Trends in Analytical Chemistry 95 (2017) 149–158

Contents lists available at ScienceDirect

Trends in Analytical Chemistry

journal homepage: www.elsevier.com/locate/trac

The role of derivatization techniques in the analysis of plant cannabinoids by gas chromatography mass spectrometry

B. Fodor [a, b], I. Molnár-Perl [a, *]

[a] Institute of Chemistry, Department of Analytical Chemistry, L. Eötvös University, H-1117, Pázmány Péter sétány 1/A, Budapest, Hungary
[b] Doctoral School of Pharmaceutical Sciences, Semmelweis University, 1085, Üllői út 26, Budapest, Hungary

ARTICLE INFO

Article history:
Available online 28 July 2017

Keywords:
Plant cannabinoids
Gas chromatography
Mass-spectrometry
FID
Derivatization
Trialkylsilylation
Acylation/esterification
Detection/acquisition studies
LOD/LOQ evaluation

ABSTRACT

Derivatization is the most powerful contribution to the identification and quantification of plant cannabinoids (p-CBDs) by gas chromatography-mass spectrometry (GC-MS): providing volatile derivatives with eminent properties (high selectivity, outstanding sensitivity and mass spectrometric peculiarities). These derivatives are excellent candidates to determine the main p-CBDs, like tetrahydrocannabivarin (THCV), tetrahydrocannabidivarin (CBDV), cannabidiol (CBD), cannabichromene (CBC), cannabicyclol (CBCL), tetrahydrocannabinol (THC), cannabigerol (CBG), cannabinol (CBN), 11-hydroxy-Δ^9-THC (11-OH-THC) and 11-nor-9-carboxy-THC (THC-COOH). Identification and quantification of p-CBDs is required – partly as trace constituents in complex biological matrices of drug users, partly as main components in seizure samples: in both cases, in extremely different ratios. GC proposals published between 2000 and 2017, along with outstanding pioneer contributions, are reviewed. Procedures, without derivatization and applying various alkylsilyl, acylation and/or esterification techniques were listed, compared and criticized. Further sorting was based on the reagent type, on examined matrices, on enrichment/detection related acquisition protocols and on analytical performance characteristics.

1. Introduction

The relevancy and continued interest towards chromatographic analyses of p-CBDs (called also marijuana) can be explained by their exponential spread in the community of drug users, started 6000 years ago [1] and continued by their partial legislation, recently [2]. Four states in the USA have legalized the cultivation, distribution and recreational use of marijuana; in addition the District of Columbia legalizes marijuana in some form. As to Europe, in the Netherland a hybrid system exists, reflecting Dutch policy [3]: in coffee shops namely, up to 5 g p-CBDs, in different forms are available; however plant's cultivation, trade and possession in the Netherlands is prohibited.

The continuously increasing popularity of p-CBDs in drug users' community is not questionable.

Literature overview of proposals – published in the time range of 2000 and 2017 [1–103] -reflects significant innovations to the field – compared to the pioneer works (Supplemental files, pioneer proposals, Graphical Abstract: 1968 [S1] 1966 [S2] 1983 [S3]).

The last comparison of GC-MS analytical techniques concerning p-CBDs' analysis was published in 1979 [S4].

It is worth mentioning that the pioneer methods dated back to the last third of the twentieth century including the activity of the pioneer 'green chemist' Rasmussen [S5, S6].

Out of reviews [4–11], appeared between 2000 and 2017 – specified exclusively to the p-CBDs [4,6,8,11] – were focused on their analysis in biological materials [4], on the efficacy of harm reduction strategy among people with schizophrenia [6], on legislation of driving under the influence of cannabis [8] and summing up micro extraction techniques for analysis of p-CBDs [11]. Reviews for illicit drugs in general, including also p-CBDs [5,7,9,10], were related to the solid phase micro extraction (SPME) techniques in analytical toxicology [5], to quantify p-CBDs in drug users' oral fluids [7], to enrich in micro-particles from the air [9], or, to perform dispersive liquid-liquid micro extraction (DLLME) in forensic toxicology [10].

The aim of this review was to pinpoint the advancements and advantages of GC analysis for the principal constituents of p-CBDs. The structure, physical chemical properties of p-CBDs, their possible reagents and the corresponding derivatized products are shown in Supplemental Files (Table S1: structure of p-CBDs in initial forms; Table S2: structure of derivatization reagents; Table S3: structure of p-CBDs as silylated and acylated derivatives).

We summed up the relevant papers into four groups according to the techniques employed and to the analytes investigated:

* Corresponding author.
E-mail address: perlne@chem.elte.hu (I. Molnár-Perl).

http://dx.doi.org/10.1016/j.trac.2017.07.022
0165-9936/

Table 1 shows methods developed for the analysis of p-CBDs in their initial forms, without derivatization [1,2,12–37], while Tables 2–4 comprises methodologies suitable to identify and quantify compounds derivatized via alkylsilylation (Tables 2 and 3; [38–86]) and combined acylation/esterification techniques (Table 4; [88–103]).

Methods were further sub grouped in Tables 1–4 according to the type of enrichment processes carried out from various, p-CBDs-containing matrices. Within such groupings, papers are listed in chronological order of their publication.

Moreover, a schematic overview of our approach is presented in Fig. 1 that visualizes the matrices, methods and citations documenting p-CBDs' analysis by gas chromatography. Numerical data indicate that the most frequently analyzed samples vary from 28.7% (whole blood, plasma, or serum) to 1.1% (bile, airborne particles, meconium, e-Cigarette, Marijuana Formulation, fingernail), expressed in the total of our paper selection.

Criticality is focused on optimized derivatization conditions, reproducibility, selectivity, sensitivity indicated with analytical performance characteristics (LOD, LOQ values), as well as with time-, labor and complexity of methodologies.

2. GC of p-CBDs

In general, it is worth mentioning that derivatization does have a particular importance in the GC based protocols. This time consuming and tedious process, by many application chemists, preferring liquid chromatographic separation of underivatized species, was and still is regarded as the main disadvantage of sample preparation, needed prior to GC analyses. However, this so called 'disadvantage' is dwarfed in comparison to several advantages. This means that (i) on the one part GC methods provide increased selectivity, sensitivity and the possible identification and quantification of numerous species on a single column, simultaneously, while, (ii) on the other part they do not suffer of ion suppression phenomena as LC analyses of underivatized species do.

It is worthy to note that recently also in LC techniques – to be comparable with the GC-MS-MS standard protocol – derivatization was reported as a not avoidable step [98]. For this purpose, prior to the LC-MS-MS analysis of the THC-COOH accumulation in hair samples, an esterification step was inserted. The methyl ester of THC-COOH manifested increased stability, selectivity and sensitivity: corresponding in thorough accordance with those results

Table 1
Analysis of CBDs by gas chromatography (GC) without derivatization.

Matrix/amount	Enriched by	Acquisition	LOD	LOQ	Aim; compounds	Ref.
			ng/mL	*ng/mg*		
Leafy material/50 mg	C_2H_5OH extr	GC-FID	–	–	Chemotype selection; THC, CBD	[12]
Resinous floral blact/50 mg	$CHCl_3$ extr	GC-FID	–	–	Chemotaxonomic analysis; THC, CBD, CBDV, THCV, CBC, CBGM, CBG	[13]
Grounded, dried marijuana/60 mg	HS-SPME 80–150°C	GC-MS	–	–	HS-SPME optimization (Swiss grown marijuana),THCV, CBCL, CBV, CBD, CBC; THC, CBG, CBN	[14,15]
Hair (3 mm cuts)/50 mg	*washing	GC-MS	THC: 414 CBN: 43.8		In vitro contamination of hair by marijuana smoke; THC, CBN	[16]
Soap bar resin/25 mg	CH_3OH	GC-MS	–	–	Cannabinoids' relative amounts definition; THC, CBN, CBD	[17]
Plasma/–	SPE	GC-MS-SIM	–	0.5–1.0	Time estimation of cannabis use; THC, THC-COOH	[18]
Hair (1 mm cuts)/10 mg	HS-SPME	GC-MS-SIM	*0.07*	*0.12*	Hair contamination study; THC, CBN, CBD	[19]
Powdered seizures/100 mg	$CH_3OH/CHCl_3$ = 9/1 extr	GC-FID	*3* μg/mg	*5* μg/mg	Seizure analysis phenotype selection; THC, CBN, CBD	[20]
Seizure resin block/100 mg	CH_3OH extr	2D-GC-FID,TOF-MS	–	–	Pixel based chemometric analysis of seizures; THC, CBD, heroine	[21]
Saliva/0.2 mL	PMME**	GC-MS-SIM	0.68	–	Method optimization, THC	[22]
Cannabis/0.1 g	$CH_3OH/CHCl_3$ = 9/1 extr	GC-FID	–	–	Propagation dependent distribution study of cannabinoids and seizure analysis; THC, THCV, CBD, CBC, CBG, CBN	[23–26]
Hair/10 mg	HS-SPME	GC-MS-MS	*0.007–0.031*	0.012–0.062	Method validation; THC, CBN, CBD	[27]
Hair/50 mg	LLE, pentane	GC-MS	*0.01*	*0.02*	Method development; THC	[28]
Milled plants/50 mg	SFE, FUSE	GC-MS	–	–	SFE, FUSE extractions' optimization/ comparison; THC, CBD, CBN	[29]
Oral fluid/0.1 mL	SPME	GC-MS	0.5–20	2–690	Drug users' testing; THC, CBN, CBD	[30]
Plant material/0.2 g	CH_3OH extr	GC-MS	–	–	Cannabis profiling; THC, CBN, CBD, CBG	[31]
Plant/50 mg	SFE; SC-CO_2	GCxGC-MS	–	–	Resolution study of *Cannabis sativa* CBDSs	[32]
Plant/25 g	C_6H_6 extr	GC-MS	–	–	Cannabis profiling; THC (THC-COOH decarboxylated)	[33]
Powdered seizures/100 mg	$CH_3OH/CHCl_3$ = 9/1 extr	GC-FID	–	–	Cannabinoids' seizure analysis; THC, THCV, CBD, CBC, CBG, CBN	[34]
Cannabis/50 mg	Vaporisers®	GC-MS-SIM	–	1–250 μg/mL	Smoke free inhalation optimization study; CBD, CBC, THC, CBN	[1]
Controlled liquid/1 mL	Tube dipping	DART-MS, GC-MS	–	–	Marijuana e-cigarette formulation study; CBDs and all and terpenes	[2]

