Fast liquid chromatography-tandem mass spectrometry method for the simultaneous determination of phytocannabinoids in oily based preparations

F. Savini[1], G.M. Merone[1], A. Tartaglia[2], S. Rossi[1], F. Santavenere[1], E. Bassotti[3], C. D'Ovidio[4], M. Bonelli[4], E. Rosato[2], U. de Grazia[5], A. Zanardo[6], M. Locatelli[2]

(1) Pharmatoxicology Laboratory, Hospital "Santo Spirito", Via Fonte Romana 8, 65124 Pescara , Italy
(2) Department of Pharmacy, University of Chieti-Pescara "G. d'Annunzio", Via dei Vestini 31, 66100 Chieti, Italy
(3) R&D Department Eureka Lab Division, Chiaravalle, Italy
(4) Department of Medicine and Aging Sciences, Section of Legal Medicine, University of Chieti–Pescara "G. d'Annunzio", Chieti 66100, Italy
(5) Fondazione IRCSS Istituto Neurologico Carlo Besta, Laboratory of Neurological Biochemistry and Neuropharmacology, Via Celoria 11, 20133 Milan, Italy
(6) Section of Pharmatoxicology – Regional Hospital Ca' Foncello; Piazzale Ospedale 1, Treviso, 31100, Italy

INTRODUCTION

The use of *cannabis* as a medicine has not been rigorously tested through clinical studies, mainly due to the production and government restriction related to its use. The pharmaceutical forms licensed for medical cannabis are oily based product, capsules, tablets, tinctures, dermal patches, etc. Oily-based products are obtained through the production of oleolites with different cannabis cultivars. For this reason, a rapid procedures that allow an accurate (precise and trueness) quali-quantitative determination of the main active ingredients were requested. In this work, a powerful method for the simultaneous determination of tetrahydrocannabinol (THC), cannabidiol (CBD), cannabinol (CBN), cannabigerol (CBG), tetrahydrocannabinolic acid (THCA),cannabidiolic acid (CBDA), and tetrahydrocannabivarin (THCV) in oily based preparations was described.

MATERIALS and METHODS

STANDARDS AND SOLVENTS EUREKA srl Lab Division (code LC88810)	SAMPLE	LC-MS/MS parameters
Chemicals calibrators (THC, CBD, CBN, CBG, THCA, CBDA, THCV)	For sample collection and storage were observed the guidelines reported directly on the products.	Initial isocratic plateau (95%:5%, v:v, M1:M2) for 0.2 min followed by a linear gradient from 95% to 25% (M1) in 7.8 min. Then the M1 % was decrease to 0% in 0.1 min. The condition 0%:100% (v:v, M1:M2) were mainteined for 2 min
Mobile Phases for LC-MS/MS (M1: H_2O + 2 mM ammonium formate + 0,2% formic acid; M2: MeOH + 2 mM ammonium formate + 0,2% formic acid)	Minimal sample handling with *diluted and shoot* procedure	Hypersil Gold PFP column Flow rate: 0.4 mL/min Sample volume: 10 µL Temperature: 40°C (± 1° C)
Internal Standards **THC-D3** for THC, THCV and THCA **CBD-D3** for CBD and CBDA **CBN-D3** for CBG and CBN	1) 100 µL of oily based formulation diluted with 990 µL of *iso* propanol 2) 10 µL of solution diluted with 990 µL of MeOH 3) 50 µL of the last solution + 950 µL of aqueous solution 4) Injection into LC-MS/MS	Electrospray ionization source (ESI) with maximum ionization efficiency at 450° C. 1500 V voltage of the source capillary

