Determination of cannabinoids in food and novel food

MERIEUX
NutriSciences

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Some facts about Cannabis sativa L.

- Very old cultivated plant

- Used for paper production since 2nd millennium B.C.

- **Cannabinoids** (> 100 substances known so far): **naturally occurring compounds** found in the Cannabis sativa plant

- All plant parts except seeds contain cannabinoids

- 2 varieties: **drug hemp** and **fibre hemp** (legal in EU if <0.2% THC)

- **Glandular hairs**
  - On the entire surface of the plant except seeds and roots
  - Secrete a resin (resin consists of 80-90% cannabinoids, the rest is made up of essential oils, phenols, terpenes, waxes)
  - Cannabinoid content correlates with the number of glandular hairs

Some facts about Cannabis sativa L.

- Cannabis sativa L. gains significance in food production
- **Hemp seeds** contain **all 8 essential amino acids** in ideal proportions for human nutrition
- **Hemp oil** has the highest content of unsaturated fatty acids of all vegetable oils.
- **Increasingly products** which contain **highly concentrated the cannabinoid cannabidiol (CBD)** are offered in internet shops and special stores.
- **Health benefits** are promoted for cannabidiol.
- **CBD extracts**, any products to which they are added, as well as synthetically obtained cannabinoids are considered **Novel Foods**
- 50 Novel Food applications were under review by the European Commission in December 2020
- Hemp seeds, hemp leaves as feed
- Transfer to food of animal origin
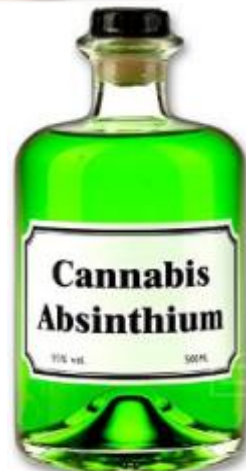
New trend: CBD stores

HEALTH BENEFITS CBD OIL

ASTHMA
CBD has potent immunosuppressive and anti-inflammatory properties

EYES
Compounds found in CBD feature neuro protection and vasodilation properties which further assist in the conservation and treatment of glaucoma

CANCER
Cannabinoids may have benefits in the Treatment of cancer-related side effects

HEART
Anti-Inflammatory, Atherosclerosis, and Anti-Ischemic

BRAIN
Anti-Anxiety, Anti-Depressant, Antioxidant, Neuroprotective

INTESTINES
Cannabidiol reduces intestine inflammation through the control of the neuroimmune system

WELL BEING
Helps to relax and to calm body and mind

SPINAL CORD INJURY
Studies have not only demonstrated CBD's pain-killing properties, but also its ability to reduce spasms and improve motor function in SCI patients

STOMACH
Antiemetic, Appetite Control

BONE STRUCTURE
CBD works by improving bone density and reducing the occurence of bone diseases. it strengths the collegen "bridge" that forms at the site of the break which then hardens with the new bone

SUPERNATURE
CBD

6

COMMISSION RECOMMENDATION (EU) 2016/2115

Monitoring of

- **Δ9-Tetrahydrocannabinol (Δ9-THC)** in *food of animal origin*

- **Δ9-Tetrahydrocannabinol (Δ9-THC), Delta-9-tetrahydrocannabinolic acids** and other cannabinoids (e.g. **Δ8-THC, cannabinol, cannabidiol** and **Delta-9-tetrahydrocannabivarin**) in *hemp-derived foods and foods containing hemp or hemp-derived ingredients*.

