

Title	Analytical method validation for assay determination of cannabidiol and tetrahydrocannabinol in hemp oil infused products by RP-HPLC
Authors	Analakkattillam, Sandhyarani;Langsi, Victor K.;Hanrahan, John P.;Moore, Eric
Publication date	2022-07
Original Citation	Analakkattillam, S., Langsi, V.K., Hanrahan, J.P. and Moore, E. (2022) 'Analytical method validation for assay determination of cannabidiol and tetrahydrocannabinol in hemp oil infused products by RP-HPLC', Scientific Reports, 12(1), 12453 (11pp). doi: 10.1038/s41598-022-13737-6
Type of publication	Article (peer-reviewed)
Link to publisher's version	10.1038/s41598-022-13737-6
Rights	© The Author(s) 2022. This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. Te images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/ . - https://creativecommons.org/licenses/by/4.0/
Download date	2026-09-07 08:02:30
Item downloaded from	https://hdl.handle.net/10468/14240



University College Cork, Ireland
Coláiste na hOllscoile Corcaigh

Title: Analytical method validation for assay determination of cannabidiol and tetrahydrocannabinol in hemp oil infused products by RP-HPLC

Sandhyarani Analakkattillam^{1,2}, Victor K. Langsi¹, John P. Hanrahan¹ and Eric Moore^{2*}

¹ *Glantreo Limited, ERI Building, Lee Road, Cork City, Ireland*

² *School of Chemistry, University College Cork, Cork, Ireland*

Supplementary Table S1: Compilation of Chromatographic conditions and percentage recovery for CBD and THC in various published methods used HPLC technique.

Supplementary Table S1

Ref. No. (References)	Author details and DOI	Chromatographic conditions							Study samples used	Percentage Recovery		Remarks
		MP	Elution Mode	Flow rate (mL/minutes)	Detection Wavelength	Column Oven (°C)	Total runtime (minutes)	Column dimensions		CBD	THC or Δ^8 -THC or Δ^9 -THC	
4	Zivovinic, S., Alder, R., Allenspach, M. D. & Steuer, C. 10.1186/s40543-018-0159-8	(A) 0.1% Formic acid in water (B) 0.1% Formic acid in Acetonitrile	Gradient	0.8	220 nm	50	20	150 x 4.6 mm 2.6 μ m (Phenomenex Kinetex XB-C18)	CBD-hemp tobacco samples and Forensic cannabis sample	106.8%	102.7%	
16	Deidda, R. et al. 10.1016/j.jpba.2019.01.032	75/25 Acetonitrile/ water containing 5 mM of K ₂ HPO ₄ adjusted to pH 3.45 v/v	Isocratic	0.38	222 nm	53	6	150 x 2.1 mm 2.7 μ m column & 5 x 2.1 mm 2.7 μ m Guard column (Agilent Poroshell 120 SB-C18)	Cannabis Olive oil extracts	98.4% – 101.4 %	99.2% - 101.6%	
25	Layton, C. E. & Aubin, A. J. 10.1080/10826076.2018.1424637	45% Acetonitrile in water	Isocratic	0.75	228 nm	40	5	75 x 2.1 mm 1.7 μ m (ACQUITY UPLC CSH Fluoro-Phenyl)	CBD Isolates	99% - 102%	Not reported	UPLC method and not transferred to HPLC

26	De Backer, B. et al. 10.1016/j.jchromb.2009.11.004	Methanol/50 mM ammonium formate buffer pH 5.19	Gradient	0.3	200 – 400nm	30	36	250 x 2.1 mm 5µm column & 10 x 2.1 mm 5µm guard column (Waters XTerra MS C18)	Cannabis plant material	109.1 ± 2.3 %	104.0 ± 2.1 %	
27	Ambach, L. et al. 10.1016/j.forsciint.2014.06.008	64/36 Acetonitrile/ 25 mM triethylammonium phosphate buffer	Isocratic	1.0	210 nm	Not mentioned	18	LiChroCart 125-4, 5mm with LiChrospher 60, RP-Select B, 5µm Pre-column	Seized cannabis product (cannabis plant and oil samples)	Not reported	Not reported	
28	Patel, B., Wene, D. & Fan, Z. T. 10.1016/j.jpba.2017.07.021	(A) 25 mM ammonium acetate pH 4.75 adjusted with acetic acid. (B) Methanol	Gradient	0.70	235 nm	30	10	75 x 3.0 mm 2.7µm (Agilent Poroshell 120 SB-C18)	Cannabis flower samples	87.6% - 107.0%	81.2% - 102.7%	
29	Fekete, S. et al. 10.1016/j.jpba.2018.03.059	(A) 20 mM ammonium acetate pH 5.8 (B) Acetonitrile	Gradient	0.57	220 nm	50	12	100 x 2.1 mm 1.7µm (BEH Shield RP18)	Cannabis sativa colas	Not reported	Not reported	Focused on Method optimization and not validation
30	Citti, C. et al. 10.1016/j.jpba.2017.11.044	(A) 0.1% Formic acid in water (B) 0.1% Formic acid in acetonitrile	Gradient	0.4	228 nm	25	15	100 x 3.0 mm 2.7µm (Agilent Poroshell 120 EC-C18)	Hemp seed oils	89.7% - 99.6%	91.0 % - 99.6%	

