Determination of Bioalcohol by GC in biological matrices and cell culture

Materials and Methods

- Reagents

  All the chemicals used were of analytical grade: ethanol (> 99.9%), methanol (> 99.9%), 1 propanol (> 99%),acetone (> 99.9%), acetaldehyde(> 99.9%), Triton X-100 , and acetonitrile(> 99.9%). William's E culture medium and supplemented with 2mM L-glutamine , 5 µg/mLPost-mortem human VH.

  Prior to analysis,each sample was injected into the GC–flame ionization detector (FID) to confirm the absence of each studied compound. Additionally, the interference between the tested compounds and some other volatile biomarkers of some diseases and with great forensic interest was excluded, such as formaldehyde, methyl and ethyl formate, ethylene glycol, propyleneglycol, glycerol, 1,4-butanediol, and 2,3-butanediol. Volatile-free B, U, VH, and CCM were used to prepare the control samples and calibration curves.

- Calibration procedures

  Concentrated solutions of 500 g/L ethanol, 50 g/L methanol, 50 g/L acetaldehyde, and 50g/L acetone were prepared daily by dilution of the commercial solutions in deionized water. Daily prepared 2.2 g/L 1-propanol in deionized water was used as internal standard (IS).A stock calibrator containing 10 g/L ethanol, 1 g/L methanol, 1 g/L acetaldehyde,and 1 g/L acetone was prepared daily in each tested matrix, from the concentrated solutions. The calibration curves were prepared. These procedures resulted in final concentration of 7.5, 15, 30,60, 120, and 240 mg/L of methanol, acetone,and acetaldehyde, and with 75, 150, 300, 600,1200, and 2400 mg/L of ethanol. These calibration standards, in B and VH, underwent a 5 times dilution with a solution containing 1.2% of Triton X-100 and 1.8 g/L of acetonitrile in water, to decrease sample viscosity and therefore facilitate volume measurements and sample injection. Each tube was vortex-mixed and centrifuged at 16,000 g for 5 min at 4°C. A fixed volume of supernatant (0.5 µL) was injected into the chromatographic system.

- Sample preparation

  One hundred microliters of B or VH samples were mixed with 10 µL of IS. The samples were diluted to 500 µL with the Triton X-100 and acetonitrile solution, centrifuged, and 0.5 µL of the supernatant were directly injected into the GC as described later. Concerning U and CCM, after adding the IS, samples were centrifuged and 0.5 µL of the supernatant was directly injected into the GC.

- Analytical instrument settings

  The GC used equipped with a FID. injection port of the chromatograph was installed with a glass liner (5-mm i.d.) appropriated for split analysis, to prevent the contamination of the GC column with non-volatile material from the tested matrices. For B and VH, the liner was replaced after 50 injections. For CCM and for U, the liner was replaced after 100 injections.

  The analyses were performed under the following chromatographic conditions:

  Column: 25 m × 0.25 mm i.d., DF = 0.2 µm. The temperature of the FID was 220ºC, and the injector temperature was 220°C. The oven temperature was programmed to 40°C (for 2 min), followed by an increase of 5ºC/min until 200ºC. The carrier gas was helium with a flow of 1.5 mL/min. The injection of B and VH was performed by means of a 10 µL Syringe with a removable needle(needle gauge 22S), cleaned under vacuum between each injection with theTriton X-100 and acetonitrile solution. On the other hand, the injection of CCM and U was performed by means of a 5-µL syringe cleaned under vacuum between each injection with deionised water. The volume of injection was 0.5 µL, with a split ratio of 100 and a split flow of 120 mL/min for Band VH; and a split ratio of 60 and a split flow of 90 mL/min for U and CCM.

- Results and Discussion

  GC separation

  Interferents were ruled out by verifying the absence of peaks in the retention times of the studies analytes (Figure).As shown in Figure , the retention times for acetaldehyde, acetone, methanol, and ethanol in all tested matrices (Figure) were 1.66, 2.02, 2.70, and 3.16 min, respectively. The IS (1-propanol) retention time was 5.26 min. The peak at 3.71 min in the chromatogram in Figure (B and VH) corresponds to the peak of acetonitrile, which is part of thetriton X-100 and acetonitrile solution used to dilute these complex matrices.