Can adulteration of urine samples mask cannabis detection by GC-MS?

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Abstract

Background: Together with coffee and tobacco, Cannabis is the most commonly used psychoactive drug worldwide, and it is the single most popular illegal drug. Recent studies have demonstrated increase in the prevalence of the use of cannabis. A limitation inherent in all urine drug testing is the possibility of sample adulteration or substitution.

Aim of study: To detect qualitative and quantitative effects of five adulterants on positive urine samples for tetrahydrocannabinol carboxylic acid (THC-COOH).

Material and Method(s): This analytical study was conducted in Clinical Toxicology Laboratory in Sohag University Hospitals. Urine samples positive to cannabis adulterated with vinegar, drano liquid hand soap, visine eye drops and bleach were tested by immunoassay (RIA) then confirmed and quantified by GC-MS.

Results: Urine samples adulterated with vinegar, drano liquid hand soap, visine and bleach generated false negative results by immunoassay testing. GC-MS confirmation showed that addition of vinegar, bleach, visine, drano and liquid hand soap decrease THC-COOH significantly with increased concentration.

Conclusion: Some adulterants make it easy to produce false negative results on RIA testing for cannabis and GC-MS is important to overcome adulteration methods in urine analysis.

Key words Drug abuse, cannabis detection, Urine adulteration, GC-MS confirmation

Introduction

S ubstance abuse in Egypt is a serious public health threat. Recent studies have demonstrated increase in the prevalence of the use of cannabis and tramadol (*Saleh*, 2015).

Together with coffee and tobacco, Cannabis is the most commonly used psychoactive drug worldwide, and it is the single most popular illegal drug. Worldwide over 160 million people are using Cannabis regularly and these numbers are still rising (*Grotenhermen and Russo, 2002*)

 Δ 9-tetrahydrocannabinol (Δ 9-THC) and cannabidiol (CBD) are the most important constituents of the plant cannabinoids. Δ 9-THC is thought to be the principal intoxicant constituent of cannabis. (*Radwan et al., 2008*).

Cannabis can impair driving skills and people driving under the influence of cannabis are 20–30% more likely to be involved in a car accident (*Hall and Degenhardt*, 2015).

The global burden of disease attributable to cannabis use disorder, expressed in disability-adjusted life years (DALYs) as 646 480 DALYs in 2016. One DALY represents one year of life lost either due to premature mortality or due to living with disability. Driving under the influence of cannabis increases the risk of accidents and is therefore a public health threat (*Kassebaum et al., 2016*).

According to *Hamdi et al.*, (2016) Cannabis was the commonest substances of abuse in Egypt, in total, 77% of the substance users were using Cannabis.

According to study conducted in Zagazig city on a sample of 130 commercial drivers, 57.7% among the participants tested positive for substance abuse and the most common abused substance was cannabis which represented 80% of the positive participants (*Hammam et al., 2018*).

A limitation inherent in all urine drug testing is the possibility of sample adulteration or substitution (*Jaffee et al.*, 2007).

The use of adulterant agents can cause false negative results in drug tests by either change PH or decrease concentration by dilution effect which leads to interfering with the screening test procedure and/or destroying the drugs present in the urine sample (*Yee et al., 2014*).

Aim of the Work

To detect qualitative and quantitative effects of five adulterants on positive urine samples for cannabis.

Materials and Methods

This analytical study was conducted in Sohag Clinical Toxicology Laboratory from January 2020 to October 2020.

Acceptable samples (inclusion criteria): the samples were from 10-100 ml urine in volume, voided in a clean dry labeled container without preservative. The samples were tested by immunoassay for tetrahydrocannabinol carboxylic acid (THC-COOH), positive samples only were included.

Five Types of adulterants were used.

- Vinegar, bleach, visine eye drops and liquid drano at 3 concentrations 10%, 20% and 40%.
- Liquid hand soap at 3 conc. 5%, 10% and 20%.

These adulterants levels were selected to obtain an accurate representation of real-world samples adulteration as it would easy to be brought in a small container and added to the urine samples.

Number of samples included:

There were 45 adulterated urine samples, one blank urine sample and one sample diluted with water 40% to exclude dilution effect (total 47 samples).

To prepare adulterated samples, 1 mL aliquots were obtained from the urine samples for each drug (adulterant or agent). 1 ml of the unadulterated urine sample was used to determine the initial concentration of the drug by GC-MS. The total volume of the adulterated samples was maintained at 1ml. The amounts of liquid adulterants were added to the urine sample to reach the 1ml limit. The 10 % v/v sample had 900 µL of urine and 100 µL of adulterant. This process was followed for the remaining concentrations: 20 % v/v (800:200) 40 % v/v (600:400) and 5% v/v (950:50).

