Creating Custom *Cannabis* CRM Mixtures: Forty-seven Phytocannabinoids, One HPLC Method

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The development of custom phytocannabinoid certified reference material (CRM) mixtures requires a robust method to resolve coeluting components. This study presents the development and validation of a method addressing the separation of 47 phytocannabinoid compounds.

INTRODUCTION

As research into *Cannabis* testing expands, interest in new phytocannabinoid compounds generates a need for diverse, multicomponent testing solutions. The production of certified reference materials (CRMs) is prescribed by the ISO 17034:2016 standard. As part of this standard, reference material producers are required to characterize CRMs using validated measurement procedures.[1,2]

The development of high-pressure liquid chromatography (HPLC) methods for multicomponent CRM mixtures may present challenges when resolving compounds within the same compound class, extending the lead times for custom CRMs. To accommodate multiple requests for custom mixtures, a general method was developed to separate several of the more commonly requested phytocannabinoid compounds.

EXPERIMENTAL CONDITIONS

HPLC analysis was performed using a Shimadzu Nexera LC-40 system with a photodiode array detector. Compounds were separated on a Millipore Ascentis® Express C18 column (150 x 3 mm, 2.7 µm). Mobile phase A was prepared as 70:30 acetonitrile:water (v/v) with 0.1% formic acid and 5 mM ammonium formate. Mobile phase B was prepared as methanol with 0.1% formic acid.

The column temperature was maintained at 25°C, and the sample temperature was held at 4°C. The flow rate was set to 0.5 ml/min, with an injection volume of 4 µl. The HPLC gradient for mobile phase B was 0% from 0-5.5 minutes, 0-85% from 5.5-19.5 minutes, 85% from 19.5-21 minutes, 0% at 21.1 to the final run time of 24 minutes. The linear calibration range for quantification was 0.05 to 1.2 mg/ml. Detection wavelengths were set to 305 nm for acidic compounds and 275 nm for neutral compounds.

RESULTS

The resulting method allows for production of customized multicomponent phytocannabinoid CRMs. Upon development, notable coelutions were observed early in the chromatography between cannabigerovarin (CBGV) and cannabidivarin (CBDV), cannabidibutol (CBDB) and cannabielsoin (CBE), and later, between the hexahydrocannabinol (HHC) and Δ^{10}-tetrahydrocannabinol (Δ^{10}-THC) isomers. The method was validated by separating the neutral phytocannabinoids in five groups to mitigate coelution. The phytocannabinoid acids were separated from the neutral compounds to enhance stability of the final multicomponent CRMs.

CONCLUSIONS

A quick turn method, meeting the ISO 17034:2016 standard, has been validated to facilitate the production of custom phytocannabinoid CRM mixtures. Coeluting neutral compounds have been identified which will expedite the production planning of multicomponent CRM solutions.

References

1. General requirements for the competence of reference material producers. In: International Organization for Standardization (2016). https://www.iso.org/standard/29357.html
2. General requirements for the competence of testing and calibration laboratories. In: International Organization for Standardization (2017). https://www.iso.org/standard/66912.html

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Figure 1 – Stacked Chromatogram. This figure represents the composition of the independent phytocannabinoid acid and neutral solutions used to validate the HPLC method. Coeluting compounds were separated among the mixtures.