Indications: extr = simple or ultrasound assisted shaking/extraction followed by filtration; BGM = cannabigerol monomethylether; *washing = exposed to marijuana smoke for 60 min, followed in three consecutive washing solutions, in total; SPE = solid phase extraction; **PMME = polymer monolith micro extraction; amount® = taken for one sample preparation; LOD/LOQ = limit of detection/limit of quantitation values; – = no data available; SPME = solid phase micro extraction; HS-SPME = headspace SPME; HS-SPDE = headspace solid phase dynamic extraction; LLE = liquid-liquid extraction; SFE = supercritical fluid extraction; SC-CO_2 = supercritical carbon dioxide; FUSE = focused ultrasound extraction; 2D = two dimensional; DART = Direct Analysis in Real Time.
ng/mg throughout the LOD and LOQ values in Table has been described in italics.

Table 2
Analysis of cannabinoids, derivatized with BSTFA, determined by GC-MS.

Matrix/amount	Enriched by	Derivatized			Acquisition; *m/z* fragments	LOD	LOQ	Aim; compounds	Ref
		With	°C	Min		ng/mL	*ng/g*		
Blood, urine/1 mL	LLE, C_6H_6/EtAC = 9/1	BSTFA	70	15	GC-MS/MS; 372 → 305, 289	–	5–50	Analysis at pg level; THC-COOH	[38]
Urine/1.5 mL	SPE	BSTFA, 1% TMCS	70	10	GC-MS; 488, 473, 398, 371	2.44	9.48	Matrix optimization; THC-COOH	[39]
Plasma/1 mL	SPE	BSTFA, 1% TMCS	80	15	SPE with a single eluant; GC-MS-SIM(PCI); THC: 387, 11-OH-THC: 459, THC-COOH: 489	0.5–1.0	0.5–1.0	SPE simplification study; THC, 11-OH-THC, THC-COOH	[40]
Rabbit plasma/0.5 mL	SPE	1. BSTFA:PYR = 1:3 2. TFAA	60 Room temp	60	1. GC-MS-EI; THC-TMS: 386 2. GC-MS-NICI; THC-TFA: 410	–	10 0.3	Two steps derivatization, two ionization techniques; THC	[41]
Hair/50 mg (5 mm cut)	Washing: 1. neutral n; 2. acidic a,	1n. THC, CBN, CBD 2a BSTFA, 1% TMCS	No derivation 60	20	GC-MS; 1n: without derivatization, 2a: as BSTFA derivative	*n: 0.012* *a: 0.024*	*n: 0.02* *a: 0.080*	Two fractions (n, a) analysis: 1. THC, CBD, CBN, 2.THC-COOH	[42]
White pig blood/5 mL	LLE, C_6H_6/EtAC = 7/1	BSTFA, 1% TMCS	70	20	GC-MS; two fractions, separately silylated, determined	–	0.5–*5*	THC metabolism study/ animal model; THC → 11-OH-THC, THC-COOH	[43]
Plasma/1 mL	Hydr, SPE	BSTFA, 1% TMCS	80	45	GC-MS-SIM; THC: 387, 11-OH-THC: 459, THC-COOH: 492	–	0.5–1	Oral administration control; THC, 11-OH-THC, THC-COOH	[44]
Whole blood/1 mL	SPE; 1. n; 2. a,	BSTFA, 1% TMCS	70	20	2D-GC-MS; two fractions, eluted into the same vial	–	1.0	Derivatization optimization; THC, THC-COOH	[45]
Urine/1 mL	LLE, C_6H_6/EtAC = 7/1	BSTFA, 1% TMCS	60	30	GC-MS-SIM: 371, 374	–	20	Administered THC-COOH in urine	[46]
Plasma/1 mL	Hydr, SPE	BSTFA, 1% TMCS	70	30	2D-GC-MS, 1D: 15 m; criofocusing, 2D: 30 m)	0.125–0.25	0.125	m.o.; THC, 11-OH-THC, THC-COOH	[47]
Hair/100 mg	HS-SPME-PDMS	BSTFA, 1% TMCS	125	20	GC-MS-SIM; THC: 303, 371, 386; CBD: 351, 390, 458; CBN: 310, 367, 382	*0.01–0.02*	*0.39–4.2*	m.o.; THC, CBD, CBN	[48]
Oral fluid/1 mL	SPE	BSTFA, 1% TMCS	60	15	GC-MS; THC: 386, 371, 303; CBN: 367, 382; THC-COOH:487, 488	–	5.0	m.o.; THC, CBD, CBN, THC-COOH	[49]
Whole blood/1 mL, urine/2 mL	SPE	BSTFA, 1% TMCS	70	45	2D-GC-MS, 1D-GC-MS: 15 m; criofocusing, 2D: 30 m)	–	0.25–0.50	m.o [47]. and abusers' urine control [48]; THC, 11-OH-THC, THC-COOH	[50,51]
Bile/1 mL	LLE; C_6H_6 EtAC/ACA = 90:10:1	ACN/BSTFA, 1% TMCS = 1/1	70	30	GC-MS-SIM; THC: 303, 371, 386; THC-COOH: 371, 473, 488	0.28	0.86	m.o.; THC, THC-COOH	[60]
Urine/1 mL	HF-LPME	BSTFA, 1% TMCS	90	15	GC-MS-SIM	1.5	2.0	THC-COOH	[68]

Indications as in Table 1, as well as: (PCI) = positive chemical ionization; HF-LPME = hollow fiber-liquid phase micro extraction; m.o. = matrix and p-CBDs related method optimization; HS-SPME-PDMS = HS-SPME-polydimethylsiloxane; NICI = negative ion chemical ionization.
ng/mg throughout the LOD and LOQ values in Table has been described in italics.

which have been obtained by the well established GC-NICI-MS-MS technique. This experience is a trend to increase selectivity and sensitivity also in the LC protocols.

2.1. Quantitation of p-CBDs in their initial forms, without derivatization (Table 1)

Concerning quantitation of plant matrices [13,23–26,34] flame ionization (FID) detection was regarded satisfactory providing excellent, fast results. Primarily GC-FID was applied when the ratios of the expected constituents had to be defined: based on the responses and retention properties of authentic standards.

Simultaneous analysis of seven [13] six [35] and five [23–26,34] p-CBDs were presented throughout the time reviewed, retaining the relevancy of this simple, cost-effective technique.

In addition, mass selective identification and quantification protocols were also employed mostly along with process validation [1,16,18,19,22,27,28,30,36,37].

2.2. Identification and quantification of p-CBDs as various derivatives (Tables 2–4)

Papers related to p-CBDs analysis in their initial forms, without derivatization (Table 1) undoubtedly confirmed that to identify and quantify their trace amounts, especially in biological matrices, quantitative enrichment protocols, followed by labeling strategy is unavoidable and obligatory.

3. The alkylsilylation techniques

In the light of p-CBDs' hydroxyl and carboxyl functions (Table S1) to trigger and exhaust the unique challenge of alkylsilylation approaches is obvious; these protocols are primarily suitable to volatilize and improve mass fragmentation properties of active proton containing groups, simultaneously [38–86]. Out of the wide choice of alkylsilyl reagents, the most commonly used BSTFA [38–69] and MSTFA [70–86] were preferred. The use of MTBSTFA, due to its assumed steric hindrance, remained of marginal importance [78,87].

3.1. Derivatization with BSTFA (Table 2); method optimization and biological activity studies

BSTFA derivations are the most popular suggestions for p-CBDs analysis by GC.

Evaluating conditions and results compiled in Table 2, it reveals that all selected proposals do provide LOQ values: promising the quantitative aspect of studies.

