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## Mass Spectrometric Differentiation of Cannabinoid-Containing Samples

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**ABSTRACT:** A direct-inlet probe mass spectrometry/multiple regression procedure is described for the confirmation of the presence of cannabinoids and the comparison of various cannabinoid-containing samples. The relative intensities of selected mass units are first established on control cannabinoids. These standards are then regressed on mass spectra of samples of interest. High values of coefficients of determination are used to confirm the presence of cannabinoid mixtures; regression coefficients are used for sample comparisons.

**KEY WORDS:** toxicology, marihuana, spectroscopic analysis

Cannabinoid-containing samples, in the forms of marihuana, hashish, and hashish oil, are commonly encountered in forensic science laboratories. While microscopic observation and the Duquenois-Levine test are effective for the examination of marihuana samples, more specific methods are generally used for hashish and hashish oil samples where morphological characteristics are lacking.

The combination of direct-inlet probe mass spectrometry and multiple regression analysis has been successfully used in the characterization of cannabinoid mixtures in marihuana [1]. The work presented here demonstrates the extension of this approach to hashish and hashish oil samples. More significantly, this procedure is used in the differentiation of cannabinoid-containing samples. Sample differentiations are based on relative component concentrations in contrast to the comparison of <sup>13</sup>C contents as established previously [2]. The combination of these two comparative approaches should contribute to achieving the goal of sample individualization.

### Materials and Methods

A Varian MAT 112S mass spectrometer and a MAT SS166/SS144 Spectro System (Bremen, West Germany) were used for this study. A Tektronix 4010-1 cathode-ray tube and a Tektronix 4631 hard-copy unit were used for data display and hard-copy production, respectively. A typical experiment used 0.2 mg of pulverized sample (or control cannabinoid), which was introduced into the mass spectrometer via a direct-inlet probe. The probe was rapidly (in less than 5 s) heated to and maintained at 100°C. The source temperature

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was maintained at 220°C and operated in an electron impact mode at 67 eV. Spectra were continuously scanned at 5 s per decade in exponential time function mode, with 0.2 s rest between each scan. Scanning was stopped when the most intense peak decreased to an insignificant intensity compared to that in the most intense spectrum.

Authentic  $\Delta$ -1-tetrahydrocannabinol ( $\Delta$ -1-THC),  $\Delta$ -6-tetrahydrocannabinol ( $\Delta$ -6-THC), cannabinol (CBN), and cannabidiol (CBD) were obtained from Applied Science Laboratories (State College, Pa.). Samples were obtained from the Chicago Police Crime Laboratory.

The Regression Subprogram in SPSS [3] was used for multiple regression analysis as described below.

## Results and Discussion

Under identical conditions, mass spectra of a cannabinoid-containing sample can be related to those of pure cannabinoids as follows [1]:

$$\begin{aligned} Y_1 &= X_{11}r_1C_1 + X_{12}r_2C_2 + \dots + X_{1m}r_mC_m \\ Y_2 &= X_{21}r_1C_1 + X_{22}r_2C_2 + \dots + X_{2m}r_mC_m \\ &\vdots \\ Y_n &= X_{n1}r_1C_1 + X_{n2}r_2C_2 + \dots + X_{nm}r_mC_m \end{aligned} \quad (1)$$

where

- $Y_n$  = observed intensity of the peak  $n$  in the sample,
- $X_{nm}$  = relative intensity of the peak  $n$  in the spectrum obtained from the cannabinoid  $m$ ,
- $r_m$  = relative sensitivity factor of cannabinoid  $m$  under the experimental conditions, and
- $C_m$  = concentration of cannabinoid  $m$  in the sample under examination.

It has been reported [1] that the regression of control cannabinoid spectra to those of cannabinoid-containing samples results in different regression coefficients ( $r_m C_m$  terms in Eq 1). The variation of these regression coefficients reflects both the variation in  $r_m$  terms under various experimental conditions and the variation in  $C_m$  terms as a result of fractionation in the sample vaporization process within the sample inlet probe. Although high values of the coefficient of determination were used to characterize the presence of cannabinoids in the sample under examination, the variation of regression coefficients precludes the comparison of cannabinoid-containing samples based on this approach.

By performing the experiment under one condition, as described in the previous section, the  $r_m$  term for each cannabinoid is kept constant. The  $C_m$  terms will vary in the vaporization process. Since each sample contains a definite concentration of each component, the sum of any individual cannabinoid vaporized during the whole process will also be definite. The  $r_m C_m$  term for each cannabinoid will then reflect its concentration in the sample if all spectra obtained from a sample are summed and used for regression analysis.

The characteristic relative intensities of selected fragments in authentic CBN, CBD,  $\Delta$ -1-THC, and  $\Delta$ -6-THC were first established and tested for their reproducibilities (Table 1). These mass units were subsequently used for multiple regression analysis as described in Eq 1.

Mass spectra obtained from cannabinoid-containing samples are characterized by the constitution and quantities of samples used. Spectra obtained in the earlier scans are substantially different from those obtained in later scans as a result of fractionation during the component vaporization process. The weighted averages of triplicate determinations of these selected mass units of the first 20, the last 20, and all spectra of a hashish oil sample are shown in Table 2. Results of multiple regression analysis using data shown in Tables 1 and 2 are presented in Table 3. Table 4 lists results obtained from various cannabinoid-containing samples.

