White Paper

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Fast, Easy, and Reliable Monitoring of THCA and CBDA Decarboxylation in Cannabis Flower and Oil Samples Using Infrared Spectroscopy

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Overview

Extraction of active ingredients such as cannabinoids, and in many cases terpene compounds, from plant matter is an important process in cannabis production that creates a more refined and potent product. There are three types of extraction typically used: hydrocarbon extraction, alcohol or ethanol extraction, and supercritical CO_2 extraction. In combination with extraction, extractors also generally carry out other postprocessing steps such as decarboxylation that are needed to prepare the extracts for consumption or further processing.

Cannabis plants naturally produce the cannabinoids tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA), but consumers are generally interested in the neutral forms, tetrahydrocannabinol (THC) and cannabidiol (CBD). To some extent, these cannabinoid acids are decarboxylated (CO_2 is eliminated) into their neutral forms by the high temperatures applied during extraction. However, without analyzing the extracts it is impossible to know whether this decarboxylation process is complete. Incomplete decarboxylation is a quality and efficiency concern for cannabis producers; however, it is also possible to let decarboxylation go for too long, resulting in higher production costs, potentially reduced product quality, and reduced return on investment.

Currently, there is no standard process to perform decarboxylation, nor is there a standard analytical method to check for complete decarboxylation. To address these gaps, DELIC Labs (formerly Complex Biotech Discovery Ventures [CBDV]) developed a near-real-time, easy, and reliable solution for monitoring decarboxylation reactions in cannabis flower and oil matrices using the Agilent Cary 630 FTIR spectrometer platform. A significant breakthrough in cannabis postprocessing, the method is applicable to monitoring the decarboxylation of both THCA and CBDA.

Decarboxylation of cannabis

Cannabinoid acids are among the main constituents of the cannabis plant. Their decarboxylation products, neutral cannabinoids, especially THC and CBD, are the desired compounds targeted by cannabis and hemp producers because of their psychoactive properties. Cannabinoid acids are thermally labile and undergo decarboxylation to their neutral forms upon heating. This process naturally occurs concurrently when cannabis flower is smoked in a joint or pipe. However, for noncombustible cannabis or hemp products, the decarboxylation reaction needs to occur prior to consumption or further processing (Figure 1).

There is debate over when and how to best decarboxylate cannabis material. Decarboxylation of cannabis material while still in its natural flower matrix has the advantage of also simultaneously removing water, which can cause problems during extraction. Processors can also decarboxylate cannabis oils after extraction, which is preferred by producers concerned with terpene stability. Terpenes degrade rapidly in the presence of heat and water. Fractionating them off in the extractor before decarboxylation better preserves them. In addition, performing decarboxylation after extraction is carried out on smaller volumes of material and is suitable for processing operations with simpler equipment.

Whether decarboxylation is performed on cannabis flower material before extraction or on oil after extraction, the extractor determines the procedure used. Because the carboxylic acid (CO_2H) group is thermally labile, a heat source, either a hot plate, oven, oil bath, or microwave, is applied to decarboxylate the cannabinoids. As shown in Figure 2, the reaction is highly temperature-

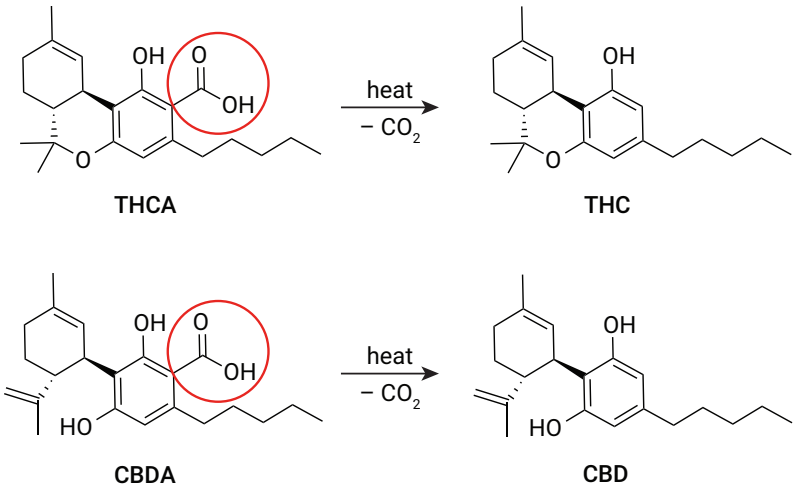

Figure 1. Decarboxylation of THCA and CBDA to THC and CBD.

