

Supporting information

Nutritive value and cannabinoid potency of diverse hemp (*Cannabis sativa* L.) varieties grown under different light conditions and harvested across multiple time points as a possible feed source for livestock

Jennifer M. Duringer,^{1} Ashley Saindon,¹ Agung Irawan,^{2‡} Serkan Ates,² Korey J. Brownstein,^{3†} Kelly M. Gude,^{3†§} Chris Ringo,⁴ Chad Alan Kinney,⁵ Mark Berhow,^{3†} Jeffrey Steiner⁴*

¹Department of Environmental & Molecular Toxicology, 139 Oak Creek Building, Oregon State University, Corvallis, OR 97333, USA

²Department of Animal and Rangeland Sciences, Oregon State University, Corvallis, OR 97331, USA

³USDA, ARS, NCAUR, Functional Foods Research Unit, Peoria, IL 61604, USA

⁴Global Hemp Innovation Center, Oregon State University, Corvallis, OR 97331, USA

⁵Institute for Cannabinoid Research, Colorado State University, Pueblo, CO 81001, USA

*Corresponding author, Email: Jennifer.Duringer@oregonstate.edu

‡Current mailing address: Universitas Sebelas Maret, Surakarta, Central Java 57216, Indonesia

§Current mailing address: Mori, Cambridge Crops, Inc., d/b/a, 500 Rutherford Avenue Ste.105, Boston, MA 02129, USA

Figure S1. Field layout showing randomized complete block design of the experiment. Red diamonds indicate placement of light fixtures.

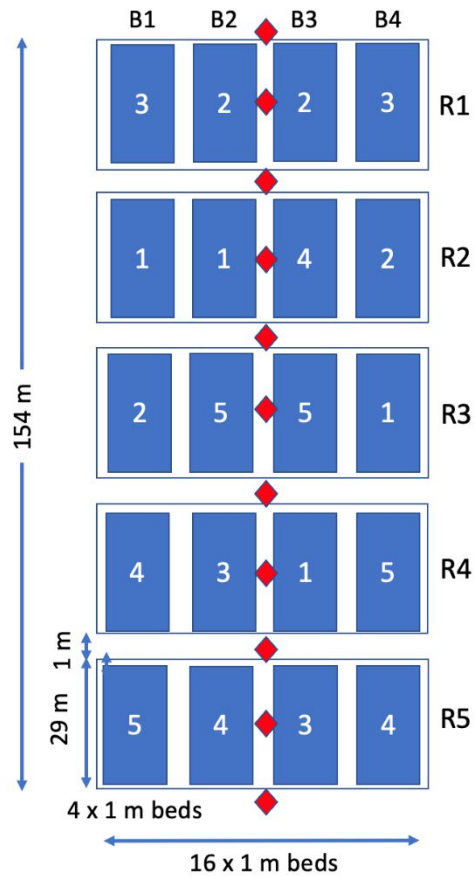


Figure S2. Comparison of the automated 20-cycle Soxhlet extractor method to the single-step sonication method. The percent totals for Δ^9 -THC (a), cannabinoids (b), CBD (c), and CBG (d), as well as the individual percentages of CBDA (e) and CBD (f) in the extracts, as measured by UPLC-DAD at 228 nm.

