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National Food Safety Authority

Central Laboratory of
Residue Analysis of Pesticides and
Heavy Metals in Food (QCAP)

Determination of Dioxins and Furans – Methodological Developments

Dr. Ashraf Sami

Deputy Head of POPs section
Central Lab of Residue Analysis of Pesticides and Heavy Metals in Food (QCAP)
&
Director General for Food Export Control
National Food Safety Authority (NFSA)

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Stockholm Convention on Persistent Organic Pollutants (POPs)

Objective: Protect human health and the environment from POPs

- Adopted in Stockholm in May 2001
- Entered into force on 17 May 2004
- Now ratified by over 140 countries

UNECE criteria for identification of POPs

Property	Criteria for definition as POP
Persistence	♦ Atmospheric half life >2 months ♦ Half-life in water >2 months ♦ Half-life in soil >6 months ♦ Half-life in sediments >6 months
Bio-accumulation	♦ Log Kow >5 ♦ Bio-concentration factor >5000
Adverse effects	♦ Potential toxic to human and/or environment
Potential for LRAT	♦ Vapour pressure <1000 Pa

12 Stockholm Convention POPs

	Chemical	Pesticide	Industrial chemical	By-product
1	Aldrin	+		
2	Chlordane	+		
3	DDT	+		
4	Dieldrin	+		
5	Endrin	+		
6	Heptachlor	+		
7	Mirex	+		
8	Toxaphene	+		
9	Hexachlorbenzene	+	+	+
10	PCB		+	+
11	PCDD			+
12	PCDF			+

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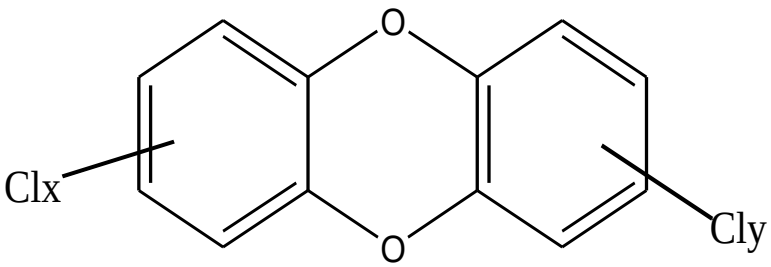
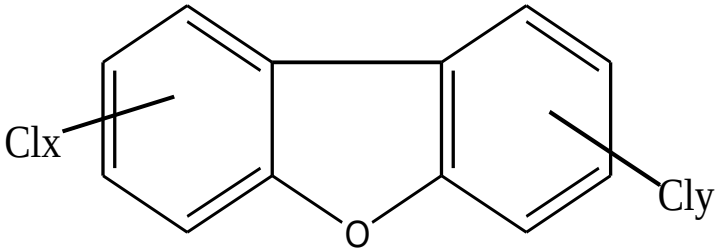
Dioxins
Chemical structures

- The term "dioxins" denotes a family of chemical compounds, known as polychlorinated dibenzo-para-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs)



Dibenzodioxin
PCDD



Dibenzofuran
PCDF

Dioxins: Congeners

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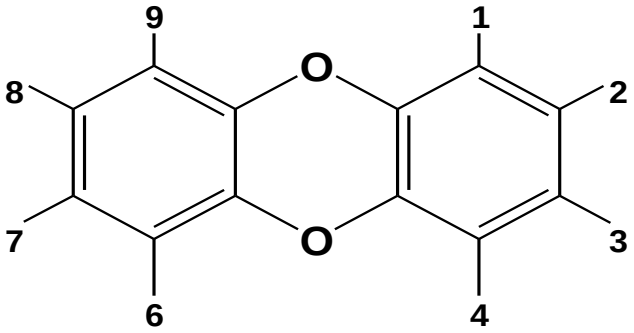
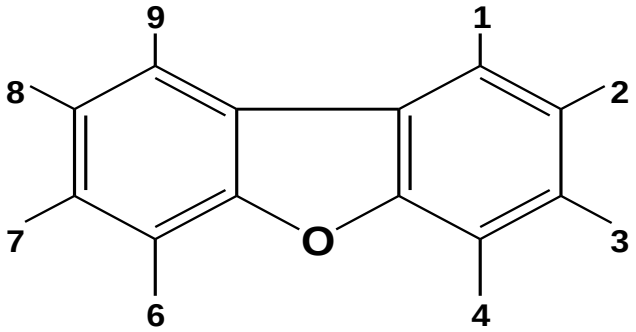
Congener: a member of the same class, or group.

Each individual PCDD or PCDF is termed a congener.

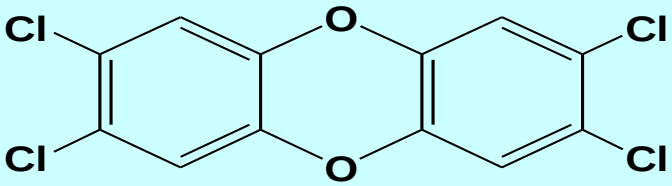
There are 75 PCDD congeners and 135 PCDF congeners.



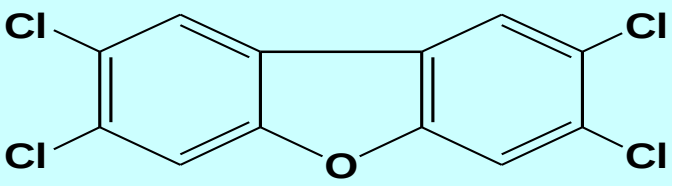
Dibenzodioxin
PCDD



Dibenzofuran
PCDF



2378-TCDD



2378-TCDF

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PCDDs

1. 2,3,7,8-TCDD
2. 1,2,3,7,8-PCDD
3. 1,2,3,4,7,8-HxCDD
4. 1,2,3,6,7,8-HxCDD
5. 1,2,3,7,8,9-HxCDD
6. 1,2,3,4,6,7,8-HpCDD
7. 1,2,3,4,6,7,8,9-OCDD

PCDFs

1. 2,3,7,8-TCDF
2. 1,2,3,7,8-PCDF
3. 2,3,4,7,8-PCDF
4. 1,2,3,4,7,8-HxCDF
5. 1,2,3,6,7,8-HxCDF
6. 2,3,4,6,7,8-HxCDF
7. 1,2,3,7,8,9-HXCDF
8. 1,2,3,4,6,7,8-HpCDF
9. 1,2,3,4,7,8,9-HpCDF
10. 1,2,3,4,6,7,8,9-OCDF

Toxicity of Dioxins

- Extraordinarily toxic to lab animals (LD_{50} in male guinea pigs is only 0.6 μg/kg)
  - Birth defects
  - Cancer
  - Skin disorders
  - Liver damage
  - Suppression of the immune systems
- Variation in toxicity among species is large.
  - Male guinea pig, oral, LD_{50} is 0.6 μg/kg.
  - Hamster, intraperitoneum, LD_{50} is 3000 μg/kg.
- Risk to humans is less clear

Biochemistry of dioxin toxicity

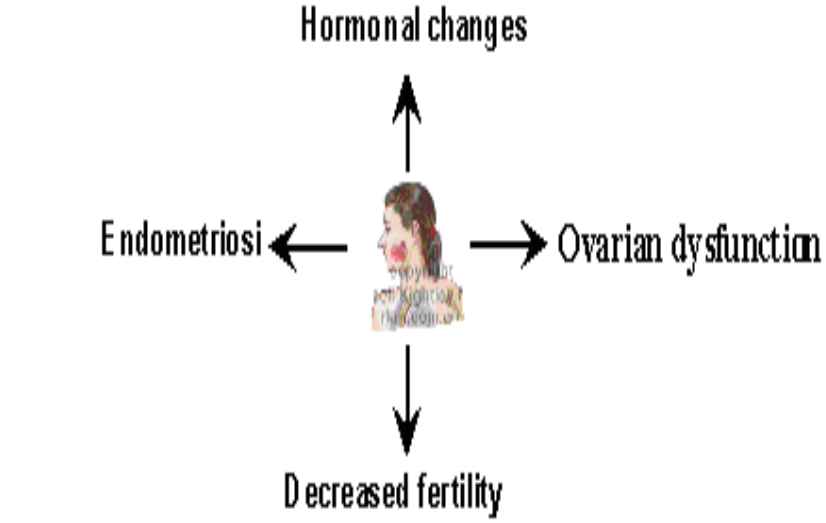
- PCDD/Fs are planar aromatic molecules.
- The planar structure allows them to bind to Ah (Aryl hydrocarbon) receptor protein that is present in all animal species.
- The Ah receptor interacts with the cell's DNA.
- Dioxin toxicity is roughly proportional to the strength of binding to the Ah receptor.
- This explains that 2378-TCDD is the most toxic one.

