B.L.T.

THE TOXICOLOGICAL SOCIETY OF BELGIUM AND LUXEMBOURG

A.S.B.L. – V.Z.W.

President:
Prof. A. VERSTRAETE

General secretary:
Dr. K. MAUDENS

Final Program

BLT scientific session and general assembly – March 8th, 2018 – UCL Saint-Luc, salle Verrière

18.30 – 18.35: welcome by our president

18.35 – 19.35: **Guidelines for Therapeutic Drug Monitoring in Psychiatry.**
Prof. C. Hiemke (University of Mainz, Germany)

19.35 – 20.05: **Royal Decree of 06.09.2017 regulating narcotics and psychotropic substances: practical implementation for toxicological laboratories.**
G. Declercq (FAGG-AFMPS)

20.05 – 20.45: **Free communications**

- **Activity-based detection and bioanalytical confirmation of a fatal carfentanil intoxication.**
  Lars Ambach, Annelies Cannaert, Peter Blanckaert and Christophe Stove

- **Ionic liquids as promising extraction solvents in toxicology.**
  Marieke De Boeck, Sophie Missotten, Lisa Dubrulle, Wim Dehaen, Jan Tytgat and Eva Cuypers

20.45 – ... : **BLT general assembly followed by reception.**

Activity-based detection and bioanalytical confirmation of a fatal carfentanil intoxication

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Keywords: synthetic opioids; untargeted screening; activity-based; bioassay; carfentanil; LC-MS/MS

Abstract

Carfentanil, one of the most potent opioids known, has recently been reported as a contaminant in street heroin in the USA and Europe, and is associated with an increased number of life-threatening emergency department admissions and deaths. Here, we report on the application of a novel *in vitro* opioid activity reporter assay and a sensitive bioanalytical assay in the context of a fatal carfentanil intoxication, revealing some of the highest carfentanil concentrations reported until now.

A 21-year-old male was found dead at home with a note stating that he had taken carfentanil with suicidal intentions. A foil bag and plastic bag labelled "C.50" were found at the scene. These bags were similar to a sample obtained by the Belgian Early Warning System on Drugs from a German darknet shop and to those found in the context of a fatality in Norway.

Blood, urine and vitreous, obtained during autopsy, were screened with a newly developed *in vitro* opioid activity reporter assay able to detect compounds based on their μ-opioid receptor activity rather than their chemical structure. All extracts showed strong opioid activity. Results were confirmed by a bioanalytical assay, which revealed extremely high concentrations for carfentanil and norcarfentanil. It should be noted that carfentanil concentrations are typically in pg/mL, but here they were mid to high ng/mL range for all three matrices. The blood and vitreous contained norcarfentanil in the high pg/mL range. No norcarfentanil was detected in urine.

This is the first report where a novel activity-based opioid screening assay was successfully deployed in a forensic case. Confirmation and quantification using a validated bioanalytical procedure revealed some of the, to our knowledge, highest carfentanil concentrations ever reported in humans.

IONIC LIQUIDS AS PROMISING EXTRACTION SOLVENTS IN TOXICOLOGY

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INTRODUCTION

Thorough clean-up of complex biological samples remains a crucial step in analytical processes. To date, solid-phase extraction is frequently applied in sample preparation, however, its main disadvantages are labor-intensive and time-consuming protocols. In this respect, dispersive liquid-liquid micro-extractions (DLLMEs) seem to offer less complex and efficient extraction protocols. Furthermore, ionic liquids (ILs) – liquid salts – are emerging as new promising extraction solvents, thanks to their non-flammable nature, negligible vapor pressures and easily adaptable physiochemical properties.

OBJECTIVES

In this study, the applicability of ILs as DLLME extraction solvents in toxicology was investigated. Moreover, two multi-analyte IL-DLLME-LC-MS/MS methods were optimized and validated for a large number of benzodiazepines (BZDs), BZD-like hypnotics and antidepressants (ADs).

METHODS

One mL whole blood was transferred into a conical bottom glass tube. Subsequently, one mL aqueous buffer and 60 µL IL (1-butyl-3-methylimidazolium hexafluorophosphate) was added. The glass tube was mixed for 5 min at 50 rpm, using a rotary mixer. Phase separation was obtained by centrifugation for 6 min at 3500 rpm. 10 µL of the lower IL layer was collected and diluted 1:10 in methanol. The final extract was analyzed using LC-ESI-MS/MS analysis in scheduled multiple reaction monitoring mode.

RESULTS

Two targeted multi-analyte procedures were successfully validated for the quantification of 19 BZDs (including 2 BZD-like hypnotics) and 18 ADs. Optimized IL-DLLME protocols for both drug classes were similar and took less than 30 minutes. Both analytical method showed good selectivity. Matrix-matched calibration curves were constructed, based on 7 concentration levels (n = 6), covering therapeutic ranges and low toxic plasma concentrations. Deuterated standards were introduced to ensure accurate quantification; 16 deuterated BZD and 3 deuterated AD analogues. Accuracy and precision results met the proposed acceptance criteria (bias, repeatability and intermediate precision < 15 % or < 20 % near LOQ) for the majority of BZDs, except for brotizolam, chlordiazepoxide, cloxazolam, flunitrazepam, loprazolam, lorazepam and nitrazepam, which could only be determined in a semi-quantitative way. Fluvoxamine was excluded from the AD quantitative method. Deviations were partly explained by incomplete dissolution of tablet extracted standards. Recoveries were within 25 % - 127 % (BZDs) and 53 % - 133 % (ADs). Matrix effects indicated ion suppression, which was attributed to the presence of IL.

CONCLUSION

Overall, the applicability of ILs as promising solvents for the extraction of BZDs and ADs in whole blood was proven. Moreover, fast and easy IL-DLLME-LC-MS/MS methods were developed for the quantification of 19 BZDs (including 2 BZD-like hypnotics) and 18 ADs in whole blood.