

A microfluidic paper-based device for on-site colorimetric detection of scopolamine in beverages, pharmaceuticals, and plant extracts

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ABSTRACT

Drug-facilitated sexual assault is a major public health and forensic concern, frequently involving central nervous system depressants such as scopolamine. This tropane alkaloid is especially hazardous due to its strong amnesic, sedative, and hallucinogenic effects, combined with its lack of color, odor, and taste. Despite its toxicological importance, there are still few methods available for the rapid on-site detection of scopolamine. Therefore, portable, low-cost analytical tools capable of detecting scopolamine in a variety of matrices at the point-of-need are still lacking. Here, we developed an autonomous microfluidic paper-based analytical device for the rapid detection of scopolamine in pharmaceuticals, plant extracts, and beverages. The device is based on Scott's colorimetric reaction, in which Cobalt(II) thiocyanate forms a visible pink-to-blue complex with scopolamine under acidic conditions. The system consists of a cellulose substrate sandwiched between hydrophilic and hydrophobic pressure-sensitive adhesive layers and a poly(methyl methacrylate) cover, enabling capillary-driven fluid transport and direct visual readout. Calibration studies demonstrated a concentration-dependent color change, achieving limits of detection of 0.13 mg mL⁻¹ for pure scopolamine and 0.11 mg mL⁻¹ for commercial formulations such as Buscapina®. The device was tested in a wide range of alcoholic and non-alcoholic beverages and produced a clear response in extracts of *Datura stramonium* and *Atropa belladonna*. This microfluidic paper-based device provides a fast, reliable, and easy-to-use approach for the on-site detection of scopolamine and related compounds. Its low cost, portability, and visual readout make it particularly suitable for forensic screening and public health protection. The platform represents a significant advancement toward practical point-of-need detection of drug-facilitated assault agents.

1. Introduction

Sexual assault is a significant public health and safety concern, with drug-facilitated sexual assault (DFSA) being a particularly relevant issue. DFSA occurs when an individual is unknowingly administered drugs or alcohol, leading to incapacitation and increased vulnerability. The substances used in these crimes, commonly referred to as date-rape drugs, are favored by perpetrators due to their ability to impair both cognitive and motor functions, rendering victims unable to resist or recall the events [1,2].

The most commonly used date-rape drugs are central nervous system (CNS) depressants, which act quickly, inducing relaxation, drowsiness, memory loss, and unconsciousness even at low doses. Their lack of taste, smell, or color allows them to be easily concealed in beverages, making detection difficult. Victims often experience rapid onset of symptoms within 30 min of ingestion, leaving them vulnerable. Among the

substances frequently used in DFSA are benzodiazepines, anesthetics (such as ketamine or fentanyl), γ -hydroxybutyric acid (GHB), and scopolamine [1,3]. Recently, our group reported the development of an autonomous, compact, and portable microfluidic device for the detection of benzodiazepines, specifically diazepam and midazolam, in beverages, offering rapid response, user-friendly operation, and on-site analytical capability [4].

Scopolamine is considered one of the most commonly used date-rape drugs because of its potent neurological effects, which include sedation, memory impairment, hallucinations, and loss of voluntary control. It is a tropane alkaloid naturally found in *Solanaceae* plants such as *Atropa belladonna* and *Datura stramonium* [5,6]. Considering the narrow therapeutic window of scopolamine, where toxic doses are close to lethal doses, timely and accurate detection is essential, especially in DFSA cases, where overdose can easily lead to fatal outcomes [6].

Typically, the detection of drugs involved in DFSA relies on

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instrumental analytical techniques such as gas chromatography-mass spectrometry (GC-MS), capillary electrophoresis (CE), high-performance liquid chromatography (HPLC), and liquid chromatography-mass spectrometry (LC-MS) [7]. While these methods provide high sensitivity and specificity, they require laboratory conditions, trained personnel, and extensive sample preparation, making them impractical for rapid, on-site analysis.

