

# Identification and Estimation of Acetaldehyde, Acetic Acid and Ethyl Acetate in Suspected Country Liquor

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## ABSTRACT

Liquor has been used in India for a very long time, but the amount consumed and problems associated have increased in recent years. Liquor is an alcoholic drink produced by distilling ethanol produced by means of fermenting grain, fruit or vegetables. In common usages, the distinction between spirits and liquors is widely unknown or ignored; consequently all alcoholic beverages other than beer and wine are generally referred to as spirits. In the human body, ethanol is first oxidized to acetaldehyde and then to acetic acid. The first step is catalyzed by the enzyme alcohol dehydrogenase and the second by acetaldehyde dehydrogenase. The acetic acid can be used to form fatty acids or can be further broken down into carbon dioxide and water. This paper analyzed 15 samples of suspected country liquor collected from the Excise department of Allahabad, using BIS methods of country liquor. In some samples, acetaldehydes, acetic acid and ethyl acetate were absent. The ethanol content varied from 33 to 44 % v/v in 15 samples, and the limit of the permissible of 36 to 42.8 % v/v. High content of acetaldehyde, above the permissible limit of 0.35 gm./liters, was observed in 13 samples. High content of acetic acid, above the permissible limit of 0.50 and 1.0 gm./liters, was observed in 13 samples. High content of ethyl acetate, above the permissible limit, was observed in 12 samples. This work evaluated the human health risk associated with the poor quality of suspected country liquor.

**Keywords:** Liquor, country liquor, beverages

## 1. Introduction

Liquor is an alcoholic drink produced by distilling ethanol produced by means of fermenting grain, fruit or vegetables. Liquor can be divided into country liquor and foreign liquor. Country liquor means “all liquors” other than power alcohol, rectified spirit and perfumed spirits. They also include “LAHAN” a fermented wash and all fermented liquors prepared according to the indigenous and can be divided into-

Plain or spiced spirit are made from mahuaflower, rice and gur or molasses. Toddy is an alcoholic drink made by fermenting the sap of a coconut palm is available in India which is white and sweet with a characteristics and is popular among the lower socio-economic groups in south India [5]. Contrary to toddy and the rice brews which are not considered harmful to health- being natural brews, the lightly fermented distillates are strong alcoholic drinks [2]. Jack- fruit wine is an alcoholic beverages made by ethnic groups in

the eastern hilly areas of India. As its name suggests, it is produced from the pulp of jack-fruit and its alcohol content is about 7 per cent to 8 per cent within fortnight [1]. Zu and Rohi are also locally brewed alcoholic beverages found in Nagaland. The liquor market in Uttar Pradesh is witnessing a radical change these days with distilleries engaged in the production of country made liquor being pushed out by IMFL units. The New Excise policy of the UP government for promoting investment in distilleries has sparked off the process of a shake up in the liquor industry. Existing distilleries were either closing down or changing hands following the new policy fixing cap of a minimum investment of Rs 100 crore.

In the human body, ethanol is first oxidized to acetaldehyde and then to acetic. The first step is catalyzed by the enzyme alcohol dehydrogenase and the second by acetaldehyde dehydrogenase. Some individuals have less effective forms of one or both of these enzymes and can experience more severe symptoms from ethanol consumption than others. Conversely, those who have acquired ethanol tolerance have a greater quantity of these enzymes, and metabolize ethanol more rapidly. The breakdown or oxidation of ethanol occurs in the liver. Alcohol dehydrogenases strip electrons from ethanol to form acetaldehyde. Another enzyme, aldehyde dehydrogenase converts the acetaldehyde, in the presence of oxygen, to acetic acid, the main component in vinegar.

Adulteration is quite frequent, which occasionally causes incidents like mass poisoning with consumer losing their lives or suffering irreversible damage to the eyes. It is a major risk factor for health harms, and it also

contributes to a personal and social burden of disease and injury [7]. This research analyzed fifteen samples of suspected country liquor and was tested for identification and estimation of acetaldehyde, acetic acid and ethyl acetate and also compared the obtained value with standard value of BIS throughout a three-month period. In the end, the research provided information about liquor, which compound was present or absent, how much adulterated beyond the permissible limit. The research also serves as a foundation for further work on identification and estimation of suspected country liquor from different methods.

## 2. Materials and Method

Fifteen samples of suspected country liquor were collected from Excise Department of Allahabad. The chemical analyses were carried out on 15 samples of suspected liquor. For identification used specific chemical test of these compounds. For quantification, the methodologies used in these analyses were based on the BIS (Bureau of Indian Standard) methodologies for analyses of country spirits (IS 3752: 2005) [3].

