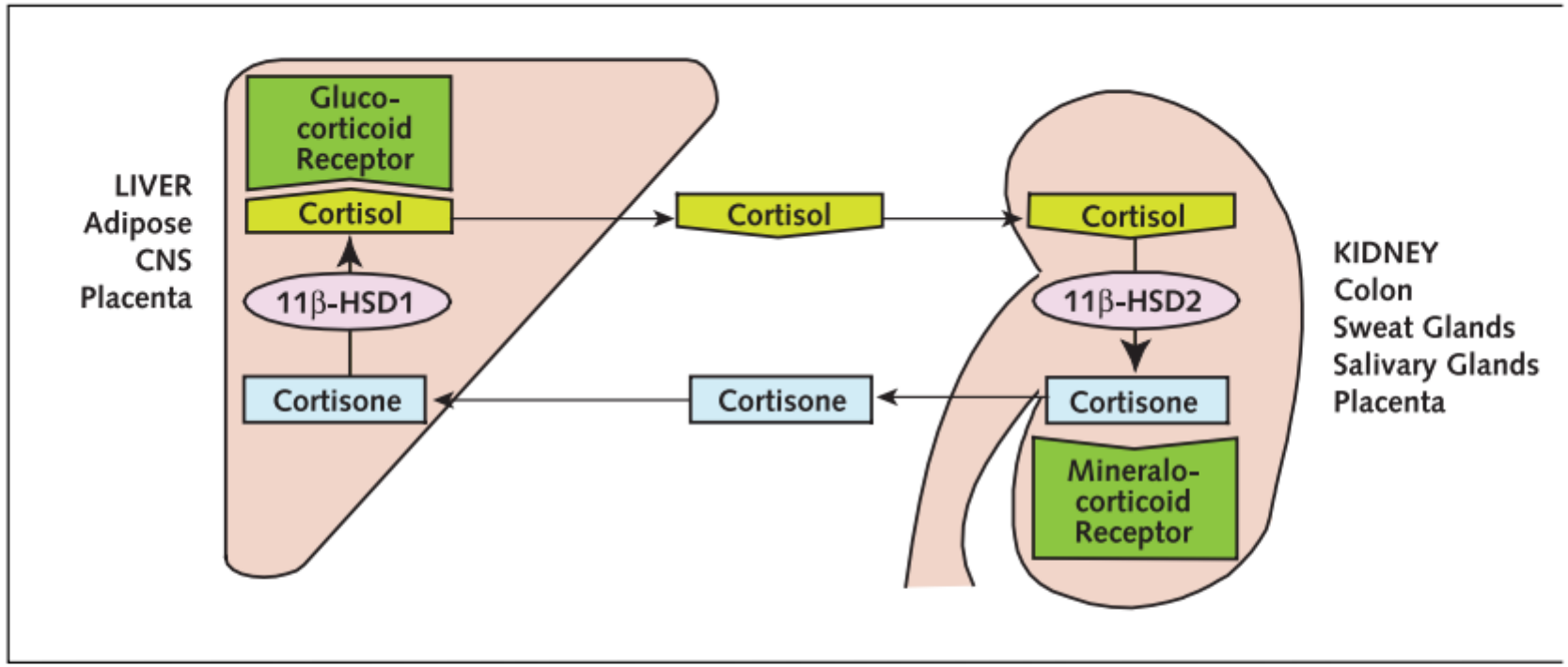
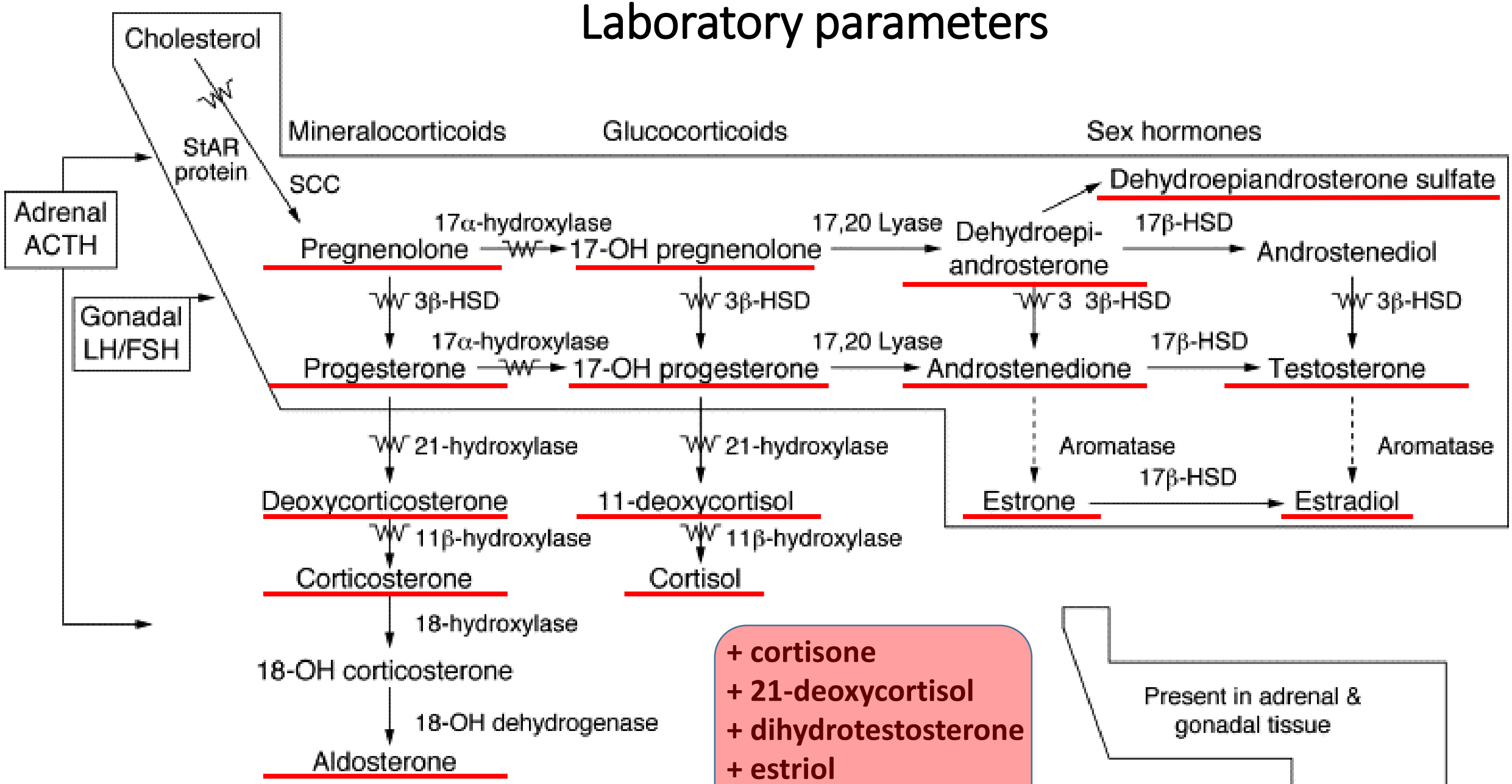


Figure 3. **The cortisol–cortisone shuttle.**



The effect of cortisol on the mineralocorticoid receptor (for example, in the kidney) is prevented by metabolism to inactive cortisone by the enzyme 11β-hydroxysteroid dehydrogenase type 2 (*11β-HSD2*). Cortisone can be reactivated to cortisol by the enzyme 11β-hydroxysteroid dehydrogenase type 1 (*11β-HSD1*). When cortisol level is very high (as in the Cushing syndrome), not all of the excess cortisol can be inactivated to cortisone, and the effects of mineralocorticoid excess (for example, hypertension and hypokalemia) can occur. Modified from Seckl JR, Walker BR. Minireview: 11β-hydroxysteroid dehydrogenase type 1—a tissue-specific amplifier of glucocorticoid action. Endocrinology. 2001;142(4):1371-6, with permission from The Endocrine Society (19). CNS = central nervous system.

