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Short communication

Gas chromatographic–mass spectrometric identification and quantitation of benzyl alcohol in serum after derivatization with perfluorooctanoyl chloride: a new derivative

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Abstract

Benzyl alcohol is commonly used as an antibacterial agent in a variety of pharmaceutical formulations. Several fatalities in neonates have been linked to benzyl alcohol poisoning. Most methods for measuring benzyl alcohol concentrations in serum utilize direct extraction followed by high-performance liquid chromatography. We describe here a novel derivatization of benzyl alcohol using perfluorooctanoyl chloride after extraction from human serum for analysis by gas chromatography–mass spectrometry (GC–MS). The derivative was eluted at a significantly higher temperature respective to underivatized molecule and the method was free from interferences from more volatile components in serum and hemolyzed specimens. Another advantage of this derivatization technique is the conversion of low-molecular-mass benzyl alcohol (M_r 108) to a high-molecular-mass derivative (M_r 504). The positive identification of benzyl alcohol can be achieved by observing a distinct molecular ion at m/z 504 as well as the base peak at m/z 91. Quantitation of benzyl alcohol in human serum can easily be achieved by using 3,4-dimethylphenol as an internal standard. The within run and between run precisions (using serum standard of benzyl alcohol: 25 mg/l) were 2.7% (mean=24.1, S.D.=0.66 mg/l, n=8) and 4.2% (mean=24.3, S.D.=1.03 mg/l, n=8), respectively. The assay was linear for the serum benzyl alcohol concentrations of 2 mg/l to 200 mg/l and the detection limit was 0.1 mg/l. We observed no carry-over (memory effect) problem in our assay as when 2 μl ethyl acetate was injected into the GC–MS system after analyzing serum specimens containing 200 mg/l of benzyl alcohol, we observed no peak for either benzyl alcohol or the internal standard in the total ion chromatogram. © 1998 Elsevier Science B.V.

Keywords: Benzyl alcohol; Perfluorooctanoyl chloride

1. Introduction

Benzyl alcohol at concentrations of 0.9–2.0% is commonly used as an antibacterial agent in many pharmaceutical formulations especially intended for intravenous administration [1]. Benzyl alcohol is widely used in organic synthesis and as a solvent for various compounds, for example cellulose [2]. Intraventricular hemorrhage and death in preterm neonates has been associated with the use of fluid containing benzyl alcohol. Exposure to benzyl alcohol was significantly associated with the develop-

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ment of kernicterus [1]. Benzyl alcohol preservatives in intravascular flash solutions has been reported to cause neurological deterioration and death in low birth weight infants [3]. Brown et al. [4] also reported symptoms of neurological deterioration, hematologic abnormalities, severe metabolic acidosis, respiratory distress, hepatic and renal failure, hypotension and cardiovascular collapse from benzyl alcohol intoxication in neonates. Garshanik et al. [5] reported blood benzyl alcohol concentrations ranging from 66 mg/l to 148 mg/l in six infants with respiratory distress resulting from benzyl alcohol toxicity. Deaths in immature animals have been reported after oral and intravenous administration of Ringer's solution containing 1.5% benzyl alcohol [6].

Different methods have been reported in the literature for identification and quantitation of benzyl alcohol, but most of the techniques utilize high-performance liquid chromatography (HPLC) and the identification of benzyl alcohol is solely based on the retention time. Tan et al. [7] reported a HPLC method for determination of benzyl alcohol using a C_{18} column and water–acetonitrile–glacial acetic acid as a mobile phase. Block and Levine [8] reported a qualitative method for the detection of benzyl alcohol, benzoic acid and nitrobenzene in benzaldehyde USP specimens by a normal-phase HPLC. Rego and Nelson [9] reported simultaneous determination of hydrocortisone and benzyl alcohol in pharmaceutical formulation using reversed-phase HPLC. Gershanik et al. [5] reported a gas chromatography (GC) method for determination of benzyl alcohol in serum.

Gjerde et al. [10] described a novel derivatization of amphetamine and methamphetamine using perfluorooctanoyl chloride. Since benzyl alcohol has a free hydroxyl group, perfluorooctanoyl chloride can potentially react with benzyl alcohol. The advantage is the significantly higher molecular mass of the derivative (benzyl alcohol, M_r 108; derivative, M_r 504). Therefore, a positive identification by mass spectrometry (MS) could be enhanced by this process which should certainly be helpful in medical–legal cases. Here we report a new derivatization of benzyl alcohol after extraction from human serum and postmortem blood using perfluorooctanoyl chloride and the subsequent analysis by GC–MS.

2. Experimental

2.1. Reagents

Benzyl alcohol, the internal standard (I.S.) 3,4-dimethylphenol and HPLC grade chloroform were purchased from Aldrich (Milwaukee, WI, USA). The derivatizing agent perfluorooctanoyl chloride was obtained from PCR (Gainsville, FL, USA).