Method

- Preferably **chromatographic separation** coupled with mass spectrometry (LC-MS or GC-MS) following an **appropriate clean-up step** (liquid-liquid (LLE) or solid phase extraction (SPE))
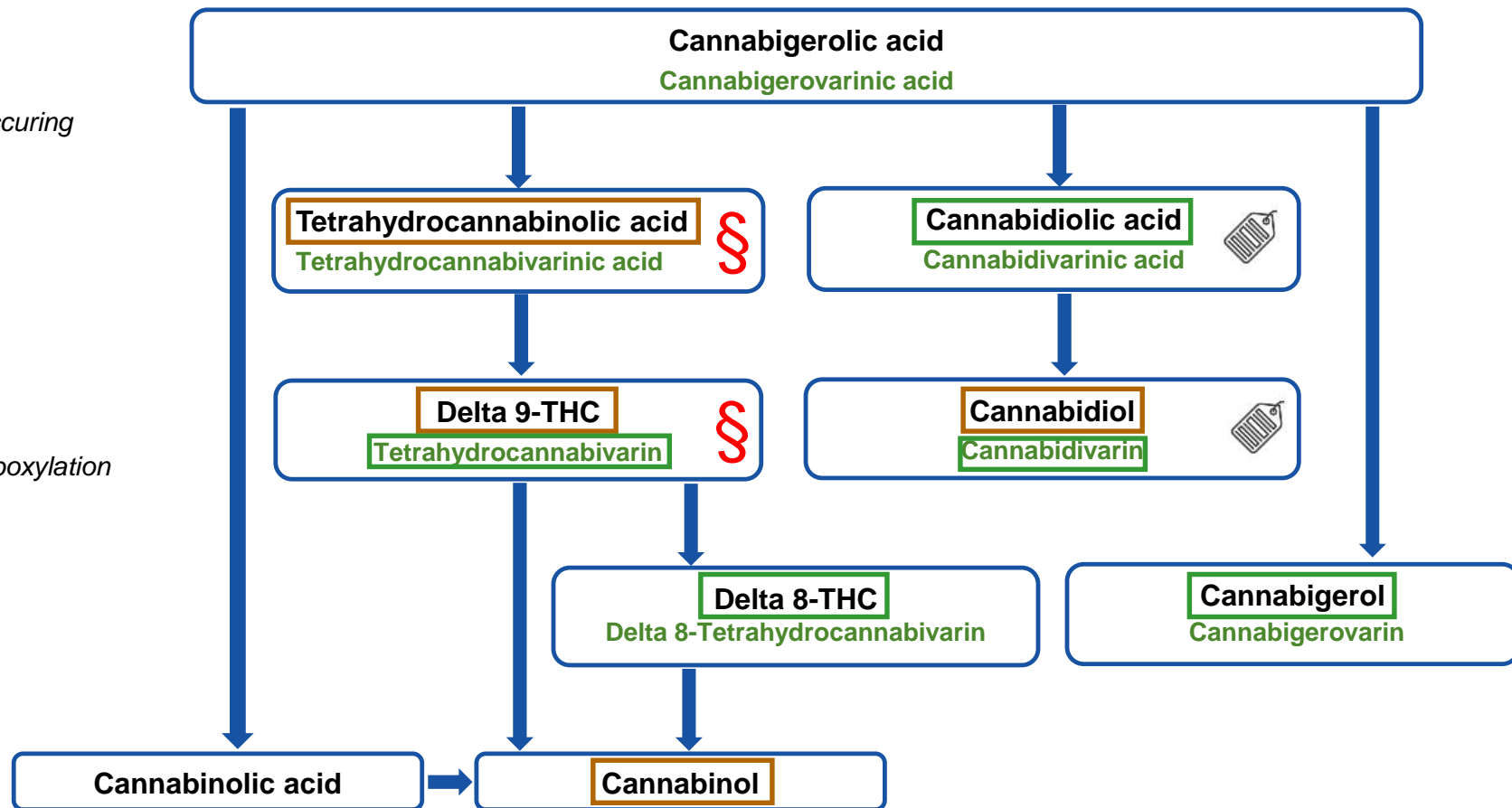- **Separate determination** of Δ^9-THC, its precursors and other cannabinoids

Cannabinoids

Raw
naturally occuring

Heated
after decarboxylation

Aged
O_2 / t

Cannabigerolic acid		
Cannabigerovarinic acid		

Tetrahydrocannabinolic acid
Tetrahydrocannabivarinic acid §

Cannabidiolic acid
Cannabidivarinic acid

Delta 9-THC
Tetrahydrocannabivarin §

Cannabidiol
Cannabidivarin

Delta 8-THC
Delta 8-Tetrahydrocannabivarin

Cannabigerol
Cannabigerovarin

Cannabinolic acid → **Cannabinol**

- extended method
  - quantification via *procedural matrix calibration* or *standard addition*

- basic method
  - quantification via labeled standard

Analytes

		Psychoactive	
Δ9-Tetrahydrocannabinol (THC)		Yes	- Regulated - Hashish: resin (THC content 5-20%) - Marijuana: resin-bonded inflorescence shoots (THC content: 0.5-7%) - Very low THC content inside the seeds (<2 mg / kg for drug hemp, <0.5 mg / kg for fiber hemp) which only result from contamination
Δ8-Tetrahydrocannabinol		Yes	Isomer of Δ9-Tetrahydrocannabinol
Cannabidiol (CBD)		Slightly	- Health benefits promoted - Currently many products which contain highly concentrated cannabidiol (CBD)
Δ9-Tetrahydrocannabinolic Acid (THCA)		No	- Precursor of THC - At higher temperatures (processing of food, smoking, but also gas chromatography) decarboxylation to THC - In fresh plant material approx. 90% of the THC as THCA-A

Analytes

		Psychoactive	
Cannabidiolic Acid (CBDA)		No	▪ Precursor of cannabidiol
Cannabigerol (CBG)		No	
Tetrahydrocannabivarin (THCV)		No	
Cannabidivarin (CBDV)		No	
Cannabinol (CBN)		No	▪ Oxidation product of THC *Characterization of phenotypes:* [1] *<u>THC + CBN</u> Drug hemp >1* * CBD Fibre hemp <1*

[1]   Anal. Bioanal. Chem. **378**, 183-189 (2004)

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Some cannabinoids have **psychoactive effects**

The most important psychoactive compound is
Δ9-Tetrahydrocannabinol (THC)

- **ARfD:** 1 µg/kg bw / day

  Acute Reference Dose: estimate of the amount of a substance in food or drinking water that can be consumed over a lifetime without presenting an appreciable risk to health

