Cannabinoid and terpenoid extraction and analysis

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Abstract

There has been an increased use of medical *Cannabis* in the United States of America as more states legalize its use. Complete chemical analyses of this material can vary considerably between producers and is often not fully provided to consumers. We report the development of a simple extraction and analysis method, amenable to use by commercial laboratories for the detection and quantification of both cannabinoids and terpenoids. Inclusion of terpenoids with cannabinoids in the analysis of medical cannabis should be encouraged, as both of these classes of compounds could play a role in the beneficial medical effects of different cannabis strains.

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Protocol

Step 1.
Cured/dried flowers or dried leaves were homogenized in 20 mL of reagent-grade acetone using a Polytron Homogenizer (Kinematica).

Step 2.
The homogenates were placed on an orbital shaker for 1 h.

Step 3.
Samples were diluted in four parts of acetone containing 1 mg/mL chlorophenol and 2 mg/mL phenacetin (ISS1 and ISS2, respectively).

Step 4.
The samples were then centrifuged briefly to remove insoluble matter and analyzed using a Varian model 3900 GC-FID with an Rxi-35 column (15 m x 0.25 mm).

Step 5.
Ultrapure nitrogen was employed as the carrier gas (flow rate: 1 mL/min).

Step 6.
The injection volume was 2.0 µL, split 10, injector temperature 250°C, FID temperature 300°C.

Step 7.
The temperature gradient for the analysis started at 45°C (with a 1 min hold), and increased to 240° at 10°/min, then increased to 280°C at 4°/min, then finally increased to 290°C at 20°/min with a one-minute hold.

Step 8.

This method proved adequate to separate six of the more abundant cannabinoids (Δ9-THC, CBD, CBC, CBG, Δ9-THCV and CBN) and 21 terpenoids.

EXPECTED RESULTS

Table 1. Linear Calibration Parameters for Quantification of Terpenoids and Cannabinoids

Peak ID	RT[a]	Analyte	Slope	Intercept	R2[b]
ISS[c]-1	3.67	Chlorobenzene	n/a	n/a	n/a
T1	4.19	a-Pinene	713	593	0.9998
T2	4.55	Camphene	729	510	0.9998
T3	4.98	b-Pinene	737	336	0.9998
T4	5.11	Myrcene	699	138	0.9998
T5	5.40	Carene	792	319	0.9998
T6	5.55	a-Terpenine	728	116	0.9998
T7	5.71	Limonene	825	186	0.9998
T8	5.89	*cis*-Ocimene[d]	857	15	0.9997
T9	5.96	p-Cymene	743	-118	0.9996
T10	6.02	*trans*-Ocimene	Combined T8 and T10 to calculate		
T11	6.27	g-Terpenine	765	81	0.9998
T12	6.72	Terpinolene	750	237	0.9998
T13	6.96	Linalool	679	-265	0.9996
T14	7.79	(-)-Isopulegol	681	-533	0.9995
T15	9.35	Geraniol	693	-575	0.9994
T16	11.07	b-Caryophyllene	789	-301	0.9996
T17	11.56	a-Humulene	814	-367	0.9997
T18	12.48	*cis*-Nerolidal[d]	752	-767	0.9993
T19	12.86	*trans*-Nerolidal	Combined T18 and T19 to calculate		
T20	13.54	(-)-Guaiol	787	-655	0.9994
T21	14.42	Bisabolol	777	-22	0.9993
ISS-2	16.12	Phenacetin	n/a	n/a	n/a
C1	21.14	THCV	662	4649	0.9998
C2	21.83	CBC	661	6232	0.9997
C3	21.96	CBD	640	5426	0.9997
C4	23.12	Δ9-THC	680	5147	0.9998
C5	23.51	CBG	660	5600	0.9997
C6	23.92	CBN	597	5061	0.9997

The regression equation parameters for each analyte standard were determined using at least 10 different concentrations between 0 and 520 ng for the terpenoids and between 0 and 3880 ng for the cannabinoids. These parameters predict the mass of the analyte from the GC-FID peak area (y = mx + b; where y is the peak area, m is the slope, x is the mass in ng, and b is the intercept on the y axis). Peak ID is the code for each of the 21 terpenoids (T1-T21) and six cannabinoids (C1-C6), for which a calibration curve was generated.