AUTONOMOUS MICROFLUIDIC DEVICE FOR THE NAKED-EYE DETECTION OF BENZODIAZEPINES IN ADULTERATED BEVERAGES

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ABSTRACT

The rise of drug-facilitated sexual assault (DFSA) has catalyzed the advancement of Lab-on-a-Chip devices for immediate detection of date-rape drugs. Here, we present the development of a microfluidic device tailored for the colorimetric detection of benzodiazepines. Layered device operates autonomously for seamless field integration. Capable of detecting benzodiazepines across a spectrum of beverages, the device exhibits a quantitative detection limit of 0.54 mg mL^{-1} for midazolam. These findings underscore the utility of the device in real-world scenarios. Furthermore, its potential for industrialization and commercialization highlights its promise in facilitating rapid, on-site, and visual detection of benzodiazepine adulteration in real-world samples.

KEYWORDS

Microfluidic device, cobalt (II) thiocyanate, benzodiazepines, colorimetric reaction

INTRODUCTION

Recently, instances of drug-facilitated sexual assault (DFSA) have surged within cases of sexual abuse, presenting a grave societal concern. DFSA entails subjecting individuals to sexual aggression while incapacitated by the effects of alcohol and/or drugs, commonly referred to as date-rape drugs. These substances, which include central nervous system depressants such as hypnotics, benzodiazepines, analgesic-anesthetics like ketamine or fentanyl, scopolamine, and γ-hydroxybutyric acid (GHB), induce a range of pharmacological effects, including relaxation, amnesia, impaired perception, and unconsciousness.

Benzodiazepines, a prominent class of central nervous system depressants, exemplify the potency of such substances, with drugs like diazepam and flunitrazepam amplifying their anxiolytic and sedative properties when combined with alcohol. Detecting these drugs promptly and accurately is critical in preventing instances of chemical submission aggression. On-site presumptive analysis of adulterated beverages offers a promising avenue for intervention, providing rapid results with minimal sample requirement.

However, identifying these substances in sexual assault cases typically relies on sophisticated analytical techniques such as gas chromatography-mass spectrometry (GC-MS), capillary electrophoresis (CE), high-performance liquid chromatography (HPLC), or liquid chromatography-mass spectrometry (LC-MS), often coupled with spectroscopic methods. Despite the precision and sensitivity of these methods, challenges persist in ensuring widespread access to such advanced analytical capabilities.

Various strategies, including electrochemical and color-based sensing methods, have been employed to develop on-site tests for detecting date-rape drugs. Color-based tests, in particular, have been utilized for decades in on-site presumptive analysis due to their simplicity, speed, and portability. Recent advancements, such as color digital analysis, the development of solid colorimetric sensors, and microfluidic devices, have fueled growing interest in deploying these tests at the point of need. Novel analytical tools for detecting date-rape drugs continue to emerge. For instance, Argente-García *et al.* [1] introduced a passive solid sensor for in-situ colorimetric detection of ketamine, offering a promising solution for rapid identification. Similarly, Rodrígez-Nuévalos *et al.* [2] demonstrated the use of gold nanoparticles for the colorimetric detection of GHB in beverages, showcasing the potential of innovative approaches in enhancing detection capabilities and addressing challenges associated with drug-facilitated sexual assault.

Microfluidic paper-based analytical devices (μPADs) have emerged as invaluable tools for rapid detection of date-rape drugs at crime scenes. These devices offer simple, on-site colorimetric tests [3] [4] capable of multiplex analysis for a wide range of analytes. Leveraging the principle of lateral-flow test strip technique, μPADs utilize capillary action to drive liquid flow, enabling easy transport and facilitating rapid analysis. One of the key advantages of μPADs is their low cost, attributed to the affordability of substrate materials and the straightforward fabrication process. These systems rely on simple chemical reactions that produce visible results, which can be easily interpreted by the naked eye. This combination of affordability, simplicity, and portability makes μPADs highly suitable for on-site detection of date-rape drugs, offering a promising solution for law enforcement and forensic investigation teams.

