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EFFECT OF TEMPERATURE, PRESERVATIVE AND TIME ON THE AFERMENTATION OF URINE AND BLOOD

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ABSTRACT

This study was designed to detect and determine the concentration of ethanol in biological samples (blood and urine). samples were collected from anon drunken, diabetic persons some of them used drugs and other not used and drunken person and divided to four plain containers. Two of them with preservative (Sodium fluoride) and others without preservative and kept at 4°C and at 40 °C for 10 days. Potassium dichromate test (Alco test) was used to detect the presence of ethanol every 24 hours for 10 days. All samples from drunken and diabetic people were negative for ethanol presence. Meanwhile, all samples from drunken persons were positive result and the colour of potassium dichromate changed to green colour indicated the presence of ethanol. In the other hand all samples were positive of ethanol with GC (FID), but they were different in their concentrations. Samples at 40 °C were showed higher concentration than other samples with preservative or at 4°C; therefore, the GC method for analysis ethanol is very important as confirmatory test and to identify the type of alcohol.

Keywords: Urine, Blood, Ethanol, Alco Test

1. INTRODUCTION

Ethanol is one of the oldest intoxicants known to man, its use or misuse was known in India long before it was known in other countries and described in Indian literature at least two thousand years before the birth of Christ (BC) Sharma (2003). It was known or used in Jezera arb before the Islamic religion (Al Quran Bee verus, 67), and its presence in intoxicating beverages e.g., Brandy, Whisky, Gin, Rum, Beers, Wines, Band and Hanson (1990) and the Country liquors, Modd (1990), Parikh (2005). In U.S.A., roughly 50% of all the persons involved in case of assault are under

the influence of alcohol, and every year 20,000 persons die, and another one million persons are injured in road accidents, Sharma (2003). Due to alcohol intoxication, various crimes are committed, criminal assaults and sexual offences are commonly committed, Modd (1990), Jahala and Raju (1997). In Sudan alcohol is associated with various crimes such as: murder, rape, burning, death, homeless, robbery, assaults, traffic accidents, insurance of companies and kidnap. Thus, in year 2004, the total number of alcohol cases were 136 (Forensic Lab, 2004). In the year 2005, the total number of alcohol crimes or cases approached 145 cases (Forensic Lab, 2005). And 2006, the total number of alcohol cases which registered in forensic laboratory were about 170, they were blood, urine, breath, stomach contents and beverages or liquors (Forensic Lab, 2006). Medically, ethanol is classified as hypnotic, Sharma (2003) and Moffat and Jaskson (1986), it is less toxic than other alcohols. Methanol, for example, it is quite poisonous drink, inhaling it for prolonged periods or allowing it to remain long on the skin can lead to blindness or death Morri and Boyd (2000).

2. MATERIALS AND METHODS

All chemicals were obtained from Riedel –De Haem AG Germany

1) Instrument

Gas Chromatograph (GC) Flame Ionization Detector (FID).

Serial No. C 1132403700SA.P/N 2217702-34.

GC-2010 ATF.230 VLV.GC-2010.

Gas Chromatograph Shimadzu.

Water bath (Kotter man-Germany).

2) Samples collection

Urine and blood samples were collected from a non-drunken, diabetic and a drunken person.

3) Blood samples

The blood samples were collected from different parts of a human body (blood from ante-cubital vein is collected with citrate as anticoagulant). Sharma (2003), Moffat et al. (2004), Dogra and Abtijitrudra (2005).

4) Urine samples

The urine samples may not give true indicators of blood alcohol at the time of arrest as the urine may be accumulating for a long time in the bladder. Therefore, a second sample was taken after half an hour. The urine was collected in disposable beakers. 0.2 percent Sodium fluoride was used as preservative Sharma (2003).

