MICROSAMPLING- AND MICROFLUIDIC-BASED STRATEGIES FOR CANNABINOID ANALYSIS IN BLOOD

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UNIVERSITÀ DI BOLOGNA

Pharmaco-Toxicological Analysis Laboratory

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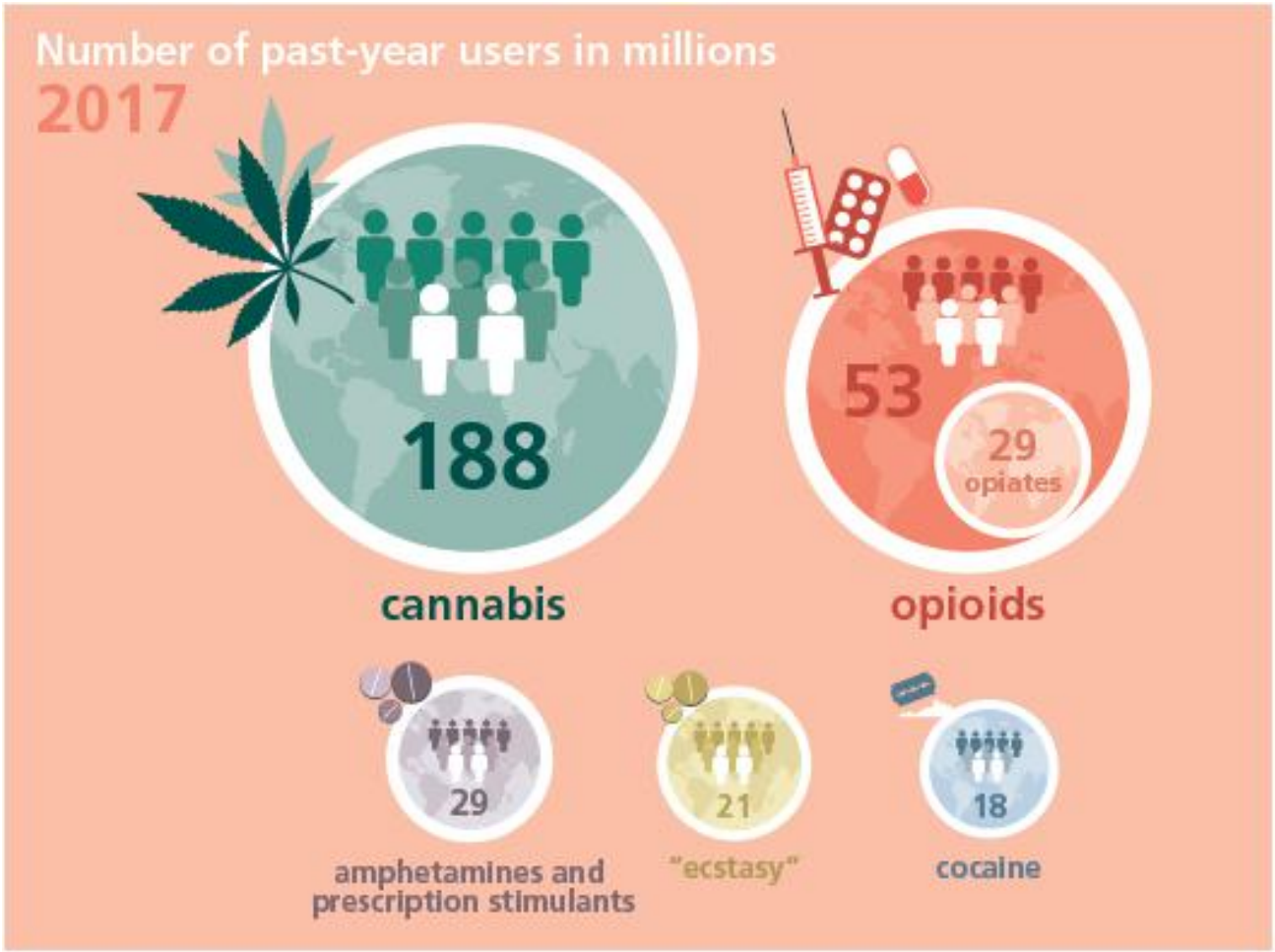
WORLDWIDE DoA USE

UNODC
United Nations Office on Drugs and Crime

2019 WORLD DRUG REPORT



Source: United Nations Office on Drugs and Crime (UNODC) World Drug Report 2019

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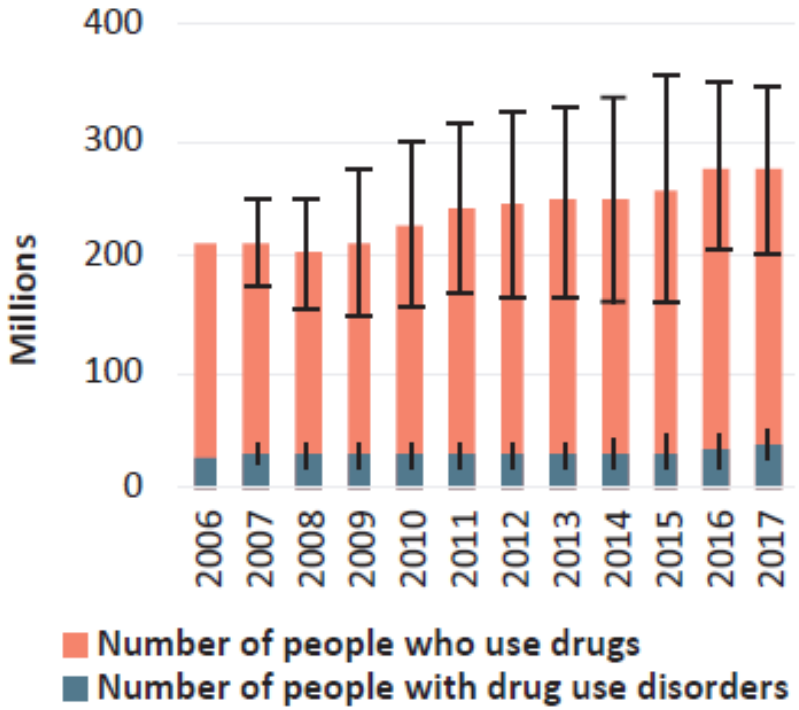
WORLDWIDE DoA USE

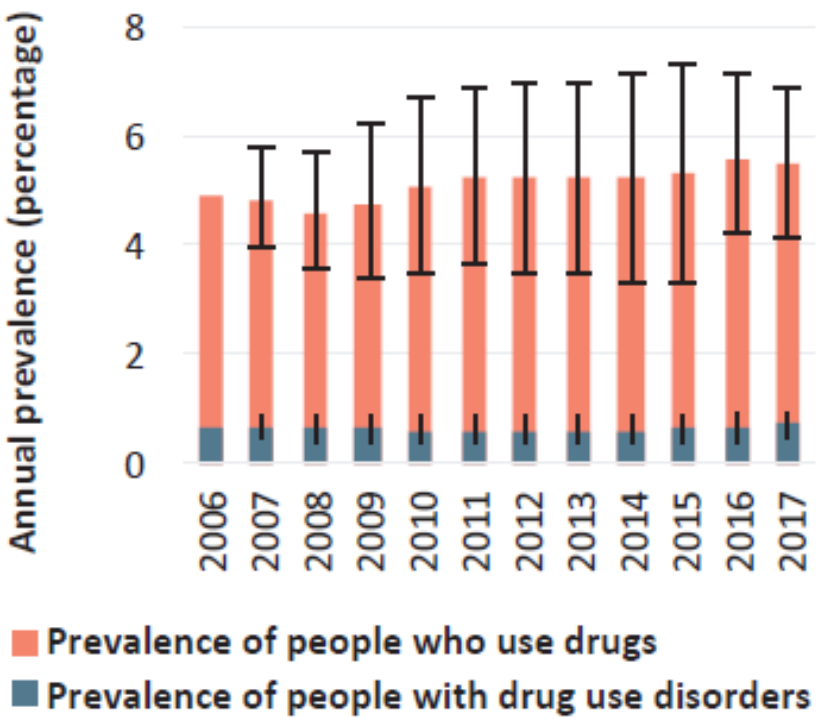
UNODC
United Nations Office on Drugs and Crime

WORLD DRUG REPORT 2019

Global trends in the estimated number of people who use drugs and those with drug use disorders, 2006–2017



Global trends in the estimated prevalence of drug use and drug use disorders, 2006–2017



Source: United Nations Office on Drugs and Crime (UNODC) World Drug Report 2019

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World Health Organization

SUSTAINABLE DEVELOPMENT GOALS

Source: United Nations: The 2030 agenda for sustainable development

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World Health Organization

SUSTAINABLE DEVELOPMENT GOALS

SUSTAINABLE DEVELOPMENT GOAL 3

Ensure healthy lives and promote well-being for all at all ages

TARGETS		INDICATORS	
3.4	By 2030, reduce by one third premature mortality from non-communicable diseases through prevention and treatment and promote mental health and well-being	3.4.1	Mortality rate attributed to cardiovascular disease, cancer, diabetes or chronic respiratory disease
		3.4.2	Suicide mortality rate
3.5	Strengthen the prevention and treatment of substance abuse, including narcotic drug abuse and harmful use of alcohol	3.5.1	Coverage of treatment interventions (pharmacological, psychosocial and rehabilitation and aftercare services) for substance use disorders
		3.5.2	Harmful use of alcohol, defined according to the national context as alcohol per capita consumption (aged 15 years and older) within a calendar year in litres of pure alcohol
3.6	By 2020, halve the number of global deaths and injuries from road traffic accidents	3.6.1	Death rate due to road traffic injuries

PTA Lab EXPERTISE IN CANNABINOID ANALYSIS

Pharmaco-Toxicological Analysis Laboratory (PTA Lab)
Department of Pharmacy and Biotechnology (FaBiT)
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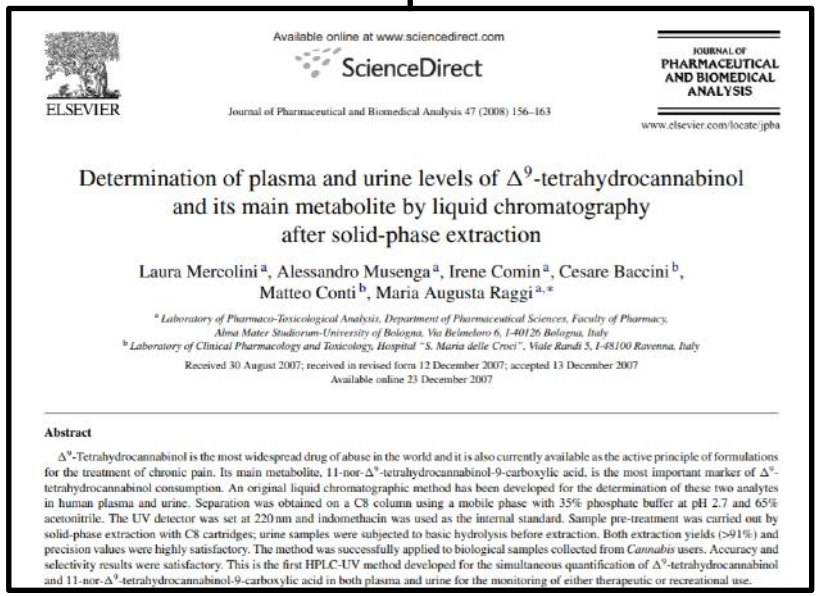

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Journal of Pharmaceutical and Biomedical Analysis 47 (2008) 156–163
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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Determination of plasma and urine levels of Δ^9-tetrahydrocannabinol and its main metabolite by liquid chromatography after solid-phase extraction

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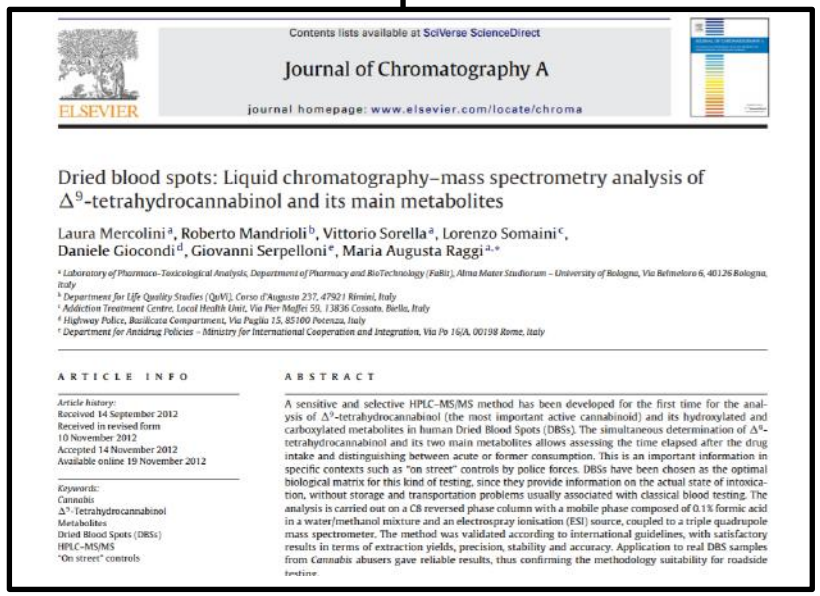
Abstract

Δ^9-Tetrahydrocannabinol is the most widespread drug of abuse in the world and it is also currently available as the active principle of formulations for the treatment of chronic pain. Its main metabolite, 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid, is the most important marker of Δ^9-tetrahydrocannabinol consumption. An original liquid chromatographic method has been developed for the determination of these two analytes in human plasma and urine. Separation was obtained on a C8 column using a mobile phase with 35% phosphate buffer at pH 2.7 and 65% acetonitrile. The UV detector was set at 220 nm and indomethacin was used as the internal standard. Sample pre-treatment was carried out by solid-phase extraction with C8 cartridges; urine samples were subjected to basic hydrolysis before extraction. Both extraction yields (>91%) and precision values were highly satisfactory. The method was successfully applied to biological samples collected from *Cannabis* users. Accuracy and selectivity results were satisfactory. This is the first HPLC-UV method developed for the simultaneous quantification of Δ^9-tetrahydrocannabinol and 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid in both plasma and urine for the monitoring of either therapeutic or recreational use.