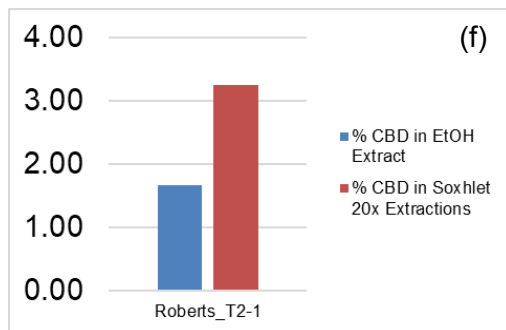
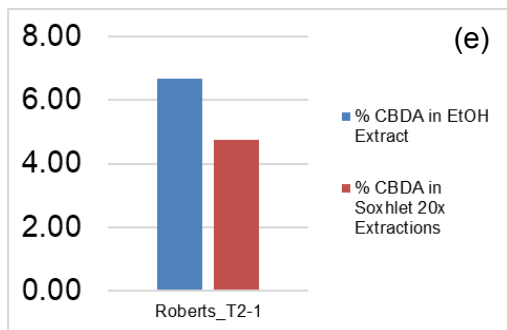
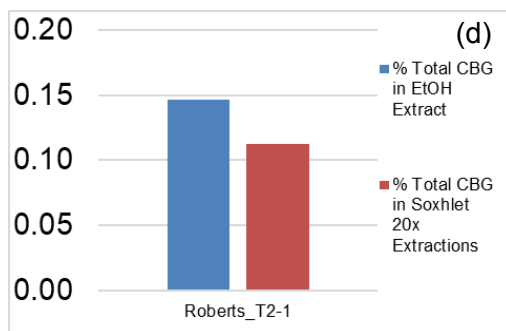
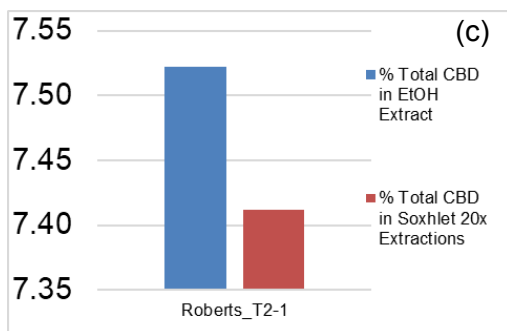
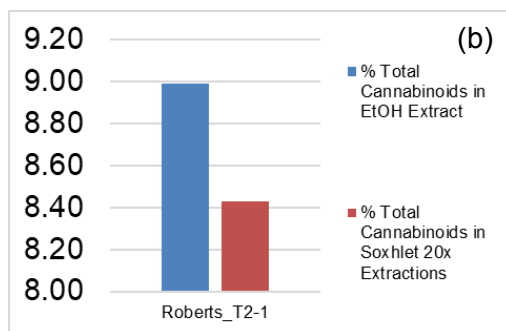
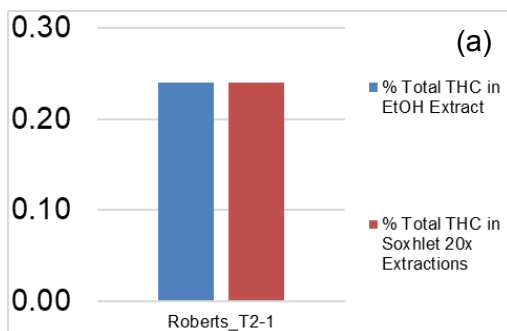


Figure S3. Analysis of percent total Δ^9 -THC data from NIST CannaQAP Exercise 2 (a, b) and Exercise 3 (c, d) samples (Wilson and Abdur-Rahman 2022). Both low (a, c) and high (b, d) Δ^9 -THC samples were investigated. The \times and horizontal line in each box plot indicate the mean and median, respectively. In (a) and (c), each point represents an individual outlier. OS corresponds to “oil” extract sample, while PS corresponds to plant sample.

