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DETECTION OF CHLORAL HYDRATE ADULTERATION IN TRADITIONAL ALCOHOLIC BEVERAGE TODDY BY HEAD SPACE-GAS CHROMATOGRAPHY

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ABSTRACT

A simple, rapid and reliable head space gas chromatographic method for the separation and determination of psychotropic substance chloral hydrate in traditional alcoholic beverages such as toddy has been developed. Separation was accomplished using head space gas chromatograph coupled with auto sampler, Elite-WAX column and flame ionization detector. The validity of the method was by analyzing different Toddy samples with spiked chloral hydrate and the extent of adulteration was determined.

KEYWORDS: HS-GC, Toddy, chloral hydrate, ethanol.

INTRODUCTION

Toddy is one of the alcoholic drinks traditionally prepared by the fermentation of sap or exudates collected by slicing off the tip of unopened flowers of either coconut or a palm tree. It is a social and local drink produced and consumed throughout Asia, particularly India, Srilanka and Bangladesh.

Due to increase in consumer demand and shorter of coconut or palm tree, it has often been diluted and adulterated with chemical substances, such as chloral hydrate belonging to non barbiturates and benzodiazepines respectively.[1,2] This drug is generally considered to be dangerous when consumed along with alcohol and have proved to be fatal poisoning. According to the recent analysis of Forensic Toxicological work conducted by Koski

et.al.[3,4] Further it was reported that the female drinkers are at increased risk to abuse benzodiazepines when compared with men.[5] This psychotropic substance has a sedative action and is added illicitly to toddy to increase its potency. The adulterated toddy is prohibited for consumption under the laws of excise, which necessitates the development of suitable analytical method for the simultaneous detection and determination of the level of Psychotropic Substance in fermented alcoholic beverages. (Toddy)

Fujiwara test, which gives intense red colour by the adding Pyridine in the presence of an alkali, is generally used to identify Chloral hydrate in toddy.[6,7] A number of assay have shown Chloral hydrate to be genotoxic[8] and Hepto- carcinogenic in male mice.[9] due to its widespread potential for human exposure, US FDA has declared chloral hydrate as a priority chemical for an in depth toxicological evaluation.

As part of this program. Head space gas chromatography with flame ionization detector was developed for the determination of chloral hydrate. The liquid chromatographic methods based on reverse phase and union exchange mechanisms were also reported in the literature.[10]

However, both of the methods are proceeds by derivatisation of chloral hydrate with dansyhydrazine and o–(4-nitrobezyl) hydroxylamine, followed by detection using Ultra-Violet and suppressed conductivity detectors. However these tests are not specific because similar colors are produced by a number of organic compounds containing halogens. Square wave voltametric studies at hangining mercury drop electrodes for the determination of 1,4-Benzodiazepines over wide range of concentrations have been conducted.[11]

Husain et. al. have studied the differential pulse polarograms of chloral hydrate in 0.1M KCl in 50 % ethanol as supporting electrolyte and tried to estimate their levels quantitatively in toddy.[12] A micellar liquid chromatographic procedure for the determination of benzodiazepines in serum has been reported[13] LC-ESI-MS and LC-MS-MS for determination of benzodiazepines in hair and plasma samples of psychiatric patients were used extensively.[14, 15] A throughout literature search has revealed that method for the detection of chloral hydrate in toddy by head space gas Chromatography has not been reported. Present paper, we describe a simple, rapid and reliable HSGC method for separation, identification and determination chloral hydrate in fermented alcoholic beverages of toddy using Elite-Wax column and flame ionization detector has been developed.

Experimental

Material and reagents

All regents were of analytical grade unless stated otherwise Glass- distilled water was deionized using (make ELGA, UK model: Pure Lab Altra model OR007xxM1). Chloral hydrate (Specrochem, Mumbai, India) was used as reference standard.

Apparatus

A Head space gas chromatograph (Make PerkinElmer, Model- Clarus 500), Auto sampler (Turbo matrix-40) coupled with GC, Flame ionization detector and Elite-Wax column was used.

Chromatographic conditions

Toddy samples thermostated at 80^{o}c for 20 minutes in Headspace auto sampler, split GC conditions: column: Elite-Wax (30 m. x 0.32mm I.D.) fused silica open tubular coated with bonded phenyl (5%) methyl silicon stationary phase. film thickness 2 mm. Column temperature. isothermal at 50^{o}c for 9 minutes. Carrier gas: Nitrogen 50Kg/cm^{2} flame ionization detector (FID) temperature 230 ^{o}c. The chromatogram, data acquisition and data processing was done using Total Chrom software.