Laboratory parameters

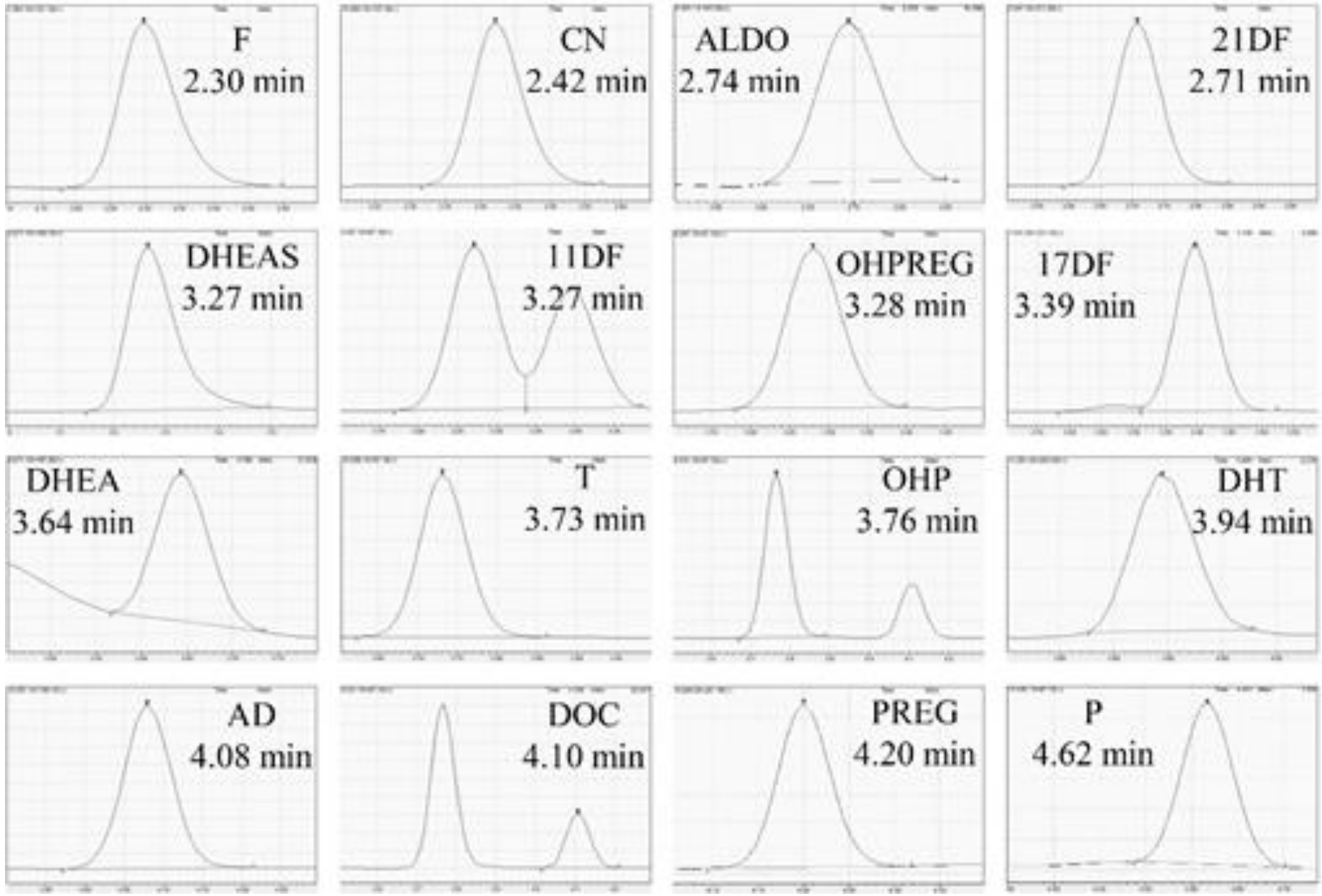


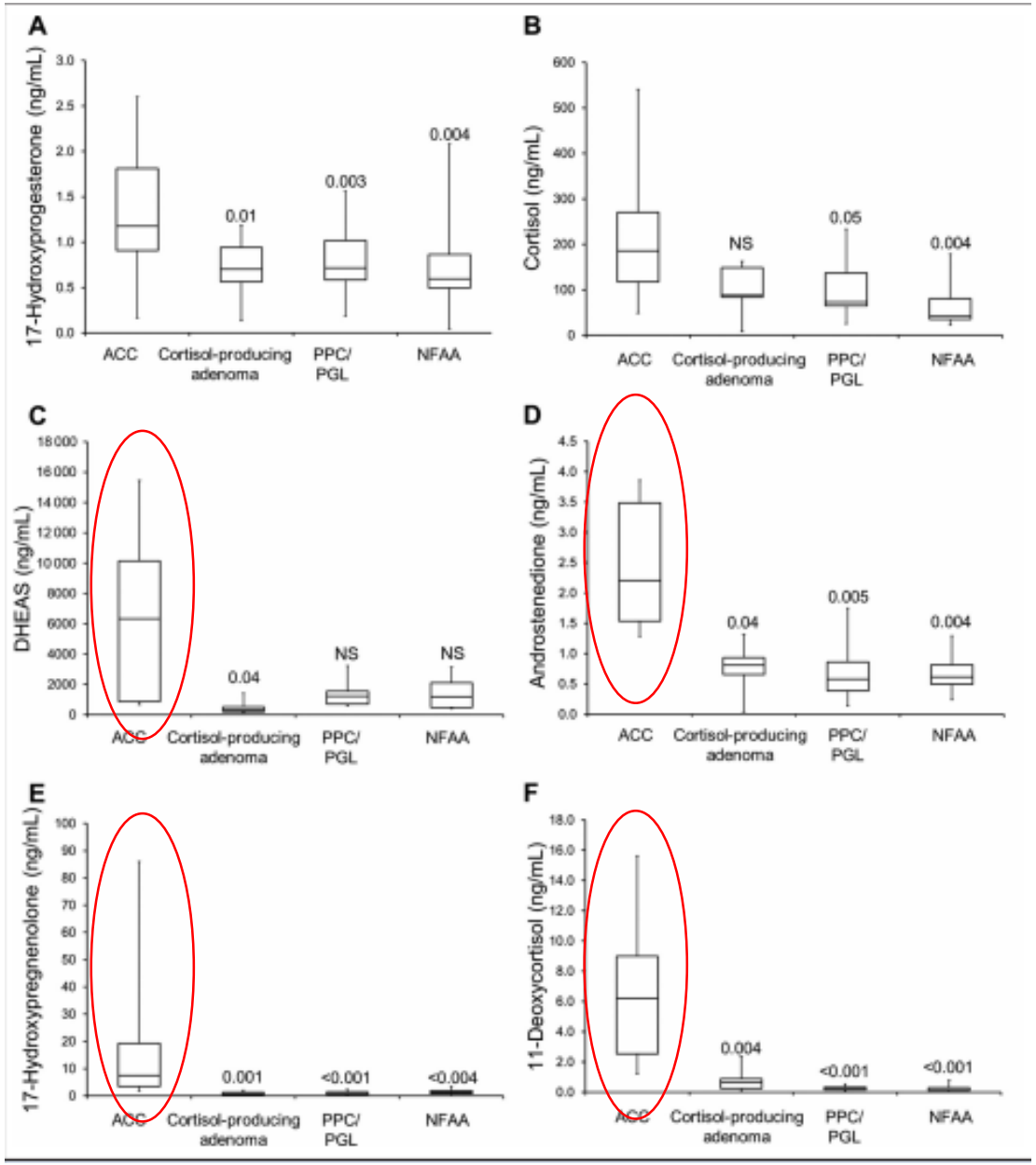
Pang S. Trends Endocrinol Metab 1998;9:82-6.

Method in serum:

- sample preparation:
  - 200 µL serum + 600 µL methanol containing internal standards
  - vortex
  - Dilute 400 µL with 400 µl water
  - Apply to Strata-X solid phase extraction cartridge
  - Wash with water-methanol 3:1
  - Elute with acetonitrile-methanol 1:1
  - Evaporate to dryness
  - Reconstitute with 50 µL water-methanol 1:1

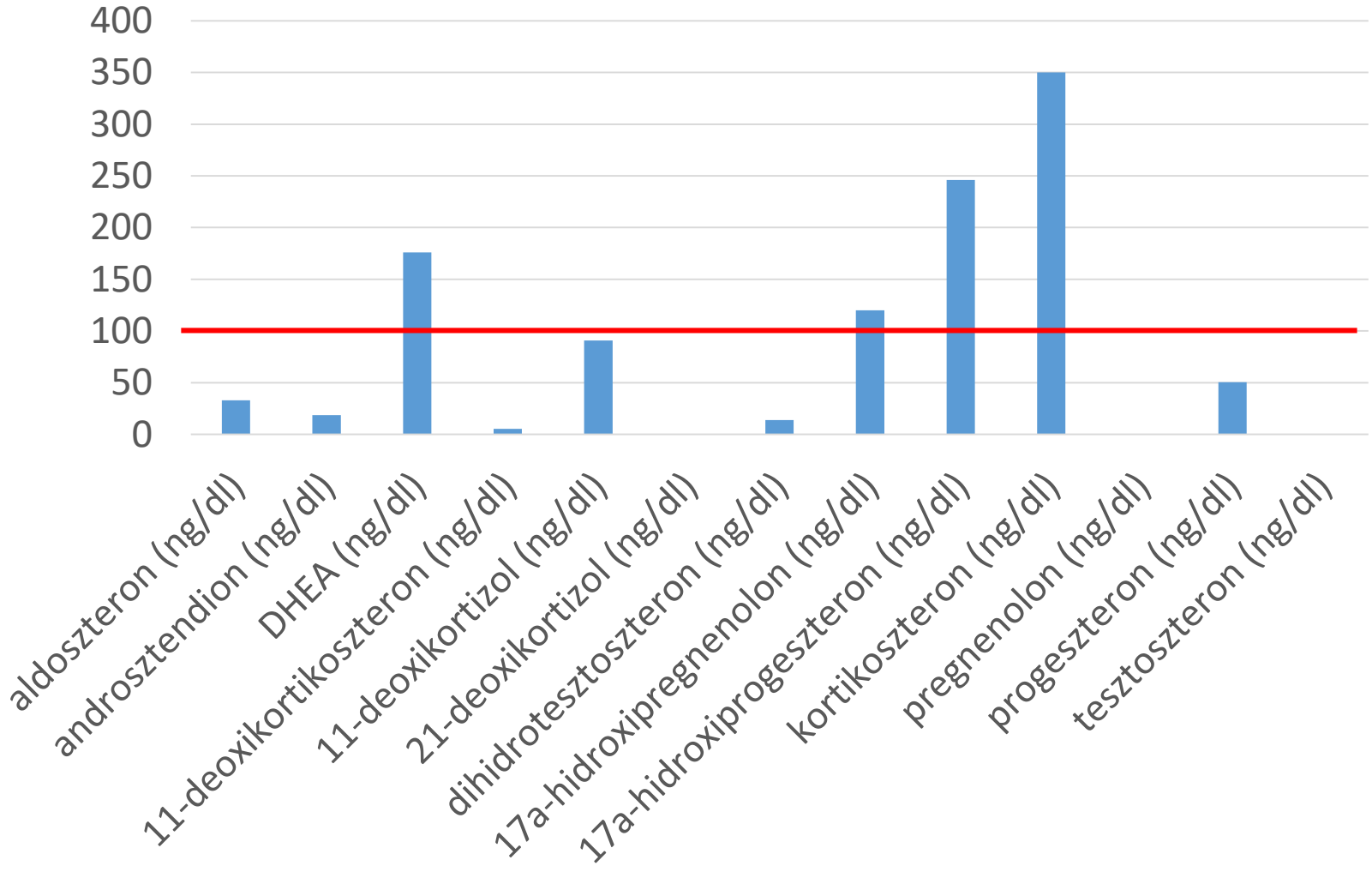
- analysis: LC-ESI(+)-MS/MS, run time: 2x6 min
  - SP: C18 50x2.1 mm, 1.7 µm + biphenyl 50x2.1 mm, 1.7 µm
  - MP: water (A), methanol (B), both containing 0.1% formic acid
  - sample volume: 5 µL, 15 °C
  - FR: 0.33 mL/min
  - CTO: 35 °C
  - MS mode: MRM

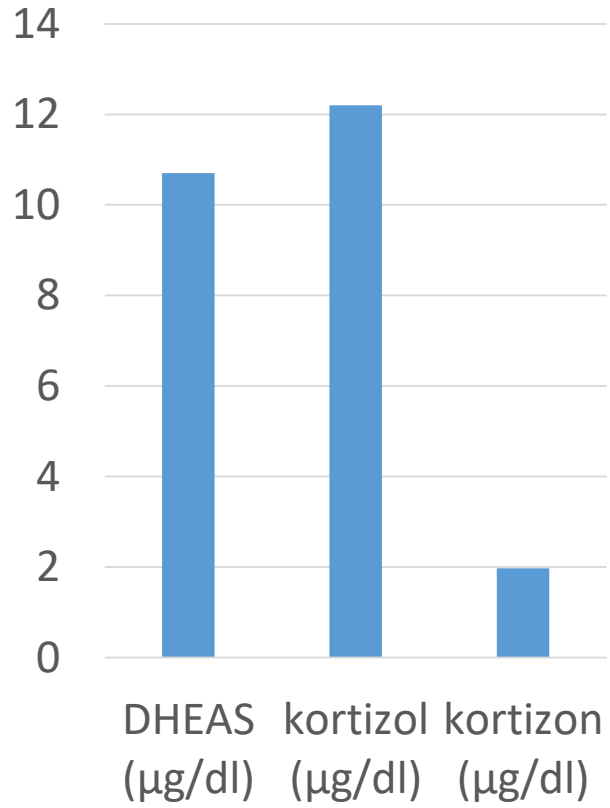

F
2.30 min
CN
2.42 min
ALDO
2.74 min
21DF
2.71 min
DHEAS
3.27 min
11DF
3.27 min
OHPREG
3.28 min
17DF
3.39 min
DHEA
3.64 min
T
3.73 min
OHP
3.76 min
DHT
3.94 min
AD
4.08 min
DOC
4.10 min
PREG
4.20 min
P
4.62 min



Taylor DR et al. Clin Chem 2017;63:1836.

