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Your Guide to Cannabis Testing by Liquid Chromatography

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Your Guide to Cannabis Testing by Liquid Chromatography

Potency Testing, Pesticide and Mycotoxin Analysis

Cannabis is a genus of flowering plants of the family of Cannabaceae containing different botanical classes including hemp which has been grown for thousands of years. Cannabis plants are known to have been used for medical purposes in China as early as 2800 B.C. [1]. Today, the application range is much wider. In the medical field, cannabis products are taken to treat chronic pain and muscle spasms, to reduce nausea and vomiting due to chemotherapy and to stimulate appetite for people with HIV/AIDS [2]. It is also used as industrial hemp for paper, textiles and ropes as well as edible products such as hemp seeds and hemp nuts. The recreational drug marijuana is made from the dried cannabis flowers, leaves, stems with the seeds being the most widely consumed illegal drug in the world [3].

Species of Cannabis

Many cannabis subspecies exist which can all be classified into the three main species: Cannabis sativa, Cannabis indica and Cannabis ruderalis [4]. Cannabis sativa was first classified in 1753 by Carl von Linné and is still the most commonly known species. In 1785, Jean Baptiste de Lamarck classified Cannabis indica which is quite different regarding its cannabinoid content and leaf shape to Cannabis sativa. Almost 150 years later, the botanist Dmitrij E. Janischwesky reported a third species: Cannabis ruderalis. The three species differ in their appearance, growing characteristics, cannabinoid content and uses which results in a broad product variety and makes the analysis quite challenging. [5]

Cannabis strains

Sativa Indica Ruderalis

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Regulatory Situation

In Europe, the regulatory situation differs among the individual states. Whereas most of the states including Portugal, Sweden or The Netherlands have legalised the use of cannabis for medical purposes, the situation for recreational use is very different [6–8]. It is still illegal in many countries, but some have already introduced a policy of decriminalisation which means that possession of small amounts is no longer prosecuted.

In the US, federal law says that cannabis is illegal [5]. However, since criminal law is the responsibility of the individual states the regulatory situation is not uniform. In many states such as California and New York cannabis is already legal for recreational use, but there are states such as Iowa or Georgia where it is still illegal with only small exceptions for medical use [9–12].

Cannabis Testing

Due to the ongoing discussion about the regulatory situation and the resulting legalisation for recreational cannabis use in more and more countries, the importance of reliable analytical methods continues to increase. Analytical methods are designed to ensure the quality, safety and efficacy of a product. Therefore, different analytical questions have to be answered in order to characterise the cannabis product completely. There are three main topics that are typically addressed by using liquid chromatography (LC):

- Potency testing
- Pesticide analysis
- Mycotoxin analysis

1. Potency Testing

Cannabinoids are a group of related compounds that are responsible for the pharmacological effect of cannabis. Cannabis sativa contains at least 100 of these cannabinoids which exhibit varied effects [13]. For example, tetrahydrocannabinol (THC) which is widely extracted from marijuana has psychoactive properties, whereas cannabidiol (CBD) provides anti-inflammatory, anti-epileptic and relaxing functions. The concentration of the individual cannabinoids differs among the species and since also minor cannabinoids can have a significant effect on the human body, a comprehensive profiling is of highest importance.

During potency testing the total amount of cannabinoids in a product is determined. In the early stages of cannabinoid analysis, gas chromatography with flame ionisation detection (GC-FID) was used as analytical technique and is still the method recommended by the European Union (EU) [14]. However, since the high temperatures used lead to decarboxylation of acidic cannabinoids and isomerisation and oxidation reactions, (U)HPLC became the gold standard for potency testing. Further, the analysis is quite challenging as cannabinoids show similar molecular structures and minor species can be present in just small amounts. (U)HPLC is an ideal method for this kind of analysis since it is highly sensitive and the structurally similar cannabinoids can be separated and identified.

In the following example, 11 common cannabinoids are analysed using LC/MS compatible conditions. A **YMC-Triart C18** column provides the optimum selectivity to separate these compounds with similar molecular structures and chemical properties. Cannabinoids are more fat soluble than water soluble resulting in high logP values. Therefore, starting the gradient with a high percentage of organic is commonly applied. The acetonitrile gradient increasing up to 80% is used to achieve high resolution for the 11 cannabinoids.

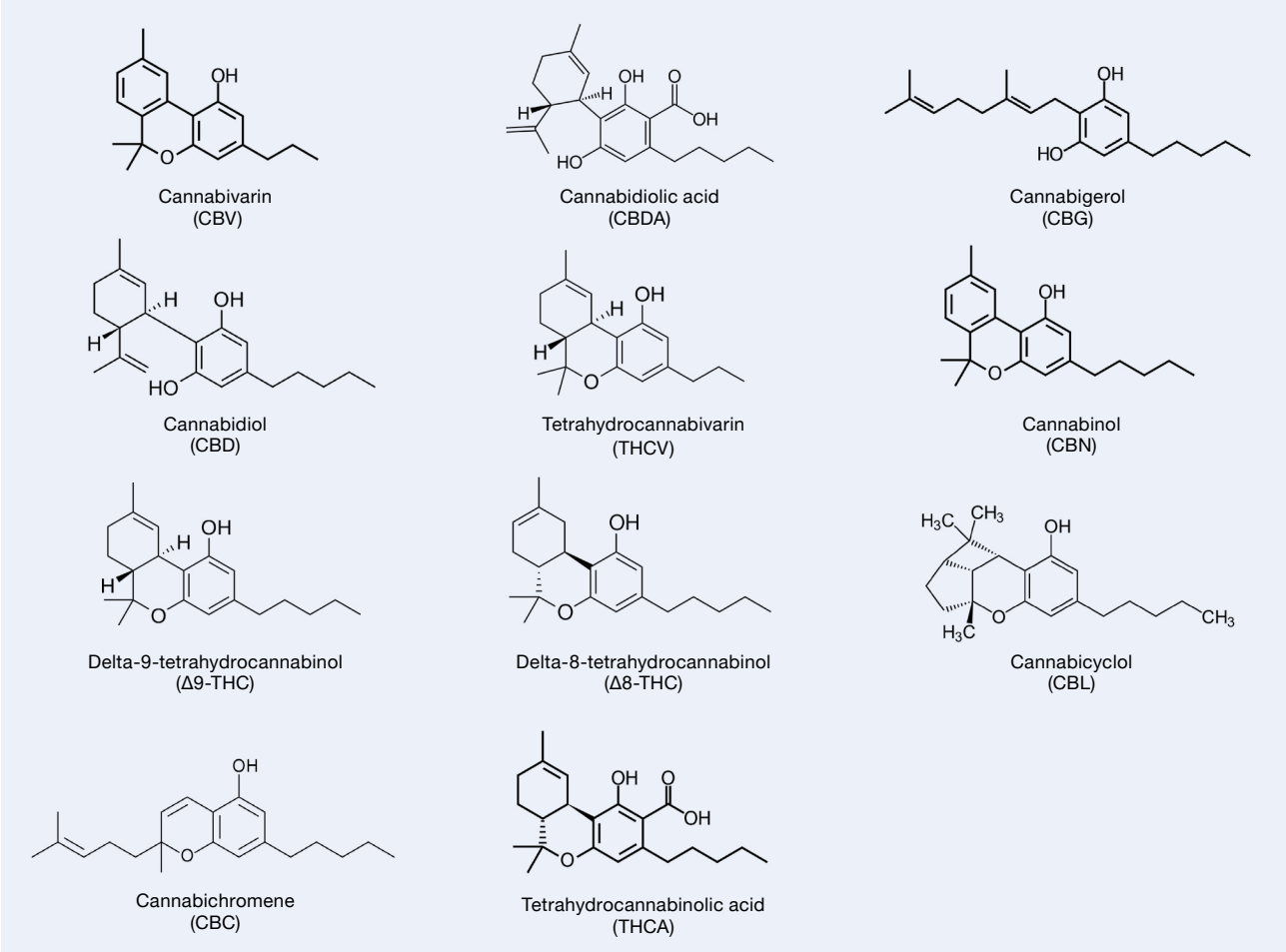

Figure 1: Cannabinoids separated in Figure 2 and 5.