3. PREPARATION OF SAMPLES

3.1. URINE SAMPLE FROM DRUNKEN PERSON

20 ml of urine was taken from drunken person and divided into four containers (5ml each). 0.5 ml of sodium fluoride was added to two of the containers while the other two containers are left without any addition of preservative. One sample with preservative (Sodium Fluoride) and other without preservative were kept in a refrigerator at 4°C for 10 days. The other two samples were kept at 40°C for 10 days. The presence of ethanol in the samples was examined by using dichromate test and gas chromatography every 24 and 48 hours for 10 days respectively Cox (2002), Moffat et al. (2004) and Sharma (2003).

3.2. BLOOD SAMPLE FROM A NON-DRUNKEN PERSON

10ml of blood was taken from a non-drunken person and divided into four containers (2.5ml each). 0.5ml of sodium fluoride was added to two of the above containers while the other two containers were left without any addition of preservative. One sample with sodium fluoride and another sample without sodium fluoride were kept in a refrigerator at 4°C for 10 days. The remaining two samples were kept at 40°C for 10 days. The presence of ethanol in the samples was examined by using dichromate test and gas chromatography every 24 and 48 hours for 10 days respectively Moffat et al. (2004).

3.3. URINE SAMPLES FROM ANON DRUNKEN PERSON

20ml of urine was taken from a non-drunken person and divided into four containers (5ml each). 0.5ml of sodium fluoride was added to two of the above samples, while the other two samples were left without any addition of preservative. One sample with preservative and another without any preservative were kept in a refrigerator at 4°C for 10 days. The remaining two samples were kept at40°C for 10 days. The presence of ethanol in the samples was examined by using dichromate test and GC every 24 and 48 hours for 10 days respectively.

3.4. BLOOD SAMPLE FROM DRUNKEN PERSON

10ml of blood was taken from drunken person and divided into four containers (2.5ml each). 0.5ml of sodium fluoride was added to two of the above samples, while the other two samples were left without any addition of preservative. One sample with preservative and the other without preservative were kept in a refrigerator at 4°C for 10 days. The remaining two samples were kept at 40°C for 10 days. The presence of ethanol in the samples was examined by using dichromate test and GC every 24 and 48 hours for 10 days respectively Moffat et al. (2004).

4. METHODS

4.1. IDENTIFICATION OF ETHANOL IN HUMAN FLUIDS (BLOOD, URINE)

1) Dichromate Test

1ml of urine and serum were placed separately in a test tube. One drop of potassium dichromate (2.5% w/v in 50% V/V Sulfuric acid) was added to a strip of glass-fibber filter paper. The paper was inserted in the neck of the test tube. Then the test tube was placed in a boiling water bath for 20 min.

A colour change to green indicates positive result. Ethanol gives a positive reaction if present above 400 mg/L, Moffat et al. (2004).

2) Gas chromatography (G C) method

A measured sample or reference sample containing alcohol was placed into the head of the column kept in a heated chamber where it is changed into vapours and carried by carrier gas through a column packed with a suitable material. Blood, urine, or other body fluids are diluted with isopropyl alcohol for example before analysis, Sharma (2003) and Moffat et al. (2004). Instrumental setup was:

Column: stain less steel, 2mm*3m. Coated with Carb Wax.

1) Temperature.

Injection Port	175°C
Oven	80 °C
Dilutor	225°C

2) Carrier gas Helium.
3) Detector FID/ TCD Sharma (2003) and (Adamovics, 1995).

5. RESULTS

The results of detection of ethanol by potassium dichromate in urine and blood were shown in Table 1, Table 2, Table 5, Table 6 (with 1% NaF) and Table 3, Table 4, Table 7, Table 8 (without 1% NaF).

