Determination of Cannabinoids in Hemp seed oil using LC/MS/MS with a Novel End-capping C18 Column

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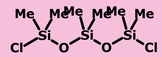
Novel End-capping

An End-capping has been recognized to be an important factor for a silica based reversed phase column. In this study, not only bonding with an end-capping reagent but also conversion of silanol groups to siloxane bond by heating were evaluated as an end-capping.

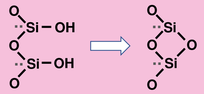
Double end-capping



Hexamethyldichlorotrisiloxane

Trimethylchrolosilane

Elevated temperature end-capping

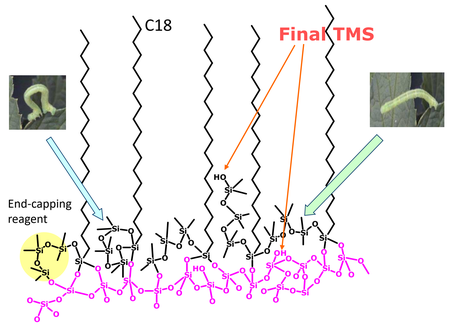


Conversion from silanol groups to siloxane bond

- **High stability (pH 1 to 10)**
- **Low bleeding**

Column end-capped by novel method:
Thunder C18 and SunShell C18

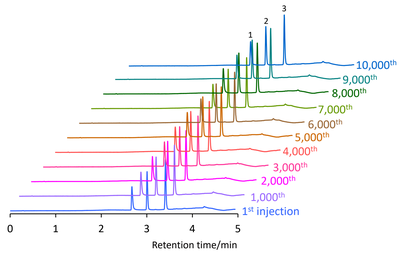
End-capping with hexamethyldichloro-trisiloxane and TMS on C18 silica



End-capping reagent moves like a *Geometrid caterpillar*, so that a functional group on the tip of the arm can bond with a silanol group which is located anywhere.

We named this end-capping method as Sunniest double end-capping.

Stability under acidic pH condition



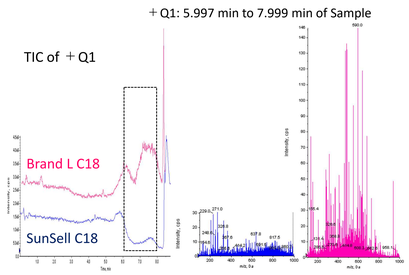
Column: SunShell C18 2.6 µm, 50 x 2.1 mm
Mobile phase: A) 0.1% trifluoroacetic acid pH 2.0
B) Acetonitrile

Gradient program:

Time (min)	0	3	3.1	5
% B	10	90	10	10

Flow rate: 0.5 mL/min
Temperature: 40 °C
Detection: UV@270nm
Injection volume: 0.5 µL
Sample: 1 = Benzydamin (0.5 mg/mL), 2 = Ketoprofen (0.04 mg/mL), 3 = Indomethacin (0.05 mg/mL)

Bleeding test using LC/MS



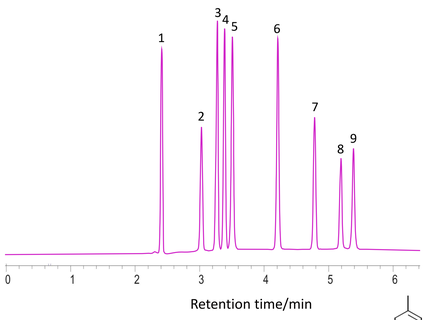
Column size: 50 x 2.1 mm
Mobile phase: A) 0.1% acetic acid
B) Acetonitrile

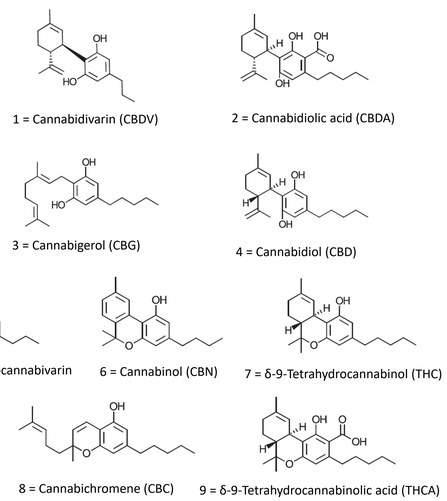
Gradient program:

