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Microextraction by packed sorbent (MEPS)-UHPLC-UV: A simple and efficient method for the determination of five benzodiazepines in an alcoholic beverage

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ABSTRACT

This article describes a nano-scale method for the determination and quantification of five benzodiazepines (BDZ) in an alcoholic grappa drink (chlordiazepoxide; lorazepam; diazepam; oxazepam; medazepam). BDZ are typically used in drug-facilitated crimes (DFC) for their accessibility and synergistic effects with alcohol. Specimens collected on the crime scene must be rapidly analyzed to prove the crime, though, in most cases, a very small amount is available. Off-line MEPS extraction of diluted grappa samples proved to be an efficient and reliable method for the recovery of the selected compounds. Requiring a very small amount of extraction solvents, MEPS is an environment-friendly technique. LC separation with UV detection was used as the analytical technique because it is simple, robust, relatively economic and easy-to-find in most laboratories. The method was validated in terms of precision, accuracy and recovery. Limits of detection and quantitation were in the range of 0.5–2 ng/μL. Linearity (R^2) spanned from 0.9994 and 1.0000. Intra- and inter-day repeatabilities were lower than 12% at any concentration. Recovery percentages of an equivalent-to-real sample at three different concentrations were between 70.7 and 74.1%.

1. Introduction

The use of benzodiazepines (BDZ) is associated to the treatment of several psychological disorders, such as anxiety, insomnia, muscle spasms, seizure and many others, so they are widely prescribed drugs. Abuse of these substances can lead to intoxication, which can be associated to alteration of the behavior. Illegal use of BDZ is often reported in drug-facilitated crimes (DFC), commonly conveyed through alcoholic drinks. The mixture of alcohol and BDZ potentiates the pharmacological effects and can intoxicate the victim, making her/him unable to resist to an aggression, stunned, in most cases with no memory of the criminal offense. For this reason, the determination of these drugs is of great importance in clinical and forensic cases. These substances and their metabolites have short-half life and are excreted rapidly from the body, therefore their detection in biological samples (urine, blood, etc.) can be impaired if the specimens are collected after many hours from the assault. In many cases, the evidence of the DFC may be found in glasses or bottles collected at the crime scene. The sample left in the container in most cases is very small, yet the amount of drugs used in DFC often exceeds the therapeutic dose, therefore it can be very informative because those analytes do not biometabolize in these kind of samples.

Though BDZ share similar chemical structures, their polarity differences are rather wide. Numerous methods can be found in literature for the determination of BDZ for forensic and clinical purposes. Gas chromatography (GC) has been widely used as well, in combination with mass spectrometry (MS) or with other detectors such as nitrogen-phosphorous (NPD) and micro-electron capture (ECD) [1 and references therein-2] Hyphenated GC techniques are very sensitive and specific, yet some BDZ are thermolabile and require derivatization prior to analysis [3,4]. Other approaches

Abbreviations: ACN, acetonitrile; BDZ, benzodiazepine; CZE, capillary zone electrophoresis; DFC, drug-facilitated crimes; DLLME, dispersive liquid-liquid microextraction; ECD, micro-electron capture detector; GC, gas chromatography; LC, liquid chromatography; LLE, liquid-liquid extraction; LOD, limit of detection; LOQ, limit of quantification; MAE, microwave-assisted extraction; MeOH, methanol; MEPS, microextraction by packed sorbent; MS, mass spectrometry; NPD, nitrogen-phosphorous detector; PDA, photo-diode array detection; RAM, restricted-access material; RSD, relative standard deviation; S/N, signal-to-noise ratio SPE solid phase extraction; SPME, Solid-phase microextraction; UHPLC, ultra-high performance liquid chromatograph; UV, ultraviolet detection.