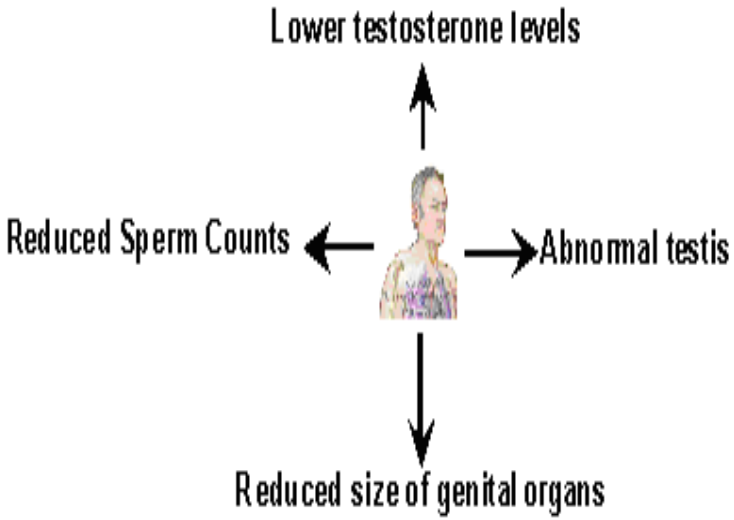
Human risk of dioxin exposure

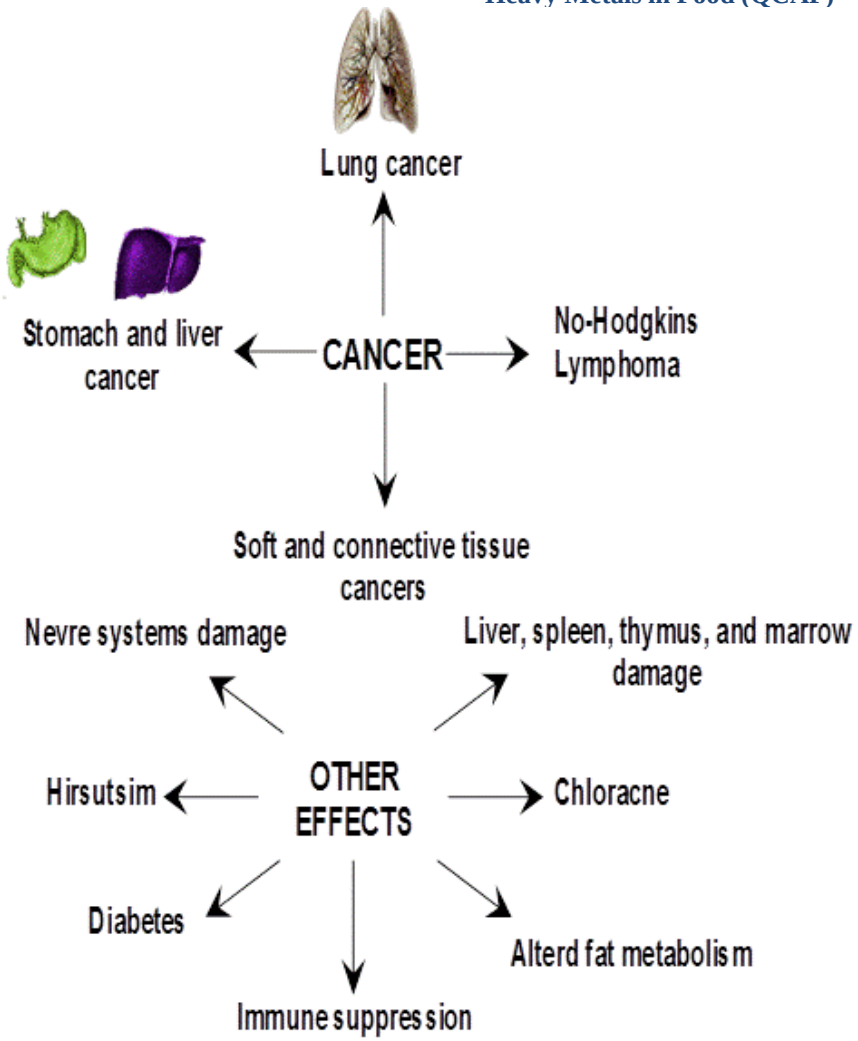
- USEPA concludes that dioxins likely increase cancer incidence.
- WHO classifies 2378-TCDD as a probable human carcinogen.
- At high levels, PCDDs cause chloracne, a painful skin inflammation.

Sources for dioxin exposure

- Risk from breathing dioxin-laden air is minimal.
- The main exposure route for human is dietary (meat, dairy products, and fish). (Due to bioaccumulation of dioxins)
- Absorption of dioxins by infant through mothers' milk is efficient.


Hormonal changes
Endometriosi
Ovarian dysfunction
Decreased fertility


Lower testosterone levels
Reduced Sperm Counts
Abnormal testis
Reduced size of genital organs


Lung cancer
Stomach and liver cancer
CANCER
No-Hodgkins Lymphoma
Soft and connective tissue cancers
Nevre systems damage
Liver, spleen, thymus, and marrow damage
Hirsutsim
OTHER EFFECTS
Chloracne
Diabetes
Alterd fat metabolism
Immune suppression

Formation of dioxins

- Combustion
  - In any situation where Cl, C, H, and O come into contact with heat, PCDD/Fs could be formed as trace by-products.
  - Dioxin emission correlates with the Cl content of the combustion feed.
- Paper pulp bleaching with chlorine
  - Chlorine is used to bleach paper pulp.
  - PCDDs are formed probably through chlorination of the phenolic groups in lignin.
- Manufacture of certain chlorophenol chemicals
  - Dioxin was produced as a contaminant of the herbicide 2,4,5-T, a component of Agent Orange

- **Fire**

  Accidental fires in homes, office and in industrial sites where chlorinated materials are burnt such PVC, PCB and solvent also leads to the emission of dioxin.

- **Recycling**

  Aluminum recycling (PVC)

  Copper recovery from cable (PVC)

  Steel and car recycling (PVC)

  Burring wood which contains chlorinated preservations (PCP)

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Formation of dioxins in combustion processes

- Incomplete combustion of organic wastes in the combustion chamber leads to the formation of organic fragments which serve as organic precursors to the PCDD/F molecule.
- The waste provides a source of chlorine, and of metals. The latter are incorporated into fly ash, which carries over to the cooler (250-400^{o}C) post-combustion zone of the incineration system.
- The organic precursors adsorb onto the surface of the fly ash in the post-combustion zone, and following a complex sequence of reactions which are catalyzed by metals (primarily copper) in the fly ash, lead to the formation of PCDD/Fs along with other chlorinated trace organics.