To overcome this limitation, alternative detection methods such as electrochemical sensors and colorimetric assays have been explored to develop portable, rapid, and cost-effective solutions [8–10]. Colorimetric tests, in particular, are widely used in on-site forensic analysis due to their simplicity, speed, and ease of interpretation [11,12]. However, despite their widespread use for detecting other substances, validated colorimetric tests for scopolamine remain insufficient, limiting their applicability for presumptive DFSA testing.

To address this research gap, various analytical approaches have been pursued for scopolamine detection. Brown et al. [13] developed electrochemiluminescent sensors as a screening strategy for scopolamine in biological matrices. Subsequently, multiple detection devices were designed based on electrophoresis [14–16] and fluorescence [17, 18] methods across different sample types. Additionally, hybrid systems combining colorimetric assays with other detection techniques were developed. For instance, Hernandez-Contreras et al. [19] introduced a BODIPY-Phen conjugated with Cu(II) probe that used a fluorescence turn-off mechanism to detect scopolamine and ketamine in beverages. However, the lack of portability and the necessity of fluorescence for analysis and quantification limited its practical application. More recently, Melo et al. [20] optimized a portable platform based on the Dragendorff's reagent for scopolamine screening, which combined colorimetric and electrochemical detection of beverages and biological samples. However, this approach lacked quantitative capabilities in the colorimetric sensor, limiting its analytical utility. In parallel, different systems relying only on colorimetric reactions for scopolamine detection have been developed. Jornet-Martinez et al. [21] designed a bicolormetric sensor using potassium permanganate (KMnO_4) in polydimethylsiloxane (PDMS) that reacted with scopolamine under alkaline conditions. Nevertheless, this system requires substantial sample pretreatment including solid-phase extraction (SPE) for beverage analysis. They also established a complementary method for scopolamine detection using a portable nano liquid chromatograph system. Similarly, Mousavizadeh et al. [22] developed an optical nanosensor by encapsulating Dragendorff's reagent within graphene oxide grafted with poly (citric acid) (GO-g-PCA) and immobilizing it on a cellulose triacetate membrane, enabling scopolamine detection in plant extracts. Although a visible color change was observed, UV-Vis spectrophotometry was necessary for quantitative analysis. Moreover, Dias et al. [23] were the first to integrate microfluidic technology with colorimetric detection for scopolamine. They developed a paper-based microfluidic platform employing six distinct chromogenic sensors (including bromocresol green, Chen's reagent, and Dragendorff's reagent) for alkaloid detection in beverages. However, the platform suffered from practical limitations since it took a long time (min. 20 min) for image acquisition, and it required multivariate analysis such as principal component analysis (PCA) and hierarchical cluster analysis (HCA) for scopolamine identification, limiting its feasible deployment in field applications.

While these methods represent notable progress, some still require sample pretreatment, specialized reagents, or fluorescence-based equipment, limiting their practicality for on-site applications. Consequently, further development of portable, reliable, rapid, and versatile colorimetric tests is needed, ideally through platforms capable of detecting multiple date-rape drugs such as scopolamine, within a single detection system. More information can be found on the table presented in [Supporting Information 1](#).

Among the various approaches for rapid, field-deployable drug detection, colorimetric assays offer an effective means of identifying scopolamine with minimal equipment and real-time response

capabilities. One particularly promising reaction for this purpose is Scott's reaction, which employs cobalt(II) thiocyanate in an acidic medium. This well-established colorimetric test has been applied to detect various illicit substances, and although its reaction with scopolamine has been documented, its full potential for this analyte detection remains largely unexplored [24].

The ideal microfluidic platform should enable simple detection of multiple drugs. Here, we present for the first time the Co(SCN)_2 reaction, previously used for cocaine and benzodiazepine detection, can also be applied to scopolamine. Building on our previous research on benzodiazepine detection [4], we adopted a similar strategy by combining a paper-based microfluidic device with a cobalt (II) thiocyanate reaction for scopolamine detection, demonstrating the platform's versatility across multiple drug targets while maintaining discrete, self-powered, and user-friendly operation.

In this study, we developed an autonomous and portable microfluidic device capable of detecting scopolamine by naked-eye through the cobalt (II) thiocyanate reaction. This approach also enables quantification through smartphone-based image analysis, thereby improving accessibility for forensic and public safety applications. The platform is designed to detect scopolamine across diverse sources, including pharmaceutical formulations, natural plant extracts, and various non-alcoholic and alcoholic beverages.