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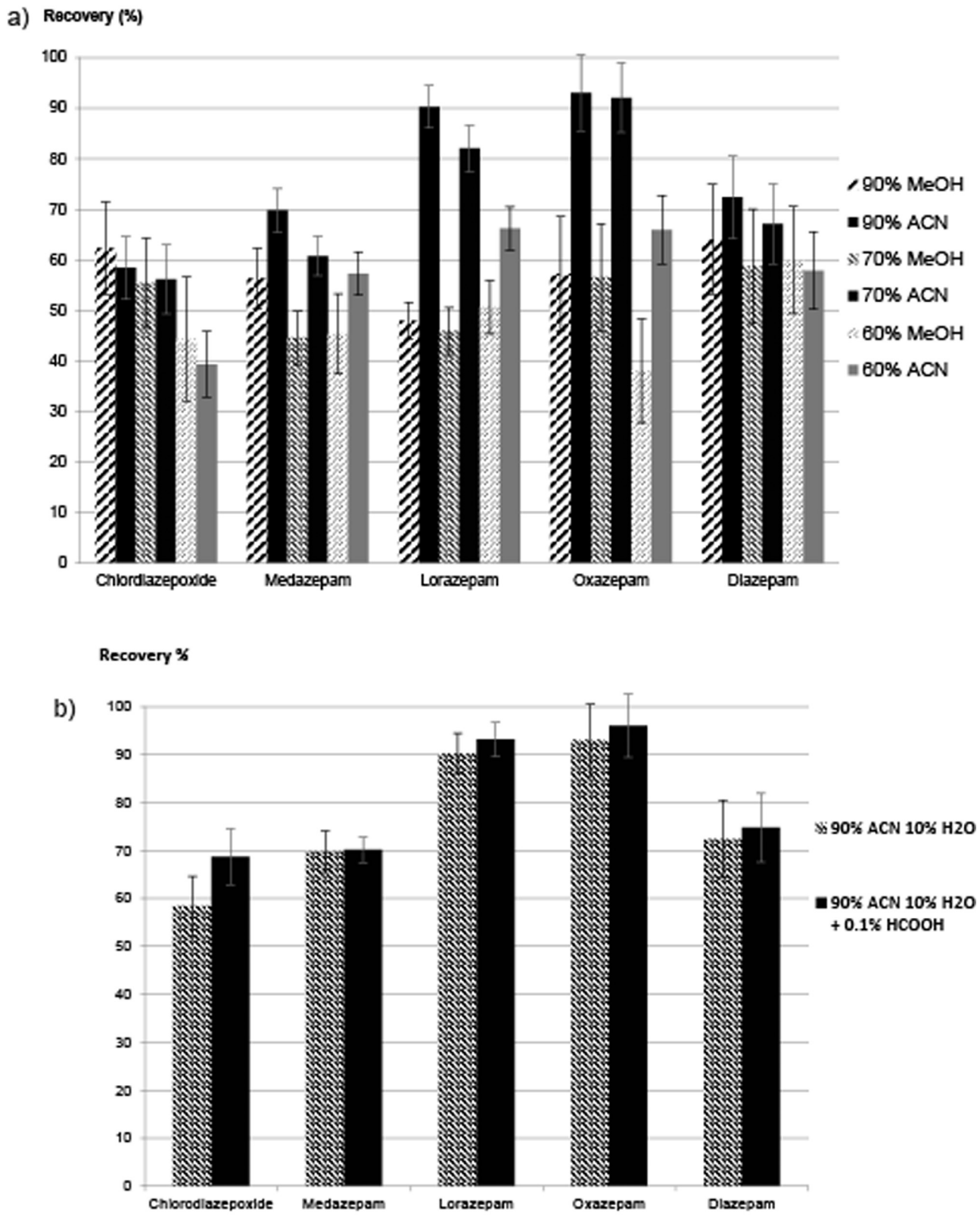

Fig. 1. Extraction mixtures tests. (a) neutral MeOH and ACN mixed with water in different proportions. (b) ACN:water 90:10 (v/v) neutral vs. acidified with 0.1% formic acid.

include micellar electrokinetic chromatography using a cyanine dye for indirect laser-induced fluorescence detection and capillary zone electrophoresis (CZE) [5]. Nonetheless, liquid chromatography (LC) is a commonly used technique, particularly when coupled with MS or ultraviolet (UV) detection [6]. Though MS is more specific and sensitive, photo-diode array (PDA) detection adds spectral information that can be valuable for the identification of the analyte. In addition, UV detection is more economical than MS, therefore more commonly available in analytical laboratories. The use of LC in the analysis of BDZ permits to overcome derivatization