قرار مجلس إدارة الهيئة القومية لسلامة الغذاء رقم 6 لسنة 2022 بشأن القواعد الفنية الملزمة للملوثات

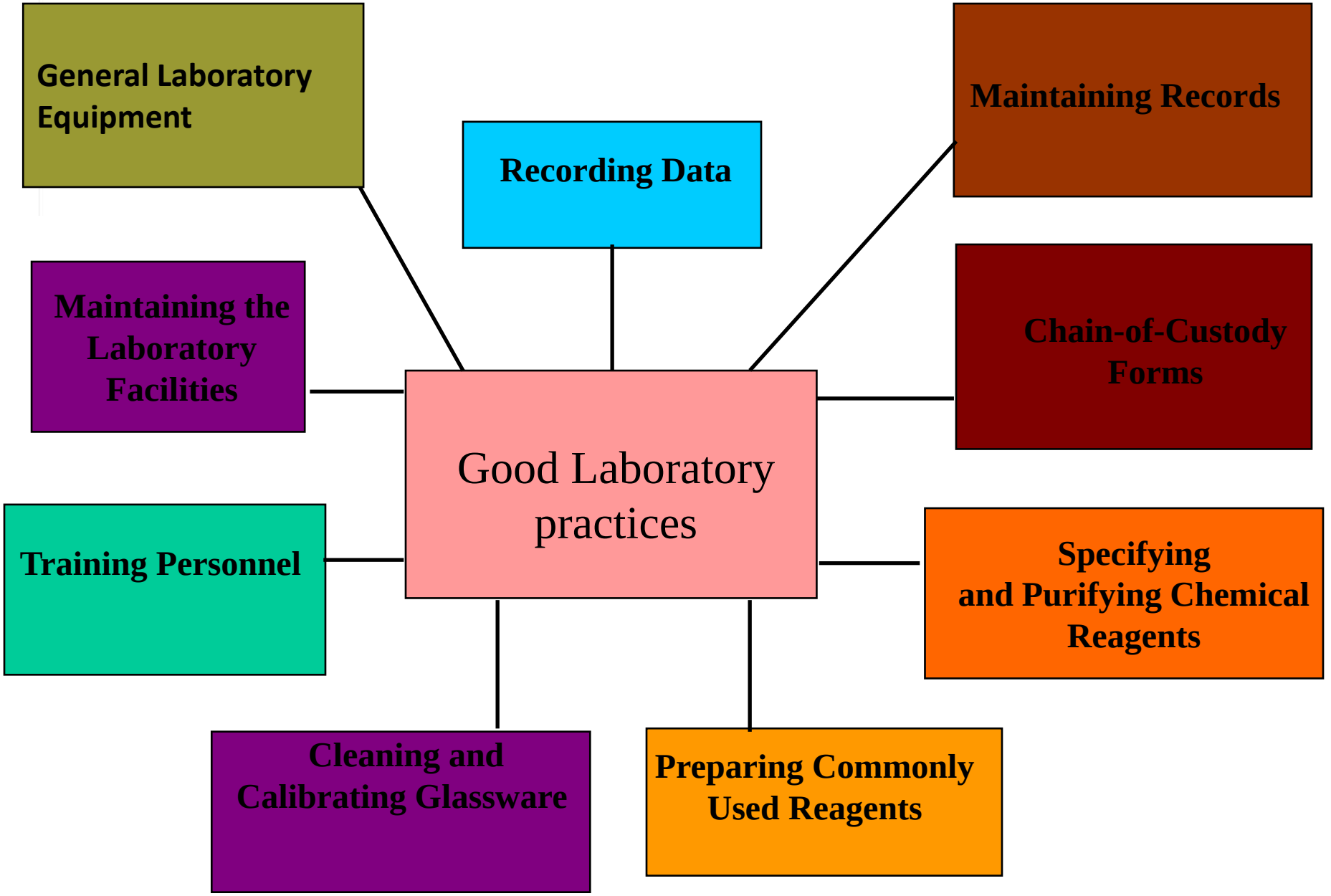
(3-E): الحدود القصوى للديوكسينات ومركبات ثنائي الفينيل متعددة الكلور Dioxins and PCBs[31]

Sum of PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 (ICES-6)[32]	Sum of dioxins and dioxin-like PCBs (WHO-PCDD/F-PCB-TEQ)[32]	Sum of dioxins (WHO-PCDD/F-TEQ)[32]	اسم المنتج (إنجليزى)	اسم المنتج (عربى)	المجموعة الغذائية (كود)
40 ng/g fat[33]	4.0 pg/g fat[33]	2.5 pg/g fat[33]	Meat and meat products of bovine animals and sheep	اللحوم ومنتجاتها من فصيلة الأبقار والأغنام	اللحوم (م 8)
40 ng/g fat[33]	3.0 pg/g fat[33]	1.75 pg/g fat[33]	Meat and meat products of poultry	اللحوم ومنتجاتها من فصيلة الدواجن	
40 ng/g fat[33]	1.25 pg/g fat[33]	1.0 pg/g fat[33]	Meat and meat products of pigs	اللحوم ومنتجاتها من فصيلة الخنازير	
3.0 ng/g wet Weight	0.50 pg/g wet Weight	0.30 pg/g wet Weight	Liver of terrestrial animals except for sheep and derived products thereof	كبدة الحيوانات الارضية باستثناء الأغنام و المنتجات المشتقة منها	الاحشاء (م 8)
3.0 ng/g wet weight	2.00 pg/g wet weight	1.25 pg/g wet weight	Liver of sheep and derived products thereof	كبدة الأغنام و المنتجات المشتقة منها	
75 ng/g wet weight	6.5 pg/g wet weight	3.5 pg/g wet weight	Muscle meat of fish and fishery products and products thereof[25][34], with the exemption of: - wild caught eel - wild caught spiny dogfish (*Squalus acanthias*)	اللحوم العضلية للأسماك ومنتجاتها والمنتجات السمكية باستثناء كل من[٢٥][٣٤]: – صيد ثعبان البحر البري – كلب البحر الشوكي الذي تم اصطياده من البرية	الأسماك ومنتجاتها (م 9)