- **LOAEL:** 2.5 mg / day

  Lowest Observed Adverse Effect Level: lowest dose at which there was an observed toxic or adverse effect

- Maximum THC-content of **state-subsidized fibre** hemp: 0.2 % (Regulation (EU) No 1307/2013); increased to 0.3%

- Regulation of Cannabis products within Europe is not harmonized

- In Germany (**German narcotics act**): classification of products as narcotics if THC-content is higher than 0.2 %

Limitation of THC content in food

- Oldest regulation within EU: German guidance values from 1999 (former BgVV - German Federal Institute for Consumer Health Protection):
  - Edible oils: 5000 µg/kg
  - Beverages: 5 µg/kg
  - Other food: 150 µg/kg

- Values referred to "total-THC" content (Δ9-THC + precursor Δ9-THCA-A)

- Opinion 006/2021 of BfR (German Federal Institute for Risk Assessment)
  - Guidance values are scientifically outdated
  - Recommendations:
    – Toxicological assessment on the basis of the ARfD of 1 µg/kg bw / day
    – Both analyte contents (Δ9-THC + precursor Δ9-THCA-A) should be determined

Legal requirements

Limitation of THC content in food

	🇩🇪 guidance values (OLD) THC+THCA-A [µg/kg]	🇮🇹 limits THC+THCA-A [µg/kg]	🇨🇭 limits THC [µg/kg]	🇪🇺 limits suggested THC+THCA-A [µg/kg]
Oil from seeds	5.000	5.000	20.000	7.500
Beverages	5		200	
Alcoholic drinks			5	
Other food	150			
Seeds		2.000	10.000	3.000 (incl. powder, cake, bran)
Supplements		2.000		
Bakery			2.000	
Herbal tea			200	
Pasta			2.000	
Plant-based food			1.000	

Evaluation of hemp food

Delta 9-THC or total THC content as worst case

< ARfD of 1 µg/kg bw / day

> ARfD of 1 µg/kg bw / day

> LOAEL (2.5 mg/day)

ALARA
As Low As Reasonably
Achievable

Art. 14, 2 b)
Reg. (EU) 178/2002
unsuitable for consumption

Art. 14, 2 b)
Reg. (EU) 178/2002
harmful

RASFF – Rapid Alert System for Food and Feed

54 alerts because of high THC contents within **last 10 years**

Product category	Number
Dietetic foods, food supplements, fortified foods	38
Fats and oils	3
Seeds	3
Cocoa, coffee, tea	2
Confectionery	2
Other	2
Fruits and vegetables	1
Cereals / bakery products	1
Honey	1
Prepared meals / snacks	1

RASFF

Rapid Alert System for *Food and Feed*

Complex legal evaluation

Crucial question for each product

Is my product food (according to Regulation (EU) 178/2002), drug or pharmaceutical?

Narcotic or psychotropic substances are not accepted as food, e.g.:

- Cannabis (flowering or fruiting tops, excluding seeds and leaves)
- Cannabis resin and extracts
- Tinctures of cannabis

In Germany: narcotics act

- Cannabis: narcotics
- Seeds (if not determined for not authorized cultivation)
- Fibre hemp (total THC < 0,2%) if trading only for industrial or scientific purposes which exclude misuse

CBD
European Union Court of Justice (19.11.2020):

- CBD is **not considered** to be a narcotic drug

European Commission (12/2020):

- CBD "*… can be qualified as food*"
- Single substance CBD and hemp extracts (CBD specifically enriched): rated as "novel" in the **Novel Food Catalogue**
- European Commission is examining approx. 50 applications for CBD products. This examination of the approval applications has not yet been completed.

> **Important!**
>
> **CBD extracts are classified as drugs by most food inspection offices,**
> **if Delta 9-THC is detectable**