Table 3
Analysis of cannabinoids, derivatized with MSTFA, determined by GC-MS.

Matrix/amount	Enriched by	Derivatized			Acquisition; *m/z* fragments	LOD	LOQ	Aim; compounds	Ref
		With	°C	Min		ng/mL	*ng/mg*		
Hair/10 mg	HS-SPME, on fiber, fully automated	MSTFA	90	8	GC-MS-SIM; THC-TMS: 303, 371, 386; CBD-di-TMS: 301, 337, 390; CBN-TMS: 367, 368, 382	*0.08–0.14*	*027–051*	mo; CBD, CBN. THC	[70]
Hair/50 mg	Alkaline digestion, SPE	MSTFA/NH_4I/DTE = 100/2/4 (v/w/w)	70	30	GC-MS-MS; THC-TMS: 371; CBN-TMS: 367, CBD-TMS: 390; THC-COOH-TMS: 371	–	–	mo; THC, CBD, CBN, THC-COOH	[71]
Hemp product/1 mL or 1 g	LLE; C_6H_6/i-PrOH = 9/1	MSTFA, 0.2% TMCS	70	30	GC-MS-SIM, THC-TMS: 303, 371, 386; CBD-TMS 337, 390, 486; CBN-TMS: 310, 367, 382	0.3–0.6	0–2	mo; THC, CBD, CBN	[72]
Urine/2 mL	LLE; $CHCl_3$/iPrOH = 9/1	MSTFA, 1% TMCS	70	20	GC-MS-SIM, THC-COOH: 371, 473, 488	3	–	mo; THC-COOH	[73]
Fingernail/30 mg	basic/acidic hydr LLE; C_6H_6/ETAC = 9/1	MSTFA, 1% TMCS	70	15	GC-MS-SIM; THC-TMS: 315, 371, 386; THC-COOH-2TMS: 371, 473, 488	<0.056–0.2	mo; THC, THC-COOH + A, MA + MDMA)		[74]
Urine/2 mL	SPE*	MSTFA/NH_4I/DTE (500:4:2, v/w/w)	60	20	GC-MS-SIM; THC: 386; OH-THC and THC-COOH: 371, 386	0.1	0.2	mo; THC, OH-THC, THC-COOH	[75]
Rat urine/1 mL	enzimatic hydr; SPE	MSTFA/ETAC = 1/1	90	45	GC-MS-SIM	–	–	metabolism study: THC-COOH to THC	[76]
River/500 mL, waste water/100–200 mL	SPE	MSTFA	80	60	GC-MS-MS; THC-TMS: 386 → 315, 330; THC-2TMS: 473 → 355	0.9–1	2.7–3.0	THC, THC-COOH + 10 others	[77]
Urine/5 mL	enzimatic hydr; LLE; C_6H_6/ETAC = 7/1	MSTFA	80	30	GC-MS; CBG-2TMS: 337, 391, 377, 460	–	–	mo; metabolism study: CBG	[78]
Blood (post mortem)/1 mL	LLE (C_6H_6: ETAC = 5/1, 2×), centrifuged	MSTFA	70	60	2D-GC-MS-SIM, CBD: 390; THC: 371; CBN: 367; 11-OH-THC: 371; THC-COOH: 371	0.25	0.25–0.50	CBD, THC, CBN, 11-OH-THC, THC-COOH	[79]
Hair/50–100 mg	LLE, 1 basic: THC; 2. acidic: THC-COOH	1.MSTFA, 0.2% TMIS 2. PFPAA/PFPOH	60 70	20 –	GC-MS/MS, (NCI)	*0.01* pg/mg	*0.04* pg/mg	two fractions analysis: THC, THC-COOH	[80]
Waste water/10 mL	SPME (60°C, 60 min)	MSTFA (on fiber)	40	10	GC-MS	1–2.5	3.3–8.3	THC, THC-COOH	[81]
Urine/1 mL	LLE	MSTFA/ethanethiol/NH_4I (500:4:2), MW:750 W, 1.5 min.			GC-MS/MS(SRM); 371 → 305, 371 → 289, 371 → 265, 371 → 95	0.057	0.19	mo; THC-COOH	[82]
Blood (post mortem)/1 mL	SPE	MSTFA	80	30	GC-MS; THC-2TMS: 315, 371, 386; THC-COOH-2TMS: 371, 474, 488	5	10	mo; THC, THC-COOH, (cocaine + amphetamines)	[83]
Plasma or serum/1 mL	SPE	MSTFA	70	20	GC-MS-SIM; THC: 303, 371, 386; OH-THC. 371, 459, 474; THC-COOH: 297, 371, 473	0.15–2.0	0.30–3.30	mo; THC, 11-OH-THC, THC-COOH	[85]
Serum/1 mL	LLE fully automated	MSTFA	80	30	GC-MS-SIM; THC: 303, 371, 386; OH-TH: 371, 459, 474; THC-COOH: 371, 473, 488	0.2–0.6	0.6–2.3	mo; THC, 11-OH-THC, THC-COOH	[86]

Indications as in Tables 1 and 2, as well as/or: *SPE = performed with calcium-hardened ß-cyclodextrin polymer; DTE = dithioerytrol; i-PrOH = isopropyl alcohol.
ng/mg throughout the LOD and LOQ values in Table has been described in italics.

Table 4
Analysis of cannabinoids, derivatized with acylation and/or esterification, determined by GC-MS.

Matrix/amount	Enriched by	Derivatized			Acquisition; *m/z* fragments	LOD	LOQ	Aim; compounds	Ref
		With	°C	Min		ng/mL	*ng/mg*		
Blood/1 mL	SPE	TFAA/$CHCl_3$ = 1/1	70	10	GC-MS-SIM; THC: 410, 11-OH-THC: 408	–	0.5	mo; THC, 11-OH-THC	[88]
Hair/50 mg	extr. $CHCl_3$/i-PrOH	PFPAA/PFPOH	65	30	GC-MS; CBD: 377, THC: 377,THC-COOH-2PFP: 489, CBD: 231, CBN: 295	–	–	mo; CBD, CBN, THC, THC-COOH	[89]
Blood/1 mL	extr. CH_6H_6	PFPAA/PFPOH = 2/1	70	25	GC-MS-SIM; THC: 417, 445, 460	0.5	–	mo; THC	[90]
Serum/1 mL	SPE	1.TBAH/DMSO = 5/1 2. CH_4I,	Room temp	→ 2 → 10	GC-MS, THC: 313, 328,245, 11-OH-THC: 313, 314, 358, THC-COOH: 313, 357, 372	0-49–0.65	0.62–3.35	mo; THC, 11-OH-THC, THC-COOH	[91]
Blood, serum/25 μL	SPE	TMAH/DMSO = 1:50, v/v, CH_3I	Room temp	15	GC-MS-SIM, THC: 313, 328, 11-OH-THC: 313, 358, THC-COOH: 313, 357	0.7–0.8	–	mo; THC, 11-OH-THC, THC-COOH	[92]
Sweat patches	extr. CH_3OH + SPE	TFAA (TEA)*	80	20	GC-MS(NICI); THC: 410	*0.2* ng/p	*0.4* ng/p	mo; THC	[93]
Blood/–	SPE	$CHCl_3$/TFAA/HFiPOH = 2/2/1	70	25	GC-MS-SIM; THC: 410, THC-COOH: 422	–	2,5	time estimation of cannabis use; THC, THC-COOH	[94]
Hair/50 mg	hydr → extr. C_7H_7/ETAC = 9/1	PFPAA/PFPOH = 5/3	70	30	GC-MS-MS(NICI); THC-COOH: 513 → 293, 363, 470	*50* pg/mg	*100* pg/mg	mo; THC-COOH	[95]
Hair/25 mg	hydr → extr. CH_6H_6/ETAC = 9/1	PFPAA/PFPOH = 2/1	70	30	GC-MS/MS(NICI); 611 → 483	*0.02* pg/mg	*0.05* pg/mg	mo; THC-COOH	[96]
Blood/0.5 mL	LLE: CH_6H_6/ETAC = 9/1	TFAA/HFIP = 2/1	70	25	GC-MS-MS/(NICI), SRM; THC: 410 → 313; THC-OH: 409 → 339; THC-COOH: 422 → 361	0.1–0.02	0.5–2.5	mo; THC, THC-OH, THC-COOH	[97]
Hair/20 mg	hydr → LLE extr. C_6H_6/ETAC = 9/1	PFPAA/PFPOH = 2/1	70	30	GC-MS-MS/(NICI), SRM; THC: 459 → 3973; THC-COOH: 602 → 474	*2.5–25*	*7.5–50*	mo; THC, THC-COOH	[98]
Hair/25 mg (mechanically pulverized)	Hydr → LLE extr. C_6H_6/ETAC = 9/1	PFPAA/PFPOH = 5/3	70	30	GC-MS-MS/(NICI), SRM; THC-COOH: 602 → 474, 602 → 513	*0.015* pg/mg	*0.05* pg/mg	mo; THC-COOH	[99]
Urine/0.5 mL	LLE, 1. basic: As 2. acidic: THC-COOH	1. TFAA 2. PFPOH	50 530	45 530	GC-MS-SIM; THC-COOH: 572	0.86	2.88	mo; THC-COOH + amphetamines	[100]
Hair/50–100 mg	MeOH extr	PFPAA/HFIPOH	–	–	GC-MS-MS/(NICI), 620 → 383, 492	–	*0.1* pg/mg	mo; comparison to LC-MS-MS; THC-COOH	[101]
Oral fliud/1 mL	SPE	TFAA/HFIP = 2/1	65	40	GC-MS-MS; THC-COOH: 522 → 490	0.007.5	0.0010	mo; THC-COOH	[102]
Urine/1 mL	NaOH hydr→acidified→ extr: C_6H_6/ETAC = 5/1	PFPAA/PF = 1/1	75	30	GC-MS-SIM; THC-COOH: 473, 459, 607, 622	1	2	THC-COOH elimination study from urine; THC-COOH	[103]