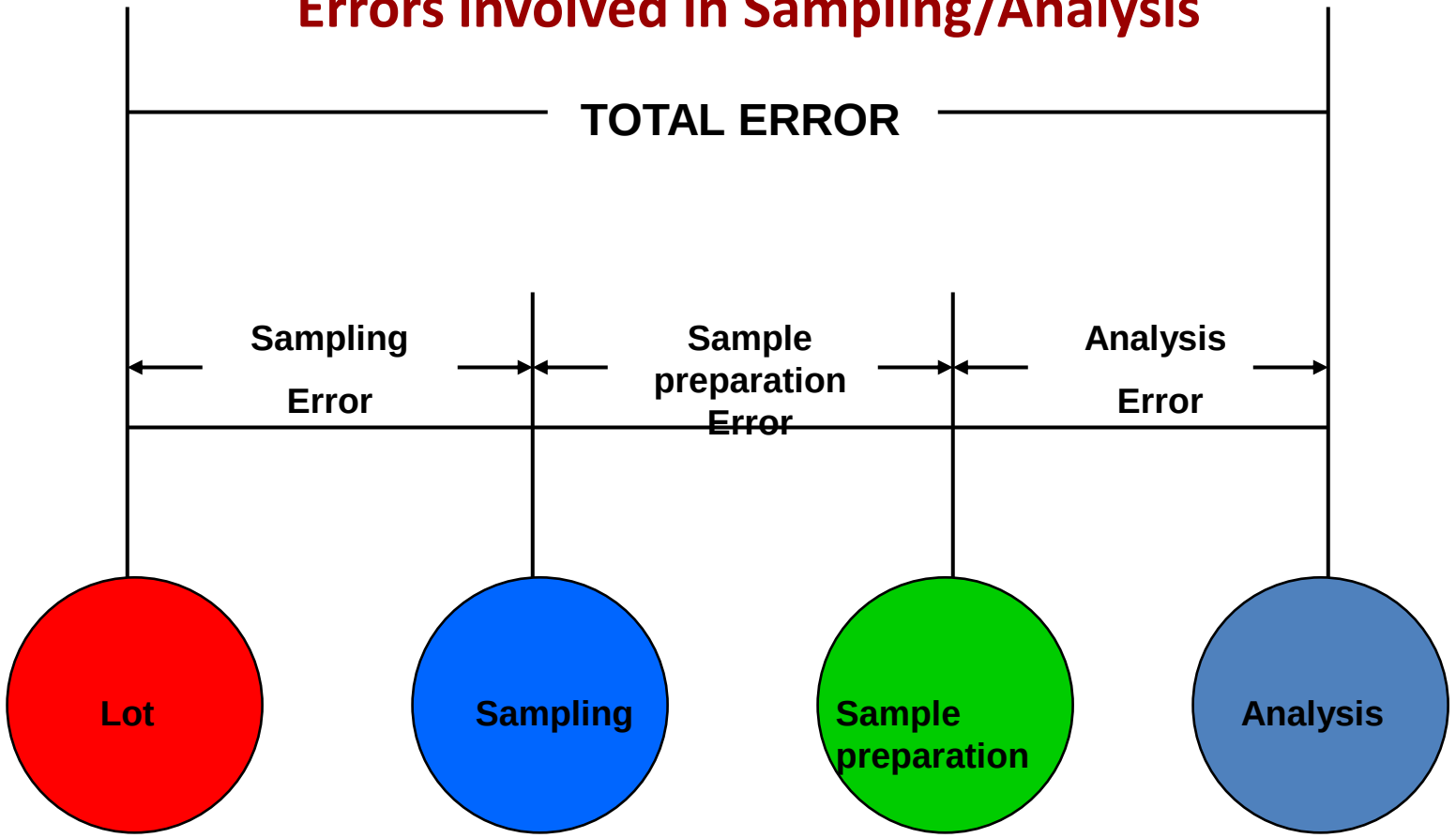
Sum of PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 (ICES-6)[32]	Sum of dioxins and dioxin-like PCBs (WHO-PCDD/F-PCB-TEQ)[32]	Sum of dioxins (WHO-PCDD/F-TEQ)[32]	اسم المنتج (إنجليزى)	اسم المنتج (عربى)	المجموعة الغذائية (كود)
			- wild caught fresh water fish, with the exception of diadromous fish species caught in fresh water - fish liver and derived products - marine oils The maximum level for crustaceans applies to muscle meat from appendages and abdomen. [21] In case of crabs and crab-like crustaceans (Brachyura and Anomura) it applies to muscle meat from appendages.	– أسماك المياه العذبة التي يتم صيدها برية، باستثناء أنواع الأسماك ثنائية الكروم التي يتم اصطيادها في المياه العذبة – كبد السمك ومنتجاته – زيوت الأسماك البحرية يسري الحد الأقصى للحوم العضلات من الانزع[٢١] والبطن واللحوم العضلات من الانزع في حالة كل من السرطانات والقشريات مثل سرطان البحر Brachyura و Anomura	
125 ng/g wet weight	6.5 pg/g wet weight	3.5 pg/g wet weight	Muscle meat of wild-caught freshwater fish, except diadromous fish species caught in freshwater, and products thereof[25]	اللحوم العضلية لأسماك المياه العذبة المصطادة ومنتجاتها[٢٥]	

المجموعة الغذائية (كود)	اسم المنتج (عربى)	اسم المنتج (إنجليزى)	Sum of dioxins (WHO-PCDD/F-TEQ)[32]	Sum of dioxins and dioxin-like PCBs (WHO-PCDD/F-PCB-TEQ)[32]	Sum of PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 (ICES-6)[32]
	لحوم عضلات كلب البحر الشوكي (*Squalus acanthias*) ومنتجاته[٢٥]	Muscle meat of wild-caught spiny dogfish (*Squalus acanthias*) and products thereof[25]	3.5 pg/g wet weight	6.5 pg/g wet weight	200 ng/g wet weight
	اللحوم العضلية لأسماك الأيل (eel) المصطادة ومنتجاتها (Anguilla)	Muscle meat of wild-caught eel (*Anguilla anguilla*) and products thereof	3.5 pg/g wet weight	10.0 pg/g wet weight	300 ng/g wet weight
	كبدة الأسماك والمنتجات المشتقة منها باستثناء الزيوت البحرية المشار اليها في النقطة التالية	Fish liver and derived products thereof except marine oils referred to in the next point	-	20.0 pg/g wet weight[35]	200 ng/g wet weight[35]
	الزيوت البحرية (زيت جسم السمك وزيت كبدة السمك وزيوت الكائنات البحرية الأخرى المعدة للاستهلاك الآدمي)	Marine oils (fish body oil, fish liver oil, and oils of other marine organisms intended for human consumption)	1.75 pg/g fat	6.0 pg/g fat	200 ng/g fat
الالبان (م ١)	اللبن الخام ومنتجات الالبان بما فيها دهن الزبد	Raw milk and dairy products, including butterfat	2.5 pg/g fat[33]	5.5 pg/g fat[33]	40 ng/g fat[33]

Sum of PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 (ICES-6)[32]	Sum of dioxins and dioxin-like PCBs (WHO-PCDD/F-PCB-TEQ)[32]	Sum of dioxins (WHO-PCDD/F-TEQ)[32]	اسم المنتج (إنجليزى)	اسم المنتج (عربى)	المجموعة الغذائية (كود)
40 ng/g fat[33]	5.0 pg/g fat[33]	2.5 pg/g fat[33]	Hen eggs and egg products	بيض الدجاج ومنتجاته	البيض (م 10)
40 ng/g fat	4.0 pg/g fat	2.5 pg/g fat	Fat of bovine animals and sheep	الدهن من الأبقار والأغنام	الزيوت والدهون (م 2)
40 ng/g fat	3.0 pg/g fat	1.75 pg/g fat	Fat of poultry	الدهن من الدواجن	
40 ng/g fat	1.25 pg/g fat	1.0 pg/g fat	Fat of pigs	الدهن من الخنازير	
40 ng/g fat	2.50 pg/g fat	1.5 pg/g fat	Mixed animal fats	خليط من الدهون الحيوانية	
40 ng/g fat	1.25 pg/g fat	0.75 pg/g fat	Vegetable oils and fats	الزيوت والدهون النباتية	
1.0 ng/g wet weigh	0.2 pg/g wet weight	0.1 pg/g wet weight	Foods for infants and young children[5]	أغذية الرضع وصغار الأطفال[٥]	الأغذية الخاصة (م 13)


General Laboratory Equipment
Recording Data
Maintaining Records
Maintaining the Laboratory Facilities
Good Laboratory practices
Chain-of-Custody Forms
Training Personnel
Specifying and Purifying Chemical Reagents
Cleaning and Calibrating Glassware
Preparing Commonly Used Reagents