Screening of THC-COOH in urine samples by immunoassay:

Apparatus: Radio immunoassay apparatus using drug analyzer: (CDx90), Thermo-fisher Scientific co. supplier AMG Company. Fully automated random access analyzer, dedicated drug testing system (photometric). Its serial number 7218-0150 present in Sohag Clinical Toxicology Lab.

i- Principle of procedure:

The DRI Cannabinoid assay is a homogenous enzyme immunoassay with liquid ready to use reagents. The assay uses a specific antibody which can detect most Cannabinoid and their metabolites as (THC-COOH) in urine. The assay is based on the competition of enzyme glucose -6- phosphate dehydrogenase (G6PDH) labeled drug and the free drug in the urine sample for the fixed amount of antibody binding sites. In the absence of the free drug in the sample, the enzyme labeled drug is bound by the specific antibody and enzyme activity is inhibited. This phenomenon creates a relationship between drug concentration in urine and enzyme activity. The enzyme (G6PDH) activity is determined at 340nm spectrophotometrically by its ability to convert of NAD to NADH (Rainey and Baird, 2012).

ii- Calibration: Figure (*A*)

- 1. For construction of the calibration curve (Linear Mode) we used the following calibrators:
 - DRI Negative Urine Calibrator.
 - DRI THC 50 ng/mL Calibrator.
- 2. For qualitative analysis we used the 50 ng/ml calibrator as a cutoff level to distinguish "positive" and "negative" specimens.

3. LQC: 40 ng/ml control negative results

HQC: 75 ng/ml control positive results.

Each conc. of each adulterant was added separately to urine sample and the samples were tested by immunoassay where cut off for THC detection was 50ng/ml above it considered positive and below it considered negative and finally the samples were confirmed by GC-MS.

<u>Confirmation and quantitatication of THC-COOH in</u> <u>urine samples by GC-MS:</u>

Apparatus: An Agilent GC-MS-5977A MSD. (USA) with an Agilent auto-sampler was used for specimen analysis. The GC was equipped with Agilent HP-5MS (5%-Phenyl-methylpolysiloxane) capillary column (30 m \times 250 μ m \times 0.25 μ m film thickness) present in faculty of science-Al Azhar University.

i- Calibrators and quality controls

i.1- Calibrators:

A stock solution of THC-COOH and delta 9-THC-COOH at concentration of 100 μ g/mL were prepared in methanol and kept stored at -20°C. Intermediate stock solution of THC-COOH at concentration of 10 μ g/mL was prepared by diluting (1:10) of THC-COOH stock standard 100 μ g/mL in methanol. Working calibrators (10, 25, 100, 500 and 1000 ng/mL) of THC-COOH were made by a serial dilution of the intermediate solution with drug free human urine.

i.2- Controls:

One negative urine control was tested with every batch. The negative control was prepared using certified blank urine.

Two positive urine controls were tested with every batch. The positive controls were prepared using certified blank urine in the same way as sample preparation.

ii- Extraction procedure

- 50 μL of delta 9-THC-COOH (IS) at concentration of 5μg/mL was added to 0.5 mL of urine samples in labeled tubes followed by adding 50 μL of 10N NaOH and shaked well by vortex for 10 sec.
- 2. The tubes incubated for a minimum of 20 minutes at 60°C.
- 3. After cooling, 50 μ l acetic acid and 4 mL of 9:1 v/v hexane, ethyl acetate was added to each tube.
- 4. The tubes were capped and placed on a rotary mixer for 30 minutes.
- 5. The tubes were centrifuged for 5 minutes at 3200 rpm to achieve separation.
- 6. The top layer was transferred (extraction solvent) to clean, labeled cap tubes containing 0.5 mL of 1N NaOH
- 7. The tubes capped and placed on a rotary mixer for 20 minutes.

- 8. Then the tubes were centrifuged for 5 minutes at 3200 rpm to achieve separation.
- 9. The top solvent layer removed using vacuum pump, to the aqueous layers, 0.5 ml of 4N HCl and 2 mL of 9:1 v/v hexane: ethyl acetate were added to each tube and shaked well by the vortex for 1 min.
- 10. The tubes then centrifuged for 5 minutes at 3200 rpm.
- 11. The organic layer transferred in to labeled glass tubes and evaporated to dryness
- iii- Derivatization

The residue samples were dissolved in 50 μ L of BSTFA (N,O-Bis (trimethylsilyl) trifluoroacetamide). Tubes were capped, mixed and incubated for 30 min at 70 °C in heater block. Samples were removed from heater block, allowed to cool at room temperature, and evaporated to dryness under nitrogen at 50 °C. Samples were reconstituted with 50 μ L ethyl acetate and transferred to GC vial insert prior to GC-EI /MS analysis.

iv- GC/MS condition

Helium was used as the carrier gas at a flow rate of 1 ml/min. The injection volume was 2.0 μ L and injections were made in splitless mode. The injector port and interface were maintained at 280 0 C, and the detector at 225 0 C. The column temperature was maintained at 150 0 C for 1 minute with a ramp of 20 0 C/min to 310 0 C and held for 3 min. The ionizing energy was 70 eV. The total run time was 12 min.

Electron Ionization (EI) mode was used and data were collected using single ion monitoring (SIM).

The principal ions at m/z 371.473, and 488 were used for THC-COO- $(TMS)_2$; and m/z 380.479, and 497 were used for delta 9-THC-COO- $(TMS)_2$, these ions were for quantification.