3 Cases

1) **Narcotic**

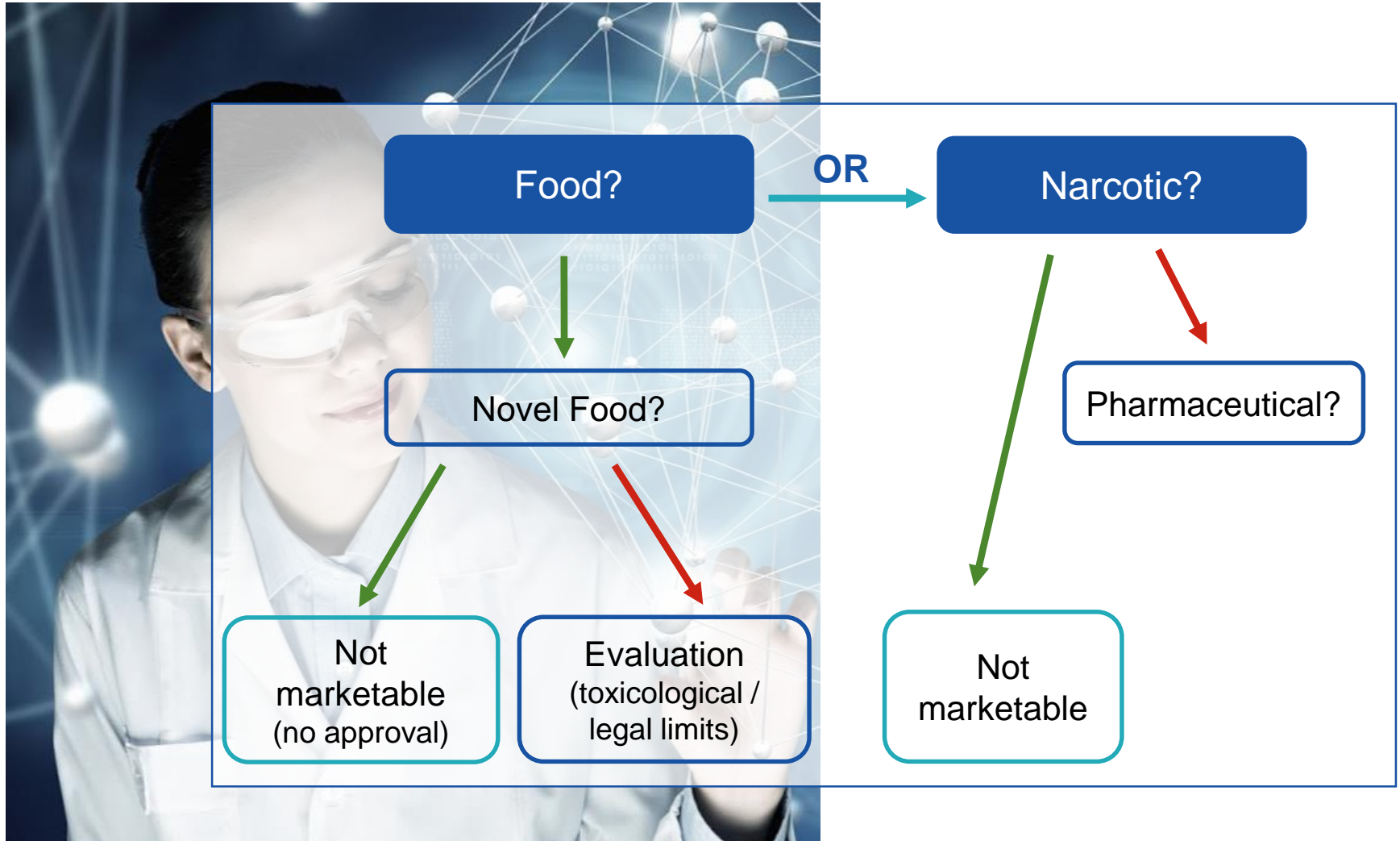   - Extracts from flowers and leaves

2) **Novel Food**

   - Products containing isolated (pure) or synthetic CBD (no Delta 9-THC detectable*)

   - Currently no approval (Art. 6 Regulation (EC) 2015/2283)

3) **Pharmaceutical**

   - Especially if health benefits are promoted

Approx. 85% of analyzed samples contain THC.

Why analyze cannabinoids?
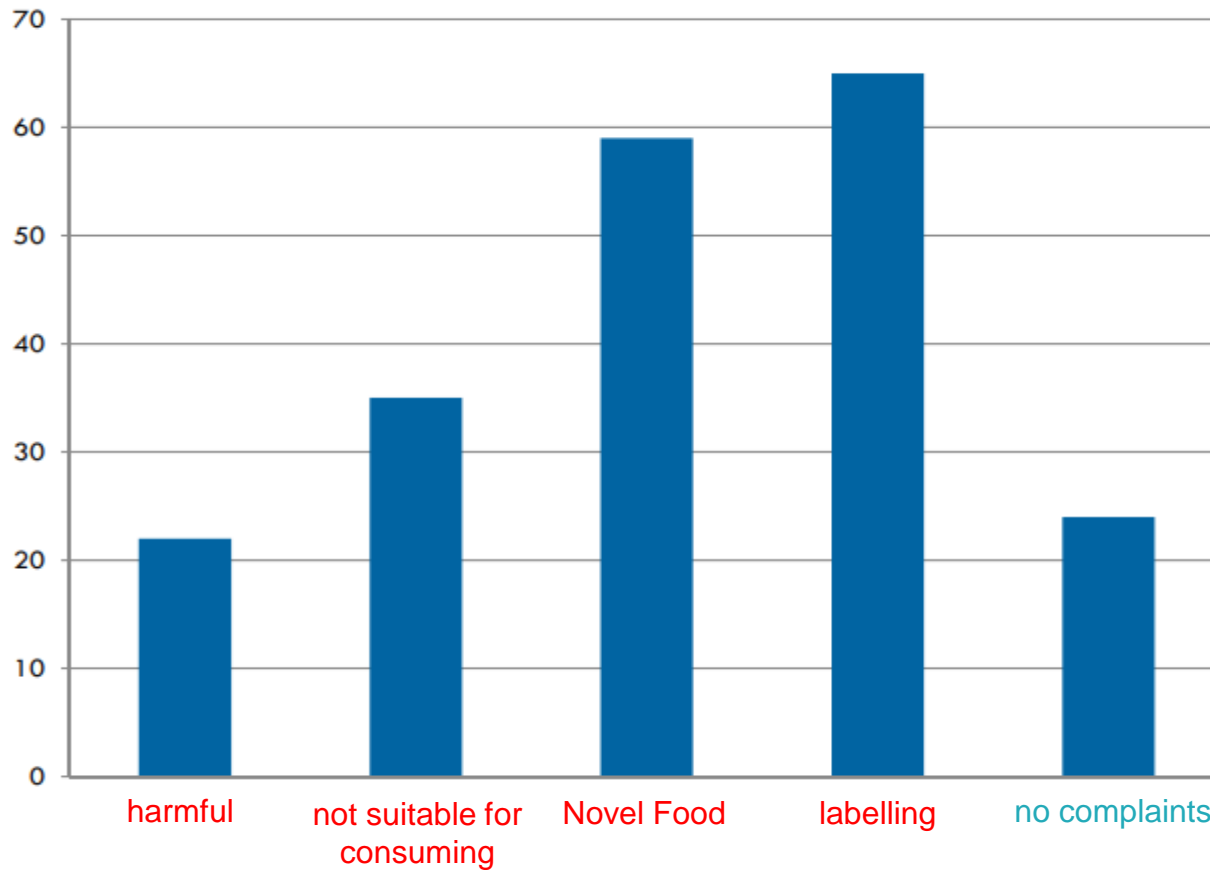
The majority of cannabinoid-containing products are NOT marketable

