International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Detection And Quantification of Alcohol in Home Made Rice Beer of Tripura Using Headspace Gc-Fid

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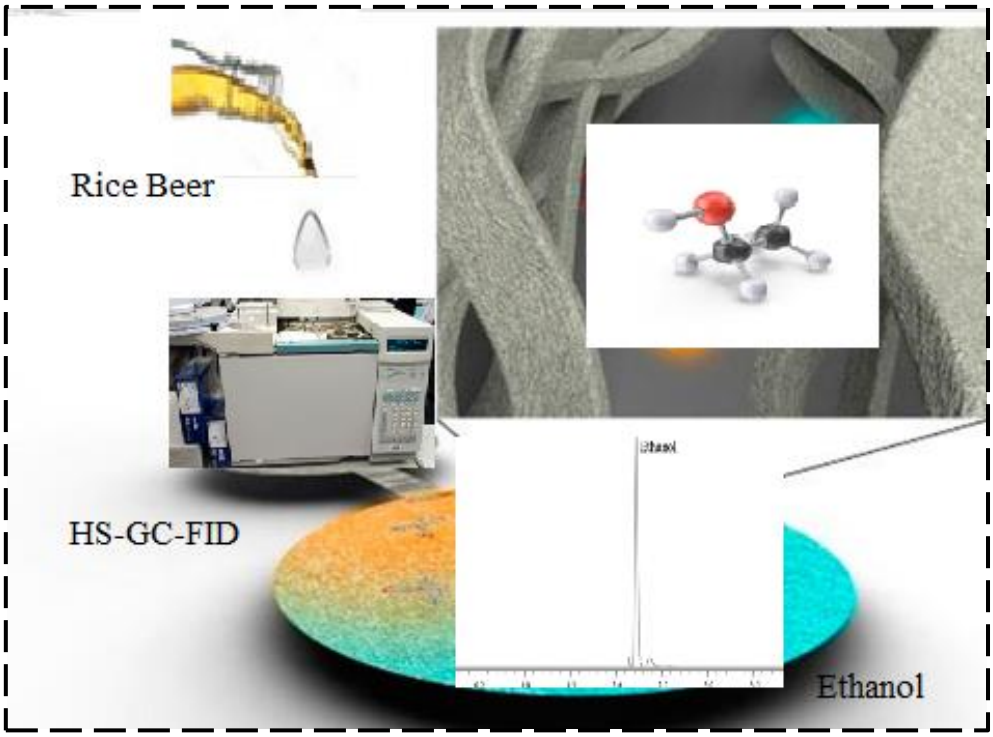
Abstract

Traditional rice beer is an indispensable part of tribal people's life, it attached economically, culturally, and spiritually with them. The excessive consumption of alcohol may lead to fatal accidents, crime of violence, antisocial activity and in some cases death also. The present study aimed to qualify and quantify alcohol from home-made rice beer of three different district (West Tripura, South Tripura and Sepahijala) of Tripura by using Headspace Gas Chromatography-Flame Ionization Detector (HS-GC-FID). Analysis was performed on 3 distinct samples of each district. The amount of ethanol present in sample 1, sample 2 and sample 3 is respectively 0.0769%, 0.1006% and 0.1057%. No evidence for presence of methanol indicates that the sample is free from adulterants like methanol.

Keywords: Ethanol, HS-GC-FID, Rice Beer, Detection



INTRODUCTION:

Tripura is the third smallest and one of the most naturally beautiful state in the country of India which covers 10.491 sq. km areas. There are 19 different tribes, namely Bill, Bhutia, Chaimal, Chakma, Garos, Halam, Khasia,Jamatia, Noatia, Orang, Reang, Munda,Tripuri, Lusai, Kukis, Uchoi, Mog, Santal and Lepcha (Debbarma et al. 2021).It relishes tropical weather, marked by heat and humidity and having an average rainfall of 2,500 mm/y. There are eight districts in Tripura, namely West Tripura, South Tripura, Gomati Tripura, North Tripura, Dhalai, Unakoti, Khowai, and Sepahijala district. According to the social composition among the total population of Tripura, around one-third of the population belongs to the Scheduled Tribes, according to the 2011 census (Ghosh et al. 2016). The rural ethnic population depends substantially on nature for their survival. They hold a strong sense of traditional knowledge for natural resources and environment. Traditional rice beer is an indispensable part of their life, attached economically, culturally, and spiritually with them, and plays a very prominent role in their socio-cultural activities (Saikia et al. 2007).