Healthy adrenal cortex (boy, 6 y)

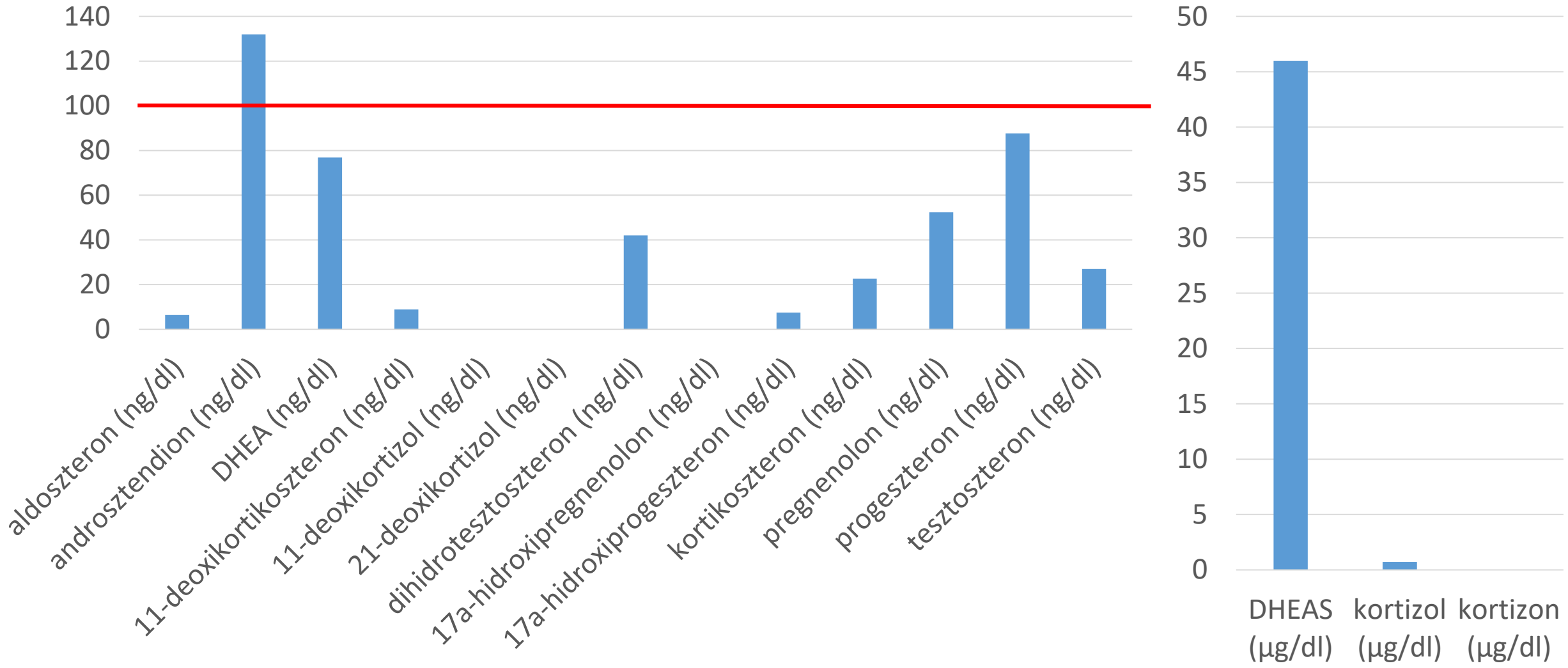




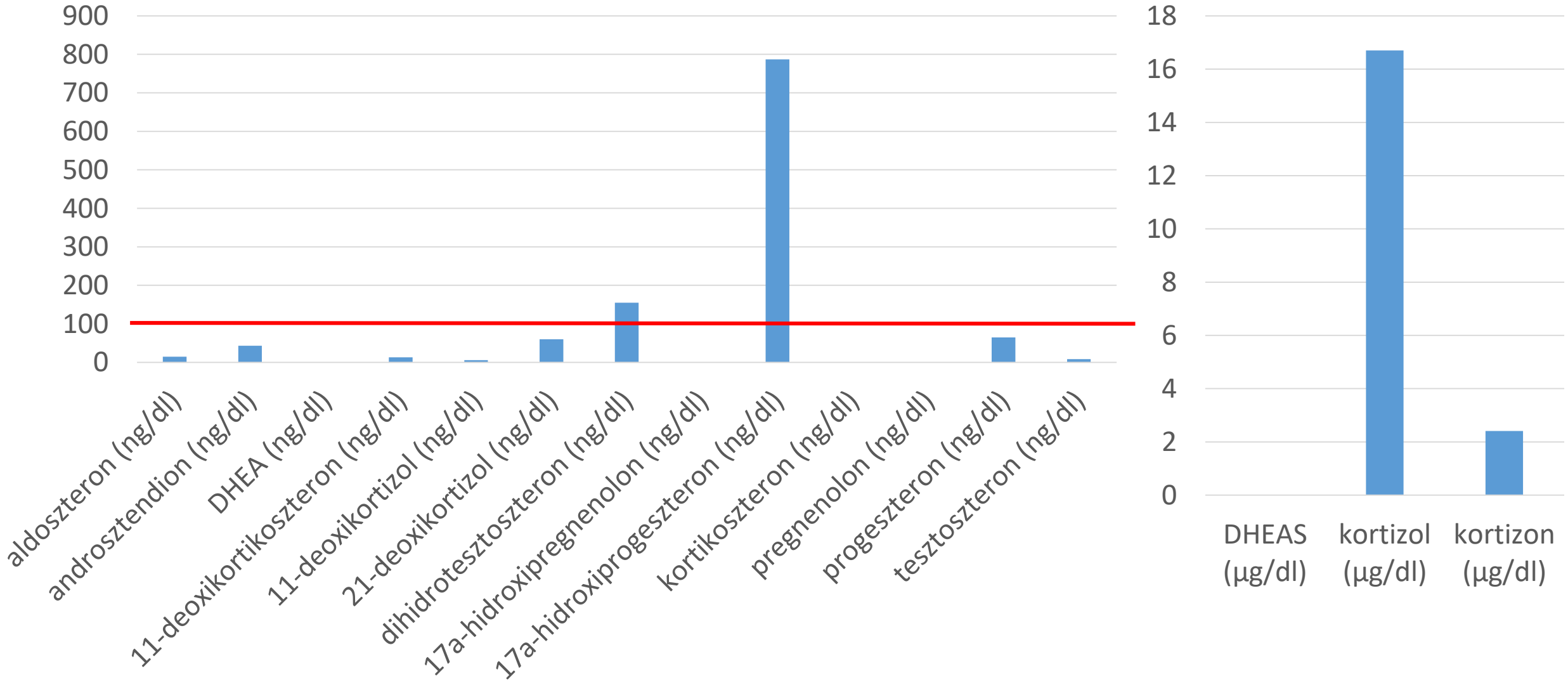
Results of ACTH stimulation test in healthy girl (11y)

Analyte	Concentration before ACTH stimulation	Concentration after ACTH stimulation
aldosterone (ng/dL)	<2.0	7.9
androstenedione (ng/dL)	120	120
DHEA (ng/dL)	315	483
DHEAS (µg/dL)	<10	14
11-deoxycorticosterone (ng/dL)	6.4	24.7
11-deoxycortisol (ng/dL)	33.9	151
21-deoxycortisol (ng/dL)	9.7	48.2
dihydrotestosterone (ng/dL)	10.9	13.1
17α-hydroxypregnenolone (ng/dL)	<30	103
17α-hydroxyprogesterone (ng/dL)	191	484
corticosterone (ng/dL)	100	1687
cortisol (µg/dL)	18.4	23.6
cortisone (µg/dL)	2.8	2.7
pregnenolone (ng/dL)	<30	<30
progesterone (ng/dL)	56.7	127
testosterone (ng/dL)	45.4	33.0

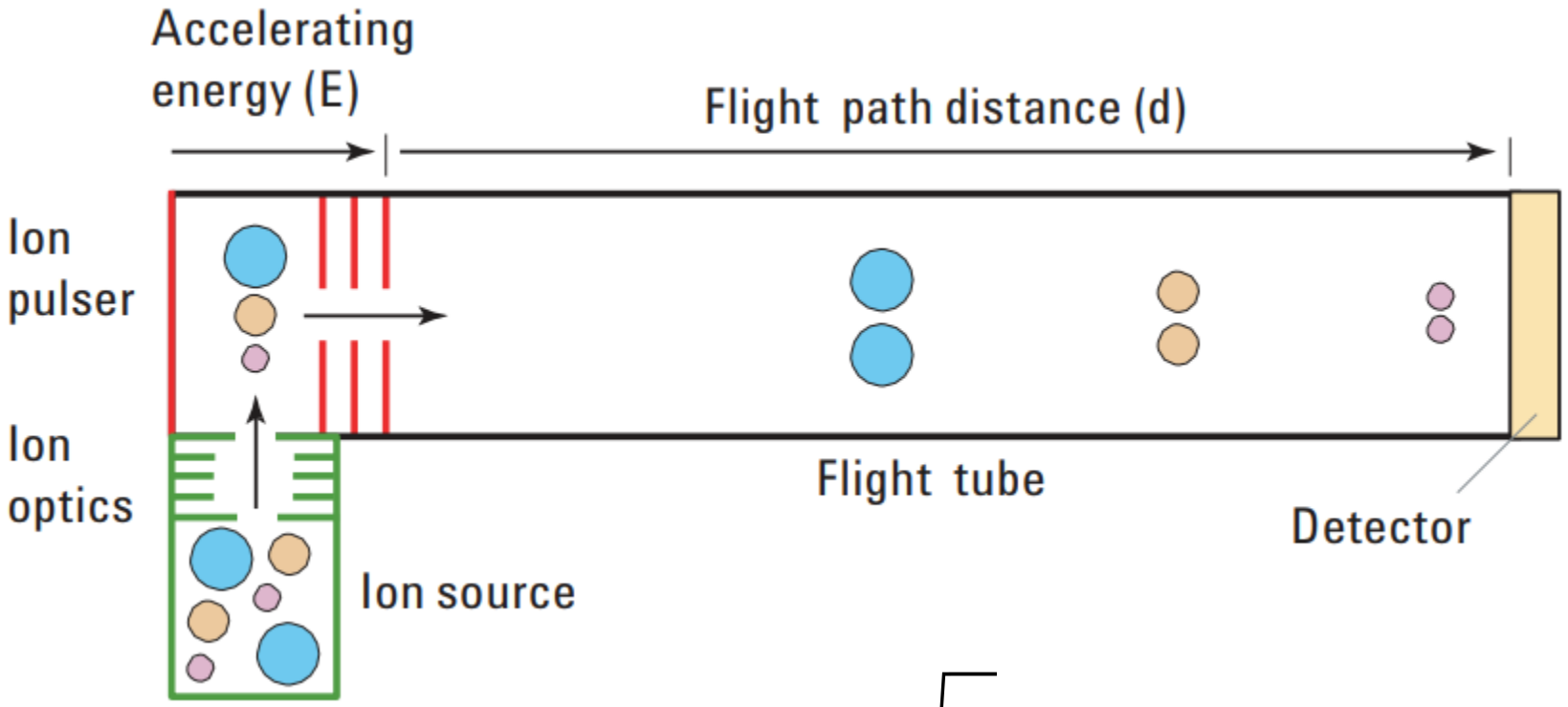
Results of a dexamethasone suppression test (girl, 16y)



