CUSTOMER APPLICATION NOTE 73324

A novel, bio-based carrier for residual solvents analysis in cannabis extracts

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Goal

To validate a modification of USP <467> for residual solvents using Cyrene™, a green alternative to DMF and DMSO for the analysis of residual solvents in cannabis extracts.

Introduction

The United States Pharmacopeia (USP) Method <467> is a widely used standard for determining residual solvents in pharmaceuticals.[1] The limits established in USP <467> have been extended to other products, such as cannabis. Health Canada's Limits for Residual Solvents in Cannabis Products is nearly identical to USP's list of Class 3 residual solvents.[2] Commercially available standards and dilutions as per USP <467> are prepared in N,N-dimethylformamide (DMF) or dimethyl sulfoxide (DMSO), both of which are harmful and potentially toxic organic solvents.[3-5]

Dihydrolevoglucosenone (Cyrene™, Figure 1), a bio-based and biodegradable solvent produced from the pyrolysis of cellulose in wood, is proposed as a less toxic and safer alternative solvent to DMF/DMSO in residual solvents analysis.[6,7] In this application note, a method adapted from USP <467> and spanning all three residual solvent hazard classes (with some compounds excluded), was validated using Cyrene.

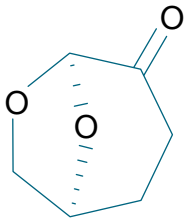

Figure 1. The chemical structure of Cyrene

Experimental

Consumables

- Dihydrolevoglucosenone (Cyrene)
- Headspace-grade DMSO
- USP <467> Residual Solvent (RS) Class 1, 2a, 2b, and 3a Mixes
- Headspace vials (20 mL) with PTFE-lined silicone septa and screw caps

Sample preparation

- Calibration standards were prepared using USP <467> residual solvent class mixes ranging from 0.2 to 2 times the USP method report limit (MRL) for Class 1-3 RS (Table 4) with Cyclohexane-d_{12} used as the internal standard.
- 250 mg of simulated cannabis extract were weighed and transferred into a 50 mL centrifuge tube for extraction in 25 mL of Cyrene then spiked with USP <467> analytical standards.
- After samples were vortexed and sonicated, 1 mL of supernatant was transferred to a HS vial and diluted by 5 mL of HPLC water for headspace analysis.
- Spike and recovery samples were prepared at low, mid, and high concentrations at 0.5×, 1×, and 3× the relevant USP limits, respectively.
- Duplicate, routine recovery samples, check standards, and method blanks were prepared with each spike and recovery sample.
- Samples were stored at 2–8 °C and protected from light until ready for processing.

Chromatography

Chromatographic separations were done using the Thermo Scientific™ TRACE™ 1300 Gas Chromatograph (GC) with the Thermo Scientific™ TriPlus™ 500 Headspace Autosampler, on a Thermo Scientific™ TraceGOLD™ TG-624SilMS 30 m × 0.25 mm ID × 1.4 µm capillary column (Figure 2). GC conditions are listed in Tables 1 and 2.

Mass spectrometry

Mass spectrometry (MS) analysis was performed on a Thermo Scientific™ ISQ™ 7000 Single Quadrupole GC-MS system. Acquisition was performed using timed selected ion monitoring (t-SIM). Two ions were monitored for each analyte with 0.80 min acquisition windows, resulting in a minimum dwell time of 16.8 ms. MS conditions are listed in Table 3.

Figure 2. TriPlus 500 Headspace Autosampler (A), TRACE 1300 Gas Chromatograph (B), and ISQ 7000 Single Quadrupole GC-MS (C)

Table 1. GC temperature gradient

Time (min)	Temp. (°C)	Rate (°C/min)	Hold (min)
0.0	35.0	-	10.0
20.0	40.0	0.5	-
40.0	300.0	15.0	2.0

Table 2. TriPlus 500 Headspace Autosampler method parameters (1000 µL sample loop)

Parameter	Value
Incubation temp (°C)	95.0
Incubation time (min)	15.0
Vial shaking	Fast
Pressurization mode	Pressure
Vial pressure (kPa)	100.0
Loop/sample path temp (°C)	110.0
Loop pressure (kPa)	50.0
Loop equilibration (min)	0.20
Injection mode	Standard
Injection time (min)	0.50
Inlet temp (°C)	140.0
Purge flow (mL/min)	5.0
Auxiliary gas	Nitrogen
Carrier gas	Helium
Carrier flow rate	CF 1.2 mL/min
Split flow	50:1

