International Journal of Medical Toxicology & Legal Medicine Volume 27, No. 4, 2024
e-ISSN: 0974-4614
p-ISSN: 0972-0448

Assessment of storage containers effect on stability of cannabis metabolites in urine using Liquid Chromatography-Tandem Mass Spectrophotometry technique

Idris Mohammed[1]*, EimanAwadh Abdullah[2], Aisha Al-Shehi[3], Mussab Osama[4], Gavash Harsha Kannikanti[1].

[1]Department of Forensic Sciences, College of Criminal Justice, Naif Arab University for Security Sciences, Riyadh, Kingdom of Saudi Arabia.
[2]Department of Medical Laboratory Science, College of Health Sciences, University of Sharjah, Emirates of Sharjah, United Arab Emirates.
[3]Forensic Toxicology Section, Sharjah Police Forensic Science Laboratory, Emirates of Sharjah, United Arab Emirates.
[4]Department of Chemistry, College of Sciences, University of Sharjah, Emirates of Sharjah, United Arab Emirates.
Email: midris@nauss.edu.sa.
*Corresponding author

Received: 10.08.2024 Revised: 16.09.2024 Accepted: 09.10.2024

ABSTRACT
Δ9-Tetrahydrocannabinol (Δ9 -THC) metabolites such as THC-OH (11-Hydroxy-Δ9-tetrahydrocannabinol), THC-COOH (11-Nor-9-carboxy-Δ⁹-tetrahydrocannabinol) and THC-COOH-glucuronide (11-Nor-9-carboxy-Δ⁹-tetrahydrocannabinol conjugate) were studied in urine samples for their stability with respect to different storage conditions involving containers, temperature and time using validated liquid chromatography – tandem mass spectrometry method. It was concluded that storing the samples for less than three months either at 4 ºC or -20ºC in the silanized clear or amber glass was desirable.

Keywords: Cannabis, Stability, containers, storage, LCMSMS, THC-COOH.

INTRODUCTION

The major active ingredient that has been found in cannabis products is Δ9-Tetrahydrocannabinol (Δ9 -THC)[1], which is accountable for its psychoactive effects. In the herbal cannabis there are other compounds such as Cannabinol (CBN), Cannabidiol (CBD) and Cannabigerol (CBG) are also present. When the body fluids or tissues are analysed for detection of cannabis we look for the metabolites of these compounds. However, the detection of Δ9 –Tetrahydrocannabinol and its metabolites, such as 11-hydroxy-THC (11-OH-THC) and 11 nor-9-carboxy-(THCCOOH) which are present as their glucuronides, are considered as strong evidence. In the forensic practice it is observed that the exhibit samples are stored for varying time durations and conditions. It is reported that there would be changes in the concentration of active ingredients which is a matter of concern. Further the cannabis used may have different concentration of cannabinoids because of various conditions such as species, soil, climate, altitude and storage etc. bringing so many variables. With a view to find the changes in the concentration of Δ9-Tetrahydrocannabinol (Δ9 -THC) and its metabolites on storage with reference to time, temperature and the container material this study was undertaken.

Usually metabolites of cannabis are measured in blood or urine as free or in conjugated form. to indicate the cannabis abuse in the subject. TheΔ9-THC can be liberated from the conjugated glucuronides by enzymatic or alkaline hydrolysis. With the advent of Liquid Chromatography – Tandem Mass spectrometry (LC-MS/MS) even conjugated form can be analysed in biological sample without hydrolysing[2-4]. The three metabolites of Δ9-THC that have been assessed in this study include the THC-OH (11-Hydroxy-Δ^9-tetrahydrocannabinol), THC-COOH (11-Nor-9-carboxy-Δ⁹-tetrahydrocannabinol) and THC-COOH-glucuronide (11-Nor-9-carboxy-Δ⁹-tetrahydrocannabinol conjugate)[5-7].

The stability of these metabolites in the sample will be having impact on the analytical findings. There are several parameters like different container types, varying temperatures and preservatives which might affect the concentration of the above compounds. In the present study we expanded the containers types compared to previous studies and studied the stability of Δ9-THC 3 major metabolites in urine samples for different

temperature and time duration using modified LC-MS/MS method[8]. The issues related to sample collection, transportation, and storage (e.g., temperature, container, time) may affect analyte concentration.

MATERIALS AND METHODS

Chemicals & Reagents

Standards of (Â±)-11-Hydroxy-delta9-THC (THC-OH, 1 mg mL-1), (Â±)-11-nor-9-Carboxy-delta9-THC (THC-COOH, 1 mg mL-1) and (+)-11-nor-9-Carboxy-delta9-THC-glucuronide (THC-COOH-glucuronide, 0.1mg mL-1), (Â±)-11-Hydroxy-delta9-THC-D3 (THC-OH, 0.1 mg mL-1), purchase from cerilliant, USA. Ammonium formate, deionized water, methanol and acetonitrile used were of LCMS grade and purchase from Sigma-Aldrich.

INSTRUMENTATION

Analytical columns used were of PFPP (PFPP (2.0 mm I.D. x 50 mm L, 2 um), UCT) and guard column (SecurityGuard C18 2.0-3.0 mm ID, Phenomenex) purchased from local suppliers. Analysis performed on LC-MS/MS (Nexera UPLC, LCMS-8050) from Shimadzu, japan. Details of the instrument parameters for performing analysis described in table-I. Total analysis run time of 10 minutes. Analytes were monitored in selected reaction monitoring mode (SRM) (Table-I& Figure-1).

URINE SAMPLE COLLECTION

Approximately 2 L negative urine collected which were received in the Sharjah Police Forensic Sciences laboratory, Emirates of Sharjah, United Arab Emirates for drug of abuse testing, screened (using Enzyme Multiplied Immunoassay Technique (EMIT) and confirmed (Gas Chromatography Mass Spectrometry metabolites (GCMS))[9] to ruled out the cannabis metabolites and other abused drugs. Above said urine was used to prepare the 500 mL of spiked solution of cannabis metabolites.

STOCK SOLUTION PREPARATION FOR STABILITY STUDY

THC-OH (100 ng mL^{-1}): Transfer 50 µL of THC-OH (1 mg mL^{-1}) solution to 500 mL volumetric flask, and make up to the mark with blank urine.

THC-COOH (100 ng mL^{-1}): Transfer 50 µL of THC-COOH (1 mg mL^{-1}) solution to 500 mL volumetric flask, and make up to the mark with blank urine.

THC-COOH-glucuronide (100 ng mL^{-1}): Transfer 500 µL of THC-COOH-glucuronide (0.1 mg mL^{-1}) solution to 500 mL volumetric flask, and make up to the mark with blank urine.