PREPARATION OF STARTER CULTURE (CHUWAN BELEB):

Except for the fundamental ingredient, "rice flour for starter culture," the raw materials used to prepare starter culture vary between tribes. Other associated raw materials, such as bark and leaves, vary by tribe. The rice is first soaked for two hours before being ground into a fine paste. Dried, finely powdered, and sieved are some of the additional associated raw materials. To make the dough, all of the ingredients are mixed together, and a small amount of water is added. In honor of God, the dough is used to make the first cake, “chuwan chwla”, and a handful of dough is used to make “chuwan beleb”. A “chuwan beleb” can weigh anywhere from 50 to 100 grams. The cakes are then left to dry in the sun and stored for later use (Ghosh et al.2016).

METHODOLOGY OF RICE BEER PRODUCTION:

Rice (Oryzae sativa L.) based alcoholic beverage preparation (after fermenting) is a most common and famous traditional drink of all the tribes of Tripura. Traditional alcoholic beverages and its nomenclature different community wise, like as “Chuwak” in Debbarma, Jamatia, Koloi, and Tripuri, “Arak” in Reang and “Zu” in Molsom. The main ingredients of Langi (undistilled) is rice, water, yeast tablet (known as Chuwan). The stepwise preparation procedure of langi is -----The rice is fully boiled for half an hour and spread over a bamboo mat for cooling. Mixing with chuwan was done to make a paste by using a traditional huller. The mixture is then tightly packed in an earthen container (locally known as Mutka) for fermentation and the mouth portion is closed by earthen lid, leaf, etc. The mutkas are left in this condition for 3 days. After that water is added up to the mouth portion of the mutka and kept for 6-8 hrs. The white coloured transparent liquid which floats at the top is known as ‘langi’ or ‘Gora’. Traditional wine (locally known as Bangla) is prepared from Langi after further processing. Langi is heated in an earthen pot having some holes at the top which helps to evaporate the vapour. Pot contains a narrow pipe from that portion which collects the vapours in droplets which is known as “local wine”. (Debbarma et al. 2021)

Alcohol is one of the most popular liquors used by community. The excessive consumption of alcohol may lead to fatal accidents, crime of violence, antisocial activity and in some cases death also. Liquors come to Forensic Chemistry laboratories as and when encountered in illegal sale and possession, adulteration, evasion of taxes, transportation from one district to another district or to other states and in cases involving alleged history of driving under the influence of alcohol. In few cases liquors are forward

to Forensic laboratory for analysis of various drugs present or not especially date rape drug like Rohypnol (Chophi R & Yadav PK, 2019).

The percentage of alcohol content also varies due to disparate fermentation process, distillation process and also with respect to community wise (specific geographical area) ingredients used for producing alcohol. Hence characterization of liquors assist not only in discerning various types of liquor brands even so also gives an impactful result about the geographical region from where it might have originated.

Alcohol may be act as poison if consumed as large amount or due to presence of some adulterants like methanol, acid, etc. Methanol poisoning causes blindness, metabolic acidosis, respiratory failure, coma, and death. Death due to toxic effect of alcohol are most common in different parts of rural areas of India (Kruse JA,1992).

Gas chromatography (GC) is a method that is frequently utilized in forensic laboratories for the purpose of analyzing alcohol in biological samples. According to Mermet (2004), this is frequently utilized in conjunction with a headspace auto sampler and flame ionization detection (FID), also known as headspace GC-FID. Headspace is based on Henry's law, which states that at a given pressure and temperature, the ratio of a volatile substance's concentration in the gas phase of a vessel to its concentration in the liquid phase is constant (Atkins, 2017).

Fig.1- The method of traditional rice beer preparation. (A) The starter culture. (B) Starter culture mix with the boiled rice. (C) Boiled rice was kept for initial fermentation. (D) The mixture of the rice covering with banana leaves. (E, F) The different traditional arrangements for the distillation of rice beer (Fi) batibakhrak (Fii) bati sabasa (Fiii) Patini (G) The traditional rice beer called "gora bwtwk" (H) chuwak after distillation (Ghosh *et al.2016).*

METHODS AND MATERIALS:

SAMPLE COLLECTION:

In the current work, 3 Samples were collected from 3 districts of Tripura namely West Tripura (1 samples), South Tripura (1 sample) and Sepahijala (1 samples) district. Distilled types of liquor were encountered, distilled liquor known as local wine (locally known as Bangla). Detail description of samples is given in Table 1.

