

Polymer-Type Chiral and Achiral Selectors for Cannabinoid Analysis

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Introduction:

- Cannabinoids form complex mixtures of structural isomers, diastereomers, and enantiomers, making chromatographic analysis challenging
- In Japan, the growing CBD market has led to strict limits for Δ^9 -THC and Δ^9 -THCA, requiring reliable quantification below regulatory thresholds
- In addition, several cannabinoids are chiral, and achiral methods alone cannot achieve stereoisomer resolution

In this study, we developed an analytical workflow for accurate quantification of Δ^9 -THC in high-CBD matrices, MS-compatible separations, and extension to chiral analysis to clarify elution behavior

Result and Discussion:

Previous Work 1:

Trace Δ^9 -THC Quantification in High-CBD Matrices

- CBD and Δ^9 -THC share the same m/z and cannot be distinguished by MS
- Conventional RP-ODS methods show strong CBD peaks that mask trace Δ^9 -THC (Fig. 1)
- A P4VP-based pre-processing protocol (A) for removing excess CBD, combined with LC-MS/MS, reduced CBD matrix effects and enabled accurate quantification¹ (Fig. 2)
- Δ^9 -THCA could be quantified using conventional methods due to its distinct m/z from CBD
- The recovery results from the spiking experiments were within the target range (70–120% recovery, RSD $\leq$ 5%)
- However, the required pre-processing step limited throughput

