

QUANTITATIVE DETERMINATION OF DELTA-9-THC AND 11-HYDROXY-DELTA-9-THC IN HUMAN PLASMA USING LC-MS/MS

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ABSTRACT

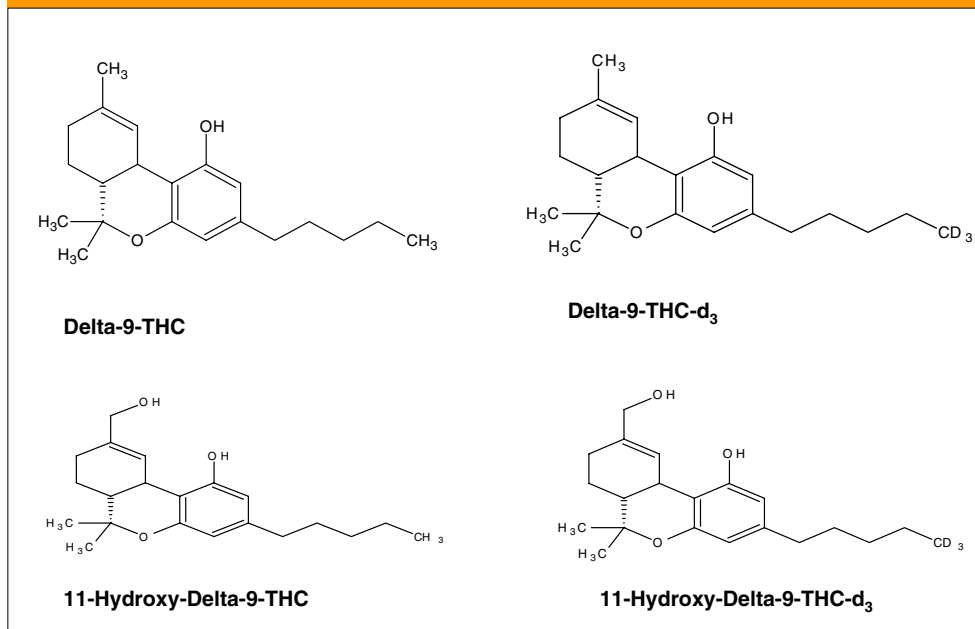
Dronabinol, delta-9-tetrahydrocannabinol (delta-9-THC) is a naturally occurring compound that has been extracted from cannabis *Salvia L.* The objective was to develop and validate a method which is selective, precise and accurate for the determination of delta-9-THC and its major metabolite, 11-hydroxy-delta-9-THC (11-hydroxy-THC) in Human plasma. An HPLC-MS/MS method was developed using a simple liquid-liquid extraction procedure and two deuterium labeled analogs as the internal standards. The method was linear over a concentration range of 0.250 to 250 ng/ml for both delta-9-THC and 11-hydroxy-delta-9-THC. This method permits for the large scale analysis of these analytes for pharmacokinetic studies.

PURPOSE:

Dronabinol is an orally active cannabinoid which, like other cannabinoids, has complex effects on the central nervous system, including central sympathomimetic activity. Cannabinoid receptors have been discovered in neural tissues. These receptors may play a role in mediating the effects of dronabinol and other cannabinoids. Several published analytical methods were developed citing derivatization and gas chromatographic procedures.

In order to simplify the method and support pharmacokinetics studies, a selective, sensitive, precise and accurate HPLC-MS/MS method was developed.

Figure 1. Chemical Structures of THC, 11-Hydroxy-THC and Internal Standards



METHODS

A sample volume of 0.500 mL of human plasma was buffered with ammonium formate followed by the addition of internal standards (deuterium labeled analogs) and chloroform to extract the analytes of interest. After vortexing, centrifuging and aspirating, the organic phase was evaporated and reconstituted with mobile phase. A sample of 20 μ L was injected on to the HPLC-MS/MS system. delta-9-THC, 11-hydroxy-delta-9-THC and their respective internal standards were separated on a C-18 analytical column using a mobile phase consisting of methanol/ammonium formate buffered with ammonium hydroxide at a flow rate of 0.325 mL/min. MS/MS detection was on a Micromass Ultima mass spectrometer in positive mode using Electrospray Ionization. Collision Energy was 25 eV for all analytes with the monitored transition ions being:

Delta-9-THC	Q1 315.0	Q3 195.0
INTSTD1	Q1 318.0	Q3 195.0
11-Hydroxy-Delta-9-THC	Q1 331.0	Q3 192.0
INTSTD2	Q1 334.0	Q3 192.0