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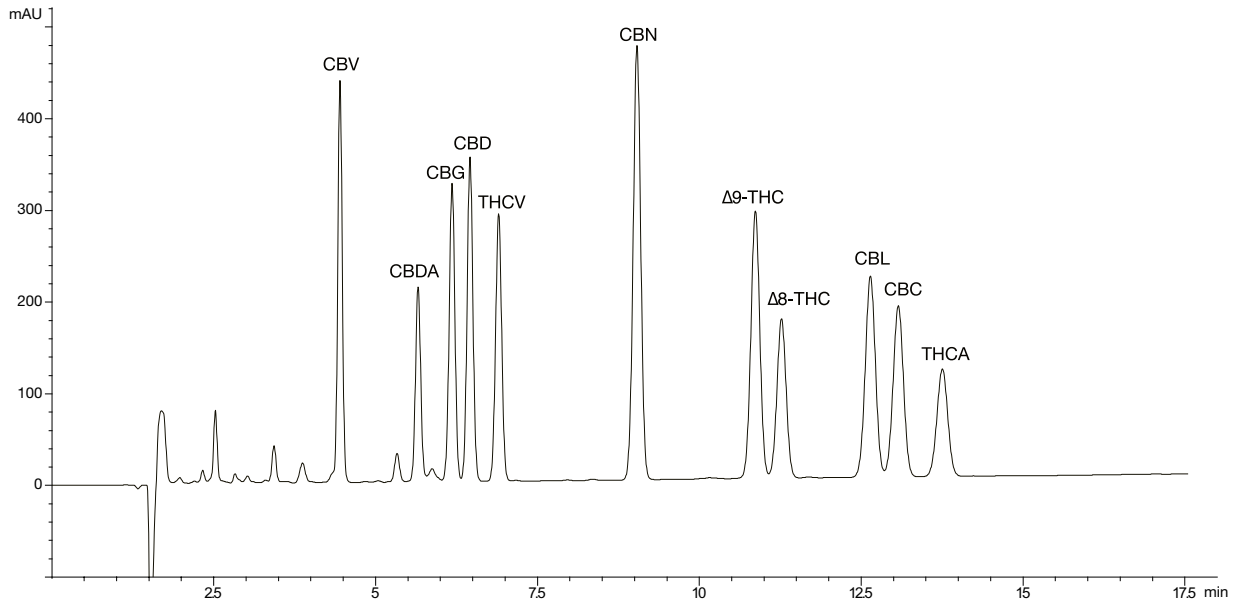

Figure 2: Separation of 11 cannabinoids using a YMC-Triart C18 column.

Chromatographic conditions

Column:	YMC-Triart C18 (3 µm, 12 nm), 150 x 4.6 mm ID
Part No.:	TA12S03-1546PTH
Eluent:	A) 0.1 % formic acid in water
	B) 0.1 % formic acid in acetonitrile
Gradient:	75–80%B (0–20 min)
Flow rate:	1.0 mL/min
Sample:	11 cannabinoids each 0.05 mg/mL diluted with acetonitrile/water (75/25)
Temperature:	35 °C
Injection:	10 µL
Detection:	UV at 220 nm

Application Data by courtesy YMC America, Inc.

By decreasing the particle size and the column dimensions (length and inner diameter) to UHPLC conditions the analysis becomes more cost-efficient, with analysis time and solvent consumption being reduced. As the following application shows, the resolution is still remarkably high for the metabolites of THC and various cannabinoids. In this application, methanol is used which has a lower elution strength compared to acetonitrile, the amount of eluent B in the gradient needs to be increased up to 100%. This ultra-fast UHPLC separation is performed with formic acid as an additive to make it LC/MS compatible.

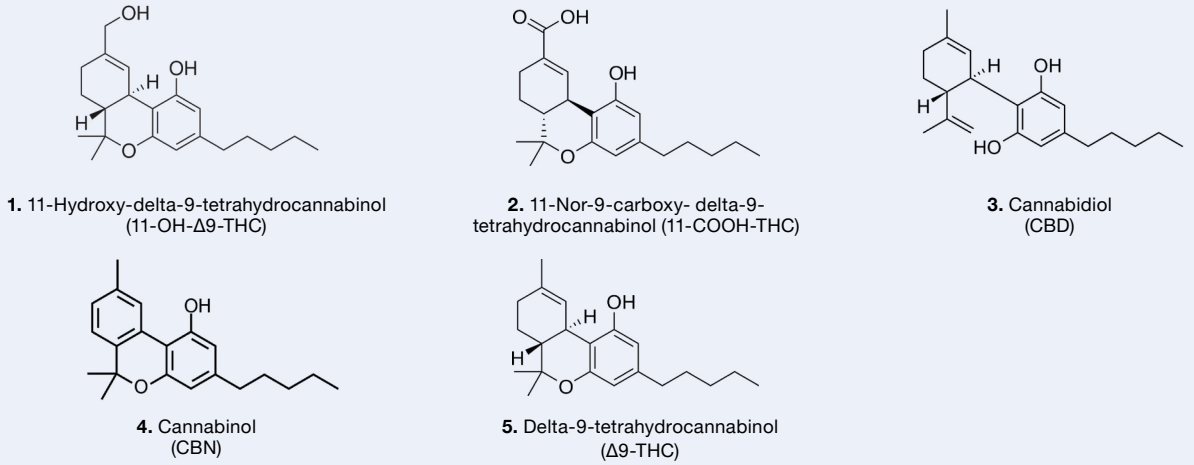

Figure 3: Cannabinoids and metabolites separated in Figure 4.

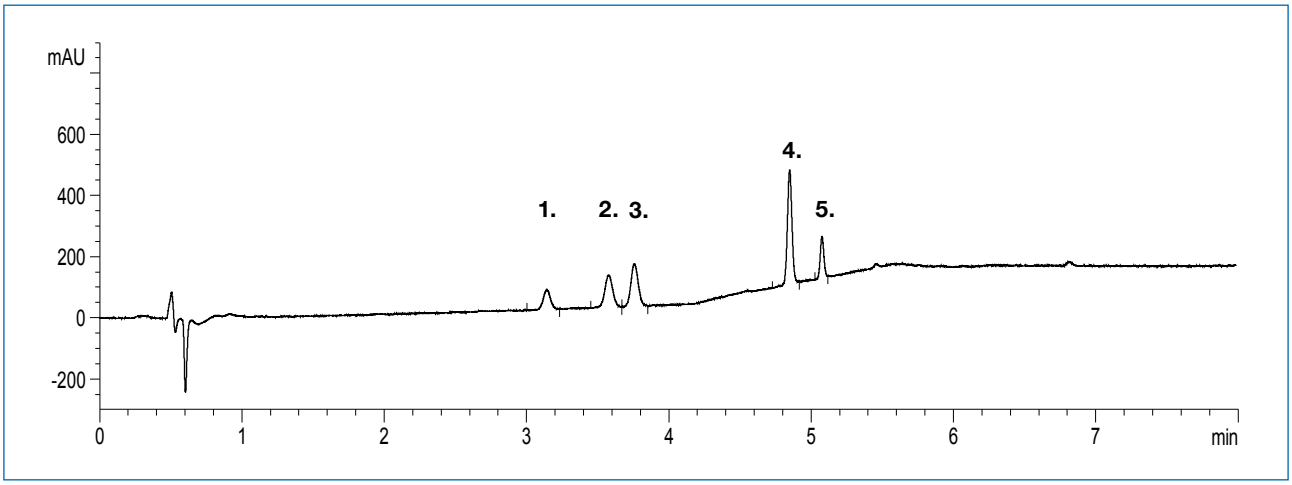

Figure 4: Separation of various cannabinoids and metabolites using a YMC-Triart UHPLC C18 column.

