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Introduction:

It has been shown for amphetamines that ¹³C labeled forensic standards are superior to the corresponding deuterium labeled compounds.

.¹³C labeled compounds have:

- Identical physicochemical properties as their native analogues, thus identical elution time response factors.
- The chemical character can be used to inhibit the negative effects of ion suppression and matrix effects in LC-MS.
- Using ¹³C labeled IS, uncertainty related to loss of sampling material, instability, matrix effects and ion suppression are negated
- Undisputable results applicable in court.

Results:

THC-analysis using ¹³C labeled compound:

 Urine analysis of THC and THC metabolites is renowned to be elusive, with unstable compounds, metabolites known to get stuck on hydrophobic surfaces and the metabolites need to be enzymatically broken down completely before analysis by LC-MS.

- Application of ¹³C labeled IS in THC analysis has a huge potential for improvements in certainty. Several of the challenges for THC-analysis can possibly be negated using ¹³C labeled standards.
- New ¹³C labeled THC and metabolites are reported here and their potential application in THC analysis is proposed.

Experimental:

• Samples from these substances were analyzed by HPLC and NMR and compared to the native substances. In **Fig. 2** ¹³C₄-THC was compared to hexane extract of a *Cannabis Sativa* hybrid strain by GC-MS, to verify identical elution. In the MS spectrum there were two sets of peaks corresponding to a mass difference of 4 due to the 4 extra neutrons in the labeled substance.





• The ¹³C labeled ISs for cannabis analysis were synthesized in our lab from ¹³C₄- labeled *n*-butanol through several advanced steps yielding isotopic pure products of the most common metabolites of THC, in addition to THC, see **Fig. 1** where the metabolic pathway is exemplified with ¹³C₄ labeling of the internal standards.



Fig 2. GC chromatogram overlay of THC extract from Cannabis Sativa and Synthetic ¹³C₄-THC

• In Fig. 3 shown the overlap of the retention times of labeled ¹³C₄-THC-COOH and the native THC-COOH in LC-MS.



Fig 3. LC chromatogram of synthetic ¹³C₄-Δ8-THC-COOH, ¹³C₄-Δ9-THC-COOH, Δ8-THC-COOH and Δ9-THC-COOH.

Conclusion:



$^{13}C_4$ -11-COOH- Δ 9-THC

Fig 1. Metabolic pathway of THC as exemplified by ¹³C₄-THC.

The physico-chemical properties of ¹³C labeled THC and THC-acid are identical with the native substances both in GC-MS and LC-MS with a mass difference of 4 from the native substances making them suitable for use as a IS and possibly giving a more robust analytical method compared to ²H. This is due to the shown equal chemical characteristics of the labeled substance compared to the native.

References:

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