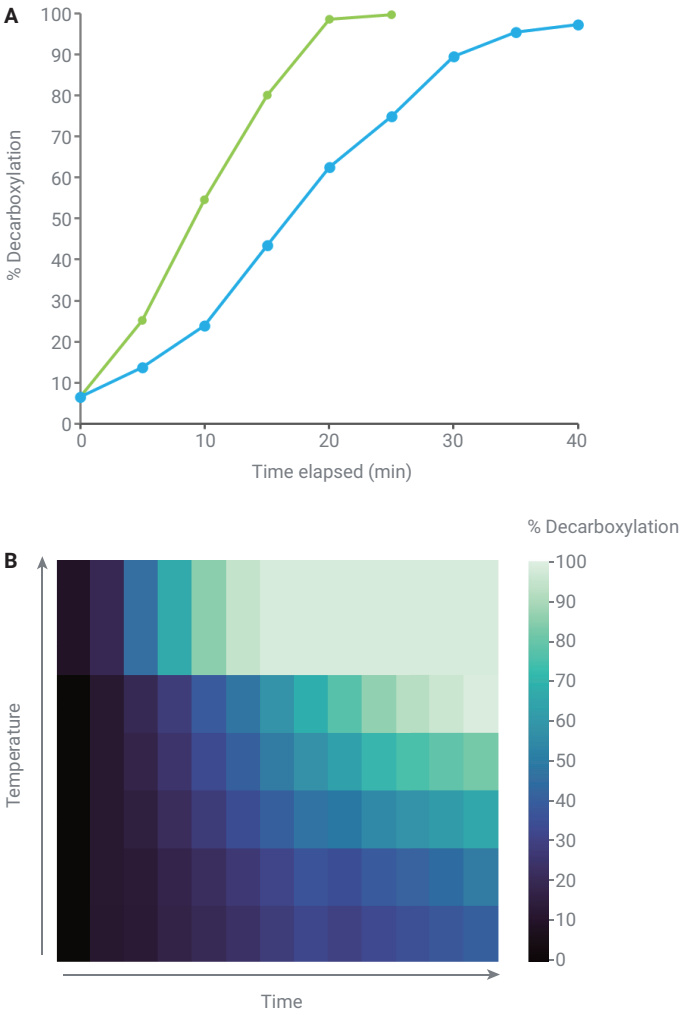

Figure 2. A plot showing the completeness of two different THCA decarboxylation reactions for similar batches of cannabis under identical reaction conditions (A), and a map of decarboxylation conditions versus percent of decarboxylation (B).

and time-sensitive. Small variations in reaction temperature and reaction time affect the completeness of the decarboxylation. Even when producers carry out decarboxylation consistently using the same method, it is important to check the composition of the final product to ensure that the reaction endpoint has been reached. However, without a standardized approach used industry-wide, understanding of the kinetics that influence the decarboxylation rate has been limited.

Decarboxylation challenges

The decarboxylation process presents three major challenges for producers:

- Different cannabinoid acids decarboxylate at different rates (Figure 3). THCA decarboxylates faster than CBDA.
- Decarboxylation rates vary between cannabis flower batches. Even if the batches are very similar and the reaction conditions are identical, reaction rates and reaction endpoints can differ by substantial amounts of time (Figure 2A).
- Applying excessive heat or letting the reaction run too long can degrade the neutral cannabinoids that are intended to be produced during the process and can result in unnecessarily long processing times and higher production costs. Neutral cannabinoids, particularly THC, are prone to isomerization, oxidation, and other degradation pathways. Excessive heating of THC (Δ9-THC) leads to isomerization to Δ8-THC or oxidization to CBN (Figure 4), which are degradation products that lower the value of the resulting product.