Data analysis was performed using the Agilent GC-MS software.

Results

Immunoassay screening for THC-COOH:

- 1. The parent sample concentration was 97 ng/ml. (as the method of detection considered semiquantitative not only screening).
- 2. Addition of vinegar at high concentration 40% was able to successfully masking positive response of THC-COOH in tested urine samples. While moderate concentration (20%) and low concentration (10%) cannot affect THC-COOH detection in urine samples as shown in table (1) and figure (1).
- 3. On other hand addition of bleach, drano whatever their concentration were able to mask THC-COOH detection by immunoassay. For visine it was effective for decreasing the response rate for THC-COOH using immunoassay method at high conc. 40% and 20% while 10% had no effect on THC-COOH result as shown in table (1) and figure (1).
- 4. Unforturely, addition of liquid hand soap by any concentration even low concentration up to 5% masked THC-COOH detection by immunoassay giving false negative results.as shown in table (2)

Detection and quantification of THC-COOH by GC-MS:

The parent positive sample concentration: 89ng/ml figure (5)

- Limit of detection (LOD): 0.875 ng/ml
- Limit of quantification (LOQ): 1.75 ng/ml
- 1. Effect of vinegar on THC-COOH detection & quantification by GC-MS

Addition of vinegar in concentration 40% leading to decrease in concentration of THC-COOH to more or less half of the actual concentration (43.3 ng/ml).

- While addition of 20% vinegar decrease THC-COOH concentration to (65.2ng/ml)
- Finally 10% has the least effect as there is minimal decrease from actual concentration (79.4ng/ml) as shown in table (3) and figures (6, 7 & 8).
- 2. <u>Effect of bleach on THC-COOH detection &</u> <u>quantification by GC-MS</u>

Addition of bleach in concentration 40% decreased THC-COOH concentration to (48.6ng/ml).

- While addition of 20% bleach decreased THC-COOH concentration to (71.3ng/ml)
- Finally 10% bleach has the least effect as there is minimal decrease from actual concentration (80.2ng/ml) as shown in table (3) and Figures (9, 10 & 11).
- 3. <u>Effect of visine on THC-COOH detection &</u> <u>quantification by GC-MS</u>
- Addition of visine in concentration 40% decreased THC-COOH concentration to (42 ng/ml).
- While addition of 20% visine decreased THC-COOH concentration to (63.6 ng/ml).
- Finally 10% has the least effect as there is minimal decrease from actual concentration (77.3ng/ml) as shown in table (3) and figures (12, 13 & 14).
- 4. Effect of drano on THC-COOH detection & quantification by GC-MS
- Addition of drano in concentration 40% leading to decreased in concentration of THC-COOH to (53.4 ng/ml).
- While addition of 20% drano cause decreased in THC-COOH concentration (72.2 ng/ml)
- Finally 10% has the least effect as there is minimal decrease from actual concentration (81.5ng/ml) as shown in table (3) and figures (15, 16 & 17).
- 5. <u>Effect of liquid hand soap on THC-COOH</u> <u>detection & quantification by GC-MS</u>
- Addition of liquid hand soap in concentration 20% leading to moderate decrease in concentration of THC-COOH (66.8 ng/ml).
- While addition of 10% liquid hand soap decreased in THC-COOH concentration to (78.3 ng/ml). Finally 5% has the least effect on THC-COOH as there is minimal decrease from actual conc. (84.5ng/ml) As shown in table (4)and figures (18, 19 & 20).

Statistical study for influence of different adulterants on THC-COOH quantification by GC-MS showing that addition of vinegar, drano, bleach, visine and liquid hand soap decreased THC-COOH concentration significantly with increased adulterant concentration as shown in table (5).

Adulterant Conc.	Vinegar	Bleach	Visine	Drano
	24 ng/ml	Zero	30ng/ml	Zero
40%	Negative	Negative	Negative	Negative
20%	62ng/ml	Zero	41ng/ml	Zero
	Positive	Negative	Negative	Negative
10%	75ng/ml	11ng/ml	79ng/ml	9ng/ml
	Positive	Negative	Positive	Negative

Table (1): Effects of different adulterants on THC metabolites screening by RIA

Table (2): Effects of Liquid hand soap on THC metabolite screening by RIA

Liquid hand soap	ТНС
20%	Zero Negative
10%	Zero Negative
5%	Zero Negative

Table (3): Effect of different adulterants on THC-COOH detection and quantification by GC MS

Adulterant Conc.	Vinegar	Bleach	Visine	Drano
40%	43.3ng/ml	48.6 ng/ml	42ng/ml	53.4ng/ml
	(Positive)	(Positive)	(Positive)	(Positive)
20%	65.2ng/ml	71.3ng/ml	63.6ng/ml	72.2ng/ml
	(Positive)	(Positive)	(Positive)	(Positive)
10%	79.4ng/ml	80.2ng/ml	77.3ng/ml	81.5 ng/ml
	(Positive)	(Positive)	(Positive)	(Positive)

Table (4): Effect of liquid hand soap on THC-COOH detection and quantification by GC.MS

Liquid hand soap conc.	ТНС-СООН	
20%	66.8ng/ml	
	(Positive)	
10%	78.3ng/ml	
	(Positive)	
5%	84.5ng/ml	
5%	(Positive)	

Table (5): Statistical study for influence of adulterants on THC-COOH quantification by GC-MS.