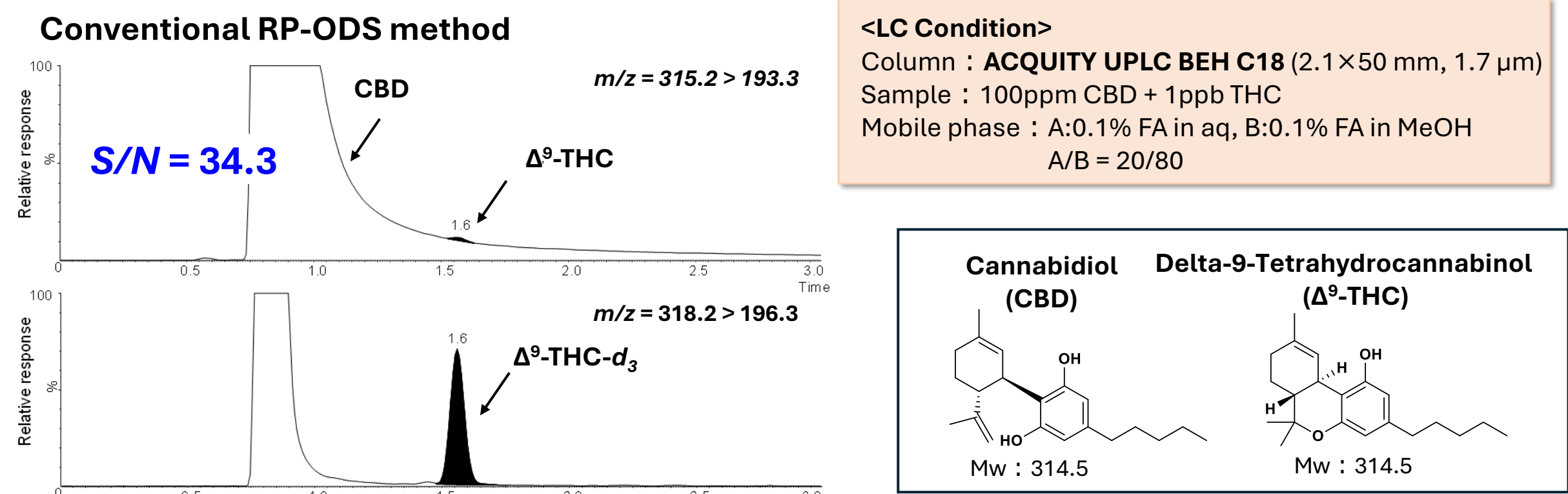


Fig.1. Challenges in Δ^9 -THC quantification in high-CBD matrices

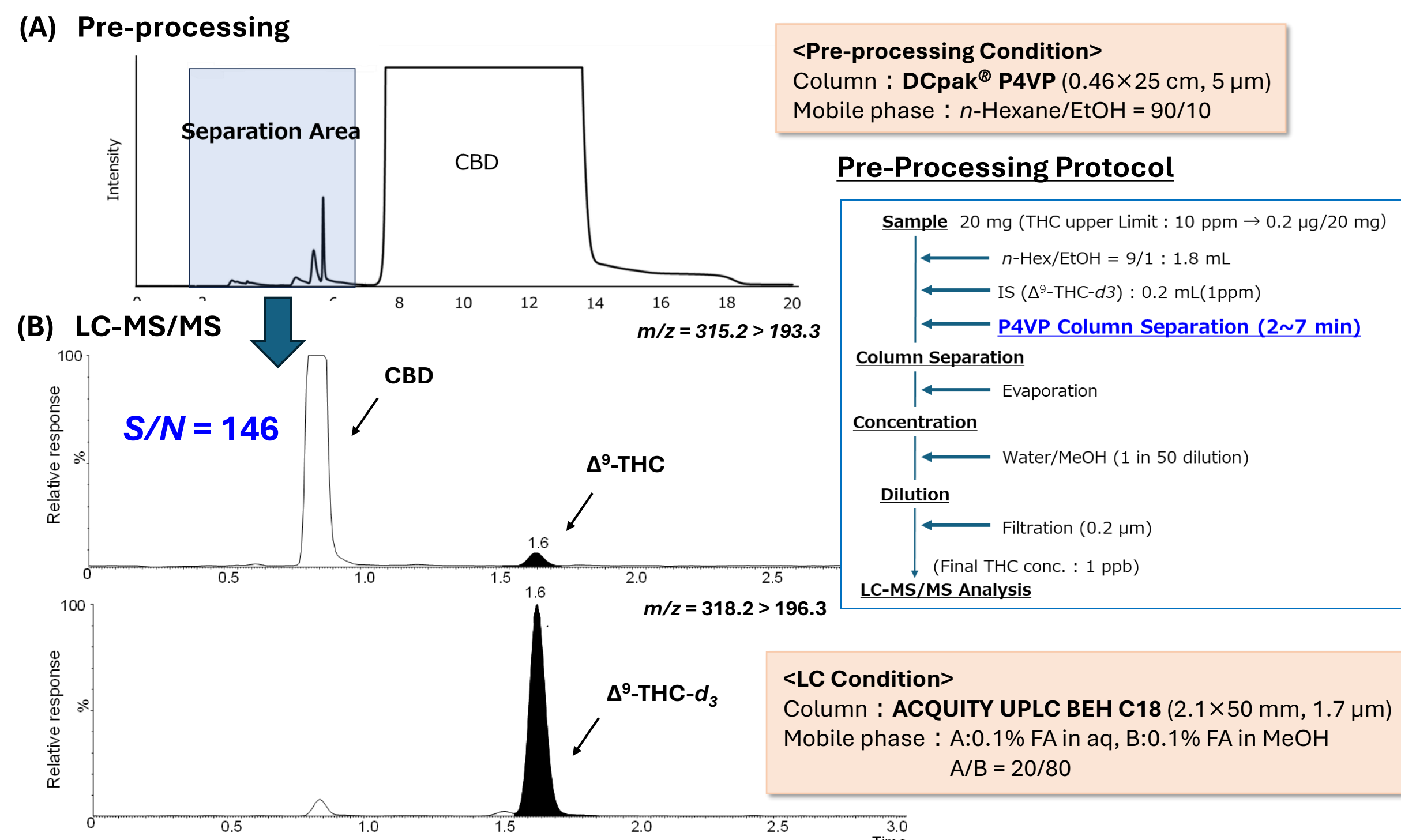


Fig.2. CBD removal and Δ^9 -THC quantification using a P4VP-based pre-processing protocol¹ Patent application filed

This Work 1:

MS-Compatible Quantification without Pre-Processing

- A Polymer-based stationary phase, DCPak® P4VP, enabled direct Δ^9 -THC quantification without pre-processing
 - CBD tailing and matrix effects were suppressed, enabling selective detection of trace Δ^9 -THC (Fig. 3)
- This approach provided a simple, robust, and MS-compatible workflow for high-throughput quantification**