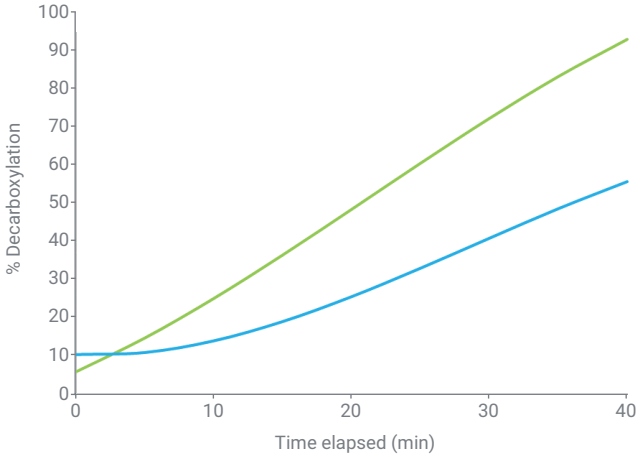

Figure 3. Decarboxylation reaction of a mixture of THCA (green) and CBDA (blue).

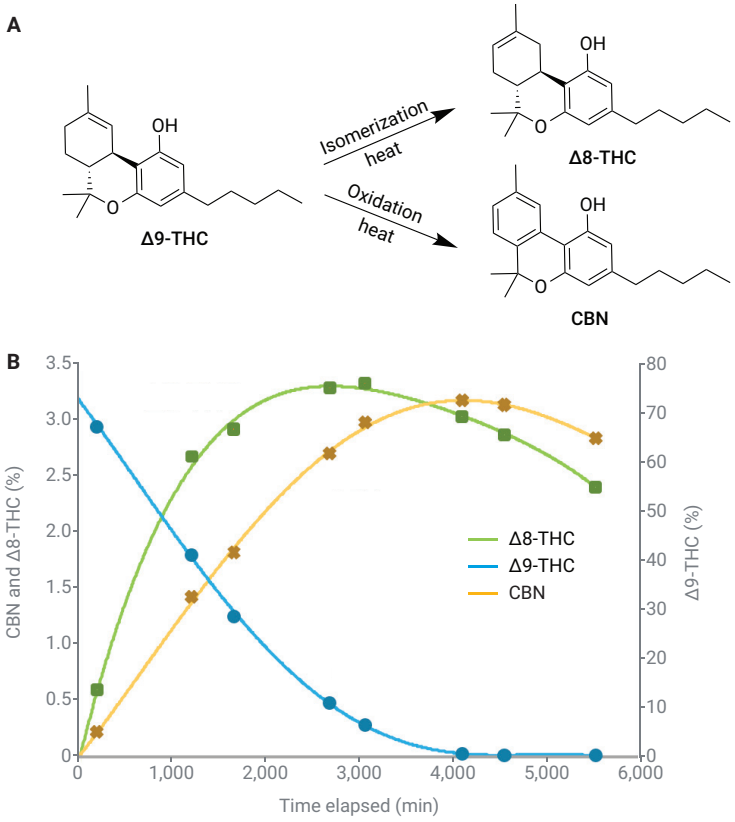

Figure 4. Degradation pathways of THC (Δ9-THC) (A) and graph showing the degradation of Δ9-THC into CBN and Δ8-THC (B).

Infrared (IR) spectroscopy offers a solution

Given these challenges, there is a need for a rapid, easy, and reliable monitoring solution that producers can use on the production floor to ensure that their decarboxylation processes reach the desired endpoint. Repeated testing must not lead to substantial product loss.

As shown in the preceding figures, decarboxylation reactions are usually complete in one hour or less, with the endpoint occurring in the final five to ten minutes of the process. The common high-performance liquid chromatography (HPLC) method to determine cannabinoid concentrations takes approximately one hour, including sample preparation and analysis, making it unsuitable for timely reaction monitoring. In addition, most producers do not have HPLC capabilities on site and thus must wait for an external laboratory to run any HPLC samples. Infrared (IR) spectroscopy instruments offer a solution because they are relatively small, inexpensive, easy to use, and, most importantly, fast. IR spectroscopy monitoring requires minimal amounts of sample (~200 mg of flower material or a few µL of oil). It is important to note that IR spectroscopy provides a process control rather than a high-precision analysis. Knowing the exact concentration of cannabinoids is not important at this stage of production.