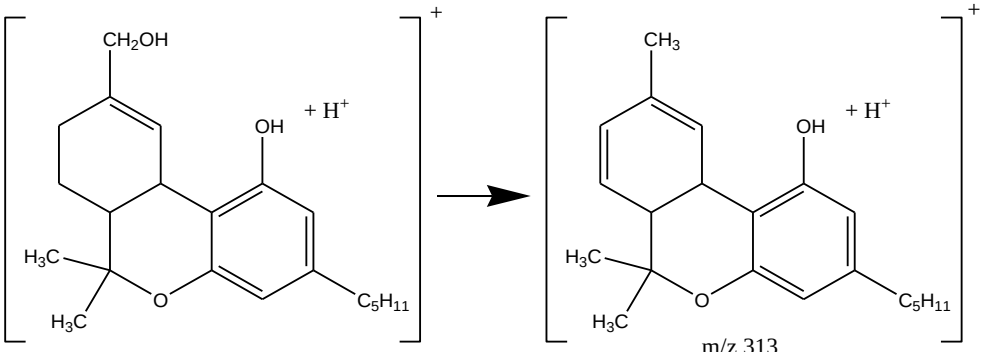
Table 1: Showing the instrumentation parameters for LCMSMS Analysis.

LC conditions				
Analytical column	UCT PFPP (2.0 mm I.D. x 50 mm L, 2 um)			
Guard column	Phenomenex SecurityGuard C18 2.0-3.0 mm ID			
Mobile phase A	10 mmol/L Ammonium formate-Water			
Mobile phase B	Methanol			
Gradient program	Step	Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
	0	0.00	70	30
	1	7.00	5	95
	2	9.00	5	95
	3	9.01	70	30
	4	10.00	70	30
Flow rate	0.3 mL/min			
Injection volume	10 ul			
Column oven temperature	40^{O}C			
MS conditions				
Nebulizing gas flow rate	1.5 L/min			
Drying gas flow rate	10 L/min			
DL temperature	250^{O}C			
Block heater temperature	400^{O}C			
Ionization Mode	ESI (Positive mode)			

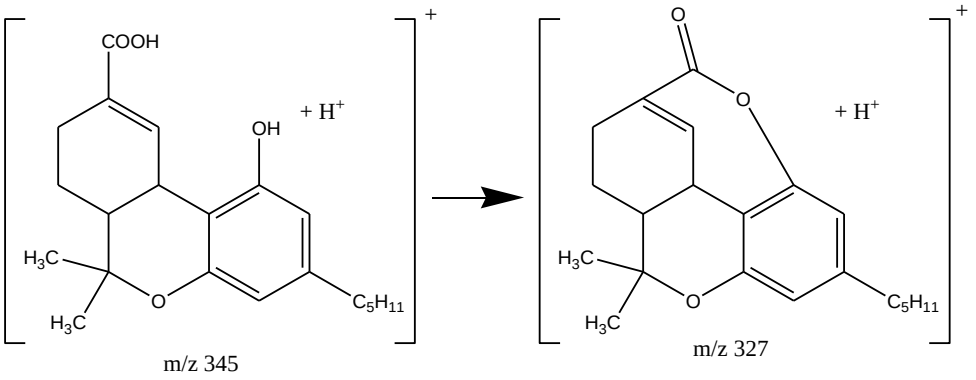
Selected Reaction Monitoring transitions (SRM)

S.N0	Compound	SRM Transitions (m/z)		Collision energy (CE) (V)	Retention time (R_t)
		Quantifier	Qualifier		

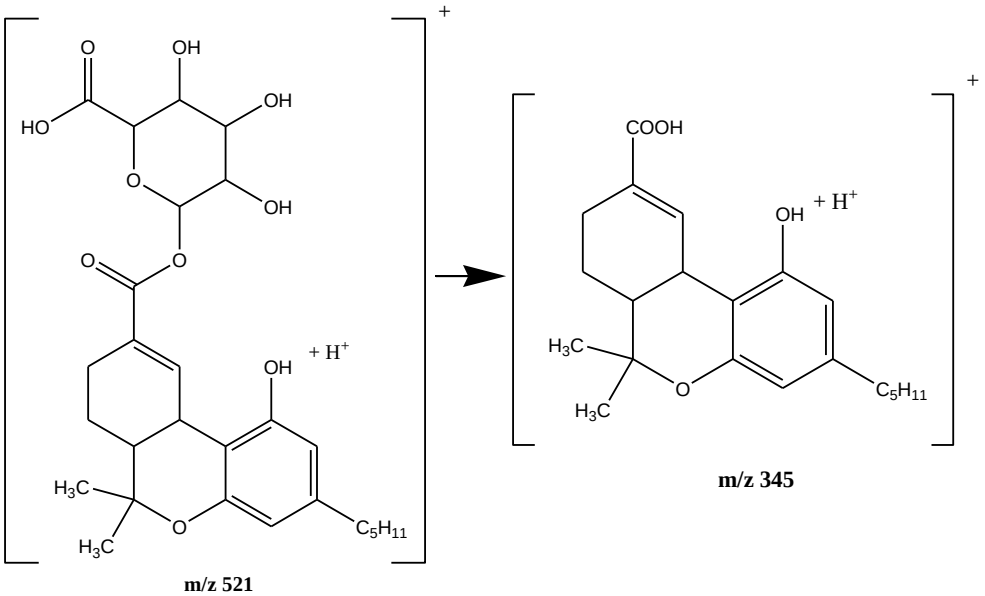
1	THC-OH	331>313	193	-16	3.82
2	THC-COOH	345>327	299	-16	3.56
3	THC-COO-glucuronide	521>345	327	-16	2.87
4	THC-OH-D3	334>316	-	-16	3.82



1.1 (THC-OH)



1.2 (THC-COOH)



1.3 (THC-COO-glucuronide)

Figure 1: Showing the Selective Reaction Monitoring (SRM) pathways for analytes for Quantifier ion.

FILLING THE CONTAINERS USED FOR THE STABILITY STUDY

Fill the different types of containers (Table II) approximately 20 mL with the above cannabis metabolites solution and stored at 4 ºC and -20 ºC respectively to perform the stability study by using LC-MS/MS.

Table 2: Showing the composition and description of different containers used for study.