2.2. Analysis conditions

GC–MS analyses were performed on a Model 5890 gas chromatograph coupled to a 5972 series mass-selective detector (Hewlett-Packard, Palo Alto, CA, USA). The capillary column (Ultra-1, 25 m×0.2 mm) also obtained from Hewlett-Packard was coated with crosslinked methyl silicone (0.33 μm film thickness). We used a splitless manual injection system with an injector port temperature of 250°C. The initial oven temperature of the gas chromatograph was 110°C. After maintaining that temperature for 3 min, the oven temperature was increased at a rate of 25°C/min to reach a final temperature of 300°C. This temperature was maintained constant for another 2 min in order to clean the column. The run time was 12.6 min. The mass spectrometer was operated in the electron impact ionization mode and selected ion monitoring (ions monitored: m/z 69, 77, 91, 105, 121, 504 and 518). For analysis of postmortem specimens, we baked the column at 310°C for an additional 10 min prior to the next injection. The carrier gas was helium. The column head pressure of helium gas was 69 kPa with a linear velocity of 31.5 cm/s. The transfer line temperature was 300°C, and the ionization energy was 70 eV.

2.3. Standards and controls

A stock solution (0.5 mg/ml) of 3,4-dimethylphenol, the I.S. was prepared in chloroform. The stock solution of benzyl alcohol (0.5 mg/ml) was prepared in water. Then a serum control containing 25 mg/l of benzyl alcohol was prepared by supplementing serum with benzyl alcohol for precision studies. Different aliquots of pooled serum and postmortem serum were supplemented with various concentrations of benzyl alcohol for further study.

The serum pools were prepared from left over specimens obtained from the local blood bank. We also pooled several postmortem blood samples which had been saved for one year and then discarded by the office of the medical examiner.

2.4. Sample preparation

Twenty-five μl of the I.S. stock solution was added to a 0.5-ml aliquot of serum, hemolyzed blood or postmortem blood (supplemented with benzyl alcohol). After that, 5 ml of chloroform was added to extract benzyl alcohol along with the I.S. from the serum. The samples were mixed for 15 min in a rotary mixer. The upper aqueous layer was separated from the lower chloroform layer by centrifugation at 1500 *g* for 10 min. Then the upper aqueous layer was discarded, and the lower organic layer was carefully transferred to a conical test tube. The chloroform extract was evaporated to dryness using air at room temperature, and 50 μl of perfluorooctanoyl chloride was added to the dry extract. After incubation at 85°C for 30 min, the excess derivatizing agent was evaporated, and the residue was reconstituted with 50 μl of ethyl acetate. Then 1–2 μl was injected into the GC–MS system. Quantitation of the benzyl alcohol peak was done by comparing the area under the curve with the area under the curve for the I.S. which eluted after the derivatized benzyl alcohol peak. The derivatization reaction was quantitative under our reaction conditions because we did not observe any underivatized benzyl alcohol or 3,4-dimethylphenol using shorter solvent delay.

3. Results and discussion

3.1. Chromatographic properties of derivatized benzyl alcohol

We observed excellent chromatographic properties of both derivatized benzyl alcohol and the I.S., 3,4-dimethylphenol. The two peaks were well separated with the retention time of derivatized benzyl alcohol being 6.4 min and the retention time of derivatized I.S. being 7.1 min. We used a relatively long solvent delay (5 min) when the mass spectrometer was off and not acquiring data. This long solvent delay on the other hand also helps to reduce useless data on the computer disk. We also observed no interfering peaks in our chromatogram when grossly hemolyzed, lipemic or postmortem specimens were supplemented with benzyl alcohol. A typical total ion chromatogram of analysis of a serum specimen supplemented with benzyl alcohol is given in Fig. 1.

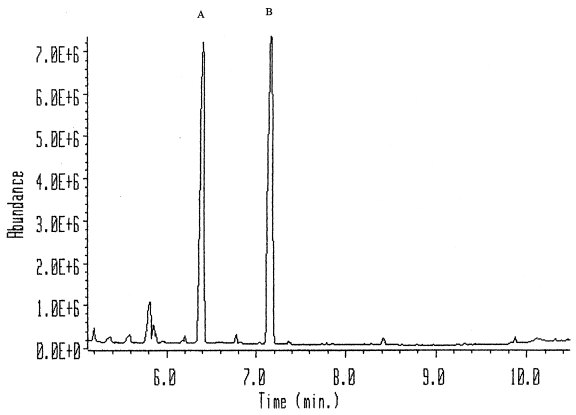

Fig. 1. Total ion chromatogram of a typical postmortem blood extract showing the separation between benzyl alcohol and the internal standard after derivatization with perfluorooctanoyl chloride. Peak A is the derivatized benzyl alcohol and peak B is the derivatized internal standard. The concentration of benzyl alcohol was 25.0 mg/l.

In order to further investigate the matrix effect from hemolyzed blood and postmortem specimens, we supplemented them with various concentrations of benzyl alcohol and compared the observed benzyl alcohol concentrations with the target concentrations. In all cases, we observed comparable values indicating that our assay can be used for identification and quantitation of benzyl alcohol in grossly hemolyzed as well as postmortem specimens (Table 1).