31	Mudge, E. M., Murch, S. J. & Brown, P. N. 10.1007/s0021 6-017-0256-3	(A) 10 mM ammonium formate, pH 3.6 (B) Acetonitrile	Gradient	0.6	220 nm	Not ment ioned	15 minute s and 7 minute s equilib ration	100 x 3.0 mm 1.7µm (Phenomenex Kinetex C18)	Cannabis flowers and oils	91.3% - 95.5%	90.7% - 99.2%	Autosampler at 4°C
32	Layton, C. & Reuter, W. M. PerkinElmer Application Note	(A) 0.1% Formic acid in water (B) 0.1% Formic acid in acetonitrile	Gradient	1.0	210 nm	50	8 minute s and 7 minute s equilib ration	150 x 4.6 mm 3.0µm (PerkinElmer Brownlee Analytical C18)	Hemp seed oil	91.0% - 95.6%	96.6% - 110.2%	
33	De Vita, D. et al. 10.1080/14786 419.2019.1601 194	80/20 Acetonitrile/ water (0.1% Trifluoroacetic acid) v/v	Isocratic	1.0	220 nm	30	11	150 x 4.6 mm 3.5µm (Waters Symmetry C18)	Industrial hemp and medicinal cannabis extracts	Not reported	Not reported	This article focused on extraction techniques, hence full validation testing not conducted.

34	Aubin, A. J., Layton, C. & Helmueller, S. Waters Application Note	59/41 Acetonitrile/ 0.1% Trifluoroacetic acid in water v/v	Isocratic	2.0	228 nm	35	26	150 x 4.6 mm 2.7µm (Cortecs Shield RP18)	Cannabis plant materials and extracts	Not reported	Not reported	This application note, only linearity test performed, and no other validation parameters studied.
35	Pellati, F. et al. 10.3390/molecules23102639	(A) 0.1% Formic acid in water. (B) 0.1% Formic acid in acetonitrile	Gradient	0.4	210 and 220 nm	30	22 minutes and 15 minutes equilibration	150 x 3.0 mm 2.7µm (Ascentis Express C18)	Hemp inflorescences	90.84 ± 0.60	Not reported	
36	Lehmann, T. & Brenneisen, R. 10.1080/10826079508009265	(A) 8.64 g/L orthophosphoric Acid (85%) in water (B) Acetonitrile	Gradient	0.2	210 and 224 nm	40	60	200 x 2.0 mm column with a 20 x 2.0 mm guard column (Spherisorb ODS-1, 3µm)	Herbal cannabis, cannabis resin or cannabis oil	Not reported	Not reported	

37	Mandrioli, M. et al. 10.3390/molecules24112113	(A) 0.085% phosphoric acid in water (B) 0.085% phosphoric acid in acetonitrile.	Gradient	1.6	220 nm	35	10	150 × 4.6 mm 2.7µm with 5 × 4.6 mm 2.7µm guard column (Nex-Leaf CBX Potency)	Cannabis sativa L. inflorescences	84.92%	99.7% - 100%	Autosampler at 4°C
38	Saingam, W. & Sakunpak, A. 10.1016/j.bjp.2018.08.001	85/15 Methanol/Water v/v	Isocratic	1.0	220 nm	25	10	100 x 4.6 mm 3.5µm (ZorbaxC-18)	Cannabis extract and oromucosal spray formulation.	95.6% - 97.1%	97.3% – 99.3%	
39	Zgair, A. et al	62/38 acetonitrile/water v/v	Isocratic	1.0	220 nm	55	20	150 x 4.6 mm 3µm column with 3µm guard cartridge (ACE C18-PFP)	Rat plasma	86.2% - 91.0%	86.7% - 94.6%	Autosampler at 4°C