Contents lists available at SciVerse ScienceDirect
Journal of Chromatography A
journal homepage: www.elsevier.com/locate/chroma

Dried blood spots: Liquid chromatography–mass spectrometry analysis of Δ^9-tetrahydrocannabinol and its main metabolites

Laura Mercolini [a], Roberto Mandrioli [b], Vittorio Sorella [a], Lorenzo Somaini [c], Daniele Giocondi [d], Giovanni Serpelloni [e], Maria Augusta Raggi [a,*]

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Cannabis
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Metabolites
Dried Blood Spots (DBSs)
HPLC–MS/MS
"On street" controls

ABSTRACT

A sensitive and selective HPLC–MS/MS method has been developed for the first time for the analysis of Δ^9-tetrahydrocannabinol (the most important active cannabinoid) and its hydroxylated and carboxylated metabolites in human Dried Blood Spots (DBSs). The simultaneous determination of Δ^9-tetrahydrocannabinol and its two main metabolites allows assessing the time elapsed after the drug intake and distinguishing between acute or former consumption. This is an important information in specific contexts such as "on street" controls by police forces. DBSs have been chosen as the optimal biological matrix for this kind of testing, since they provide information on the actual state of intoxication, without storage and transportation problems usually associated with classical blood testing. The analysis is carried out on a C8 reversed phase column with a mobile phase composed of 0.1% formic acid in a water/methanol mixture and an electrospray ionisation (ESI) source, coupled to a triple quadrupole mass spectrometer. The method was validated according to international guidelines, with satisfactory results in terms of extraction yields, precision, stability and accuracy. Application to real DBS samples from *Cannabis* abusers gave reliable results, thus confirming the methodology suitability for roadside testing.

Contents lists available at SciVerse ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis
journal homepage: www.elsevier.com/locate/jpba

Monitoring of chronic *Cannabis* abuse: An LC–MS/MS method for hair analysis

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Δ^9-Tetrahydrocannabinol
11-Nor-9-carboxy-Δ^9-tetrahydrocannabinol

ABSTRACT

An advanced analytical method based on liquid chromatography–tandem mass spectrometry (LC–MS/MS), has been developed for the identification and determination in hair of Δ^9-tetrahydrocannabinol together with its major metabolite 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol. Since the latter is formed endogenously, it allows the assessment of chronic use excluding passive exposure to *Cannabis*. The sample pre-treatment procedure is based on a feasible incubative extraction followed by a liquid–liquid extraction step. Chromatographic separation was performed using a reversed-phase column and gradient elution with a formic acid/acetonitrile/water mobile phase. The limits of quantitation and of detection were 3 pg/mg and 1 pg/mg, respectively, for both analytes. The method was successfully applied to the analysis of hair samples from *Cannabis* abusers; the analyte concentrations found ranged from 55 to 100 pg/mg for Δ^9-tetrahydrocannabinol and from 5 to 10 pg/mg for 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol. Accuracy studies also gave satisfactory results (recovery > 87%), thus confirming the suitability of the assay for chronic consumption monitoring.

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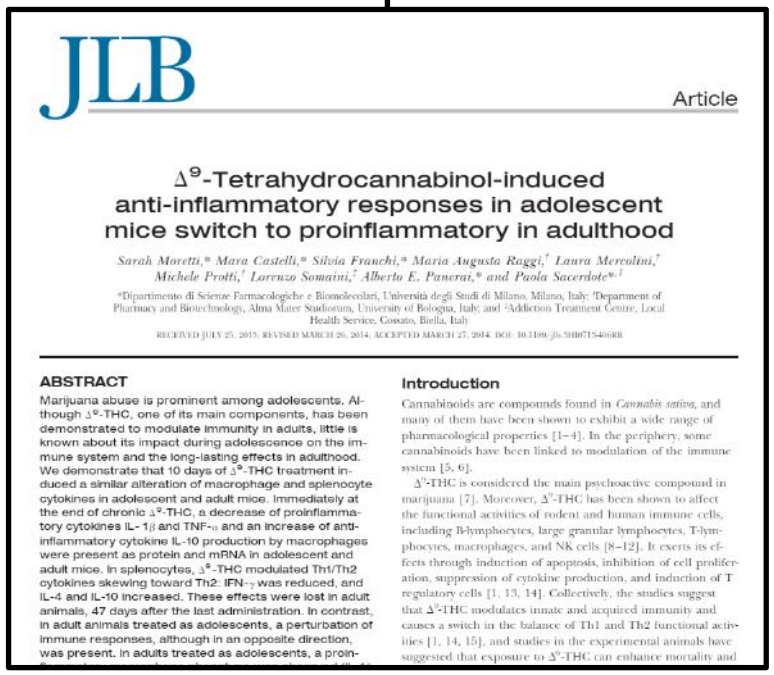

JLB

Article

Δ^9-Tetrahydrocannabinol-induced anti-inflammatory responses in adolescent mice switch to proinflammatory in adulthood

Sarah Moretti,[a] Mara Castelli,[a] Silvia Franchi,[a] Maria Augusta Raggi,[b] Laura Mercolini,[b] Michele Protti,[b] Lorenzo Somaini,[c] Alberto E. Panerai,[a] and Paola Sacerdote[a,c]

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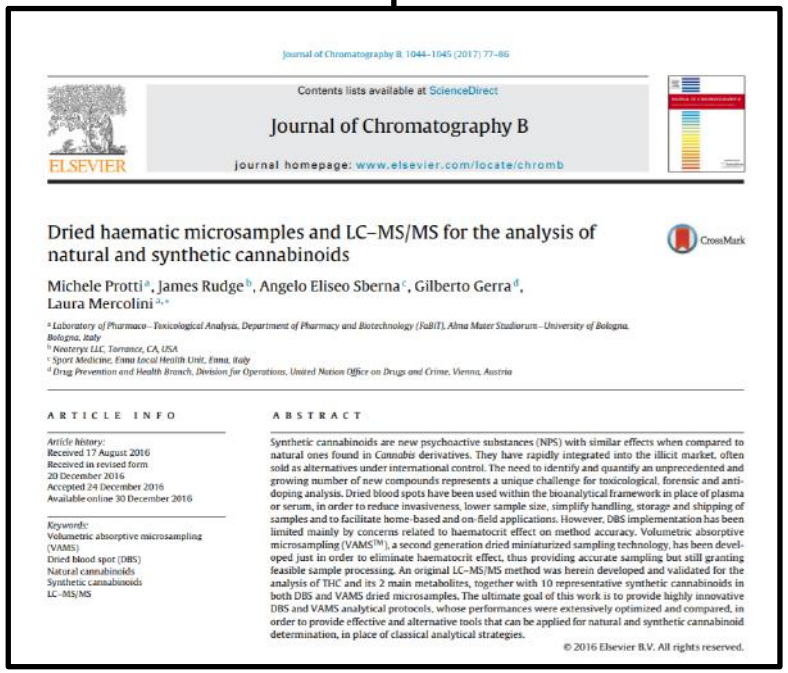
ABSTRACT

Marijuana abuse is prominent among adolescents. Although Δ^9-THC, one of its main components, has been demonstrated to modulate immunity in adults, little is known about its impact during adolescence on the immune system and the long-lasting effects in adulthood. We demonstrate that 10 days of Δ^9-THC treatment induced a similar alteration of macrophage and splenocyte cytokines in adolescent and adult mice. Immediately at the end of chronic Δ^9-THC, a decrease of proinflammatory cytokines IL-1β and TNF-α and an increase of anti-inflammatory cytokine IL-10 production by macrophages were present as protein and mRNA in adolescent and adult mice. In splenocytes, Δ^9-THC modulated Th1/Th2 cytokines skewing toward Th2: IFN-γ was reduced, and IL-4 and IL-10 increased. These effects were lost in adult animals, 47 days after the last administration. In contrast, in adult animals treated as adolescents, a perturbation of immune responses, although in an opposite direction, was present. In adults treated as adolescents, a proin-

Introduction

Cannabinoids are compounds found in *Cannabis sativa*, and many of them have been shown to exhibit a wide range of pharmacological properties [1–4]. In the periphery, some cannabinoids have been linked to modulation of the immune system [5, 6].

Δ^9-THC is considered the main psychoactive compound in marijuana [7]. Moreover, Δ^9-THC has been shown to affect the functional activities of rodent and human immune cells, including B-lymphocytes, large granular lymphocytes, T-lymphocytes, macrophages, and NK cells [8–12]. It exerts its effects through induction of apoptosis, inhibition of cell proliferation, suppression of cytokine production, and induction of T regulatory cells [1, 13, 14]. Collectively, the studies suggest that Δ^9-THC modulates innate and acquired immunity and causes a switch in the balance of Th1 and Th2 functional activities [1, 14, 15], and studies in the experimental animals have suggested that exposure to Δ^9-THC can enhance mortality and



Journal of Chromatography B, 1044–1045 (2017) 77–86

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Dried haematic microsamples and LC–MS/MS for the analysis of natural and synthetic cannabinoids

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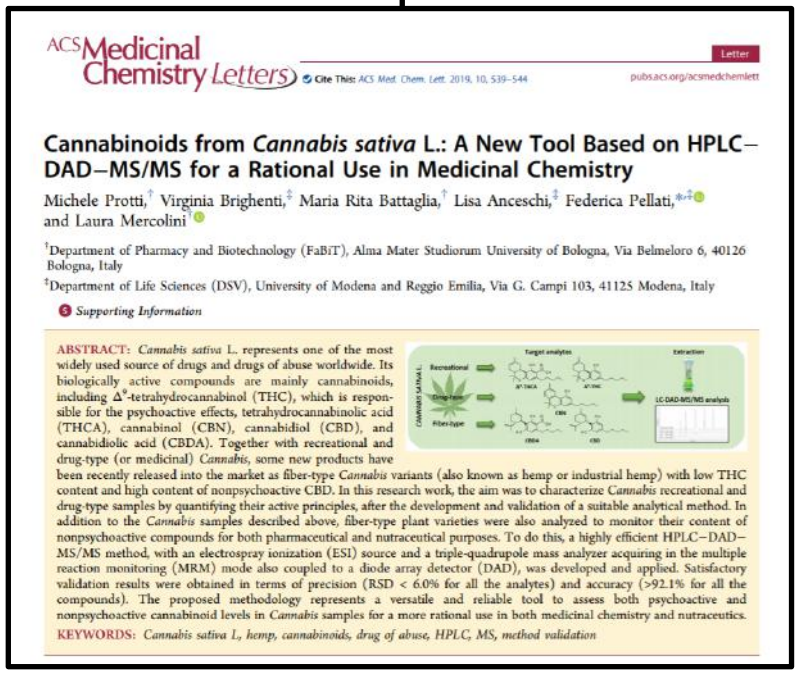
Dried blood spot (DBS)
Natural cannabinoids
Synthetic cannabinoids
LC–MS/MS

ABSTRACT

Synthetic cannabinoids are new psychoactive substances (NPS) with similar effects when compared to natural ones found in *Cannabis* derivatives. They have rapidly integrated into the illicit market, often sold as alternatives under international control. The need to identify and quantify an unprecedented and growing number of new compounds represents a unique challenge for toxicological, forensic and anti-doping analysis. Dried blood spots have been used within the bioanalytical framework in place of plasma or serum, in order to reduce invasiveness, lower sample size, simplify handling, storage and shipping of samples and to facilitate home-based and on-field applications. However, DBS implementation has been limited mainly by concerns related to haematocrit effect on method accuracy. Volumetric absorptive microsampling (VAMS™), a second generation dried miniaturized sampling technology, has been developed just in order to eliminate haematocrit effect, thus providing accurate sampling but still granting feasible sample processing. An original LC–MS/MS method was herein developed and validated for the analysis of THC and its 2 main metabolites, together with 10 representative synthetic cannabinoids in both DBS and VAMS dried microsamples. The ultimate goal of this work is to provide highly innovative DBS and VAMS analytical protocols, whose performances were extensively optimized and compared, in order to provide effective and alternative tools that can be applied for natural and synthetic cannabinoid determination, in place of classical analytical strategies.



ACS Medicinal Chemistry Letters

Letter

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Cannabinoids from *Cannabis sativa* L.: A New Tool Based on HPLC–DAD–MS/MS for a Rational Use in Medicinal Chemistry

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Supporting Information

ABSTRACT: *Cannabis sativa* L. represents one of the most widely used source of drugs and drugs of abuse worldwide. Its biologically active compounds are mainly cannabinoids, including Δ^9-tetrahydrocannabinol (THC), which is responsible for the psychoactive effects, tetrahydrocannabinolic acid (THCA), cannabinol (CBN), cannabidiol (CBD), and cannabidiolic acid (CBDA). Together with recreational and drug-type (or medicinal) *Cannabis*, some new products have been recently released into the market as fiber-type *Cannabis* variants (also known as hemp or industrial hemp) with low THC content and high content of nonpsychoactive CBD. In this research work, the aim was to characterize *Cannabis* recreational and drug-type samples by quantifying their active principles, after the development and validation of a suitable analytical method. In addition to the *Cannabis* samples described above, fiber-type plant varieties were also analyzed to monitor their content of nonpsychoactive compounds for both pharmaceutical and nutraceutical purposes. To do this, a highly efficient HPLC–DAD–MS/MS method, with an electrospray ionization (ESI) source and a triple-quadrupole mass analyzer acquiring in the multiple reaction monitoring (MRM) mode also coupled to a diode array detector (DAD), was developed and applied. Satisfactory validation results were obtained in terms of precision (RSD < 6.0% for all the analytes) and accuracy (>92.1% for all the compounds). The proposed methodology represents a versatile and reliable tool to assess both psychoactive and nonpsychoactive cannabinoid levels in *Cannabis* samples for a more rational use in both medicinal chemistry and nutraceutics.