Time (min)	0	1	5	7
% B	5	5	100	100

Flow rate: 0.4 mL/min
Temperature: 40 °C
MS: ABI API-4000
Ionization: Turboionspray (cation)
Measurement mode: Q1 Scan m/z 100-1000

Separation of 9 kinds of cannabinoids





CONDITIONS
Column: SunShell C18 2.6 µm, 100 x 2.1 mm
Mobile Phase: A) 0.1% formic acid in water
B) 0.1% formic acid in acetonitrile

Time (min)	0	1	8	10
%B	70	70	100	100

Flow rate: 0.4 mL/min
Temperature: 40 °C
Detection: UV@210 nm

Measurement of CBD and THC in hemp seed oil

Table 1. Preparation of samples

Sample pretreatment

500mg sample
↓
Add Methanol — 10 mL
↓
shake for 10 minutes
↓
Centrifuge — 4000 rpm, 10 min, 4 °C
↓
Collection of supernatant
↓
0.25 µm syringe filtering (PTFE)
↓
LC/MSMS

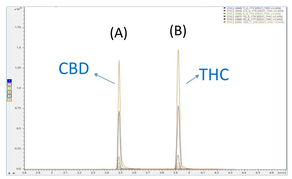


Fig 1. (A), (B) : 1, 2.5, 5, 10, 50, 100 ppb STD Overlay

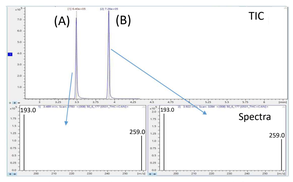
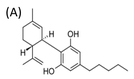
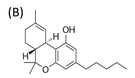


Fig 2. (A), (B) : TIC (Total Ion Chromatogram) / Spectra

(A) 
Cannabidiol (CBD)

(B) 
δ-9-Tetrahydrocannabinol (THC)

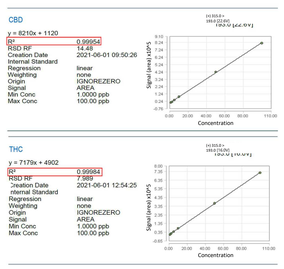


Fig 3. Verification of Calibration Curve (1 ~ 100 ppb)

Table 2. CBD / THC MRM Transitions

Compound Name	R.T.	Precursor ion	Product ion	CE (V)	Polarity
CBD	3.49	315	193	16.0	Positive
CBD	3.49	315	259	16.9	Positive
THC	3.92	315	193	22.6	Positive
THC	3.92	315	259	22.5	Positive

Table 3. Measurement result of hemp seed oil

	CBD	THC
Average (ppb)	44.2	3.0
Dilution	20	20
Calculation of concentration (ppb)	884 (n=10)	60

Column: Thunder C18 2.7 µm, 150 x 2.1 mm
Mobile phase: A) 0.1% Formic acid in water
B) 0.1% Formic acid in acetonitrile

Time (min)	0	0.5	2.5	6	6.1	10
%B	30	30	80	80	30	30

Flow rate: 0.4 mL/min
Temperature: 40 °C
Injection Volume: 5 µL
Peaks: A = Cannabidiol (CBD, 1000 µg/mL)
B = δ-9-Tetrahydrocannabinol (THC, 10 µg/mL)
Sample: Hemp seed oil

MS conditions
Source type: HESI
Spray voltage: 4500 V (Positive)
Cone temperature: 250 °C
Cone gas pressure: 20 psi
Heated probe temperature: 300 °C
Probe gas pressure: 40 psi
Nebulizer gas pressure: 40 psi
Active exhaust: ON

Conclusion

- ◆Hexamethyldichlorotrisiloxane and trimethylchlorosilane were used as end-capping reagents. Silanol groups were changed to siloxane bonding at an elevated temperature. As a result, stability increased drastically.
- ◆Nine kinds of cannabinoids were successfully separated using the C18 with novel end-capping.
- ◆The concentrations of CBD and THC in hemp seed oil determined using LC/MS/MS were 884 ppb and 60 ppb, respectively.