Adulterant	Sample Size(N)	Pearson Correlation
Vinegar	12	-0.763
Bleach	12	-0.989
Visine	12	-0.891
Drano	12	-0.992
Liquid hand soap	12	-0.968

Significance at 1 % level

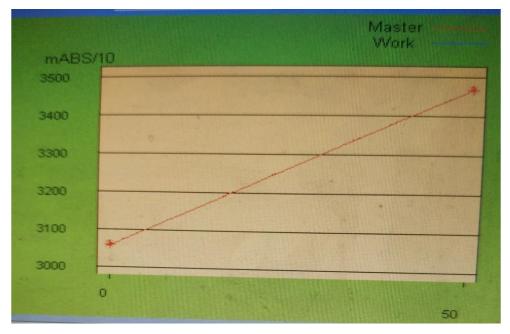


Figure (A): THC calibration curve on Radioimmunoassay.

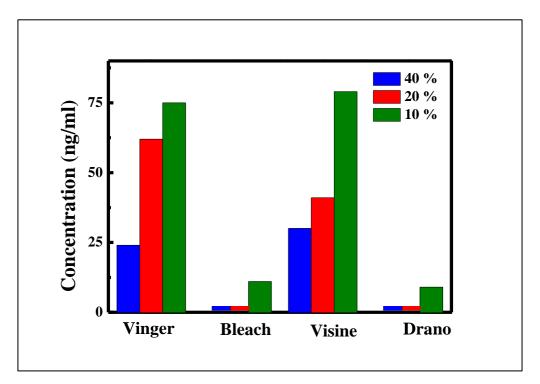


Figure (1): Effects of different adulterants on THC detection by RIA

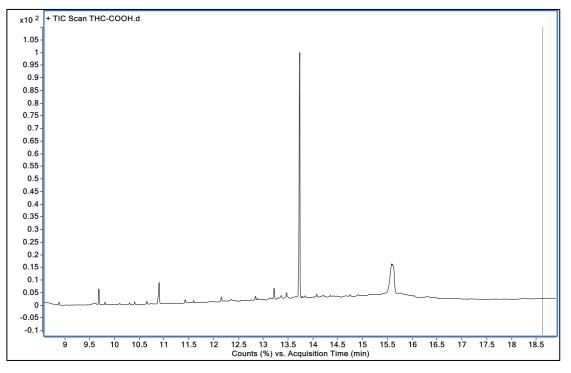


Figure (2): GC-MS chromatogram for the analysis of TMS derivative of THC-COOH

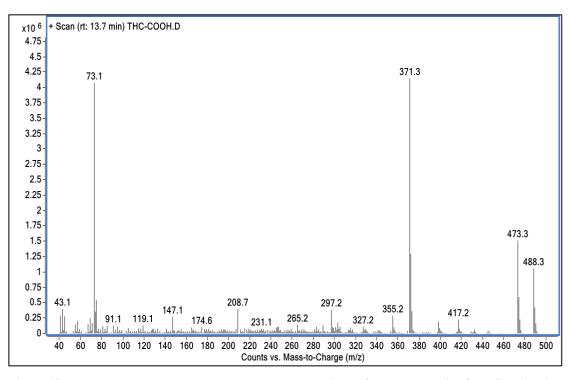


Figure (3): Full scan mass spectrum chromatogram obtained after the analysis of TMS derivative of THC-COOH

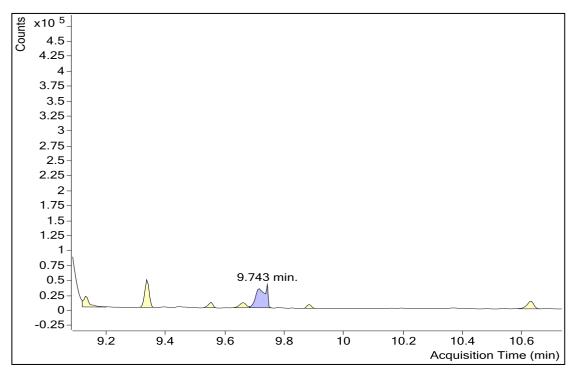


Figure (4): SIM chromatograms obtained after the analysis of blank urine

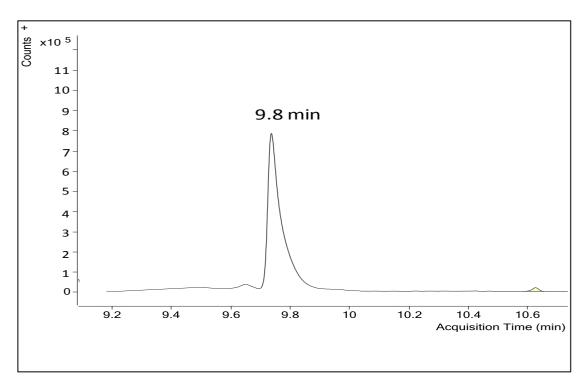


Figure (5): GC-MS chromatograms obtained after the analysis of parent sample (THC-COOH conc 89 ng/ml)