S.NO	Containers description	Component of containers	Manufacturer
1	Simport Urine Container, 60mL, Tamper Evident, Cyan, Sterile (Container1) (Cat-no: C566-60CYS)	Sterile, High-Clarity Polypropylene	Simport Scientific, Canada
2	Simport Eco-Friendly SpecTainer™, 90mL, Tamper Evident, Yellow, Non-Sterile (Container 2) (Cat-no: C566- 90DOECO)	Non-sterile, Polypropylene-Biodegradable	Simport, Scientific, Canada
3	SimportSecurTainer III Specimen Container, 60mL, (Container 3) (Cat-no: C577-60W)	High-Clarity Polypropylene	Simport, Scientific, Canada
4	MACHEREY-NAGEL Vials N24 screw, 60 mL, 27.5 x 140.0 mm, clear, flat bottom, 1st hydrol. Class (Container 4) (Cat-no: 702074)	Clear glass, 1st hydrol class	Machery-Nagel, USA
5	MACHEREY-NAGEL Vials N24 screw, 60 mL, 27.5 x 140.0 mm, amber, flat bottom, 1st hydrol. Class (Container 5) (Cat-no: 702131)	Amber glass, 1st hydrol class	Machery-Nagel, USA
6	Clear Glass Vial with Solid Closed Top Septa Closure, 20mL, 24-414mm (Container 6) (Cat-no: C39- 20C/CT-S)	Clear glass, Silanized	EP Scientific Products, USA
7	Amber Glass Vial with Solid Closed Top Septa Closure, 20mL, 24-41.4mm (Container 7) (Cat-no: C39- 20A/CT-S)	Amber glass, Silanized	EP Scientific Products, USA

CALIBRANTS, QUALITY CONTROL AND INTERNAL STANDARD PREPARATIONS

Internal standard preparation

Spike suitable amount of THC-OH-D3 (0.1 mg mL^{-1}) to prepare the 50 ng mL^{-1} solution in 10 mM Ammonium formate in water: Methanol (70:30).

Calibrants preparation

Spike suitable amount of THC-OH (1 mg mL^{-1}), THC-COOH (1 mg mL^{-1}) and THC-COO-glucuronide (1 mg mL^{-1}) into the blank urine collected earlier for stability study to prepare the six calibration levels 20 ng mL^{-1}, 100 ng mL^{-1}, 1000 ng mL^{-1}, 2000 ng mL^{-1}, 4000 ng mL^{-1} and 5000 ng mL^{-1}.

Quality control (QC) preparation

Three quality control levels 80 ng mL^{-1} (LOQ-QC), 2500 ng mL^{-1} (Mid-QC) and 4500 ng mL^{-1} (High-QC) by spiking suitable amount of THC-OH (1 mg mL^{-1}), THC-COOH (1 mg mL^{-1}) and THC-COO-glucuronide (1 mg mL^{-1}) into the blank urine collected earlier for stability study.

METHOD VALIDATION

Limit of detection (LOQ), Limit of quantitation (LOQ) and Linearity

For the LOD (3.3 x S/N) and LOQ (10 x S/N) determination, different concentrations of analytes (1, 2, 3, 5, 10, 20, 30, 50, 100 ng mL^{-1}) were spiked in the blank urine. Whereas for linearity study varying concentration level like 20, 50, 100, 1000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10000 ng mL^{-1} were prepared in the blank urine.

Precision and Accuracy study

Earlier prepared (Section 2.6.3) QCs were used to evaluate the precision and accuracy study. For intraday precision five replicates (n=5) of each level of QCs were injected and for intraday precision the same study has been carried out for 3 consecutive days. For accuracy study these QCs concentration were evaluated to check the %recovery of the analytes.

Carry over

For carry over study the blank urine sample spiked with the internal standard was injected (n=3) after each calibration levels. Any detectable concentration in blank urine more than 20% is considered as carry over[10].

Ionization Suppression/matrix effect

To study the matrix effect processed blank urine and neat solvent (mobile phase, 10 mM Ammonium formate in water: Methanol (70:30)) were fortified with 20 ng mL^{-1} and 5000 ng mL^{-1} of each analytes (n=5).

SAMPLE PREPARATION FOR LCMSMS

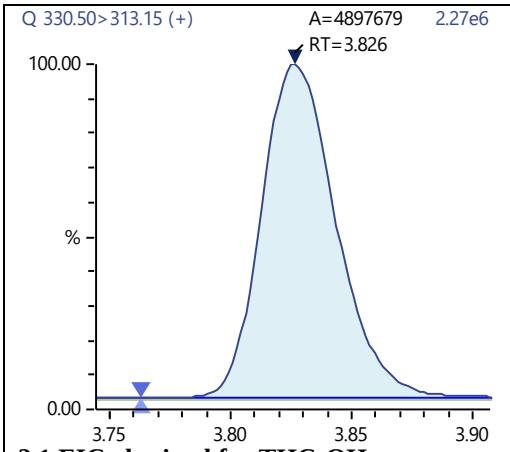
Take out the stored samples from the refrigerator and deep freezer at least 1 hour before the analysis. Thawed the frozen urine sample by sonication. Modified sample preparation method was used for the analysis as reported earlier[10]. Transfer 50 µL of urine from containers into the 1 mL eppendorf centrifuge tubes, then add 450 µL of internal standard (50 ng mL^{-1}) prepared earlier. Vortex it and centrifuge at 13000 RPM and transfer the top solution to the LC-MS/MS auto sampler vial with 250 µL insert. Sampling has to be done from the above urine filled containers at different interval of time up to 3 months. And the concentration (ng mL^{-1}) of cannabis metabolites has been measured by LC-MS/MS. Freshly prepare all the calibrants and QCs in the same way as above for stability study samples. All the samples, calibrants and QCs were prepared in triplicate for the LCMSMS analysis.

RESULTS AND DISCUSSION

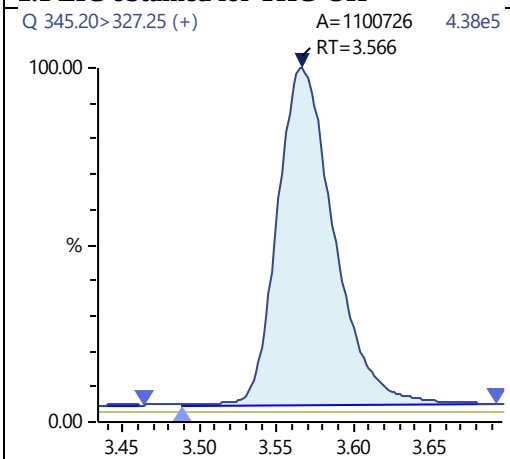
Method Validation

The Chromatograms obtained for the quantifier ion in sample also shown in figure-2.The LOD and LOQ value for the analytes were 5 ng mL^{-1} and 20 ng mL^{-1} and the method was linear in the concentration range of 20 ng mL^{-1} to 5000 ng mL^{-1} for all three analytes. All the precision and accuracy data was in the acceptable range and displayed in the table-III. And also the R^2 value for all the analytes were >0.99 (figure 3)

Carry over for the analytes in developed methods was less than 5% of the lowest calibrator level (20 ng mL^{-1}) and also the ion suppression was negligible.