RESULTS

Specificity, Sensitivity, Recovery, Stability and Linearity

Specificity

To ensure specificity of the extraction procedure six human plasma samples from different donors were assessed. Aliquots of each plasma lot containing spiked amount of delta-9-THC and 11-hydroxy-delta-9-THC were analyzed. No significant interference was observed at the retention time of delta-9-THC, 11-hydroxy-delta-9-THC or the internal standards in all of the blank human plasma samples. Figure (2)

The lower limit of quantitation of 0.250 ng/mL was chosen for delta-9-THC and 0.250 ng/mL for 11-hydroxy-delta-9-THC from 0.500 mL aliquots of human plasma (Figure 2). Signal-to-noise (S:N Ratio > 20:1) was calculated using 3 times the standard deviation of the root mean square noise. A representative chromatogram (Figure 3) of the upper limit of quantitation is also presented.

Recovery

Recovery was determined for delta-9-THC and 11-hydroxy-delta-9-THC and the internal standards by comparing the respective peak areas. The peak area of the human plasma quality control samples at low, middle and high concentrations were compared to the peak area of the recovery samples. The latter consisted of extracted plasma blanks with internal standard and mobile phase solutions containing delta-9-THC and 11-hydroxy-delta-9-THC that were added during the reconstituting step in place of 100 μ L of mobile phase. The recoveries of delta-9-THC, 11-hydroxy-delta-9-THC were acceptable at the concentration studied. The mean recovery for delta-9-THC was 60.06%, mean recovery for 11-hydroxy-delta-9-THC was 79.71%.

Figure 2. Representative Matrix Blank with Internal Standard for Delta-9-THC (upper two traces) and 11-Hydroxy-THC (lower two traces)

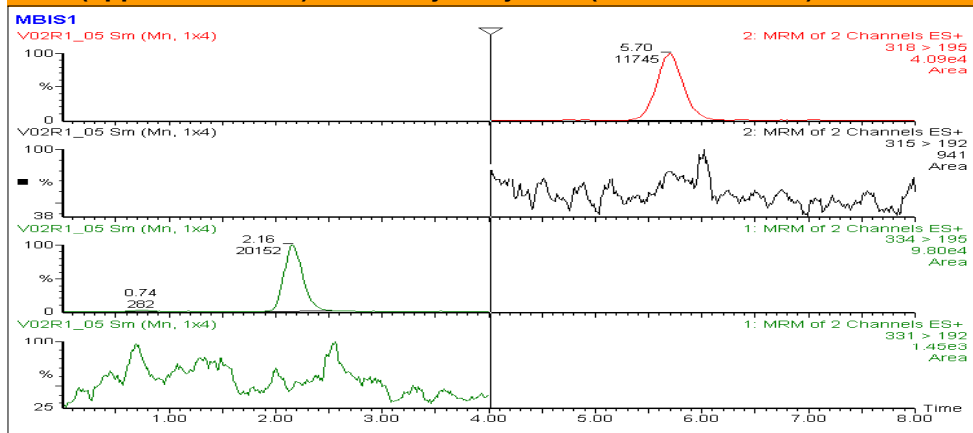


Figure 3. Representative Low Standard (0.500 ng/mL) of Delta-9-THC (upper two traces) and 11-Hydroxy-THC (lower two traces).