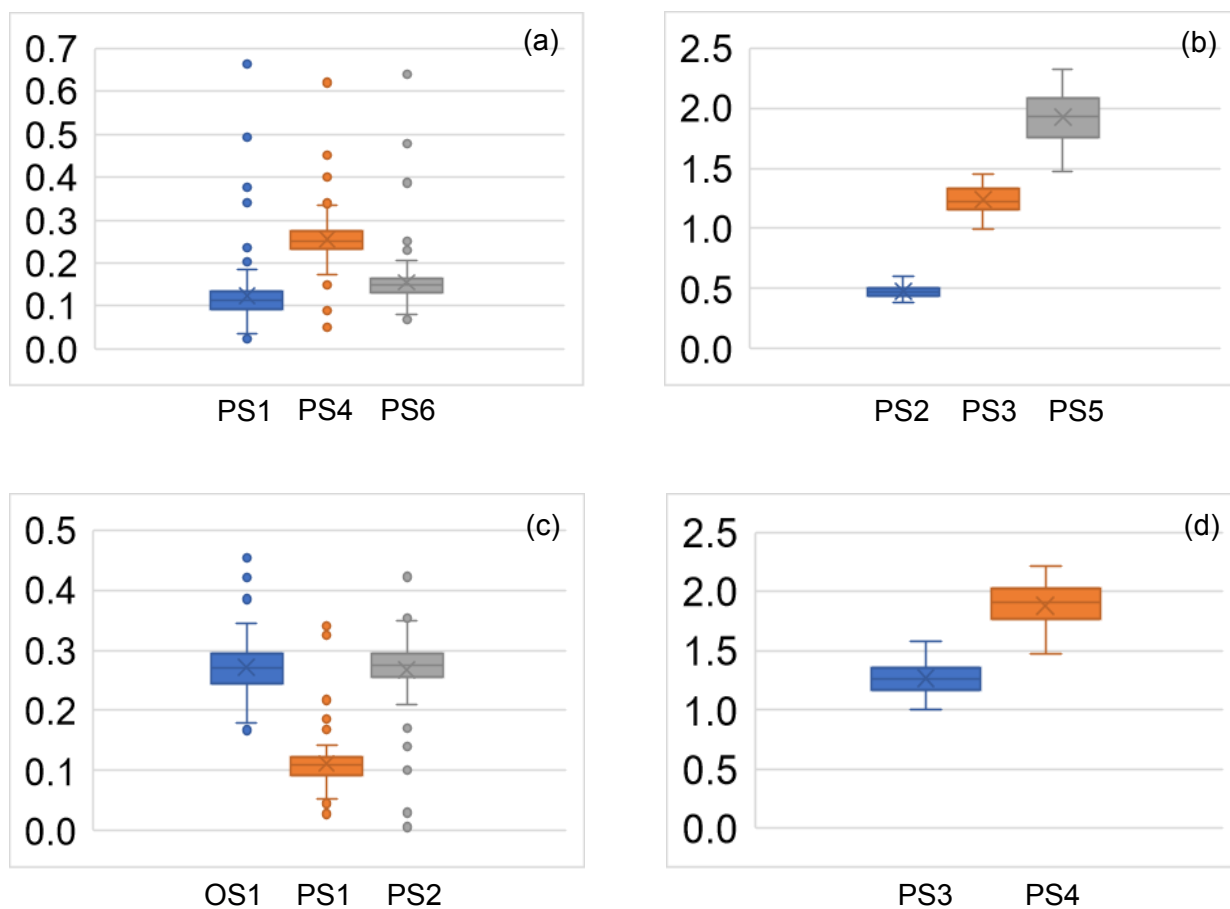


Figure S4. Agronomic characteristics of photoperiod-sensitive and photoperiod-insensitive hemp flowers sampled at 116, 133 and 152 days under two light conditions significant for **Entry x Light x Harvest** interaction. Compact letter display values above bars, bars sharing a letter are not significant different ($\alpha = 0.05$); L=light (L1=no additional light, L2=application of lamp energy); H=harvest time point (H1=day 116, H2=day 133 and H3 = day 152).