2.1 EIC obtained for THC-OH



2.2 EIC obtained for THC-COOH

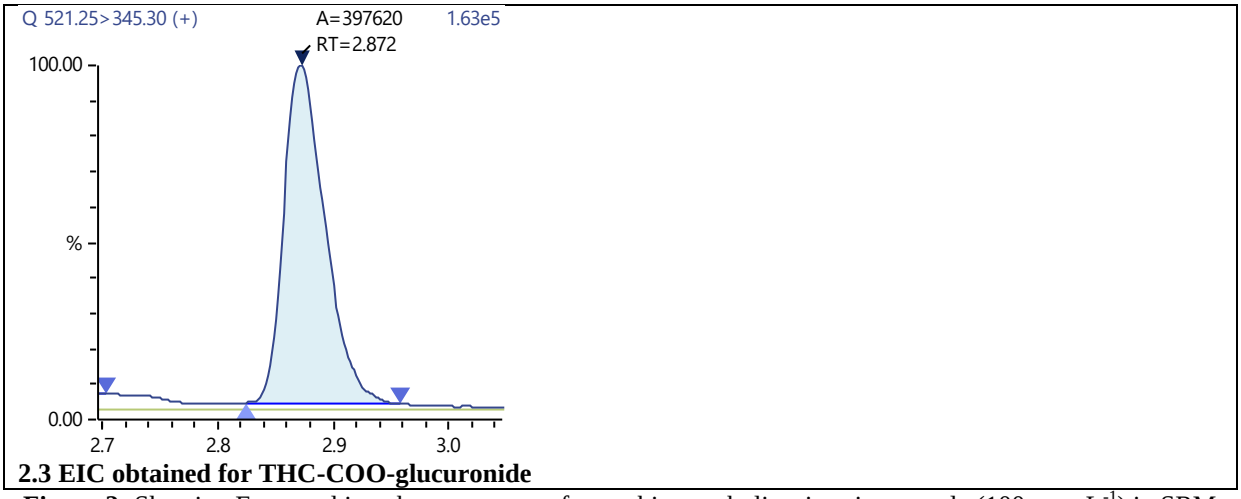


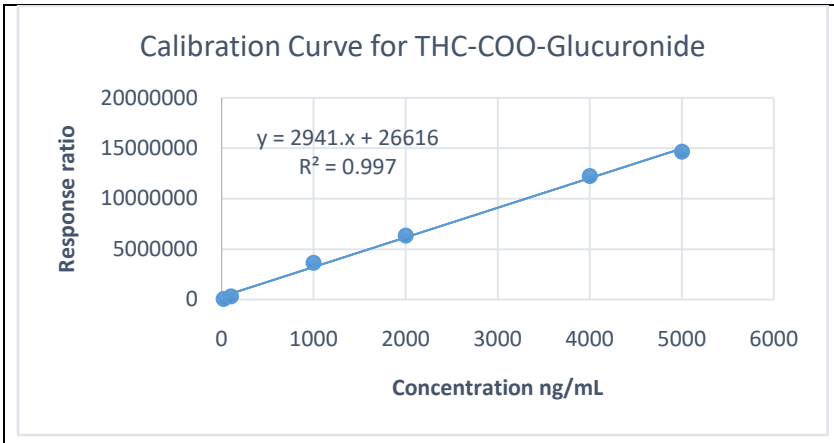
Figure 2: Showing Extracted ion chromatogram of cannabis metabolites in urine sample (100 ng mL^{-1}) in SRM mode (Quantifier ion).

Table 3: Shows the accuracy, precision data for interday and intraday study for the developed method.

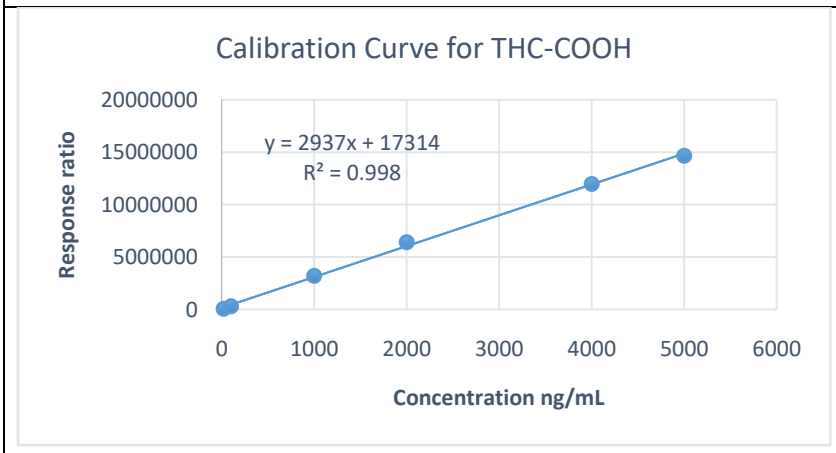
Analyte	Experiment	Low QC (80 ng/mL)			Mid QC (2500 ng/mL)			High QC (4500 ng/mL)		
		Amount detected	Accuracy (%)	RSD (%)	Amount detected	Accuracy (%)	RSD (%)	Amount detected	Accuracy (%)	RSD (%)
THC-OH	Day 1 (n=5)	78.22	97.77	3.27	2350.6	94.02	7.86	4400	97.78	5.24
	Day 2 (n=5)	80.86	101.07	3.98	2562.4	102.49	6.68	4515.6	100.34	8.05
	Day 3 (n=5)	79.32	99.15	3.78	2449.8	97.99	9.61	4685.8	104.12	3.67
	Interday (n=3)	79.46	99.33	1.66	2454.26	98.17	4.31	4533.86	100.75	3.16
THC-COOH	Day 1 (n=5)	81.13	101.41	7.3	2543.6	101.74	5.87	4260.8	94.68	4.22
	Day 2 (n=5)	80.54	100.68	6.1	2156	86.24	7.37	4708.8	104.64	10.8
	Day 3 (n=5)	81.70	102.13	2.92	2562.6	102.5	11	4447	98.82	7
	Interday (n=3)	81.12	101.4	0.71	2420.7	96.82	9.47	4472.2	99.38	5
THC-COOH-Glucuronide	Day 1 (n=5)	81.762	102.2	6.9	2800	112	12.5	4685.8	101.12	6.5
	Day 2 (n=5)	86.58	108.21	4.8	2635	105.4	8.2	4869.4	108.2	5.1
	Day 3 (n=5)	82.86	103.6	8.1	2521.4	100.9	9.9	4972.4	110.5	3.5
	Interday (n=3)	83.73	104.7	3	2652.1	106.1	5.3	4842.5	107.6	2.99