In recent years, there has been a surge in the development of microfluidic devices tailored for the naked-eye detection of psychotropic compounds through colorimetric reactions. A notable example is the work by Musile *et al.* [5], who engineered a six-channel paper device for detecting ketamine and other psychotropic drugs like morphine, epinephrine, and codeine. The microfluidic device featured wax channels imprinted on chromatographic paper, each hosting different reagents necessary for the detection process. Despite the promising approach, this method encountered a significant limitation: a high and inadequate limit of detection. This drawback hinders its practical application in real-life scenarios, where sensitivity and accuracy are paramount.

Although several benzodiazepine detection assays in solution have been reported, no microfluidic device has yet demonstrated sufficient efficiency. One notable example is the complexation reaction involving Cobalt (II) thiocyanate in an acid medium, known as Scott's reaction, which serves as a universal colorimetric sensor for benzodiazepines in solution. However, this method has not yet been adapted for use on a solid support or incorporated into a microfluidic device. [6]

In this work, we introduce the creation of an autonomous, compact, and portable microfluidic device designed for the detection of benzodiazepines such as diazepam, midazolam, and lorazepam in beverages. This device offers the potential for real-world applications due to its rapid response time, user-friendly operation, and capability for on-site analysis (see Figure 1). Notably, the Microfluidics Cluster UPV/EHU recently submitted a European patent application (EP22382986.2) for a microfluidic device specifically engineered to detect date-rape drugs.

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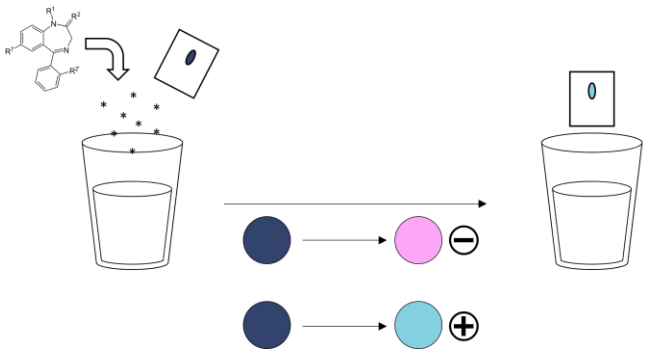

Figure 1: Scheme of the protocol followed for analysis. Color changes in the presence of benzodiazepines, from dark blue to light pink in a negative result, and to light blue in a positive result.

EXPERIMENTAL

The benzodiazepine microfluidic device is composed of several overlapping layers, Figure 2. From bottom layer to top layer: hydrophilic pressure-sensitive adhesive (PSA), a double layer double side PSA, where the paper strip fits, and a layer of Poly(methyl methacrylate) (PMMA), 175 µm thick, as a cover.

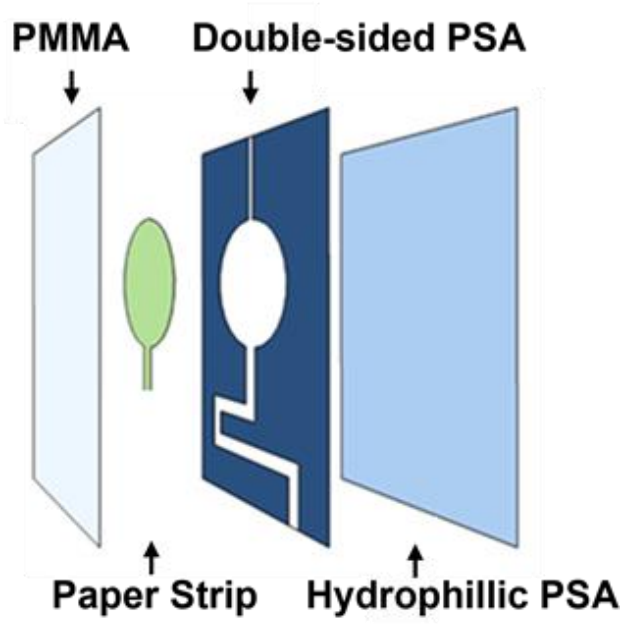

Figure 2: Scheme of the different layers forming the benzodiazepines microfluidic device test.

PSA was used since it is an adhesive material, where the paper strip can be glued and thus fix the sensing area position for visualization. Moreover, the hydrophilic PSA was used since it enables a small sample volume of the drink to flow up from the beverage to the strip by capillarity forces.