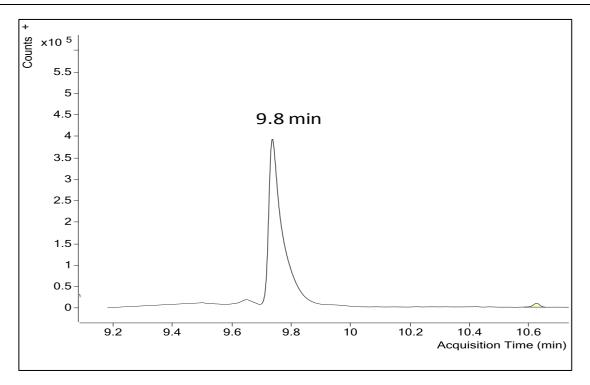


Figure (6): GC-MS chromatogram for parent sample after addition of 40% vinegar (THC-COOH conc.43.3ng/ml)

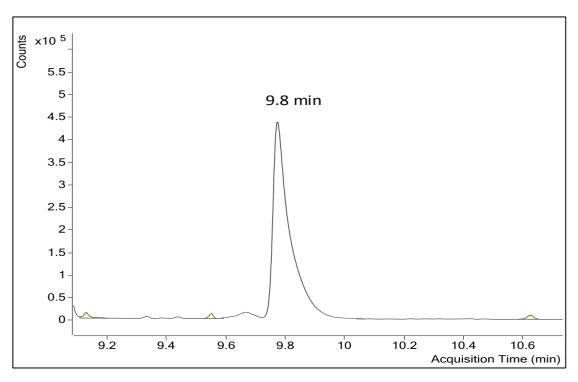


Figure (7): GC-MS chromatogram for parent sample after addition of 20% vinegar (THC-COOH conc.65.2ng/ml)

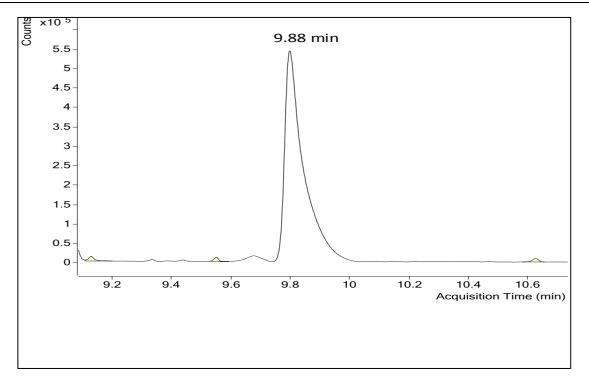


Figure (8): GC-MS chromatogram for parent sample after addition of 10% vinegar (THC-COOH conc.79.4ng/ml)

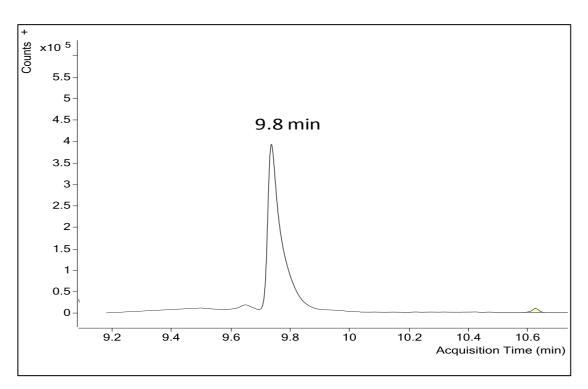


Figure (9): GC-MS chromatogram for parent sample after addition of 40%bleach (THC-COOH conc.48.6ng/ml)

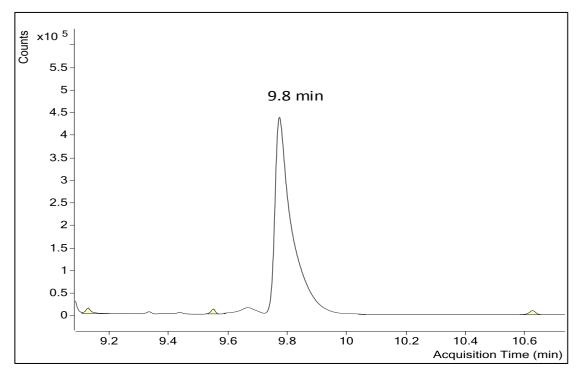


Figure (10): GC-MS chromatogram for parent sample after addition of 20% bleach (THC-COOH conc.71.3ng/ml)