2. Methodology

2.1. Reagents

Hydrochloric Acid (HCl) 1 M and Cobalt (II) Thiocyanate complex (Co(SCN)_2) were purchased from Sigma-Aldrich, Spain. Scopolamine (Scopolamine Hydrobromide Trihydrate) was purchased from Sigma-Aldrich, Spain. Buscapina® injectable solution (Scopolamine Butylbromide 20 mg) was purchased from Opella Healthcare, Spain. *Datura stramonium* and *Atropa belladonna* dry plants were freely provided by the Neuropsychopharmacology Group of the University of the Basque Country (UPV/EHU), Spain. Schweppes Tonic, Lemon KAS, Orange KAS, Coca-Cola, Red-Bull, San Miguel beer, Don Simon white and red wine, Mayaray Rum, Smirnoff Vodka and Steward Gin were all purchased from Eroski, Spain. Poly methyl methacrylate (PMMA) Plexiglas 175 μm , was purchased from Evonik Industries AG, Germany. Pressure Sensitive Adhesive (PSA), ARcare 8939 and ARFlow 93049, were purchased from Adhesive Research, Ireland. The cellulose paper (Whatman qualitative paper, Grade 1) was purchased from Merck, Spain.

2.2. Solutions

20 % Co(SCN)_2 stock solutions were prepared dissolving Co(SCN)_2 powder in distilled water. The scopolamine sensor consisted of a mixture of Co(SCN)_2 stock solution and HCl (1 M) on a ratio of 3:1(v:v) respectively. Scopolamine solutions (0.5, 1.0, 2.0, 3.0, 4.0, 5.0 mg mL^{-1}) were prepared by diluting scopolamine powder in distilled water or in different beverages. Buscapina® solutions (0.5, 1.0, 2.0, 3.0, 4.0, 5.0 mg mL^{-1}) were prepared by diluting the stock solution (20 mg) in distilled water. Solutions of *Datura stramonium* and *Atropa belladonna* were prepared using solid-liquid extraction. For each, 3 g of dried plant material were combined with 50 mL of HCl (5 %). The mixture was heated for 10 min, allowed to cool, and then filtered twice to separate the plant material from the liquid extract. A portion of the resulting solution was then diluted to half its concentration with distilled water.

2.3. Fabrication of the microfluidic device

The microfluidic devices were multilayered, including PMMA layers for support, shape-cut Whatman cellulose paper for containing the scopolamine sensor solution, and PSA layers to bind the different components. PSA layers were cut using a Graphtec CE6000-40 cutting