Errors involved in Sampling/Analysis



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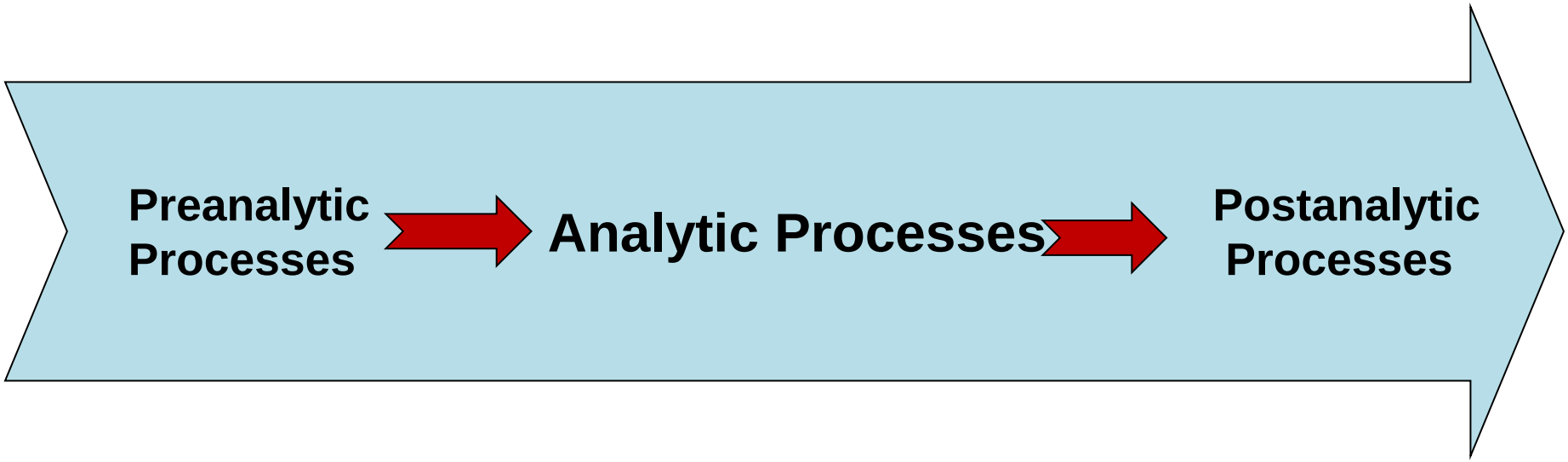
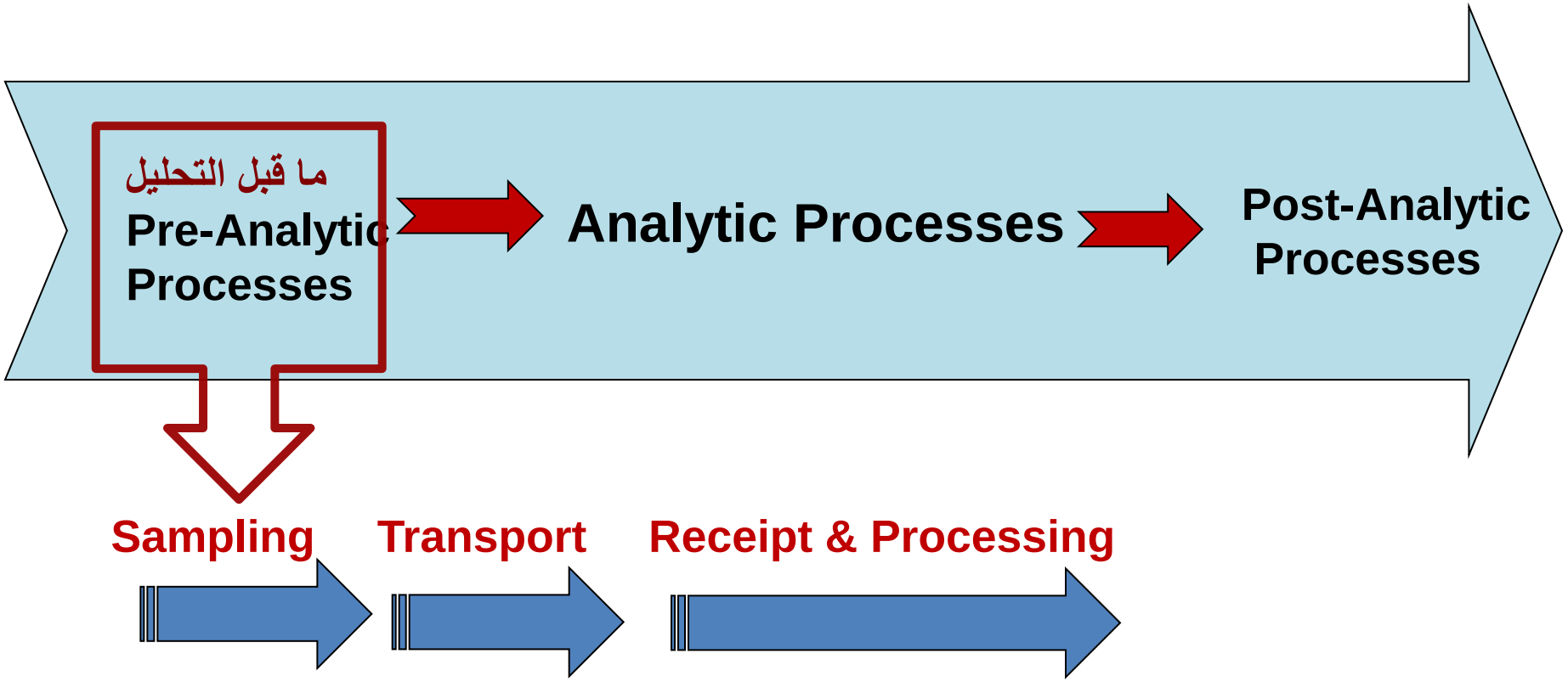
Overview on Analytical Processes

As for any other chemical analysis, Dioxins analysis includes the following three main steps



Overview on Analytical Processes



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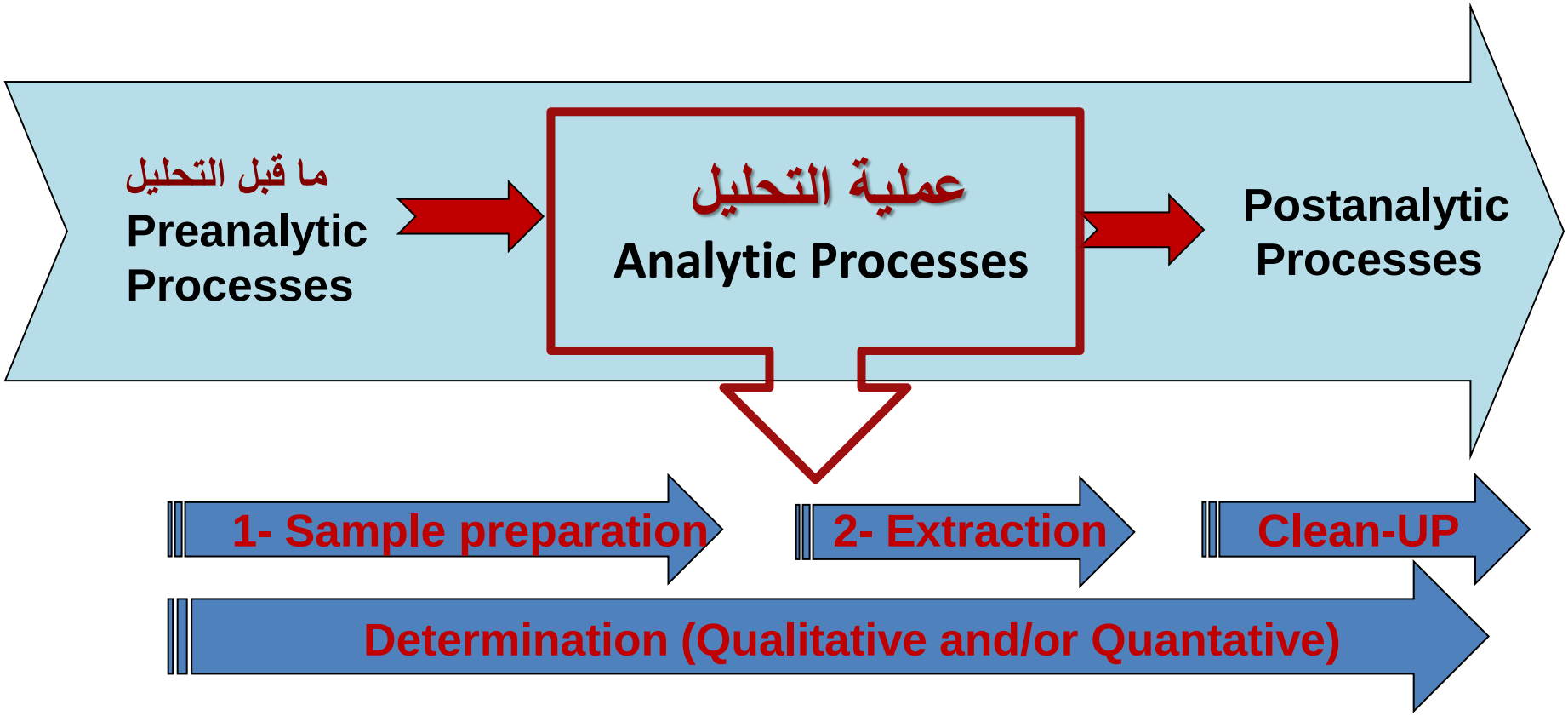
Overview on Analytical Processes