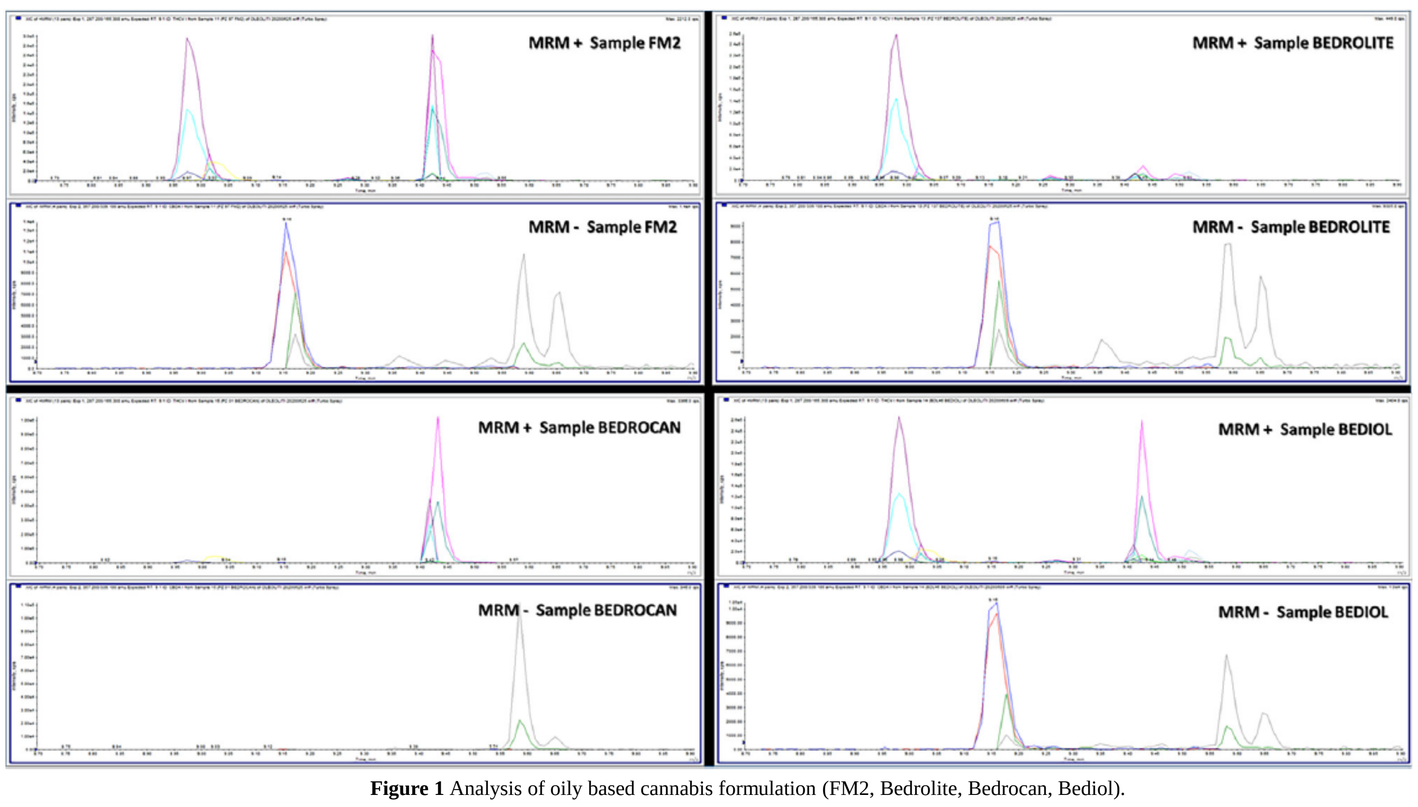

Figure 1 Analysis of oily based cannabis formulation (FM2, Bedrolite, Bedrocan, Bediol).

The reported method was fully validated in terms of linearity, limit of detections and quantifications (LODs and LOQs), accuracy (precision and trueness, both intra and interday), selectivity and matrix effects according to the International Guidelines. All the analytical parameters of the method have been shown in **Table 1**.

REAL SAMPLES ANALYSIS

The validated method was tested to real oily-based pharmaceutical formulation (more of 70 different formulation) in order to verify the cannabis preparation. In **Figure 1** were reported the MRM chromatographic profiles for oily-based formulations (FM2, Bedrolite, Bedrocan, Bediol).
All the analyzed samples fall within the product acceptability values. The analyses with the validated method were performed based on the current regulations in force for the quantification of active ingredients.

