

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

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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 Δ8-THC or Δ9-THC | |
| 4 | Zivovinovic, 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 2.6µm (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 K2HPO4 adjusted to pH 3.45 v/v | Isocratic | 0.38 | 222 nm | 53 | 6 | 150 x 2.1 mm 2.7µm column & 5 x 2.1 mm 2.7µm 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 1.7µm (ACQUITY UPLC CSH Fluoro-Phenyl) | CBD Isolates | 99% - 102% | Not reported | UPLC method and not transferred to HPLC |

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| 26 | De Backer, B. et al. 10.1016/j.jchroomb.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% | |

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| 31 | Mudge, E. M., Murch, S. J. & Brown, P. N. 10.1007/s00216-017-0256-3 | (A) 10 mM ammonium formate, pH 3.6 (B) Acetonitrile | Gradient | 0.6 | 220 nm | Not mentioned | 15 minutes and 7 minutes equilibration | 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 minutes and 7 minutes equilibration | 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. 10.1080/14786419.2019.1601194 | 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. |

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| 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 | |

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| 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 |