KEYWORDS: *Cannabis sativa L., hemp, cannabinoids, drug of abuse, HPLC, MS, method validation*

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CLASSICAL ANALYSIS ISSUES

- Invasiveness
- Low subject compliance
- Need for trained personnel
- Sample-operator cross- contamination
- Logistical issues
- Need for temperature-controlled storage and shipping
- Analysis costs
- Stability

Clinical Chemistry 46:9
1384–1386 (2000)

Hematology

Stability of Common Analytes in Urine Refrigerated for 24 h before Automated Analysis by Test Strips

PAUL FROOM,* BARBARA BIEGANIEC, ZAHAVA EHRENRICH, and MIRA BARAK

The effect of antioxidants on the long-term stability of THC and related cannabinoids in sampled whole blood

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Stability of analytes in biosamples—an important issue in clinical and forensic toxicology?

Frank T. Peters

Stability of Drugs, Drug Candidates, and Metabolites in Blood and Plasma

Gregory A. Reed[1]

[1]Department of Pharmacology, Toxicology, and Therapeutics and the University of Kansas Cancer Center, Kansas City, Kansas

Special Issue

Cannabinoid Stability in Antemortem and Postmortem Blood

Vanessa Meneses* and Dani Mata

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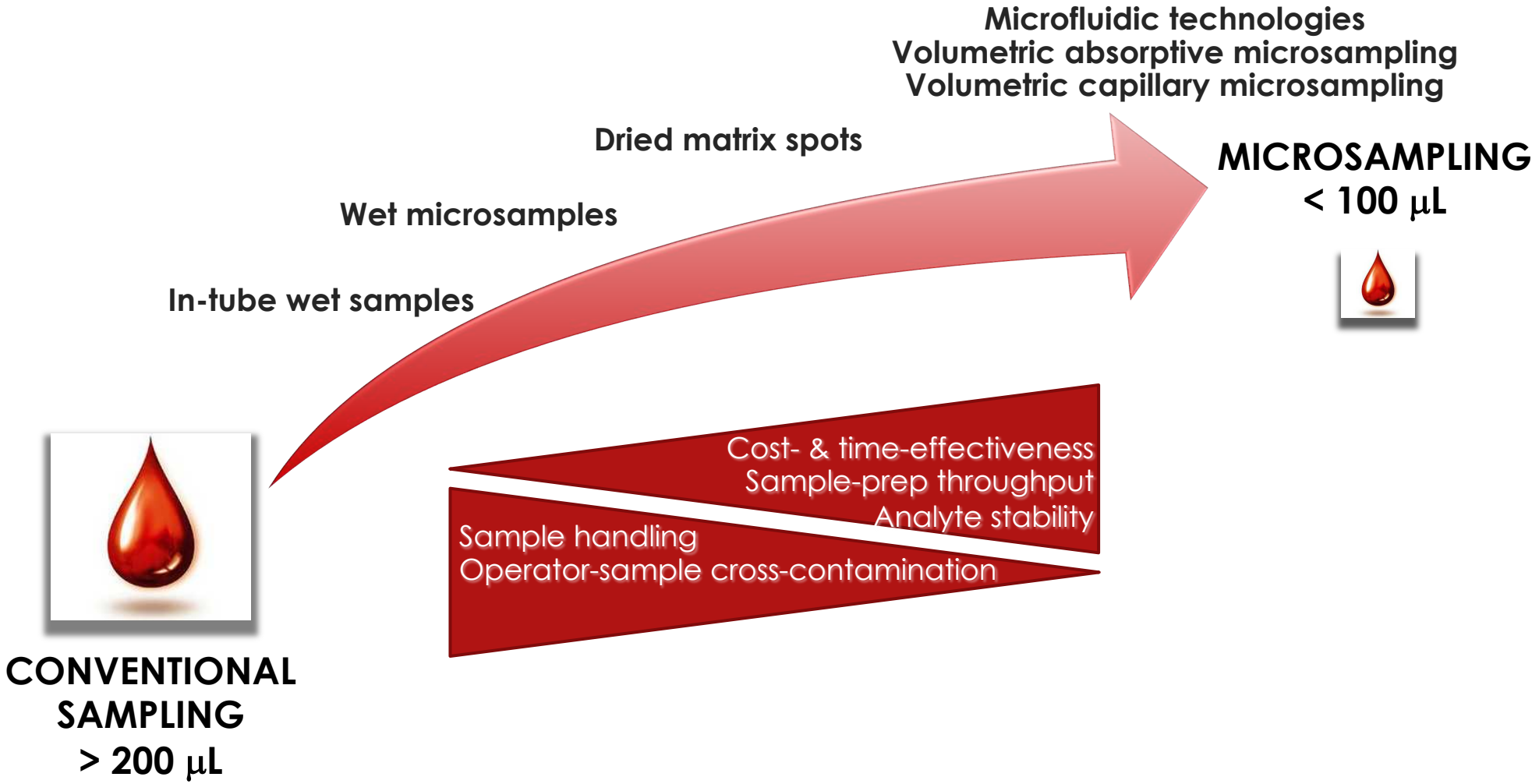
Clinical Interpretation of Urine Drug Tests: What Clinicians Need to Know About Urine Drug Screens

Karen E. Moeller, PharmD, BCPP; Julie C. Kissack, PharmD, BCPP;

NOVEL DEVELOPMENTS IN SAMPLING AND SAMPLE HANDLING



Mercolini L., Protti M., J. Pharm. Biomed. Anal. 130 (2016)202-219.
Mercolini L., Saracino M.A., Protti M., Bioanalysis. 7 (2015),1925-1942.

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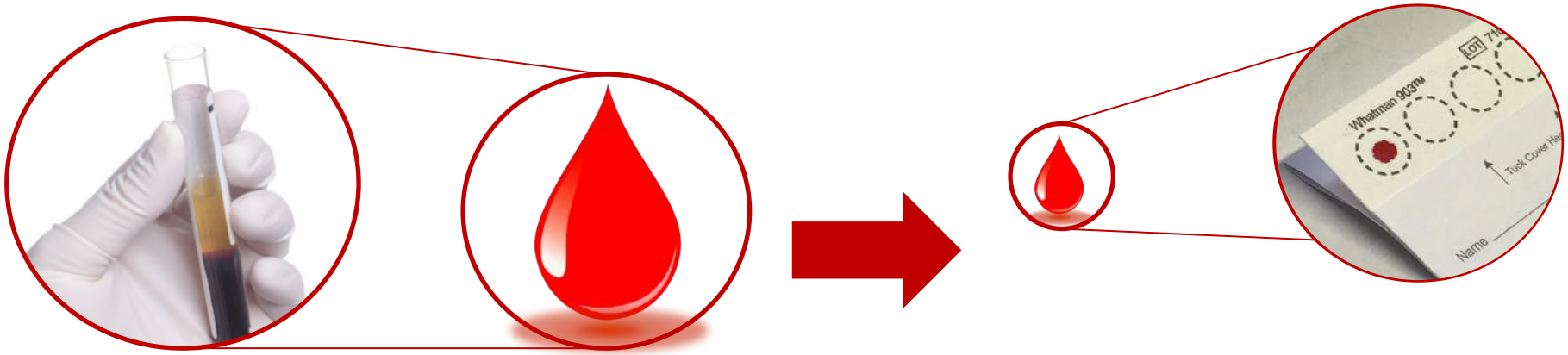
WHAT IS MICROSAMPLING?



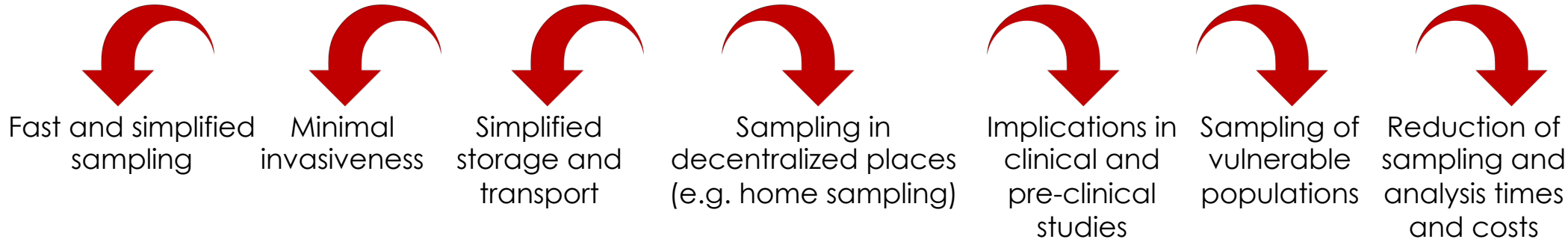
Technologies for the collection and treatment of small volumes of blood / plasma / serum / urine etc.

for the accurate determination of circulating concentrations of drugs,

metabolites and biomarkers

Mercolini L., Protti M., J. Pharm. Biomed. Anal. 130 (2016)202-219.
Mercolini L., Saracino M.A., Protti M., Bioanalysis. 7 (2015),1925-1942.

ADVANTAGES OF MICROSAMPLING
-when properly developed and validated-



Minimal invasiveness	Sample storage and transport at T amb.	Minimal sample handling	Stop of bacterial and enzymatic reactions	Fast and simplified procedures
• Finger / heel prick sampling	• No freezer • No dry ice • Overall analysis time and costs considerably reduced	• Lower risk of sample contamination • Lower biohazard • Simplified chain of custody	• Increased analyte stability • Possibility of delayed analysis	• Lower analysis times • High throughput

Mercolini L., Protti M., J. Pharm. Biomed. Anal. 130 (2016)202-219.
Mercolini L., Saracino M.A., Protti M., Bioanalysis. 7 (2015),1925-1942.

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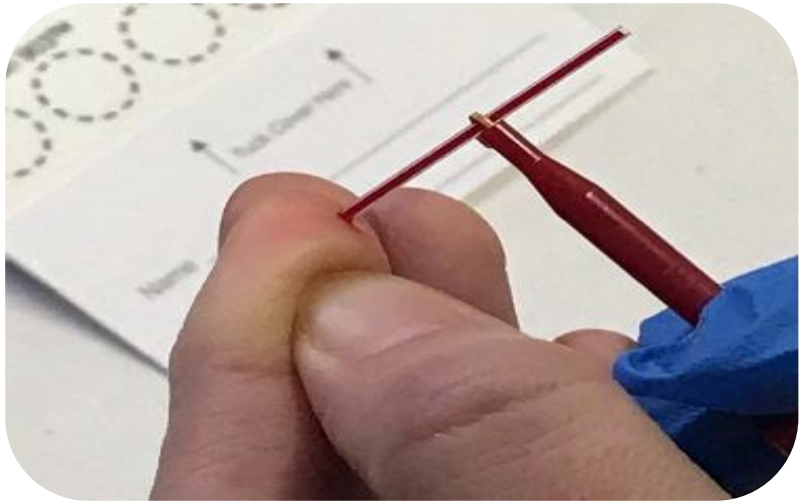
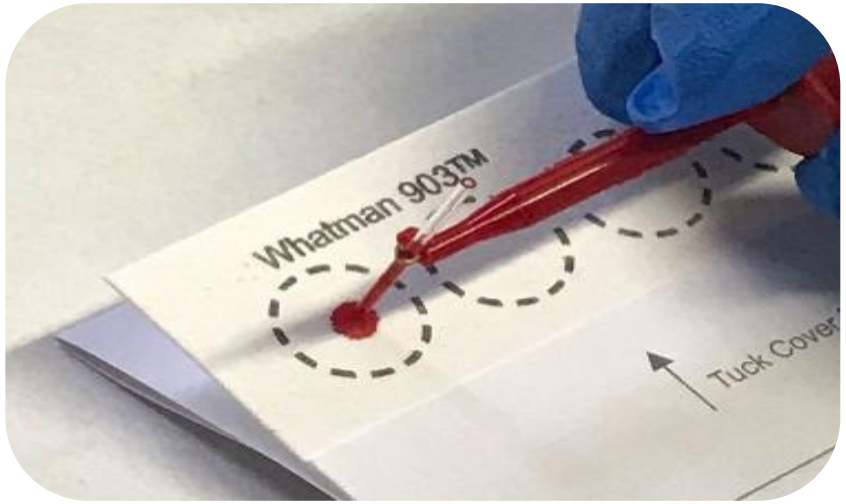
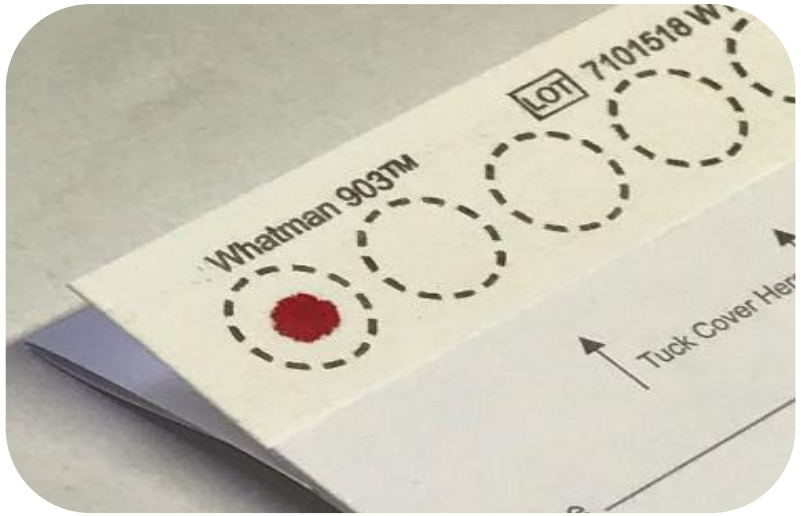
DRIED BLOOD MICROSAMPLING STARTED WITH DBS

DRIED BLOOD SPOT (DBS)