Indications as in Tables 1–3, as well a/or: TBAH/DMSO = tertabutylammmonium hydroxide/dimethylsulfoxide reagent; CH_4I = iodomethane derivatization reagent; TMAH = tetramethylammonium hydroxide; HFIPOH = hexafluoroisopropanol; TFAA (TEA)*100 μL of 0.01 mol/L triethylamine in heptane, and 20 μL of TFAA; p = patch.
ng/mg throughout the LOD and LOQ values in Table has been described in italics.

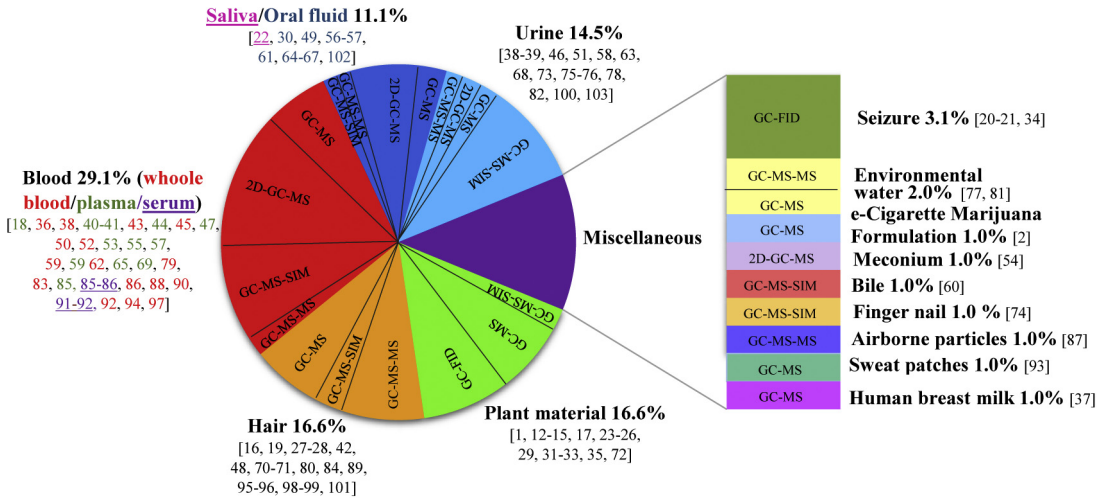

Fig. 1. Matrix, method and citations related analysis of cannabinoids by gas chromatography. Note: Literature overview of proposals was selected on Web of Science, Science Direct and Scopus basis obtained with the key words of Cannabinoids, gas chromatography in the publication time range of 2000–2017, including the novelty containing ones. Excluding papers that (i) did not give detailed sample preparation protocol, simply referred to a previously published article, as well as (iii) related to synthetic-, and/or endo-CBDs.

With two exceptions [41,42] analyses were carried out in one fraction. Independently of the species to be labeled, the use of 1% trimethylchlorosilane (TMCS) containing BSTFA was preferred. Also, BSTFA of its own [38], or in dilutions with pyridine (PYR) [41] was applied. Conditions for temperature (60–125°C) and time (10–60 min) of derivatizations were varied: consequently in the frame of this review optimized derivatization conditions can not be suggested.

Due to the intrinsic peculiarities of microextractions they are only suitable to determine the neutral [48] and acidic species [68], separately. GC-MS-SIM [40,44,47,48,60,68]; and GC-MS-MS acquisition was performed in a single case, only [38].

It is worth to note that this type of method developments regarding basic researches and innovative practical proposals equally, are significantly associated with the activities of NIH experts, leaded by Huestis [40,44,47,50–59,61–67,69]. It means, in addition to the details listed in Table 2, additional studies [61–67,69] manifested increased method selectivity performed via two dimensional (2D) GC-MS. This concept was extended and many sided utilized. Because of their relevant content, considered to be interesting in the community of analysts, biochemists and toxicologists, these are summed up briefly.

a) Providing a scientific database to asses p-CBDs in oral fluids (OF) [61].
b) Characterizing p-CBD elimination from blood of daily cannabis smokers by highlighting the usefulness of improving the accuracy of results interpretation [62].
c) Optimizing the alkaline and enzymatic hydrolysis conditions to the highest p-CBDs recovery in urine [63].
d) Following THC concentrations under therapy: meaning 4–5 days of monitored abstinence from smoking [64].
e) Evaluating the relationship between OF and plasma p-CBDs concentration depending on dose, route of administration and time after dosing [65].
f) Studying OF collection device impact on p-CBDs stability [66]. Since, reliable analytical and technical conditions were of primary importance in specifying time courses of THC, 11-OH-THC, THC-COOH, CBN and CBD concentrations during multiple and ad libitum smoking periods [67].
g) Defining pharmacokinetics of p-CBDs in plasma after controlled and ad libitum smoking making possible differentiation between chronic/frequent and single users [69].

3.2. Derivatizations with MSTFA (Table 3)

MSTFA, due to its stronger silyl donor behavior – next to BSTFA, out of seventeen cases in seven – was used without TMCS and solvents [67,77–79,83,86]. In two cases MSTFA was completed with 0.2% [72] or 1% [73,74] TMCS. Forced conditions were ensured applying the MSTFA 0.2% TMIS [80], or MSTFA/NH_4I/DTE [71,75], or MSTFA/C_2H_5SH/NH_4I [82] reagents. As solvent, in 1/1 vol ratios, ethyl acetate [76,84] was performed. Unfortunately the advantages of the use of special catalysts and solvents were not detailed.

Regarding the analytical performance characteristics – we have to accept that the comparisons after various enrichment working strategies, performed from different matrices are not, or moderately comparable. The optimum method selection is a matter of the technical and personal preparedness of the laboratory where the task is to be solved.

3.3. Derivatization via N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA)

A relevant topic was introduced to determine THC, CBD and (CBN), the primary active constituents of cannabis preparation, in airborne particulates [87]. The specific procedure consists of soot extraction by ultrasonic bath, purification by solvent partitioning, derivatization with MTBSTFA, and analysis by applying an optimized tandem GC-MS-MS technique. The proposal proved to be suitable to quantitate the three psychotropic substances at concentrations ranging from ~0.001 to ~5.0 ng/cm air. The procedure was performed on field in Rome and Bari (Italy), demonstrating that all three compounds contaminate the air in Italian cities. The comparison of MTBSTFA with MSTFA in the analysis of THC and 11-OH-THC revealed the preference of trimethylsilylation [81].

4. Acylation and/or esterification methods (Table 4)

All proposals in this section are matrix and method related optimization studies taking into consideration in particular the quantitation of THC-COOH.

Analyte enrichment processes are similar to detailed before (Tables 2 and 3).

Characteristic, combined derivatizations consist of acylation and esterification [89–92,94–103]. In our evaluation all these processes are overcomplicated; mostly aiming the full derivation of THC-

COOH. Two exceptions [88,93], in favor to quantify 11-OH-THC and THC [88] or THC [93] exclusively, acylation was performed.

These two reagents applying techniques, based on separate reactions with the hydroxyl and carboxyl moieties with one exception [93] were suggested in the analysis of biological matrices.

5. Comparison of derivatization approaches (Tables 2–4)

Reaction temperature and time of reagent depending techniques are not comparable: from these points of view even within the same reagent, basic researches fail. Especially as suggested reaction times and temperatures are varying inexplicably. BSTFA and MSTFA were applied in wide temperature and time ranges: BSTFA (Table 2) between 60°C for 15 min [49] and 125°C, for 20 min [48], while MSTFA (Table 3) between 40°C for 10 min [81] and 90°C, for 45 min [76].

Acquisition techniques' types were applied prominently in different ratios comparing the BSTFA, MSTFA and acylation/esterification derivatizations. Beside the simple GC-MS protocols [89,91,93], both the GC-MS-SIM [88,90,92,94,100,103] and the GC-MS-MS [95–100] techniques were represented. GC-MS-MS protocols, in increasing order of listing, in 20% (BSTFA: 3/15 × 100), in 40% and 40% (in MSTFA and in acylation/esterification, cases, equally: 6/15 × 100), respectively.

