Original Article

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Determination of Ethanol in Blood and Urine Samples- A Case Study Showing the Significance of (i) Application of One Method Over the Other and (ii) Inclusion of Uncertainty of Measurement

Kumar Raj*, Jaiswal A.K., Yadav Anita**, Singh Dalbir***

Authors Affiliation: *Chemistry Division, Forensic Science Laboratory (H), Madhuban, Karnal, Haryana 132037, India. **Dept. of Forensic Medicine and Toxicology, All India institute of Medical Sciences, New Delhi, Delhi 110029, India.

Reprints Requests: Raj Kumar, Chemistry Division, Forensic Science Laboratory (H), Madhuban, Karnal, Haryana 132037, India.

E-mail: dahiya.rajk@gmail.com

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Abstract

Drunken drivers in India always pose a high risk to other users of the roads. Nearly 0.14 million people lost their lives every year in last three years in India, which is more than all casualties of wars of India [6]. Statistics derived from postmortem reports from road traffic fatalities in various countries show that 20-50% of drivers had been drinking alcohol before the crash and level of consumed alcohol in their body was above the legal limit for driving [2,3]. The influence of alcohol or drugs, many a times, goes to the extent that driver becomes incapable of having control of vehicle leading to the ill fate [2,3]. Seeing the intensity of the matter, Government of India amended the relevant laws from time to time to make it more stringent to deter the offenders. The Motor Vehicle (Amendment) Bill 2016 is such an effort by the Government of India, which was approved by the Union Cabinet of India on 3rd of August 2016 [7]. Such cases, when passes through the process of Criminal Justice Delivery System, the quantity of ethanol determined by a forensic chemist in blood sample of the accused plays a vital role in fair trial. There are several methods available for determination of quantity of ethanol in blood and urine samples. Two such methods were used for ethanol determination in blood and urine samples by the authors. The objective of this paper is to describe the preferential selection of one method over the other in some cases and inclusion of the value of uncertainty of measurement while reporting the result. The available blood and urine samples were analyzed to determine the ethanol contents with the help of two methods. The contents of ethanol determined with two methods were found different to the extent of about 20 to 30 mg%. The results of the method with lower values were found justified with the case history and were reported along with inclusion of value of uncertainty of measurement.

Keywords: Ethanol; Cavett Cup; Kojelka-Hine Apparatus; mg%; UOM-Uncertainty of Measurement.

Introduction

The blood and urine samples of a person were received in Forensic Science laboratory (H), Madhuban, Haryana to find out the level of ethanol consumption. As per the police investigation findings, the person was driving in his car on a national highway. His wife was also sitting on the front side seat of the car. Suddenly, the person lost the balance and his car smashed into a pole on side of the road, crashing the driver side of the car. The driver was badly injured while his wife also sustained some injuries. Both of them were admitted into the nearby hospital where the lady was relieved with first aid and some minor medication while the driver lost his life after about 24 hours of treatment. The blood samples of both of them were collected by the medical officer, at the time of their admission in the hospital for determination of ethanol. When the case was taken up for examination, it was observed from the medico-legal report that the driver was a diabetic

person and hence there was a big possibility of presence of acetone in the blood and urine samples of the driver, which is readily oxidized by Potassium dichromate thus contributing in the enhanced value of resultant ethanol contents. So the application of cavett method for determination of ethanol in such cases would have led to injustice to the person. Hence alternatively, Kojelka-Hine method was used to eliminate the contribution of acetone towards oxidation of dichromate [1,4,5]. At the same time, cavett method was also applied to blood and urine samples to see the amount of conribution of acetone towards the oxidation of dichromate. The preference of application of Kojelka-Hine method over the cavett method becomes important for fair trial in such cases where the person is diabetic or the patients who have been medicated with urotropine, salicylates or other such derivatives [1,4,5].

Thus the health record or treatment history of the person can influence the route of examination of scientific evidences, and it becomes necessary for the forensic toxicologist to get such type of information from the investigating agency for fair delivery of the justice to the public. The blood and urine samples were analyzed in quadruplet sets at the same time i.e. four sets of blood sample and four sets of urine sample of each person were analyzed by Kojelka-Hine method to give four concordant readings in each case. Their mean value was taken to reach the conclusion.

The uncertainty of measurement of a value plays a crucial role in fair trial in a court of law and it should be included while reporting the results because it can be helpful in acquittal or conviction of the person [8,9].

If the blood alcohol concentration during driving is punishable beyond 70mg% and the result comes out to be 73 mg% with UOM ±5mg%, then lower side of the result i.e. 68mg% (73-5= 68) shall be considered in court of law so as to adhere to basic principle that None of the innocent should be punished, whatever number of accused get acquitted'. In the present work also, UOM was calculated and final result was reported [8,9].

