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Short communication

Determination of Terpene Profiles in Medical Cannabis

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Introduction

Terpenes are volatile and semi volatile chemicals that engender flavor and aroma organoleptic properties to cannabis and cannabinoid products. Cannabis growers and producers may use terpene profiles to characterize specific strains of cannabis plant, they may wish to make a specific labelling claim to terpene content, and/or they may wish to establish an end product quality control function so that materials supplied to market are consistent. To this end, a robust analytical method is necessary to chemically profile terpenes in cannabis, and cannabinoid products, prior to use in medicinal programs.

At time of publication, the terpene profile does not come under the scrutiny of regulatory agencies such as California Bureau of Cannabis Control (BCC) (2019). Likewise, those laboratories working in the European supply line do not have an obligation to declare a terpene profile prior to going to market unless a specific claim is made. However, for reasons of plant science studies, product quality control and any material claim for product labelling, most laboratories engaged in medical cannabis analysis do run terpene profile analysis. It is expected that as the use of cannabis products increases, an even closer examination of terpene content will be required, possibly aligning with the European regulation for fragrance allergens which sees a list of 26 terpenes, (some in common with those found in cannabis), and other related compounds, having a maximum concentration threshold of 0.001% within the go to market product.

The most common approach to terpenes analyses in these laboratories is headspace gas chromatography (GC) with flame ionization detection (FID), mass spectrometry (MS), or both (FID/MS). Over the past several years, issues such as experimental loss of sesquiterpenoids, for example α-bisabolol, has been observed in high-potency cannabis samples using headspace methodologies. This has led to a need for liquid injection terpenes analysis. A selective, sensitive, and robust method for the analysis of 40 chromatographically resolved terpenes common to Cannabis *spp*, using liquid injection GC/MS, has been developed.

Methodology

The final sample solutions presented for analysis nominally represented 0.5 g of plant sample in 50 mL final volume, (= 0.010 g/mL), obtained by solvent addition, agitation and filtration. For solutions of cannabis resin, where terpene levels might exceed 5.0%, an additional 10-fold dilution with ethyl acetate was made. To enhance the quality control and data precision, 2-fluorobiphenyl was considered a low cost, chemically viable internal standard.

Terpene calibration standards were made to be as best matrix matched as possible, experience showing that food grade hemp oil served that purpose.

An Agilent gas chromatograph configured with a proprietary mid-column backflush mechanism, and a multimode inlet (MMI) was used. The Agilent automatic liquid sampler, configured with a 10.0 µL syringe, was installed. A 4 mm Ultra Inert, low pressure drop, glass wool split liner and a DB-Select 624 Ultra Inert column (30 m × 0.25 mm id, 1.4 µm film thickness) were used for all analyses. The GC system was connected to an Agilent 5977B mass selective detector with EI Extractor source, the latter having a 9mm Extractor lens to accommodate the required dynamic range. Data were collected using Agilent MassHunter B.10 GC/MS Acquisition software. All data

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analyses were performed using MassHunter Quantitative Software.

For Limit of Detection and Limit of Quantitation determinations, two independent datasets were collected using eight replicate injections at 50% of the lowest calibrator concentration for each target terpene. The intraday and interday Limits of Detection were calculated statistically with a Student t-statistic of 2.998 for n – 1 degrees of freedom at the 99% confidence level. Limits of Quantitation for each analyte were determined statistically from this dataset using (10 × standard deviation) for both the intraday and interday data. Intraday and interday precision were also determined in this dataset as %RSD.

Accuracy and precision were determined by three independent batches of eight calibrator levels prepared in hempseed oil matrix. Each batch was collected over the course of 5 days and designated P1, P2, and P3, respectively. Each calibrator level in each batch was injected five times. The intraday and interday accuracy was determined. Percent accuracy acceptability criteria was defined as an average percent accuracy greater than 80% and less than 120%. The intraday batch precision was determined as %RSD

Results and discussion

To test the validity of the analytical approach, a "commercially available terpene content sample" was purchased. According to the product insert, approximately 94% of this product was comprised of (+)-limonene, β-caryophyllene, β-myrcene, α-pinene, linalool, β-pinene, α-humulene, terpinolene, and α-bisabolol with (+)-limonene, β-caryophyllene, and β-myrcene amounting to 71% of the total. The product also contained at least 31 other known terpenes with concentrations < or << than 1.0% (wt/wt). No lot or analytical description was provided in the product insert. We therefore surmised that the product insert was more of a "general description" of the terpene content rather than a true certificate of analysis.

In each of the three datasets used to determine method performance, the sample was analyzed twice (n = 6) in SIM/SCAN mode. Each replicate was defined as P1R1, P1R2, P2R1, P2R2, P3R1, and P3R2. The quantitative results for target terpenes in % (wt/wt) were compared to the values provided by the manufacturer. β-caryophyllene, α-pinene, linalool, β-pinene, terpinolene, and α-bisabolol (6/9 of the major terpenes in the product) were determined to be within ±10% of the % (wt/wt) provided in the product insert.

The use of SIM/Scan data collection with the mass spectrometer enabled targeted quantitative analysis and interrogation of the full m/z scan spectra to search for nontargeted terpenes and putatively identify them using a known mass spectral database. We used the NIST Mass Spectral Search Program within the MassHunter Unknowns Analysis 10.1 software that is part of the MassHunter Quantitative Analysis package to identify other terpenes in the sample. In addition to nine major components in the product noted above, the spectral library search identified four trace terpenes listed in the product insert: γ-terpinene 0.04% (wt/wt), bornyl acetate 0.23% (wt/wt), camphene 0.45% (wt/wt), and α-cubebene 0.05% (wt/wt).

Conclusion

This work developed and verified method parameters and outcomes for the liquid injection analysis of 40 chromatographically resolved terpenes in cannabis and in cannabinoid products using the Agilent GC/MS system. All data were matrix-matched and used an internal standard. This novel method used capillary flow technology to backflush matrix and other unwanted compounds before the next injection. Accuracy, precision, range, linearity, limits of detection (defined as MDL), and limits of quantitation were determined through both intraday and interday studies.

References

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Article 19 (1) Regulation Number 1223/2009 Annex III of the cosmetics regulation