### Procedure of Identification Test

#### 1. Tollen's test for aldehyde as Acetaldehyde

A small quantity of  $\text{AgNO}_3$  was taken in a test tube. Dil.  $\text{NaOH}$  (few drops) was added in it. Brown precipitate of silver oxide was formed. Then, few drops of  $\text{NH}_4\text{OH}$  were added in it. After that, precipitate got dissolved. Then, small quantity of suspected country liquor was added

in it and it was heated for some time. Aldehyde was confirmed when silver mirror was appeared.

## 2. Ester test for volatile acids as Acetic acid

A small quantity of suspected country liquor was taken in a test tube. A small quantity of ethyl alcohol and conc.  $H_2SO_4$  (few drops) were added in it. After that, heated the mixture in the water bath for two minutes. Then, the mixture was poured into the distilled water. Acetic acid was confirmed when fruity smell was produced.

## 3. Hydroxylamic Acid test for ester as Ethyl Acetate

A small quantity of suspected country liquor was taken in a test tube. Then, hydroxylamine HCL (1 mL, 0.5M solution) and NaOH (0.2 mL, 6M) were added in it. The mixture was boiled for some time. After that, cooled it and HCL was added in it. If the solution was cloudy then 1-2 mL of ethanol was added to make it clear and also ferric chloride solution (1-2 drops) was added in it. Ester was confirmed when magenta color was appeared.

### Procedure of Estimation Method

#### 1. Estimation of Ethyl Alcohol by Specific Gravity method

The pycnometer was filled with alcoholic Liquid. The thermometer was dipped in to liquid and the exact temperature of liquid was noted in pycnometer before the performed

stopper was inserted then weighed the pycnometer or specific gravity with alcoholic liquid. This filled at  $t^0$  c the pycnometer by subtracting the weight of empty specific gravity bottle or pycnometer. The weight so obtained was divided by water equipment. That was the weight in air of some value of water contact in the pycnometer at  $15^0$  C. That gave the specific gravity of alcohol. Liquid in air at  $t^0C / 15^0C$ . The temperature was recorded and specific gravity was calculated.

#### Observation:-

Weight of empty pycnometer =  $w_1$  (in gm.)

Weight of pycnometer + distill water =  $w_2$  (in gm.)

Weight of pycnometer + liquid =  $w_3$  (in gm.)

#### Calculation:-

$$w_3 - w_1 / w_2 - w_1$$

The specific gravity of the alcohol was calculated at the required temperature. Obtained corresponding alcohol percent by volume, from the table given in IS 3506-1967 (Indian standard table for alcoholometry by pycnometer).

#### 2. Estimation of Aldehydes as Acetaldehydes

Firstly, distillate of liquor (50 mL) was taken in a 250 mL iodine flask and added 10 ml of bisulphite solution. The flask was kept in a dark place for 30 min with occasional shaking. Then, standard iodine solution was added in it and back titrated excess iodine against standard sodium thiosulphate solution using starch as indicator. Then, ran a blank distilled water (50mL) was taken in place of the distillate of the liquor in the same way. The different in titration

value in milliliter's of sodium thiosulphate solution were given the equivalent aldehydes.(one milliliter of standard solution of thiosulphate is equal to 0.0011g of acetaldehyde.).

### Calculations

Aldehydes expressed as acetaldehydes, gm./ liters of absolute alcohol.

$$= (0.0011 \times 2 \times V \times 1000) / V_1$$

Where,

V = Difference in ml of standard sodium thiosulphate solution used for blank and experiment,

$V_1$  = Alcohol percentage by volume.

### 3. Estimation of Volatile Acids as Acetic Acid

Firstly, distillate of liquor (50mL) was taken in a flask and titrated against standard sodium hydroxide solution using phenolphthalein as indicator. (one milliliters of standard hydroxide is equivalent to 0.003g of acetic acid.)

#### Calculation

Volatile acids expressed as acetic acid, grams per liters of absolute alcohol

$$= (V \times 0.003 \times 1000 \times 2) / V_1$$

Where,

V= volume of standard sodium hydroxide used for titration in ml

$V_1$  = alcohol percent by volume.

### 4. Estimation of Esters as Ethyl Acetate

To the neutralized distillate from the volatile acidity determination, 10 mL of standard alkali was added in it and refluxed it for an hour. Cooled and back titrated the excess of alkali with standard sulphuric acid. Simultaneously ran blank, distilled water (50ml) was taken in place of the distillate of the sample in the same way. The difference in titration value in milliliters of standard acid solution was given the equivalent ester.