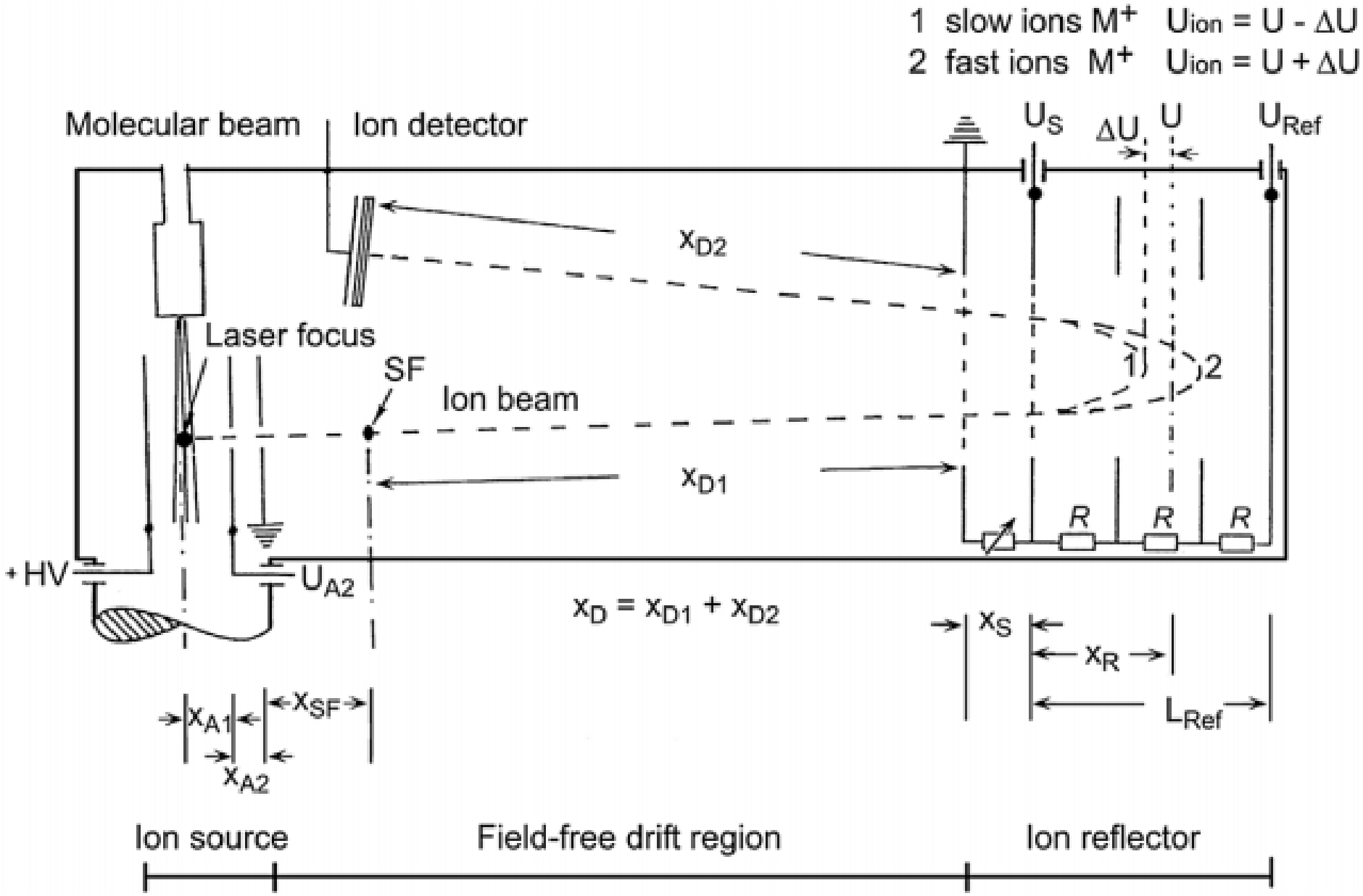
Classical congenital adrenal hyperplasia (boy, 11y)



Time-of-flight mass analyzers

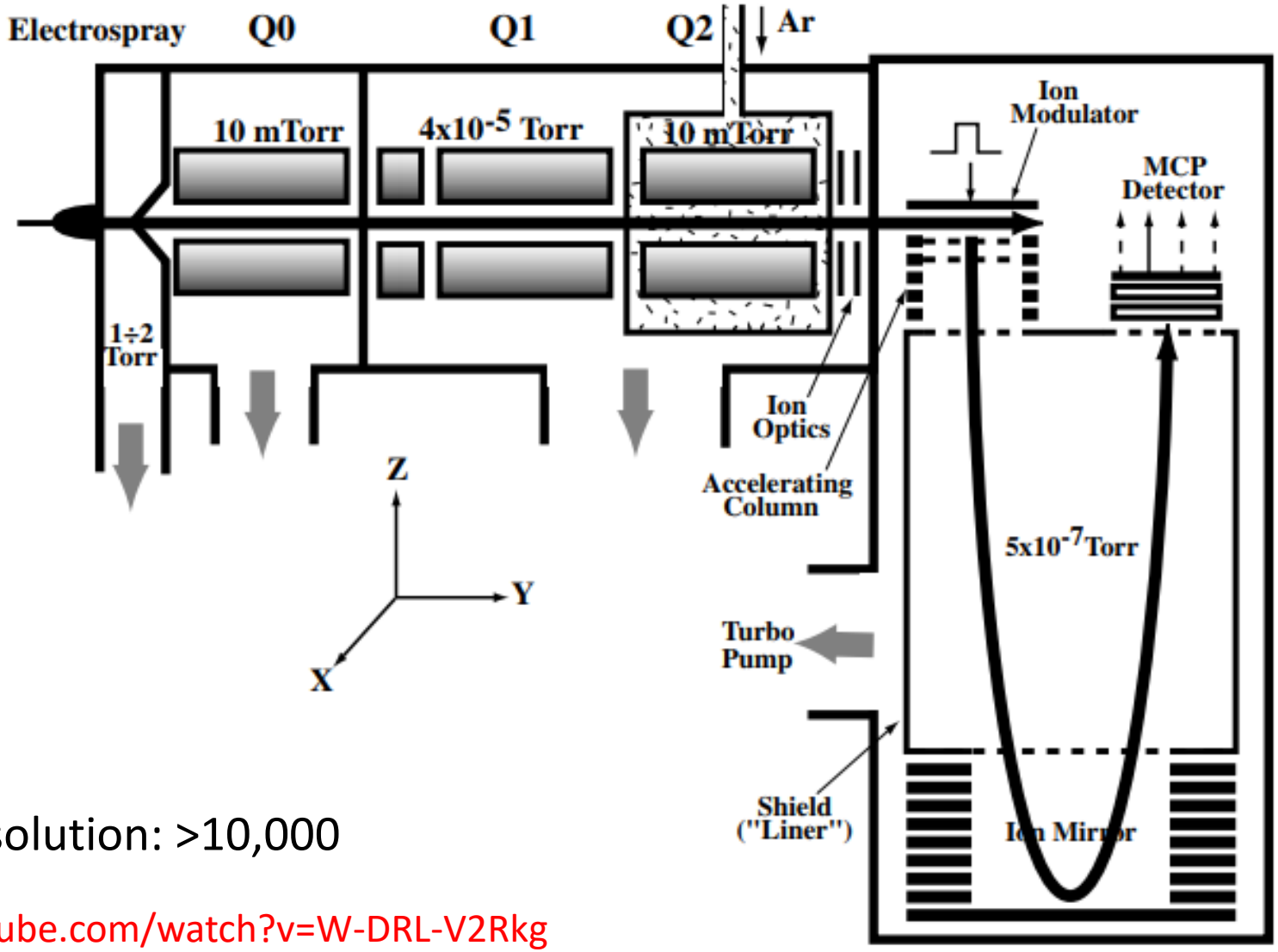


$$t \sim \sqrt{\frac{m}{q}}$$



Boesl U. Mass Spectrom Rev 2017; 36:86.