Chromatographic conditions

Column:	YMC-Triart C18 (1.9 µm, 12 nm) 100 x 2.0 mm ID
Part No.:	TA12SP9-1002PT
Eluent:	A) water/formic acid (100/0.1) B) methanol/formic acid (100/0.1)
Gradient:	75–82%B (0–3.5 min), 82–100%B (3.6–5.0 min), 100%B (5.0–8.0 min)
Flow rate:	0.5 mL/min
Temperature:	25 °C
Detection:	UV at 220 nm
Injection:	2 µL (17 µg/mL)

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Another option to save analysis time is to choose a Core-Shell stationary phase. Due to the particles' solid core higher flow rates compared to fully porous particles can usually be used which makes the analysis highly efficient.

In Figure 5 the separation of 7 commonly known cannabinoids is shown using the Core-Shell column **Meteoric Core C18**. Isocratic conditions using water + formic acid (0.1%) and acetonitrile allow a high-resolution separation in less than 9 minutes.

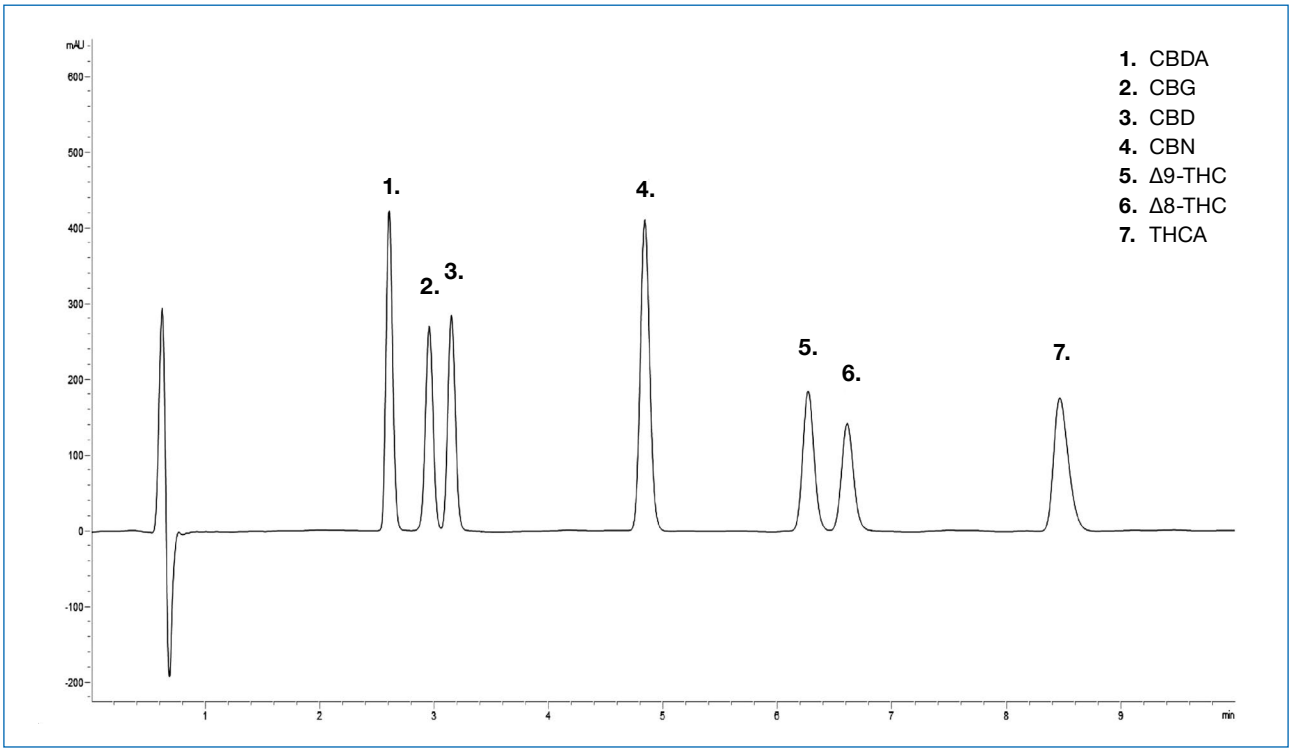

Figure 5: Separation of 7 cannabinoids using a Meteoric Core C18 column .

Chromatographic conditions

Column:	Meteoric Core C18 (2.7 µm) 100 x 4.6 mm ID
Part No.:	CAS08SQ7-1046PT
Eluent:	water+formic acid (0.1%) / acetonitrile (27/73)
Flow rate:	1.25 mL/min
Detection:	UV at 220 nm
Injection:	25 µL (0.05 mg/mL)
Temperature:	35 °C

Application Data by courtesy YMC America, Inc.

The cannabinoid which is most commonly known for its psychoactive properties is THC. It has two chiral centres and various double bond isomers with additional stereoisomers. Since the isomers can influence the pharmacological activity, chiral analysis of cannabinoids is also an important part of potency testing. In Figure 6 the chiral separation of THC is shown.

(+)trans-9-THC and (-)trans-9-THC were successfully separated with a resolution of 8.5. The separation is achieved in less than 3 minutes by using a coated polysaccharide stationary phase: CHIRAL ART Amylose-C. The resolution can further be increased by using the recently introduced **CHIRAL ART Amylose-C Neo** column.

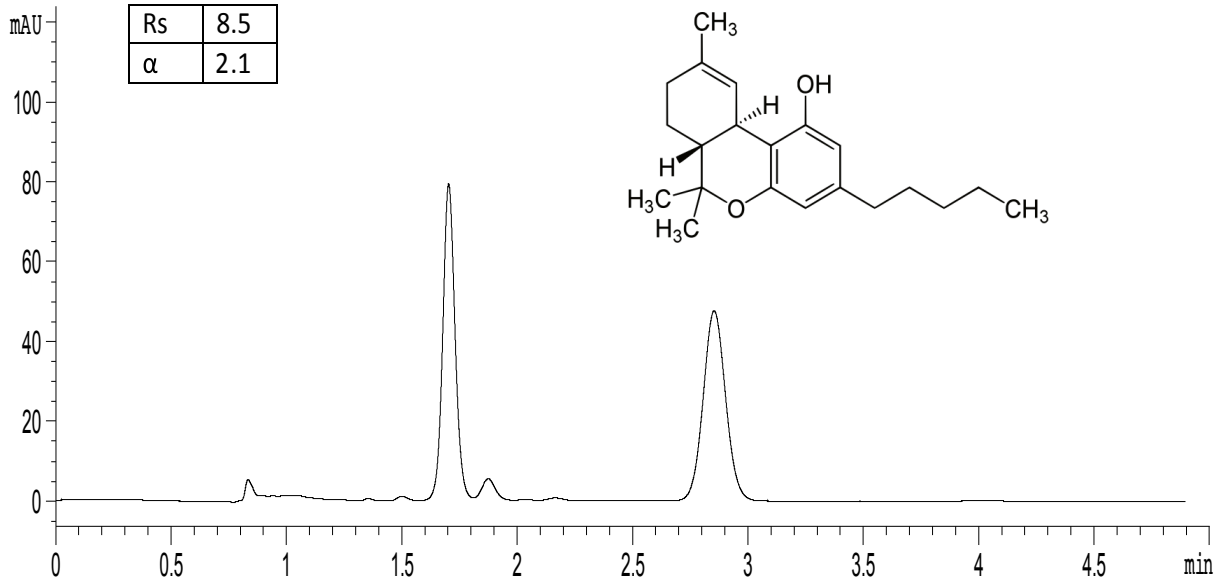

Figure 6: Chiral separation of THC using a CHIRAL ART Amylose-C column.