Decarboxylation monitoring using the Cary 630 FTIR spectrometer

Shown in Figure 5, the decarboxylation monitoring workflow using the Cary 630 FTIR spectrometer equipped with the DELIC Labs-developed decarboxylation model is remarkably rapid and easy to carry out during the decarboxylation process. If oil extracts are monitored, no sample preparation is needed. Cannabis flower material is prepared for FTIR analyses by adding 2 mL pentane to 200 mg sample followed by 2 minutes of sonication. Then, the sample is placed on the Cary 630 FTIR spectrometer's ATR crystal. Agilent MicroLab software provides step-by-step guidance using instructive images and its intuitive software design guides users through the entire analytical workflow from cleaning and sample loading to data acquisition. The software performs all calculations. Results are immediate and presented in an unambiguous color-coded format with result-dependent recommendations (Figure 6).

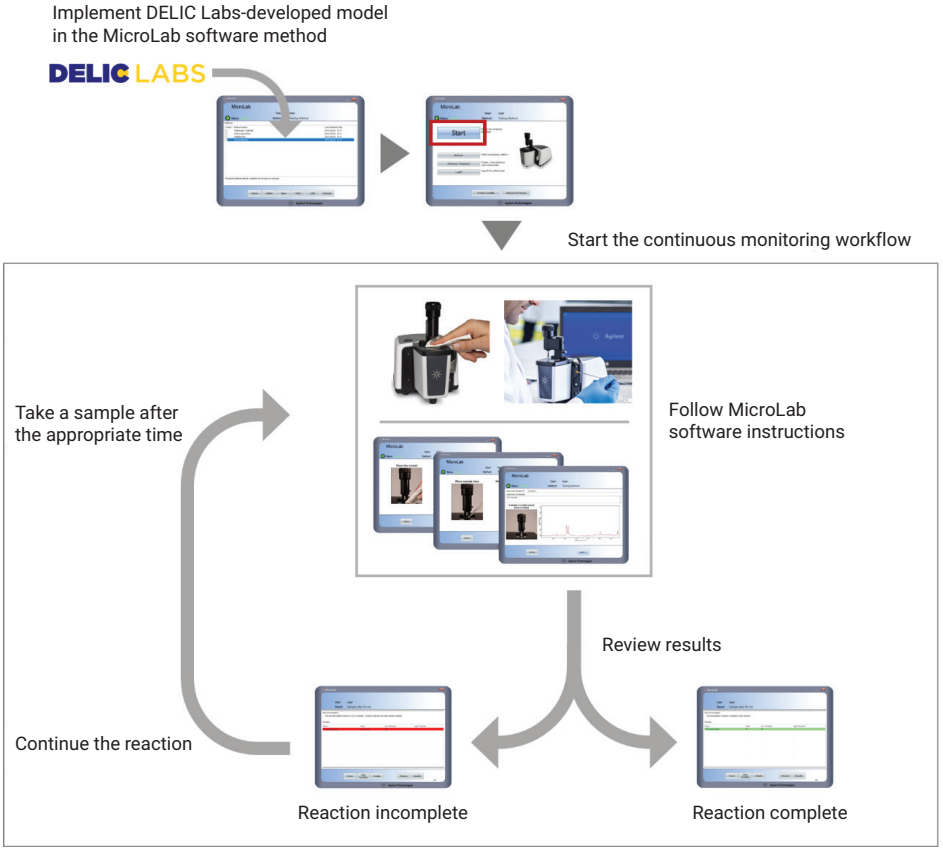

Figure 5. The Agilent Cary 630 FTIR spectrometer platform with the DELIC Labs-developed decarboxylation model offers a rapid, easy, and reliable three-step analytical approach to monitoring cannabinoid decarboxylation reactions.

The Cary 630 FTIR spectrometer is a flexible benchtop instrument offering high performance and extraordinary simplicity in an ultra-compact design. At just 20 × 20 cm and 3.6 kg, it is the world's smallest and lightest benchtop FTIR spectrometer, making the most of lab space. The field-proven, robust optomechanical system delivers outstanding performance and reproducibility, even in humid environments. The intuitive and easy-to-use design of the Cary 630 FTIR is matched by the Agilent MicroLab software suite, where built-in workflows with picture guidance and color-coded reporting make finding answers impressively easy–even for untrained users. The decarboxylation model is available from DELIC Labs and can be easily implemented in the MicroLab software.

Model and method development experiments

DELIC Labs built the FTIR prediction model that relates the IR spectral data to the decarboxylation of THCA and CBDA in both cannabis flower batches and oil by running a series of experiments using the Cary 630 FTIR spectrometer and the Agilent 1220 Infinity II LC System. The Cary 630 FTIR spectrometer with ZnSe optics was equipped with a diamond ATR module, and MicroLab software.