The microfluidic channel, forming the liquid reservoir, was defined using double-sided PSA. A double layer was necessary to provide the channel with the required height for accommodating the paper strip and to create sufficient space to hold the precise volume of liquid necessary for wetting the entire strip. Additionally, the double-sided PSA was employed to establish an air channel at the top of the strip, enabling liquid flow through the microfluidic channel, functioning as a vent and preventing the buildup of excessive pressure inside the device.

Cellulose paper was selected for constructing the paper strip to serve two purposes: firstly, to physically position the sensing area, and secondly, to facilitate the flow of the sample towards the sensing area for colorimetric determination. The design of the paper strip was inspired by the natural capillary action of solutions in paper, aiming to enhance its ability to continuously absorb liquid and thereby regulate the operation time of the device. Furthermore, the shape and dimensions of the circular area housing the sensing region were carefully chosen to ensure a discernible color change, Figure 3.

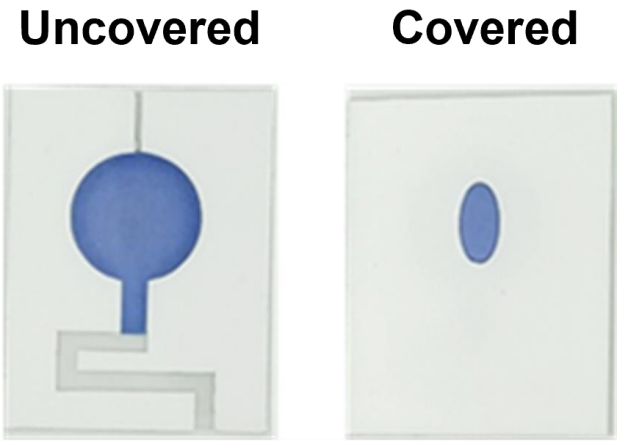

Figure 3: Pictures of two benzodiazepines microfluidic devices, uncovered (left) and covered (right).

RESULTS AND DISCUSION

A calibration curve was generated for the benzodiazepines microfluidic device to assess the sensitivity of the reaction with midazolam, Figure 4. To accomplish this, five midazolam solutions including 0.0, 0.5, 1.0, 2.0, 3.0 mg mL^{-1} in distilled water were prepared and tested with the device. Observations were made with the naked-eye, revealing the formation of a cyan coloration on the bottom half of the microfluidic device and a darkening of the pink color on the upper part. This color change commenced slightly at a midazolam concentration of 0.5 mg mL^{-1}, became distinctly apparent at 1.0 mg mL^{-1}, and increased in intensity with higher midazolam concentrations. The quantitative limit of detection was determined to be 0.54 mg mL^{-1}, Figure 5.

Taking into consideration the effective oral dosage for midazolam when used as hypnotic (7.5 mg), and the possible volume of 1 to 5 full sips (20 – 100 mL), the minimum effective concentration of midazolam that can be found in adulterated beverages can range from 0.08 mg mL^{-1} to 0.75 mg mL^{-1}. In its present form, both the qualitative and quantitative limit of the detections of the benzodiazepines microfluidic device falls within the upper range of midazolam concentrations that can be found in real scenarios.

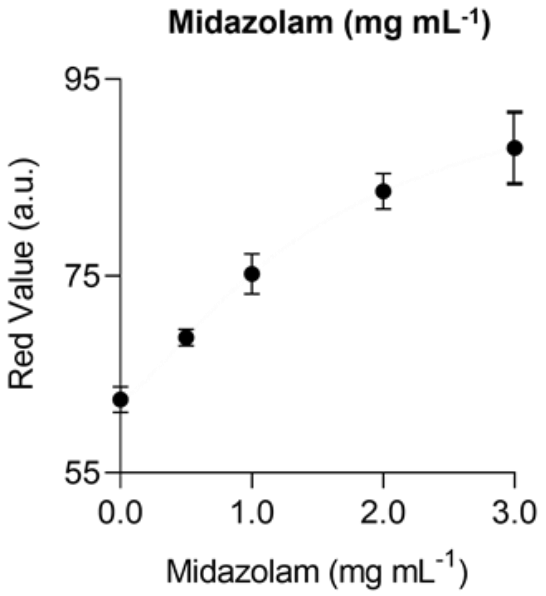

Figure 4: Calibration curve for midazolam using the microfluidic device. Red Values were obtained for different concentrations of midazolam. Error bars mean SEM (n = 3).