Sl. no	Place of Collection	Sample code
1	Agartala	WT
2	Manu Bazar	ST
3	Nalchar	SJ

WT-West Tripura, ST- South Tripura, SJ- Sepahijala

Table1: Description of liquor samples

SAMPLE PREPARATION:

1µLof samples have been taken in 22 ml vial and sealed. The vial changed into then heated at 100° C for five min. 1µLof headspace changed into injected into the injection port of GC thru head area switch line which changed into maintained at 110°C.

HS-GC-FID parameters:

The sample was analyzed using Shimadzu GC-2014. The injection port used was Spl 1, and Injection heat port was Inj 1. The injection mode split ratio was kept at 5.0, and column oven temperature was kept at 40°C. The sampling time was 1minute. and the carrier gas used was Helium. The pressure was adjusted to 85 KPa and the total flow was 3mL/min. The column flow, purge flow, was 2.78 ml/min, 3ml/min, respectively. The linear velocity was 42.30 cm/sec. The detector temperature was kept at 280° C and the analysis time was 4 minutes.

RESULT AND DISCUSSION:

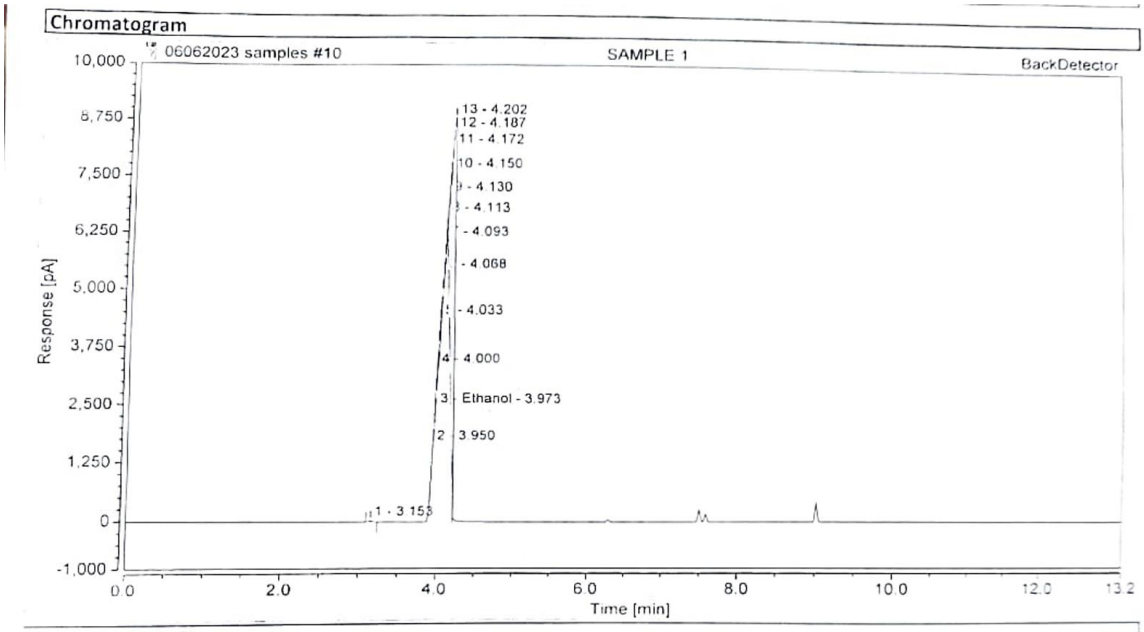
The home-made rice beers were investigated on the aspect of chemistry and forensic science. The samples Showed their characteristic colour and odour. All samples were having burning taste and strong smell.

The HS-GC-FID proved the presence of most effective ethanol. The percent of ethanol is one-of-a-kind on district-smart pattern, the ethanol content material in pattern 1 is 0.0769%, pattern 2 is 0.100 %, pattern 3 is 0.1057 % and given in fig. 1, fig. 2, fig. 3 respectively. From this recorded percent of ethanol will effortlessly apprehend the quantity of ethanol.

From the analysis of the 3 spectra of sample, there is only one prominent peak near the retention time about 4 mins, before that no peak is appeared. Hence, it can be concluded that methanol is not present in the samples. So, we will affirm that the rice beer samples of Tripura are loose from adulterant like methanol.