TABLE 1—Relative intensities of selected mass units of CBN, CBD,  $\Delta$ -1-THC, and  $\Delta$ -6-THC.<sup>a</sup>

Compound	Number of Spectra	Parameter	m/e														
			314	310	299	295	271	258	246	243	238	231	193				
CBN	17	RI	0	13.0	0	100	0	0	0	0	0	0	0	0	30.9	0	0
		SD	...	1.1	...	...	...	...	...	...	...	...	...	...	0.7	...	...
CBD	15	RI	8.2	0	1.3	0	0	1.1	22.8	0	0	100	13.7	...	0	100	13.7
		SD	2.0	...	0.1	...	...	0.1	1.7	...	...	...	2.9	...	...	...	...
$\Delta$ -1-THC <sup>b</sup>	16	RI	43.7	0	49.7	0	37.5	33.0	15.3	37.9	0	100	31.4	...	0	100	31.4
		SD	2.9	...	5.2	...	3.4	3.9	5.2	3.9	...	...	4.7	...	...	...	...
$\Delta$ -6-THC	9	RI	41.5	0	5.6	0	27.1	33.9	17.9	4.9	...	100	25.3	...	...	100	25.3
		SD	1.4	...	0.4	...	1.0	1.3	0.4	0.3	...	...	1.7	...	...	...	1.7

<sup>a</sup>RI = relative intensity and SD = standard deviation.

<sup>b</sup>Mass units 310, 295, and 238 were observed as the result of the thermal conversion of this compound to CBN [1]. For regression purpose, these peaks were eliminated.

TABLE 2—The weighted average of the first 20, the last 20, and all mass spectra obtained from a hashish oil sample (triplicate determinations).<sup>a</sup>

Spectra Numbers	m/e												
	314	310	299	295	271	258	246	243	238	231	193		
					First 20								
554-573	14.2	0.6	11.0	4.2	7.5	4.9	17.4	6.8	1.3	100	13.2		
704-723	14.9	0.6	11.3	4.4	7.3	5.0	17.5	6.5	1.4	100	13.2		
825-844	14.9	0.7	11.5	4.4	7.5	5.1	17.4	6.9	1.4	100	13.5		
					Last 20								
640-659	20.1	20.1	1.5	9.9	10.6	7.7	15.0	9.5	3.2	100	15.7		
785-804	20.5	20.5	1.5	9.9	10.3	7.5	15.1	9.6	3.2	100	16.2		
902-921	20.7	20.7	1.6	9.5	10.4	7.6	15.6	10.5	3.3	100	16.5		
					All								
554-659	14.0	0.8	11.9	5.4	7.8	5.5	15.6	7.6	1.9	100	15.7		
704-804	14.6	0.8	12.5	5.5	7.9	5.5	15.7	7.5	1.9	100	15.6		
825-921	15.3	0.9	13.0	5.9	8.2	5.8	15.7	7.8	2.1	100	16.1		

<sup>a</sup> Values in the body of the table are relative intensities.

TABLE 3—Multiple regression analysis parameters of a hashish oil sample.

Spectra Numbers	Regression Coefficient				Coefficient of Determination	Constant Term
	CBN	CBD	$\Delta$ -1-THC	$\Delta$ -6-THC		
First 20						
554-573	0.075	0.79	0.23	0	0.998	-2.51
704-723	0.076	0.79	0.23	0	0.998	-2.51
825-844	0.076	0.78	0.24	0	0.998	-2.49
Last 20						
640-659	0.14	0.66	0.37	0	0.995	-3.29
785-804	0.14	0.65	0.37	0	0.994	-3.27
902-921	0.13	0.65	0.37	0	0.995	-2.84
All						
554-659	0.090	0.76	0.26	0	0.997	-2.77
704-804	0.092	0.76	0.26	0	0.997	-2.81
825-921	0.094	0.75	0.27	0	0.997	-2.69

TABLE 4—Comparison of multiple regression analysis parameters obtained from the first 20 spectra of various cannabinoid-containing samples.

Sample	Regression Coefficient				Coefficient of Determination	Constant Term
	CBN	CBD	$\Delta$ -1-THC	$\Delta$ -6-THC		
Hashish oil	0.76	0.79	0.23	0	0.998	-2.50
Hashish-1	0.68	0.91	0.10	0.032	0.996	-5.80
Hashish-2	1.0	0.50	0.071	0	0.997	-4.00
Hashish-3	1.0	0.20	0.35	0.28	0.987	-3.86
Hashish-4	0.63	0.90	0.092	0.050	0.996	-5.18
Leaf	0.50	0.17	0.99	0	0.942	-8.97

Results presented in Table 3 indicate that, under the described experimental conditions, good precision was obtained in repeated experiments. This good precision and the high values of coefficients of determination allow a meaningful comparison of results obtained from various samples (Table 4). Among the four hashish samples investigated, the relative concentrations of CBN, CBD, and  $\Delta$ -1-THC in Samples 2 and 3 are distinct from each other and from Samples 1 and 4. Samples 1 and 4 are similar.

The described approach is useful for confirmation of the presence of cannabinoid mixtures and comparison of various samples based on their relative concentration of cannabinoids. The automation of the data reduction process in a mass spectrometer/data system will be pursued in this laboratory.

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