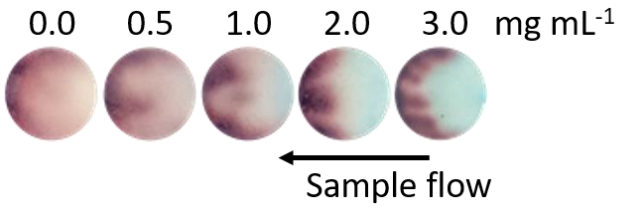

Figure 5: Photographs of the reaction section of the microfluidic device loaded with different concentrations of midazolam. Photographs were taken 5 min after the device performance.

In order to test the device in different matrices that can be found in real scenarios, a variety of conventional beverages were tested, such as coke, orange soda, lemon soda, tonic water, energy drink, white wine, red wine, beer rum + coke (3:7), vodka + lemon soda (3:7) and gin + tonic water (3:7) were chosen. The matrices were adulterated with different drugs such as midazolam (0.5, 1.5, 2.5 mg mL^{-1}). In Figure 6 (soft drinks) y Figure 7 (alcoholic drinks) it was demonstrated that the quantification of the intensity of red value on each of the cases showcased those increasing concentrations of benzodiazepines, such as midazolam, corresponded to higher red intensity values.

CONCLUSION

In conclusion, this study presents the development of an autonomous and portable microfluidic device capable of detecting benzodiazepines in various common beverages, achieving an adequate limit of detection. The novel multilayered microfluidic device introduced here holds promise for direct application in real date-rape cases. Whether through visual inspection or combined with image analysis software, this device emerges as a potential valuable tool in sexual assault prevention. However, further optimization is necessary to effectively implement the device in real-case scenarios.

Future research should prioritize testing a broader range of benzodiazepines and exploring the detection of other complex substances to enhance the limit of detection. Additionally, owing to its exceptional performance and straightforward fabrication process, the device exhibits strong scalability and stands poised for commercialization.

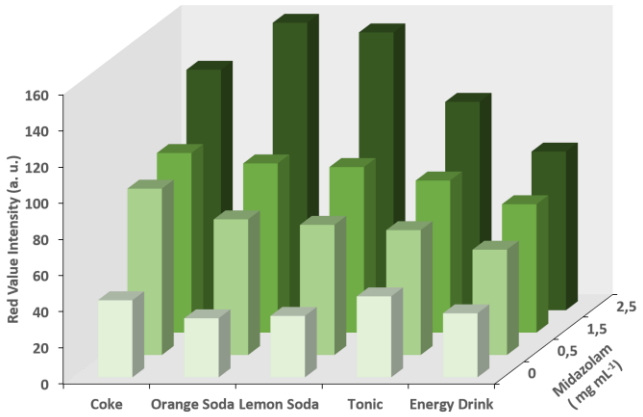

Figure 6: Colorimetric analysis of midazolam sensing on different soft drinks. Comparative diagram of the mean red value, z-axis, (n = 3) according to different beverages (coke, orange soda, lemon soda, tonic water, energy drink) and midazolam concentration (0.0, 0.5, 1.5, 2.5 mg mL^{-1}).

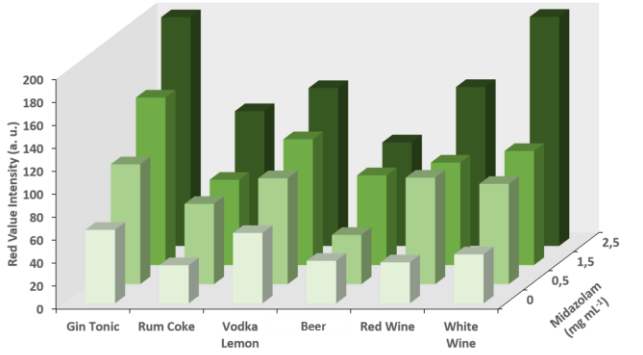

Figure 7: Colorimetric analysis of midazolam sensing on different alcoholic drinks. Comparative diagram of the mean red value, z-axis, (n = 3) according to different beverages (gin tonic, rum coke, vodka lemon, beer, red wine, white wine) and midazolam concentration (0.0, 0.5, 1.5, 2.5 mg mL^{-1}).

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