Table 1

Table 1 Urine of Anon Drunken Person (40°C)		
After every 24 hours	**Colour changes**	**Result**
For Ten Days	None	-Ve

Table 2

Table 2 Urine of Anon Drunken Person (4°C)		
After Every 24 Hours	**Colour Changes**	**Result**
For Ten Days	None	-Ve

Table 3

Table 3 Urine of Anon Drunken Person (40°C)		
After Every 24 Hours	**Colour Changes**	**Result**
For Ten Days	None	-Ve

Table 4

Table 4 Urine of Anon Drunken Person (4 °C)		
After Every 24 Hours	**Colour Changes**	**Result**
For Ten Days	None	-Ve

Table 5

Table 5 Blood of Anon Drunken Person (40°C)		
After Every 24 Hours	**Colour Changes**	**Result**
For Ten Days	None	-Ve

Table 6

Table 6 Blood of Anon Drunken Person (4°C)		
After Every 24 Hours	**Colour Changes**	**Result**
For Ten Days	None	-Ve

Table 7

Table 7 Blood of Anon Drunken Person (40°C)		
After Every 24 Hours	**Colour Changes**	**Result**
For Ten Days	None	-Ve

Table 8

Table 8 Blood of Anon Drunken Person (4 °C)		
After Every 24 Hours	**Colour Changes**	**Result**
For Ten Days	None	-Ve

The results of detection of ethanol by potassium dichromate in urine and blood from diabetic persons were shown in tables Table 9, Table 10, Table 13, Table 14 (with 1% NaF) and Table 11, Table 12, Table 15, Table 16 (without 1%NaF)

Table 9

Table 9 Urine of Diabetic Person (40°C)		
After Every 24 Hours	**Colour Changes**	**Result**
For Ten Days	None	-Ve

Table 10

Table 10 Urine of Diabetic Person (4 °C)		
After Every 24 Hours	**Colour Changes**	**Result**
For Ten Days	None	-Ve

Table 11

Table 11 Urine of Diabetic Person (40°C)		
After Every 24 Hours	**Colour Changes**	**Result**
For Ten Days	None	-Ve

Table 12

Table 12 Urine of Diabetic Person (4 °C)		
After Every 24 Hours	**Colour Changes**	**Result**
For Ten Days	None	-Ve

Table 13

Table 13 Blood of Diabetic Person (40 °C)		
After Every 24 Hours	**Colour Changes**	**Result**
For Ten Days	None	-Ve

Table 14

Table 14 Blood of Diabetic Person (4°C)		
After Every 24 Hours	**Colour Changes**	**Result**
For Ten Days	None	-Ve

Table 15

Table 15 Blood of Diabetic Person (40°C)		
After Every 24 Hours	**Colour Changes**	**Result**
For Ten Days	None	-Ve

Table 16

Table 16 Blood of Diabetic Person (4°C)		
After Every 24 Hours	**Colour Changes**	**Result**
For Ten Days	None	-Ve

Table 17

Table 17 Detection of Ethanol from Biological Fluids (Blood and Urine) Samples by Potassium Dichromate Test. Cases Admitted to Forensic Laboratory

No.	Sample	Colour	Result
1	Urine	Green	+Ve
2	Blood	Green	+Ve
3	Blood	Green	+Ve
4	Urine	Green	+Ve

All samples (urine and blood) showed positive test for alcohol by Potassium dichromate test.

Table 18

Table 18 Determination of Ethanol Concentration by GC in Urine Sample from Anon Drunken Person Treated with 1% Sodium Fluoride (40°C)

Standard Solutions of Ethanol	Retention Time (Rt)	Peak Area	Concentration
100µl	1.775	123457	0.4 G/L
250µl	1.775	86737	1 G/L
500µl	1.722	334786	2 G/L
750µl	1.784	479666	3 G/L
1000µl	1.792	684906	4 G/L
Urine Sample (1)	**Retention Time (Rt)**	**Peak Area**	**Concentration**
After 48 Hours	1.806	5717	0.034 G/L
After 96 Hours	1.88	9453	0.067 G/L
After 144 Hours	1.798	5507	0.033 G/L
After 240 Hours	1.802	9727	0.058 g/L

From this table we deduced that at high temperature the concentration of ethanol by auto fermentation of urine sample with a preservative is low even after 10 days.

