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LETTER TO THE EDITOR

Response to letter to the editor: "Comments on 'discrepancies between validated GC-FID and UHPLC-DAD methods for the analysis of Δ-9-THC and CBD in dried hemp flowers'"

Dear editor,

We appreciate the comments of our colleagues Tsujikawa and Iwata on our article "Discrepancies between validated GC-FID and UHPLC-DAD methods for the analysis of Δ-9-THC and CBD in dried hemp flowers".[1,2]

First of all, we were aware that the article could cause some discussion, since it is a very actual topic and most authors suggested liquid chromatography (LC-UV) as the better option for this kind of analysis.

A little bit of context, in Belgium, we have to check each year the harvest of agricultural hemp as well as a series of herbal smoking products, claimed to contain only cannabidiol (CBD) and less than 0.2% m/m of delta-9-tetrahydrocannabinol (Δ9-THC). It is agreed upon with the justice departments that only results of gas chromatography (GC) would be taken into account in court. Therefore, our gas chromatography-flame ionization detector (GC-FID) method was used as gold standard in this research. The idea of the research performed results from the observations that, regularly, certificates of analysis from companies were received, where the levels of Δ9-THC as determined with LC-UV were lower than the results obtained with GC-FID. The latter was also observed during the analysis of the real samples in the original article.[2]

The conclusion of this article[2] concerning the fact that LC-UV tends to overestimate the total-CBD and total-THC and GC-FID to underestimate is based on the objective observations obtained during the validation of both methods. During validation, spiked samples were used containing the herbal matrix, so it was exactly known what result should be obtained. The article states clearly that the validation results are not significantly different but still may be at the basis of the discrepancies found between analysis certificates and regulatory analysis. Also, in order to validate, we had to set the β-expectation tolerance limit to 90% and the acceptance limits to 15% for the LC-UV method, compared with 95% and 10% for the GC-FID method. This again is a pure observation and is probably due to the low concentrations, especially of Δ9-THC and Δ9-THCA. The situation could be entirely different if, instead of UV, a mass spectrometric detector was used, which would indeed also identify the compounds and eliminate the risk of false positive or overlapping peaks. Though the goal of the research was to investigate discrepancies between companies' certificates of analysis and the results obtained by the regulatory laboratories and for the moment the certificates are either based on GC-FID or MS and LC-UV.

A first comment of Tsujikawa and Iwata[1] was on the fact that systematically higher Δ9-THC values were obtained with GC-FID compared with LC-UV. The authors from the letter to the editor suggest that the incomplete decarboxylation of Δ9-THCA in Δ9-THC in the injector would lead to a lower recovery, and therefore, our method would overestimate the levels of Δ9-THC. As was written in the original article,[2] the used GC-FID was completely validated and accredited under ISO17025.[3] Also, all adaptions performed, compared with the European method, were tested and validated using the European method as standard. Validation parameters as well as the results of the yearly ring tests, in which we participate for both drug like as CBD like cannabis, suggest that our method has a systematic negative bias, though resulting in acceptable *z*-scores in the ring test. In our laboratory, this small negative bias is considered an advantage, especially for the release of industrial hemp and the check of CBD herbal smoking products. We therefore are positive that our method is not overestimating the Δ9-THC content.

A first possible reason for overestimation, suggested by Tsujikawa and Iwata,[1] was the thermal decomposition of CBD to Δ9-THC in the injector of the GC. As described by Tsujikawa et al.,[4] this phenomenon occurs in splitless mode with a deteriorated liner. As the readers probably know, the design of a liner for split injection is different as the one for a splitless injection, that is, the liner for split injection has a different form and a protective filtering system in order to protect it from deterioration. As mentioned in the original article,[2] all injections were performed using split mode (1:20) with an injector temperature of 225°C (below 250°C). As suggested by Tsujikawa et al.[4] themselves, there should not be any problem here. The validation results of our GC-FID method never showed this issue, and also, our quality system demands that the liner is changed every 4 months, rendering this explication for higher Δ9-THC not probable.

Another suggestion explaining a possible overestimation by the presented GC-FID method is the peak overlap between Δ9-THC and a presumptive cannabielsoin (CBE) isomer, formed by heating cannabidiolic acid (CBDA). This is indeed something that was never tested during the validation of the GC-FID method, and therefore, a standard solution of CBDA of 0.2 mg/ml in methanol, corresponding to a herbal sample containing 5% of CBDA, was injected on the

GC-FID and analyzed according to the method described in the original article.[2] Figure 1 shows the chromatogram of this analysis, together with a chromatogram of a real sample, found positive for Δ9-THC. As can be seen, no peak was detected at the retention time of Δ9-THC. Although a quick test and therefore not a conclusive proof, this seems to suggest that for our study, this isomer is not interfering with the Δ9-THC determination.

We want to point at the fact that the research referenced by our colleagues[4–6] was all performed in the context of the analysis of e-liquids, which is generally a matrix containing 30% to 50% glycerine and 50% to 70% propylene glycol, often containing some ethanol and flavoring substances. The CBD used here is the purified substance, often obtained from herbal extracts. The original article, however, deals with dried herbal material, which is a completely different situation. It is possible that both the thermal decomposition of CBD as the formation of the presumptive CBE isomer is somehow triggered or catalyzed by the matrix of the e-liquid and therefore is less or not present when analyzing alcoholic extracts of dried cannabis flowers. More general, the degradation and the influence of the matrix on the analysis and the instrument is to be taken into account. It is, for instance, a fact that the liner in GC will deteriorate much faster when analyzing e-liquids compared with the analysis of alcoholic *extracts*.

