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Analysis of cannabinoids by liquid chromatography–mass spectrometry in milk, liver and hemp seed to ensure food safety

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ABSTRACT

A method for determining cannabinoids, Δ9-tetrahidrocannabinol (THC), 11-nor-9-carboxy-Δ^9-THC (THC-COOH) and 11-hidroxy-Δ^9-THC (THC-OH) in milk, liver and hemp seeds based on liquid chromatography tandem mass spectrometry has been optimized and validated. Analytes were extracted with methanol and the extracts cleaned-up by solid-phase extraction using Oasis HLB (60 mg). The developed method was validated according to the Commission Decision 2002/657/EC. The decision limit (CCα) and detection capability (CCβ) ranged from 3.10–10.5 ng g^{-1} and 3.52–11.5 ng g^{-1}, the recoveries were 76–118% and matrix effect ranged from −17.8% to 19.9% in the three matrices studied. The method was applied to food samples obtaining positive results for THC in hemp seeds (average 0.82 μg g^{-1}) and three brands of junior formula milk at concentrations from 4.76 to 56.11 ng g^{-1}. The developed method was suitable achieving identification and quantification of cannabinoids in food matrices.

1. Introduction

Hemp (*Cannabis sativa*) is a plant able to synthetize more than 60 cannabinoids being the main active component the Δ9-tetrahidrocannabinol (THC), followed by the cannabidiol and the cannabinol (Lachenmeier, Kroener, Musshoff, & Madea, 2004). The hemp varieties allowed for cultivation in Europe have less than 0.2% THC, which is mostly present as Δ9-tetrahydrocannabinol acid (THC-A) a non-psychotropic constituent that account for 90% of total cannabinols in fiber-type cannabis plant (EFSA, 2011; Grotenhermen, 2003; Huestis, 2007; Takeda et al., 2012). However, hemp seeds have lower THC content, mainly in the external surface, as result from physical contamination with plants debris, products like hemp straw or hemp oil seed-cakes are a suitable feed material for livestock due to its high fiber content. After harvest, the THC-A begins its transformation into THC, a process quickened by heat and sunlight. Most of the acid form will be transform in THC of hemp oil seed-cakes that are obtained at high temperatures (EFSA, 2011). The noticed practice indicates that a daily amounts of 0.5 to 1.5 kg whole hemp plant dry matter can be incorporated in the daily ration of dairy cows (EFSA, 2011). The scientific opinion of the European Food Safety Authority (EFSA) on the safety of hemp (*Cannabis genus*) recommended to put whole hemp plant-derived feed materials in the list of materials banned for animal nutritional purposes and to introduce a maximum THC content of 10 mg kg^{-1} to hemp seed-derived feed material (EC., 2009; EFSA, 2011).

According to the limited number of studies performed in farm animals, after oral exposition [biodisponibility of the THC is from 6 to 30% (Ashton, 2001)], THC is metabolized in liver by oxidation through CYP 2C9 to its principal active metabolite (±)-11-hidroxy-Δ^9-THC (THC-OH) that presents higher psychotropic activity. Then, THC-OH is oxidized again to form the inactive metabolite 11-nor-9-carboxy-Δ^9-THC (THC-COOH). THC is excreted within days and weeks, mainly as metabolites, about 20–35% in urine and 65–80% in feces. Mainly the THC-COOH glucuronide is excreted in urine, and contrarily, the metabolites in the feces are only present as the non-conjugated form (Grotenhermen, 2003; Huestis, 2007). THC and its metabolites due to their lipophilic character, are distributed in the different tissues and organs, and can be excreted into milk (EFSA, 2011) as already reported in humans (Plotka, Narkowicz, Polkowska, Biziuk, & Namiesnik, 2014), squirrel monkeys (Chao et al., 1976), ruminants (Beltrán, Althaus, Molina, Berruga, & Molina, 2015), buffalos (Ahmad & Ahmad, 1990) and cows (Guidon & Zoller, 1999). In view of THC psychological effects and the EFSA concern, the quantification of the parent compounds and its metabolites in milk, liver and hemp-seeds is essential in order to ensure safe intake levels.

A summary of the analytical methods recently developed in a variety of matrices that include urine, blood, liver and milk is

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Table 1
Summary of different studies determining cannabinoids in several complex matrices.