Agenda

Analytical diversity

Extraction solvent	Extraction procedure	Cleanup	Detection procedure
• Acetonitrile / water / citrate-buffer • Methanol[1][2] • Methanol / Dichloromethane • Benzene • Ethyl acetate • Hexane[3]	• Shaking[1] • Ultrasonic[2][3]	• „dilute and shoot"[2][3] • QuEChERS • Headspace • SPME • SPE • Liquid/liquid-separation[1]	• GC-FID[3] • GC-MS(/MS)[1][2] • LC-MS/MS • LC-UV

Official methods:

[1] = §64 LFGB (German Food and Feed Act), ASU L 13.04.19-1:   Determination of total Δ9-Tetrahydrocannabinol (THC) in hemp oil

[2] = §64 LFGB (German Food and Feed Act), ASU L 47.00-9:   Determination of total Δ9-Tetrahydrocannabinol (THC) in hemp containing tea-like products

[3] = Regulation (EU) No. 809/2014, Appendix: Community method for the quantitative determination of the delta-9 tetrahydrocannabinol content in hemp varieties

§64 LFGB (German Food and Feed Act) committee affiliation of MXNS Berlin:
Participation in development of an official method

GC(-MS/MS) or LC(-MS/MS)

GC-MS/MS

+ Fewer matrix effects

– No dinstinction between acids
(e.g. Δ9-THC and Δ9-THC-acid) possible
due to decomposition of acids in liner
– Less volatile analytes cannot be analyzed

→ Sufficient for determination of „total THC"
(guidance values)

LC-MS/MS
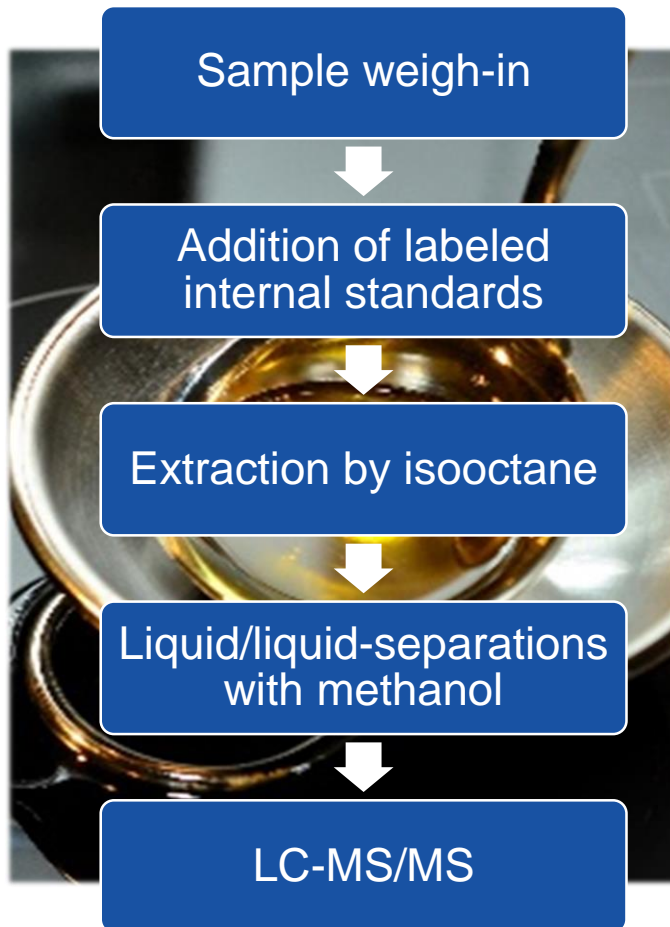

+ Determination of single compounds possible

– Compensation of matrix effects necessary
(labeled internal standard or standard
addition, respectively matrix calibration)