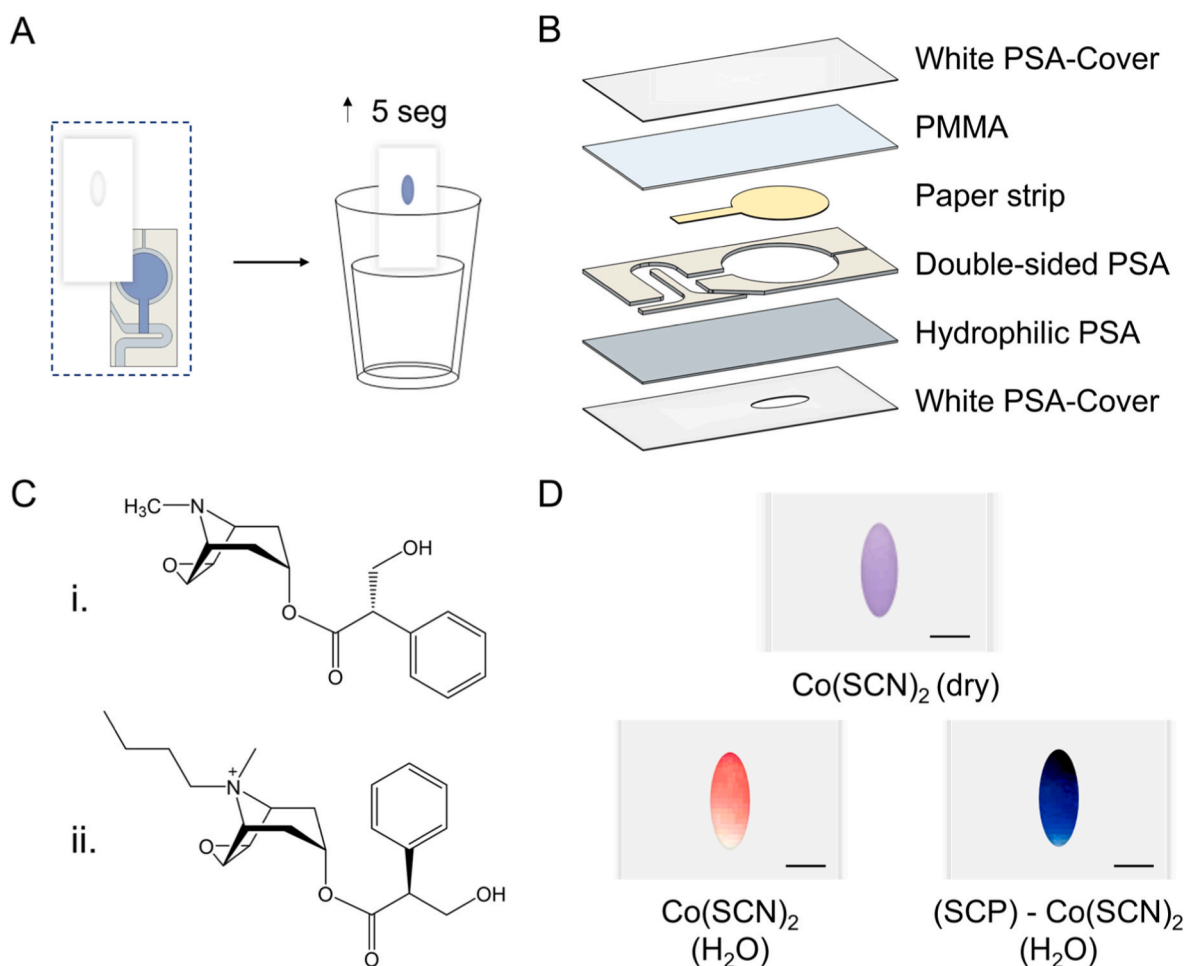


Fig. 1. Scopolamine detection on microfluidic device. A) Illustration depicting the operational steps of the microfluidic device in a real scenario. B) Schematic representation of the different layers forming the scopolamine microfluidic test device. C) Chemical structures of (i.) scopolamine and (ii.) scopolamine *N*-butyl bromide, known as Buscapina®. D) Zoomed images of three scopolamine microfluidic devices, showing the dry sensing area before use (top), the sensing area after distilled water loading (left) and the sensing area after loading with scopolamine (SCP) solution (right). Scale bars represent 250 μm .

plotter (CPS Cutter Printer Systems, Spain), while PMMA and Whatman paper were cut with a CO₂ VERSA VLS2.30 Desktop Universal Laser System (USA). For the microfluidic device, 5 μL of the sensor solution was applied to the paper and allowed to dry completely at room temperature.

The microfluidic device was manually aligned and assembled from the bottom to the top layer. The hydrophilic PSA was first attached to the double-sided hydrophobic PSA. After placing the paper-circles embedded with the sensor solution in the respective space, the circles were closed with the top PMMA layer. The careful manual assembly of the microfluidic device ensured proper layer alignment and prevented leaks.

2.4. Detection of scopolamine and its derivatives using the microfluidic device

Microfluidic devices were immersed in the beverages for 5 s and tested with various concentrations of scopolamine in different matrices, including Milli-Q water, soft drinks, and alcoholic beverages mixed with soft drinks. In addition, the microfluidic devices were tested with Buscapina®, *Datura stramonium* and *Atropa belladonna* solutions following the same protocol.