As to the analytical performance characteristics – due to the intrinsic peculiarities of the task to be solved – derivatization techniques' unambiguous comparison is impossible. Notwithstanding, contrasting the same sample type and size (blood/serum/urine, 0.5–2 mL), applying the GC-MS-SIM [47,75,103] and the tandem GC-MS-MS [38,82,97] acquisition protocols, LOQ values proved to be as follows:

GC-MS-MS: 5 ng/mL [38], 0.19 ng/mL [82] and 0.5 ng/mL [97];

GC-MS-SIM: 2.0 ng/mL [46], 0.2 ng/mL [75], 2.0 ng/mL [103];

In the light of these values MSTFA derivatization might be preferred.

Authors of this review are convinced that the use of two reagents in order to obtain full derivatization of p-CBDs and/or to perform analysis in two fractions is needless: (i) resulting in complexity of processes and (ii) leading to increased time and work consume. (iii) In addition, two fractions' analysis involves a potentially increased loss of the analyte.

6. Final remarks and future trends

Accordingly the aim and scope of this review specific attention was paid to consider sample related optimized conditions, and to define the necessary future round of the duties to point out contradictory literature suggestions.

Summing up experiences of GC proposals for p-cannabinoids' analysis – published in the time range of 2000–2017 – in order of importance, it can be stated that

a) gas chromatography retained its relevancy as standard separation technique.
b) In cases of plant matrices, including seizures, FID detection of underivatized species – even at the present – proved to be satisfactory. Excellent, fast results can be obtained primarily when the ratios of the constituents had to be defined, only. In these cases, due to the availability of plenty of materials, analytical performance characteristics were of marginal importance. Simultaneous GC-FID analysis of seven p-CBDs was presented throughout the time reviewed, saving the timeliness of this simple, cost-effective technique.
c) The importance of derivatization approaches providing outstanding mass spectrometric properties along with increased selectivity and sensitivity is not questionable. Out of the most common alkylsilylation and acylation/esterification processes, in authors understanding, alkylsilylation labeling is the methods of choice, because hydroxyl and carboxyl moieties are reacting at once.
d) Acquisition techniques were applied prominently in different ratios comparing the BSTFA, the MSTFA and the acylation/esterification processes. Beside the simple GC-MS protocols, the GC-MS-SIM and the GC-MS-MS techniques were contrasted. Distribution of these protocols, indicating in total of papers commented, shows up in 20% (BSTFA: 3/15 × 100) and in 40% (both MSTFA and acylation/esterification: 6/15 × 100), respectively.
e) In order to designate derivatization/acquisition techniques of choice it is impossible: an approximate comparison has been provided. As a possible approach, considering the similar sample type and size (blood/serum/urine, 0.5–2 mL) the LOQ values – obtained in the GC-MS-SIM and the tandem GC-MS-MS acquisition protocols of variously derivatized species – have been contrasted. In this correlation the following LOQ values can be compared:

GC-MS-MS: 5 ng/mL (BSTFA), 0.19 ng/mL (MSTFA) and 0.5 ng/mL (acylation/esterification);

GC-MS-SIM: 2.0 ng/mL (BSTFA), 0.2 ng/mL (MSTFA) and 2.0 ng/mL (acylation/esterification);

f) On the basis of this literature overview and self experiences – because of their many-sided suitability and unique efficiency – we are convinced on the general advantages of trimethylsilyl processes: introduced by Pierce [104], followed by thousands of researchers and confirmed also in our earlier approaches [105–108] associated with the analysis of various, active proton containing species.
g) As an endpoint of this review authors state that
   – in cases of plant matrices [13,23–26,34,35] flame ionization (FID) detection was regarded satisfactory providing excellent, fast results. Primarily GC-FID was applied when the ratios of the expected constituents had to be defined. Simultaneous analysis of seven [13], six [35] and five [23–26,34] p-CBDs were presented throughout the time reviewed, saving the relevancy of this simple, cost-effective technique.
   – In contrary when selectivity, sensitivity, reliability and reproducibility are of primary importance, (infinitive amounts of species are to be isolated, identified and quantified from biological tissues) independent of the type of matrix, trialkylsilylation and tandem mass spectrometry should be the method of choice.
h) As future perspective authors of this review are convinced that alkylsilyl derivatization optimization should be performed in the same laboratory, on the same apparatus to be comparable. The p-CBDs' trialkylsilylation related, optimized working strategy, using GC-MS and/or GC-MS-MS, in authors' laboratory is in progress.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.trac.2017.07.022.

References

[1] C. Lanz, J. Mattsson, U. Soydaner, R. Brenneisen, Medicinal cannabis: in vitro validation of vaporizers for the smoke-free inhalation of cannabis, PLoS One 11 (2016) 1–18.