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Samples preparation

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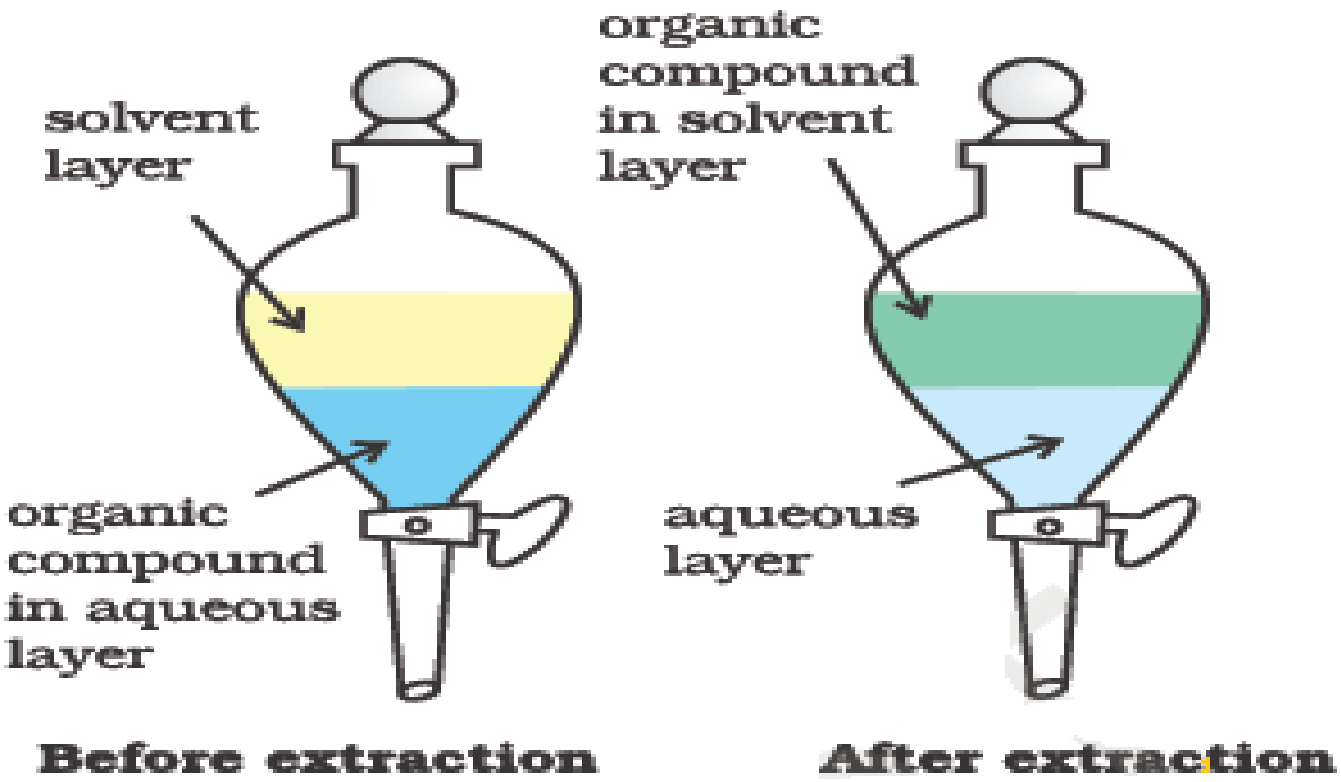
Extraction

Extracting compounds of dioxins, furans and dioxin like-PCBs from samples collected by using the device extraction. **Addition of C13-labeled standard solution** (Dioxins & PCBs) prior to extract for all samples.

Liquid-Liquid Extraction



Differential Extraction

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Automated Soxhelt Extractions

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Accelerated Solvent Extractor

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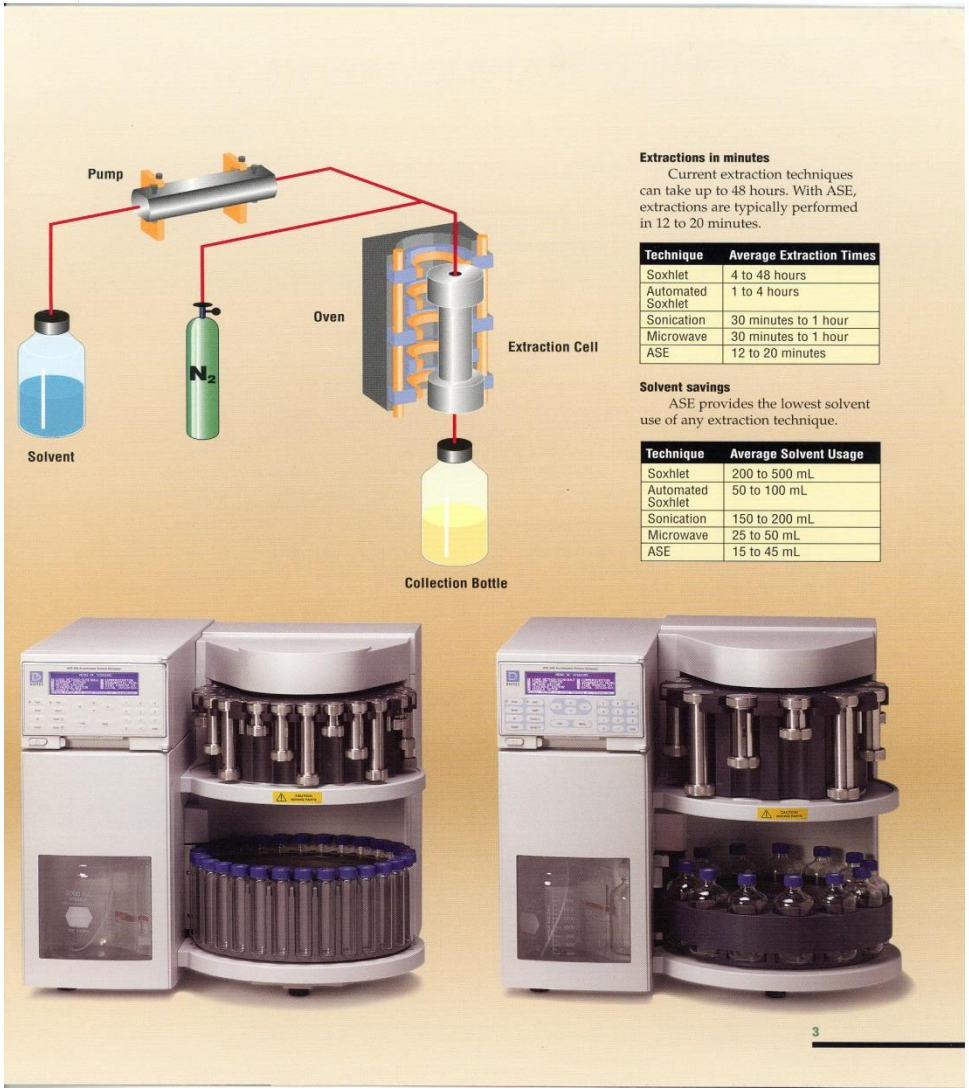


Extractions in minutes

Current extraction techniques can take up to 48 hours. With ASE, extractions are typically performed in 12 to 20 minutes.