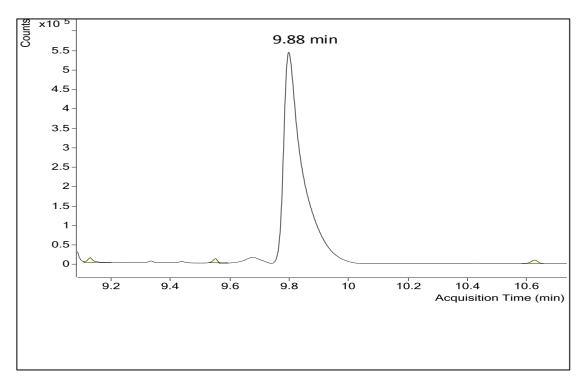


Figure (11): GC-MS Chromatogram for parent sample after addition of 10% bleach (THC-COOH conc.82.2ng/ml)

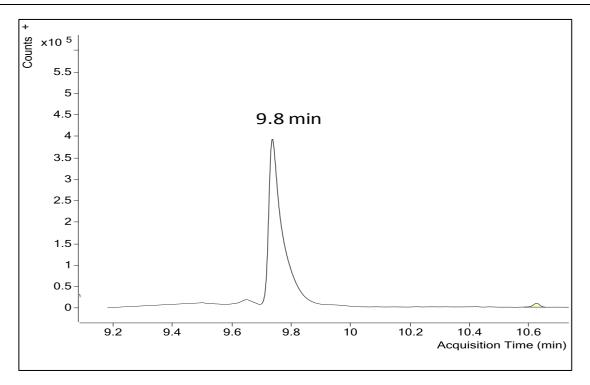


Figure (12): GC-MS chromatogram for parent sample after addition of 40% visine (THC-COOH conc.42ng/ml)

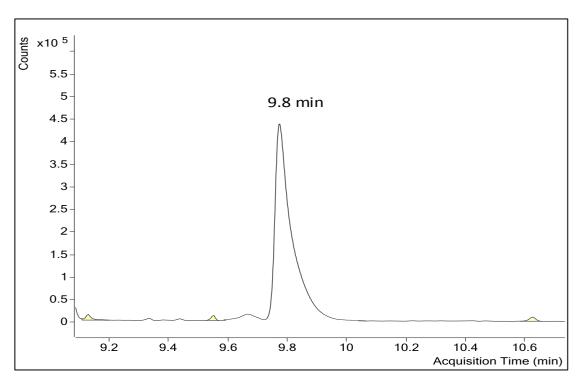


Figure (13): GC-MS Chromatogram for parent sample after addition of 20% visine (THC-COOH conc.63.6ng/ml)

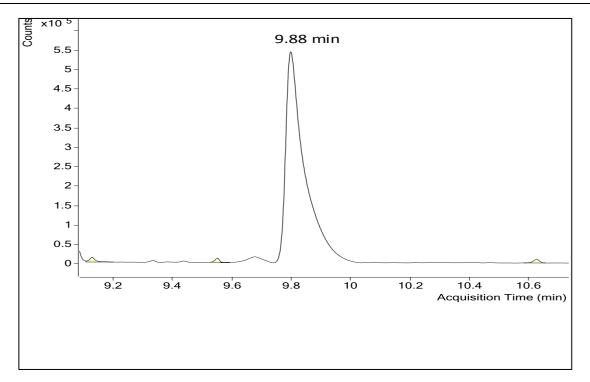


Figure (14): GC-MS Chromatogram for parent sample after addition of 10% visine (THC-COOH conc.77.3ng/ml)

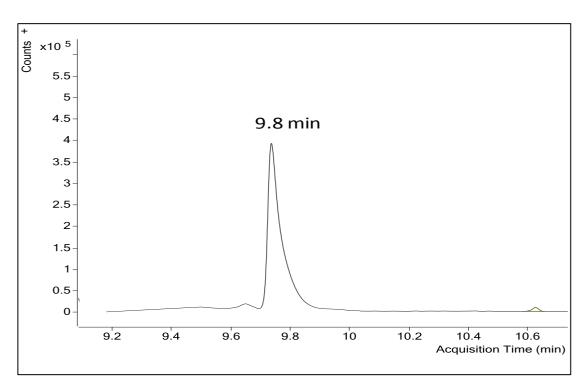


Figure (15): GC-MS chromatogram for parent sample after addition of 40%drano (THC-COOH conc.53.4ng/ml)

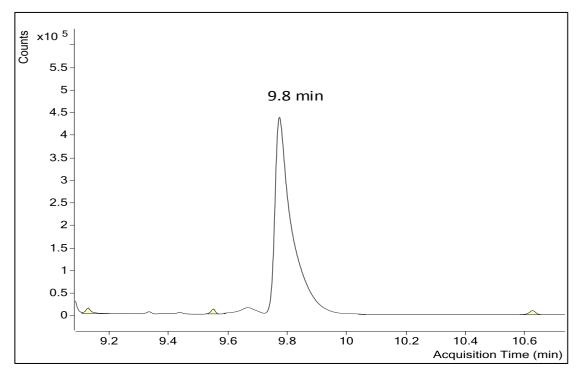


Figure (16): GC-MS Chromatogram for parent sample after addition of 20% drano (THC-COOH conc.72.2ng/ml)