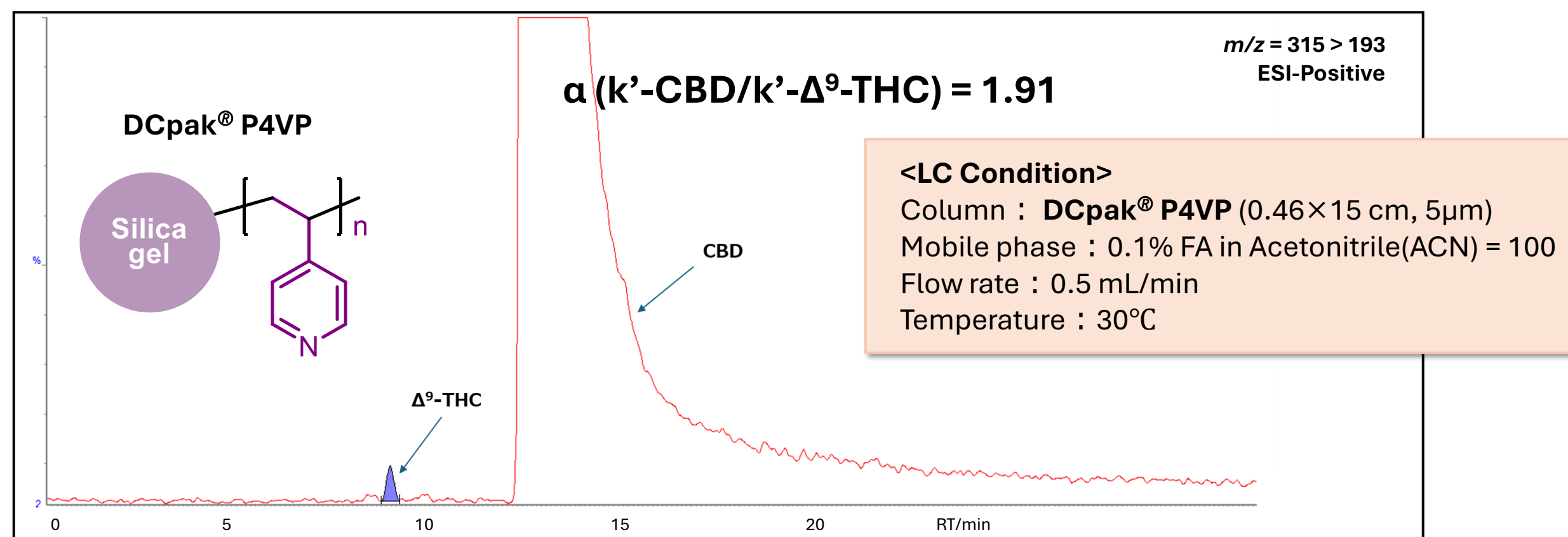


Fig.3. Chromatogram for Δ^9 -THC separation in high concentration CBD matrices on DCPak® P4VP.

Previous Work 2:

Chiral Separation Using Polysaccharide Chiral Stationary Phases (CSPs)

- Polysaccharide CSPs enable separation of enantiomers and isomers
- A previously reported method² used TFA, limiting MS compatibility (Fig. 4, 5) (Table1)
- Practical conditions combining chiral separation and MS compatibility remain a requirement.
- Elution order under MS-compatible conditions has not been fully established yet.