Table 19

Table 19 Determination of Ethanol Concentration by GC in Urine Sample from Anon Drunken Person Treated with 1%Sodium Fluoride (4°C)

Standard Solutions of Ethanol	Retention Time (RT)	Peak Area	Concentration
100µl	1.775	123457	0.4 G/L
250µl	1.775	86737	1 G/L
500µl	1.722	337786	2 G/L
750µl	1.784	479666	3 G/L
1000µl	1.792	684906	4 G/L
Urine Sample (2)	**Retention Time (RT)**	**Peak Area**	**Concentration**
After 48 Hours	1.8	13041	0.078 G/L
After 96hours	1.794	10615	0.063 G/L

After 144 Hours	1.803	5380	0.032 G/L
After 240hours	1.829	8463	0.051 G/L

From this table we deduced that at low temperature the concentration of ethanol by auto fermentation of urine sample with a preservative is low even after 10 days.

Table 20

Table 20 Determination of Ethanol Concentration by GC in Urine Sample from a non-Drunken Person (40°C)

Standard Solutions of Ethanol	Retention Time (RT)	Peak Area	Concentration
100µl	1.775	123457	0.4 G/L
250µl	1.775	86737	1 G/L
500µl	1.722	334786	2 G/L
750µl	1.784	479666	3 G/L
1000µl	1.792	684906	4 G/L
Urine Sample (3)	**Retention Time (RT)**	**Peak Area**	**Concentration**
After 48 Hours	1.798	4136	0.025 G/L
After 96hours	1.799	10559	0.063 G/L
After 144 Hours	1.812	6333	0.038 G/L
After 240hours	1.822	46063	0.275 G/L

From this table we deduced that at high temperature, the concentration of ethanol by auto fermentation of urine sample without a preservative became very high after 10 days.

Table 21

Table 21 Determination of Ethanol Concentration by GC in Urine Sample from a Non-Drunken Person (4°C)

Standard Solutions of Ethanol	Retention Time (RT)	Peak Area	Concentration
100µl	1.775	123457	0.4 G/L
250µl	1.775	86737	1 G/L
500µl	1.722	334786	2 G/L
750µl	1.784	479666	3 G/L
1000µl	1.792	684906	4 G/L
Urine Sample (4)	**Retention Time (RT)**	**Peak Area**	**Concentration**
After 48 Hours	1.801	6011	0.034 G/L
After 96hours	1.794	6383	0.038g/L
After 144 Hours	1.789	6438	0.038 G/L
After 240hours	1.805	7372	0.044 G/L

From this table we deduced that at low temperature the concentration of ethanol by auto fermentation of urine sample without a preservative is low even after 10 days.

Table 22

Table 22 Determination of Ethanol Concentration by GC in Blood Sample from a Non-Drunken Person Treated with 1% Sodium Fluoride (40 °C)

Standard Solutions of Ethanol	Retention Time (RT)	Peak Area	Concentration
100µl	1.775	123457	0.4 G/L
250µl	1.775	86737	1 G/L
500µl	1.722	334786	2 G/L
750µl	1.784	479666	3 G/L
1000µl	1.792	684906	4 G/L
Blood Sample (1)	**Retention Time (RT)**	**Peak Area**	**Concentration**
After 48 Hours	1.794	3485	0.021 G/L
After 96 Hours	1.793	11394	0.068 G/L
After 144 Hours	1.801	6420	0.038 G/L
After 240 Hours	1.816	7420	0.045 G/L

At high temperature the auto fermentation of blood sample from anon drunken person with a preservative gave low concentration of ethanol even after 10 days.

Table 23

Table 22 Determination of Ethanol Concentration by GC in Blood Sample from a Non-Drunken Person Treated with 1% Sodium Fluoride (4°C)

Standard Solutions of Ethanol	Retention Time (RT)	Pea K Area	Concentration
100µl	1.775	123457	0.4 G/L
250µl	1.775	86737	1 G/L
500µl	1.722	334786	2 G/L
750µl	1.784	479666	3 G/L
1000µl	1.792	684906	4 G/L
Blood Sample (2)	**Retention Time (RT)**	**Peak Area**	**Concentration**
After 48 Hours	1.804	4161	0.025 G/L
After 96 Hours	1.787	6047	0.075g/L
After 144 Hours	1.806	1769	0.033 G/L
After 240 Hours	1.805	8695	0.05 G/L 2

At low temperature the concentration of ethanol by auto fermentation of blood sample from anon drunken person with a preservative is low even after 10 days.