Table 3. Selected ion monitoring MS acquisition parameters

Parameter	Value
Transfer line (°C)	250
Source temp. (°C)	300
Ionization mode	EI, 70 eV

Results and discussion

Calibration curves for all analyzed compounds in Cyrene were linear except for methanol, which had a quadratic fit. The spike and recovery results for the USP <467> compounds extracted in Cyrene for low, mid, and high fortifications ranged from 86 to 122%, 76 to 112%,and 81 to 112%, respectively. Carryover was less than 0.1% of the highest calibration concentration for all analyzed compounds. On average MDLs were 4.5× lower than the MRL, demonstrating that USP <467> residual solvents can be reliably quantified using Cyrene as the solvent. Single day precisions (RSD_r) ranged from 1.4 to 5.5% and inter-day precision (RSD_R) from a single analyst ranged from 3.2 to 21.8%. The following compounds were excluded due to poor method performance: acetic acid, formic acid (Class 3); 1,2-dimethoxyethane, pyridine, 2-ethoxyethanol, 2-methoxyethanol, ethylene glycol, formamide, N-methylpyrrolidine, N,N-dimethylacetamide, sulfolane (Class 2); 1,2-dichloroethane (Class 1). DMSO (Class 3) and DMF (Class 2) were also excluded, as purchased standard solutions were prepared in these solvents. All other residual solvents included in USP <467> were validated by this method (Figure 3 and Table 4).

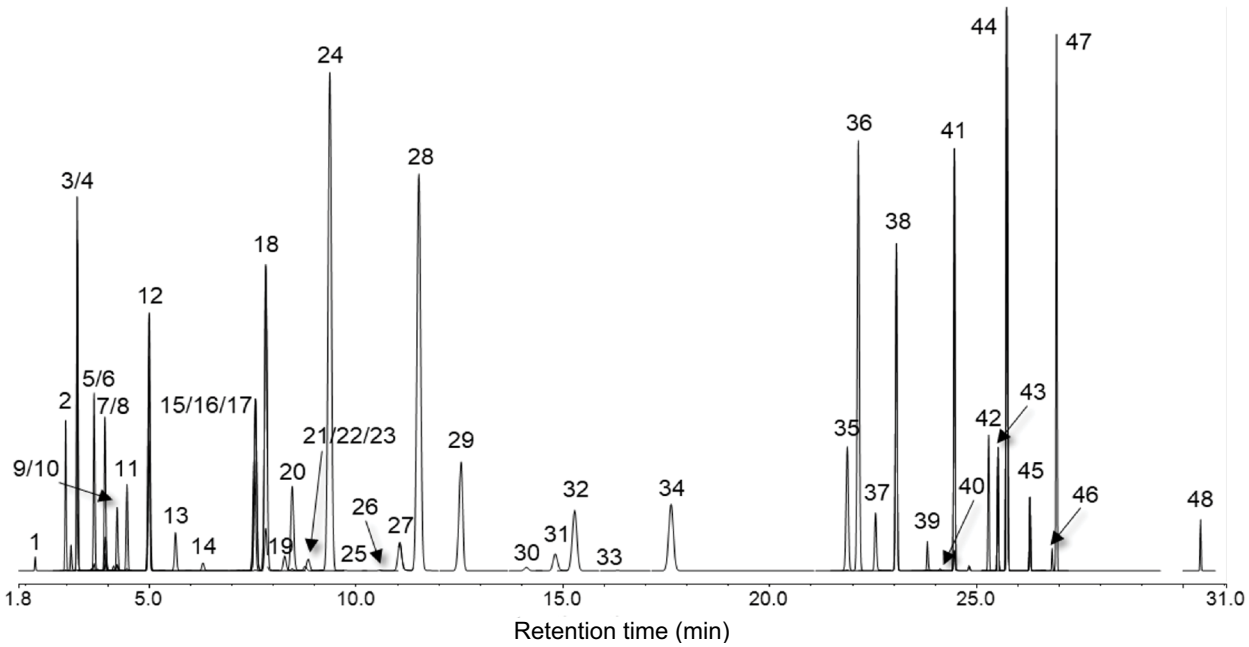

Figure 3. Sample chromatogram of target analytes at their USP limits (see Table 4) on a TraceGOLD TG-624SilMS 30 m × 0.25 mm ID × 1.4 µm capillary GC column at 1.2 mL/min and 50:1 split ratio. Analytes that co-elute were all mass resolved using selected ion monitoring.