Quadrupole-time-of-flight mass analyzers



Resolution: >10,000

https://www.youtube.com/watch?v=W-DRL-V2Rkg

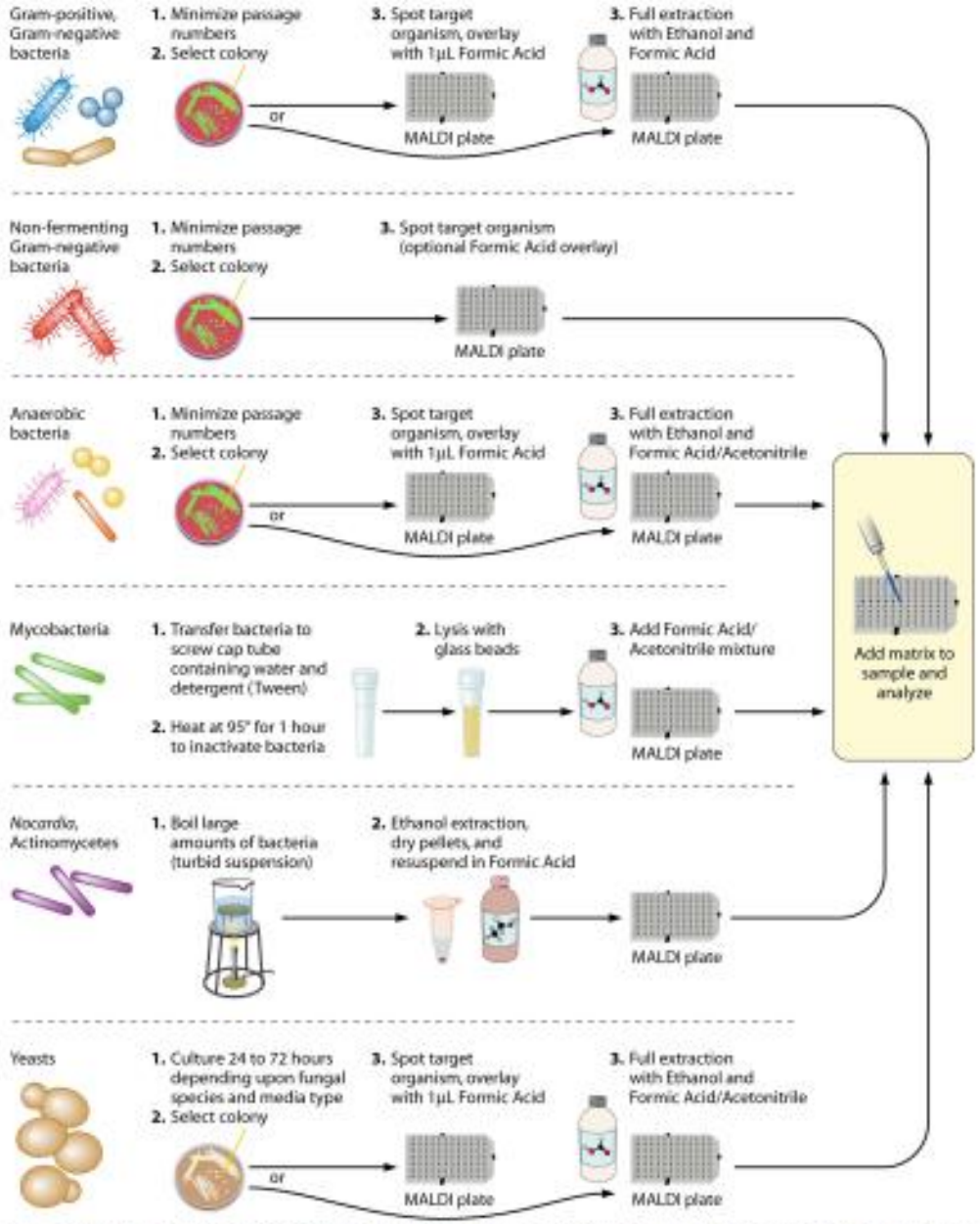


FIG 3 Additional suggestions for MALDI-TOF MS sample preparations for use with different classes of microbes. Different groups of microorganisms vary fundamentally in their cellular composition and architecture. These differences have been demonstrated to affect the quality of spectra generated during MS experiments and, thus, the accuracy of MALDI-TOF MS-derived identifications. As such, investigators from a number of independent studies have evaluated different methods for sample preparation of different groups of microorganisms, ranging directly from intact-cell to full-protein-extraction-based methodologies. Results from these studies are summarized here. Proper biological safety precautions should be followed with respect to dangerous members of these groups of organisms.

Clarke AE et al. Clin Microbiol Rev 2013;26:547-603.

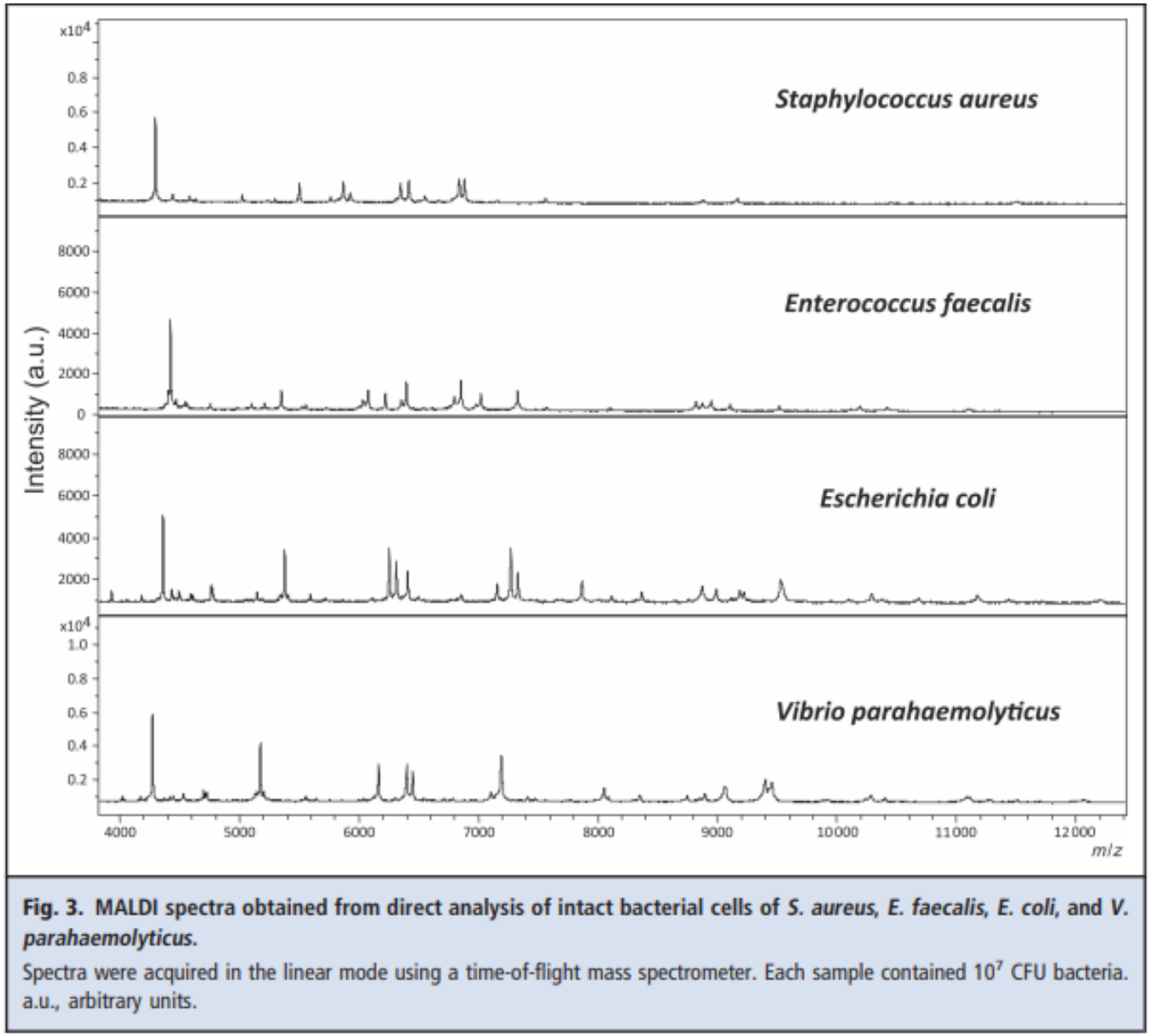


Fig. 3. MALDI spectra obtained from direct analysis of intact bacterial cells of *S. aureus*, *E. faecalis*, *E. coli*, and *V. parahaemolyticus*.

Spectra were acquired in the linear mode using a time-of-flight mass spectrometer. Each sample contained 10^7 CFU bacteria. a.u., arbitrary units.

Ho YP, Reddy PM. Clin Chem 2010;56:525.

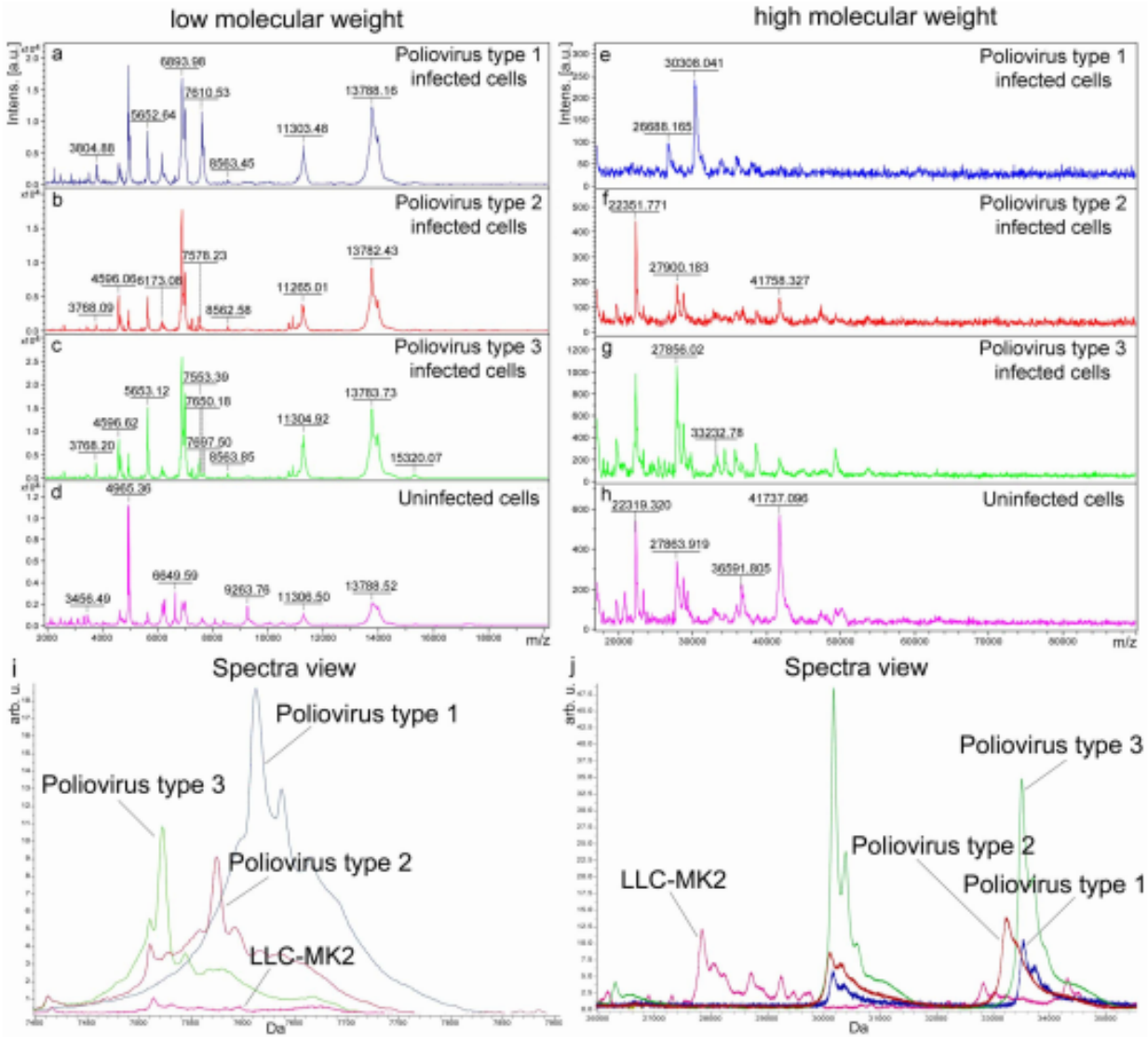


Figure 2 | Representative MALDI-TOF mass spectra of Sabin poliovirus-infected and uninfected LLC-MK2 cells and comparative analysis by ClinPro Tools software. The spectra of LLC-MK2 cells infected with human poliovirus types 1, 2, 3 ("Poliovirus type 1-infected cells", "Poliovirus type 2-infected cells", "Poliovirus type 3-infected cells"), with pointed average masses, are compared in the m/z range 2 to 20 KDa (a–c) and 17 to 90 KDa (e–g) with the spectra of LLC-MK2 uninfected cells (d,h). (i,j) Spectra view of the Average spectra profiles in the mass range 7,400–7,900 Da (i) and in the mass range 26,000 to 35,000 Da (j) of LLC-MK2 cells infected with the 3 different poliovirus serotypes with LLC-MK2 uninfected cells.