Table 24

Table 23 Determination of Ethanol Concentration by GC in Blood Sample from Anon Drunken Person (40°C)

Standard Solutions of Ethanol	Retention Time (RT)	Peak Area	Concentration
100µl	1.775	123457	0.4 G/L
250µl	1.775	86737	1 G/L
500µl	1.722	334786	2 G/L
750µl	1.784	479666	3 G/L
1000µl	1.792	684906	4 G/L
Blood Sample (3)	**Retention Time (RT)**	**Peak Area**	**Concentration**
After 48 Hours	1.791	2281	0.014 G/L
After 96 Hours	1.795	10573	0.063 G/L

After 144 Hours	1.812	19768	0.118 G/L
After 240 Hours	1.884	19388	0.116 G/L

At high temperature the auto fermentation of blood sample from anon drunken person without a preservative gave high concentration of ethanol after 10 days.

Table 25

Table 24 Determination of Ethanol Concentration by GC in Blood Sample from Anon Drunken Person (4°C)

Standard Solutions of Ethanol	Retention Time (RT)	Peak Area	Concentration
100µl	1.775	123457	0.4 G/L
250µl	1.775	86737	1 G/L
500µl	1.722	334786	2 G/L
750µl	1.784	479666	3 G/L
1000µl	1.792	684906	4 G/L
Blood Sample (4)	**Retention Time (RT)**	**Peak Area**	**Concentration**
After 48 Hours	1.798	2925	0.017 G/L
After 96 Hours	1.795	5118	0.024 G/L
After 144 Hours	1.802	5262	0.016 G/L
After 240 Hours	1.834	6570	0.039

At low temperature the auto fermentation of blood sample from anon drunken person without a preservative lead to low concentration of ethanol even after 10 days.

Table 26

Table 25 Determination of Ethanol Concentration in Biological Fluids (Blood and Urine) Samples by Using GC FID. Cases Admitted to Forensic Laboratory

No.	Sample	Retention Time	Peak Height	Peak Area	Ethanol Concentration
1	Urine	1.783	3501	43926	0.263 G/L
2	Blood	1.769	32589	4902	0.195g/L
3	Blood	1.813	9619	44641	0.267 G/L
4	Urine	1.781	6770	66827	0.399 G/L

Figure 1

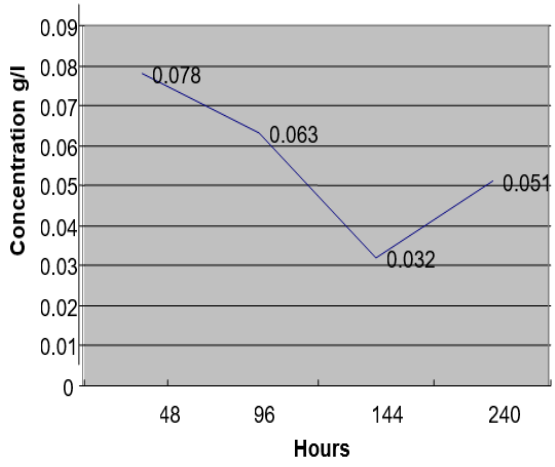

Figure 1 Concentration of Ethanol in Urine Sample (At 4 °C +1% Na F) from a Non-Drunken Person for 10 Days

Figure 2

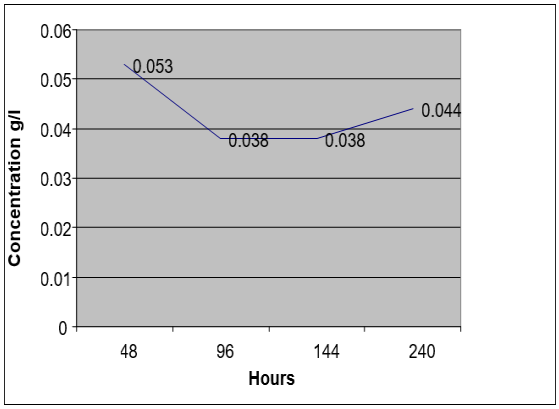

Figure 2 Concentration of Ethanol in Urine Sample (At 4 °C) from a Non-Drunken Person for 10 Days