Chromatographic conditions

Column:	CHIRAL ART Amylose-C (3 µm) 150 x 3.0 mm ID
Part No.:	KAN99S03-1503WT
Eluent:	*n*-heptane / 2-propanol (92/8)
Flow rate:	1.0 mL/min
Temperature:	40 °C
Detection:	UV at 228 nm
Injection:	10 µL (50 µg/mL)

YMC stationary phases for cannabis potency testing

Mode	Phase	Base Particle/ Modification	Particle Sizes	pH Range	YMC's Recommended Alternative
RP	YMC-Triart C18	Hybrid silica/C18	1.9, 3, 5 µm	1–12	–
RP	Meteoric Core C18	Core-Shell silica/C18	2.7 µm	1.5–10	–
Chiral	CHIRAL ART Amylose-C Neo	Silica/Amylose tris (3,5-dimethyl-phenylcarbamate)	3, 5, 10, 20 µm	3.5–6.5	–
Chiral	CHIRAL ART Amylose-C	Silica/Amylose tris (3,5-dimethyl-phenylcarbamate)	3, 5 µm	3.5–6.5	CHIRAL ART Amylose-C Neo

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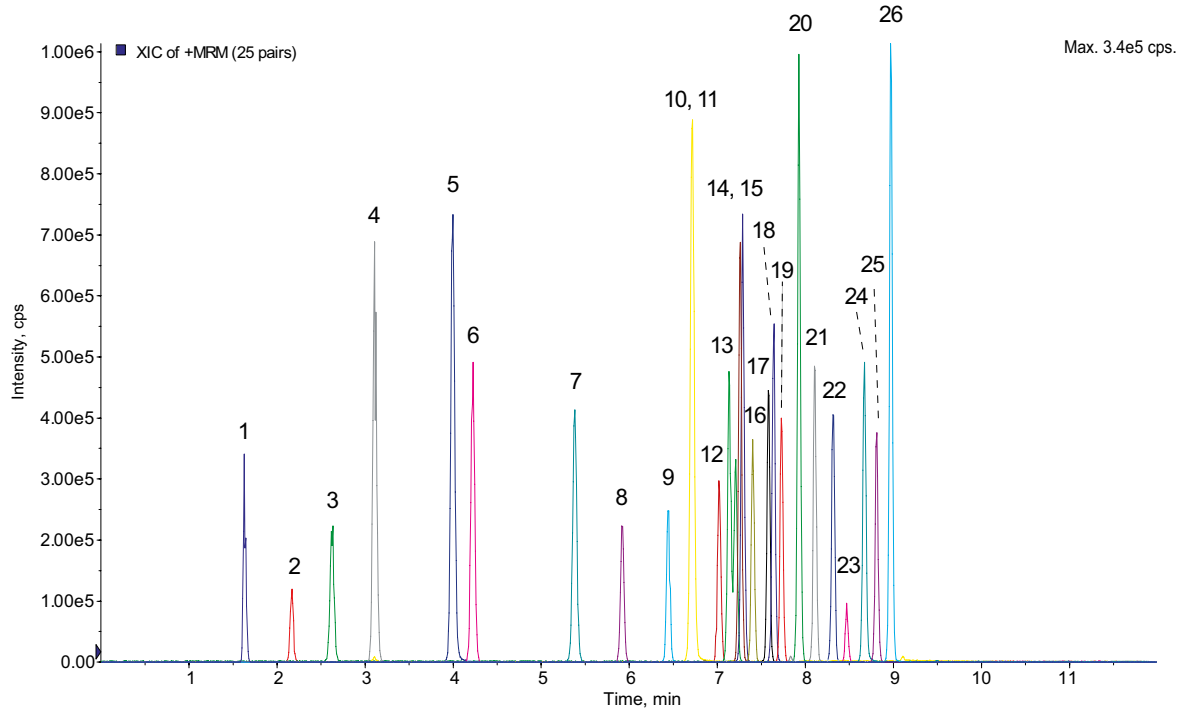
2. Pesticide analysis

Pesticides are a group of compounds which protect plants from insects, weeds or fungi. This also includes the protection of cannabis. Since the demand in cannabis products has grown for several years, the industrial cultivation has also increased and therefore the use of pesticides.

Whilst insecticides, herbicides, fungicides etc. protect the plants against harmful impact resulting in higher yields, they also pollute the environment, disturb the balance of the ecosystem and are a human health risk. To ensure the consumer's safety reliable and fast methods for the analysis of pesticides are required.

Pesticide analyses benefit from using mass spectrometry as a highly accurate detection technique. Because very low levels of pesticides need to be identified in different matrices, the complete analysis has to be highly sensitive. In Figure 7 the separation of various pesticides, including insecticides, herbicides and fungicides is shown.

By using a stationary phase with 2 µm particle size and MS as the detection technique a reliable and ultra-fast analysis of 26 pesticides is obtained.
The **YMC-UltraHT*Pro* C18** column makes it possible to separate these compounds with sharp peaks in only 9 minutes.



1. Thiamethoxam (Q1/Q3 : 292.3/211.3)
2. Clothianidin (Q1/Q3 : 250.3/169.1)
3. Chloridazon (Q1/Q3 : 222.3/77.0)
4. Thiacloprid (Q1/Q3 : 253.3/126.0)
5. Thiabendazole (Q1/Q3 : 202.3/175.2)
6. Azamethiphos (Q1/Q3 : 325.2/183.0)
7. Dimethirimol (Q1/Q3 : 210.4/71.1)
8. Isoxaflutole (Q1/Q3 : 360.2/251.1)
9. Pyriftalid (Q1/Q3 : 319.3/139.1)
10. (*E*)-Ferimzone (Q1/Q3 : 255.4/91.1)
11. (*Z*)-Ferimzone (Q1/Q3 : 255.4/91.1)
12. Methoxyfenozide (Q1/Q3 : 369.4/149.3)
13. Iprovalicarb (Q1/Q3 : 321.4/119.3)
14. Chromafenozide (Q1/Q3 : 395.4/175.1)
15. Butafenacil (Q1/Q3 : 492.1/331.1)
16. Simeconazole (Q1/Q3 : 249.3/70.1)
17. Cyazofamid (Q1/Q3 : 325.2/108.0)
18. Naproanilide (Q1/Q3 : 292.3/171.3)
19. Fenoxycarb (Q1/Q3 : 302.3/88.1)
20. Anilofos (Q1/Q3 : 368.2/199.1)
21. Cyflufenamid (Q1/Q3 : 431.3/295.2)
22. Pyrazolynate (Q1/Q3 : 439.1/91.0)
23. Indoxacarb (Q1/Q3 : 528.1/203.2)
24. Benzofenap (Q1/Q3 : 431.2/105.1)
25. Furathiocarb (Q1/Q3 : 383.3/195.2)
26. Cloquintocet-mexyl (Q1/Q3 : 336.3/238.2)

Figure 7: Separation of 26 pesticides using a YMC-UltraHT Pro C18 column .

Chromatographic conditions

Column:	YMC-UltraHT *Pro* C18 (2 µm, 12 nm) 75 × 2.0 mm ID
Part No.:	AS12S02-L502WT
Eluent:	A) 5 mM CH_3COONH_4 in water
	B) 5 mM CH_3COONH_4 in methanol
Gradient:	15–40%B (0–0.5 min), 40%B (0.5–1.75 min), 40–50%B (1.75–3 min), 50–55%B (3–4 min), 55–95%B (4–8.75 min), 95%B (8.75–11 min)
Flow rate:	0.4 mL/min
Temperature:	ambient
Detection:	API5000, ESI, Positive, MRM
Injection:	3 µL (10 ng/mL)
Sample:	Pesticide Mixture Standard Solution PL-7-2, manufactured by Wako Pure Chemical Industries, Ltd.

Courtesy of J.Watanabe, TAKARA BIO INC.

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Atrazine is one of the best-known herbicides in the world [15]. In the EU its use is prohibited, but in the US its use is still allowed [16]. Therefore, it still can be found in plants such as cannabis and needs to be strictly monitored.

A **YMC-Triart C18** column is the perfect choice to analyse atrazine and its metabolites. By using the UHPLC particle size the compounds can be separated in only 2 minutes!