Analytical Procedure

Toddy samples were filtered through whatman No-1 filter paper and 1 ml of the filtrate was thermostated on autosampler at 80^{o}c for 20 minutes and all of vapour is injected onto the chromatograph. The standard chloral hydrate sample was analyzed under the identical condition. The presence of chloral hydrate was determined by comparing the rotation times with that of the standards and their quantity was estimated from the areas of the respective peaks

RESULT AND DISCUSSION

The chemical structure of chloral hydrate is shown in the figure 1 The optimum separation conditions for standard chloral hydrate the column temperature was kept at 50^{o}c. The HSGC chromatogram of ethyl alcohol eluted at 4.31 minutes and chloral hydrate eluted at 7.31 minutes. The peaks were identified by injecting authentic compounds. The peaks were resolved with excellent symmetry and reproducibility. Further, the effect of the temperature on the retention time of the compounds are also studied. As the column temperature increased the compound was found to be less retained. Due to volatile nature of chloral hydrate. The

different concentrations of chloral hydrate in ethanol were injected and calibration curve was plotted from he calibration curve unknown samples could be analysed by adapting the same procedure. The amount of chloral hydrate found in different toddy samples ranged from0.01mg% to1mg% the compound eluted was at 50^{o}c. This condition was found more suitable for the present study.

The accuracy of the method was determined by the standard addition technique. The subsequent addition of small amounts of analyses was accuracy reflected in their peak areas known amounts of chloral hydrate was added to toddy to yield concentration in range of 0.01 to 1 mg/ml. and used for calibration. The linearity between the mass and the integral response was found to be quite good. The standard curve for chloral hydrate was found to be linear. The precision of the analysis was accessed by five replicate analysis of toddy containing chloral hydrate in the concentration ranges of 0.01 to 1mg/ml. The quantity of toddy samples suspected to be adulterated with chloral hydrate were thoroughly analyzed by HSGC. The relative response factors were determined and used to quantity the levels of adulterants in different sample of toddy.

Conclusion: The HSGC method is suitable rapid and easily adoptable for the for qualitative and quantity determination of chloral hydrate adulteration in toddy. It is reliable and convenient for forensic analysis.

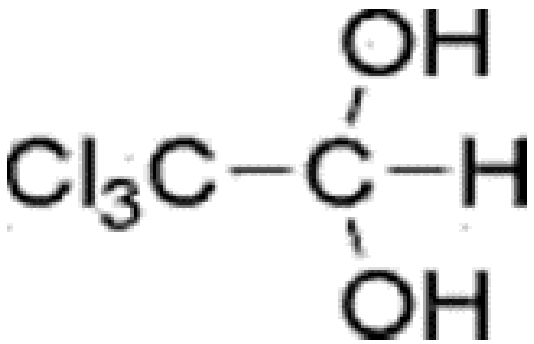

Fig.1 Chemical structure of Chloral hydrate.

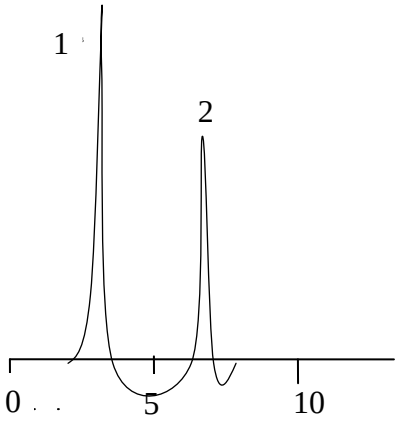

Fig-2

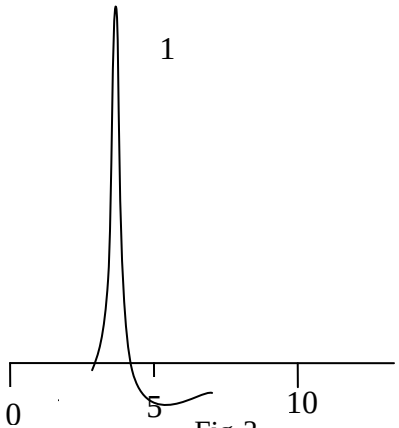

Fig-3

Fig.2 Typical HS-GC chromatogram of Toddy, with adulteration of chloral hydrate (1-ethanol & 2 -chloral hydrate).

Fig.3 Typical HS-GC chromatogram of Toddy, without adulteration of chloral hydrate (1- ethanol).

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