Table 1
Observed and target benzyl alcohol concentrations in grossly hemolyzed and postmortem specimens

Specimen	Benzyl alcohol (mg/l)	
	Target	Observed
Gross hemolysis	25.0	27.5
Gross hemolysis	100.0	89.8
Lipemic (triglyceride: 874 mg/dl)	100.0	117.8
Lipemic (triglyceride: 4496 mg/dl)	50.0	48.4
Postmortem specimen 1	100.0	98.9
Postmortem specimen 2	100.0	108.9

3.2. Mass spectral characteristics of derivatized benzyl alcohol

Benzyl alcohol is a small molecule with a molecular mass of 108. In our new derivatization protocol, the molecular mass of perfluorooctanoyl derivative of benzyl alcohol was 504. We observed a distinct molecular ion at m/z 504 (relative abundance 23.3%) in the mass spectrum of the perfluorooctanoyl derivative of benzyl alcohol. The base peak was observed at m/z 91 because of the stability of $C_6H_5CH_2^+$ ion (Fig. 2). It may be possible to observe m/z 108 using a soft ionization technique employing ammonia as a reagent gas.

The perfluorooctanoyl derivative of 3,4-dimethylphenol, the I.S., showed a strong molecular ion at m/z 518. The base peak observed at m/z 121 was due to the loss of perfluorooctanoyl moiety. Other characteristic peaks were observed at m/z 105, 91 and 77 (Fig. 3).

3.3. Precision, linearity and detection limit

The within-run and between-run precision of the assay were determined by analyzing a serum pool supplemented with benzyl alcohol to achieve a final concentration of 25 mg/l. The within-run coefficient of variation (C.V.) was 2.7% (mean=24.1, S.D.=0.66 mg/l, n=8), while the between-run C.V. was 4.2% (mean=24.3, S.D.=1.03 mg/l, n=8). The assay was linear for a serum benzyl alcohol concentration between 2 mg/l and 200 mg/l. Using the x-axis as the target concentration and the y-axis as the observed concentration in the linearity study, we obtained the following regression equation: $y=0.93x+3.6$, ($r=0.99$).

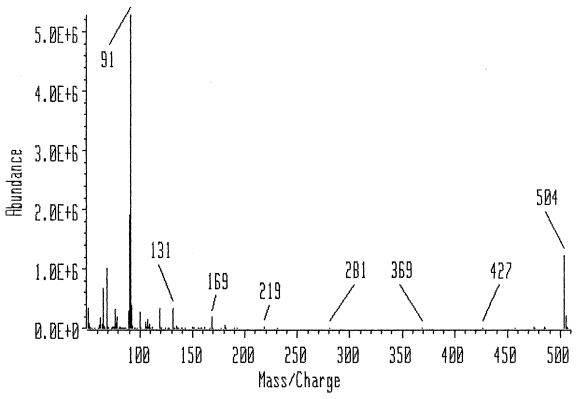

Fig. 2. Mass spectrum of perfluorooctanoyl derivatives of benzyl alcohol.

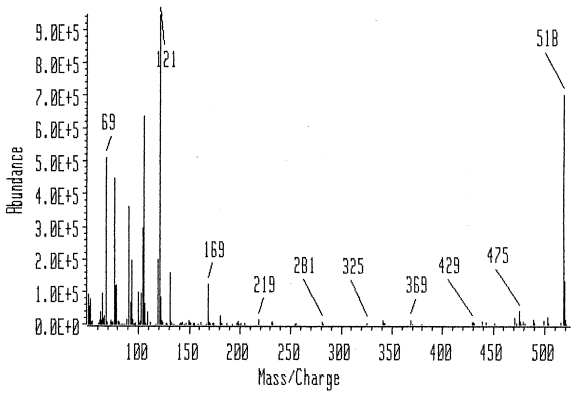

Fig. 3. Mass spectrum of perfluorooctanoyl derivative of 3,4-dimethylphenol, the internal standard.

The detection limit was 0.1 mg/l serum benzyl alcohol concentration. Block and Levine [8] reported a detection limit of 6 ng of benzyl alcohol (using HPLC) in 1 μl, which was 6 mg/l. Rego and Nelson [9] reported a detection limit of 0.125 mg/l using HPLC assay.

3.4. Memory effect

In order to study any carry over problem, we analyzed a serum specimen supplemented with benzyl alcohol to achieve a final concentration of 200 mg/l. Immediately after the GC–MS run, we injected 2 μl of ethyl acetate into the GC–MS system. We observed no peak for either derivatized benzyl alcohol or the I.S. We performed our analysis in duplicate. As expected, we did not observe any peaks with our blank injection after analyzing another serum specimen supplemented with 150 mg/l of benzyl alcohol (duplicate measurement). We also observed no carry-over problem after analysis of hemolyzed specimens. However, with one occasion with postmortem blood, we observed two peaks which were eluted at a very high temperature (no interfering peaks for benzyl alcohol or I.S.). The problem can be easily circumvented if the column was baked for 10 min at 310°C. Therefore, we concluded that the GC–MS assay for derivatized

benzyl alcohol does not have any carry-over problems.

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