In the present case, the person, who expired, was under insurance cover and when his wife filed claim from the insurance company, the claim was rejected on the grounds of drunken driving. The lady also lost the case in the court of law despite inclusion of uncertainty of measurement in the result as the blood and urine alcohol concentration determined was far beyond the set limit of 30 mg% as per Section 185 of 'The Motor Vehicle Act, 1988' of Government of India.

Materials and Methods

• *Apparatus and Accessories*

a. Kojelka-Hine Glass apparatus [1,4,5].
b. Cavett cup apparatus [1,4].
c. Micropipette (LABQUEST) of Borosil make, with variable volume capacity of 100-1000 µl was used.

• *Chemicals and Reagents*

Potassium dichromate, Sodium thiosulphate, Conc. Sulphuric acid, Mercuric chloride, Potassium iodide from Merck Germany, freshly prepared water, starch solution were used.

• *Glassware*

Burette 50ml and 10ml capacity, test tube, Erlenmeyer flask of 150ml capacity, Volumetric flasks 500ml & 1 liter from Borosil India were used. Prior to use, all glassware were thoroughly washed and rinsed 2-3 times with water and dried in oven.

• *Preparation of Reagents*

a. 0.1N acidified Potassium Dichromate: This solution was prepared by dissolving 4.903 gm K_2Cr_2O7 in 1 liter solution of 60% v/v Sulphuric acid in water.
b. 0.1N Sodium Thiosulphate: This solution was prepared by dissolving 24.818 gm of Sodium Thiosulphate ($Na_2S_2O_3.5H_2O$) in water.
c. Saturated solution of mercuric chloride: This solution was prepared by mixing 7gm of $HgCl_2$ per 100 ml of water.
d. Saturated solution of Sodium Hydroxide: 600 gm NaOH in 500 ml water.
e. Potassium Iodide Solution: 20% w/v of KI in water.
f. Starch solution: 1% in water.
g. Sodium Tungstate solution: 10% in water.
h. Sulphiric acid, Approx. 1N: 30ml of conc. Sulphuric acid dissolved in 1liter water.

Note: The term water mentioned in this text means double distilled water.

• *Method*

Kojelka-Hine glass apparatus and Cavett apparatus were cleaned with chromic acid and then

rinsed with water six times. The cleaned apparatus were dried in oven. The kojelka-Hine and Cavett apparatus were set up as shown in Fig.1 and Fig.2 respectively.

a. Kojelka & Hine Method [1,4,5]: Kojelka & Hine apparatus was set up as shown in Figire 1. One ml of blood sample (with the help of micropipette), 5 ml of Sodium Tungstate and 5 ml of the 1N Sulphuric acid were introduced in the distillation tube D. 10 ml of saturated solution of $HgCl_2$ and 10 ml of saturated solution of NaOH were added to tube E and mixed. Inserted the stopper (Tube C), lubricating the joint with graphite and connected the train as shown in Figure 1. The tubes were kept in beaker of boiling.

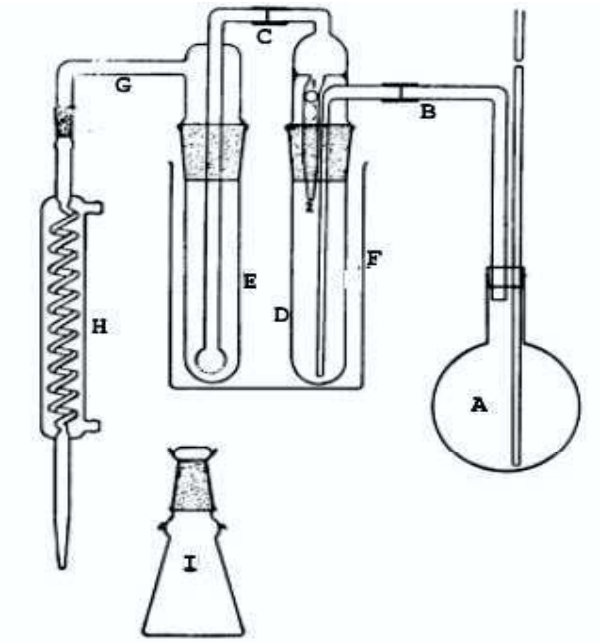

Fig. 1: Assembled apparatus of Kojelka and Hine

A. Round bottom flask 500ml (a steam generator) with air pipe; B. U shaped glass tube (having ground joint in middle) with one end opening in flask A near its neck and other end opening at the bottom of Large tube D; C. U shaped glass tube (having ground joint in middle) with one end opening in tube D near its neck and other end (porous bulb) opening at the bottom of Large tube E; D & E. Large tubes with ground glass mouth; F. Water bath, 2 litre beaker; G. Glass tube connecting C & condenser as shown; H. Glass Condenser; I. Receiver-150 ml Erlenmeyer flask with cap.] water and passed the steam originating from generator A through heated tubes D & E. Put the flask I to receive the distillate from condenser H, with condenser tube well inside the flask I. 25 ml of distillate was collected. The volatile compounds like acetone, formaldehyde are retained behind from the distillate. Ethanol from blood reaches in distillate.