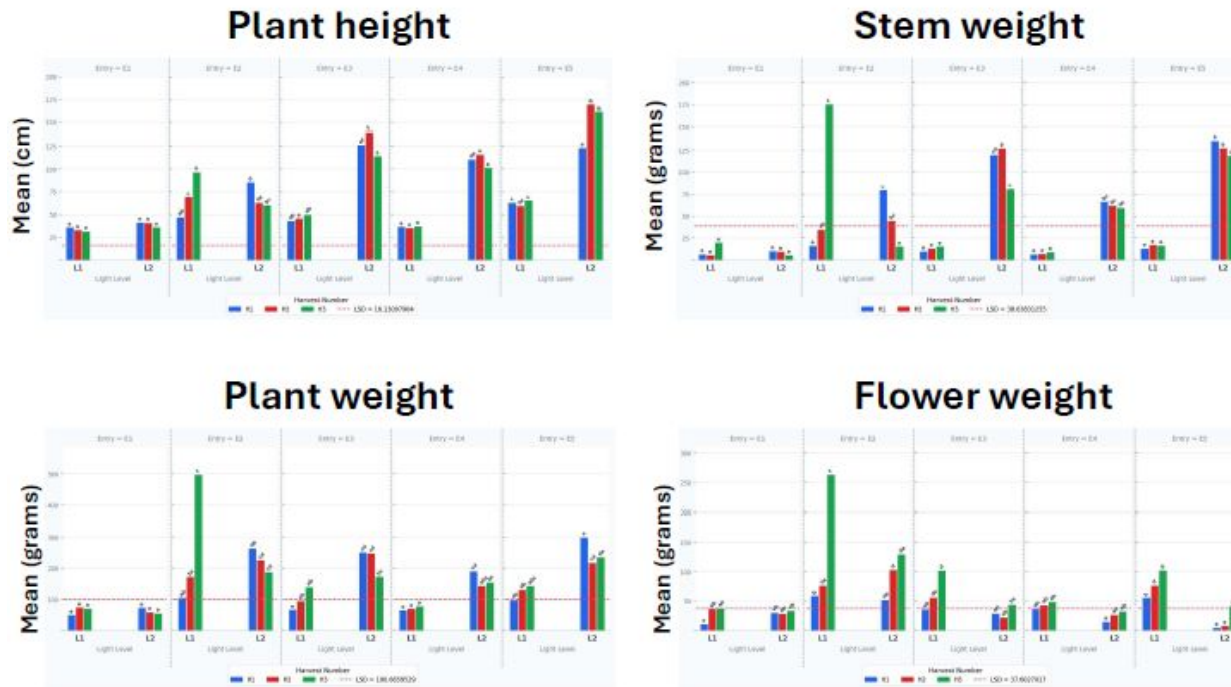


Figure S5. Nutritive values of photoperiod-sensitive and photoperiod-insensitive hemp flowers, leaves and stems sampled at 116, 133 and 152 days under two light conditions significant for **Entry x Light x Harvest** interaction. Compact letter display values above bars, bars sharing a letter are not significant different ($\alpha = 0.05$); L=light (L1=no additional light, L2=application of lamp energy); H=harvest time point (H1=day 116, H2=day 133 and H3 = day 152).

