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Short communication

Determination and stability of chloroform and diethyl ether on textile evidence in forensic toxicology

Zorica Bozhinoska*, Stefanija Ubavkova, Marija Bujaroska Perkovikj, Natasha Bitoljanu, Rosica Chakar, Viktorija Belakaposka Srpanova, Aleksandar Stankov

Institute of Forensic Medicine, Criminology and Medical Deontology, Medical Faculty, Ss. Cyril and Methodius University in Skopje, Mother Theresa 19, 1000, Skopje, Republic of North Macedonia

Introduction

Historically, chloroform ($CHCl_3$) and diethyl ether ($(C_2H_5)_2O$) were used as anesthetics, due to their sedative properties. Despite being abandoned in modern medicine, these volatile substances remain of significant interest in forensic investigations considering their involvement in accidental or intentional poisonings through forced inhalation, usually associated with homicides, suicides, or drug-facilitated sexual assaults (Gaillard et al., 2005; Monticelli, et al., 2011; Richeval et al., 2016). Both compounds are classified as "knock-out drugs"as a result of their ability to incapacitate victims through sedations, altered behavior, rendering them defenseless and vulnerable to exploitation (Madea and Musshoff, 2009). In cases where volatile substances are suspected, analyzing non-biological matrices recovered near the victim, such as textiles, containers, or similar items is crucial. The aim of this study was to assess the applicability of an analytical method intended for whole blood to the analysis of alternative textile matrices and to determine the time window during which these samples retain evidentiary value for forensic analysis.

Materials and methods

Analytical grade (p.a.) chloroform and diethyl ether were purchased from MERCK (Germany). HPLC-grade methanol (99.8% purity) was obtained from Fisher Chemical (Belgium) and acetone (p.a.) was procured from Carlo Erba (France). Analyses were performed using a GC-MS-TQ8050 NX equipped with a HS-20 NX headspace sampler (SHIMADZU, Japan).

Stock solutions of chloroform and diethyl ether (100 mg/L) were prepared in methanol and working solutions were diluted with 20% methanol. Aqueous acetone solution was used as internal standard (IS). Drug-free whole blood served as the matrix for calibrators and quality control (QC) samples across a concentration range of 1-100 mg/L.

To simulate a forensic case scenario, two 40 × 40 cm cotton cloth samples were completely soaked by applying diethyl ether and chloroform. The treated textiles were stored within a laboratory safety cabinet maintained at room temperature with no active ventilation. Every 24 h over a ten-day monitoring period, a 2 × 2 cm sample was cut from each cotton cloth and immediately sealed in a headspace vial.

*zorica.stojcheska@medf.ukim.edu.mk

Before the analysis, 0.1 mL calibrator, QC, or sample, and 0.4 mL of the IS solution were added to each headspace vial. Chromatographic separation was achieved using a SH-BAC2 column (30 m × 0.32 mm × 1.20 µm) with helium as carrier gas at a constant flow rate of 1.5 mL/min. Headspace equilibration was conducted at 60 °C, with a total GC cycle time of 21 min. The GC oven temperature program started at 35 °C (2 min), ramped at 35°C/min to 220 °C, and held for 2 min. The interface and ion source temperatures were maintained at 230 °C and 200 °C. Target and qualifier ion were monitored in SIM mode: chloroform (*m/z* 83, 47), diethyl ether (*m/z* 59, 74), and acetone (*m/z* 43, 58).

Results and discussion

Under optimized chromatographic conditions, chloroform, diethyl ether, and IS were well separated without matrix interference at their respective retention times. The analytes were eluted at 4.11 min (chloroform), 2.23 min (diethyl ether), and 3.15 min (IS), demonstrating excellent peak repeatability throughout the analytical runs.

The method demonstrated strong linear relationship over the concentration range of 1–100 mg/L, with R^2 greater than 0.99 for both compounds. The criteria for precision and accuracy were fulfilled according to SWGTOX guidelines. Furthermore, the method exhibited high analytical sensitivity, with a limit of detection of 0.2 mg/L and limit of quantification of 1 mg/L.

The applicability of the method for the analysis of alternative samples was evaluated using two simulated forensic textile specimens, achieving satisfactory analytical response for both analytes. Furthermore, the temporal stability of these samples was evaluated over a 10-day period, demonstrating that chloroform and diethyl ether remained detectable within the cotton cloth matrix throughout the entire monitoring interval. Despite their high volatility, the prolonged detection of chloroform and diethyl ether indicates that textile fibers actively adsorb these analytes, slowing evaporation and extending the forensic detection window (Dąbrowski, 2024). Consequently, these findings confirm the method's viability for analyzing alternative, non-biological matrices in forensic and toxicological investigations involving volatile agents. However, more extensive research is needed to determine the usefulness of this kind of forensic evidence when collected from different places and stored under different conditions.

Conclusion

The analysis of alternative non-biological matrices like textiles presents a challenge regarding analyte evaporation. Adapting existing protocols for the analysis of volatiles in whole-blood to alternative samples matrices provide a rapid, sensitive, and reproducible analytical approach. Demonstrating that chloroform and diethyl ether remain detectable on cloth matrices for several days significantly expands the scope of forensic scene-of-crime investigations by extending the time window for collecting valuable physical evidence in suspected poisoning cases.

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