The cannabis flower materials used in these experiments were milled and then decarboxylated by heating on a hotplate at temperatures ranging from 75 to 150 °C and for reaction times ranging from 25 to 90 minutes. Various flower types were used to ensure the model is robust and ready for routine use.

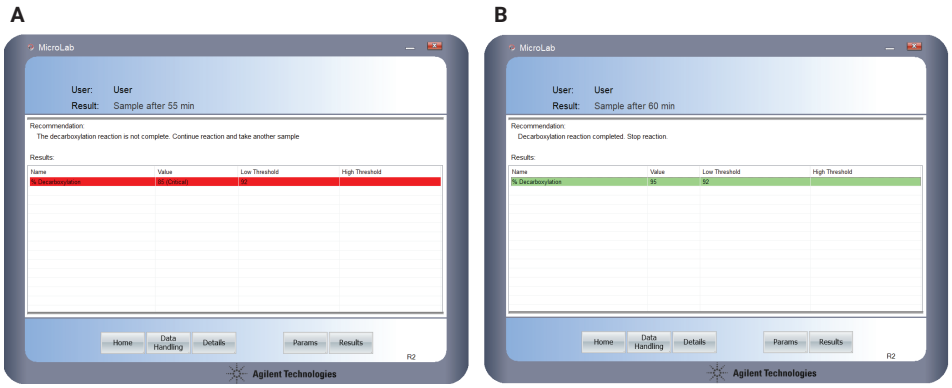

Figure 6. The DELIC Labs-developed decarboxylation model is used within Agilent MicroLab Software. After data acquisition, the software directly reports the percent decarboxylation of the sample. Color-coding facilitates results interpretion and subsequent decision making, while result dependend recommendations help the operator optimize the decarboxylation process.

To produce the oils for decarboxylation, various cannabis flower batches were extracted using various solvents (ethanol, hexane, and dichloroethane.) After removing the solvent, the neat cannabis oil was heated in a round-bottom flask over the same temperature (75 to 150 °C) and time (25 to 90 minutes) ranges to cause decarboxylation.

Samples of both flower material and oil were taken every 5 minutes for both IR and HPLC analyses. For the flower samples, a short sample preparation was performed to extract the relevant substances from the sample. Two mL of pentane were added to a sample of approximately 200 mg flower material and the mixture was sonicated for two minutes. After previously testing various solvents, it was determined that pentane provided the best combination of complete extraction and fast evaporation from the ATR crystal.

For the IR analysis of the flower samples, a droplet of the sonicated flower material solution was placed onto the ATR crystal and the solvent evaporated until a good IR signal was visible. Because the solvent evaporates quickly, it leaves behind a clear signal of the neat cannabis extract. For the IR analysis of the oil samples, the oil was placed directly onto the ATR crystal.

THC/THCA and CBD/CBDA concentrations and reaction temperatures were recorded. The number of runs performed for model building and evaluation are listed in Table 1.

Table 1. Decarboxylation experiments, number of runs for model building and evaluation.

Oil Type	Model Runs	Evaluation Runs
THC	8	4
CBD	5	2
Mixed	5	0
Flower Type	**Model Runs**	**Evaluation Runs**
THC	8	3
CBD	7	2

Model development and evaluation

Using the IR spectra and HPLC reference data from the analysis of the cannabis flower material and oils, the model was built in the Agilent MicroLab Expert software using principal component analysis (PCA) for both the THCA to THC and the CBDA to CBD decarboxylation reactions (Figures 7 and 8). The model expresses reaction progress as % decarboxylation, where 100% signifies complete conversion of THCA to THC, and CBDA to CBD. Expressing the reaction progress as one simple number permits easy observation of the reaction's progress and is independent of absolute cannabinoid concentration.

The model was loaded into MicroLab software and then evaluated by monitoring additional decarboxylation experiments using the Cary 630 FTIR spectrometer and then the Agilent 1220 Infinity II LC System to confirm results. The results of these analyses are represented by the red dots shown in Figures 7 and 8. The linear fit of the model yielded a correlation coefficient (R^2) of 0.961 for the decarboxylation of THCA and 0.987 for CBDA in cannabis oils, and 0.953 for the decarboxylation of THCA in flower material, demonstrating that the model reliably predicts the endpoint of the decarboxylation reaction. Especially at the later stages of decarboxylation – the most important timeframe to monitor – the IR spectroscopy model was in good agreement with the HPLC results.