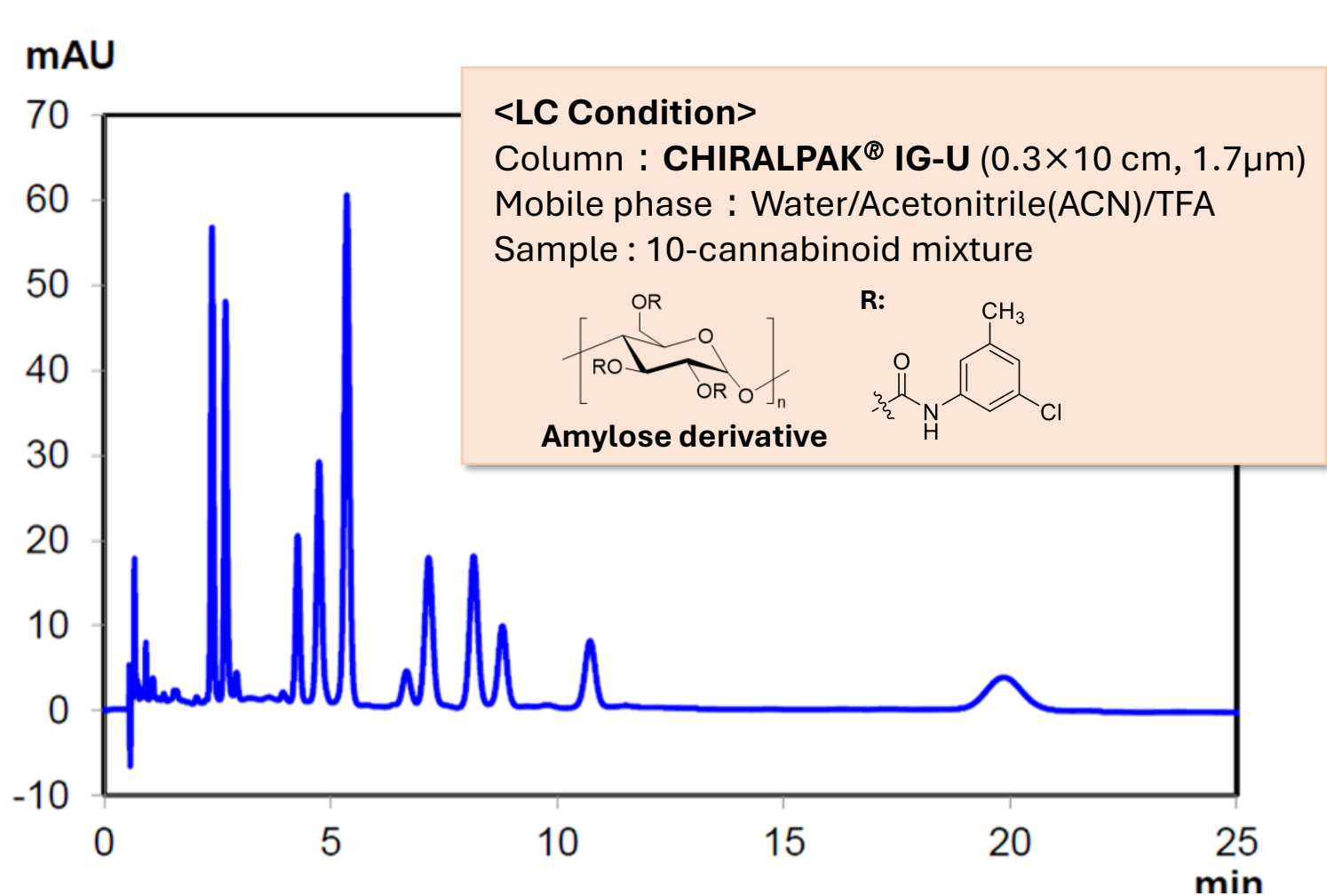


Fig.4. 10-cannabinoid mixture separation under RP-conditions with CHIRALPAK® IG-U.