SL. NO.	Sample Code	Retention time	Percentage of Ethanol	Methanol
1	WT	3.973	0.0769	Absent
2	ST	3.965	0.1006	Absent
3	SJ	3.962	0.1057	Absent

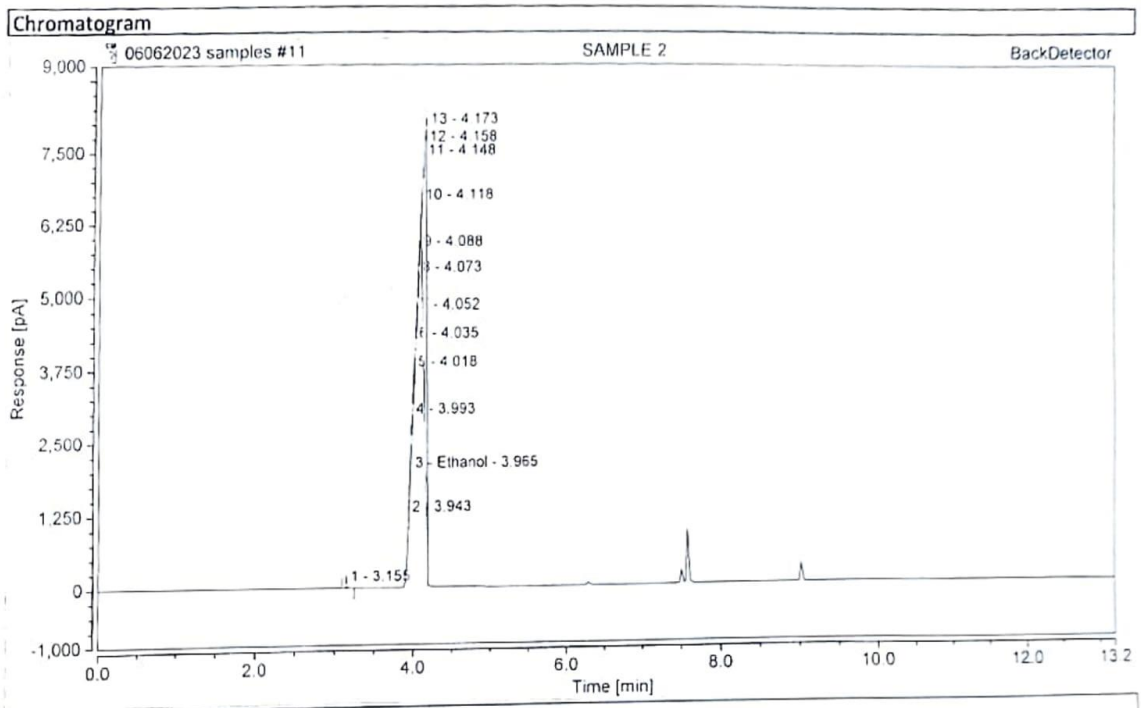
Table .2- Summary of results of 3 home-made liquor's samples



Integration Results

No.	Peak Name	Retention Time min	Area pA*min	Height pA	Relative Area %	Relative Height %	Amount %
n.a.	Methanol	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
1		3.153	1.085	27.858	0.23	0.10	n.a.
2		3.950	2.280	85.214	0.48	0.31	n.a.
3	Ethanol	3.973	3.600	170.317	0.76	0.62	0.0769
4		4.000	7.222	221.848	1.53	0.81	n.a.
5		4.033	8.155	231.830	1.73	0.85	n.a.
6		4.068	3.472	143.440	0.74	0.53	n.a.
7		4.093	1.147	72.647	0.24	0.27	n.a.
8		4.113	11.617	703.930	2.47	2.58	n.a.
9		4.130	39.134	2083.267	8.32	7.64	n.a.
10		4.150	81.429	3704.345	17.30	13.58	n.a.
11		4.172	98.345	5439.682	20.90	19.94	n.a.
12		4.187	76.508	6632.072	16.26	24.31	n.a.
13		4.202	136.616	7768.745	29.03	28.47	n.a.
Total:			470.610	27285.194	100.00	100.00	