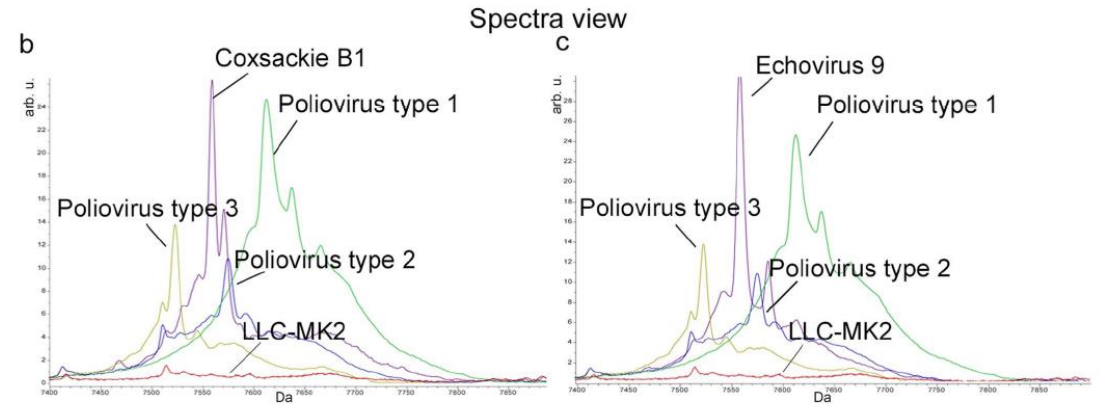


Figure 4 | Representative MALDI-TOF mass spectra of LLC-MK2 cells infected with the *Picornaviridae* family members human coxsackievirus B1 and human echovirus 9 and uninfected cells, and ClinPro Tools comparative analysis with the Sabin poliovirus type 1, 2, and 3 strains. (a) Spectra of LLC-MK2 cells infected with human coxsackievirus B1 and human echovirus 9 ("Coxsackie B1-infected cells" and "Echovirus 9-infected cells") compared with the protein profile of LLC-MK2 uninfected cells in the m/z range of 2 to 20 KDa. Molecular weight values are indicated on peak tops. (b,c) Spectra view of the average spectra of human coxsackievirus B1 (b) and human echovirus 9 (c) compared by ClinPro Tools in the mass range 7,400–7,900 Da with the average spectra of the 3 Sabin poliovirus strains (poliovirus type 1; poliovirus type 2; poliovirus type 3) and uninfected LLC-MK2 cells.

Calderaro A et al. Sci Rep 2014;4:6803.

TABLE I. Performance of matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) identification with routine samples in clinical microbiology laboratories

Number of isolates tested	Manufacturer of the MALDI-TOF MS system	Overall correct identification at the species level (%)	Overall correct identification at the genus level (%)	Correct identification of Gram-negative bacteria at the species level (%)	Correct identification of Gram-positive bacteria at the species level (%)	References
1660[a]	Bruker Daltonics GmbH	84.1	11.3	NA	NA	[11]
1371[a]	Bruker Daltonics GmbH	91.7	2.8	88.8	88.0	[14]
720[a]	Bruker Daltonics GmbH	93.6	NA	98.2	83.9	[13]
720[a]	Shimadzu Corporation	88.3	NA	94.8	75.6	[13]
1116[b]	Bruker Daltonics GmbH	95.2	4.8	93.8	97.7	[10]

NA, not available.
[a]Prospective study.
[b]Retrospective study.

Bizzini A, Greub G. Clin Microbiol Infect 2010;16:1614.

web.expasy.org/tagident/

Szüneteltetve

Alkalmazások | AbsoluteIDQ Stero... | Dinamikus Periódus... | LabArchives, Your E... | Statistical Methods... | Authorea | Quartzy | The free a... | Digital tools for res... | Biorepository Regul...

ExPASy Bioinformatics Resource Portal

Home | Contact

TagIdent tool (formerly GuessProt)

TagIdent is a tool which allows

1. the generation of a list of proteins close to a given pI and Mw,
2. the identification of proteins by matching a short sequence tag of up to 6 amino acids against proteins in UniProtKB/Swiss-Prot close to a given pI and Mw,
3. the identification of proteins by their mass, if this mass has been determined by mass spectrometric techniques

for one or more species and with an optional keyword [references].

Documentation is available.

Fill out in the following form the *pI* (isoelectric point) and *Mw* (molecular weight) you wa
values in which the search should take place. Leave empty if you don't want to specify

The "Organism Name or Classfication or NCBI_TaxID" field allows you to specify terms
will only be returned, if all the given terms appear in either the *OS* or *OC* or *OX* lines of
particular be aware that if you enter "rat", all taxonomical terms containing the substring
even better its TaxID '10116') narrows down your search and probably corresponds bet

Scan in UniProtKB/Swiss-Prot can be restricted by the use of a keyword. This should b
keywords.

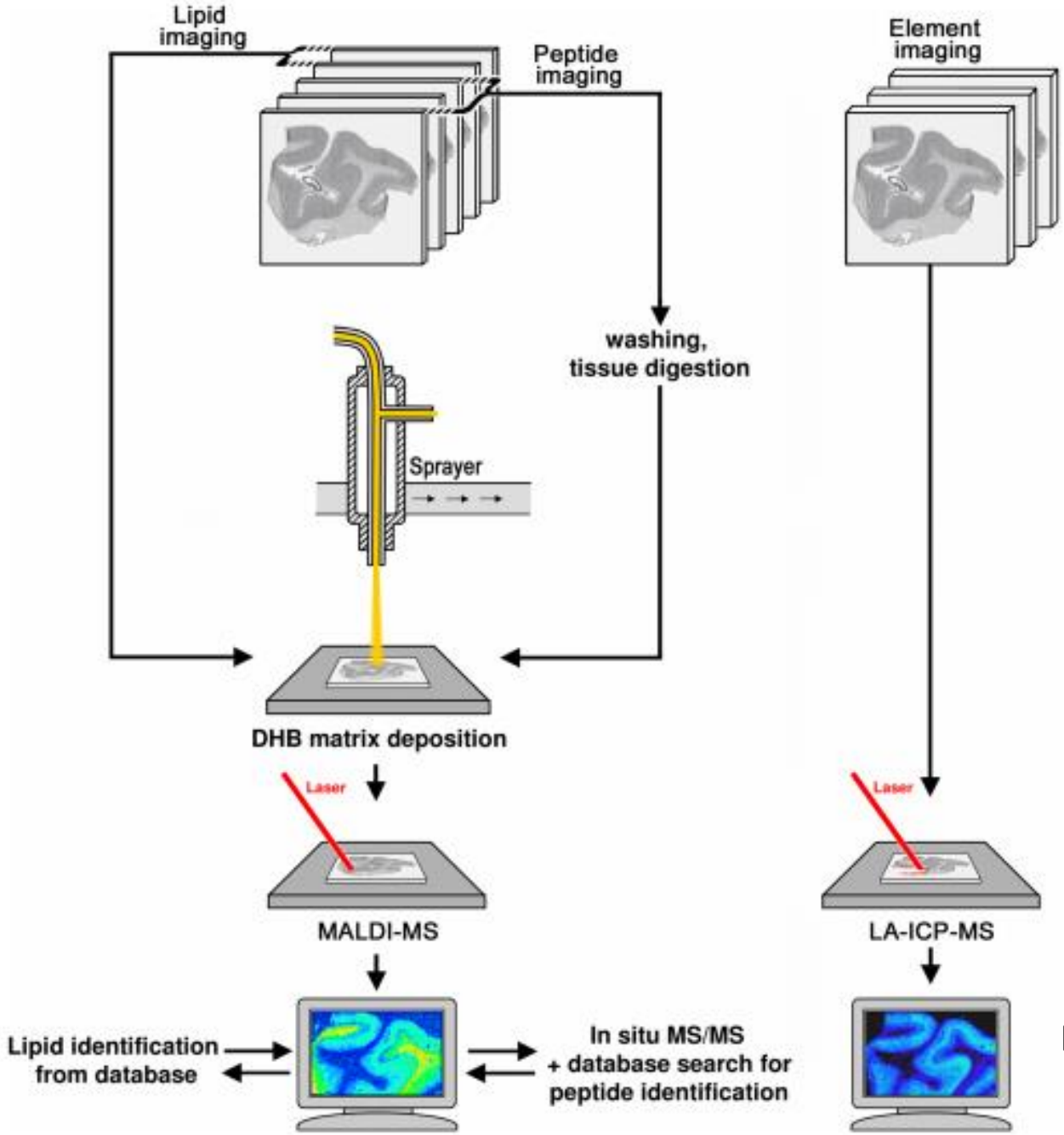
For example, entering the following values:

```
pI min = 5.15
```

Table 1. Peaks identified to separate the clusters.