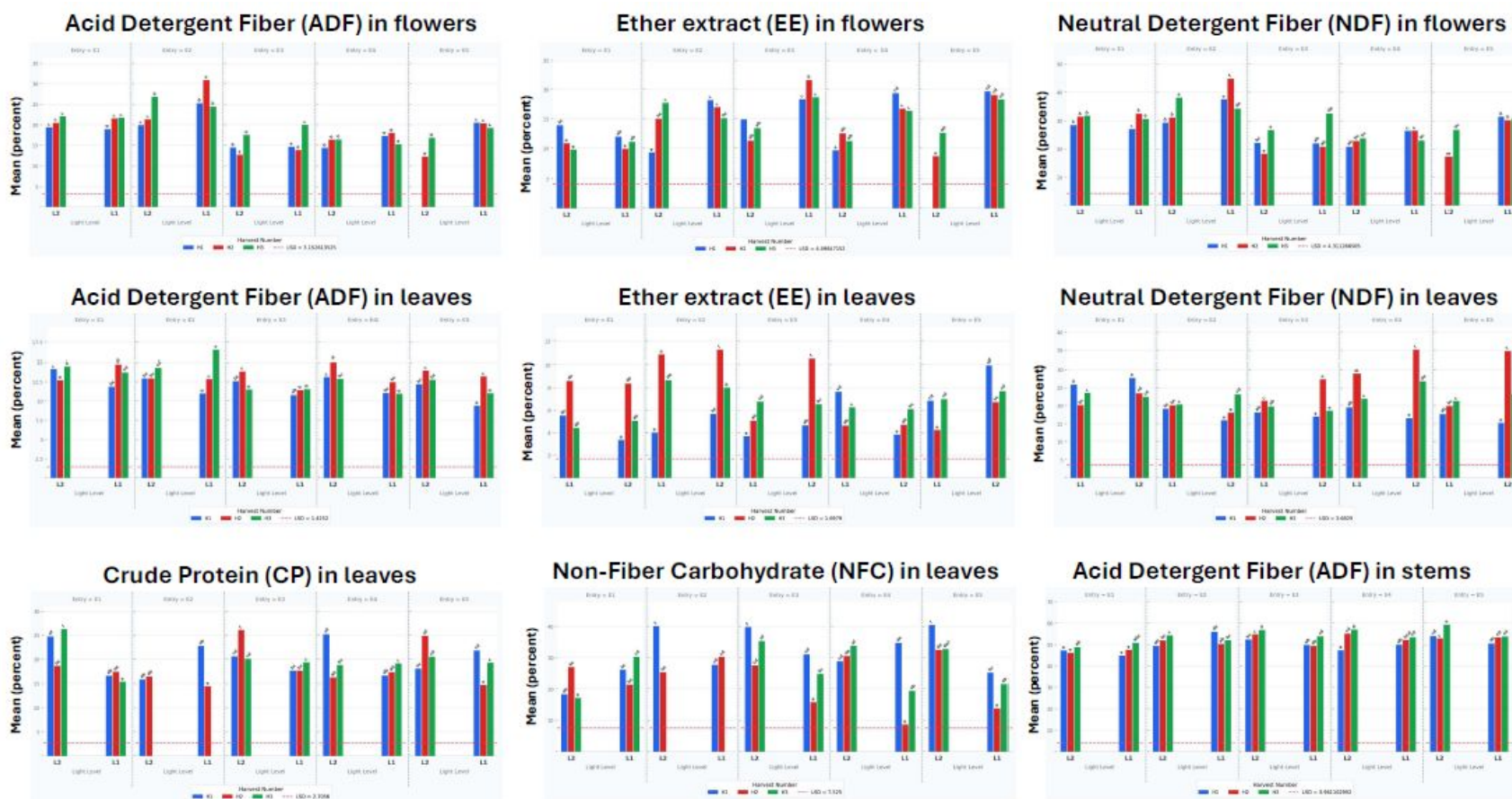


Table S1. Comparison of extraction solvents and volume.

| Fraction^a | Solvent^b | Total Cannabinoids (%) | Total CBD (%) | Total Δ^9-THC (%) |
|-----------------------------|-----------------------------------|-------------------------------|----------------------|--|
| Extract 1+2 | 1. 200x EtOH (sonicate) | 16.13 | 12.65 | 0.45 |
| Extract 1+2 | 2. 200x ACN (sonicate) | 13.81 | 10.94 | 0.37 |
| Extract 1+2 | 3. 200x MeOH (sonicate) | 14.29 | 11.15 | 0.39 |
| Extract 1+2 | 4. 200x 80:20 MeOH:H2O (sonicate) | 12.79 | 10.45 | 0.35 |
| Extract 1+2 | 5. 200x DMSO/MeOH (sonicate) | 15.01 | 11.77 | 0.41 |
| Extract 1+2 | 6. 200x EtOH (orbital shaker) | 13.53 | 10.60 | 0.34 |
| Extract 1+2 | 7. 200x ACN + 0.1% FA (sonicate) | 14.13 | 11.08 | 0.39 |
| Extract 3 | 1. 200x EtOH (sonicate) | 0.02 | 0.02 | 0.00 |
| Extract 3 | 2. 200x ACN (sonicate) | 0.02 | 0.02 | 0.00 |
| Extract 3 | 3. 200x MeOH (sonicate) | 0.03 | 0.03 | 0.00 |
| Extract 3 | 4. 200x 80:20 MeOH:H2O (sonicate) | 0.06 | 0.06 | 0.00 |
| Extract 3 | 5. 200x DMSO/MeOH (sonicate) | 0.03 | 0.03 | 0.00 |
| Extract 3 | 6. 200x EtOH (orbital shaker) | 0.03 | 0.03 | 0.00 |
| Extract 3 | 7. 200x ACN + 0.1% FA (sonicate) | 0.03 | 0.03 | 0.00 |