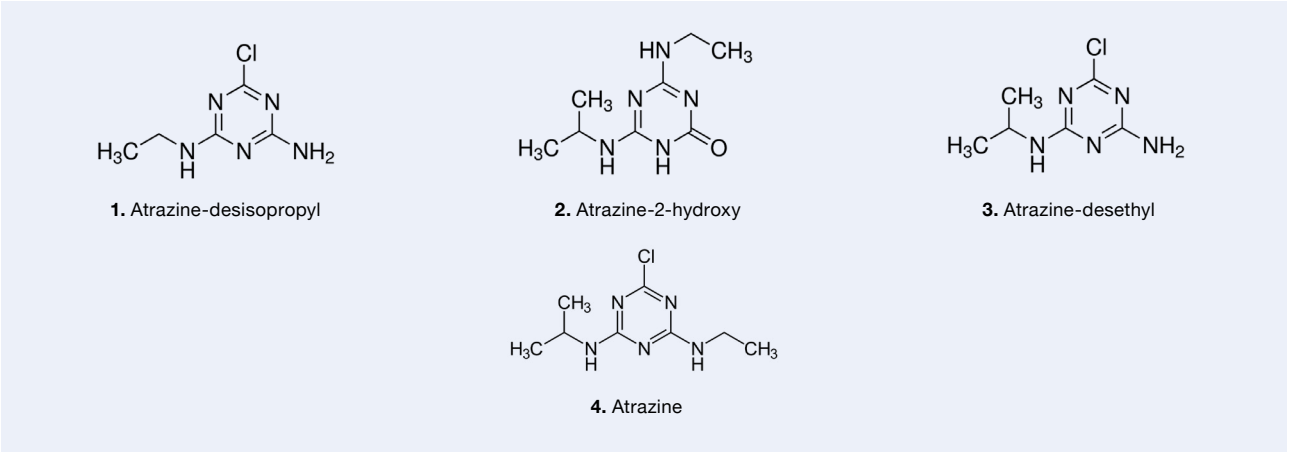

Figure 8: Atrazine and its metabolites.

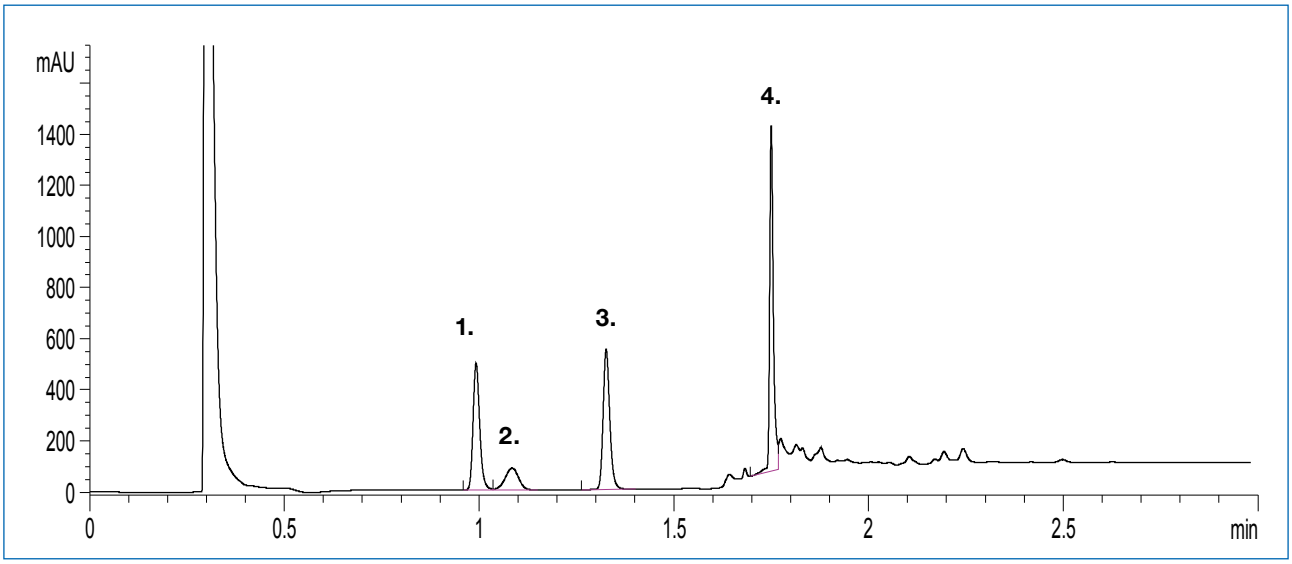

Figure 9: Separation of atrazine and metabolites using a YMC-Triart C18 column.

Chromatographic conditions

Column:	YMC-Triart C18 (1.9 µm, 12 nm) 75 x 2.0 mm ID
Part No.:	TA12SP9-L502PT
Eluent:	A) water/TFA (100/0.1)
	B) acetonitrile/TFA (100/0.1)
Gradient:	15-40%B (0-1.2 min), 90%B (1.3–2.0 min)
Flow rate:	0.6 mL/min
Temperature:	25 °C
Detection:	UV at 230 nm
Injection:	1 µL (0.2 mg/mL)

Since pesticides can also have hydrophilic properties, stationary phases showing a good selectivity for polar substances are required. YMC's **Hydrosphere C18** columns are based on the same ultra-high purity silica as **YMC-Pack *Pro* C18** and have been designed especially for the separation of polar compounds. The hydrophilic insecticide oxamyl, which has a logP value below 0, shows good retention on a Hydrosphere C18 column as shown in Figure 11. Excellent resolution is obtained for all five insecticides.

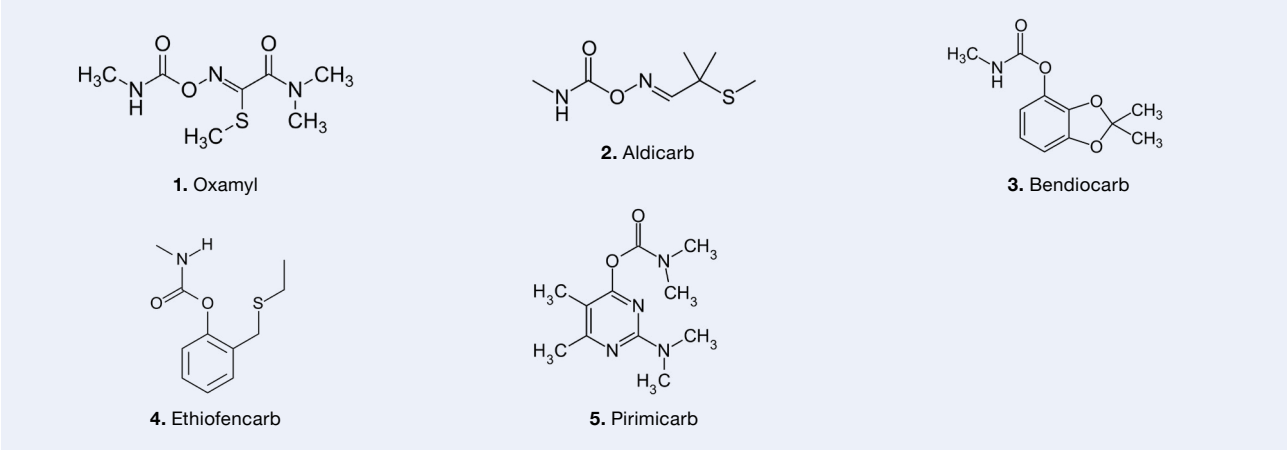

Figure 10: 5 insecticides analysed in Figure 11.

