Supporting Information

Cannabinoids from *Cannabis sativa* L.: a new tool based on HPLC-DAD-MS/MS for a rational use in medicinal chemistry

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S1. Experimental procedures for method validation

Linearity. Calibration curves were prepared by diluting analytes at seven different concentrations with the IS at a constant concentration. The analysis was carried out in triplicate for each concentration level. The resulting solutions were injected into the HPLC. Analyte peak areas were plotted against nominal concentrations to obtain the calibration curves. The values of limit of quantification (LOQ) and limit of detection (LOD) were calculated according to the international guidelines, as the analyte concentrations which generating chromatographic peaks with signal-to-noise (S/N) ratios of 10 and 3, respectively.

Precision. To test intra- and interday precision related to analyte peak areas, the assays described above under "linearity" were repeated six times within the same day and six times over six different days, respectively. Both values were expressed as percentage relative standard deviation (%RSD).

Accuracy. Accuracy was evaluated with analytes standard solutions at three different concentrations and IS at constant concentration, which were added before extraction to *Cannabis* samples whose analyte levels were already been determined. Added concentrations corresponded to the lower limit, a middle point and the high value of each calibration curve. These assays were repeated three times to calculate the percentage recovery and the standard deviation (SD) of the standard addition.

Extraction yield. Extraction yield was determined by consecutive extractions carried out as previously described on the same sample and each extract was analysed. This process was repeated until analyte signals were lower than the respective LOQ values. The sum of all extractions was then considered as the 100% yield and the percentage recoveries of the fist extraction step was evaluated for all the analytes. Moreover, ISs absolute recovery was obtained by adding the ISs at constant concentration to *Cannabis* specimens before extraction. To calculate percentage absolute recovery values, analytes and IS peak areas were compared to those obtained by injecting standard solutions at the same theoretical concentrations.

Matrix effect. Matrix effect was assessed by fortifying post-extraction samples with known amount of the analytes at three concentration levels and ISs at constant concentration right before injection in the analytical system. Matrix effect was expressed as percentage recovery values by comparing the analyte peak areas of the standard addition with those obtained from standard solutions at the same nominal concentrations, in order to assess any ion suppression or enhancement due to residual matrix components.

Table S2: Validation parameters

Analyte	Linearity [a]				Extraction yield (% ± SD) [a]	Concentration (ng/mL)	Precision		Matrix effect (% ± SD) [a]
	Linearity range (ng/mL)	r^2	LOQ (ng/mL)	LOD (ng/mL)			Intra-day (%RSD) [b]	Inter-day (%RSD) [b]	
						0.1	4.1	4.5	90.4 ± 2.1
THC	0.1-100	0.9992	0.1	0.03	99.8 ± 1.9	10	2.9	3.4	91.3 ± 2.0
						100	1.9	2.6	98.0 ± 1.4
						0.2	4.4	4.6	86.7 ± 3.0
THCA	0.2-100	0.9998	0.2	0.06	93.9 ± 2.2	10	3.1	3.8	90.9 ± 2.5
						100	2.7	3.2	97.5 ± 2.2
						0.1	4.7	5.2	92.1 ± 2.4
CBD	0.1-200	1.0000	0.1	0.03	93.9 ± 2.5	10	3.1	3.5	95.3 ± 2.0
						200	2.2	2.9	99.0 ± 1.4
						0.2	5.1	5.9	89.9 ± 2.7
CBDA	0.2-200	0.9992	0.2	0.06	90.6 ± 2.7	10	2.3	3.0	91.3 ± 1.7
						200	1.7	2.0	98.2 ± 1.5
						0.5	5.0	5.5	90.5 ± 4.0
CBN	0.5-100	0.9995	0.5	0.15	99.7 ± 3.0	10	3.4	4.0	93.2 ± 2.7
						100	1.9	2.4	96.4 ± 2.2
THC-D_3	-	-	-	-	97.3 ± 1.4	50	1.5	1.9	98.5 ± 3.1
CBD-D_3	-	-	-	-	92.1 ± 1.2	50	1.6	2.2	97.3 ± 2.5
CBN-D_3	-	-	-	-	94.6 ± 1.5	50	1.8	2.6	95.2 ± 2.0

[a] *n=3* [b] *n=6*