Technique	Average Extraction Times
Soxhlet	4 to 48 hours
Automated Soxhlet	1 to 4 hours
Sonication	30 minutes to 1 hour
Microwave	30 minutes to 1 hour
ASE	12 to 20 minutes

Solvent savings

ASE provides the lowest solvent use of any extraction technique.

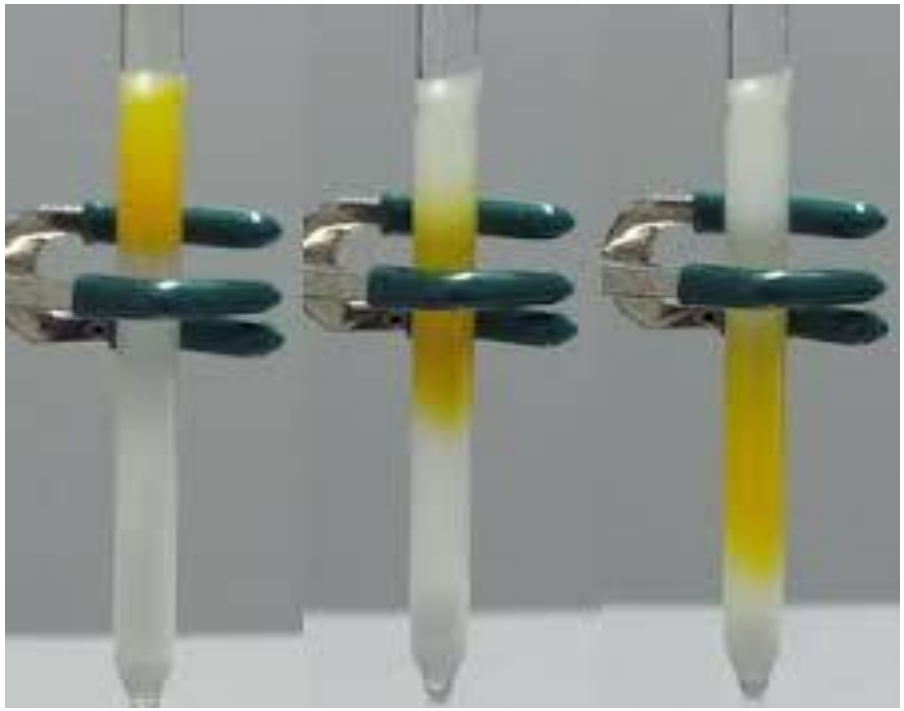
Technique	Average Solvent Usage
Soxhlet	200 to 500 mL
Automated Soxhlet	50 to 100 mL
Sonication	150 to 200 mL
Microwave	25 to 50 mL
ASE	15 to 45 mL

Clean-up

Fractionation column of dioxins & PCBs

Used for removal of high molecular weight interferences (fat & colors)

Used for removal of non-polar and polar interferences compounds.



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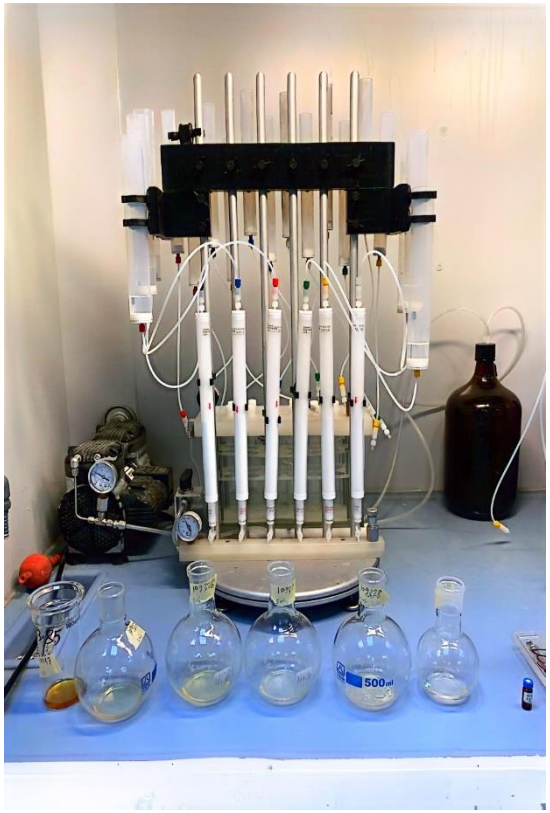
Automated Clean-up



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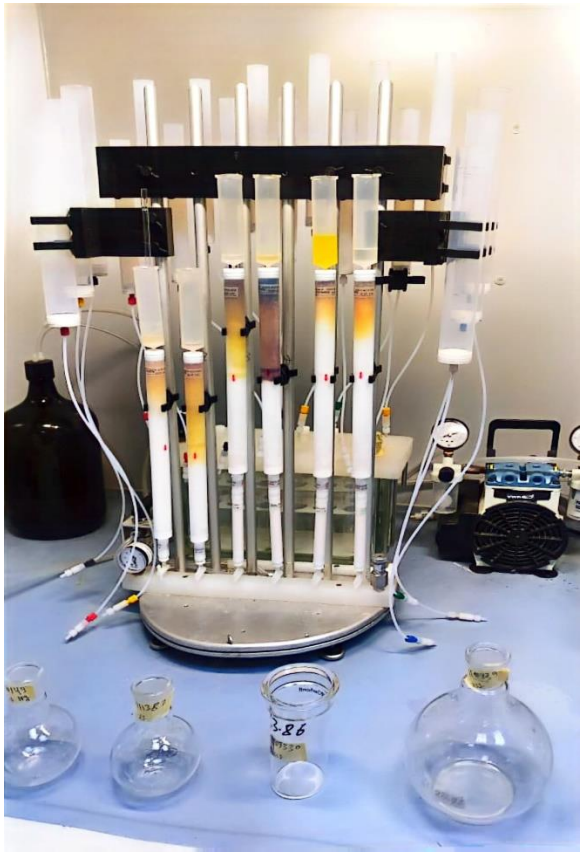

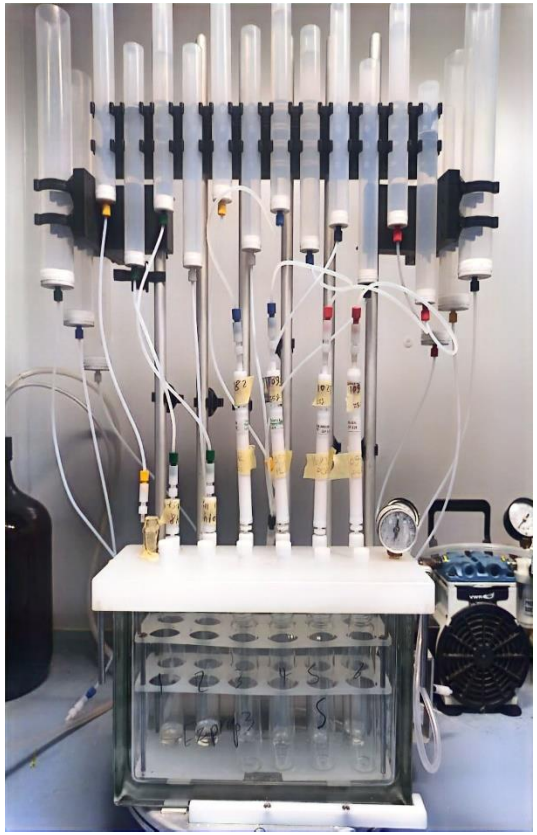

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Fluid Management System

Central Laboratory of
Residue Analysis of Pesticides and
Heavy Metals in Food (QCAP)