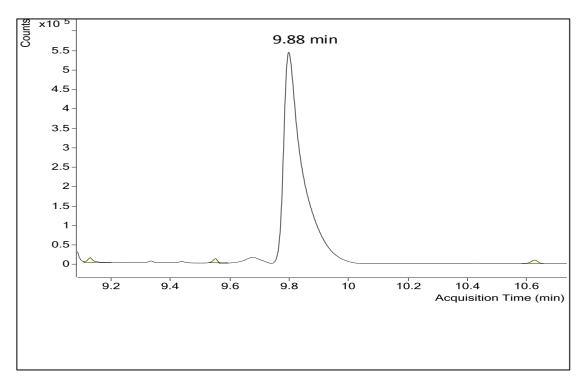


Figure (17): GC-MS chromatogram for parent sample after addition of 10% drano (THC-COOH conc.81.5ng/ml)

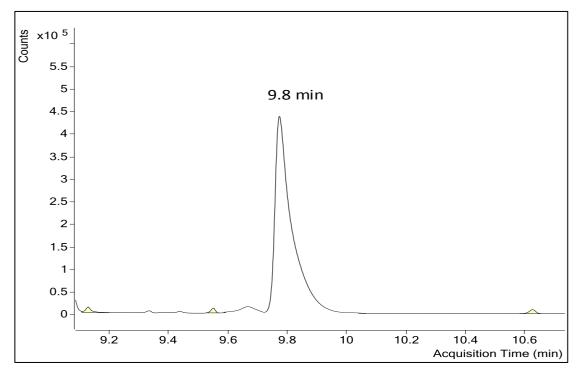


Figure (18): GC-MS Chromatogram for parent sample after addition of 20% liquid hand soap (THC-COOH conc.66.8ng/ml)

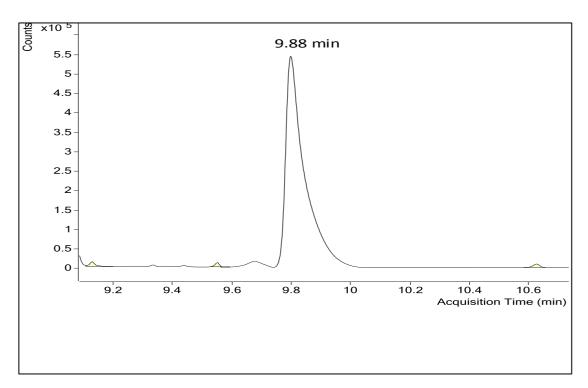


Figure (19): GC-MS chromatogram for parent sample after addition of 10%liquid hand soap (THC-COOH conc.78.3ng/ml)

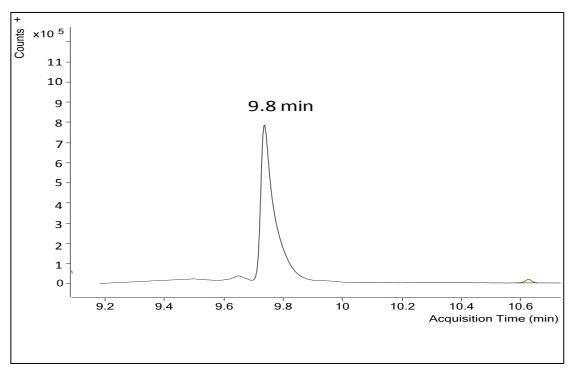


Figure (20): GC-MS chromatogram for parent sample after addition of 5% liquid hand soap (THC-COOH conc.84.5ng/ml)

Discussion

Effect of different urine adulterants on radioimmunoassay screening for cannabis:

Addition of vinegar at high concentration 40% was able to successfully masking positive response of cannabis in tested urine samples, while moderate and low concentration (20%, 10%) didn't affect cannabis detection in urine samples.

This was in agreement with *Dasgupta*, (2010) who report that vinegar is effective adulterant for many drugs of abuse as cannabis.

Addition of bleach whatever its concentration was able to mask cannabis detection by immunoassay.

These results were in agreement with *Uebel and Wium, (2002)* who reported that bleach at high concentration can decrease response rate for cannabis by immunoassay techniques

Addition of visine at concentration 40% and 20% was able to successfully masking positive response of cannabis in tested urine while 10% had no effect.

The active ingredient in Visine eye drops is tetrahydrozo-line hydrochloride, which relieves redness and irritation by constricting blood vessels. However, *Dasgupta, (2007)* found that the mechanism of adulteration is most likely due to the inactive ingredients benzalkoniumchloride and borate.

Similar to *Jaffee et al., (2007)* uptake of THC through benzalkoniumchloride reduces the binding in immunoassay drug screens, causing false-negative results on immunoassays.

Addition of drano by any concentration was able to mask cannabis detection by immunoassay. Also addition of liquid hand soap by any concentration even low concentration up to 5% masked cannabis detection by immunoassay giving false negative results. These results were in agreement with *Uebel and Wium, (2002)* who reported that drano is very effective in masking cannabis detection by immunoassay techniques. Also in consistent with *Bronner et al., (1990) and Wu, (2003)* who stated that hand soap detergent adulteration has caused false-negative results across a variety of drug assays using the cloned enzyme donor immunoassay (CEDIA) including screens for THC, amphetamine, barbiturates, cocaine, opiates, and PCP. <u>Effect of different adulterants on THC-COOH</u> <u>detection and quantification by GC-MS:</u>

Addition of vinegar in conc.40% leading to decrease in concentration of THC-COOH to more or less half the actual concentration (43.3ng/ml).While addition of 20% vinegar decreased THC-COOH concentration.to (65.2ng/ml). Finally addition of 10% vinegar had the least effect as there was minimal decrease from actual concentration (79.4ng/ml).