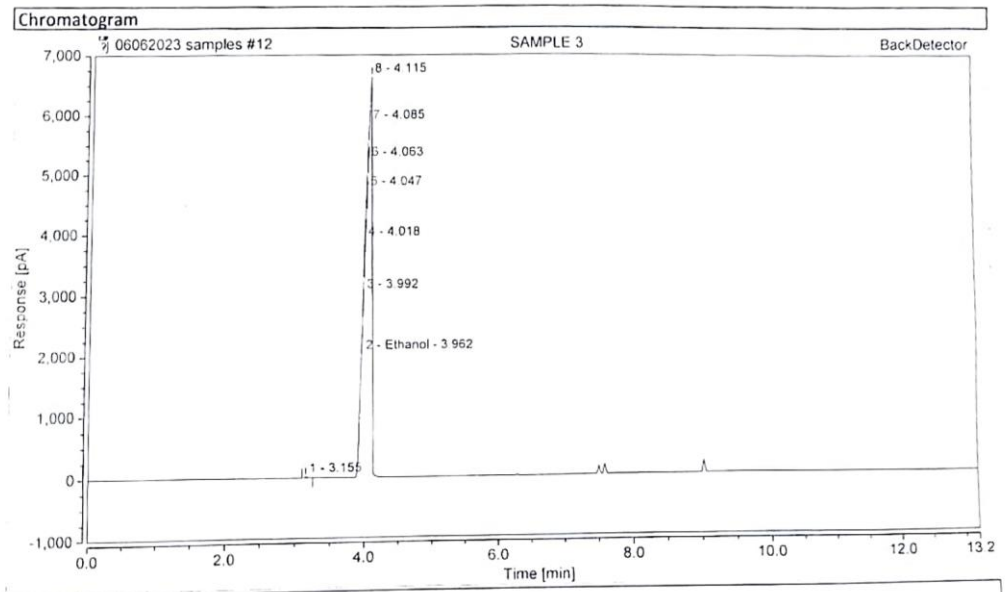
Fig.2 --- Headspace GC Spectra of Sample 1



Integration Results

No.	Peak Name	Retention Time min	Area pA*min	Height pA	Relative Area %	Relative Height %	Amount %
n.a.	Methanol	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
1		3.155	0.956	24.353	0.31	0.13	n.a.
2		3.943	0.002	0.615	0.00	0.00	n.a.
3	Ethanol	3.965	4.396	114.715	1.43	0.62	0.1006
4		3.993	2.887	176.494	0.94	0.96	n.a.
5		4.018	6.536	218.585	2.12	1.18	n.a.
6		4.035	0.932	189.241	0.30	1.02	n.a.
7		4.052	3.521	151.033	1.14	0.82	n.a.
8		4.073	1.963	111.555	0.64	0.60	n.a.
9		4.088	1.131	75.002	0.37	0.41	n.a.
10		4.118	49.431	1330.023	16.05	7.20	n.a.
11		4.148	81.711	4261.258	26.54	23.08	n.a.
12		4.158	35.807	5214.728	11.63	28.24	n.a.
13		4.173	118.663	6595.418	38.53	35.72	n.a.
Total:			**307.938**	**18463.020**	**100.00**	**100.00**	

Fig.3 --- Headspace GC Spectra of Sample 2



Integration Results

No.	Peak Name	Retention Time min	Area pA*min	Height pA	Relative Area %	Relative Height %	Amount %
n.a.	Methanol	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
1		3.155	0.732	18.478	1.05	0.54	n.a.
2	Ethanol	3.962	4.569	125.961	6.57	3.66	0.1057
3		3.992	3.760	198.082	5.40	5.76	n.a.
4		4.018	7.574	224.061	10.89	6.52	n.a.
5		4.047	3.302	174.238	4.75	5.07	n.a.
6		4.063	2.369	138.059	3.41	4.01	n.a.
7		4.085	1.637	85.072	2.35	2.47	n.a.
8		4.115	45.618	2474.859	65.58	71.97	n.a.
Total:			69.561	3438.809	100.00	100.00	

Fig.4 --- Headspace GC Spectra of Sample 3

CONCLUSION:

The home-made liquor samples collected from the state of Tripura have been analysed and characterized in this study. When it comes to characterizing alcoholic beverages, HS-GC-FID demonstrates that it is a very sensitive method. This study's findings will be useful in a variety of alcoholic beverage-related forensic cases. Additional research is needed to identify components that can better differentiate between alcoholic beverage samples**.**

When law enforcement encounters cases of illicit liquor, illegal trading and transportation, tax evasion, and other illegal activities like adulteration, the current profile of components in various liquor samples would be helpful. The study is also helpful in case of methanol poisoning, death due to alcohol consumption.

Conflict of interest: The authors declare that there is no conflict of interest.

Acknowledgement: The authors would like to thank Dr. Rito Chophi, TRA and Dr. Sanay Naha, Assistant Professor, in the Department of Forensic Science, National Forensic Sciences University, Tripura Campus, Agartala for valuable guidance.

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