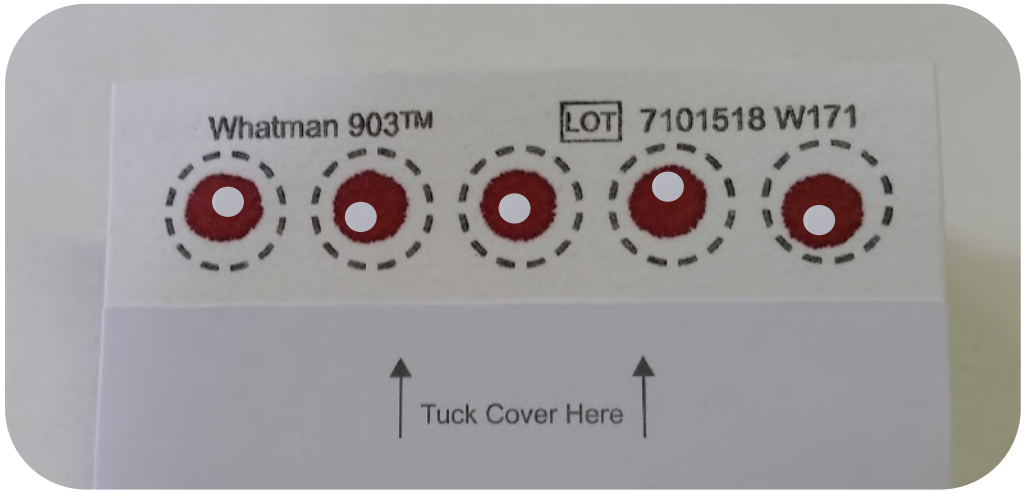
Capillary blood from finger pricking, adsorbed on dedicated support





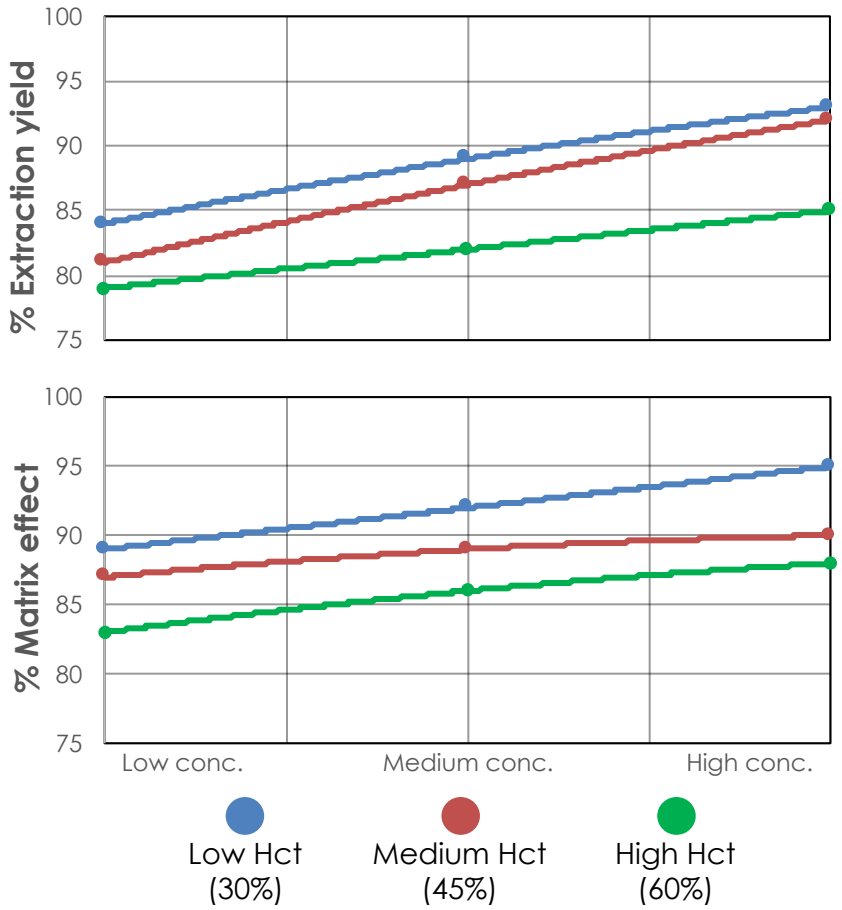
DBS can be stored for months at room temperature with minimal risk of sample degradation: most enzymatic and non-enzymatic reactions are blocked due to water loss

DBS DRAWBACKS: HEMATOCRIT EFFECT



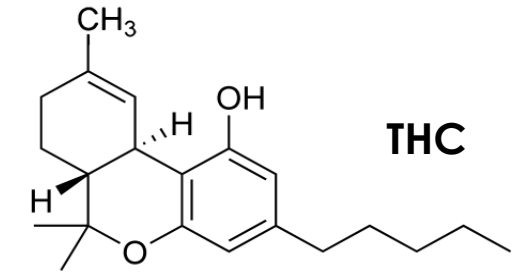
If a **fixed diameter sub-punch** is taken from the sample, a different volume of blood is sampled depending on HCT and thus blood density

– It introduces assay bias
– Recovery and suppression may also play a role

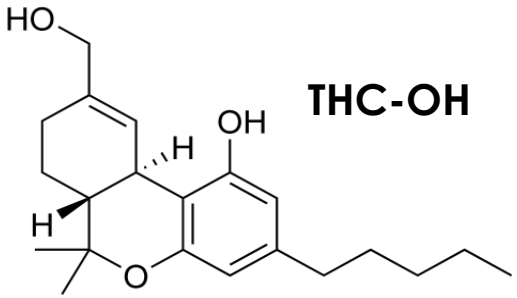


Protti M, Rudge J, Sberna AE, Gerra G, Mercolini L.
J Chromatogr B, 1044 (2017) 77-86.

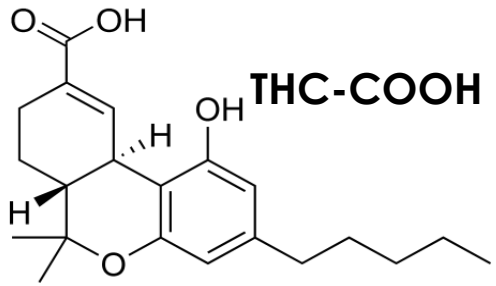
Blood microsampling approaches for THC and endogenous metabolites



THC



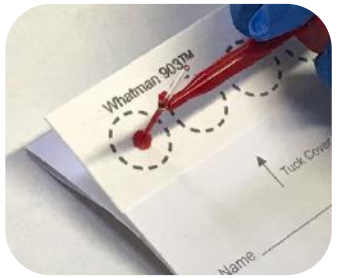
THC-OH

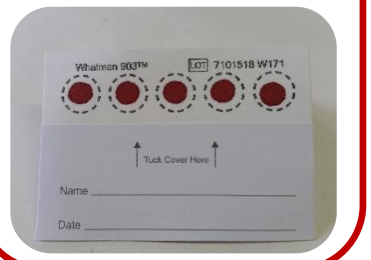


THC-COOH

- **Dried blood spots (DBS)**
- **Volumetric absorptive microsampling, VAMS (Mitra® technology)**
- **Capillary volumetric blood microsampling (hemaPEN® technology)**

DBS





VAMS

HemaPEN

Protti M, Mandrioli R., Mercolini L.
Anal. Chim. Acta, 1046 (2019) 32-47

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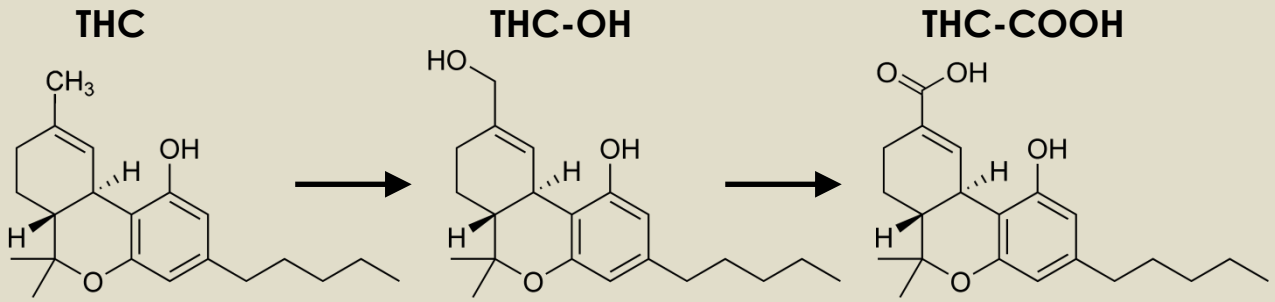
HPLC-MS/MS for THC and endogenous metabolites

Analytes



Parent compound ~24h → Active metabolite <5h → Inactive metabolite >>48h

Mass spectrometry

Triple quadrupole; MRM mode, ESI+/ESI- ionisation source

Chromatographic system

Column:	RP C18, 3.5 µm
Dimensions:	50 x 2.1 mm I.D.
Flow Rate:	0.3 mL/min
Mobile Phase:	0.1 % F.A. in ACN/ 0.1 % F.A. in H_2O, gradient elution

Pretreatment procedure

Solvent: methanol

Extraction method:

- UAE (5 min)
- Vortex mixing (1 min)

Vacuum drying

Reconstitution in mobile phase

Mass spectrometry parameters

Compound	Q1 (m/z)		Q3 (m/z)	Cone voltage (V)	Coll. energy (eV)
THC	315.53	>	193.2	50.00	30.00
THC-D3	318.53	>	196.2	50.00	30.00
THC-OH	331.52	>	313.3	30.00	20.00
THC-OH-D3	334.52	>	316.3	30.00	20.00
THC-COOH	343.52	>	297.2	50.00	30.00
THC-COOH-D3	346.52	>	300.2	50.00	30.00

Protti M, Rudge J, Sberna AE, Gerra G, Mercolini L.
J Chromatogr B, 1044 (2017) 77-86.

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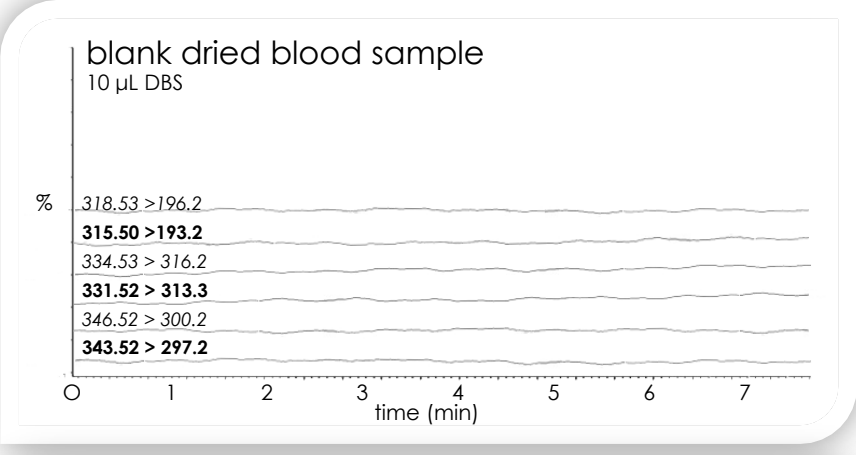
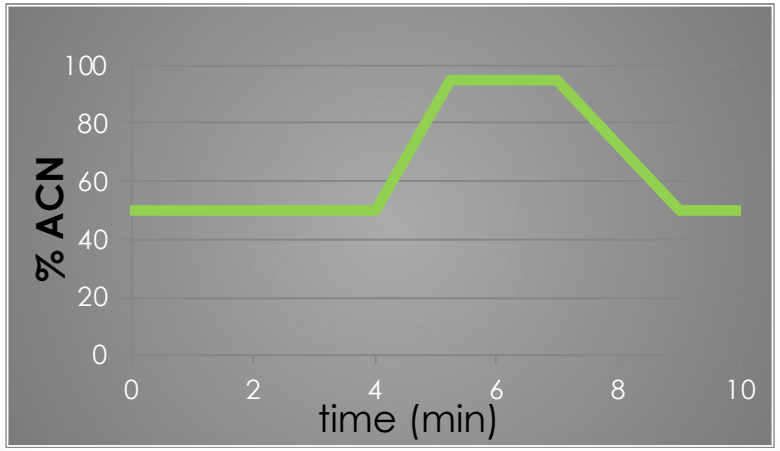
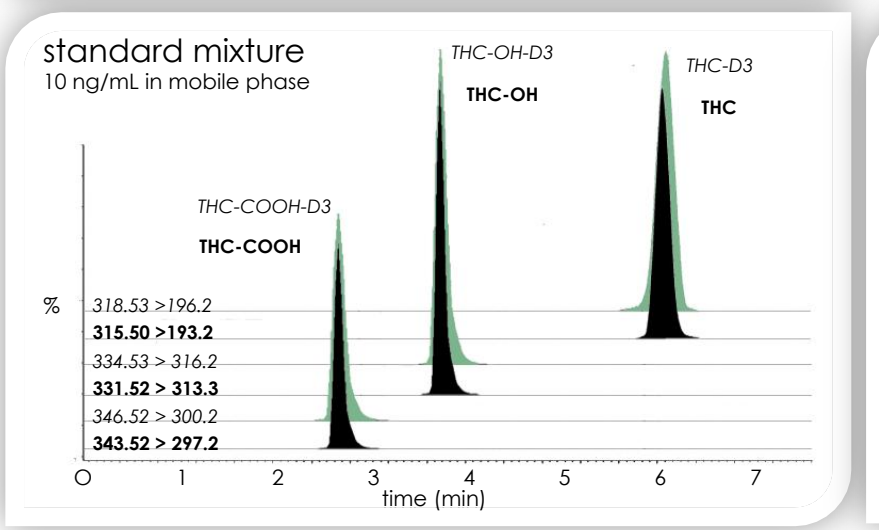
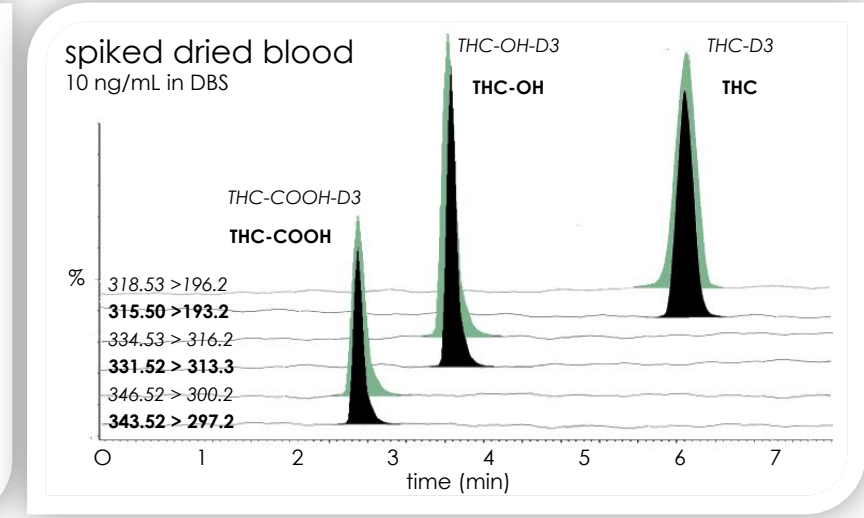
HPLC-MS/MS for THC and endogenous metabolites