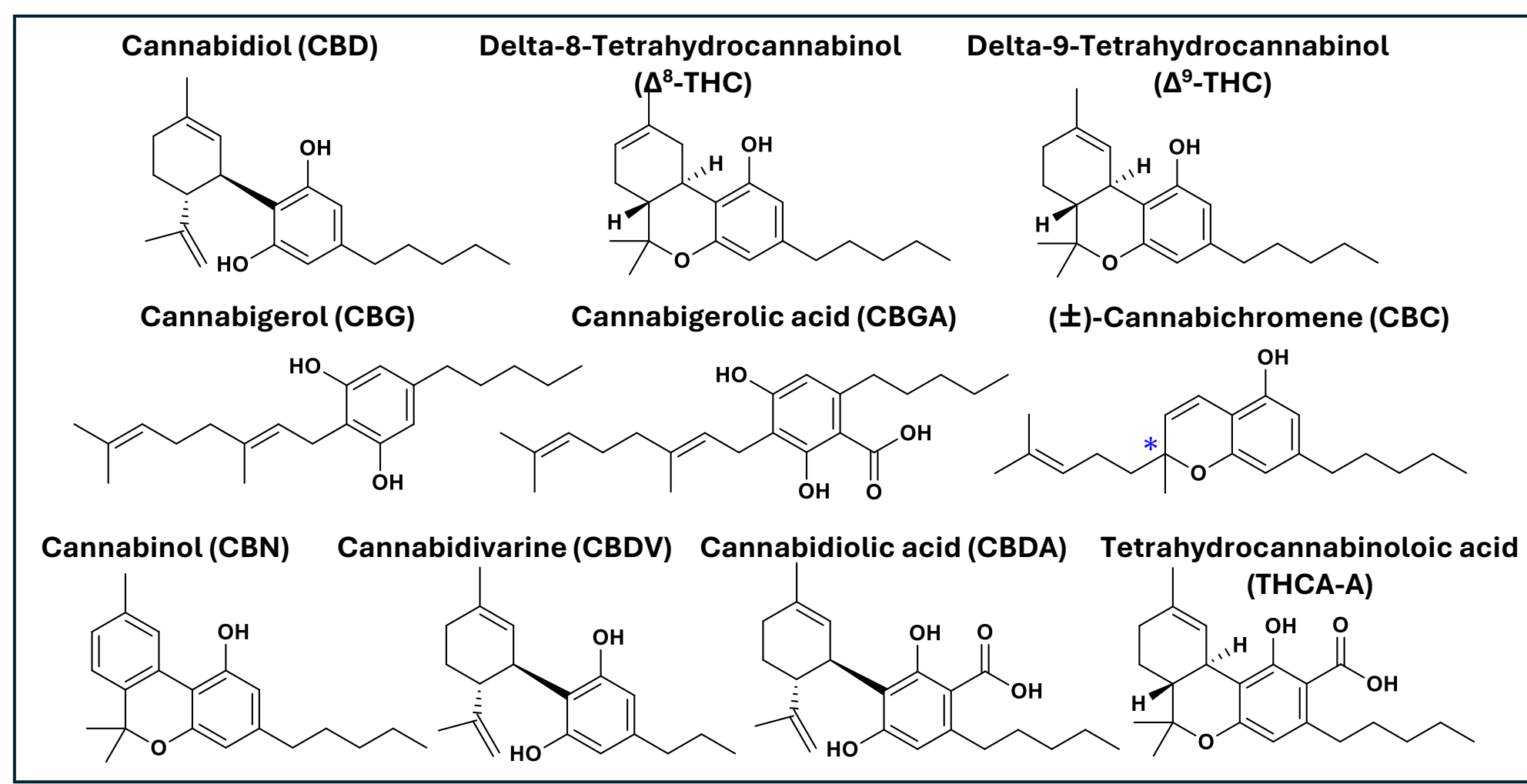


Fig.5. Structures of cannabinoids in 10-cannabinoid mixture (purchased from Cayman Chemical Company in Ann Arbor, MI, USA)

Table1. Elution times for 10-cannabinoid RP-phase separation on CHIRALPAK® IG-U.

	CBDA	CBG	CBN	CBD	Δ^8 THC	Δ^9 THC	CBC	THCA-A	Total Time
Elution Time (min)	2.39	4.28	5.37	6.68	7.17	8.16	8.79 10.74	19.81	25.00

² Onishi, T.; Umstead, W. J. Pharmaceuticals **2021**, *14*, 1250.

This Work 2: MS-Compatible Chiral Separation

- MS-compatible reversed-phase (RP) method development screening was performed on CHIRALPAK® IC-3, ID-3, and IG-3. (Fig. 5) (Table2)
- The mixture is reported as 10 compounds, however 11 peaks were observed in most methods due to the separation of CBC enantiomers.
- The elution order of CBGA and CBDV, previously unclear, was clarified, improving peak assignment
- Column-dependent selectivity indicated that IC, ID, and IG columns provided complementary separation behavior, enabling flexible method design

This approach enabled simultaneous characterization of both structural and stereochemical information under MS-compatible conditions

