On the use of fast-HPLCDAD for the analysis of cannabinoids: A quantitative method

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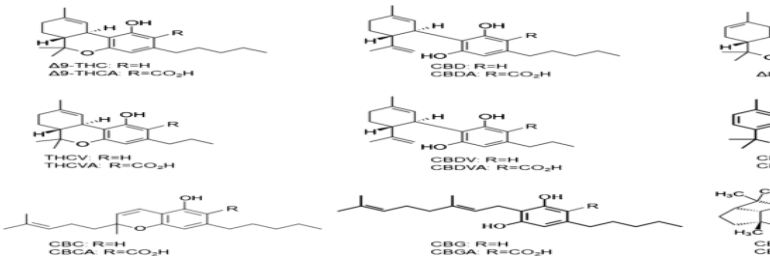
Abstract

Forensic laboratories commonly use GC-MS for cannabis research, as they do for a variety of other products. Even though GC has many benefits, it has limitations when it comes to analysing thermally instable materials, such as THC-A and??9-THC. In the GC injector, both compounds degrade. After that, a fast-HPLC-DAD methodology was proposed to achieve efficient separation of major cannabinoids in 5 minutes and to provide accurate THC quantification in plant extracts. The validation procedure was also carried out in accordance with international standards, with excellent accuracy being noted.

With the legalisation of recreational cannabis in Canada and medical cannabis in many other countries, a slew of analytical laboratories specialising in cannabis research have sprung up to meet the demand. Because of the lack of standardisation in cannabis research, a wide range of approaches have been used, which has undoubtedly led to the high variability of findings between testing laboratories. However, organisations such as the American Association for the Advancement of Science, the American Society for Testing and Materials, and the United States Pharmacopeia are working to establish systematic methods to help address this issue.

To fulfil testing and labelling criteria for cannabis and to follow regulatory standards for hemp, many of the existing approaches concentrate mainly on the four main cannabinoids, 9-THC, 9-THCA, CBD, CBDA, and CBN. Many other cannabinoids found in cannabis and hemp have commercial reference levels, including cannabigerol (CBG), cannabigerolic acid (CBGA), cannabibinolic acid (CBNA), cannabichromene (CBC), cannabichromenic acid (CBCA), tetrahydrocannabivarin (THCV), tetrahydrocannabivarinic acid (THCVA), cannabidivarin acid (CBLA), and Δ^8-tetrahydrocannabinol (Δ^8-THC) (Fig. 1). Cannabigerol (CBG), cannabigerolic acid (CBGA), cannabibinolic acid (CBNA), cannabichromene (CBC), cannabichromenic acid (CBCA), tetrahydrocannabivarin (THCV), tetrahydrocannabivarinic acid (THCVA), cannabidivarin. HPLC-UV (high-performance liquid chromatography with ultraviolet detection), LC-MS/MS (liquid chromatography tandem mass spectrometry), and nuclear magnetic resonance (1H-NMR) spectroscopy are among the techniques used. Many of these methods have limitations in terms of susceptibility and accuracy, and GC's capacity to specifically quantify acidic cannabinoids without derivatization is one of them .Many approaches are quantitative for the main cannabinoids (THC, THCA, CBD, and CBDA) and certain minor cannabinoids; however, due to susceptibility and precision limits, many minor neutral and acidic cannabinoids are not quantified

Fig. 1



Chemical structures of the 17 cannabinoids targeted in the reported method

While the broad variety of cannabinoid concentrations found in cannabis and, to a lesser degree, hemp is challenging to cover in a single study, one method is to use a wide-range calibration curve in combination with acceptable extract dilutionsThe quantifiable spectrum is limited by the use of a single sample dilution, which typically comes at the expense of lower concentration ranges. The low cost of laboratory set-up and service makes HPLC-UV a popular option. While this method provides sufficient quantitative

results for the main cannabinoids at higher concentration levels, it loses sensitivity and specificity for cannabinoids at lower concentrations, reducing the LLOQ in matrix that can be achieved. Though complete isolation of up to 8 or 12 cannabinoids is possible, LC-UV is not capable of resolving larger suites of cannabinoids. In certain cases, precise pH regulation is needed to resolve difficult cannabinoid pairs, but this can be troublesome and limit the method's robustness .However, the LOQ is elevated at 10 g/mL, CBDA and most of the minor cannabinoids were not tested, and there is no proof that many of the cannabinoids do not co-elute. LC-MS/MS is a responsive and precise technique that allows for the simultaneous study of major and minor cannabinoids at low LLOQs. Though it is not yet widely used for routine cannabinoid analysis in plant material, it is the method of choice for cannabinoids and metabolites analysis in other complex matrices such as urine, blood, plasma, and oral fluid. By combining a wide calibration range with sufficient sample extraction and dilution, the LC-MS/MS approach discussed here achieves low LLOQs for 17 cannabinoids with a range of 0.002 to 200 mg/g in matrix. In both cannabis and hemp matrices, the procedure has been tested according to AOAC and ASTM guidelines.

The exponential rise in demand for cannabinoid analysis in cannabis and hemp has sparked a parallel demand for analytical approaches that can satisfy current regulatory criteria while still being adaptable to potential needs, such as cannabinoid analysis in edibles. To satisfy this need, HPLC-UV has been and continues to be a popular technique; however, it has limitations in terms of sensitivity, accuracy, and peak separation requirements. Because of its superior sensitivity, accuracy, and less strict peak separation criteria, LC-MS/MS has become a popular analytical technique in many laboratories. Additional cannabinoids may be applied to LC-MS/MS methods more quickly than HPLC-UV methods, which involve full chromatographic isolation of all cannabinoids when standards become accessible. The advantages offered by LC-MS/MS allowed for the low quantitation limits and wide range of quantitation in matrix stated here for 17 cannabinoids. We anticipate that this approach can be readily applied to more difficult matrices like oils and edibles, allowing for the use of a standardized procedure across all matrices, making it a reasonable alternative for cannabinoid analysis.

This work is partly presented at International Conference on CHROMATOGRAPHY & SPECTROMETRY on March 20-21, 2019 held in New York, USA