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Case Report: Detection of Neo-Formation of Ethanol
in a Postmortem Blood Sample
Using N-Propanol and a Urine Sample

by
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TECHNICAL REPORT

CASE REPORT: DETECTION OF NEO-FORMATION OF ETHANOL IN A POSTMORTEM BLOOD SAMPLE USING N-PROPANOL AND A URINE SAMPLE

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ABSTRACT

A case report of a suicidal fall in which a postmortem blood sample collected from the victim was placed in a jar without preservative and a urine sample was placed in a tube containing at least 1% w/v sodium fluoride preservative. The samples were received at the Centre of Forensic Sciences nineteen days later. The ethanol concentration of the blood as determined by headspace GC, was 96 mg/dL. No ethanol was detected in the urine. In addition the blood sample was found to contain 4 mg/dL of n-propanol. This paper discusses the use of n-propanol as an indicator of neo-formation of ethanol and cautions against use of this compound as an internal standard for postmortem ethanol analysis unless previous screening for it has been conducted.

RÉSUMÉ

Cet article porte sur un cas de suicide par chute mortelle où un échantillon de sang postmortem de la victime a été entreposé dans un bocal sans agent de conservation alors que le tube utilisé pour la collection d'urine contenait au moins 1% de fluorure de sodium. Les échantillons ont été reçus au Centre of Forensic Sciences dix neuf jours plus tard. Une concentration sanguine d'éthanol de 96 mg/dL a été trouvée à l'aide d'un chromatographe à phase gazeuse et à espace de tête. Aucun éthanol n'a été décelé dans l'urine. De plus, une concentration sanguine de n-propanol de 4 mg/dL a été trouvée. Une discussion portant sur l'utilisation du n-propanol comme indicateur de néo-formation d'éthanol s'en suit. On déconseille également d'utiliser ce produit comme étalon interne pour des analyses d'éthanol sur des échantillons postmortem sauf si un criblage initial a révélé l'absence du n-propanol.

INTRODUCTION

Neo-formation of ethanol due to microbial action either postmortem in the body or from improper storage or preservation of the biological samples is a common problem in forensic toxicology (1). Whether the victim consumed ethanol antemortem or whether the ethanol formed due to putrefactive processes is an important forensic issue and much research has been made into this area. Various indicators have been proposed to identify postmortem ethanol neoformation. In this case report the usefulness of n-propanol as such an indicator is illustrated.

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CASE HISTORY

A 31-year-old male was found dead several hours after a suicidal fall. An autopsy was conducted the next day and the cause of death was due to multiple traumatic injuries. A blood sample was collected from the peritoneal cavity, as no vascular blood was available. The blood was placed in a jar with no preservative. A urine sample was also collected and placed in a tube, which contained at least 1% sodium fluoride as preservative. Nineteen days later the samples were received at the Centre of Forensic Sciences. The storage conditions of these samples are not known but the samples were in transit during late summer.

The blood and urine were analysed for ethanol using headspace gas chromatography according to the method of Machata (2). In this method, tertiary-butanol is used as an internal standard. A Hewlett Packard 5890 Series II gas chromatograph using a flame ionization detector was employed with a 183 cm long by 0.3 cm internal diameter 0.2% Carbowax 1550 on 100 Carbopak C column. A Tekmar 7000 automatic headspace sampler was used, which heats all samples to 45°C for a uniform time of 30 minutes prior to sample injection. In addition to ethanol, acetaldehyde, methanol, acetone, isopropanol and n-propanol are analysed. All analyses were performed in duplicate.

In addition, a general drug screen for chemically basic drugs was conducted on the blood sample using a Hewlett Packard 5890 Series II gas chromatograph equipped with a nitrogen/phosphorous detector using 2 columns. This method has been previously published in detail (3).

RESULTS AND DISCUSSION

There were no significant findings in the drug screen of the blood sample. The mean results of the headspace analysis of the blood and urine are shown in Table 1. Figure 1 shows a gas chromatogram for the blood sample. Three volatile substances were detected in the blood sample but none in the urine sample. The blood ethanol concentration was found to be 96 milligrams of alcohol in one decilitre of blood (mg/dL). If this result alone was accepted, it could indicate that the person was under the influence of ethanol before the fall.

N-propanol was also detected in the blood sample, which can be produced by micro-organisms in putrefying blood samples (4,5). It has been proposed that n-propanol is an indicator of the neo-formation of ethanol and that the maximal neo-formation of ethanol to n-propanol is approximately 20:1 to 25:1 (6,7,8). The ratio of ethanol to n-propanol detected in this case was 24:1 which could indicate that all the ethanol could have been produced by micro-organisms probably during transit.

N-propanol has been commonly used as an internal standard for postmortem ethanol analysis (9,10). As shown in this case and in other published studies, n-propanol should not be used for postmortem ethanol analysis (1,11).

TABLE 1
Mean Results of Duplicate Headspace GC Analysis. N.D. = Not Detected

Volatile Substance	Blood (mg/dL)	Urine (mg/dL)
Acetaldehyde	3	N.D.
Ethanol	96	N.D.
N-Propanol	4	N.D.