After collection of 25 ml of distillate, process of steam distillation was stopped and receiver I was removed. To the distillate, 10 ml of 0.1N Potassium Dichromate solution was added slowly, and stopper was put in place. The mixture was shaken slowly to mix the contents and then placed in boiling water for 20 minutes. Then, cooled the solution of receiver I and diluted with about 10 ml of water. To it, 10 ml of Potassium Iodide solution was added and wait for 20 minutes to allow it to completely react with unused Potassium Dichromate and then titrated the liberated iodine with 0.1N Sodium Thiosulphate, using starch solution as indicator (with light blue coloured end point). Subtracted the average blank value for normal blood. Total four such readings were taken for each blood and urine sample with this method.

Chemical Reactions Involved

$$K_2Cr_2O_7 + 4H_2SO_4 \rightarrow K_2SO4 + Cr_2(SO_4)_3 + 4H_2O + 3O$$
$$CH_3CH_2OH + 2O \rightarrow CH_3COOH + H_2O$$

Each milliliter of 0.1N $K_2Cr_2O_7$ used is equivalent to 1.15 mg alcohol.

Observations & Calculation

Table 1: Data of blood/Urine Sample of Man (Driver)

Kojelka & Hine method	Blood/Urine Sample of Man (Driver)							
	Blood sample Replica 1	Blood sample Replica 2	Blood sample Replica 3	Blood sample Replica 4	Urine sample Replica 1	Urine sample Replica 2	Urine sample Replica 3	Urine sample Replica 4
Amount of sample taken	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml
Amount of 0.1N Potassium Dichromate taken	10 ml	10 ml	10 ml	10 ml	10 ml	10 ml	10 ml	10 ml
Value of Blank (B)	10.1 ml	10.1 ml	10.1 ml	10.1 ml	10.1 ml	10.1 ml	10.1 ml	10.1 ml
Amount of 0.1N Sodium Thiosulphate consumed (T)	9.3 ml	9.3 ml	9.2 ml	9.3 ml	8.8 ml	8.9 ml	8.9 ml	8.8 ml
Amount of 0.1N Potassium Dichromate consumed by test sample Y= (B-T)	0.8 ml	0.8 ml	0.9 ml	0.8 ml	1.3 ml	1.2 ml	1.2 ml	1.3 ml
Conc. Of ethanol in test sample = Y×1.15×100/volume of test sample	92 mg%	92 mg%	103.5 mg%	92 mg%	149.5 mg%	138 mg%	138 mg%	149.5 mg%

Table 2: Data of Blood/Urine Sample of Lady

Kojelka & Hine method	Blood/Urine Sample of Lady							
	Blood sample Replica 1	Blood sample Replica 2	Blood sample Replica 3	Blood sample Replica 4	Urine sample Replica 1	Urine sample Replica 2	Urine sample Replica 3	Urine sample Replica 4
Amount of sample taken	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml
Amount of 0.1N Potassium Dichromate taken	10 ml	10 ml	10 ml	10 ml	10 ml	10 ml	10 ml	10 ml
Value of Blank (B)	10.2 ml	10.2 ml	10.2 ml	10.2 ml	10.2 ml	10.2 ml	10.2 ml	10.2 ml
Amount of 0.1N Sodium Thiosulphate consumed (T)	10.1 ml	10.2 ml	10.2 ml	10.1 ml	10.2 ml	10.2 ml	10.2 ml	10.1 ml
Amount of 0.1N Potassium Dichromate consumed by test sample Y= (B-T)	0.1 ml	Nil	Nil	0.1 ml	Nil	Nil	Nil	0.1 ml
Conc. Of ethanol in test sample = Y×1.15×100/volume of test sample	11.5 mg%	Nil	Nil	11.5 mg%	Nil	Nil	Nil	11.5 mg%

Table 3: Calculations of Uncertainty of Measurement (UOM)[8,9]