[2] M.R. Peace, J.W. Stone, J.L. Poklis, J.B.M. Turner, A. Poklis, Analysis of a commercial marijuana e-cigarette formulation, J. Anal. Toxicol. 40 (2016) 374–378.
[3] https://sensiseeds.com/en/blog/legal-status-cannabis-netherlands-overview/.
[4] T.J. Raharjo, R. Verpoorte, Methods for the analysis of cannabinoids in biological materials: a review, Phytochem. Anal. 15 (2004) 79–94.
[5] F. Pragst, Application of solid-phase microextraction in analytical toxicology, Anal. Bioanal. Chem. 388 (2007) 1393–1414.
[6] C.J. Laker, A literature review to assess the reliability and validity of measures appropriate for use in research to evaluate the efficacy of a brief harm reduction strategy in reducing cannabis use among people with schizophrenia in acute inpatient settings, J. Psychiatr. Ment. Health Nurs. 15 (2008) 777–783.
[7] W.M. Bosker, M.A. Huestis, Oral fluid testing for drugs of abuse, Clin. Chem. 55 (2009) 1910–1931.
[8] S.M. Ville, M. del Mar Ramirez Fernades, N. Samim, G. De Boeck, Conventional and alternative matrices for driving under the influence of cannabis: recent progress and remaining challenges, Bioanalysis 2 (2010) 791–806.
[9] A. Cecinato, C. Balducci, M. Perilli, Illicit psychotropic substances in the air: the state-of-art, Sci. Total Environ. 539 (2016) 1–6.
[10] R. Jain, R. Singh, Applications of dispersive liquid-liquid micro-extraction in forensic toxicology, Trac-Trends Anal. Chem. 75 (2016) 227–237.
[11] R. Jain, R. Singh, Microextraction techniques for analysis of cannabinoids, Trac-Trends Anal. Chem. 80 (2016) 156–166.
[12] E.P.M. de Meijer, M. Bagatta, A. Carboni, P. Crucitti, V.M.C. Moliterni, P. Ranalli, G. Mandolino, The inheritance of chemical phenotype in *Cannabis sativa* L, Genetics 163 (2003) 335–346.
[13] K.W. Hillig, P.G. Mahlberg, A chemotaxonomic analysis of cannabinoid variation in Cannabis (Cannabaceae), Am. J. Bot. 91 (2004) 966–975.
[14] Y. Ilias, S. Rudaz, P. Mathieu, J.L. Veuthey, P. Christen, Analysis of cannabis material by headspace solid-phase microextraction combined with gas chromatography-mass spectrometry, Chimia 58 (2004) 219–221.
[15] Y. Ilias, S. Rudaz, P. Mathieu, P. Christen, J.L. Veuthey, Extraction and analysis of different Cannabis samples by headspace solid-phase microextraction combined with gas chromatography-mass spectrometry, J. Sep. Sci. 28 (2005) 2293–2300.
[16] J. Thorspecken, G. Skopp, L. Potsch, In vitro contamination of hair by marijuana smoke, Clin. Chem. 50 (2004) 596–602.
[17] R. Lewis, S. Ward, R. Johnson, D.T. Burns, Distribution of the principal cannabinoids within bars of compressed cannabis resin, Anal. Chim. Acta 538 (2005) 399–405.
[18] M.A. Huestis, M. ElSohly, W. Nebro, A. Barnes, R.A. Gustafson, M.L. Smith, Estimating time of last oral ingestion of cannabis from plasma THC and THCCOOH concentrations, Ther. Drug Monit. 28 (2006) 540–544.
[19] C.D.R. de Oliveira, M. Yonamine, R.L.D. Moreau, Headspace solid-phase microextraction of cannabinoids in human head hair samples, J. Sep. Sci. 30 (2007) 128–134.
[20] G.L. de Oliveira, M.H. Voloch, G.B. Sztulman, O.N. Neto, M. Yonamine, Cannabinoid contents in cannabis products seized in Sao Paulo, Brazil, 2006–2007, Forensic Toxicol. 26 (2008) 31–35.
[21] T. Gröger, M. Schäffer, M. Pötz, B. Ahrens, K. Drew, M. Eschner, R. Zimmermann, Application of two-dimensional gas chromatography combined with pixel-based chemometric processing for the chemical profiling of illicit drug samples, J. Chromatogr. A 1200 (2008) 8–16.
[22] D. Luo, F. Chen, K. Xiao, Y.Q. Feng, Rapid determination of Delta(9)-Tetrahydrocannabinol in saliva by polymer monolith microextraction combined with gas chromatography-mass spectrometry, Talanta 77 (2009) 1701–1706.
[23] H. Lata, S. Chandra, N. Techen, I.A. Khan, M.A. ElSohly, Assessment of the genetic stability of micropropagated plants of *Cannabis sativa* by ISSR markers, Planta Med. 76 (2010) 97–100.
[24] S. Chandra, H. Lata, Z. Mehmedic, I.A. Khan, M.A. ElSohly, Assessment of cannabinoids content in micropropagated plants of *Cannabis sativa* and their comparison with conventionally propagated plants and mother plant during developmental stages of growth, Planta Med. 76 (2010) 743–750.
[25] H. Lata, S. Chandra, I.A. Khan, M.A. ElSohly, High frequency plant regeneration from leaf derived callus of high delta(9)-tetrahydrocannabinol yielding *Cannabis sativa* L, Planta Med. 76 (2010) 1629–1633.
[26] Z. Mehmedic, S. Chandra, D. Slade, H. Denham, S. Foster, A.S. Patel, S.A. Ross, I.A. Khan, M.A. ElSohly, Potency trends of delta 9-THC and other cannabinoids in confiscated cannabis preparations from 1993 to 2008, J. Forensic Sci. 55 (2010) 1209–1217.
[27] E.S. Emídio, V. de Menezes Prata, H.S. Dórea, Validation of an analytical method for analysis of cannabinoids in hair by headspace solid-phase microextraction and gas chromatography-ion trap tandem mass spectrometry, Anal. Chim. Acta 670 (2010) 63–71.
[28] S.E. Breidi, J. Barker, A. Petroczi, D.P. Naughton, Enzymatic digestion and selective quantification of underivatised delta-9-tetrahydrocannabinol and cocaine in human hair using gas chromatography-mass spectrometry, J. Anal. Methods Chem. (2012) 1–8.
[29] J. Omar, M. Olivares, M. Alzaga, N. Etxebarria, Optimisation and characterisation of marihuana extracts obtained by supercritical fluid extraction and focused ultrasound extraction and retention time locking GC-MS, J. Sep. Sci. 36 (2013) 1397–1404.
[30] L. Anzillotti, E. Castrignano, S.S. Rossi, M. Chiarotti, Cannabinoids determination in oral fluid by SPME-GC/MS and UHPLC-MS/MS and its application on suspected drivers, Sci. Justice 54 (2014) 421–426.
[31] K.W. Chan, Validating a gas chromatography-mass spectrometric method and sample classification procedure for cannabis profiling using cannabinoids from case samples, Aust. J. Forensic Sci. 46 (2014) 424–432.
[32] J. Omar, M. Olivares, J.M. Amigo, N. Etxebarria, Resolution of co-eluting compounds of *Cannabis sativa* in comprehensive two-dimensional gas chromatography/mass spectrometry detection with Multivariate Curve Resolution-Alternating Least Squares, Talanta 121 (2014) 273–280.
[33] M. Tayyab, D. Shahwar, GCMS analysis of *Cannabis sativa* L. from four different areas of Pakistan, Egypt. J. Forensic Sci. 5 (2015) 114–125.
[34] M.A. ElSohly, Z. Mehmedic, S. Foster, C. Gon, S. Chandra, J.C. Church, Changes in cannabis potency over the last 2 decades (1995–2014): analysis of current data in the United States, Biol. Psychiatr. 79 (2016) 613–619.
[35] A. Hazekamp, K. Tejkalova, S. Papadimitriou, Cannabis: from cultivar to chemovar II—a metabolomics; approach to cannabis classification, Cannabis Cannabinoid Res. 1 (2016) 202–215, http://dx.doi.org/10.1089/can.2016.0017.
[36] P.B. Chase, J. Hawkins, J. Mosier, E. Jimenez, K. Boesen, B.K. Logan, F.G. Walter, Differential physiological and behavioral cues observed in individuals smoking botanical marijuana versus synthetic cannabinoid drugs, Clin. Toxicol. 54 (2016) 14–19.
[37] S.G. de Oliveira, S. Loddi, C.D.R. de Oliveira, C. Dizioli, A.D. Zukoloto, A. Dias, L.V.G. Fruchtengarten, M. Yonamine, Headspace solid-phase microextraction and gas chromatography-mass spectrometry for determination of cannabinoids in human breast milk, Forensic Toxicol. 35 (2017) 125–132.
[38] M. Chiarotti, L. Costamagna, Analysis of 11-nor-9-carboxy-delta(9)-tetrahydrocannabinol in biological samples by gas chromatography tandem mass spectrometry (GC/MS-MS), Forensic Sci. Int. 114 (2000) 1–6.
[39] O. Boldis, Gy. Kocsis, A. Gachályi, J. Fűrész, Gas chromatography-mass spectrometry-single ion monitoring measurement of 11-nor-d^9-tetrahydrocannabinol-carboxylic acid in urine, J. Chromatogr. Sci. 41 (2003) 190–194.
[40] R.A. Gustafson, E.T. Moolchan, A. Barnes, B. Levine, M.A. Huestis, Validated method for the simultaneous determination of Delta(9)-tetrahydrocannabinol (THC), 11-hydroxy-THC and 11-nor-9-carboxy-THC in human plasma using solid phase extraction and gas chromatography-mass spectrometry with positive chemical ionization, J. Chromatogr. B 798 (2003) 145–154.
[41] J. Mannila, M. Lehtonen, T. Jarvinen, P. Jarho, Determination of Delta9-tetrahydrocan-nabinol from rabbit plasma by gas chromatography-mass spectrometry using two ionization techniques, J. Chromatogr. B 810 (2004) 283–290.
[42] J.L. Villamor, A.M. Bermejo, M.J. Tabernero, P. Fernandez, Determination of cannabinoids in human hair by GC/MS, Anal. Lett. 37 (2004) 517–528.
[43] B. Brunet, C. Doucet, N. Venisse, T. Hauet, W. Hebrard, Y. Papet, G. Mauco, P. Mura, Validation of Large White Pig as an animal model for the study of cannabinoids metabolism: application to the study of THC distribution in tissues, Forensic Sci. Int. 161 (2006) 169–174.
[44] R.S. Goodwin, R.A. Gustafson, A. Barnes, W. Nebro, E.T. Moolchan, M.A. Huestis, Delta(9)-tetrahydrocannabinol, 11-hydroxy-delta(9)-tetrahydrocannabinol and 11-nor-9-carboxy-delta(9)-tetrahydrocannabinol in human plasma after controlled oral administration of cannabinoids, Ther. Drug Monit. 28 (2006) 545–551.
[45] R.D. Scurlock, G.B. Ohlson, D.K. Worthen, The detection of Delta9-tetrahydrocannabinol (THC) and 11-nor-9-carboxy-Delta9-tetrahydrocannabinol (THCA) in whole blood using two-dimensional gas chromatography and EI-mass spectrometry, J. Anal. Toxicol. 30 (2006) 262–266.
[46] L. Dietz, A. Glaz-Sandberg, H. Nguyen, G. Skopp, G. Mikus, R. Aderjan, The urinary disposition of intravenously administered 11-nor-9-carboxy-delta-9-tetrahydrocannabinol in humans, Ther. Drug Monit. 29 (2007) 368–372.
[47] R.H. Lowe, E.L. Karschner, E.W. Schwilke, A.J. Barnes, M.A. Huestis, Simultaneous quantification of Delta 9-tetrahydrocannabinol, 11-hydroxy-Delta 9-tetrahydrocannabinol, and 11-nor-Delta 9-tetrahydrocannabinol-9-carboxylic acid in human plasma using two-dimensional gas chromatography, cryofocusing, and electron impact-mass spectrometry, J. Chromatogr. A 1163 (2007) 318–327.
[48] T. Nadulski, F. Pragst, Simple and sensitive determination of Delta(9)-tetrahydrocannabinol, cannabidiol and cannabinol in hair by combined silylation, headspace solid phase microextraction and gas chromatography-mass spectrometry, J. Chromatogr. B 846 (2007) 78–85.
[49] C. Moore, S. Rana, C. Coulter, Simultaneous identification of 2-carboxy-tetrahydrocannabinol, tetrahydrocannabinol, cannabinol and cannabidiol in oral fluid, J. Chromatogr. B 852 (2007) 459–464.
[50] E.L. Karschner, E.W. Schwilke, R.H. Lowe, W.D. Darwin, H.G. Pope, R. Herning, J.L. Cadet, M.A. Huestis, Do Delta 9-tetrahydrocannabinol concentrations indicate recent use in chronic cannabis users? Addiction 104 (2009) 2041–2048.
[51] R.H. Lowe, T.T. Abraham, W.D. Darwin, R. Herning, J.L. Cadet, M.A. Huestis, Extended urinary Delta 9-tetrahydrocannabinol excretion in chronic cannabis users precludes use as a biomarker of new drug exposure, Drug Alcohol Depend. 105 (2009) 24–32.
[52] E.W. Schwilke, E.L. Karschner, R.H. Lowe, A.M. Gordon, J.L. Cadet, R.I. Herning, M.A. Huestis, Intra- and intersubject whole blood/plasma cannabinoid ratios determined by 2-dimensional, electron impact ggms with cryofocusing, Clin. Chem. 55 (2009) 1188–1195.