Stability Study

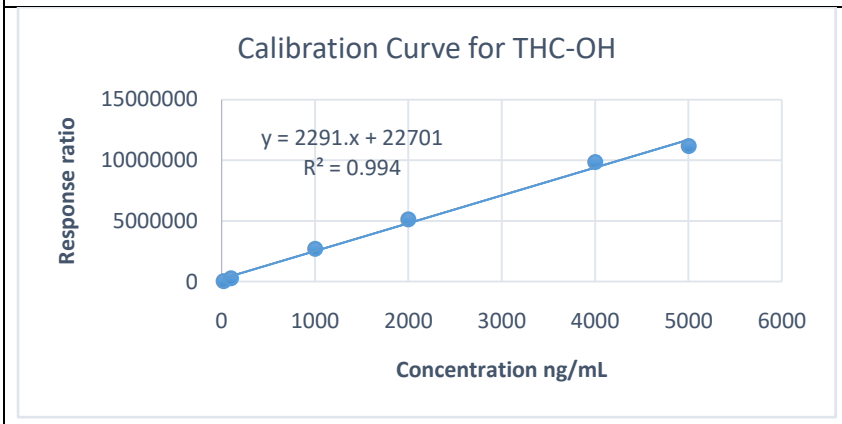
Decrease in the metabolites concentration is observed during long term storage as these hydrophobic cannabinoids will interact (surface adsorption) with the containers or sample handling devices[11-14]. Drug stability can also be affected by other parameters like types of preservative used specially for blood sample, microbial contamination during specimen collection, physiochemical properties of molecules, specimen or matrix type, pH of the sample, tendency of the molecule to conjugate/deconjugate or intermolecular interaction. Metabolic degradation orchemical transformation can also lead to the variation in analytes instability[15]. Decrease in concentration up to 89% observed due to strong sample mixing and due to glass surface adsorption 27% reduction in concentration observed. In the refrigerated samples up to 8%declined in concentration observed while room temperature storage caused up to 22% declined



3.1 Showing the Calibration curve obtained for THC-COO-Glucuronide.



3.2 Showing the Calibration curve obtained for THC-COOH



3.3 Showing the Calibration curve obtained for THC-OH

Figure 3: Showing the calibration curves obtained for analytes.

in observed concentration. Even within an hour of collection declined in the concentration of THC-COOH (THCA) observed due to adsorptive loss to storage container. And also THCA was greatly loss when the urine pH is acidic in nature[16]. Hara et. Al.[17] observed rapid loss for THC-COOH concentration for samples stored at 4˚C with polypropylene containers shows 14% and polyethylene containers shows 17% loss[18]. The results specify that polypropylene and polyethylene have low affinity binding THC-COOH which may compromise the integrity of specimens. It also indicates that the low affinity of THC-COOH binding may be due to decreased solubility of THC-COOH at lower temperatures as well as interaction with lipophilic plastic. Researchers[17,18,19-21] founds that there is a loss around 8% for frozen samples that stored for 4 weeks where as 22.4% in THC-COOH concentration in urine sample that stored at room temperature for 10 days. This indicate a significant loss of THC-COOH concentration in short duration at high temperature. Thomas et. al. stated a decreased

concentration for THC-COOH from solutions stored in borosilicate glass comparatively with silanized glass type containers[19, 20, 22-24].

We carried out a set of stability experiments by determining the content of three cannabinoids metabolites (THC-OH, THC- COOH and THC-COOH glucuronide) in urine during different storage conditions and containers. As per the UAE federal law, the cannabis cut/off value in urine is set on 50 ng mL^{-1} assessed by immunoassay. Due to cannabinoids adsorption[21] to the surface of urine storage containers, their concentration varies in reanalysis made after a certain duration of time. By demonstrating potential analytes loss at different determination times, this study will help to find proper storage containers and conditions.

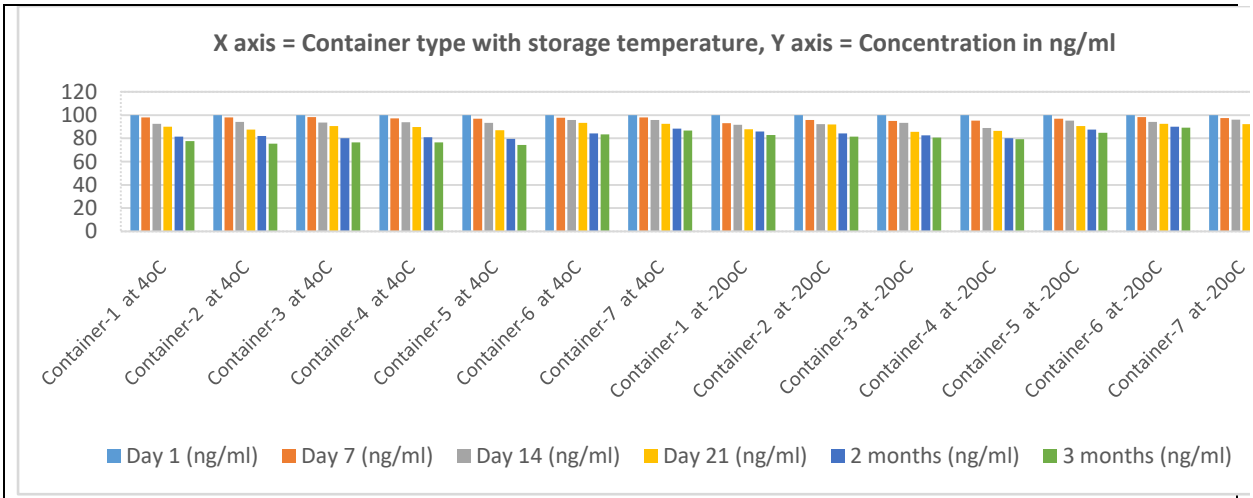
THC-OH (11-hydroxy-THC)

Table-IV shows the concentration loss of THC-OH, THC-COOH, and THC-COOH Glucuronide in urine during different temperature storage conditions (4 ºC and -20 ºC) in seven different types of containers during three months period (Figure 4). At 4 ºC and -20 ºC container-7 (Amber glass, Silanized) presented the minor concentration loss of THC-OH with approximately 3% compared