Figure 3

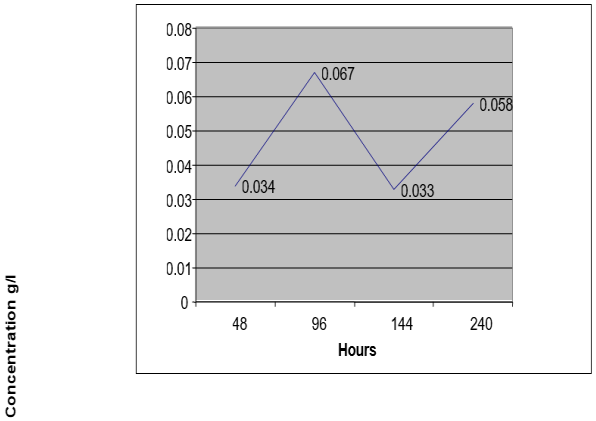

Figure 3 Concentration of Ethanol in Urine Sample (At 40 °C +1% Na F) from a Non-Drunken Person for 10 Days

Figure 4

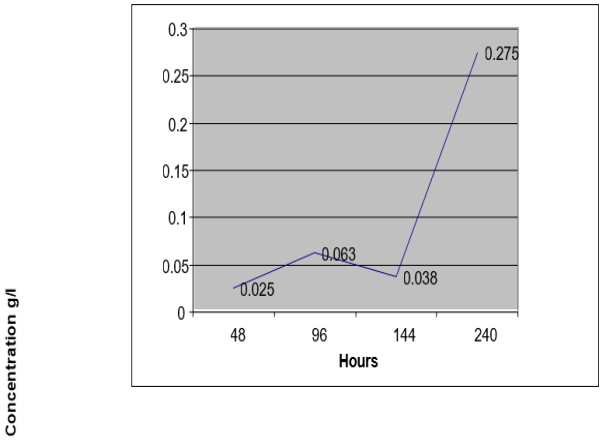

Figure 4 Concentration of Ethanol in Urine Sample (At 40°C) from a Non-Drunken Person for 10 Days

Figure 5

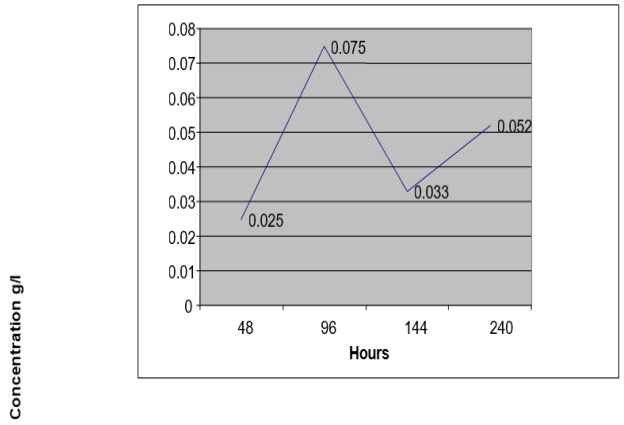

Figure 5 Concentration of Ethanol in Blood Sample (At 4 °C+ 1%Na F) from a Non-Drunken Person for 10 Days

Figure 6

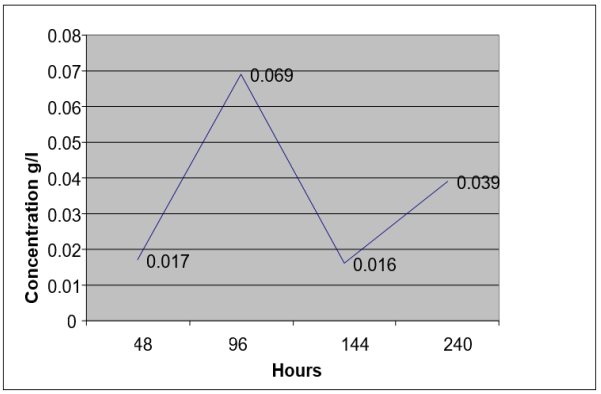

Figure 6 Concentration of Ethanol in Blood Sample (At 4 °C) from a Non-Drunken Person for 10 Days