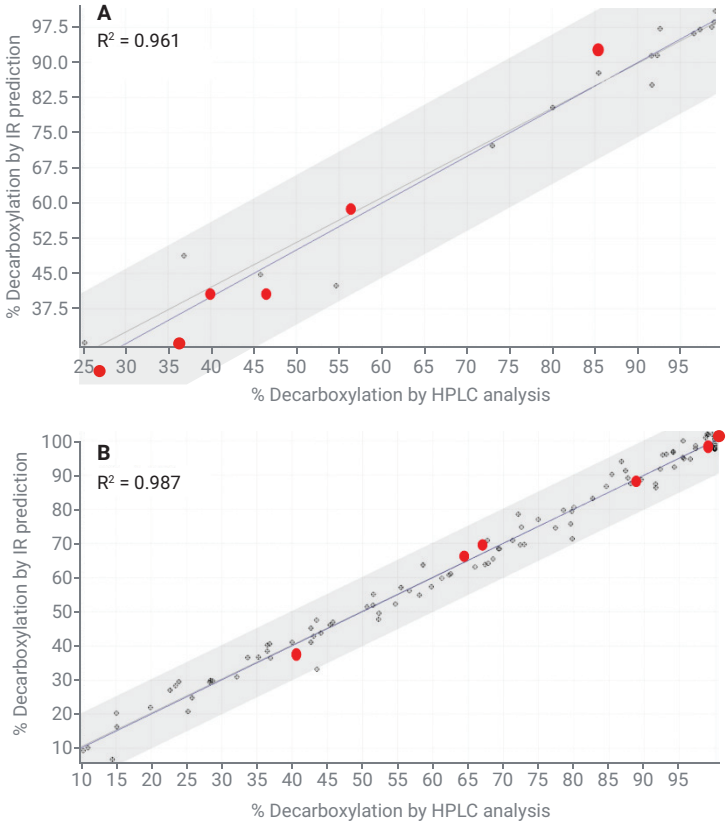

Figure 7. Visual PCA representation of the DELIC Labs-developed model for decarboxylation of THCA (A) and CBA (B) in oils, showing the experiments used to create the model (black dots), the certainty factor range (grey shading), and evaluation runs to test the model (red).

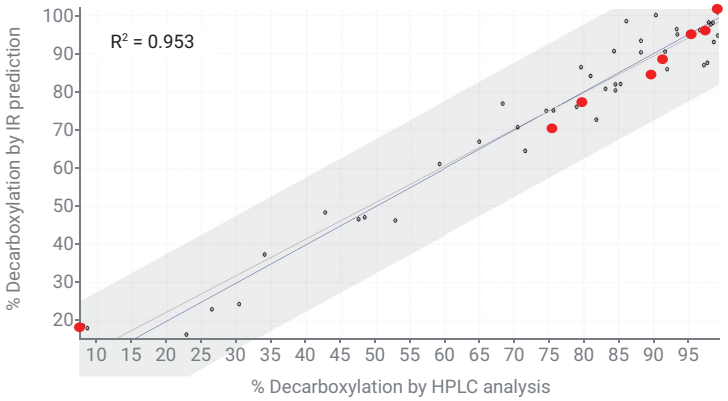

Figure 8. Visual PCA representation of the DELIC Labs-developed model for decarboxylation of THCA in cannabis flowers, showing the experiments used to create the model (black dots), the certainty factor range (grey shading), and evaluation runs to test the model (red).

Conclusion

Noncombusted cannabis and hemp products rely on decarboxylation to convert THCA to THC and CBDA to CBD, the desired compounds targeted by cannabis and hemp producers. Both incomplete decarboxylation and overprocessing are concerns that make reaction monitoring an imperative. The Agilent Cary 630 FTIR spectrometer platform with the DELIC Labs-developed decarboxylation model offers a rapid, easy, and reliable analytical approach for monitoring of cannabinoid decarboxylation reactions.

Agilent products and solutions are intended to be used for cannabis quality control and safety testing in laboratories where such use is permitted under state/country law.

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