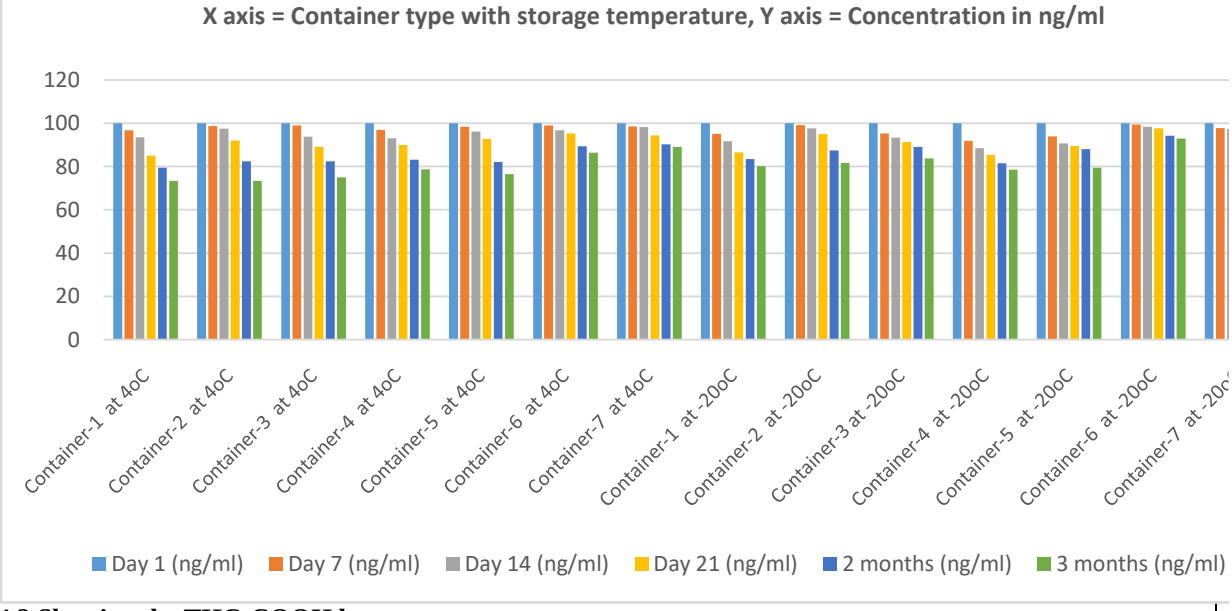
Table 4: Shows the concentration loss of THC-OH, THC-COOH and THC-COOH-Glucuronide in urine at different storage conditions.

Container information	Compound Name	Day 1 (ng/ml)	Day 7 (ng/ml)	Day 14 (ng/ml)	Day 21 (ng/ml)	2 Months (ng/ml)	3 Months (ng/ml)
Container-1 at 4°C	THC-OH	100	95.88	91.49	88.5	80.41	78.63
	THC-COOH	100	96.81	93.54	85.1	79.48	73.42
	THC-COO-	100	97.92	92.43	90.02	81.44	77.66
Container-2 at 4°C	THC-OH	100	97.98	94.1	89.75	80.66	79.63
	THC-COOH	100	98.76	97.55	92.01	82.41	73.42
	THC-COO-	100	98.05	94.16	87.51	82.09	75.34
Container-3 at 4°C	THC-OH	100	97.78	95.6	92.52	81.45	77.32
	THC-COOH	100	98.92	93.76	89.13	82.41	74.98
	THC-COO-	100	98.21	93.5	90.62	80.14	76.64
Container-4 at 4°C	THC-OH	100	96.91	94.52	92.06	86.42	81.42
	THC-COOH	100	96.88	93.14	89.9	83.1	78.69
	THC-COO-	100	97.21	93.9	89.86	80.82	76.42
Container-5 at 4°C	THC-OH	100	97.95	94.93	90.18	83.42	81.42
	THC-COOH	100	98.36	96.14	92.72	82.11	76.45
	THC-COO-	100	96.8	93.26	86.97	79.45	74.21
Container-6 at 4°C	THC-OH	100	98.26	96.25	93.35	86.94	84.75
	THC-COOH	100	99.03	96.76	95.29	89.34	86.41
	THC-COO-	100	97.8	95.67	93.37	84.32	83.42
Container-7 at 4°C	THC-OH	100	99.33	97	96.62	91.45	88.97
	THC-COOH	100	98.49	98.25	94.34	90.23	89.01
	THC-COO-	100	98.03	95.76	92.44	88.45	86.74
Container-1 at -20°C	THC-OH	100	96.34	95.63	87.58	84.63	82.42
	THC-COOH	100	95.16	91.7	86.61	83.45	80.14
	THC-COO-	100	93.16	91.75	87.71	85.74	82.74
Container-2 at -20°C	THC-OH	100	97.01	91.9	87.63	84.45	78.46
	THC-COOH	100	99.19	97.67	94.95	87.45	81.74
	THC-COO-	100	95.7	92.32	91.94	84.16	81.36
Container-3 at -20°C	THC-OH	100	97.16	92.45	88.11	84.65	81.47
	THC-COOH	100	95.28	93.36	91.31	89.04	83.74
	THC-COO-	100	94.83	93.25	85.47	82.47	80.63
Container-4 at -20°C	THC-OH	100	96.15	87.25	82.79	80.11	79.42
	THC-COOH	100	91.9	88.44	85.32	81.48	78.63
	THC-COO-	100	95.34	88.81	86.43	80.04	79.24

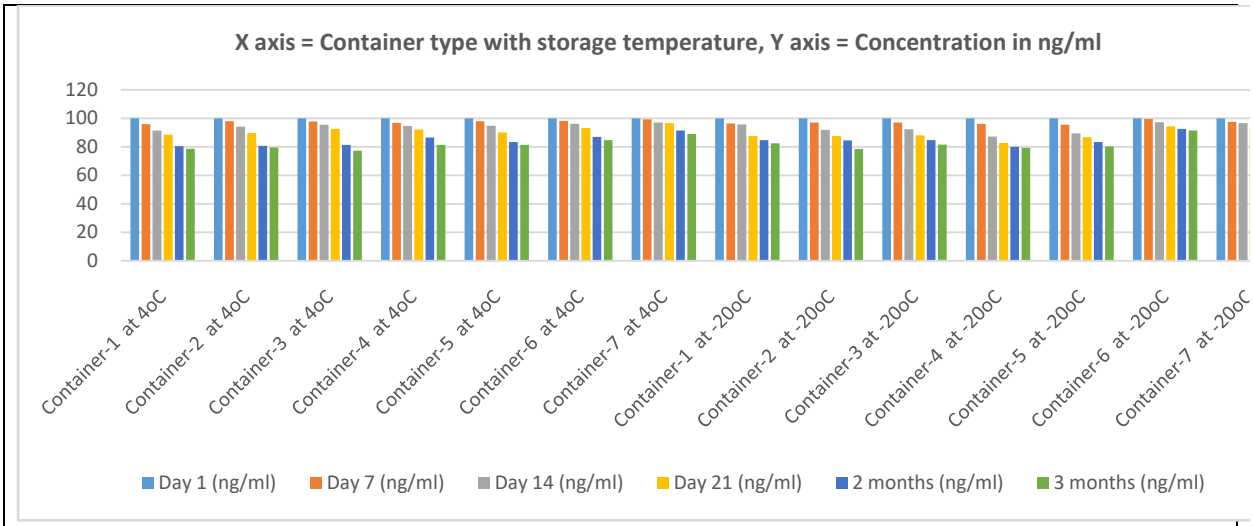
Container-5 at -20°C	THC-OH	100	95.51	89.51	86.82	83.47	80.14
	THC-COOH	100	93.98	90.64	89.49	88.05	79.45
	THC-COO-	100	96.81	95.23	90.65	87.6	84.66
Container-6 at -20°C	THC-OH	100	99.66	97.21	94.46	92.64	91.4
	THC-COOH	100	99.39	98.33	97.62	94.3	92.9
	THC-COO-	100	98.2	94.25	92.6	90.05	89.15
Container-7 at -20°C	THC-OH	100	97.45	96.72	94.56	93.9	90.1
	THC-COOH	100	97.86	97.43	95.89	95	93.4
	THC-COO-	100	97.47	96.06	92.18	91.2	90.06



