Analysis of certain cannabinoid metabolites in urine samples from Hungary by GC/MS after alkaline and enzymatic hydrolysis

ELŐD HIDVÉGI[1], EDIT PENCZI[1], ZSUZSA HIDEG[1], DOUWE DE BOER[2], GÁBOR SOMOGYI[1],

[1] National Institute of Forensic Toxicology, Budapest, Hungary

[2] Department of Clinical Chemistry, University Hospital Maastricht, Maastricht, The Netherlands

Cannabinoids mainly occur in the form of glucuronide conjugates in the human urine after the consumption of hashish or marijuana. Glucuronidation takes place on hydroxyl or carboxyl groups. While the conjugates of carboxylic acids can be liberated by alkaline hydrolysis, enzymatic hydrolysis is necessary for the formation of free neutral cannabinoids from conjugates. Subsequently, an appropriate derivatisation makes the GC/MS analysis of an extensive urinary cannabinoid profile achievable and indirect investigation of plant source material feasible.

We found in our previous work1 that 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (Δ9-THC-COOH), the carboxylic acid metabolite of Δ^9-tetrahydrocannabinol which is the main psychoactive component of hashish and marijuana, and 11-nor-Δ^9-tetrahydrocannabivarin-9-carboxylic acid (Δ^9-THCV-COOH), the carboxylic acid metabolite of the minor cannabinoid Δ9-tetrahydrocannabivarin can be investigated simultaneously by GC/ion trap-MS after alkaline hydrolysis, liquid-liquid extraction and derivatisation with N-methyl-N-(trimethylsilyl)trifluor oacetamide/trimethylchlorosilane mixture. The ratio of the carboxylic acid metabolites (Δ9-THC-COOH/Δ^9-THCV-COOH) based on base peak fragments of derivatives showed lognormal distribution in the investigated sample populations. Moreover, the mean of the distribution was significantly different between two investigated sample populations (n=30) originating from Hungary and Portugal, indicating indirectly the use of hashish and marijuana of different plant material sources.

In this study we show our results on a more numerous (n=49) Hungarian sample population with an adapted method. For the analysis we selected urine samples previously screened for Δ^9-THC-COOH by HPLC/DAD analysis and showing high (>1µg ml^{-1}) Δ^9-THC-COOH concentration. The samples were diluted by distilled water prior to extraction in order to get Δ^9-THC-COOH concentration of approximately 1 µg ml^{-1}. The sample preparation included alkaline hydrolysis, solid phase extraction of urine samples, derivatisation with N-methyl-N(trimethylsilyl)-trifluoroacetamide and analysis with GC/quadrupole-MS.

According to the Kolmogorov-Smirnov normality test, the logarithmically transformed target peak area ratios of the base peaks of the bis-trimethylsilyl derivative of the carboxylic acid metabolites (Δ^9-THC-COOH at *m/z* 371 and Δ^9-THCV-COOH at *m/z* 343, respectively) showed normal distribution. This confirms previous results and shows that the type of distribution was unaffected by the mode of extraction and instrumental analysis. Some samples were screened for neutral cannabinoids after enzymatic hydrolysis with type IX-A ß -glucuronidase of Escherichia coli with the modified method of Kemp *et al.*[2]. In two samples of high Δ^9-THC-COOH concentration we detected cannabigerol (CBG), a neutral cannabinoid which is the precursor of cannabinoid biosynthesis in hemp (Cannabis sativa L.). CBG was identified with the help of the reference substance of CBG. The candidate peak had the same retention time and mass spectrum as the reference substance. Main characteristic ions were at *m/z* 337, 391, 377, 321 and 460 for the bis-trimethylsilyl derivative detected at 21.2 min, and at *m/z* 221, 275, 344, 207 and 261 for the bis-methyl derivative after flash methylation with trimethylanilinium hydroxide detected at 21.0 min. The gas chromatographic parameters were as follow: *capillary column:* HP-5MS of 25 m length, 0.2 mm inner diameter and 0.33 µm film thickness. *Injector:* 270°C, oven initial temperature: 100°C, rate: 10° min^{-1}, final temperature: 290°C, final time: 25 min. Carrier gas (He) pressure: 120 kPa, splitless time: 0.6 min, total flow: 17.2 ml min^{-1}.

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Corresponding author: hidvegielod@freemail.hu