^aSamples were extracted in sequence three times. Extracts 1 and 2 were combined; the third extraction was run separately. All extractions used the same sample.

^bEtOH=ethanol, ACN=acetonitrile, MeOH=methanol, H2O=water, DMSO=dimethylsulfoxide, FA=formic acid

Table S2. Comparison of extraction solvents using multiple cultivars (N=3, performed in triplicate).

| Solvent | Total Cannabinoids (%) | CBD-A (%) | THC-A (%) | Total CBD (%) | Total Δ^9-THC (%) |
|----------------|-------------------------------|------------------|------------------|----------------------|--|
| Methanol | 7.64 | 7.04 | 0.15 | 7.22 | 0.19 |
| Ethanol | 7.99 | 7.30 | 0.17 | 7.50 | 0.21 |
| Acetonitrile | 7.10 | 6.32 | 0.17 | 6.66 | 0.21 |

Table S3. Comparison of Soxhlett extraction for 20 cycles (1 gram by 200 mL ethanol total volume) to single step extraction (20 mL ethanol for 100 mg dried sample).

| Extraction Method | Total Cannabinoids (%) | Total CBD (%) | Total CBG (%) | Total Δ^9-THC (%) |
|--------------------------|-------------------------------|----------------------|----------------------|--|
| Sonication 18 h | 11.53 | 10.51 | 0.16 | 0.27 |
| Soxhlett 20x | 11.57 | 10.32 | 0.12 | 0.33 |

Table S4. Method validation parameters.

| Cannabinoid | RT^a | Slope | Intercept | R² | LOD (ug/mg)^b | LOQ (ug/mg)^c | RSD (%)^d | <i>U = k</i> * RSD^e |
|--------------------------|-----------------------|--------------|------------------|----------------------|------------------------------------|------------------------------------|--------------------------------|---|
| CBDV-A | 2.119 | 82.374 | 0.571 | 0.9956 | 0.002 | 0.005 | 5.196 | 11.140 |
| CBDV | 2.449 | 90.430 | 0.000 | 0.9928 | 0.001 | 0.004 | 5.662 | 12.139 |
| CBD-A | 3.078 | 103.86 | 0.072 | 0.9999 | 0.001 | 0.004 | 7.897 | 16.932 |
| CBG-A | 3.269 | 76.666 | 0.526 | 0.9884 | 0.002 | 0.005 | 5.662 | 12.139 |
| CBG | 3.416 | 84.251 | 0.000 | 0.994 | 0.001 | 0.005 | 5.267 | 11.293 |
| CBD | 3.505 | 80.708 | 0.000 | 0.9934 | 0.002 | 0.005 | 5.378 | 11.531 |
| THCV | 3.616 | 78.489 | 0.000 | 0.9936 | 0.002 | 0.005 | 5.346 | 11.462 |
| THCV-A | 3.731 | 73.809 | 0.472 | 0.9931 | 0.002 | 0.005 | 5.543 | 11.885 |
| CBN-A* | 4.339 | 62.064 | 0.336 | 0.9938 | 0.002 | 0.008 | 5.875 | 12.595 |
| CBN* | 4.350 | 197.15 | 0.000 | 0.9934 | 0.001 | 0.003 | 5.293 | 11.349 |
| Δ⁹-THC | 5.044 | 75.083 | 0.000 | 0.9933 | 0.002 | 0.006 | 5.479 | 11.748 |
| Δ⁸-THC | 5.223 | 65.863 | 0.000 | 0.9933 | 0.002 | 0.008 | 5.754 | 12.336 |
| THC-A | 5.574 | 70.281 | 0.489 | 0.9889 | 0.002 | 0.007 | 5.274 | 11.307 |
| CBL | 5.728 | 52.836 | 0.000 | 0.9916 | 0.002 | 0.010 | 5.522 | 11.840 |
| CBC | 5.945 | 167.32 | 0.000 | 0.9933 | 0.001 | 0.003 | 5.395 | 11.567 |
| CBC-A | 6.083 | 28.852 | 0.175 | 0.9906 | 0.004 | 0.023 | 7.524 | 16.132 |
| CBL-A | 6.246 | 45.888 | 0.677 | 0.9969 | 0.003 | 0.011 | 5.212 | 11.175 |