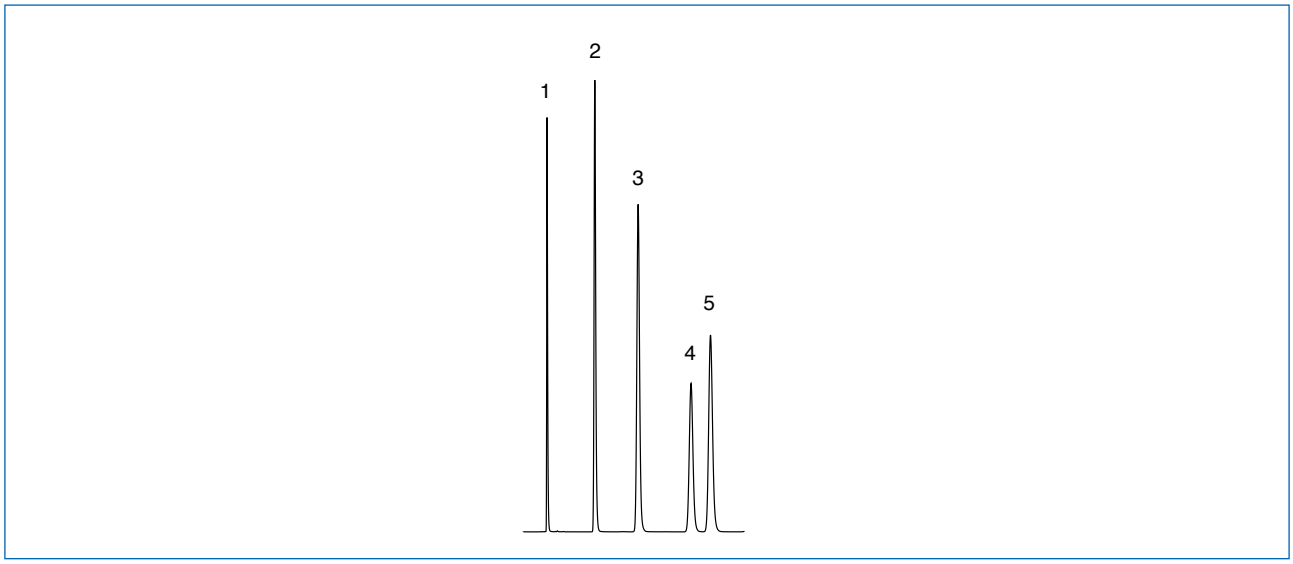

Figure 11: Separation of 5 insecticides using a Hydrosphere C18 column.

Chromatographic conditions

Column:	Hydrosphere C18 (3 µm, 12 nm) 75 x 4.6 mm ID
Part No.:	HS12S03-L546WT
Eluent:	methanol / 20mM KH_2PO_4 (45/55)
Flow rate:	1.0 mL/min
Temperature:	30 °C
Detection:	UV at 240 nm, 0.64 AUFS
Injection:	5 µL (0.1~1.5 mg/mL)

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The world's most widely used herbicide is glyphosate [17]. Since the 1970s it has been used to eliminate weeds. Due to its extensive use in agriculture, fields where cannabis is harvested may be contaminated with it even though it has not been directly applied. Therefore, highly sensitive analytical methods are required. Aminomethylphosphonic acid (AMPA) is the main metabolite of glyphosate. Due to the reported risks to human health for both compounds, their analysis is an important topic.

Since both compounds are highly polar they are difficult to retain on C18 columns. The derivatisation with FMOC-Cl (fluoromethyloxycarbonyl chloride) is used to lower the analytes' polarity and therefore increase their retention. The analysis of FMOC-derivatised glyphosate and AMPA at a concentration of 800 ng/L is shown in Figure 13. A **YMC-Triart C18** UHPLC column is used to obtain these highly sensitive results. Stable isotope M-AMPA-FMOC and M-glyphosate-FMOC were used as internal standards.

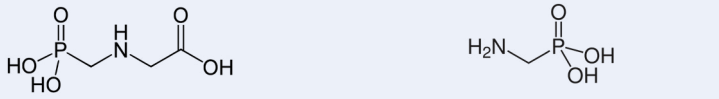

Figure 12: Structures of glyphosate and AMPA.

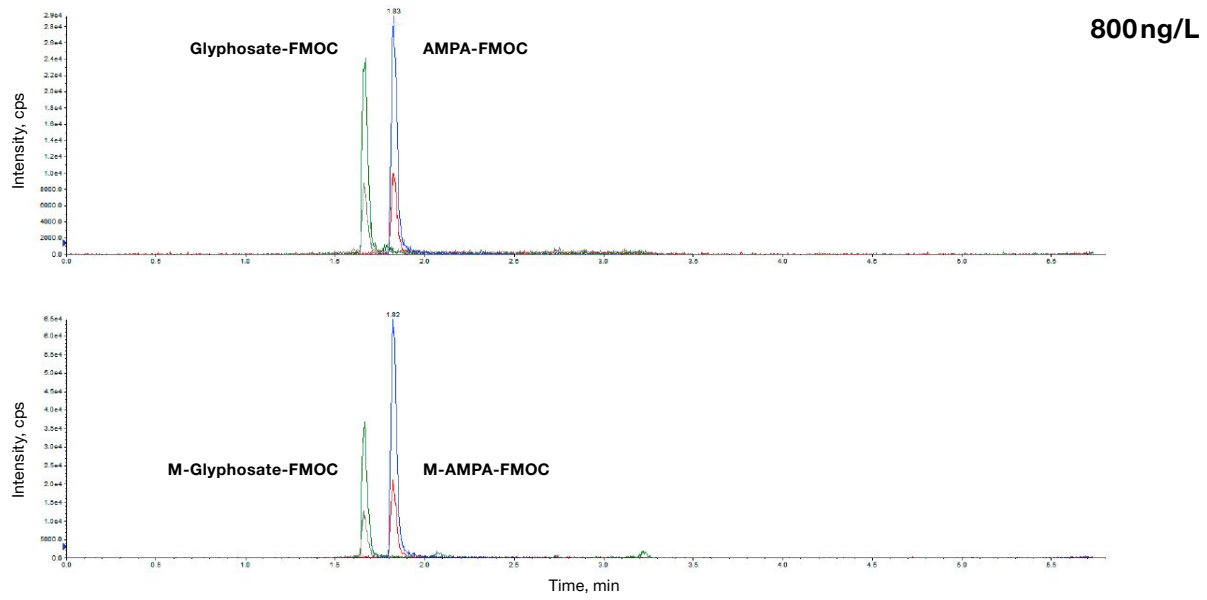

Figure 13: Extracted-ion chromatograms at m/z=332 and m/z=334 for glyphosate-FMOC and AMPA-FMOC and the internal standards at a concentration of 800 ng/L.

Chromatographic conditions.

Column:	YMC-Triart C18 (1.9 µm, 12 nm) 75 x 2.1 mm ID
Part No.:	TA12SP9-L5Q1PT
Eluent:	A) 0.1% triethylamine in H_2O (adjusted to pH 9.5 with glacial acetic acid) B) acetonitrile
Gradient:	5%B (0–2.1 min), 5–65%B (2.1–4 min), 65–95%B (4–4.8 min), 95–5%B (4.8–5 min), 5%B (5–10 min)
Flow rate:	0.4 mL/min
Temperature:	40 °C
Detection:	MS (ABSciex QTrap 6500+) in negative MRM mode
Injection:	20 µL

ESI source conditions.

Curtain gas:	25 psi	Spraying gas (GS1):	40 psi
CAD gas:	Medium	Drying gas (GS2):	60 psi
Ion spray voltage:	-4,500 V	Entrance potential:	-10 V
Drying temperature:	350 °C		

Compound-related MS-conditions.