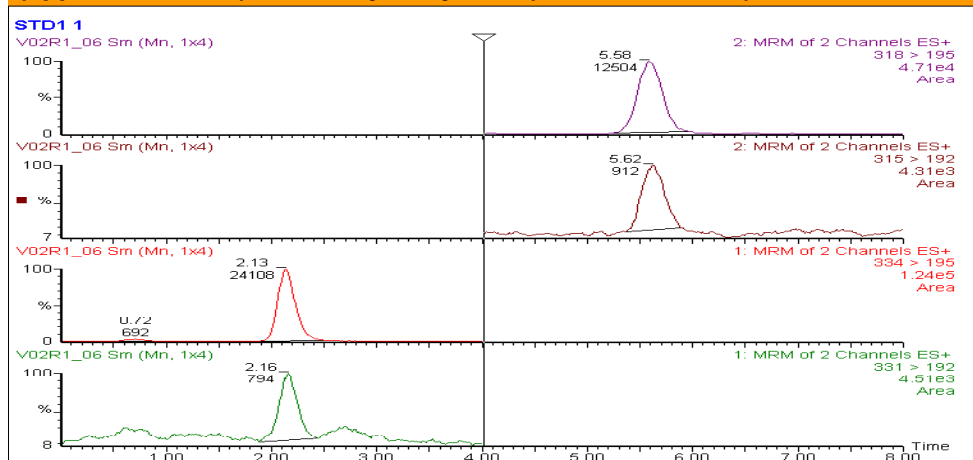


Figure 4. Representative Calibration Curve for Delta-9-THC

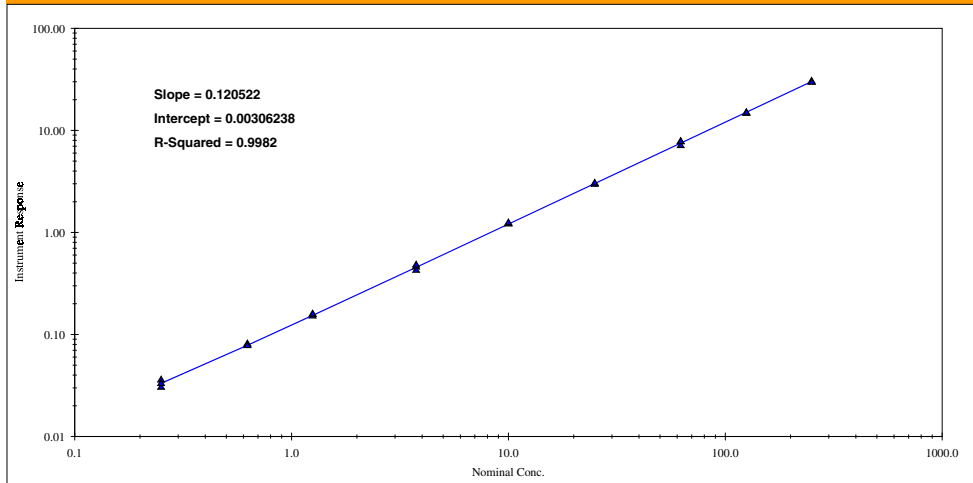


Figure 5. Representative Calibration Curve for 11-Hydroxy-THC

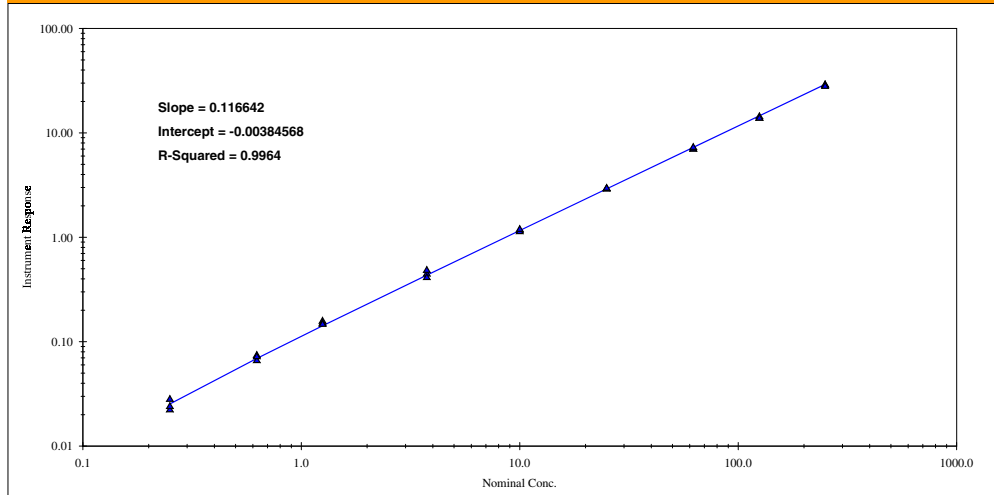


Table 1. Delta-9-THC Freeze/Thaw Stability

	Freeze Thaw Cycle 1		Freeze Thaw Cycle 2		Freeze Thaw Cycle 3	
	Theoretical Concentration		Theoretical Concentration		Theoretical Concentration	
	0.625	213	0.625	213	0.625	213
Mean Measured Concentration	0.635	190	0.686	216	0.646	210
Precision (% C.V.)	4.20	9.72	2.12	5.26	10.44	2.35
Stability (%)	101.60	89.36	109.81	101.56	103.36	98.75
Number of Samples	3	3	3	3	3	3