الهيئة القومية لسلامة الغذاء
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HRGCMS

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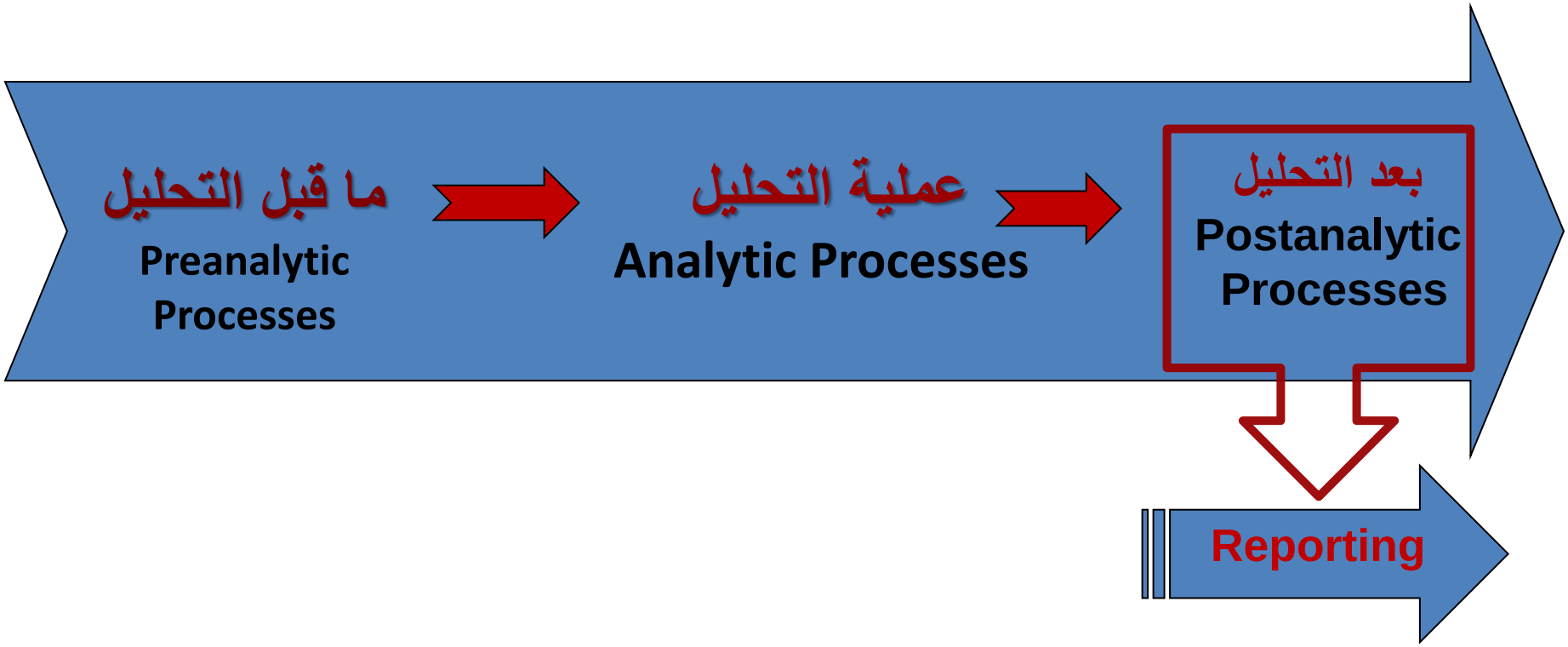
HRGCMS


Agilent Technologies 7693
Agilent Technologies 7010 GC/MS Triple Quad
DELL
Orbitrap

Overview on Analytical Processes



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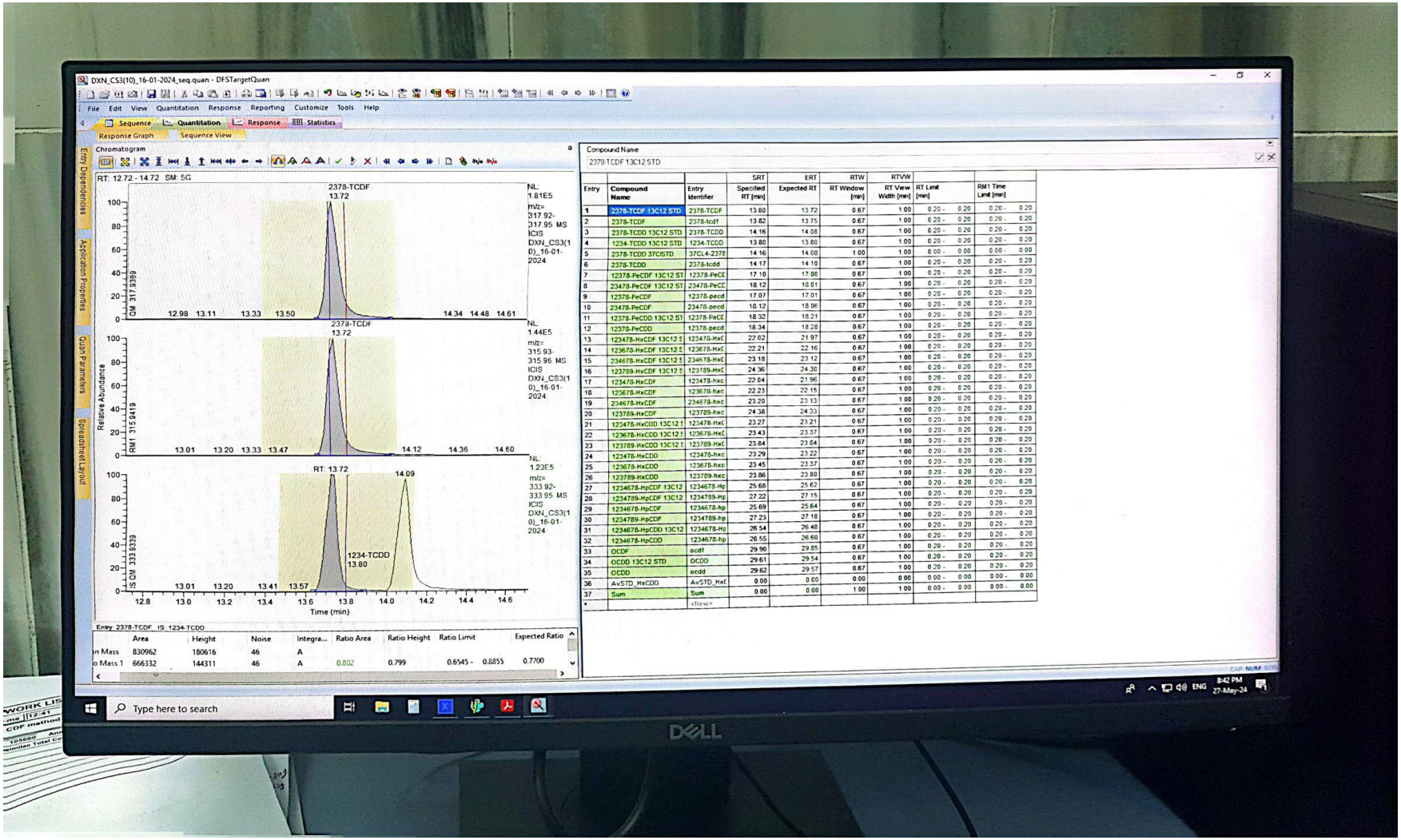

Quality Control of Analytical Method

- Spike Sample
- Control Chart
- Blank Sample
- Repeatability
- PT
- CRM
- Calibration
- Swap sample
- Blind sample
- Quality of solvent and reagents
- Avoid contamination

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THANK YOU