Table 4 (part 1). Method validation results for residual solvents in simulated cannabis extracts (hops extracts). All calibration functions were weighted 1/×, with a linear fit (except methanol, which was quadratic). Carryover was <0.1% of the highest calibration concentration for all compounds. Compounds without a resolution value had no interfering peaks. Low, mid, and high spike recovery samples were fortified at 0.5×, 1×, and 3× the relevant USP limits, respectively.

ID	Analyte (USP Class)	RT min	R_s [a]	Low %	Mid %	High %	RSD_r [b] %	RSD_R [c] %	MDL [d] ppm	MRL [e] ppm
1	Methanol (2)	2.22	-	122	109	86	5.6	7.9	921	3000
2	*n*-Pentane (3)	2.98	5.0	94	95	81	3.0	5.9	981	5000
3	Ethanol (3)	3.11	2.7	105	105	112	5.5	8.3	1540	5000
4	Diethyl ether (3)	3.25	-	96	100	97	1.4	3.2	465	5000
5	1,1-Dichloroethene (1)	3.60	2.5	101	98	98	2.0	4.8	1.14	8
6	Acetone (3)	3.66	3.8	100	103	108	3.4	5.6	1050	5000
7	Ethyl formate (3)	3.93	4.7	102	98	95	3.1	5.3	898	5000
8	2-Propanol (3)	3.95	2.7	105	102	103	4.8	7.9	1480	5000
9	Acetonitrile (2)	4.13	1.3	100	102	107	5.3	8.6	130	410
10	Methyl acetate (3)	4.22	-	103	102	103	2.9	6.6	1170	5000
11	Methylene chloride (2)	4.47	-	109	106	102	3.2	6.0	131	600
12	Methyl *t*-butyl ether (3)	4.99	-	107	104	97	2.0	3.3	613	5000
13	*trans*-1,2-Dichloroethene (2)	4.99	-	103	103	102	3.6	6.0	375	1870
14	Hexane (2)	5.63	-	95	76	84	1.4	17	124	290
15	1-Propanol (3)	6.30	-	86	97	101	5.4	6.9	1270	5000
16	Nitromethane (2)	7.36	1.8	106	100	102	4.3	7.3	13.7	50
17	*cis*-1,2-Dichloroethene (2)	7.54	-	105	102	103	3.0	5.0	318	1870
18	2-Butanone (3)	7.57	2.2	105	105	103	3.6	6.1	1150	5000
19	Ethyl acetate (3)	7.79	7.7	101	103	102	3.0	5.3	964	5000
20	Tetrahydrofuran (2)	8.27	1.4	104	103	105	3.4	5.6	145	720
21	2-Butanol (3)	8.46	-	98	101	106	4.5	6.1	1110	5000
22	Chloroform (2)	8.76	-	103	100	103	3.4	5.6	11.9	60
23	1,1,1-Trichloroethane (1)	9.16	-	104	102	104	3.0	4.7	232	1500
24	Cyclohexane (2)	9.36	-	101	103	101	2.7	6.5	869	5000

[a] R_s – Chromatographic resolution (based on FWHM)
[b] RSD_r % – Single day precision (n = 5)
[c] RSD_R % – Interday precision from a single analyst (n = 5/day over three days)
[d] MDL – The method detection limit, calculated using replicate extractions over three days (n = 7), with two analysts preparing at least one replicate[8]
[e] MRL – The method reporting limit, as verified using the prediction interval of results outlined, for example, in EPA water quality methods[9] (The MRL is equivalent to the USP limit for all analytes in the developed method.)

Table 4 (part 2). Method validation results for residual solvents in simulated cannabis extracts (hops extracts). All calibration functions were weighted 1/×, with a linear fit (except methanol, which was quadratic). Carryover was <0.1% of the highest calibration concentration for all compounds. Compounds without a resolution value had no interfering peaks. Low, mid, and high spike recovery samples were fortified at 0.5×, 1×, and 3× the relevant USP limits, respectively.