This was in agreement with *Wu et al., (1999)* who noticed considerable decrease in THC-COOH at a lower pH.

Addition of bleach in concentration 40% decreased THC-COOH concentration to (48.6ng/ml). While addition of 20% bleach decreased THC-COOH concentration to (71.3ng/ml). Finally 10% bleach has the least effect as there is minimal decrease from actual concentration (80.2ng/ml).

This means that change in pH either by increase or decrease can affect THC-COOH *concentration* by GC-MS.Similar to *Baiker et al., (1994)* who reported that bleach adulteration of urine samples caused a decreased concentration of THC-COOH as measured by GC/MS. Addition of visine in concentration 40% decreased THC-COOH concentration to (42/ml), while addition of 20% visine decreased THC-COOH concentration to (63.6ng/ml). Finally 10% has the least effect as there is minimal decrease from actual concentration (77.3ng/ml)

In consistent with *Dasgupta, (2007)* who stated that visine has been reported to cause marked adulterating effects on THC-COOH by GC-MS

Addition of drano in conc.40% leading to decrease in concentration of THC-COOH to (53.4 ng/ml).While addition of 20% drano caused decrease in THC-COOH concentration (72.2ng/ml). Finally 10% has the least effect as there is minimal decrease from actual concentration (81.5ng/ml).

In consistent with *Fu et al., (2014)*, Drano may affect GC-MS confirmation method for THC-COOH.

Addition of liquid hand soap in concentration 20% leading to moderate decrease in concentration of THC-COOH (66.8ng/ml). While addition of 10% liquid hand soap cause decreased in THC-COOH conc. to (78.3ng/ml). Finally 5% has the least effect on THC-COOH as there is minimal decrease from actual concentration (84.5ng/ml).

In consistent with *Wu et al.*, (1999), in the gas chromatographic-mass spectrometric (GC-MS) confirmation method, oxidizing agents as sodium hypochlorite in liquid hand soap interfere with the detection of THC-COOH.

The major problem which faced during examination of samples was the loss of THC-acid and the internal standard in the extraction process. To alleviate this problem, reducing agents as sodium hydrosulfite or sulfamic acid can be used. These methods only allowed detection of the remaining THC-acid in the urine. Generally, to save the drug from the oxidizing agents, addition of carbonate as buffering agent prior to or following urine void was also suggested.

Conclusion

The current study concludes that some adulterants make it easy to produce false negative results on RIA testing for cannabis and GC-MS is important to overcome adulteration methods in urine analysis.

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هل يمكن ان يمنع غش عينات البول الكشف على الحشيش باستخدام جهاز كروماتوجرافيا الغاز

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الملخص العربي

مقدمة: يُعدالحشيش، إلى جانب القهوة والتبغ، أكثر العقاقير ذات التأثير النفسى استخدامًا في جميع أنحاء العالم، وهو العقار غير القانوني الأكثر شيوعًا، وقد أظهرت الدراسات الحديثة زيادة في انتشار تعاطي الحشيش. ومن اهم المشكلات التي تواجه اختبارات الكشف عن المخدرات في البول هي امكانية غش العينات.

الهدف من البحث: در اسة التاثير الكمى والنوعى لخمسة مواد شائبة على عينات البول الإيجابية للحشيش.

طريقة البحث: أجريت هذه الدراسة التحليلة على عينات بول إيجايبة للحشيش جمعت من المترددين على معمل السموم الاكلينيكية بمستشفيات جامعة سوهاج. تم غش العينات باستخدام الخل، والدرانو، وصابون اليد السائل، وقطرة الفايزين، والكلور ثم اختبار العينات بواسطة جهاز المقايسة المناعية ثم تأكيدها وتحديد كميتها بواسطة جهاز كروماتو غرافيا الغاز مقياس الطيف.

النتائج: أظهرت عينات البول المغشوشة بالخل وصابون اليد السائل والدرانو والكلوروقطرة الفايزين نتائج سلبية كاذبة للحشيش عن طريق اختبار المقايسة المناعية كما أظهرت تأكيد الاختبار بواسطة جهاز كروماتو غرافيا الغاز مقياس الطيف أن إضافة الخل والكلور والفايزين والدرانو وصابون اليد السائل يقلل تركيز الحشيش بشكل ملحوظ مع زيادة التركيز.

الخلاصة: بعض المواد الغاشة تؤدى الى نتائج سلبية خاطئة في اختبارات المقايسة المناعية للحشيش، كما ان تاكيد النتائج بواسطة جهاز كروماتو غرافيا الغاز مقياس الطيف مهم للتغلب على طرق الغش في تحليل البول.

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