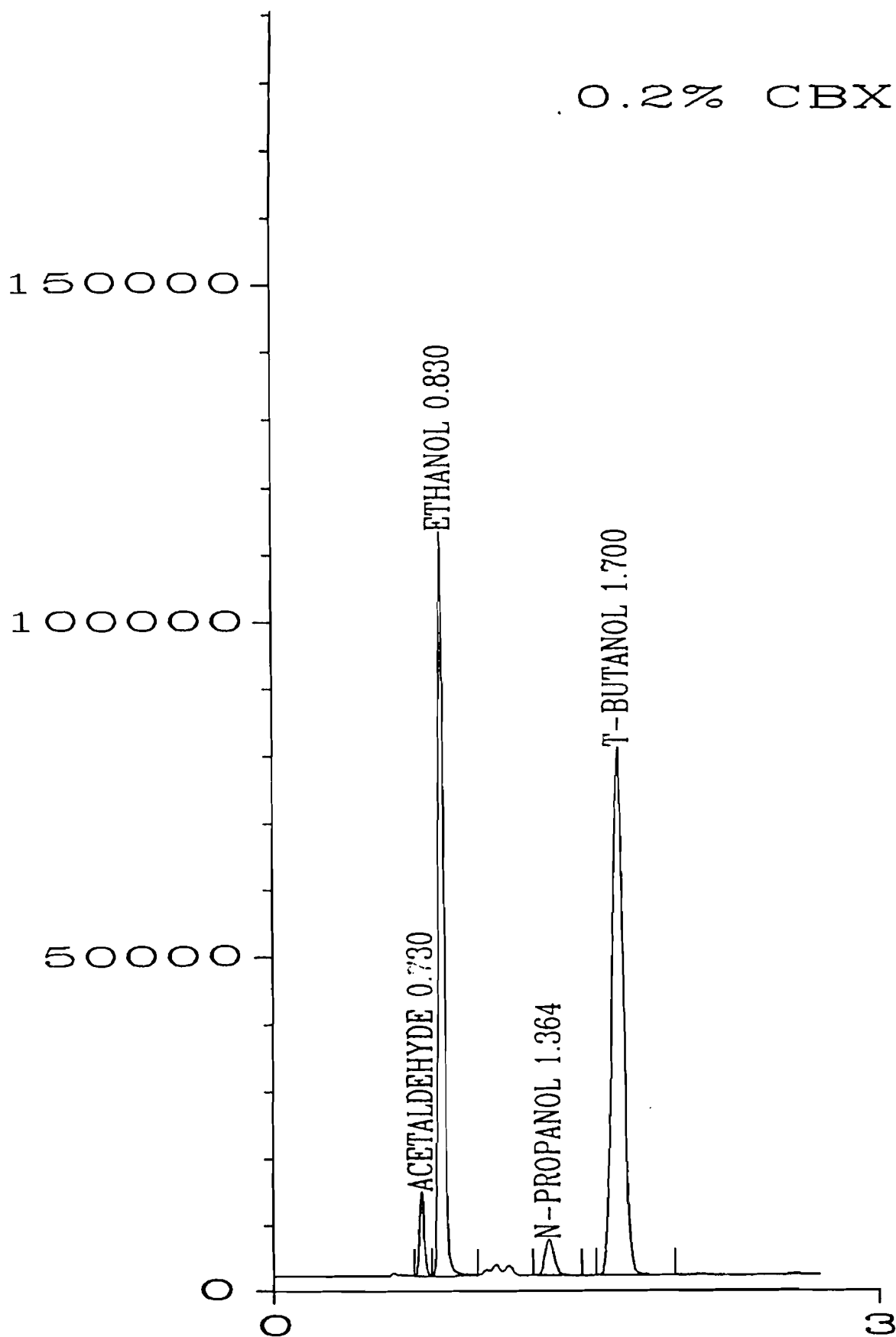


Figure 1. A gas chromatogram of the analysis of the blood sample. The vertical axis is the peak area and the horizontal axis is the analysis run time in minutes.

A concentration of 3 mg/dL acetaldehyde was also detected in the blood. Elevated post-mortem blood acetaldehyde concentrations have been found to be due to a temperature dependent oxyhemoglobin-catalysed conversion of ethanol to acetaldehyde in vitro (12). The higher the temperature the faster the production of acetaldehyde occurs. To minimize the formation of acetaldehyde during headspace analysis, at this laboratory, the automatic headspace sampler is set at a lower temperature of 45°C and the samples are only heated for 30 minutes (13). The acetaldehyde concentration detected in blood may, therefore, indicate that the sample was not completely refrigerated during transit.

No acetaldehyde was detected in the urine for two reasons, one is that the urine sample contains no hemoglobin for the reaction to occur, and the other is that no ethanol was detected in the urine to be converted into acetaldehyde.

The urine was preserved with a least 1% sodium fluoride which has been found to prevent neo-formation of ethanol even when the sample is not refrigerated (14). This also illustrates the importance of obtaining multiple biological samples in postmortem cases. In this case the urine result is a more reliable indicator of ethanol use antemortem than the blood sample.

The blood ethanol concentration, therefore, is considered unreliable due to the n-propanol and acetaldehyde concentrations detected in the blood, the lack of preservation, the long transit time, the apparent lack of refrigeration, and the lack of ethanol or other volatile substances detected in the preserved urine sample. The victim was, therefore, not under the influence of ethanol or other common drugs at the time of the fall.

CONCLUSIONS

This case illustrates the usefulness of n-propanol as an indicator of neoformation of ethanol. In combination with other biological samples (e.g. urine, vitreous humor) and a closer examination of the circumstances of the case this confounding problem can be detected. N-propanol is not recommended as an internal standard for postmortem ethanol analysis unless previous screening for it has been conducted.

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