Table 4. Between Run Precision and Percent Bias for Delta-9-THC QC Samples

Run Date	Curve Number	Low (0.250 ng/mL)	Mid (0.625 ng/mL)	Mid (6.25 ng/mL)	High (213 ng/mL)
02-Jul-2003	1	0.237	0.605	6.36	203
		0.207	0.623	6.24	210
		0.259	0.562	6.47	211
		0.235	0.663	6.30	210
		0.249	0.616	6.61	216
		0.256	0.667	6.45	221
03-Jul-2003	2	0.266	0.642	6.26	199
		0.254	0.640	6.33	204
		0.248	0.672	6.16	209
		0.247	0.706	6.26	202
		0.231	0.731	6.39	200
		0.272	0.708	6.65	209
07-Jul-2003	3	0.253	0.599	6.43	215
		0.260	0.636	6.62	222
		0.247	0.640	6.83	240
		0.251	0.611	6.58	216
		0.251	0.650	6.62	244
		0.258	0.612	6.83	242
Mean		0.249	0.644	6.47	215
S.D.		0.0147	0.0426	0.199	14.0
% CV		5.90	6.61	3.08	6.51
% Theoretical		99.60	103.04	103.52	100.94
% Bias		-0.40	3.04	3.52	0.94
n		18	18	18	18
Overall % CV		5.53			

Table 5. Between Run Precision and Percent Bias for 11-Hydroxy-THC QC Samples

Run Date	Curve Number	Low (0.250 ng/mL)	Mid (0.625 ng/mL)	Mid (6.25 ng/mL)	High (213 ng/mL)
02-Jul-2003	1	0.26	0.703	5.88	205
		0.287	0.687	6.16	196
		0.285	0.717	6.21	197
		0.295	*0.848	6.18	206
		0.251	0.686	6.35	204
		0.282	0.659	6.46	207
03-Jul-2003	2	0.239	0.677	5.98	199
		0.265	0.563	5.73	199
		0.270	0.711	5.98	196
		0.237	0.691	6.14	197
		0.263	0.763	6.04	190
		0.266	0.657	5.77	198
07-Jul-2003	3	0.268	0.605	6.40	220
		0.267	0.636	5.98	219
		0.249	0.668	6.15	228
		0.256	0.635	6.30	219
		0.284	0.648	6.40	230
		0.257	0.646	6.21	231
Mean		0.266	0.668	6.13	208
S.D.		0.0164	0.0461	0.214	13.2
% CV		6.17	6.90	3.49	6.35
% Theoretical		106.40	106.88	98.08	97.65
% Bias		6.40	6.88	-1.92	-2.35
n		18	17	18	18
Overall % CV		5.73			
* Sample Deactivated		Grubbs Outlier			

Stability

The stability of spiked human plasma samples following three freeze/thaw cycles was determined. Triplicate samples spiked at known concentration for delta-9-THC and 11-hydroxy-delta-9-THC were subjected to three freeze/thaw cycles. The mean concentrations of the stability samples were compared to the theoretical concentrations. Table (1) represent the means and standard deviations.

The stability of delta-9-THC and 11-hydroxy-delta-9-THC in spiked human plasma after 23 hours at room temperature was determined. Triplicate samples spiked at known concentrations of delta-9-THC and 11-hydroxy-delta-9-THC were kept at room temperature for approximately 23 hours before extraction. The mean concentrations of the stability samples were compared to the theoretical concentrations. The 24 hour stability samples quantitated within 15% of theoretical.

The stability of the extracted samples on the autosampler at ambient temperature was measured by comparing the mean concentration of reinjections at known concentrations of delta-9-THC and 11-hydroxy-delta-9-THC to the theoretical concentration of delta-9-THC and 11-hydroxy-delta-9-THC after 27 hours at ambient temperature. (Table 3) represent the mean measured concentrations.

Linearity

During the validation of delta-9-THC and 11-hydroxy-delta-9-THC, the calibration curves were shown to be acceptable from 0.250 ng/mL to 250 ng/mL for both delta-9-THC and 11-hydroxy-delta-9-THC using a weighted least square regression analysis. Best-fit calibration lines of chromatographic response versus concentration were determined by weighted least square linear regression analysis with a weighting factor of 1/concentration squared. The assay was linear over the course of the validation with the regression coefficients R^2 being greater than or equal to 0.9970 for delta-9-THC (Table 4) and greater than or equal to 0.9968 for 11-hydroxy-delta-9-THC (Table 5).

Precision and Accuracy

Within and between run precision and accuracy was assessed by comparing the response ratios of replicate quality control samples (QC) at four levels to a calibration standard curve. Within run data was assessed using six replicate QC samples for a given run (Table 6).

Conclusion

The analytical method described is suitable for the analysis of delta-9-THC and 11-hydroxy-delta-9-THC in human plasma. The method is selective, sensitive, precise and accurate. A reproducible and selective method for the analysis of delta-9-THC and 11-hydroxy-delta-9-THC in human plasma using HPLC-MS/MS has been developed and validated. A run time of 8 minutes per sample allows for high throughput of bioanalytical samples, makes full use of the HPLC-MS/MS system. This analytical method has been successfully used for the routine analysis of clinical samples.

Acknowledgements

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