4.1 Showing the THC-COO-Glucuronide loss



4.2 Showing the THC-COOH loss



4.3 Showing the THC-COOH loss

Figure 4: Chart Showing analytes concentration loss during storage at different temperature and containers.

to the initial concentration (Day 1= 100 ng mL-1). In other words, the adsorption to this container surface is less with these storage temperatures, compared to different types of containers. In contrast, container-2 (Polypropylene-Biodegradable-Non-sterile) displayed a higher THC-OH concentration loss at 4 ºC (around 8%) than other container types. On the other hand, container-4 (Clear glass, 1st hydrol class) showed more THC-OH loss at – 20 ºC, roughly 14% at day 21 compared to the initial concentration (Day 1=100 ng mL^{-1}). There could be high adsorption to the container's surface which leads to the loss of compounds of interest. As previously observed, adsorption of free THC to plastic tubes during storage might yield false-negative results. Nevertheless, THC-glucuronide remained stable. Previous studies indicate that false-negative results occur when samples are stored in polypropylene containers at room temperature for less than 16 h and ii) at 4 ºC for less than 72 h[5].

THC-COOH (11-nor-9-carboxy-THC)

THC-COOH shows a concentration loss in urine specimens during different storage conditions (4 ºC and -20 ºC) and seven container types during three months period. Both containers-6 (Clear glass, Silanized) and container-7 (Amber glass, Silanized) displayed the same percentage loss (about 4% and 2 % at 4ºC and -20ºC respectively), which may be the minimum loss as compared to other types. Moreover, container-1 (Polypropylene-Sterile) showed the maximum loss of THC-COOH concertation at both at 4 ºC and -20 ºC (approximate 11% and 9% respectively) when compared to initial concentration (Day 1=100 ng mL^{-1}).

THC- COOH- Glucuronide

THC-COOH-glucuronide shows a concentration loss in urine specimens during different storage conditions (4 ºC and -20 ºC) in seven container types during three months period. Both containers-6 (Clear glass, Silanized) and container-7 (Amber glass, Silanized) showed the least concentration loss (about 4% and 6 % at 4ºC and -20ºC respectively) compared to the initial concentration (Day 1= 100 ng mL^{-1}). However, container-2 (Polypropylene-Biodegradable-Non-sterile) displayed the concentration loss of at 4ºC (11%) compared to the initial concentration (Day 1= 100 ng mL^{-1}). Nevertheless, container-2 showed the minimum amount of loss (around 4%) when stored at -20 ºC compared to the initial concentration (100 ng mL^{-1}). In addition, container-3 (Polypropylene) and container-4 (Clear glass, 1st hydrol class) showed the higher percentage of loss (9%) when stored at – 20 ºC compared to initial concentration (Day 1= 100 ng mL^{-1}). Only two studies appear to evaluate the stability of THCCOOH-glucuronide in authentic urine specimens. Authors indicated more than 25 % of THC-COOH-glucuronide loss when sample is stored at room temperature for five days. On the other hand, the loss was minimal when samples were stored for five days at 4ºC.Moreover, THC-COOH and THC-COOH-glucuronide results did not change and metabolites remained stable when samples were stored at 4ºC for seven days as well as for 120 days at -20ºC[16]. A minimal loss in the concentration of total THC, cannabidiol and cannabinol was observed when samples were stored at -70 ºC for five months in silanized glass vials, indicating that adsorption of THC is limited in glass tubes[6].

CONCLUSION

As compared to initial concentration (Day 1=100 ng mL^{-1}), container-7 (Amber glass, Silanized) has the lower concentration loss (both at 4 ºC and -20 ºC) for both metabolites THC-OH and THC-COOH showing around 4% to 7% loss during three months period. Moreover, a minimum loss was observed at 4ºC for THC-COOH

Glucuronide. Based on these findings we may recommend storing the samples either at 4ºC or -20ºC as minimum loss has been displayed for some containers, such as container-7 (Amber glass, Silanized) and container-6 (Clear glass, Silanized). The drawback of using glass containers is that during storage at -20º C there is a possibility of cracking of the glass containers. Our findings indicate that both storage temperatures, whether at 4ºC or -20ºC, are fine as both displayed the minimum loss for the compounds of interest. The container-2 (Polypropylene-Biodegradable-Non-sterile) displayed the higher THC-OH, THC-COOH glucuronide concentration loss at 4 ºC, while container-1 (Polypropylene-Sterile) showed the maximum THC-COOH concentration loss at both 4 ºC and -20 ºC with 11% and 9% respectively. Due to the importance of specimen transportation and storage conditions, we believe this work may provide essential conditions to set suitable container, storage and time condition in order to get reliable results.

FINANCIAL SUPPORT
This research did not receive any financial support from any public and private agencies.

CONFLICTS OF INTEREST
No conflicts of interest.