Validation results

Linearity range
THC: 2.5-200 ng/mL
THC-OH &
THC-COOH: 5-200 ng/mL
$r^2 > 0.9988$

Sensitivity
LLOQ = 2.5 ng/mL

Precision
RSD% < 6.1%

Accuracy
> 90%

Recovery
> 85 %

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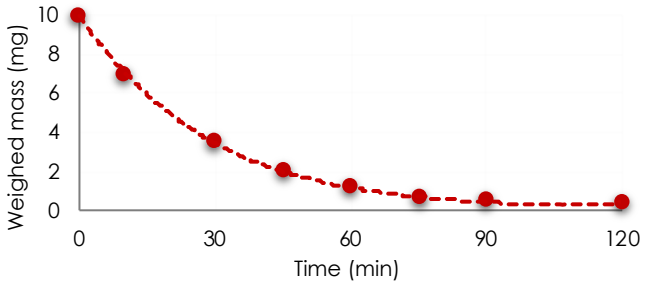
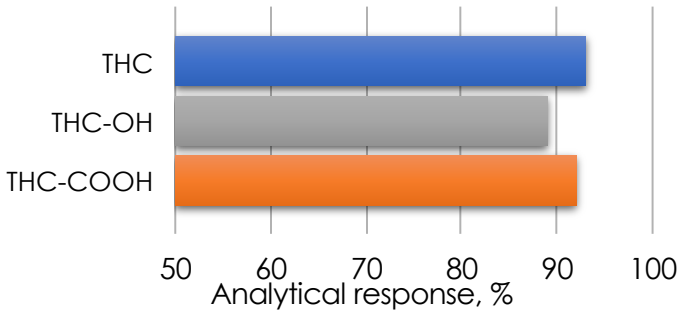
THC and endogenous metabolites: DBS process optimization

DRYING

Room conditions

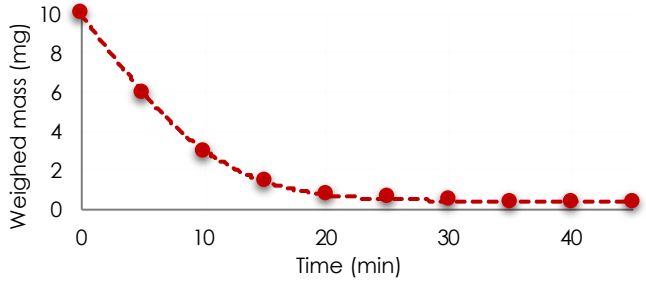
25 °C, 55% humidity

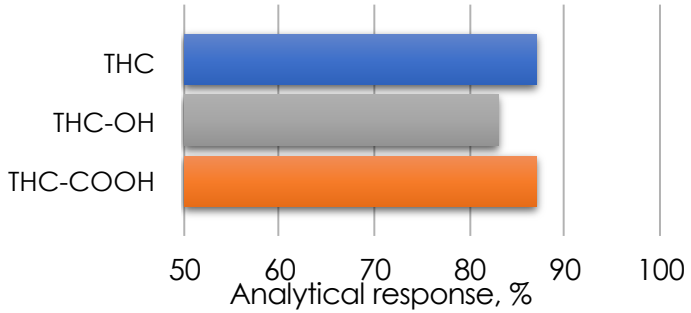




Heat-assisted drying

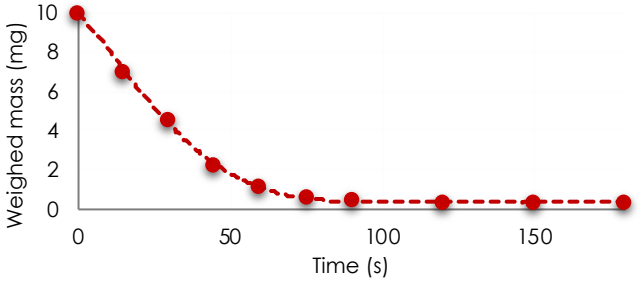
Ventilated oven, 60 °C

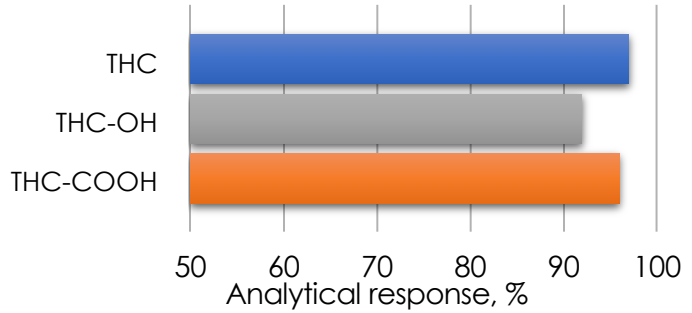




Microwave-assisted drying

700 W power





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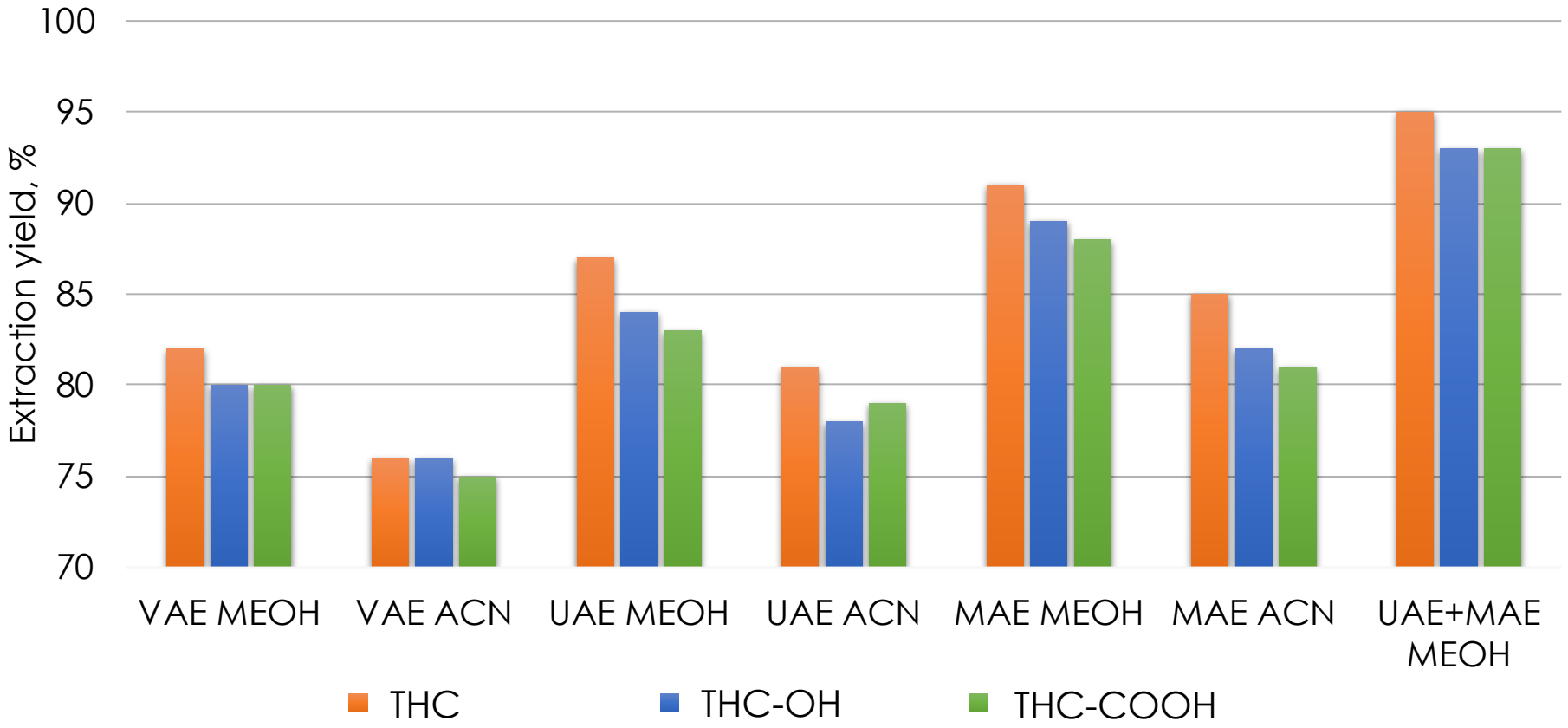
THC and endogenous metabolites: DBS process optimization

EXTRACTION



VAE: Vortex-assisted extraction, 10 min; **UAE**: Ultrasound-assisted extraction, 15 min, 40 kHz;
MAE: Microwave-assisted extraction, 2 min, 700W

DEALING WITH HEMATOCRIT BIAS: VOLUMETRIC ABSORPTIVE MICROSAMPLING

VAMS (Mitra® technology)

Courtesy of Neoteryx

- Consecutive collection of 2/3/4/96x unique samples (depending on format)
- 10/20/30 µL
- Volumetric accuracy by absorption on porous polymeric tip
- Amenable to automation with standard equipment

Protti M, Mandrioli R., Mercolini L.
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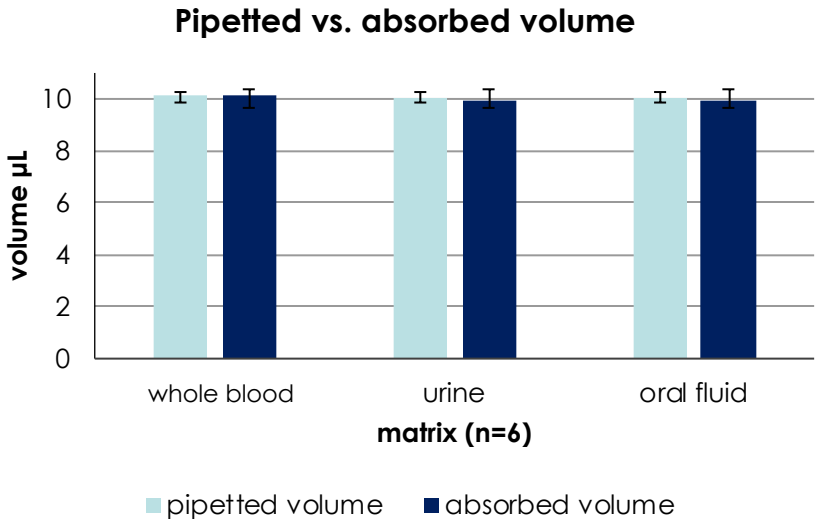
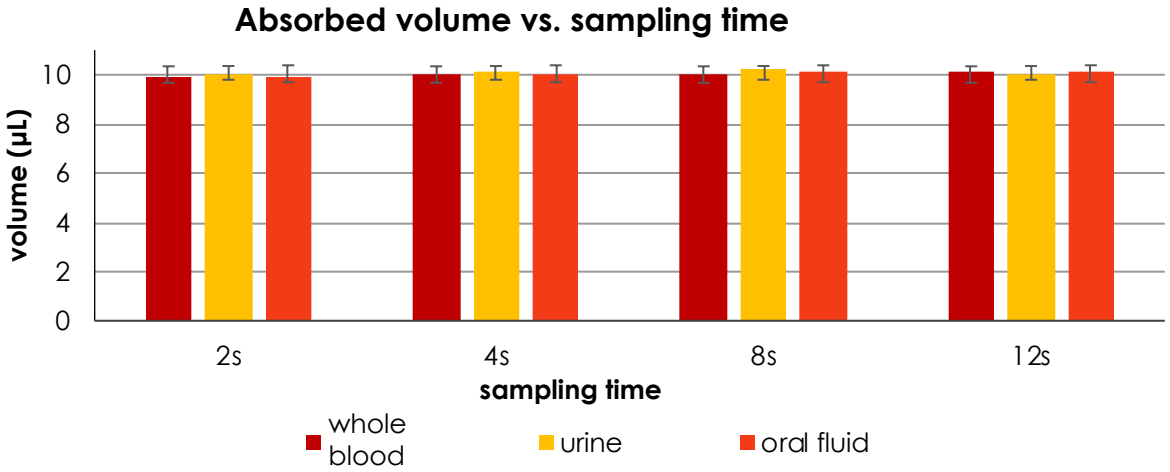
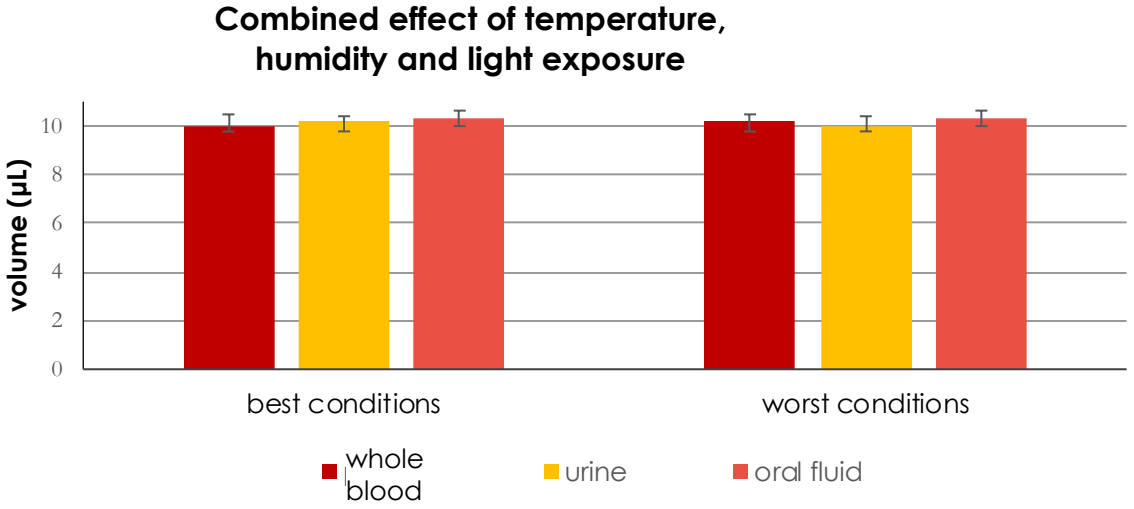
THC and endogenous metabolites: VAMS sampling performance