Kojelka & Hine method	Blood sample of Man (Driver)	Urine sample of Man (Driver)
Measured values (n) 1, 2, 3 & 4	92, 92, 103.5, 92	149.5, 138, 138, 149.5
Mean of all measured values (A)	94.875	143.75
Deviations from Mean value (B)	-2.875, -2.875, -2.875, 8.625	5.75, -5.75, -5.75, 5.75
Value of Square of all B values	8.265625, 8.265625, 8.265625, 74.390625	33.0625, 33.0625, 33.0625, 33.0625
Sum of square of all B values (C)=	99.1875	132.25
Standard Deviation (S)=√(C/n-1)	5.75	6.6395
Standard uncertainty (UA)= S/√n	2.875	3.32
Degree of freedom	3	3
Uncertainty due to burette 50 ml (From Calibration certificate) (UB1)	0.05	0.05
Uncertainty due to measuring cylinder10 ml (From Calibration certificate) (UB2)	0.05	0.05
Uncertainty due to micropipette 1 ml (From Calibration certificate) (UB3)	0.01	0.01
Total uncertainty (UOM)= √(UA²+UB1²+UB2²+UB3²)	2.49	3.32
Expression of the result of Ethanol concentration with UOM	94.9mg% ± 2.5	140.9mg% ± 3.3

b. Cavett Method[1,4]: The blood and urine samples of the Man and Lady were also analyzed with cavett method using the chemicals of same strength as mentioned in Kojelka & Hine method. The apparatus was setup as shown in Figure 2.

The 10 ml of acidified 0.1N Potassium Dichromate was taken in flask A. One ml of blood/urine sample was taken in Cavett cup B and immediately put into the flask as shown in Figure 2. The joint was sealed with water. The apparatus was kept in incubator at 45°C for two and a half hour. Then it is taken out and cooled at room temperature. The Cavett cup was taken out and 10 ml of Potassium Iodide solution

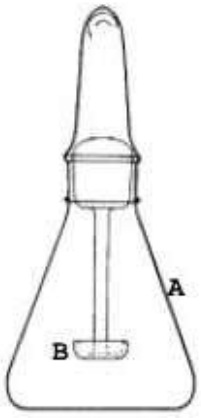

Fig. 2: Assembled apparatus of Cavett Cup; A. 150 ml Flask; B. Cavett Cup attached with glass top with a glass rod.

was added and wait for 20 minutes to allow it to completely react with unused Potassium Dichromate and then titrated the liberated iodine with 0.1N Sodium Thiosulphate, using starch solution as indicator (with light blue coloured end point). Subtracted the average blank value for normal blood, as was done in Kojelka & Hine method. Due to limited availability of samples, only single reading of each of blood & urine sample of Man (Driver) and Lady was taken with this method.

Observations & Calculation

Table 4: Data of blood/Urine Sample of Man (Driver)

Cavett cup method	Blood/Urine Sample of Man (Driver)		Blood/Urine Sample of Lady	
	Blood sample	Urine sample	Blood sample	Urine sample
Amount of sample taken	1 ml	1 ml	1 ml	1 ml
Amount of 0.1N Potassium Dichromate taken	10 ml	10 ml	10 ml	10 ml
Value of Blank (B)	10.1 ml	10.1 ml	10.2 ml	10.2 ml
Amount of 0.1N Sodium Thiosulphate consumed (T)	9.1 ml	8.6 ml	10.1 ml	10.2 ml
Amount of 0.1N Potassium Dichromate consumed by test sample Y= (B-T)	1.0 ml	1.5 ml	0.1 ml	0
Conc. Of ethanol in test sample = Y×1.15×100/volume of test sample	115 mg%	172.5 mg%	11.5	NIL

Results and Discussion

The value of alcohol contents reported by a Forensic Chemist in his report has a direct impact in fair trial of accused in Motor Vehicle Tribunals. The value of measurement of Uncertainty plays a vital role in cases where the reported value of ethanol contents lies near the threshold value of 30 mg% in Indian context.

The person gets acquitted easily, if the inclusion of measurement of Uncertainty lowers down the ethanol contents of blood and urine samples to below 30 mg%. But in this case, the values of ethanol content in blood and urine samples of Man (Driver) were 115 mg% and 172.5 mg% respectively by Cavett cup method; and 94.9 mg% & 140.875 mg% in blood and urine samples with uncertainty of measurement ±2.5 and ±3.3 respectively by Kojelka & Hine method. The higher values of results of Cavett cup method can be accounted for diabetic condition of the person.

Though, in this case, the results of both the methods were far beyond the set limit of 30mg% as per Motor Vehicle Act of India and it made no difference for the accused to be prosecuted whether the results were reported on the basis of Cavett method or Kojelka & Hine method, a forensic scientist should prefer the Kojelka & Hine method over Cavett method while reporting the results of ethanol contents in blood/urine samples in cases where the accused/ patient is suffering from diabetes for a fair trial in the Criminal Justice Deliver System.

Conclusion

For fair trial in Indian Criminal Justice Delivery System, the reports by Forensic scientists should be prepared objectively, without polarizing the mind. This article describes the preference of one method over the other in a particular condition of the person. Kojelka & Hine method's applicability over the Cavett method helps the Forensic scientists to prepare the results of ethanol contents in blood/urine samples more judicially.

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