REFERENCES

1. Nahar, L.; Onder, A.; Sarker, S. D. A Review on the Recent Advances in HPLC, UHPLC and UPLC Analyses of Naturally Occurring Cannabinoids (2010-2019). Phytochem. Anal. PCA**2020**, 31 (4), 413–457. https://doi.org/10.1002/pca.2906.
2. Kim, J.; Choi, K.; Chung, D. S. Sample Preparation for Capillary Electrophoretic Applications; Elsevier, 2012; pp 701–721. https://doi.org/10.1016/B978-0-12-381373-2.00110-1.
3. F, M.; B, M. Review of Biologic Matrices (Urine, Blood, Hair) as Indicators of Recent or Ongoing Cannabis Use. Ther. Drug Monit.**2006**, 28 (2). https://doi.org/10.1097/01.ftd.0000197091.07807.22.
4. Pitt, J. J. Principles and Applications of Liquid Chromatography-Mass Spectrometry in Clinical Biochemistry. Clin. Biochem. Rev.**2009**, 30 (1), 19.
5. Disposition of Toxic Drugs and Chemicals in Man, Seventh Edition. Randall C. Baselt. Foster City, CA: Biomedical Publications, 2004, 1250 pp., $139.50, hardcover. ISBN 09626523-6-9. | Clinical Chemistry | Oxford Academic. https://academic.oup.com/clinchem/article/51/3/680/5629777 (accessed 2024-10-17).
6. Scheidweiler, K. B.; Desrosiers, N. A.; Huestis, M. A. Simultaneous Quantification of Free and Glucuronidated Cannabinoids in Human Urine by Liquid Chromatography Tandem Mass Spectrometry. Clin. Chim. Acta Int. J. Clin. Chem.**2012**, 413 (23–24), 1839–1847. https://doi.org/10.1016/j.cca.2012.06.034.
7. Huestis, M. A. Pharmacokinetics and Metabolism of the Plant Cannabinoids, Delta9-Tetrahydrocannabinol, Cannabidiol and Cannabinol. Handb. Exp. Pharmacol.**2005**, No. 168, 657–690. https://doi.org/10.1007/3-540-26573-2_23.
8. Screening analysis for drugs of abuse by LC-MS/MS enables fast polarity switching MRM triggered product ion scanning on the fly. LabRulez LCMS. https://lcms.labrulez.com/paper/10073 (accessed 2024-10-17).
9. Marijuana and the Cannabinoids | SpringerLink. https://link.springer.com/book/10.1007/978-1-59259-947-9 (accessed 2024-10-17).
10. Young, B. L.; Victoria Zhang, Y. A Rapid Dilute-and-Shoot LC-MS/MS Method for Quantifying THC-COOH and THC-COO(Gluc) in Urine. J. Chromatogr. B Analyt. Technol. Biomed. Life. Sci.**2022**, 1211, 123495. https://doi.org/10.1016/j.jchromb.2022.123495.
11. Kneisel, S.; Speck, M.; Moosmann, B.; Auwärter, V. Stability of 11 Prevalent Synthetic Cannabinoids in Authentic Neat Oral Fluid Samples: Glass versus Polypropylene Containers at Different Temperatures. Drug Test. Anal.**2013**, 5 (7), 602–606. https://doi.org/10.1002/dta.1497.
12. Fort, C.; Jourdan, T.; Jesse Kemp, null; Curtis, B. Stability of Synthetic Cannabinoids in Biological Specimens: Analysis Through Liquid Chromatography Tandem Mass Spectrometry. J. Anal. Toxicol.**2017**, 41 (5), 360–366. https://doi.org/10.1093/jat/bkx015.
13. Toennes, S. W.; Kauert, G. F. Importance of Vacutainer Selection in Forensic Toxicological Analysis of Drugs of Abuse. J. Anal. Toxicol.**2001**, 25 (5), 339–343. https://doi.org/10.1093/jat/25.5.339.
14. The role of time and storage conditions on the composition of hashish and marijuana samples: A four-year study - PubMed. https://pubmed.ncbi.nlm.nih.gov/30901710/ (accessed 2024-10-17).
15. Biswas, P.; Mishra, P.; Bose, D.; Durgbanshi, A. Cannabis: A Neurological Remedy or a Drug of Abuse in India. CNS Neurol. Disord. Drug Targets**2017**, 16 (5), 576–584. https://doi.org/10.2174/1871527316666170424115008.

16. Stout, P. R.; Horn, C. K.; Lesser, D. R. Loss of THCCOOH from Urine Specimens Stored in Polypropylene and Polyethylene Containers at Different Temperatures. J. Anal. Toxicol.**2000**, 24 (7), 567–571. https://doi.org/10.1093/jat/24.7.567.
17. Hara, M.; Nakanishi, H.; Yoneyama, K.; Saito, K.; Takada, A. Effects of Storage Conditions on Forensic Examinations of Blood Samples and Bloodstains Stored for 20 Years. Leg. Med. Tokyo Jpn.**2016**, 18, 81–84. https://doi.org/10.1016/j.legalmed.2016.01.003.
18. Drug of abuse. A DEA resource guide: Drug Enforcement Administration; U.S Department of Justice, **2020** Edition, 88-93 pages.
19. Pawula, M.; Hawthorne, G.; Smith, G. T.; Hill, H. M. Best Practice in Biological Sample Collection, Processing, and Storage for LC-MS in Bioanalysis of Drugs. In Handbook of LC-MS Bioanalysis; John Wiley & Sons, Ltd, 2013; pp 139–164. https://doi.org/10.1002/9781118671276.ch13.
20. Desrosiers, N. A.; Lee, D.; Scheidweiler, K. B.; Concheiro-Guisan, M.; Gorelick, D. A.; Huestis, M. A. In Vitro Stability of Free and Glucuronidated Cannabinoids in Urine Following Controlled Smoked Cannabis. Anal. Bioanal. Chem.**2014**, 406 (3), 785–792. https://doi.org/10.1007/s00216-013-7524-7.
21. Surface adsorption of cannabinoids in LC–MS/MS applications | Forensic Toxicology. https://link.springer.com/article/10.1007/s11419-019-00505-x (accessed 2024-10-17).
22. Nadulski, T.; Sporkert, F.; Schnelle, M.; Stadelmann, A. M.; Roser, P.; Schefter, T.; Pragst, F. Simultaneous and Sensitive Analysis of THC, 11-OH-THC, THC-COOH, CBD, and CBN by GC-MS in Plasma after Oral Application of Small Doses of THC and Cannabis Extract. J. Anal. Toxicol.**2005**, 29 (8), 782–789. https://doi.org/10.1093/jat/29.8.782.
23. Maralikova, B.; Weinmann, W. Simultaneous Determination of Delta9-Tetrahydrocannabinol, 11-Hydroxy-Delta9-Tetrahydrocannabinol and 11-nor-9-Carboxy- Delta9-Tetrahydrocannabinol in Human Plasma by High-Performance Liquid Chromatography/Tandem Mass Spectrometry. J. Mass Spectrom. JMS**2004**, 39 (5), 526–531. https://doi.org/10.1002/jms.616.
24. Li, W.; Zhang, J.; Tse, F. L. S. Strategies in Quantitative LC-MS/MS Analysis of Unstable Small Molecules in Biological Matrices. Biomed. Chromatogr. BMC**2011**, 25 (1–2), 258–277. https://doi.org/10.1002/bmc.1572.