SAMPLING ACCURACY







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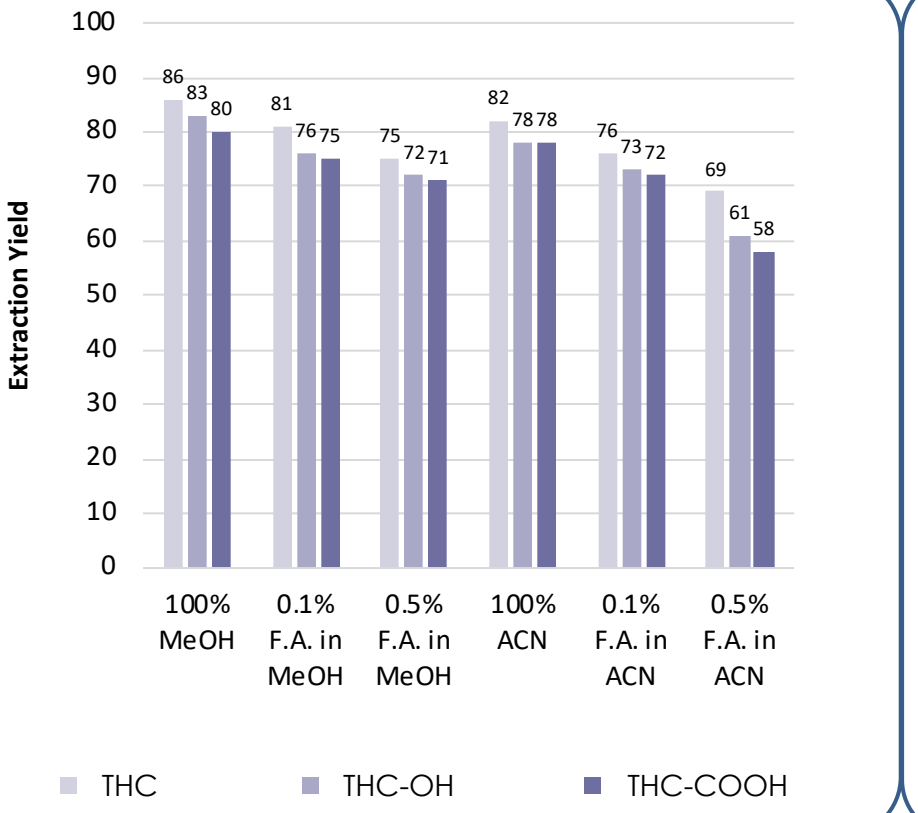
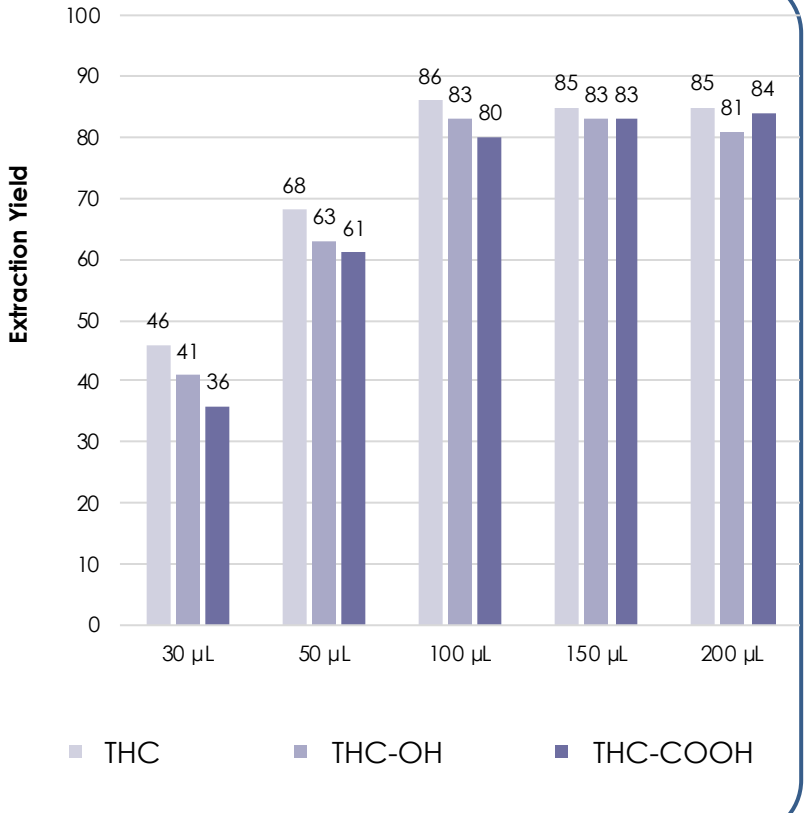
L. Mercolini, M. Protti, M.C. Catapano, J. Rudge, A.E. Sberna
Journal of Pharmaceutical and Biomedical Analysis 123 (2016) 123-186.

THC and endogenous metabolites: VAMS process optimisation

Extraction solvent



Extraction volume



Optimised protocol

- Sampling with VAMS device for 2s
- Drying at RT for 1h
- Extraction in a vial
  - 100 µL of 100% MeOH
  - Ultrasound-assisted extraction 20 min
- Drying (N_2 stream) and reconstitution in MP

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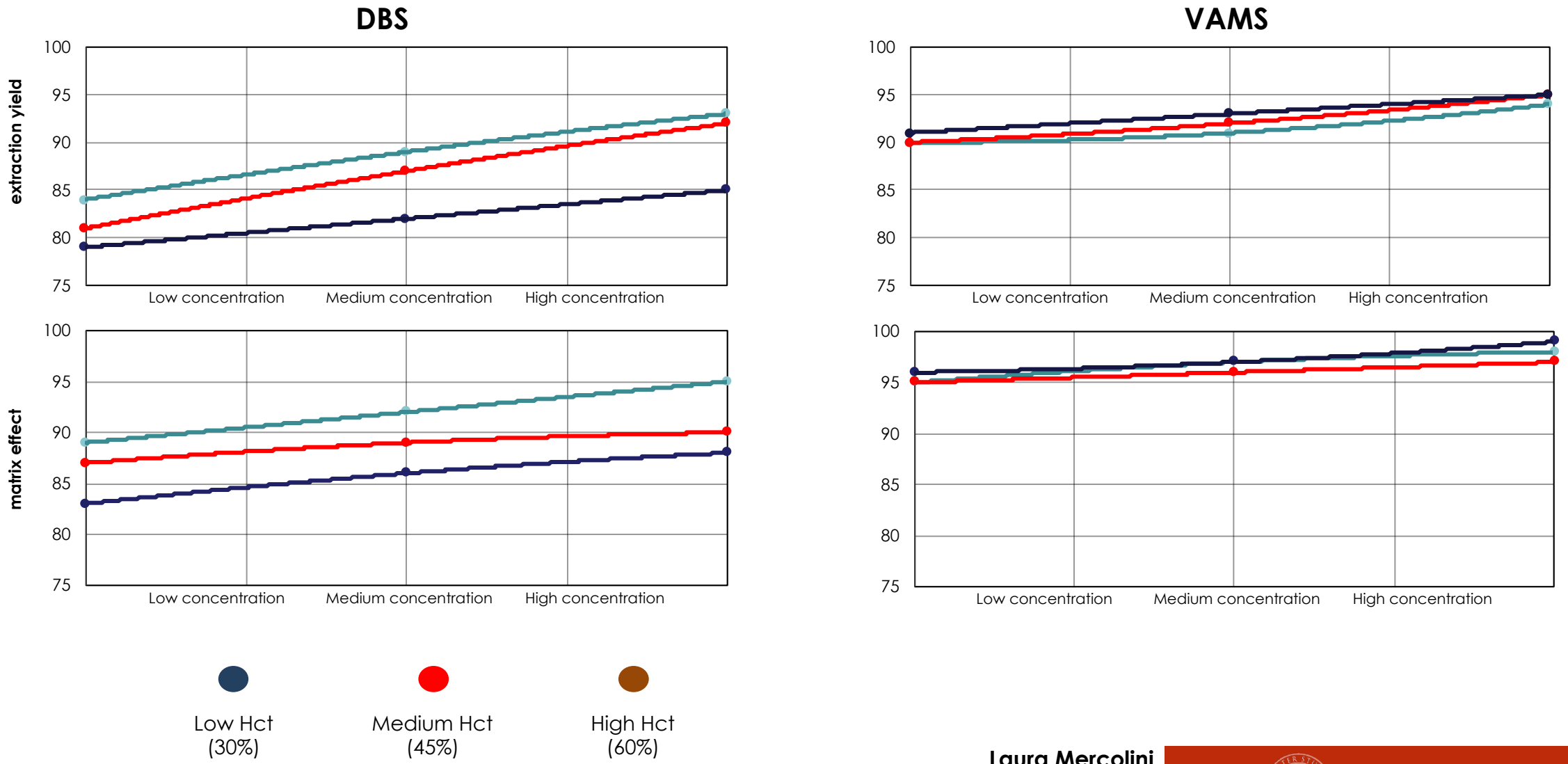
THC and endogenous metabolites: DBS and VAMS performances at different HCT levels



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Journal of Chromatography B 1044 (2017) 77-86.

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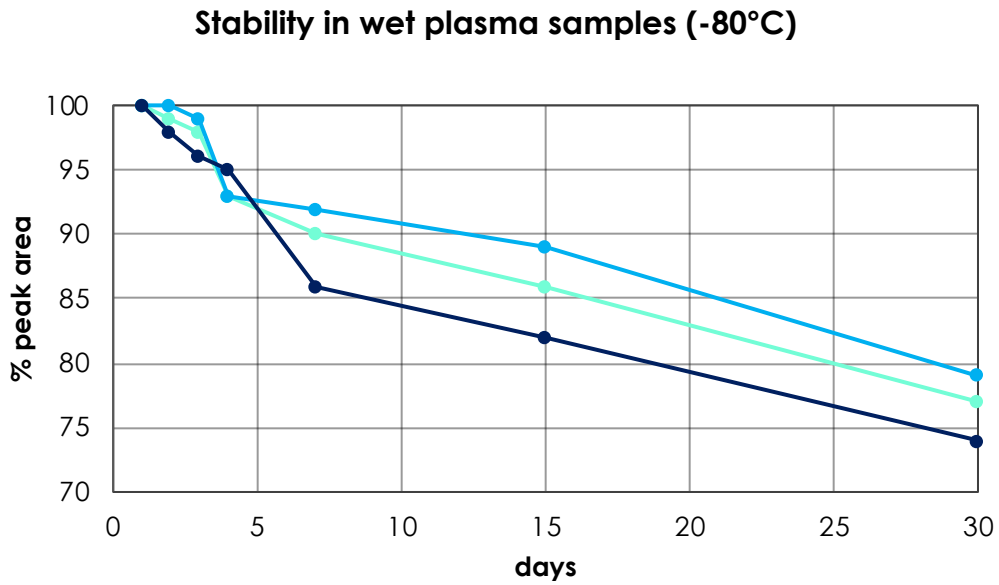
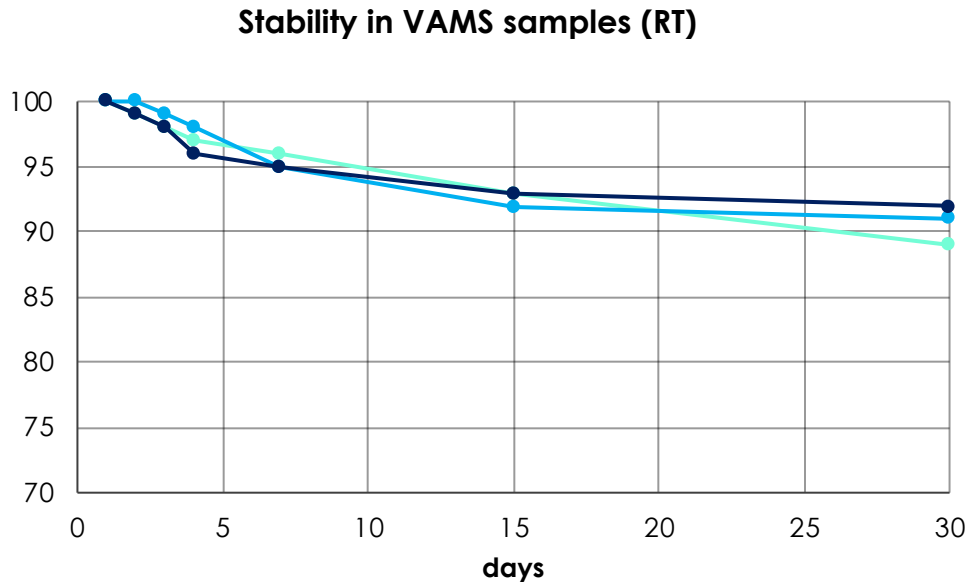
THC and endogenous metabolites: stability in VAMS samples



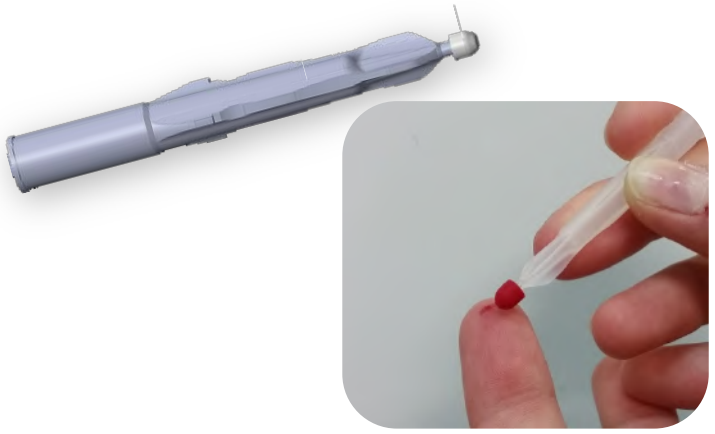


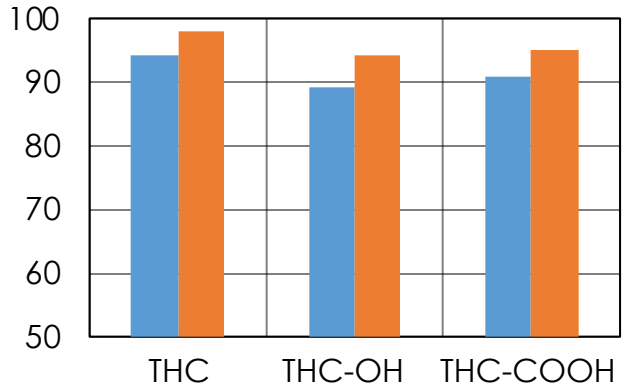
THC THC-OH THC-COOH

L. Mercolini, M. Protti, M.C. Catapano, J. Rudge, A.E. Sberna
Journal of Pharmaceutical and Biomedical Analysis 123 (2016) 123-186.