Compound	MRM transitions	Dwell time	Declustering potential	Collision energy	Collision cell exit potential
AMPA-FMOC	332 → 110 (332 → 136)	20 ms (20 ms)	-35 V (-35 V)	-10 V (-18 V)	-15 V (-13 V)
Glyphosate-FMOC	390 → 168 (390 → 150)	20 ms (20 ms)	-50 V (-50 V)	-16 V (-30 V)	-17 V -9 V
M-AMPA-FMOC ($^{13}C^{15}N$-AMPA)	334 → 112 (334 → 138)	20 ms (20 ms)	-35 V (-35 V)	-10 V (-18 V)	-13 V (-15 V)
M-Glyphosate-FMOC (1,2-$^{13}C^{15}N$-glyphosate)	393 → 171 (393 → 153)	20 ms (20 ms)	-65 V (-65 V)	-16 V (-30 V)	-9 V (-17 V)

YMC stationary phases for pesticide analysis

Phase	Base Particle/ Modification	Particle Sizes	pH Range
YMC-Triart C18	Hybrid silica/C18	1.9, 3, 5 µm	1–12
YMC-Pack *Pro* C18	Silica/C18	2*, 3, 5 µm	2–8
Hydrosphere C18	Silica/C18	2*, 3, 5 µm	2–8

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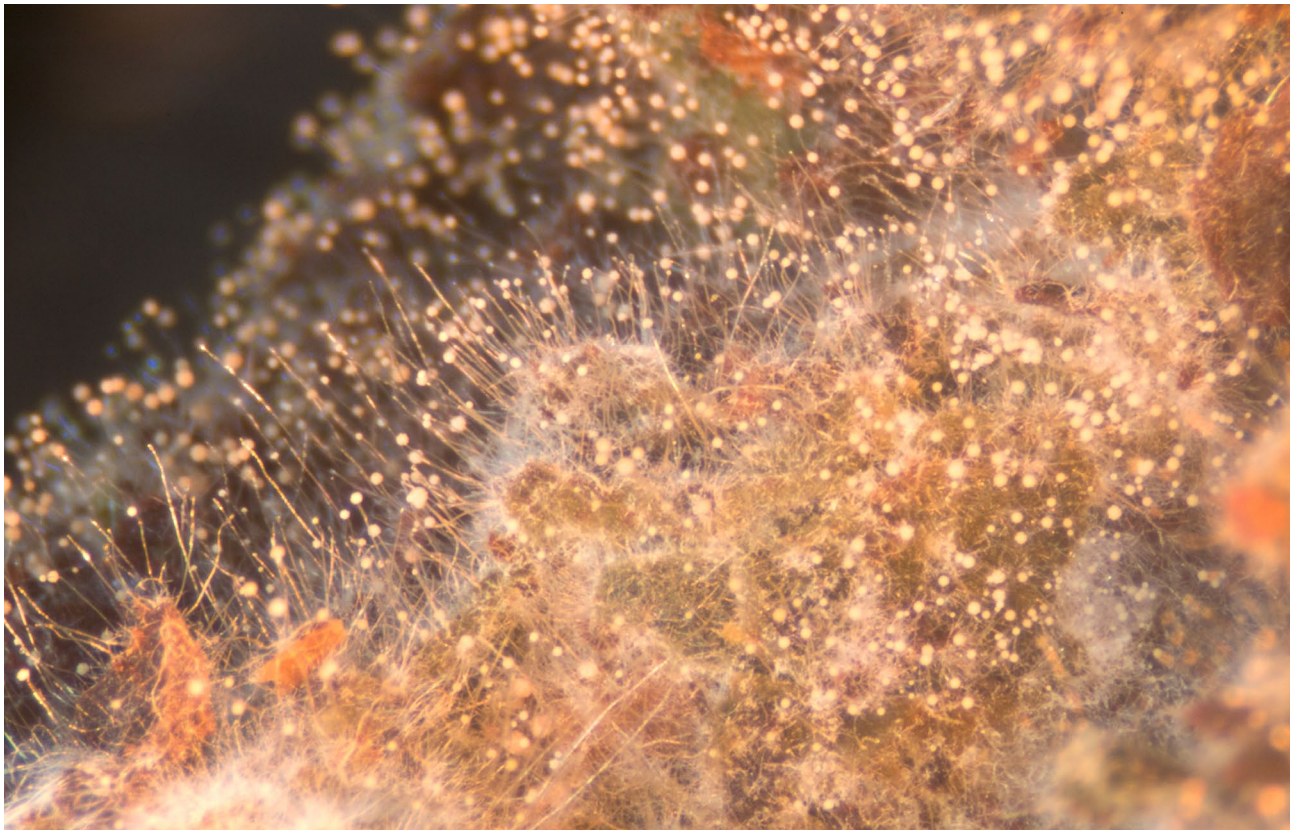
3. Mycotoxin analysis

Besides cannabinoid and pesticide analysis, the detection of mycotoxins is an important part of cannabis testing. Mycotoxins are a health risk for both humans and other animals as they are toxic secondary metabolites produced by fungi and can be carcinogenic, immunosuppressive or mutagenic [18]. They occur in cannabis because of mouldy plants, which either turned mouldy in the field or during storage or transport. Commonly known mycotoxins are aflatoxins, fumonisins and ochratoxin A.



Since fumonisins have coordinating groups in their molecular structure they show poor peak shapes and carryover with conventional HPLC columns due to their interaction with the stainless steel column hardware.
YMC's metal-free hardware which is made of a PEEK-lined stainless steel body and a PEEK frit is the ideal choice for the analysis of such coordinating compounds. Due to the fully inert nature of the columns, the sample does not come into contact with any metal inside the column resulting in sharp peaks and no carryover. By using hyphenated **YMC-Triart C18 metal-free** columns with MS, highest sensitivity results are obtained.

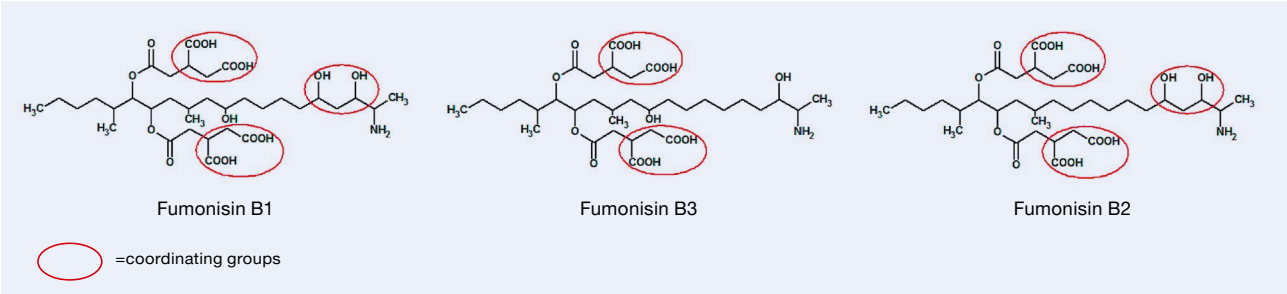


Figure 14: The fumonisins separated in Figure 15.