It can be said that this is a textbook example, why each method needs a thorough validation in function of the matrix that is analyzed. The original article only claims the use of validated methods for the analysis of dried herbal material and compared the methods for this purpose. It was never claimed that the methods were valid for the analysis of, for example, oils or e-liquids, wherefore the results of the comparison could be different and even favor the use of an LC-UV method.

To conclude, in the case of the analysis of herbal products, as was done in the original article, the hypothesis of the influence of the low concentrations still stands. It is a fact that in LC-UV, Δ9-THC and Δ9-THCA are quantified separately, leading to higher measurement errors and also the fact that often the Δ9-THCA amount is lower than the LOQ and therefore is not taken into account in contrast with the GC-FID analysis. This means that, in these cases, even if the decarboxylation of Δ9-THCA would not be complete, more of the Δ9-THCA is taken into account by GC-FID than with LC-UV. Inhomogeneity will probably play a secondary, less important role in these observations.

In the context of the original paper, we still tend to favor the GC-FID method for the analysis under discussion, though more importantly standardization is necessary. Regulators and producers/distributors should come to a standard set of methods to ensure the quality of their products in analogy with what is done for pharmaceuticals, where the European Pharmacopeia just presented their draft monograph[7] for cannabis flowers for medicinal use. A monography valid for the entire European Union or broader, stipulating to what norms products have to conform and which methods should be used to test it, would solve the problem once and for all. Producers/distributors will know in advance to what tests the products could be submitted by regulatory authorities and adapt their choice of analytical methods or most often the choice of the analytical laboratory with whom they work. Finally, producers/distributors should always work with laboratories, accredited under ISO17025 by their respective national accreditation body.

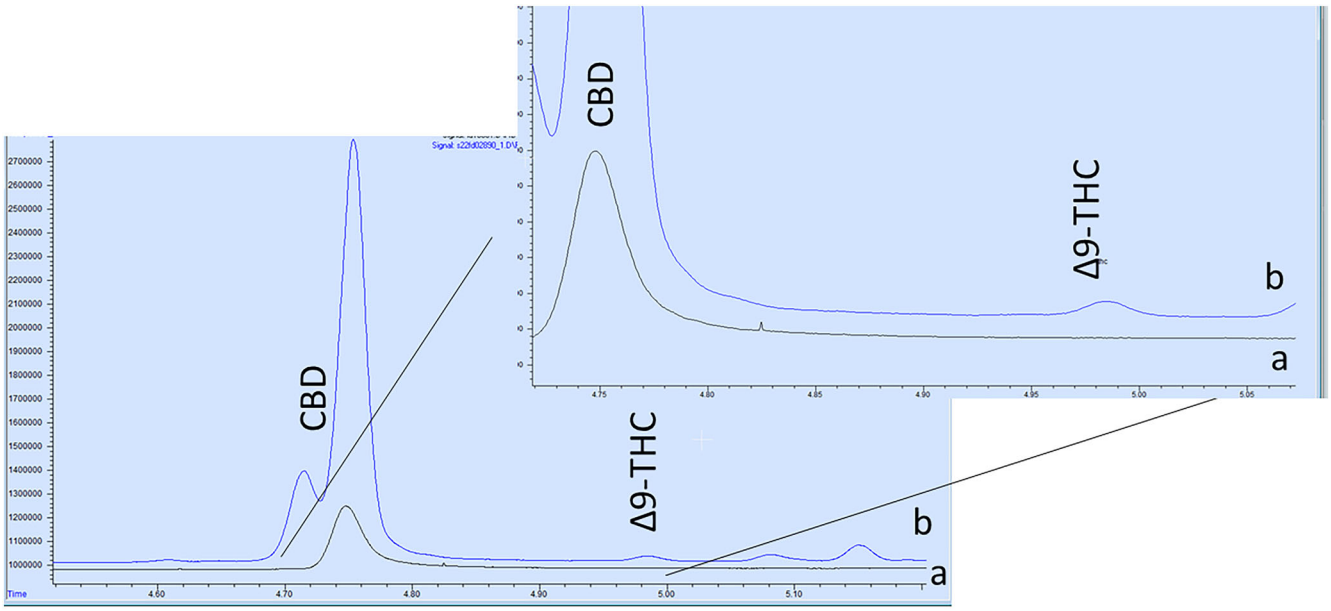

FIGURE 1 Analysis of a cannabidiolic acid (CBDA) reference standard with gas chromatography-flame ionization detector (GC-FID) under analytical conditions described in Duchateau et al.[2] (a) Chromatogram for the standard solution; (b) chromatogram of a herbal sample found positive for Δ9-THC [Colour figure can be viewed at wileyonlinelibrary.com]

Eric Deconinck[1,2]
Michaël Canfyn[1]
Céline Duchateau[1,2]
Kris De Braekeleer[2]

[1]*Scientific Direction Chemical and Physical Health Risks, Service of Medicines and Health Products, Brussels, Belgium*
[2]*Pharmacognosy, Bioanalysis & Drug Discovery, Faculty of Pharmacy, ULB, Brussels, Belgium*

Correspondence
Eric Deconinck, Scientific Direction Chemical and Physical Health Risks, Service of Medicines and Health Products, Sciensano, J. Wytsmanstraat 14, B-1050 Brussels, Belgium.
Email: eric.deconinck@sciensano.be

ORCID

Eric Deconinck https://orcid.org/0000-0001-6980-6684
Kris De Braekeleer https://orcid.org/0000-0002-3910-2990

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