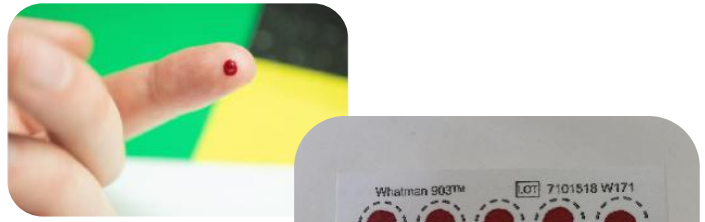
THC and endogenous metabolites: VAMS vs. DBS vs. wet plasma

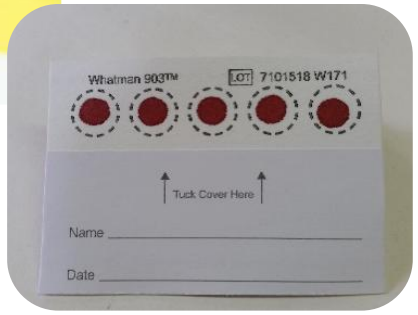
10 µL blood VAMS

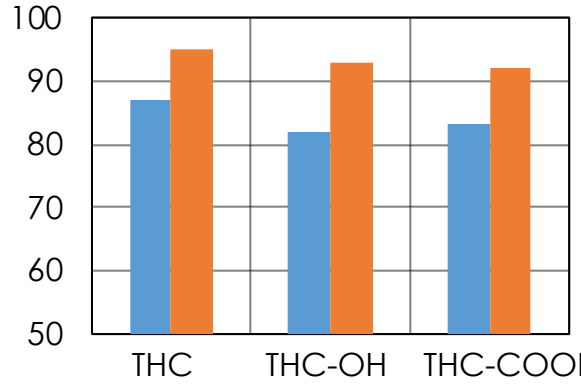




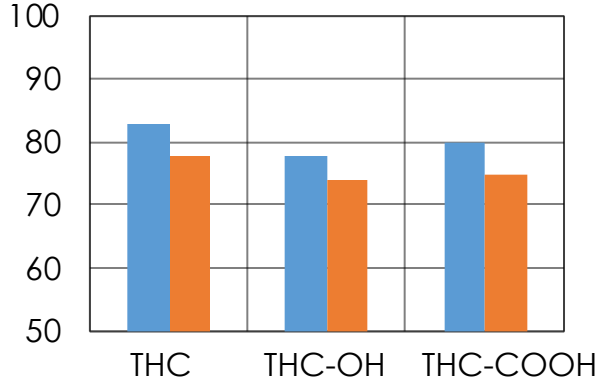
10 µL DBS







Wet plasma



Matrix effect

Extraction yield

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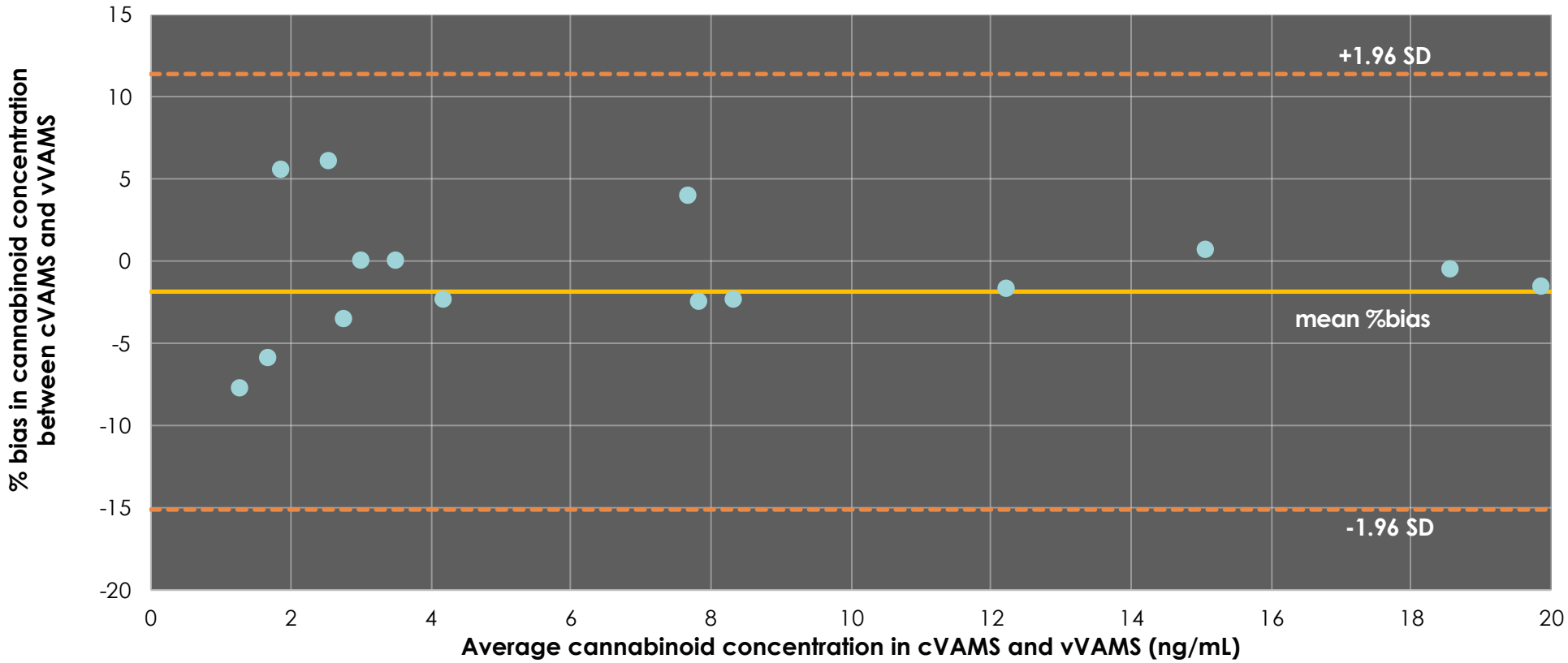
THC and endogenous metabolites: capillary vs. venous blood VAMS

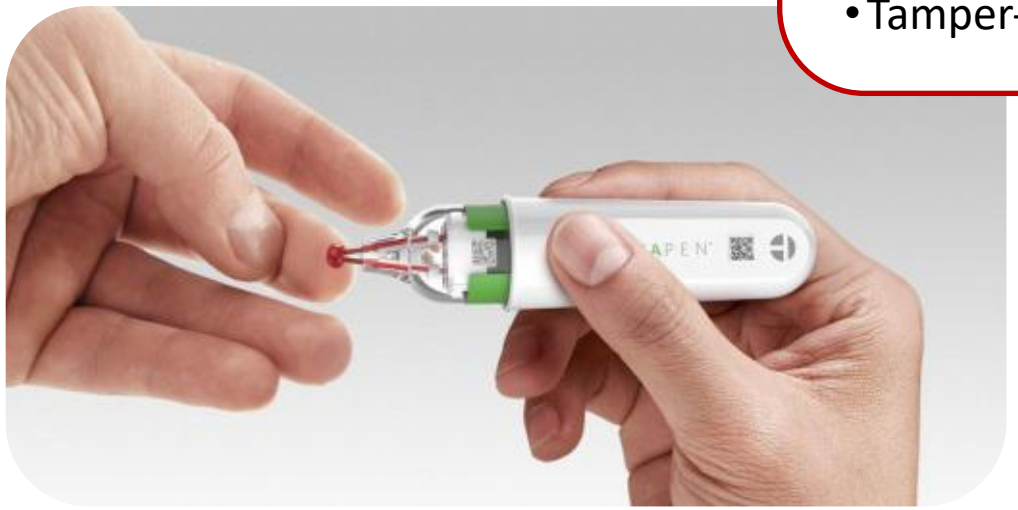


M. Protti, J. Rudge, A.E. Sberna, G. Gerra, L. Mercolini
Journal of Chromatography B 1044 (2017) 77-86.

DEALING WITH HEMATOCRIT BIAS: CAPILLARY VOLUMETRIC BLOOD MICROSAMPLING

- Simultaneous collection of 4x 2.74 µL replicates
- Generation of DBS on pre-punched disks
- Volumetric accuracy by glass capillaries
- Integrated storage
- Tamper-proof

hemaPEN® technology



Courtesy of Trajan

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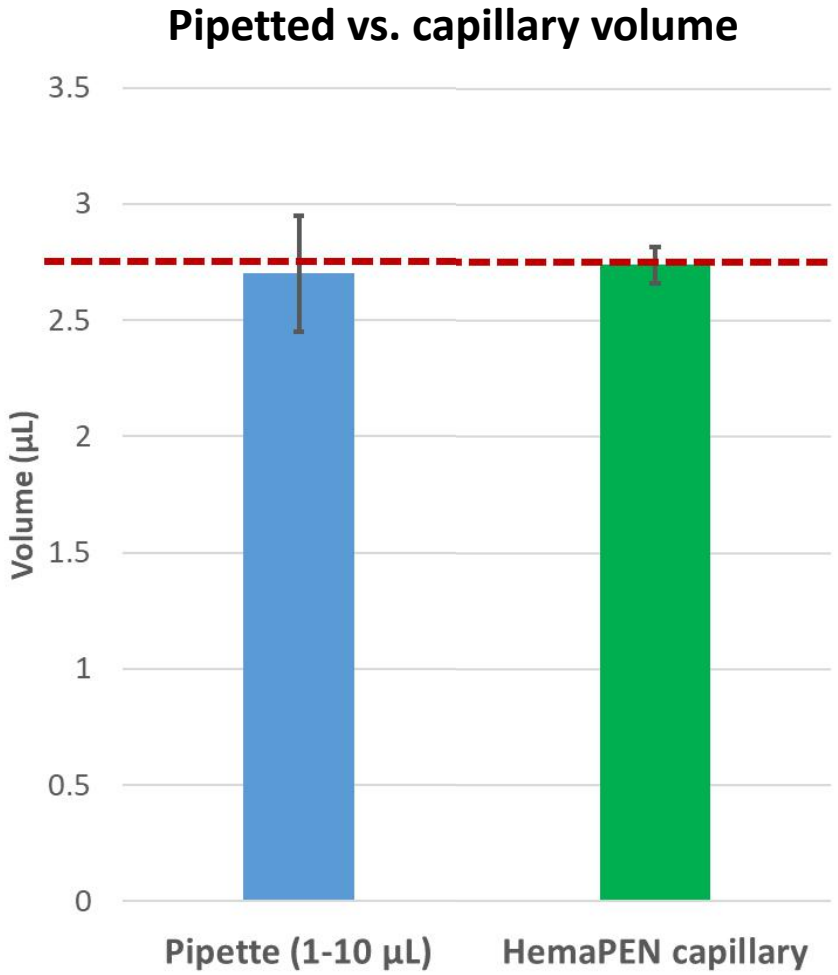
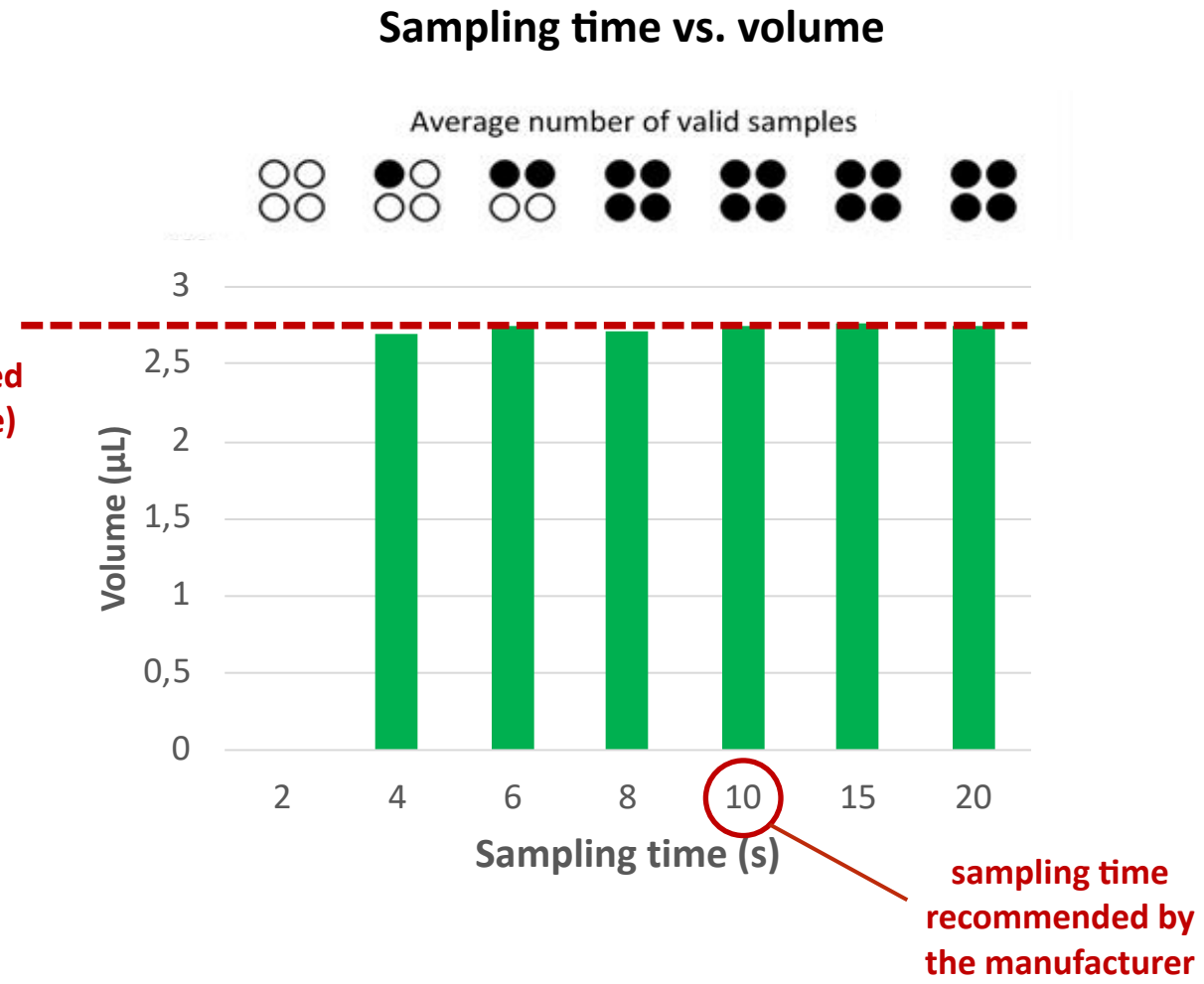
THC and endogenous metabolites: hemaPEN sampling performance