^aRT = retention time

^bLOD (Limit of Detection) = 3.3 x SD /Slope (SD: standard deviation) (Lee et al., 2019)

^cLOQ (Limit of Quantification) = 10 x SD /Slope (SD: standard deviation) (Lee et al., 2019).

^dRSD (%) = Percent relative standard deviation, intra-day precision.

^e $U = k$ * RSD provided by the FDA Office of Regulatory Affairs (ORA Laboratory Manual, 2019). The relative standard deviation used in this calculation was the one generated from the intra-day validation. With a 95% confidence level, a coverage factor of $k = 2.144$ for $N = 15$ was used (Berthold et al., 2020).

Table S5. Weather data for Imperial, California (USA) during months experiment was conducted^a

| | Dec 2019 | Jan 2020 | Feb 2020 | Mar 2020 | Apr 2020 |
|-----------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Avg. temp | 54.80 | 57.90 | 60.40 | 62.20 | 72.10 |
| Min. temp | 44.70 | 44.80 | 46.60 | 51.20 | 57.90 |
| Max temp. | 64.80 | 71.00 | 74.00 | 73.20 | 86.20 |
| Precipitation (in.) | 0.59 | 0.02 | 0.46 | 1.78 | 0.38 |
| <hr/> | | | | | |
| 1901-2000 avg. temp | 54.47 | 54.17 | 58.44 | 63.09 | 69.84 |
| Historic low temp | 35.00 | 32.50 | 38.30 | 42.30 | 49.60 |
| Historic high temp | 75.10 | 76.90 | 80.40 | 88.70 | 92.90 |
| 1901-2000 avg. precipitation (in) | 0.55 | 0.53 | 0.50 | 0.37 | 0.10 |

^aInformation obtained from the Western Regional Climate Center, <https://wrcc.dri.edu/cgi-bin/cliMAIN.pl?caimpe+sca>

Table S6. Description of hemp varieties used in evaluation of agronomic, nutritive and chemical attributes grown under two light conditions, harvested across three time points

| Entry Number | Variety^a | Company | Light sensitivity |
|---------------------|----------------------------|---|--------------------------------------|
| E1 | Autopilot 1.0 | HiLo | Photoperiod-insensitive (autoflower) |
| E2 | Blue Genius | Unique Botanicals | Photoperiod-insensitive (autoflower) |
| E3 | NS-26 | Phytonyx | Photoperiod-sensitive (full season) |
| E4 | NS-52 | Phytonyx | Photoperiod-sensitive (full season) |
| E5 | Siskiyou OG | Phytonyx (distributed by Cascade Hemp Collective) | Photoperiod-sensitive (full season) |

^aAll varieties are chemotype III.