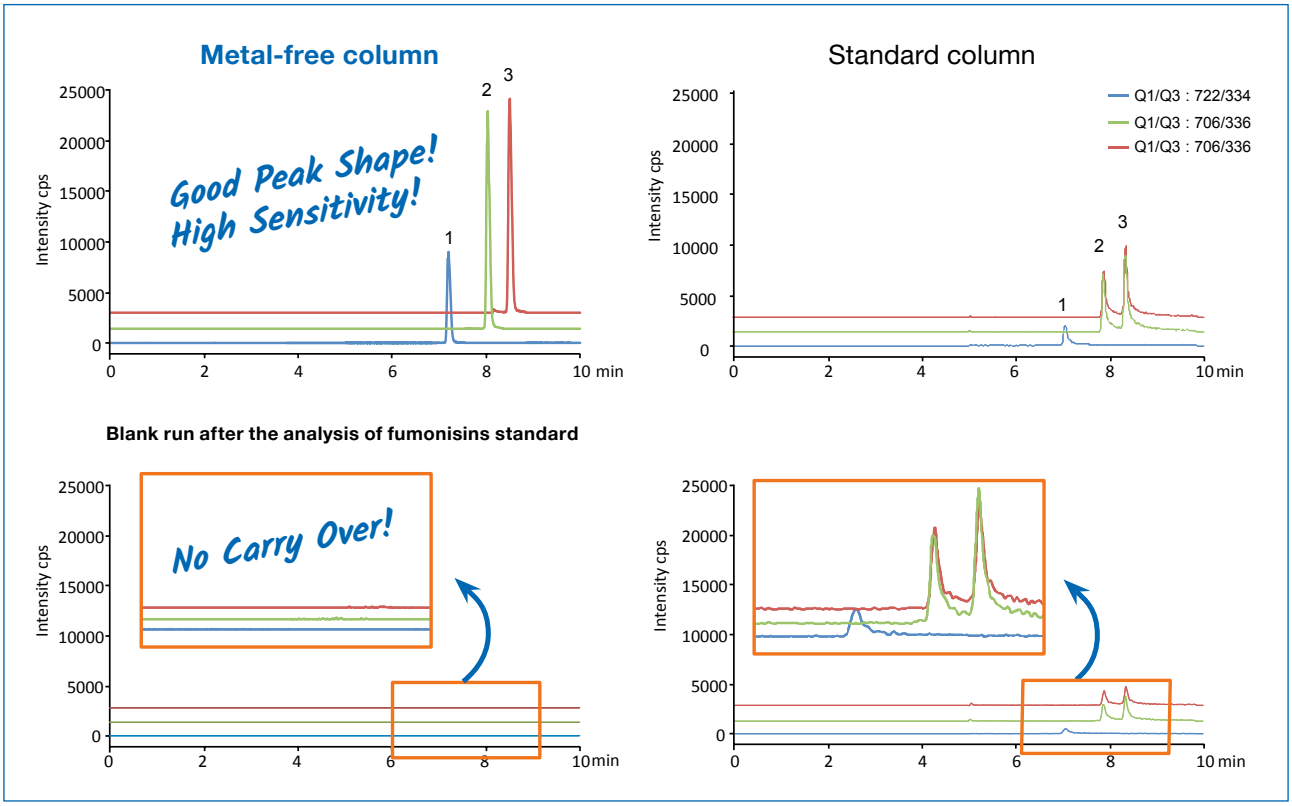


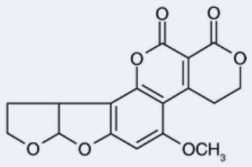
Figure 15: Comparison of the separation of fumonisins using a YMC-Triart C18 metal-free and a standard column .

Chromatographic conditions

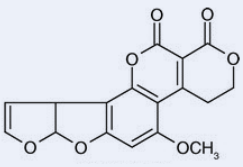
Column:	YMC-Triart C18 (metal-free/standard) (3 µm, 12 nm) 150 x 2.1 mm ID
Part Nos.:	TA12S03-15Q1PTP/TA12S03-15Q1PTH
Eluent:	A) water/HCOOH (100/0.1)
	B) acetonitrile
Gradient:	25–50%B (0–5 min), 50%B (5–8 min), 50–90%B (8–10 min)
Flow rate:	0.2 mL/min
Temperature:	40 °C
Detection:	ESI, positive
	Scheduled MRM (Metal-free column)
	MRM (Standard column)
Injection:	5 µL (0.1 mg/mL)
Instrument:	LC) Shimadzu Prominence UFLC, MS) AB Sciex 3200 QTRAP

Aflatoxins are very similar in hydrophobicity and structure which makes their separation very challenging. However, due to their toxic properties, their analysis needs to be highly reliable. Therefore, highly selective and reliable stationary phases are required. Columns with an intermediate hydrophobicity are a good choice for this analysis.

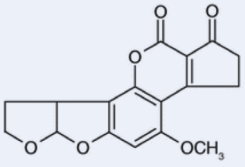
Sharp peaks can be obtained with high resolution for these structurally similar compounds as shown in Figure 17. To have more flexibility in method development particularly with regards to available particle sizes and applicable pH range, the use of modern stationary phases with similar properties such as **Hydrosphere C18** is to be recommended.



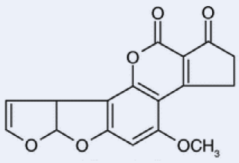
1. Aflatoxin G2



2. Aflatoxin G1



3. Aflatoxin B2



4. Aflatoxin B1

Figure 16: The aflatoxins separated in Figure 17.

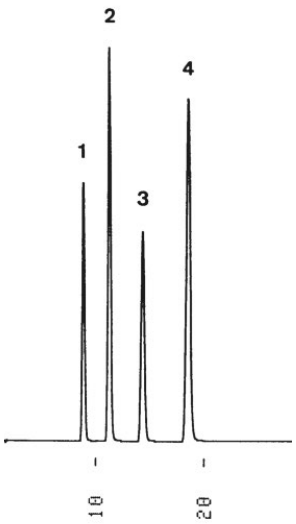


Figure 17: Separation of aflatoxins using a column with moderate hydrophobicity.

Chromatographic conditions

Columns:	J'sphere ODS-M80 (4 µm, 8 nm) 150 x 4.6 mm ID
Part No.:	JM08S04-1546WT
Eluent:	methanol/water (40/60)
Flow rate:	1.0 mL/min
Temperature:	37 °C
Detection:	UV at 365 nm, 0.04 AUFS
Injection:	10 µL (7~10 ppm)

YMC stationary phases for mycotoxin analysis

Phase	Base Particle/ Modification	Particle Sizes	pH Range	YMC's Recommended Alternative
YMC-Triart C18 (Metal-free hardware available)	Hybrid silica/C18	1.9, 3, 5 µm	1–12	–
Hydrosphere C18	Silica/C18	2*, 3, 5 µm	2–8	–
J'sphere ODS-M80	Silica/C18	4 µm	2–7.5	Hydrosphere C18

* UltraHT

Conclusion

Cannabis products have to be characterised in a comprehensive way in order to ensure the consumer safety. In addition to potency testing, contaminants such as pesticides and mycotoxins have to be fully determined due to their health risk for humans. This Whitepaper shows how to overcome the many existing challenges in cannabis analyses. **YMC's stationary phases provide:**

- ✔ **high resolution for structurally similar cannabinoids in potency testing**
- ✔ **excellent selectivity for non-polar and polar pesticides**
- ✔ **good peak shapes for coordinating mycotoxins**

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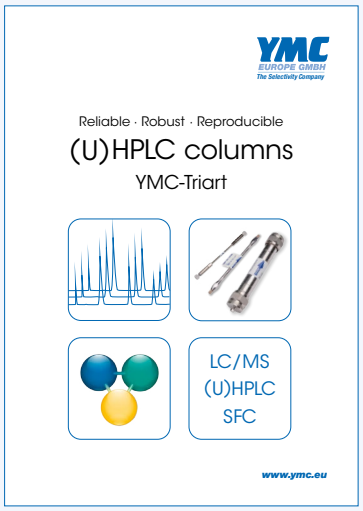
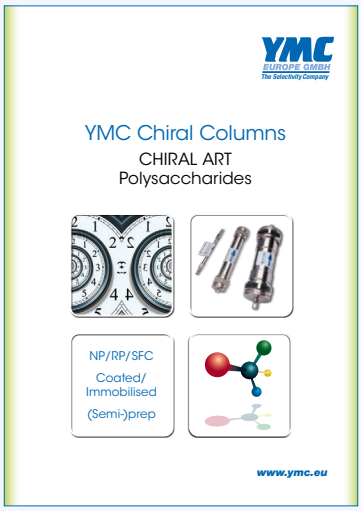
Further information



Brochure
YMC-Triart



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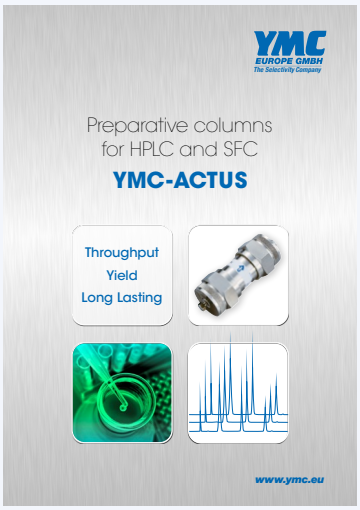
YMC Chiral Columns



Brochure
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