ID	Analyte (USP Class)	RT min	R_s [a]	Low %	Mid %	High %	RSD_r [b] %	RSD_R [c] %	MDL [d] ppm	MRL [e] ppm
25	Carbon tetrachloride (1)	9.72	-	102	100	102	3.7	5.3	0.665	4
26	Benzene (1)	10.59	-	106	101	103	3.8	5.9	0.382	2
27	Isobutanol (3)	11.05	-	94	99	107	4.9	4.8	887	5000
28	Isopropyl acetate (3)	11.51	-	107	106	103	2.8	5.1	972	5000
29	*n*-Heptane (3)	12.54	-	114	112	104	2.8	14.8	2280	5000
30	Trichloroethylene (2)	14.11	-	104	100	106	3.4	5.9	16.5	80
31	1-Butanol (3)	14.84	-	99	93	101	5.5	6.1	1050	5000
32	Methylcyclohexane (2)	15.28	-	99	100	108	3.1	8.7	290	1180
33	1,4-Dioxane (2)	16.33	-	104	100	107	5.3	8.0	113	380
34	Propyl acetate (3)	17.61	-	106	101	105	2.4	6.0	1100	5000
35	4-Methyl-2-pentanone (3)	21.87	-	108	103	104	2.8	5.5	1050	5000
36	Toluene (2)	22.15	-	97	99	107	3.4	7.2	224	890
37	3-Methyl-1-butanol (3)	22.57	-	97	93	101	5.1	5.5	972	5000
38	Isobutyl acetate (3)	23.05	24	108	105	105	2.5	6.4	1250	5000
39	1-Pentanol (3)	23.84	-	94	95	102	5.4	6.6	1190	5000
40	2-Hexanone (3)	24.12	4.8	106	100	104	3.1	6.6	12.4	50
41	Butyl acetate (3)	24.46	6.6	109	106	105	2.6	6.8	1350	5000
42	Chlorobenzene (2)	25.29	-	99	99	107	2.4	7.4	94.7	360
43	Ethylbenzene (1)	25.52	3.8	100	102	109	3.4	10.6	137	368
44	*m/p*-Xylene (1)	25.73	4.1	100	106	108	3.0	10.4	576	1606
45	*o*-Xylene (1)	26.29	9.8	102	99	108	3.2	10.7	70.2	196
46	Cumene (2)	26.84	-	104	101	109	2.9	13.0	30.8	70
47	Anisole (3)	26.94	-	97	103	108	3.0	6.0	1130	5000
48	Tetralin (2)	30.41	-	103	99	107	3.7	21.8	68.2	100

[a] R_s – Chromatographic resolution (based on FWHM)
[b] RSD_r % – Single day precision (n = 5)
[c] RSD_R % – Interday precision from a single analyst (n = 5/day over three days)
[d] MDL – The method detection limit, calculated using replicate extractions over three days (n = 7), with two analysts preparing at least one replicate[8]
[e] MRL – The method reporting limit, as verified using the prediction interval of results outlined, for example, in EPA water quality methods[9] (The MRL is equivalent to the USP limit for all analytes in the developed method.)

Beyond safety and environmental concerns, using Cyrene instead of DMSO or DMF offers other advantages. While it is currently unfeasible due to the availability of standards, using Cyrene as the primary solvent for this method could allow DMSO and DMF to be quantitated in the same method. This is a necessary step towards an ultimate goal of covering all USP <467> residual solvents in a minimal number of injections. The importance of this objective is underscored by the trend of standard-setting organizations to use USP <467> as the basis for residual solvent testing in cannabis extracts.

Cyrene has a higher boiling point and lower vapor pressure than either DMSO or DMF. This suggests that using it as a solvent for headspace analysis would allow high-temperature headspace work to be pushed further; the upper temperature limit of a headspace method is often dictated by the head pressure generated by the bulk solvent (i.e., what the headspace vial and injection system can handle).

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Conclusion

Dihydrolevoglucosenone (Cyrene) is a biodegradable and safer alternative to DMF and DMSO for the analysis of residual solvents in cannabis extracts. A modification of USP <467> for residual solvents was validated using Cyrene as a solvent. This approach may improve laboratory throughput by minimizing the number of analyses needed to cover USP <467> analytes, including DMR and DMSO, and allow headspace analysis to occur at higher temperatures, increasing sensitivity of analysis.

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