2.5. Image analysis

Photographs of the microfluidic device were captured before and

after 10 min exposure to the different solutions. The photographs were taken with the front camera of a POCO X3 NFC phone, equipped with a 64 MP Sony IMX682 sensor (1/1.73", f/1.89 aperture, and PDAF, under a photographic setup with controlled and constant light intensity to ensure consistency and reproducibility of the results. The images were analyzed using Fiji ImageJ. All images were converted to RGB, and red values were chosen for the analysis. A 2×6 mm region of interest, corresponding to the viewing window, was applied to each image, and "Color Intensity" was defined as 255 - Red Value.

3. Results and discussion

This study presents the development of a microfluidic device for the rapid detection of scopolamine and its derivatives in adulterated beverages. The device consists of a paper-based sensing area enclosed within multiple polymeric layers, forming a compact microfluidic system. The operation is straightforward: the device is immersed in the beverage for 5 s, allowing the sample reservoir to fill. The sensing area, embedded in a paper disc, absorbs the liquid, initiating self-driven capillary flow toward the detection zone. This configuration ensures optimal interaction between the sensing reagents and the target analyte, enabling clear colorimetric detection, as illustrated in Fig. 1A. Additionally, the multilayer structure prevents reagent leakage, protects the sensing area from external contamination, and maintains a controlled liquid volume necessary for consistent sensor performance.

The device architecture comprises multiple overlapping layers, each

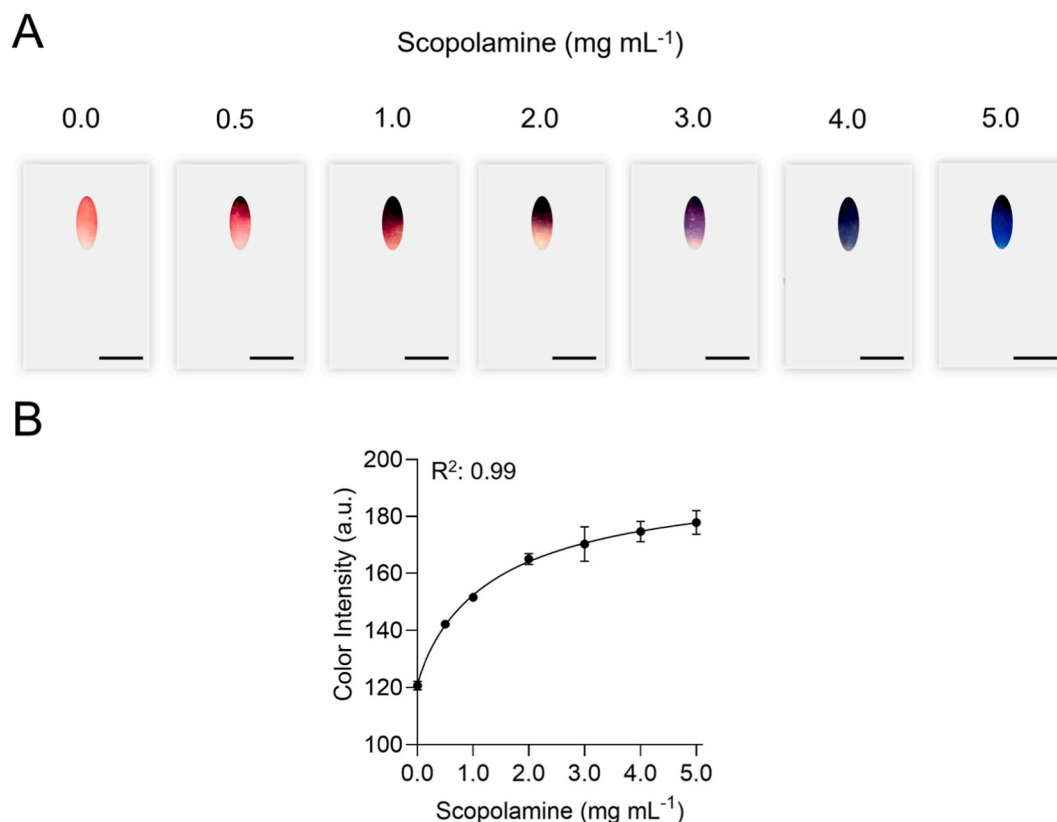


Fig. 2. Calibration curve for scopolamine. A) Images of microfluidic devices loaded with different concentrations of scopolamine. Photographs were taken 10 min after the device performance. Scale bars represent 500 μm . B) Graphical representation of Red Values obtained for different concentrations of scopolamine. Error bars represent the standard error of the mean (SEM) ($n = 3$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