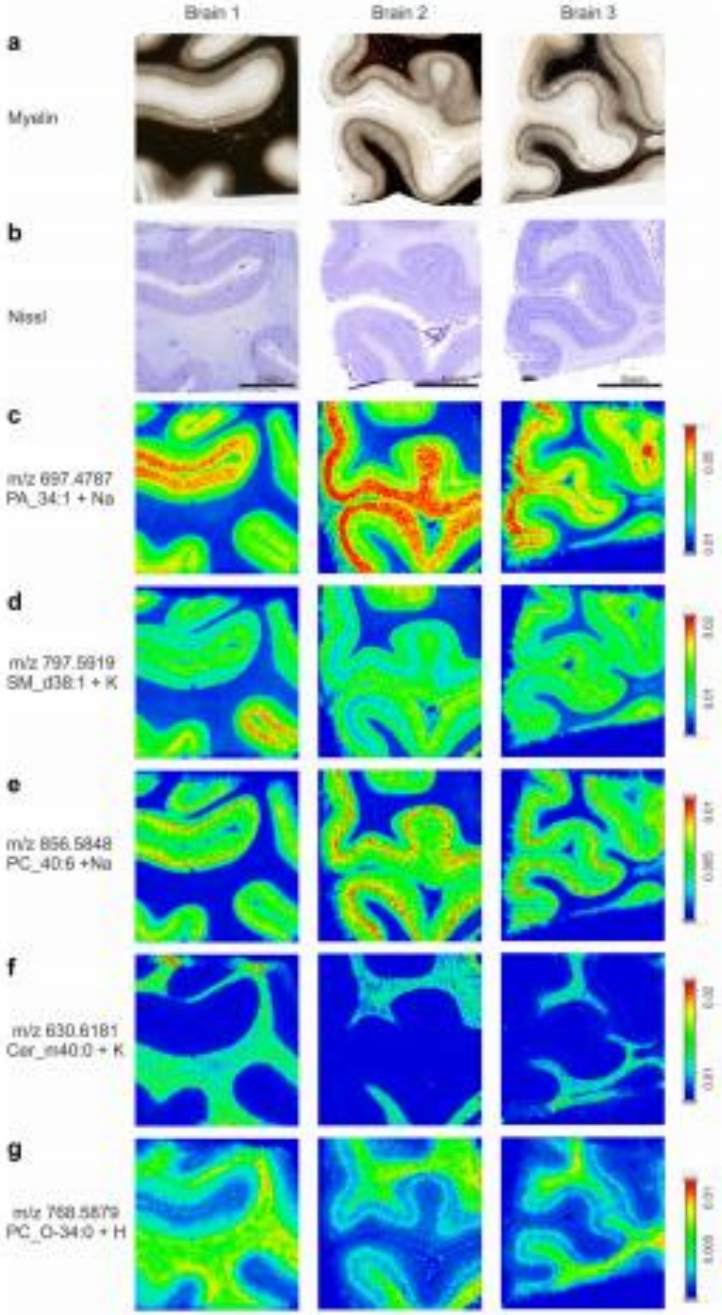
Peak Position	Cluster 1	isolates 11 and 12	Cluster 2	Possible proteins (from TagIdent)
3444	Yes	Yes	No	Protamine-like protein
5873	Yes	Yes	No	Regulatory protein MokB
6539	Yes	No	No	50S ribosomal protein L30
7173	Yes	No	No	Pilin; Protein CopA/IncA
7650	No	No	Yes	Response regulator inhibitor for tor operon; Protein KleB; Protein IscX; Cold shock-like protein CspH
7708	Yes	Yes	No	Response regulator inhibitor for tor operon; Protein KleB; Protein IscX; Cold shock-like protein CspH
8326	Yes	Yes	No	Tautomerase PptA; Dihydrofolate reductase type 2; Ferrous iron transport protein A
8350	No	No	yes	Tautomerase PptA; Dihydrofolate reductase type 2; Ferrous iron transport protein A
9712	No	No	yes	30S ribosomal protein S17; Regulatory protein AriR; UPF0386 protein YjhX; Acid stress chaperone HdeA
9739	Yes	Yes	No	30S ribosomal protein S; Regulatory protein AriR 17; UPF0386 protein YjhX; Acid stress chaperone HdeA
10463	Yes	Yes	No	30S ribosomal protein S19; Sugar fermentation stimulation protein B
10489	No	No	Yes	30S ribosomal protein S19; Sugar fermentation stimulation protein B

doi:10.1371/journal.pone.0164260.t001



De San Román EG et al. Brain Struct Funct 2018;223:2767.

Fig. 5 Lipid distributions in the human primary visual cortex. **a** Myelin staining. **b** Nissl staining of sections adjacent to (**a**). **c**–**g** Lipid distribution images measured by MALDI-MSI tissue sections directly adjacent to the Nissl staining shown in (**b**). Images show the distributions of ions at **c** *m/z* 697.4787, identified as PA_34:1 + Na, **d** *m/z* 797.5919, identified as SM_d38:1 + K, **e** *m/z* 856.5848, identified as PC_40:6 + Na, **f** *m/z* 630.6181, identified as Cer_m40:0 + K and **g** *m/z* 768.5879, identified as PC_O-34:0 + H. Images were recorded in positive ion mode at 100 µm lateral resolution. Scale bar in **b** 5 mm, applies vertically to all images of the corresponding specimen. Color bars indicate normalized lipid ion intensities (arbitrary units, applies horizontally to the corresponding lipid across all specimens)



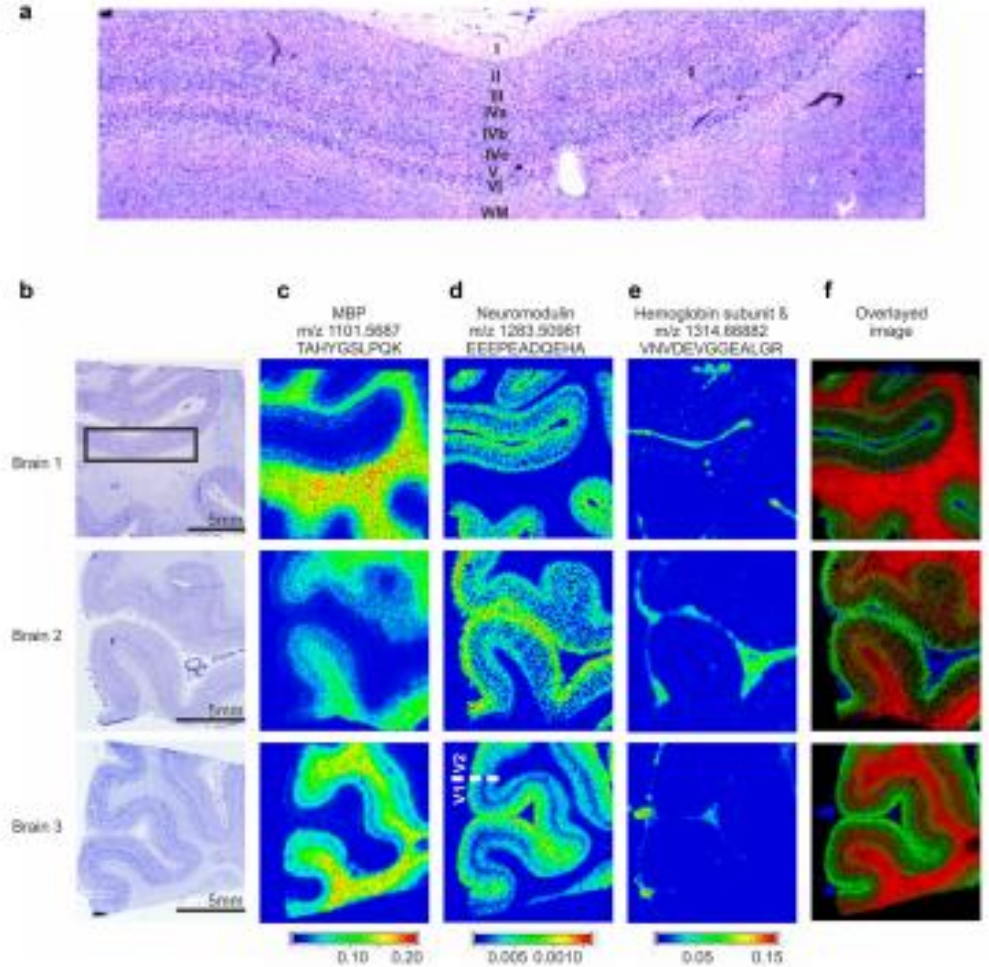


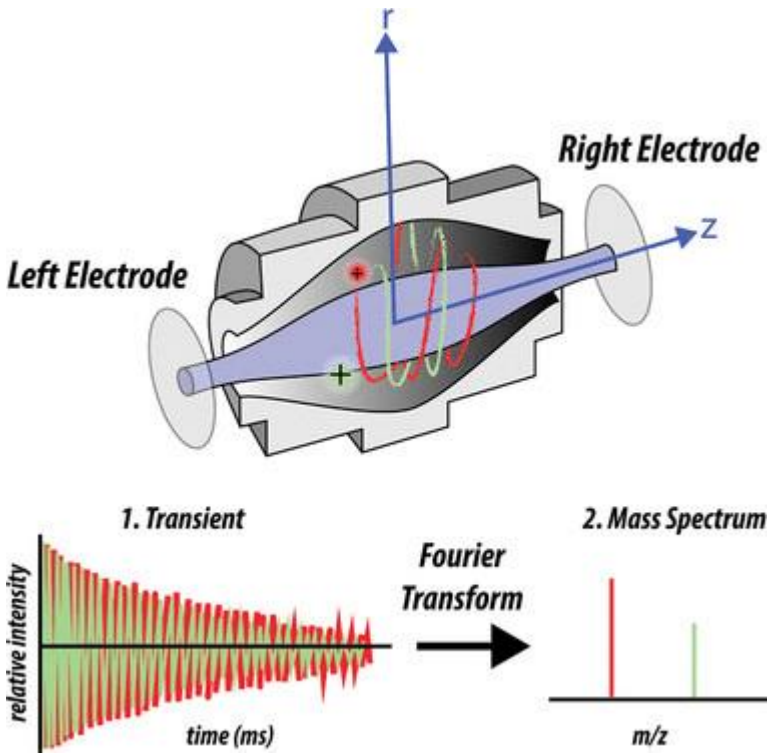
Fig. 2 Protein distributions in the human primary visual cortex determined by MALDI-MSI. **a** High-resolution image of a Nissl-stained section. Layer I, II, III, IVa–c, V, VI and white matter are indicated. **b** Nissl-stained sections from three different post-mortem brains. **c**–**e** Molecular feature images at **c** *m/z* 1101.5687, identified by MS/MS as a tryptic peptide of myelin basic protein, **d** *m/z* 1283.5998, identified as tryptic peptide of neuromodulin, **e** *m/z* 1314.6688, identified as tryptic peptide of hemoglobin β. **f** Overlay of the three peptide images highlighting their discrete distribution, MBP as red, neuromodulin as green and hemoglobin ß as blue. Spectra were recorded in positive ion mode at 100-µm lateral resolution. Black scale bar in panel **b**: 5 mm (applies horizontally to all images of the corresponding specimen). Color scales: Peptide ion intensity in arbitrary units (applies vertically to all images of the corresponding peptide)

adduct of ceramide (Cer)_m40:0, was enhanced along WM, but absent or reduced to non- detectable amounts in layer IVb (Fig. 5g). In contrast, the ion at *m/z* 768.5879, the protonated ion of PC_O-34:0, was abundant in WM including layer IVb (Fig. 5f). In summary, we were able to identify lipids specifically accumulating in different cortical layers and even sublayers of V1 or the WM.