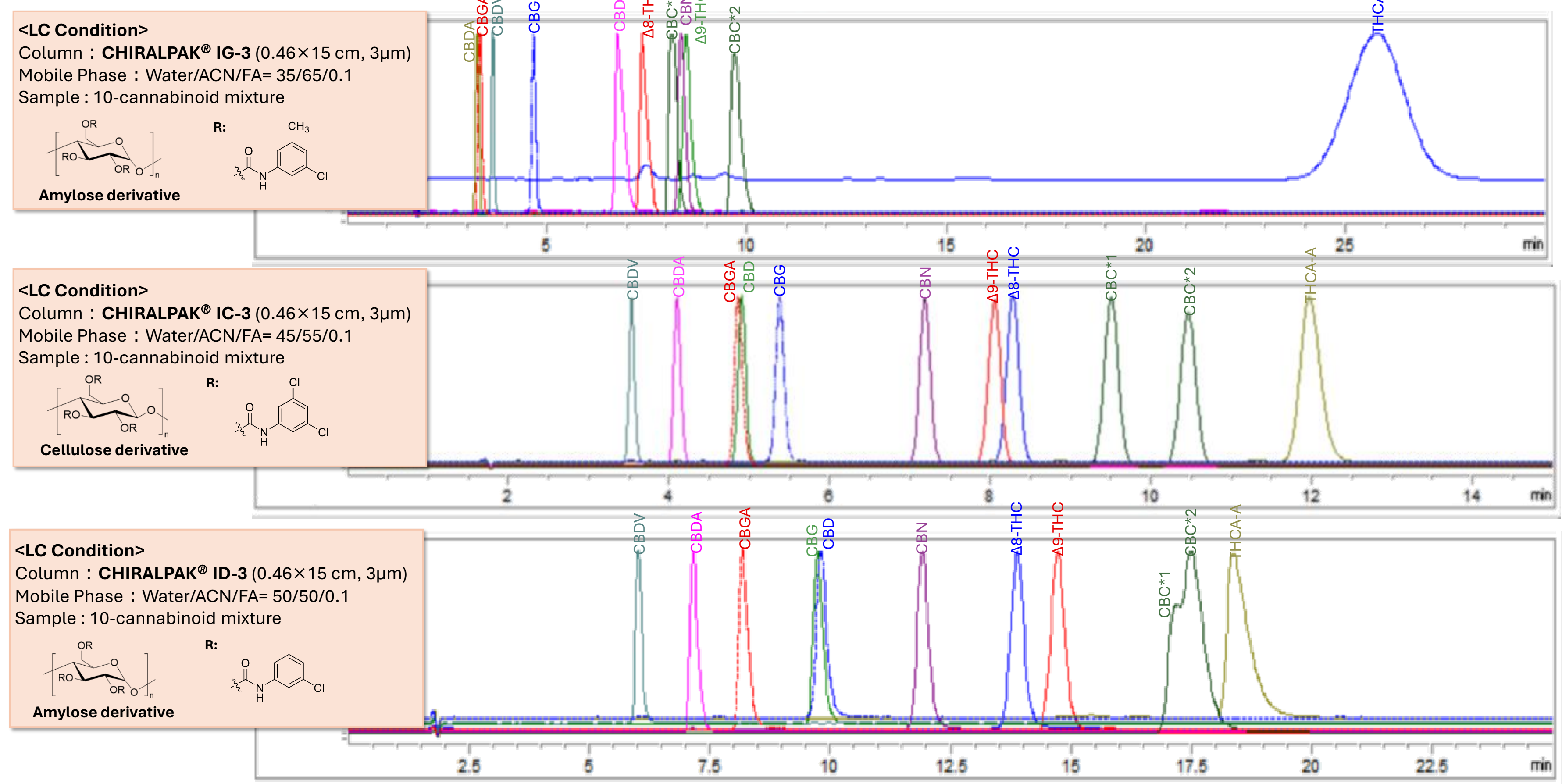


Fig.6 Chromatograms for 10-cannabinoid MS-compatible RP-phase separation on CHIRALPAK® IG-3 / IC-3 / ID-3.

Table2. Elution orders for 10-cannabinoid separation on CHIRALPAK® IG-3 / IC-3 / ID-3.

Column	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11	
CHIRALPAK® IG-3	CBDA	CBGA	CBDV	CBG	CBD	Δ^8 -THC	CBC* 1	CBN	Δ^9 -THC	CBC* 2	THCA-A	* : enantiomer pair
CHIRALPAK® IC-3	CBDV	CBDA	CBGA	CBD	CBG	CBN	Δ^9 -THC	Δ^8 -THC	CBC* 1	CBC* 2	THCA-A	: coeluted
CHIRALPAK® ID-3	CBDV	CBDA	CBGA	CBG	CBD	CBN	Δ^8 -THC	Δ^9 -THC	CBC* 1	CBC* 2	THCA-A	

Conclusion :

- ✓ Direct MS-compatible quantification of Δ^9 -THC in high-CBD matrices was achieved using DCPak® P4VP without pre-processing
- ✓ Chiral separations on CHIRALPAK® IC-3, ID-3, and IG-3 resolved multiple components and clarified the elution order
- ✓ The complementary selectivity of IC-3/ID-3/IG-3 enabled flexible and application-specific method design
- ✓ The approach provides a reliable platform for regulatory testing under MS-compatible conditions
- ✓ In the future, the method can be extended to other cannabinoids and more complex matrices for broader analytical applications