→ Necessary if contents of single
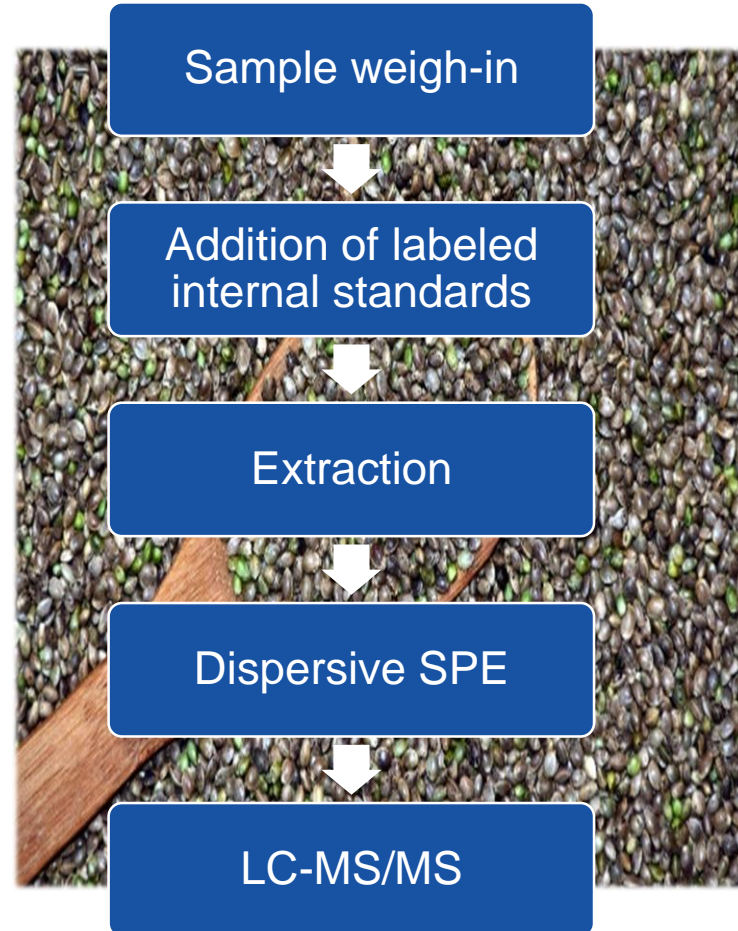compounds are to be determined

Sample preparation at MXNS Berlin

Hemp oil
on the basis of the official method §64 LFGB ASU L 13.04.19-1

- Sample weigh-in
- Addition of labeled internal standards
- Extraction by isooctane
- Liquid/liquid-separations with methanol
- LC-MS/MS

Food matrices with high fat content
(e. g. hemp seed)

- Sample weigh-in
- Addition of labeled internal standards
- Extraction
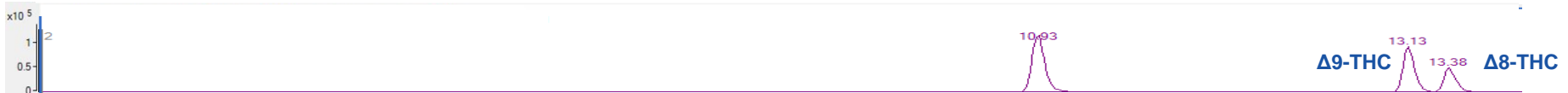- Dispersive SPE
- LC-MS/MS