Figure 7

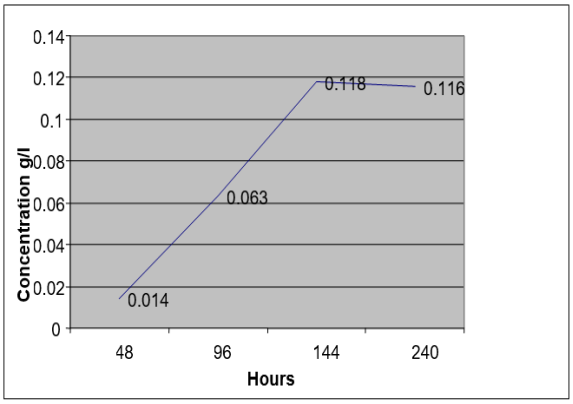

Figure 7 Concentration of Ethanol in Blood Sample (At 4°C + 1%Naf) from a Non-Drunken Person for 10 Days

Figure 8

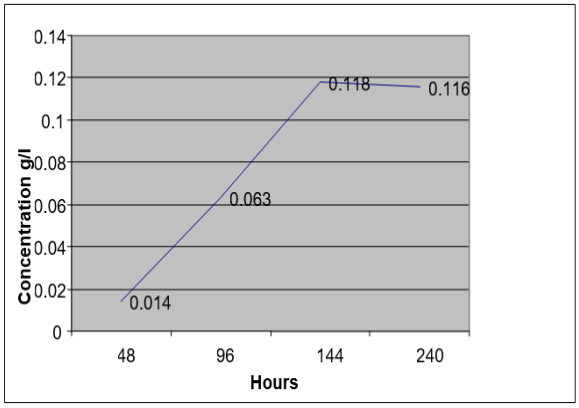

Figure 8 Concentration of Ethanol in Blood Sample (At 40°C) from a Non-Drunken Person for 10 Days

6. DISCUSSION

Potassium dichromate test (Alco test) for blood and urine samples from anon drunken and diabetic persons gave a negative result, so there was no color change. But at the same time the gas chromatography (GC) flame ionization detector (FID) test for these samples was positive result for ethanol that means GC is more sensitive than Alco test.

While blood and urine samples from a drunken person showed positive result for ethanol presence (the colour changed from yellow to green indicated the presence of ethanol). The GC FID showed high concentrations of ethanol in the samples (blood and urine), these results were similar to Moffat et al. (2004) and Sharma (2003). Urine samples at 40°C without preservative gave high concentration of ethanol because at this temperature microorganisms can act more; therefore, the auto fermentation occur in the samples. All samples from anon drunken or diabetics persons at 4°C with preservative (Na F) have shown low concentration of ethanol this reflex the important of preservative, time, and the temperature conditions. Samples are capable to remain for long period without impair or auto ferment due to their conditions (temperature, preservative, and the period). The blood samples at 4 °C with preservative showed low concentration than the urine samples at the same conditions, this because the preservative is most suitable for blood samples. This was similar to Vij (2005) results. Traces of ethanol are found in all persons (even a non-drunken person), this is corresponding to Readdy (2004) result.

7. CONCLUSION

All biological fluids (blood and urine) that need examination for ethanol must keep under good conditions such as preservative (NaF), temperature and limit of period. The temperature below 4°C is the most suitable to keep biological samples (blood and urine). The low concentration of ethanol which cannot appear or detect under the potassium dichromate test, can be detected by using the GC FID instrument.

CONFLICT OF INTERESTS

None.

ACKNOWLEDGMENTS

None.

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