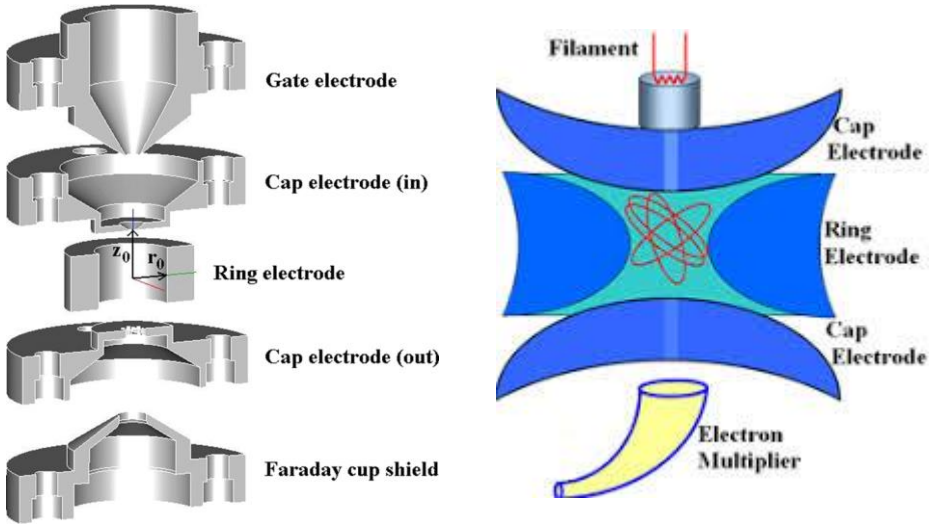
To clarify if and how lipids differed between V1 and neighboring area V2, a tissue section containing the border between V1 and V2 was measured with the highest spatial resolution possible of 30 µm. Two lipid ions, the potassium

MALDI-Orbitrap MS

Orbitrap mass analyzer

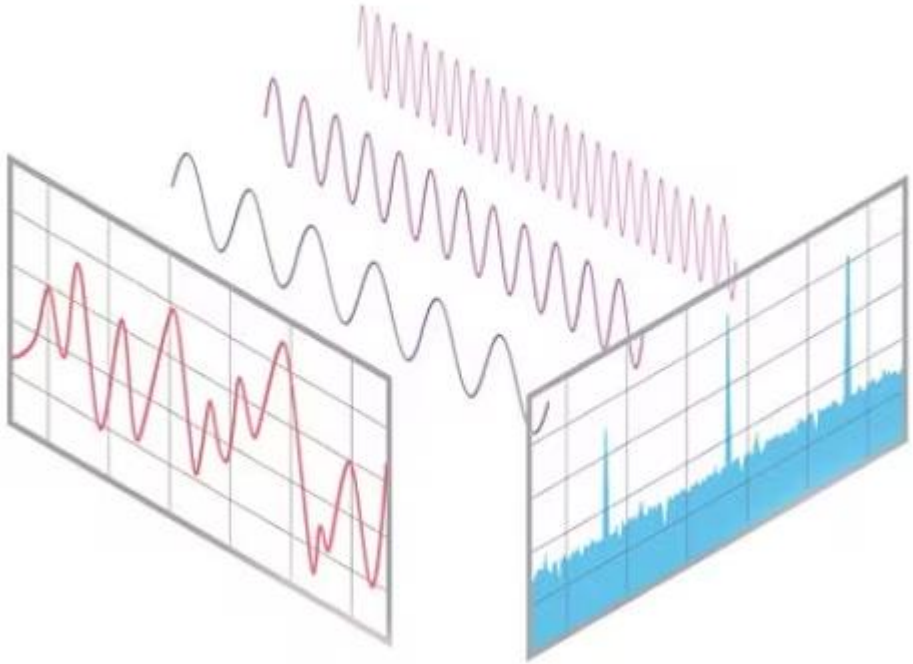


Savaryn JP et al. Proteomics 2016;16:2435.

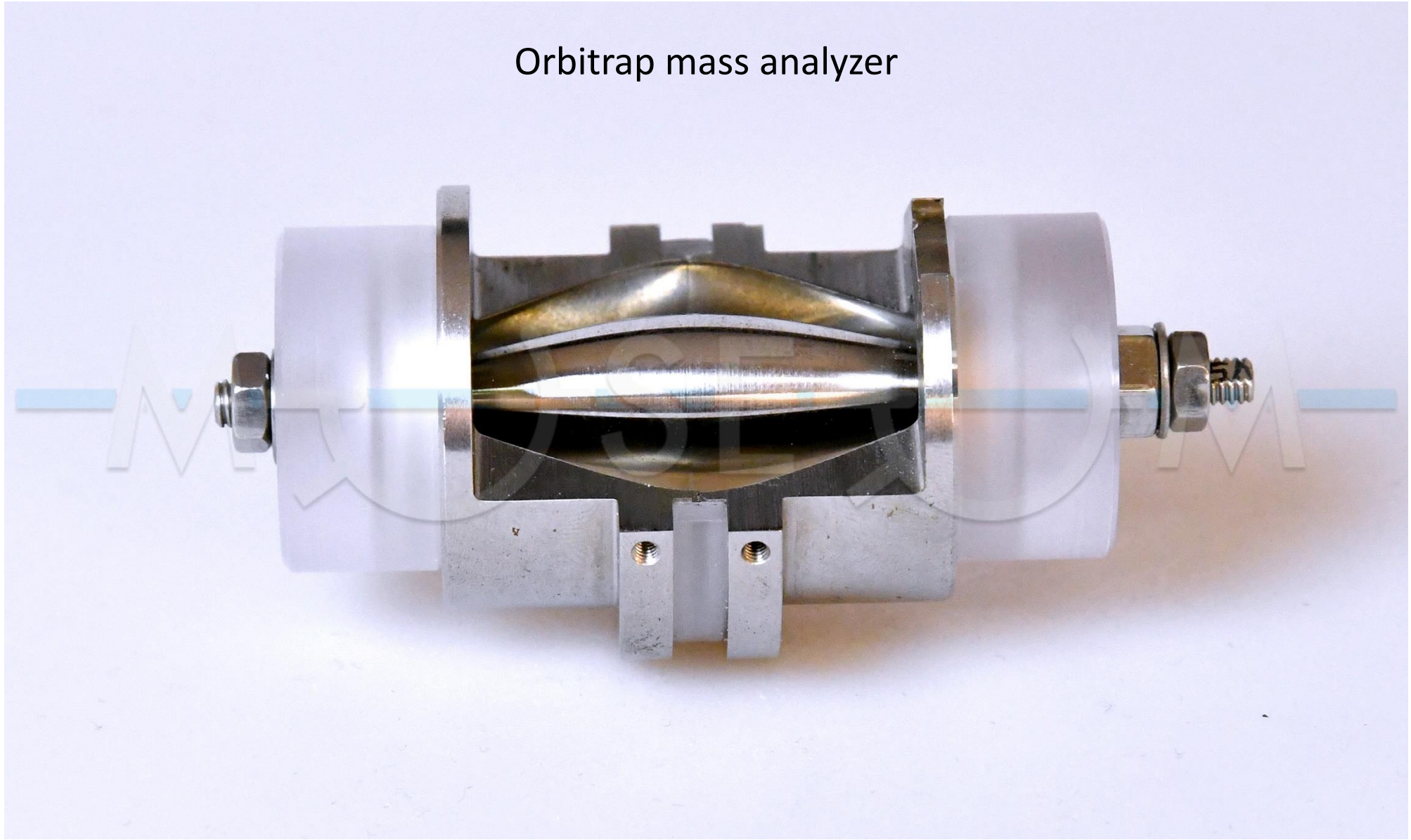


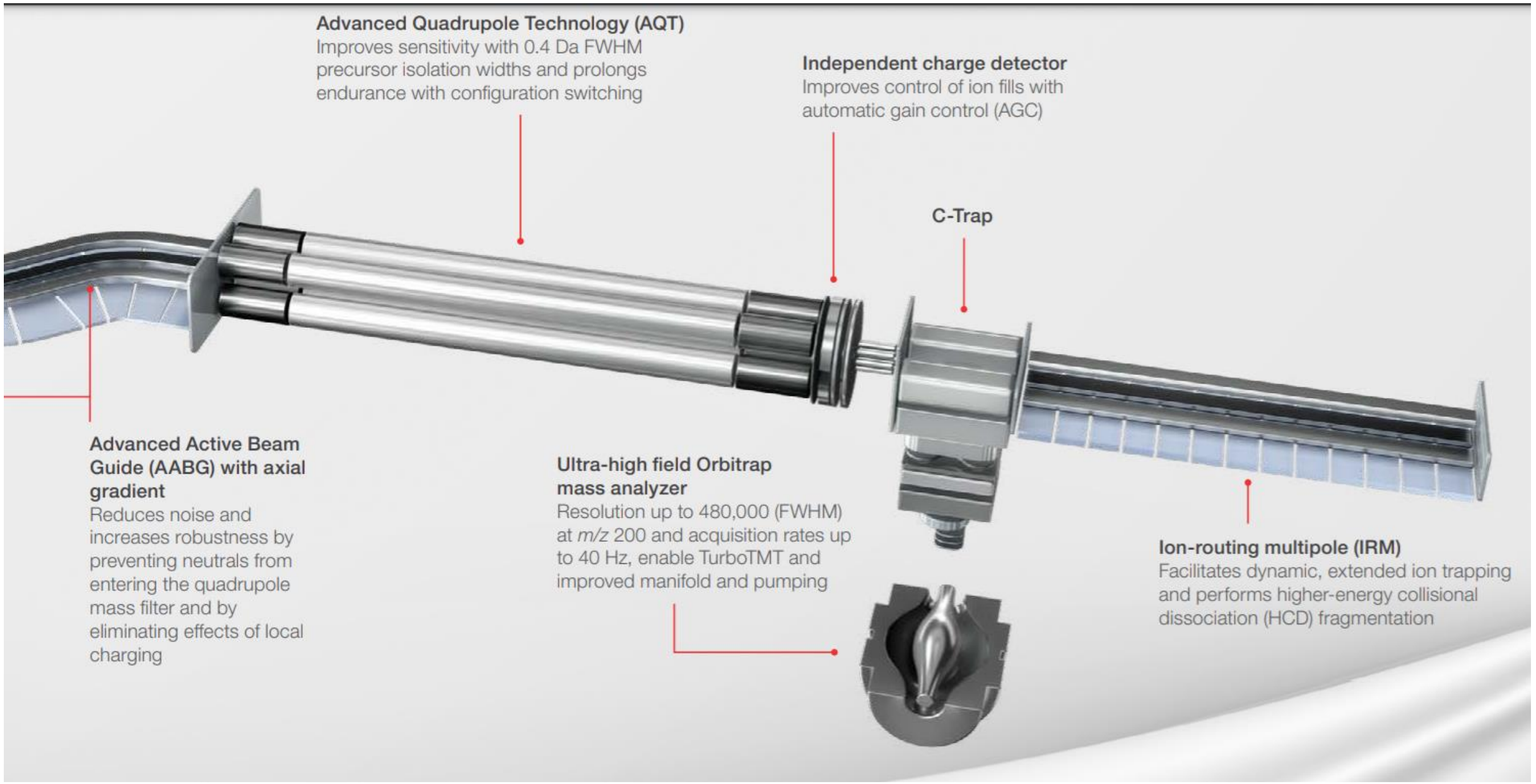
Ion trap mass analyzer

Concept of the Fourier transformation of time series data



Orbitrap mass analyzer





https://assets.thermofisher.com/TFS-Assets/CMD/brochures/br-65448-ms-orbitrap-exploris-480-br65448-en.pdf

https://www.youtube.com/watch?v=RsFsaCkVqxM