Standard solution 50 ng/mL
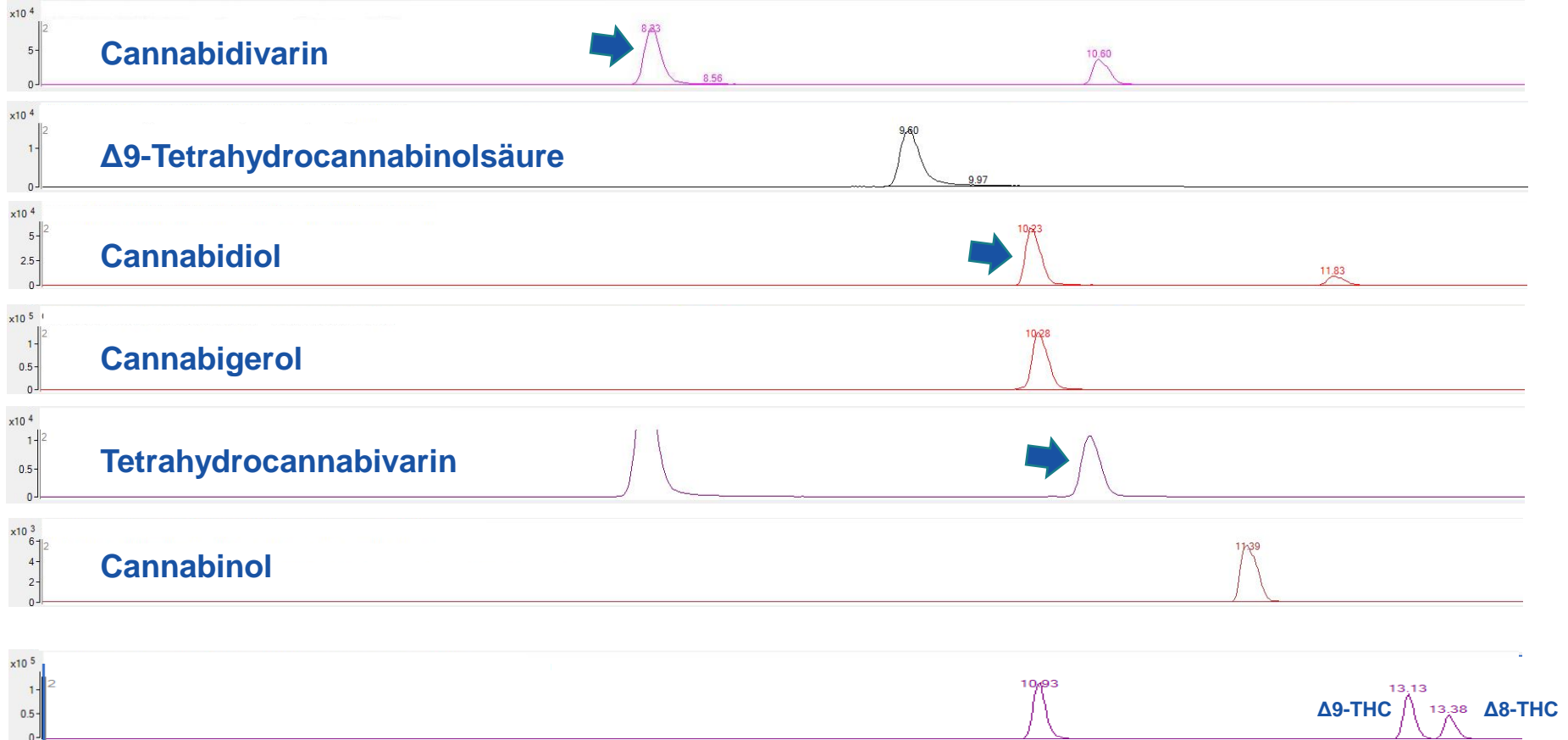
Standard solution 50 ng/mL

Chromatographic separation of Δ9-Tetrahydrocannabinol and Δ8-Tetrahydrocannabinol is necessary because MRMs are identical and limits only refer to Δ9-Tetrahydrocannabinol

Analytical challenges

High range of concentrations between different analytes

Example: CBD-oil

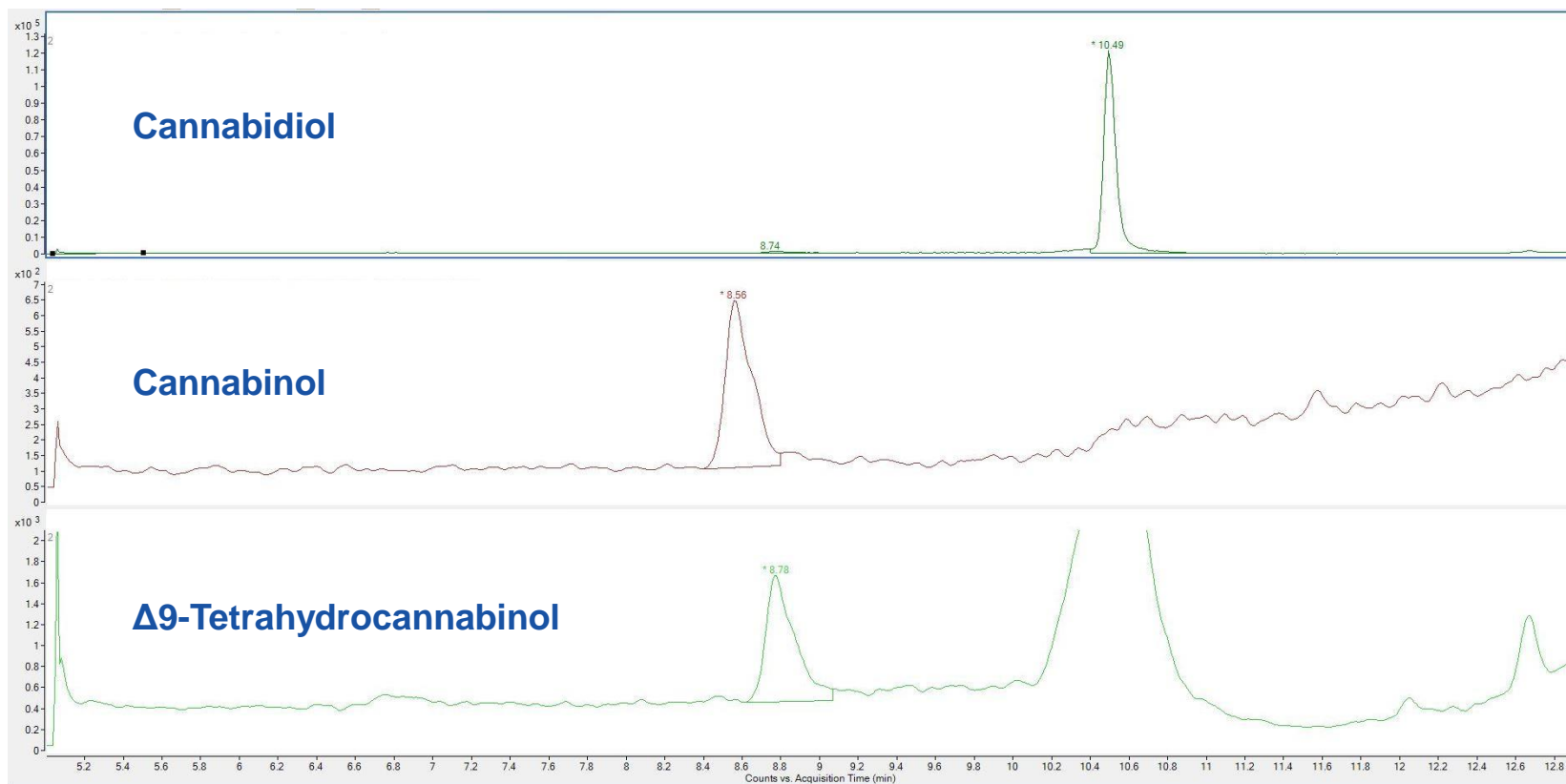|	
Cannabidiol:	45 g/kg
Δ9-THC:	3.3 mg/kg
Δ9-THC-acid:	1.6 mg/kg
Cannabinol:	<1 mg/kg