Matrix	Compound	Extraction	Determination	Chromatographic time	LOD (ng mL^{-1})	LOQ (ng mL^{-1})	Recovery (%)	References
Liver, plasma, urine and other tissues	THC, THC-OH and THC-COOH	- Previous enzymatic hydrolysis (plasma and urine) - Alkaline extraction (NaOH) + Extraction with hexane: ethyl acetate (7:1, v/v)) for THC and THC-OH - Acidic extraction (HCl) + Extraction with hexane:ethylacetate (7:1, v/v) for THC-COOH	GC–MS Derivatization (BSTFA–TMCS)	- Alkaline fraction: 20.5 min - Acidic fraction: 9.33 min	–	0.5 plasma 5 other tissues	–	Brunet et al. (2006)
Nail	THC and THC-COOH	- Alkaline hydrolysis (NaOH) + Extraction with ethyl acetate - Acidic hydrolysis (acetic acid) + Extraction with *n*-hexane: ethyl acetate (9:1, v/v)	GC–MS Derivatization (MSTFA)	16 min	*0.035–0.044	*0.2	76.3–86.6	Kim et al. (2008)
Hemp plant	THC and others cannabinoids	Methanol/chloroform (9:1, v/v)	HPLC-DAD	36 min	62.5–250	125–250	97.2–109.6	De Backer et al. (2009)
Blood	THC, THC-OH, THC-COOH and others cannabinoids	Automated on-line SPE (acetonitrile for elution)	LC–MS ESI+	10 min	0.5–3	2–8	–	Jagerdeo et al. (2009)
Hair	THC and other cannabinoids	- Alkaline hydrolysis (NaOH) + LLE with *n*-hexane: ethyl acetate (9:1, v/v) - Methanol extraction + LLE with acetonitrile	GC–MS Derivatization (MSTFA + ethyl acetate).	28.3 min	*0.02–0.05	*0.05–0.15	–	Auwarter et al. (2010)
Plasma and urine	THC, THC-OH, THC-COOH	- Plasma: Enzymatic hydrolysis and automated SPE (acetone for elution) - Urine: Enzymatic hydrolysis and automated SPE (hexane: ethyl acetate 8:2, v/v, for elution)	GC–MS Derivatization with BSTFA–TMCS	- Plasma: 31.5 min - Urine: 22.5 min	0.1	0.1 urine 0.75 plasma	61–76 (Plasma) 68–88 (Urine)	Brenneisen et al. (2010)
Milk	THC, THC-OH, THC-COOH and other illicit drugs	SPE (Methanol + dichloromethane: isopropanol 8:2, v/v with 2% ammonium hydroxide for elution)	LC–MS/MS ESI+	20 min	1.0–1.5	5.0	53.2–63.5	Marchei et al. (2011)
Urine	THC, THC-OH THC-COOH	Alkaline hydrolysis + SPE (chloroform: ethyl acetate 6:4, v/v for elution)	GC–MS Derivatization with BSTFA-1%TMCS	25 min	1.0–2.5	2.0–3	71.9–78.6	Nestic et al. (2013))
Cannabis sativa L. plant	THC and other cannabinoids	SFE (CO2 was used as extraction solvent and ethanol (20%) as co-solvent in order to modify polarity)	LC–MS/MS APCI +	28 min	0.05–2	–	–	Aizpurua-Olaizola et al. (2014)
Urine	THC, THC-OH, THC-COOH and other cannabinoids	Enzymatic hydrolysis + μ-SPE (methanol for elution)	LC–MS/MS ESI + (Except THC-COOH in ESI -)	5.8 min	2.0–4.0	6.0–10.0	65–85	Montesano et al. (2014)
Urine	Synthetic cannabinoids	Enzymatic hydrolysis + SPE (chloroform/acetone 1:1, v/v + ethyl acetate/ammonia water 94:4, v/v)	LC–MS/MS ESI+	12 min	0.1–1	0.25–1	65–99	Jang et al. (2015)

THC: Δ^9-tetrahydrocannabinol; THC-OH: (±)-11-hydroxy-Δ^9-THC; THC-COOH: 11-nor-9-carboxy-Δ^9-THC; LOD: Limit of detection; LOQ: Limit of quantification; LLE: Liquid-liquid extraction; SPE: Solid phase extraction; SFE: Supercritical fluid extraction; GC–MS: Gas chromatography-mass spectrometry; HPLC-DAD: High performance liquid chromatography with photodiode array detection; LC–MS: Liquid chromatography mass spectrometry; LC–MS/MS: Liquid chromatography–tandem mass spectrometry; MSTFA: *N*-methyl-(trimethylsilyl) trifluoroacetamide; TMCS: trimethylchlorosilane; BSTFA: N,O-Bis (trimethylsilyl) trifluoroacetamide; ESI: electrospray ionization; APCI: atmospheric pressure chemical ionization.

* Concentration in ng mg^{-1}.