2.74 µL
(nominal declared capillary volume)



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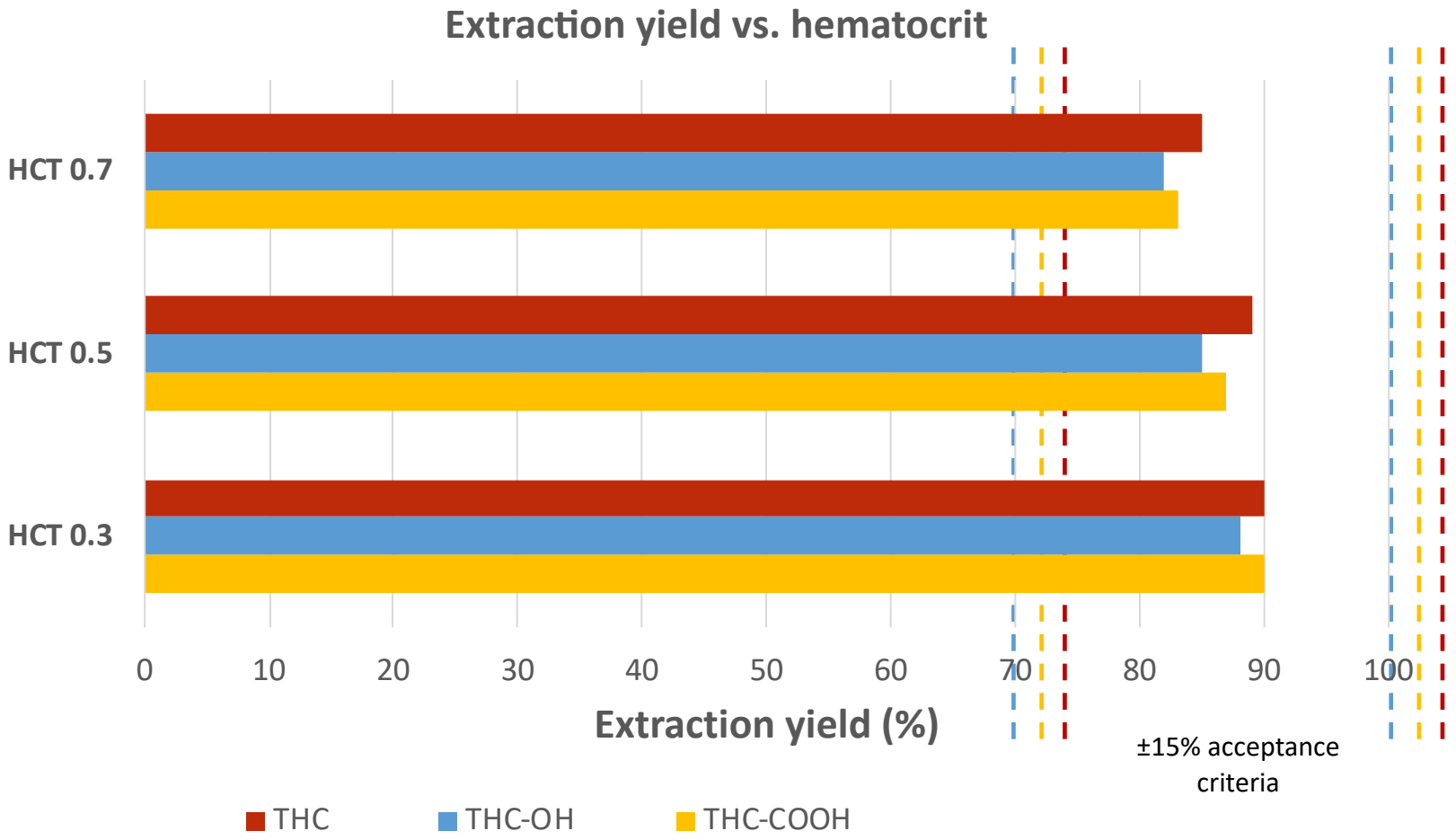
THC and endogenous metabolites: hemaPEN sampling performance



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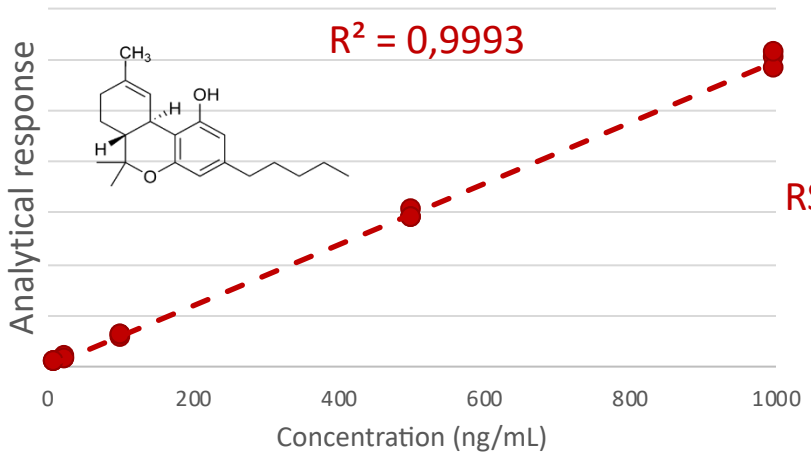
DEALING WITH HEMATOCRIT BIAS: MICROFLUIDIC TECHNOLOGY

HemaXis® technology

- Consecutive collection of 4 DBS samples on a single device
- Classic DBS card format
- 5/10 µL
- Volumetric accuracy by microfluidic channel chip
- Compatible with automated handling process

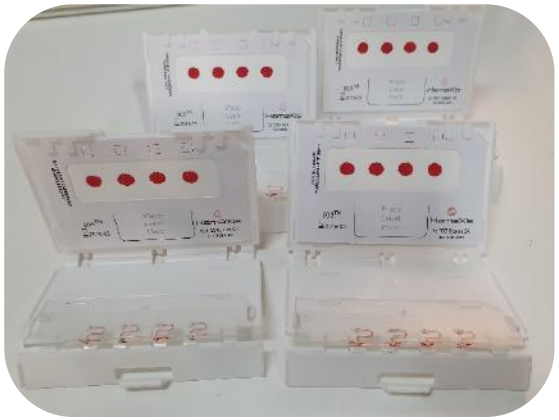
THC and endogenous metabolites: HemaXis performance

THC

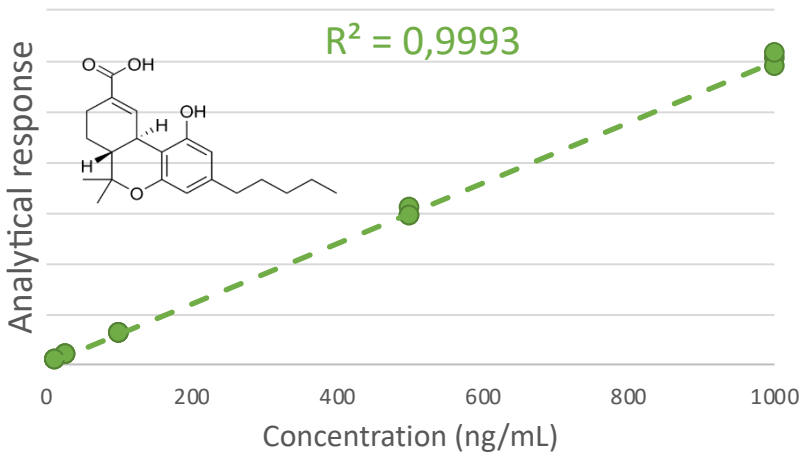


RSD% < 6.8

CALIBRATION CURVES AND PRECISION

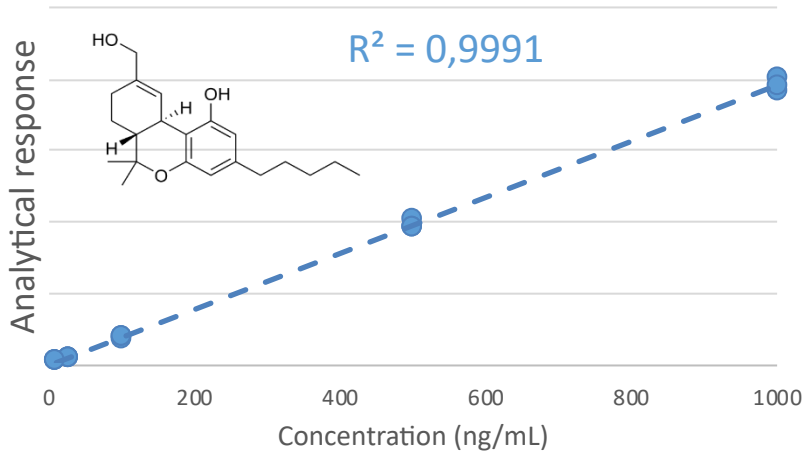


THC-COOH



RSD% < 7.3

THC-OH



RSD% < 7.9

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Take-home message

Advantages of microsampling over classic fluid matrix for cannabinoid analysis

- Higher cannabinoid stability: samples are dried, stored and shipped at RT
- Lower contamination risks (operator-sample, sample-sample and sample-operator)
- Lower analysis costs and logistical savings by storage and shipping without the need for cryopreservation
- Analysis feasibility even when small sample amounts are available and extraction is performed by using small solvent volumes
- Significant simplification of sampling and pretreatment steps:

  no sub-sampling, centrifugation, isolation, nor further clean-up is needed
- High-throughput, automated sample handling and analysis

Conclusion

- Microsampling approach coupled to LC-MS/MS analysis has proven to be a suitable and advanced tool for the analysis of THC together with their main metabolites.
- The proposed miniaturised strategies are feasible, straightforward and reliable.
- Microsampling devices allow accurate collection of microvolumes of biological matrices with minimal invasiveness and in a feasible way, suitable for in-situ, point-of-care sampling.
- Dried microsamples guarantee enhanced stability of cannabinoids at room temperature, providing good results in terms of extraction yield and precision.
- Innovative promising method for the investigation of possible consumption scenarios.

The PTA Lab at Unibo

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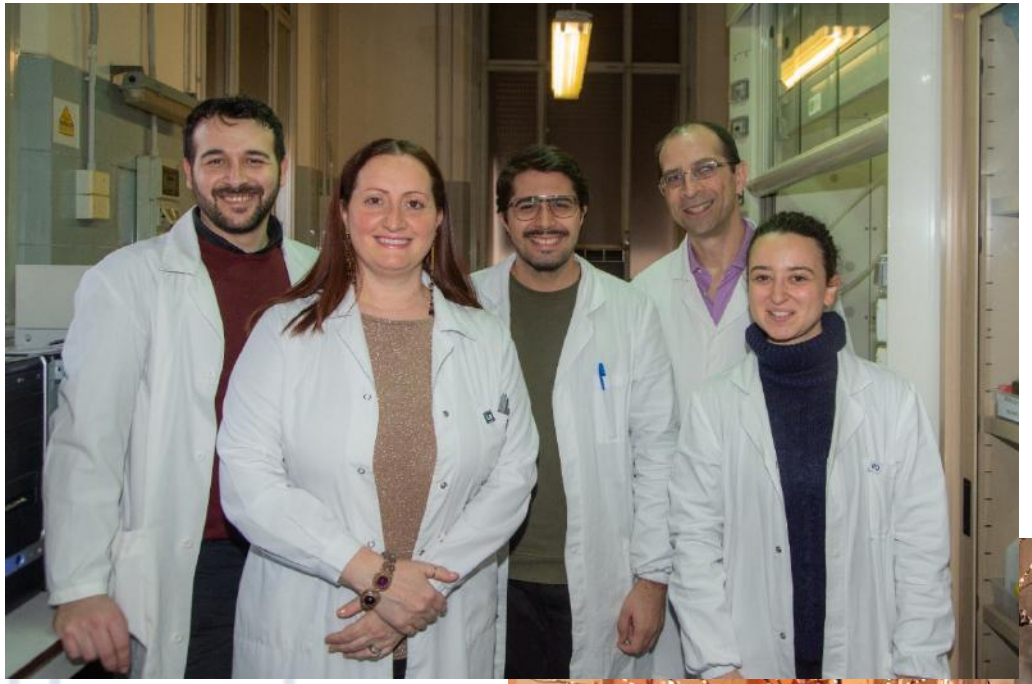

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