if necessary LOQ of 10 µg/kg each

Picture from pixabay

❗ Different dilutions have to be analyzed (due to limited linear range of mass spectrometer); for CBD very high dilution, for traces of cannabinol rather concentrated extract solution

❗ HPLC-system has to be rinsed intensively after analyzing concentrated sample solution (if not: carryover-effects possible)

High range of concentrations between different analytes

Analytical challenges

Acid-precursors should not be neglected

Example: CBD-oil

declared
Cannabidiol: 5 g/kg

analyzed
Cannabidiol (CBD): 2.5 g/kg

Cannabidiolic acid (CBDA): 3.0 g/kg
CBDA calculated as CBD-equivalents: *2.6 g/kg*

Sum of CBD + CBDA (calculated as CBD): 5.1 g/kg

> The determination of Δ9-Tetrahydrocannabinolic acid (THCA-A)
> is mandatory for calculation of „total THC".

Analytical challenges

Need for internal standards due to matrix effects

- Matrix components cause **ion suppression effects** → quantification via external calibration leads to false results

Signal intensity / concentration

● matrix cal. ● ext. cal.
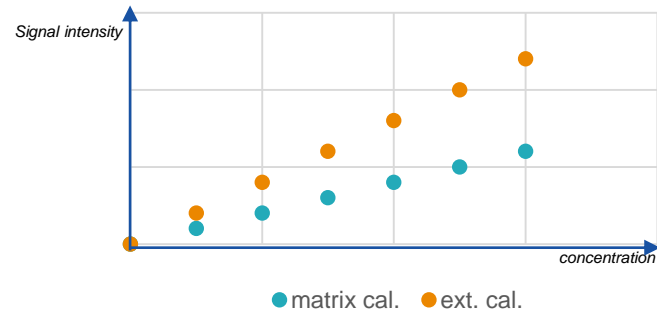
- Labeled internal standard for **correction** necessary; if too expensive or not available at all: matrix calibration or standard addition (= time-consuming) necessary

- Some years ago: labeled Δ9-Tetrahydrocannabinolic acid was not yet commercially available; standard addition for every sample not praticable; custom synthesis very expensive; affordable as centralized buying with colleagues from other laboratories

Need for authorization due to drug regulations

- **Δ9-Tetrahydrocannabinol** and **Δ8-Tetrahydrocannabinol** are regulated (in Germany according to German Narcotics Act)

- Laboratories have to apply for authorization (in Germany at BfArM - Federal Institute for Drugs and Medical Devices) and have to establish strict regulations in sample and standard management

- Authorizations restrict the **field of activity** (e. g. food analysis) and the **amount** of yearly purchasable reference substance.

Bundesinstitut für Arzneimittel und Medizinprodukte

Summary / Outlook

Analysis of cannabinoids is

up-to-date
(growing markets)

crucial
(for producers, retailers and customers)

elaborate
(matrix effects, diversity of concentrations, analyte selection, authorization)

challenging
(experienced laboratory necessary)

Our method can be expanded for further cannabinoids on demand in a short time.

MXNS is your competent partner in cannabinoid analysis

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