[53] E.W. Schwilke, D.M. Schwope, E.L. Karschner, R.H. Lowe, W.D. Darwin, D.L. Kelly, R.S. Goodwin, D.A. Gorelick, M.A. Huestis, Delta(9)-Tetrahydrocannabinol (THC), 11-hydroxy-THC, and 11-nor-9-carboxy-THC plasma pharmacokinetics during and after continuous high-dose oral THC, Clin. Chem. 55 (2009) 2180–2189.
[54] T.R. Gray, A.J. Barnes, M.A. Huestis, Effect of hydrolysis on identifying prenatal cannabis exposure, Anal. Bioanal. Chem. 397 (2010) 2335–2347.
[55] E.L. Karschner, A.J. Barnes, R.H. Lowe, K.B. Scheidweiler, M.A. Huestis, Validation of a two-dimensional gas chromatography mass spectrometry method for the simultaneous quantification of cannabidiol, Delta(9)-tetrahydrocannabinol (THC), 11-hydroxy-THC, and 11-nor-9-carboxy-THC in plasma, Anal. Bioanal. Chem. 397 (2010) 603–611.
[56] G. Milman, A.J. Barnes, R.H. Lowe, M.A. Huestis, Simultaneous quantification of cannabinoids and metabolites in oral fluid by two-dimensional gas chromatography mass spectrometry, J. Chromatogr. A 1217 (2010) 1513–1521.
[57] G. Milman, D.M. Schwope, E.W. Schwilke, W.D. Darwin, D.L. Kelly, R.S. Goodwin, D.A. Gorelick, M.A. Huestis, Oral fluid and plasma cannabinoid ratios after around-the-clock controlled oral delta(9)-tetrahydrocannabinol administration, Clin. Chem. 57 (2011) 1597–1606.
[58] E.W. Schwilke, R.G. Gullberg, W.D. Darwin, C.N. Chiang, J.L. Cadet, D.A. Gorelick, H.G. Pope, M.A. Huestis, Differentiating new cannabis use from residual urinary cannabinoid excretion in chronic, daily cannabis users, Addiction 106 (2011) 499–506.
[59] E.L. Karschner, D.M. Schwope, E.W. Schwilke, R.S. Goodwin, D.L. Kelly, D.A. Gorelick, M.A. Huestis, Predictive model accuracy in estimating last Delta(9)-tetrahydrocannabinol (THC) intake from plasma and whole blood cannabinoid concentrations in chronic, daily cannabis smokers administered subchronic oral THC, Drug Alcohol Depend. 125 (2012) 313–319.
[60] I. Papoutsis, P. Nikolaou, C. Pistos, M. Stefanidou, C. Spiliopoulou, S. Athanaselis, A validated GC-MS method for the determination of Delta(9)-tetrahydrocannabinol and 11-nor-Delta(9)-tetrahydrocannabinol-9-carboxylic acid in bile samples, Forensic Toxicol. 30 (2012) 51–58.
[61] S. Anizan, G. Milman, N. Desrosiers, A.J. Barnes, D.A. Gorelick, M.A. Huestis, Oral fluid cannabinoid concentrations following controlled smoked cannabis in chronic frequent and occasional smokers, Anal. Bioanal. Chem. 405 (2013) 8451–8461.
[62] M.M. Bergamaschi, E.L. Karschner, R.S. Goodwin, K.B. Scheidweiler, J. Hirvonen, R.H.C. Queiroz, M.A. Huestis, Impact of prolonged cannabinoid excretion in chronic daily cannabis smokers' blood on per se drugged driving laws, Clin. Chem. 59 (2013) 519–526.
[63] M.M. Bergamaschi, A. Barnes, R.H.C. Queiroz, Y.L. Hurd, M.A. Huestis, Impact of enzymatic and alkaline hydrolysis on CBD concentration in urine, Anal. Bioanal. Chem. 405 (2013) 4679–4689.
[64] D. Lee, R. Vandrey, D.R. Mendu, S. Anizan, G. Milman, J.A. Murray, A.J. Barnes, M.A. Huestis, Oral fluid cannabinoids in chronic cannabis smokers during oral delta(9)-tetrahy-drocannabinol therapy and smoked cannabis challenge, Clin. Chem. 59 (2013) 1770–1779.
[65] D. Lee, R. Vandrey, G. Milman, M. Bergamaschi, D.R. Mendu, J.A. Murray, A.J. Barnes, M.A. Huestis, Oral fluid/plasma cannabinoid ratios following controlled oral THC and smoked cannabis administration, Anal. Bioanal. Chem. 405 (2013) 7269–7279.
[66] S. Anizan, M.M. Bergamaschi, A.J. Barnes, G. Milman, N. Desrosiers, D. Lee, D.A. Gorelick, M.A. Huestis, Impact of oral fluid collection device on cannabinoid stability following smoked cannabis, Drug Test. Anal. 7 (2014) 114–120.
[67] D. Lee, R. Vandrey, D.R. Mendu, J.A. Murray, A.J. Barnes, M.A. Huestis, Oral fluid cannabinoidsin chronic frequent cannabis smokers during ad libitum cannabis smoking, Drug Test. Anal. 6 (2014) 88–111.
[68] S. Eller, L.G. Flaiban, B. Paranhos, J.L. da Costa, F.R. Lourenco, M. Yonamine, Analysis of 11-nor-9-carboxy-Delta(9)-tetrahydrocannabinol in urine samples by hollow fiber-liquid phase microextraction and gas chromatography-mass spectrometry in consideration of measurement uncertainty, Forensic Toxicol. 32 (2014) 282–291.
[69] D. Lee, M.M. Bergamaschi, G. Milman, A.J. Barnes, R.H.C. Queiroz, R. Vandrey, M.A. Huestis, Plasma cannabinoid pharmacokinetics after controlled smoking and ad libitum cannabis smoking in chronic frequent users, J. Anal. Toxicol. 39 (2015) 580–587.
[70] F. Musshoff, H.P. Junker, D.W. Lachenmeier, L. Kroener, B. Madea, Fully automated determination of cannabinoids in hair samples using headspace solid-phase microextraction and gas chromatography-mass spectrometry, J. Anal. Toxicol. 26 (2002) 554–560.
[71] C. Gambelunghe, R. Rossi, C. Ferranti, R. Rossi, M. Bacci, Hair analysis by GC/MS/MS to verify abuse of drugs, J. Appl. Toxicol. 25 (2005) 205–211.
[72] M. Pellegrini, E. Marchei, R. Pacifici, S. Pichini, A rapid and simple procedure for the determination of cannabinoids in hemp food products by gas chromatography-mass spectrometry, J. Pharm. Biomed. Anal. 36 (2005) 939–946.
[73] S. Strano-Rossi, F. Molaioni, F. Rossi, F. Botre, Rapid screening of drugs of abuse and their metabolites by gas chromatography/mass spectrometry: application to urinalysis, Rapid Commun. Mass Spectrom. 19 (2005) 1529–1535.
[74] J.Y. Kim, J.C. Cheong, M.K. Kim, J.I. Lee, M.K. In Simultaneous determination of amphetamine-type stimulants and cannabinoids in fingernails by gas chromatography-mass spectrometry, Arch. Pharm. Res. 31 (2008) 805–813.
[75] J.Y. Moon, J.Y. Kim, M.H. Moon, B.C. Chung, M.K. In, M.H. Choi, Validated gas chromatographic-mass spectrometric analysis of urinary cannabinoids purified with a calcium-hardened beta-cyclodextrin polymer, J. Chromatogr. A 1204 (2008) 87–92.
[76] J. Jung, M.R. Meyer, H.H. Maurer, C. Neususs, W. Weinmann, V. Auwarter, Studies on the metabolism of the Delta 9-tetrahydrocannabinol precursor Delta 9-tetrahydrocannabinolic acid A (Delta 9-THCA-A) in rat using LC-MS/MS, LC-QTOF MS and GC-MS techniques, J. Mass Spectrom. 44 (2009) 1423–1433.
[77] I. Gonzalez-Marino, J.B. Quintana, I. Rodriguez, R. Cela, Determination of drugs of abuse in water by solid-phase extraction, derivatisation and gas chromatography-ion trap-tandem mass spectrometry, J. Chromatogr. A 1217 (2010) 1748–1760.
[78] E. Hidvégi, G.P. Somogyi, Detection of cannabigerol and its presumptive metabolite in human urine after Cannabis consumption, Pharmazie 65 (2010) 408–411.
[79] R. Andrews, S. Paterson, A validated method for the analysis of cannabinoids in post-mortem blood using liquid-liquid extraction and two-dimensional gas chromatography-mass spectrometry, Forensic Sci. Int. 222 (2012) 111–117.
[80] M. Minoli, I. Angeli, A. Ravelli, F. Gigli, F. Lodi, Detection and quantification of 11-nor-Delta 9-tetrahydrocannabinol-9-carboxylic acid in hair by GC/MS/MS in Negative Chemical Ionization mode (NCI) with a simple and rapid liquid/liquid extraction, Forensic Sci. Int. 218 (2012) 49–52.
[81] I. Racamonde, E. Villaverde-de-Saa, R. Rodil, J.B. Quintana, R. Cela, Determination of Delta 9-tetrahydrocannabinol and 11-nor-9-carboxy-Delta 9-tetrahydrocannabinol in water samples by solid-phase microextraction with on-fiber derivatization and gas chromatography-mass spectrometry, J. Chromatogr. A 1245 (2012) 167–174.
[82] N. De Brabanter, W. Van Gansbeke, F. Hooghe, P. Van Eenoo, Fast quantification of 11-nor-Delta 9-tetrahydrocannabinol-9-carboxylic acid (THCA) using microwave-accelerated derivatisation and gas chromatography-triple quadrupole mass spectrometry, Forensic Sci. Int. 224 (2013) 90–95.
[83] F.S. Pelição, M.D. Peres, J.F. Pissinate, B.S. De Martinis, A one-step extraction procedure for the screening of cocaine, amphetamines and cannabinoids in postmortem blood samples, J. Anal. Toxicol. 38 (2014) 341–348.
[84] B. Moosmann, N. Roth, M. Hastedt, A. Jacobsen-Bauer, F. Pragst, V. Auwarter, Cannabinoid findings in children hair – what do they really tell us? An assessment in the light of three different analytical methods with focus on interpretation of 9-tetrahydrocannabinolic acid A concentrations, Drug Test. Anal. 7 (2015) 349–357.
[85] A. Gasse, H. Pfeiffer, H. Kohler, J. Schurenkamp, Development and validation of a solid-phase extraction method using anion exchange sorbent for the analysis of cannabinoids in plasma and serum by gas chromatography-mass spectrometry, Int. J. Leg. Med. 130 (2016) 967–974.
[86] K. Purschke, S. Heinl, O. Lerch, F. Erdmann, F. Veit, Development and validation of an automated liquid-liquid extraction GC/MS method for the determination of THC, 11-OH-THC, and free THC-carboxylic acid (THC-COOH) from blood serum, Anal. Bioanal. Chem. 408 (2016) 4379–4388.
[87] C. Balducci, G. Nervegna, A. Cecinato, Evaluation of principal cannabinoids in airborne particulates, Anal. Chim. Acta 641 (2009) 89–94.
[88] M.V. Doig, R. Andela, Analysis of pharmacologically active cannabinoids by GC-MS, Chromatographia 52 (2000) S101–S102.
[89] M.J. Baptista, P.V. Monsanto, E.G. Pinho Marques, A. Bermejo, S. Avila, A.M. Castanheira, C. Margalho, M. Barroso, D.N. Vieira, Hair analysis for delta(9)-THC, delta(9)-THC-COOH, CBN and CBD, by GC/MS-EI. Comparison with GC/MS-NCI for delta(9)-THC-COOH, Forensic Sci. Int. 128 (2002) 66–78.
[90] M.H. Chu, O.H. Drummer, Determination of delta9-THC in whole blood using gas chromatography-mass spectrometry, J. Anal. Toxicol. 26 (2002) 575–581.
[91] S. Steinmeyer, D. Bregel, S. Warth, T. Kraemer, M.R. Moeller, Improved and validated method for the determination of Delta(9)-tetrahydrocannabinol (THC), 11-hydroxy-THC and 11-nor-9-carboxy-THC in serum, and in human liver microsomal preparations using gas chromatography-mass spectrometry, J. Chromatogr. B 772 (2002) 239–248 (TMAH/DMSO).
[92] J. Teske, K. Putzbach, W. Engewald, R.K. Muller, Determination of cannabinoids by gas chromatography-mass spectrometry and large-volume programmed-temperature vaporiser injection using 25 μL 1 of biological fluid, J. Chromatogr. B 772 (2002) 299–306.
[93] T. Saito, A. Wtsadik, K.B. Scheidweiler, N. Fortner, S. Takeichi, M.A. Huestis, Validated gas chromatographic-negative ion chemical ionization mass spectrometric method for Delta(9)-tetrahydrocannabinol in sweat patches, Clin. Chem. 50 (2004) 2083–2090.
[94] M.A. Huestis, A. Barnes, M.L. Smith, Estimating the time of last cannabis use from plasma Delta(9)-tetrahydrocannabinol and II-nor-9-carboxy-Delta(9)-tetrahydrocannabinol concentrations, Clin. Chem. 51 (2005) 2289–2295.
[95] R. Marsili, S. Martello, M. Felli, S. Fiorina, M. Chiarotti, Hair testing for Delta(9)-THC-COOH by gas chromatography/tandem mass spectrometry in negative chemical ionization mode, Rapid Commun. Mass Spectrom. 19 (2005) 1566–1568.
[96] J.Y. Kim, M.K. In, Determination of 11-nor-Delta(9)-tetrahydrocannabinol-9-carboxylic acid in hair using gas chromatography/tandem mass spectrometry in negative ion chemical ionization mode, Rapid Commun. Mass Spectrom. 21 (2007) 1339–1342.
[97] A. Thomas, C. Widmer, G. Hopfgartner, C. Staub, Fast gas chromatography and negative-ion chemical ionization tandem mass spectrometry for forensic analysis of cannabinoids in whole blood, J. Pharm. Biomed. Anal. 45 (2007) 495–503.