#### Calculation:-

Esters expressed as ethyl acetate, grams /liters of absolute alcohol

$$= (V \times 0.0088 \times 1000 \times 2) / V_1$$

Where,

V= difference of standard acid used for blank and samples, in ml

$V_1$  = alcohol percent by volume

### 3. Results

This work was carried out to identify and estimate the quantity of acetaldehyde, acetic acid and ethyl acetate and compare with their standard value which was given by BIS. After experiment following results were obtained:-

**Table 3.1:- Reading of hydrometer and result for identification test of aldehyde as Acetaldehyde , volatile acids as Acetic acid and ester as Ethyl Acetate in given suspected country liquor:-**

S.No.	Samples of country Liquor	Aldehyde	Acetic acid	Ester	Reading on hydrometer
1	Sample-1	+ve	+ve	- ve	930
2	Sample-2	+ve	+ve	-ve	960
3	Sample-3	+ve	+ve	+ve	970
4	Sample-4	+ve	+ve	-ve	950
5	Sample-5	+ve	+ve	+ve	980
6	Sample-6	+ve	+ve	+ve	930
7	Sample-7	+ve	-ve	+ve	920
8	Sample-8	+ve	+ve	+ve	975
9	Sample-9	-ve	+ve	+ve	990
10	Sample-10	+ve	+ve	+ve	940
11	Sample-11	+ve	+ve	+ve	965
12	Sample-12	+ve	+ve	+ve	980
13	Sample-13	+ve	+ve	+ve	960
14	Sample-14	-ve	+ve	+ve	950
15	Sample-15	+ve	-ve	+ve	970

The silver mirror (brown) indicates positive test for the presence of aldehyde by Tollen's test. Fruity smell indicates positive test for the presence of acetic acid by ester test. Appearance of magenta color indicates the presence of ester by Hydroxylamic acid test. Aldehydes were present in all the sample except 9 and 14. Acetic Acid were present in all the sample except 7 and 15. Esters were present in all the sample except 1,2 and 4. The reading on the hydrometer showed the values of specific gravity less than 1000 for each sample. It implies that these liquor samples were distilled in nature.

**Table 3.2:- Result for the estimation of Ethyl alcohol, Acetaldehyde, Acetic acid and Ethyl acetate in given samples:-**

S.No	Samples of liquor	Types of country Liquor	Ethyl Alcohol (% v/v)		Aldehydes as Acetaldehyde (in gm./liters)		Volatile acids as Acetic acid (in gm./liters)		Ester as Ethyl acetate (in gm./liters)	
			Obtained Value	BIS Value	Obtained Value	BIS Value	Obtained Value	BIS Value	Obtained Value	BIS Value
1	Sample-1	Blended	42.8	36% and 42.8 % v/v	2.45	0.35 gm. / liters	3.65	1.0 and 0.5 gm / liters	.....	1.50 and 1.0 gm /liters
2	Sample-2	Blended	42.8		1.90		1.90		.....	
3	Sample-3	Plain	37.25		3.00		2.40		4.2	
4	Sample-4	Spiced	36		2.80		1.76		.....	
5	Sample-5	Spiced	43.5		1.85		2.80		2.6	
6	Sample-6	Blended	36		2.00		3.5		3.52	
7	Sample-7	Spiced	42.8		4.25		.....		4.32	
8	Sample-8	Blended	42.8		3.40		1.53		0.90	
9	Sample-9	Plain	44.25		.....		4.25		1.93	
10	Sample-10	Blended	36		3.20		3.28		2.63	
11	Sample-11	Blended	42.8		2.40		2.35		1.25	
12	Sample-12	Blended	33.45		4.75		2.0		1.76	
13	Sample-13	Plain	42.8		0.90		1.98		3.0	
14	Sample-14	Plain	39.3		.....		2.85		4.65	
15	Sample-15	Blended	36		2.50		.....		2.80	

According to State Excise Authority, the standard value of the Ethyl alcohol is 36 and 42.8 % v/v. The amount of the Ethyl Alcohol present in the sample 3, 5, 9 and 14 were beyond the permissible limit. In sample 12, the ethyl alcohol content was lower than the standard value. According to BIS (IS 5287: 2005) [4], the standard value of Aldehydes as Acetaldehyde of Plain, Blended and Spiced country liquor are 35 gm./100 liters (for this study, gm./100 liters was converted into gm./liters). Acetaldehyde were

absent in the sample no- 9 and 14. The standard value of volatile acids as Acetic acid of Plain and Spiced country liquor are 100 gm./100 liters and blended country liquor is 50gm./100 liters (for this study, gm./100 liters was converted into gm./liters). Acetic acid were absent in the sample no- -7 and 15.). The standard value of ester as Ethyl acetate of Plain, and Spiced country liquor are 150 gm./100 liters and blended country liquor is 100 gm./100 liters (for this study, gm./100 liters was converted into gm./liters). Ethyl acetate were absent in the sample -1, 2 and 4. According to Bureau of Indian Standard, the amount of ester as ethyl acetate, acetaldehyde and acetic acids present in each sample were beyond the permissible limit. It implies that these samples were illicit and non-consumable.