CONCLUSIONS

The reported method aims to be a powerful tool for the simultaneous determination of tetrahydrocannabinol (THC), cannabidiol (CBD), cannabinol (CBN), cannabigerol (CBG), tetrahydrocannabinolic acid (THCA), cannabidiolic acid (CBDA) and tetrahydrocannabivarin (THCV) in oily based preparations.
The accuracy, sensitivity and the absence of matrix effects allowed the use for routine analyses and the quantification of seven phytocannabinoids in less than 10 min. Moreover, the proposed method shows a very *green* profile based on Green Analytical Procedure Index (GAPI).

Table 1. Analytical parameters of the validated method

Parameters			THC	CBD	CBG	CBN	THCV	CBDA	THCA
	Slope[a]		3.2561±0.33	2.0129±0.20	16.3238±0.16	1.9824±0.02	4.1579±0.04	3.4770±0.03	1.1856±0.02
	Intercept[a]		0.3251±0.03	0.9276±0.09	0.1253±0.01	0.5297±0.05	0.0157±0.01	0.1272±0.01	0.0018±0.01
	Correlation coeff. (r^2)[a]		0.9966±0.0022	0.9971±0.0014	0.9947±0.0036	0.9950±0.0043	0.9964±0.0024	0.9949±0.0012	0.9942±0.0018
	Matrix effect (%)		125.0	117.1	115.7	83.0	122.7	116.7	114.4
	Expressed in mg/mL of the original sample	Range	0.1–200	0.1–200	0.05–200	0.5–200	0.1–200	0.05–200	0.05–200
	LLOD		0.03	0.03	0.01	0.1	0.03	0.01	0.01
	LLOQ		0.1	0.1	0.05	0.5	0.1	0.05	0.05
	Expressed in ng/mL of the diluted sample (1:200000)	Range	0.5–1000	0.5–1000	0.25–1000	2.5–1000	0.5–1000	0.25–1000	0.25–1000
	LLOD		0.15	0.15	0.05	0.5	0.15	0.05	0.05
	LLOQ		0.5	0.5	0.25	2.5	0.5	0.25	0.25
	Expressed in total mass injected (ng)	Range	0.005–10	0.005–10	0.0025–10	0.025–10	0.005–10	0.0025–10	0.0025–10
	LLOD		0.0015	0.0015	0.0005	0.005	0.0015	0.0005	0.0005
	LLOQ		0.005	0.005	0.0025	0.025	0.005	0.0025	0.0025
Intraday	Trueness (Bias%)	LLOQ	4.02	5.22	2.48	5.97	6.19	3.03	3.64
		C_m	2.71	5.38	4.19	6.15	1.99	6.64	3.40
		C_h	2.68	3.29	2.83	4.69	2.98	1.37	5.01
	Precision (Std. Dev.)	LLOQ	7.70	11.52	4.28	6.97	7.89	6.11	7.16
		C_m	2.73	4.47	4.31	6.59	1.03	3.42	6.41
		C_h	2.93	4.92	5.48	4.26	2.36	4.22	4.91
Interday	Trueness (Bias%)	LLOQ	6.26	6.46	5.59	6.67	7.59	7.33	6.82
		C_m	4.88	7.16	7.41	8.95	5.40	8.77	8.63
		C_h	5.08	5.94	6.62	6.53	5.69	5.57	6.54
	Precision (Std. Dev.)	LLOQ	13.25	14.8	10.8	8.54	12.6	12.8	12.9
		C_m	6.84	8.96	9.87	6.56	6.52	6.06	6.15
		C_h	9.16	8.88	8.46	9.55	6.06	5.30	6.38

[a] Average of six determinations ± standard deviation; LLOQ = lower limit of quantification; C_l = lower concentration; C_m = medium concentration; C_h = higher concentration; LLOD = lower limit of detection.

CONTACT

Dott. SAVINI Fabio
Pharmatoxicology and Analytical Quality Laboratory
Hospital "Santo Spirito", Pescara, Italy;
Mail: fabio.savibi@ausl.pe.it

Società Chimica Italiana *Divisione di Chimica Analitica*

Le indagini forensi ed il contributo della spettrometria di massa

Società Chimica Italiana *Divisione di Spettrometria di Massa*

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