[98] E. Han, Y. Park, E. Kim, S. In, W. Yang, S. Lee, H. Choi, S. Lee, H. Chung, J.M. Song, Simultaneous analysis of Delta(9)-tetrahydrocannabinol and 11-nor-9-carboxy-tetrahydrocan-nabinol in hair without different sample preparation and derivatization by gas chromatography-tandem mass spectrometry, J. Pharm. Biomed. Anal. 55 (2011) 1096–1103.
[99] J.Y. Kim, J.C. Cheong, J.I. Lee, M.K. In, Improved gas chromatography-negative ion chemical ionization tandem mass spectrometric method for determination of 11-nor-Delta(9)-tetrahydrocannabinol-9-carboxylic acid in hair using mechanical pulverization and bead-assisted liquid-liquid extraction, Forensic Sci. Int. 206 (2011) E99–E102.
[100] S.Y. Kim, J.Y. Kim, W. Kwon, M.K. In, Y.E. Kim, K.J. Paeng, Method development for simultaneous determination of amphetamine type stimulants and cannabinoids in urine using GC-MS, Microchem J. 110 (2013) 326–333.
[101] D. Thieme, H. Sachs, M. Uhl, Proof of cannabis administration by sensitive detection of 11-nor-Delta(9)-tetrahydrocannabinol-9-carboxylic acid in hair using selective methylation and application of liquid chromatography-tandem and multistage mass spectrometry, Drug Test. Anal. 6 (2014) 112–118.
[102] A.J. Barnes, K.B. Scheidweiler, M.A. Huestis, Quantification of 11-nor-9-carboxy-delta9-tetrahydrocannabinol in human oral fluid by gas chromatography-tandem mass spectrometry, Ther. Drug Monit. 36 (2014) 225–233.
[103] J. Lewis, A. Molnar, D. Allsop, J. Copeland, S.L. Fu, Rapid elimination of Carboxy-THC in a cohort of chronic cannabis users, Int. J. Leg. Med. 130 (2016) 147–152.
[104] A.E. Pierce, Silylation of Organic Compounds, Pierce Chemical Company, Rockford, IL, 1968.
[105] B. Molnár, A. Csámpai, I. Molnár-Perl, Hexamethyldisilazane as an acylation generator for perfluorocarboxylic acids in quantitative derivatization of primary phenylalkyl amines confirmed by GC-MS and computations, Anal. Chem. 87 (2015) 848–852.
[106] B. Molnár, B. Fodor, I. Boldizsár, I. Molnár-Perl, Quantitative silylation speciations of primary phenylalkyl amines, amphetamine and 3,4-methylenedioxy amphetamine prior to their analysis by gas chromatography mass spectrometry, Anal. Chem. 87 (2015) 10188–10192.
[107] B. Molnár, B. Fodor, I. Boldizsár, I. Molnár-Perl, Trimethylsilyl speciations of cathine, cathinone and norephedrine followed by gas chromatography mass spectrometry: direct sample preparation and analysis of khatamines, J. Chromatogr. A 1440 (2016) 172–178.
[108] B. Molnár, B. Fodor, E. Hidvégi, I. Molnár-Perl, Derivatization-related direct sample preparation of khatamine type designer drugs: followed by gas chromatography mass spectrometry, J. Chromatogr. A 1477 (2016) 70–75.