#### 4. Discussion

The suspected country liquor have attracted the attention of BIS, due to the presence of compounds such as acetaldehydes, acetic acid, ethyl acetate and other toxic compounds. There have been discussions on whether or not these substances can produce serious illness effects in humans. Many studies were carried out to evaluate the chemical analysis of suspected country liquor with the aim to investigate the possible health impact of country liquor. A study was carried out the analyses of methanol, ethanol and other compounds content in 27 different illicit liquor in the northern region of the India. Ethanol content varied drastically and methanol was not present in any of the samples. The components found can be categorized into different groups, namely alcohols, esters, acids, ketones and aldehydes [6].

In this study, the contamination with acetaldehydes, acetic acids and ethyl acetate was analyzed in 15 samples of suspected country liquor. Besides this the content of ethyl alcohol, was also evaluated. A similar extensive study was carried out in the northern region of the India 27 samples of illicit liquor [6].

As shown in Table 3.1, the reading on the hydrometer shows the values less than 1000 for

each sample. It implies that these liquor samples were distilled in nature. As shown in Table 3.2, some of the suspected country liquor, the content of ethyl alcohol was beyond the permissible limit which was also observed by the other authors. These samples may be injurious to health.

Acetaldehyde is the more toxic than ethanol with documented cases of poisonings, including headache, nausea and liver cirrhosis etc. In this study, the acetaldehyde were beyond the permissible limit in all the suspected country liquor samples except, two samples, acetaldehyde were absent as shown in Table 3.2. Volatile acids as acetic acids, its legal limit are 0.5 gm. /liters and 1.0gm./liters. The acetic acid can be used to form fatty acids or can be further broken down into carbon dioxide and water. As shown in Table 3.2, the acetic acid were beyond the permissible limit in all the suspected samples. In two samples, acetic acid were absent.

The contamination with ethyl acetate was also found in many liquors. This problems occurs when the homemade liquor are produced from fermented materials such as grains, potatoes etc and by adding flavoring agent. As shown in Table 3.2, the ethyl acetate were beyond the permission limit in all the suspected liquor

samples. In three samples, ethyl acetate were absent.

## 5. Conclusion

During thesis work, 15 samples were analyzed by chemical test and quantification was also carried out for acetaldehyde, acetic acid and ethyl acetate using BIS methodologies for country liquor. Most of the samples were showed positive (+ve) presence of aldehyde as acetaldehyde, volatile acids as acetic acid and ester as ethyl acetate using specific test. Some of the samples were showed negative (-ve)

presence in the qualitative test. Aldehyde were absent in the sample no.-9 and 14. Acetic Acid were absent in the sample no. - 7 and 15. Ester were absent in the sample no-1, 2 and 4. In all samples, acetaldehyde, acetic acid and ethyl acetate were present beyond the permissible limit as per BIS. On the basis of the analysis, it was concluded that all the 15 samples were illicit in nature, non-consumable for the society and should be prohibited. Further, the population should be informed about the risk of consumption of spurious liquor.

## 6. References

1. Battcock, M. and Azam-Ali, S. (1998) Fermented fruits and vegetables: a global perspective. *Food and Agriculture Organization of the United Nations, Rome, Italy*, <http://www.fao.org/docrep/x0560e/x05609.htm>.
2. Chakraverti, S. (2004) Prohibitions. (<http://www.ccsindia.org/antidote/chapter%207.doc>).
3. IS 3752 (2005) Alcoholic Drinks-Country Spirit. (<https://archive.org>is.5287.2005.djvu.txt>).
4. IS 5287 (2005) Alcoholic Drinks-Country Spirit. (<https://archive.org>is.5287.2005.djvu.txt>).
5. Lal, J., Kumar, C.V., Suresh, M.V., Indira, M. and Vijayammal, P.L. (2001) Effect of exposure to country liquor during gestation on lipids metabolism in rats. *Plant Foods for Human Nutrition, In National Institute on Alcohol Abuse and Alcohol Problems Science Database (online database). National Institute on Alcohol Abuse and Alcoholism, Bethesda, 56 (2), 133-143.*
6. Punia, B.S., Yadav, P.K., Bumrah, G.S. and Sharma, R.M. (2017) Analysis of illicit liquor by HS-GC-MS. *Journal of the Association of Official Analytical Chemists International*, 100, 109-125.
7. Rehm, J., Mathers, C., Popova, S., Thavorncharoensap, M., Teerawattananon, Y. and Patra, J. (2009) Global burden of disease and injury and economic cost attributable to alcohol use and alcohol use disorders. *Lancet*; 373, 2223-2233.