serving a specific function (Fig. 1B). From bottom to top, the layers include a hydrophilic pressure-sensitive adhesive (PSA), a double layer of hydrophobic PSA, and a 175 μm thick polymethyl methacrylate (PMMA) cover. The hydrophilic PSA allows liquid to flow into the paper disc while securely binding it to the microfluidic structure. The paper disc is further stabilized by the double-layered hydrophobic PSA, which defines the microfluidic channel and supports fluid transport while preventing uncontrolled diffusion. This layer also incorporates an air channel at the top, preventing overpressure buildup and ensuring smooth liquid movement. Additionally, an external air vent allows bubbles to escape, further stabilizing fluid flow toward the sensing area. The cellulose paper disc plays a critical role in sample transport and detection. Its intrinsic capillary action enables continuous fluid absorption, regulating flow and ensuring controlled interaction with the sensing reagents. The shape and dimensions of the circular sensing area are specifically designed to enhance colorimetric contrast, facilitating visual detection. A PMMA layer is incorporated for its transparency, structural integrity, and non-adhesive properties. A cover was incorporated into the device design, as depicted in Fig. 1. Each cover, fabricated from double-sided white pressure-sensitive adhesive, incorporated a precision-aligned viewing window over the region of maximal color change, thereby enhancing signal discrimination and suppressing surface non-uniformity artifacts.

For scopolamine detection, the paper discs were preloaded with a cobalt(II) thiocyanate ($\text{Co}(\text{SCN})_2$) solution in an acidic medium. $\text{Co}(\text{SCN})_2$ is widely recognized for its ability to form colored complexes with various drugs, including cocaine, benzodiazepines, and ketamine. Haddoub et al. [25] described its mechanism as a complexation reaction between the alkaloid and the $\text{Co}(\text{SCN})_2$ complex, $[\text{Co}(\text{SCN})_2(\text{H}_2\text{O})_4]$, which appears pink in solution and undergoes a blue color change upon interaction with the target compound. Through IR spectroscopy and

theoretical studies, they further demonstrated the formation of a hexacoordinated cobalt(II) complex, $[\text{Co}(\text{NCS})(\text{H}_2\text{O})_3(\text{cocaine-HCl})]$ (SCN), involving one bis-chelating cocaine molecule and three coordinated water molecules. According to D. M. de Souza et al. [24] cocaine behaves as a Lewis base by donating pairs of non-ligand electrons present in the amino and carbonyl groups; in the same way, scopolamine contains both donor groups in its structure, which allow it to react in a similar way to cocaine with $\text{Co}(\text{II})$ (Fig. 1C).

Although $\text{Co}(\text{SCN})_2$ has been widely studied for its interactions with various alkaloids, its reaction with scopolamine has been less explored. Inside the microfluidic device, exposure to an adulterated beverage triggers the interaction between $\text{Co}(\text{SCN})_2$ and scopolamine, inducing a visible color change in the embedded reagent, as shown in Fig. 1D, which propagates across the entire paper disc for easy detection. This result confirms the capability of the $\text{Co}(\text{SCN})_2$ reaction for the colorimetric detection of scopolamine, expanding its applicability beyond previously reported targets such as ketamine [10,26] and benzodiazepines [4,27], and highlighting its broader potential for drug screening.

3.1. Sensitivity of scopolamine detection

To evaluate the sensitivity of the microfluidic device, a calibration curve was established using seven scopolamine solutions at concentrations of 0.0, 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 mg mL^{-1} ($n = 3$). Fig. 2A displays images of the strips 10 min after sample application, revealing a distinct color change across all tested concentrations. The negative controls exhibited a light pink hue, indicative of the baseline color of the reagent in the absence of scopolamine. As the scopolamine concentration increased, a gradual shift in coloration was observed, transitioning through pale violet and moderate purple before reaching a deep purple-blue at the highest concentration. This progressive darkening suggests a

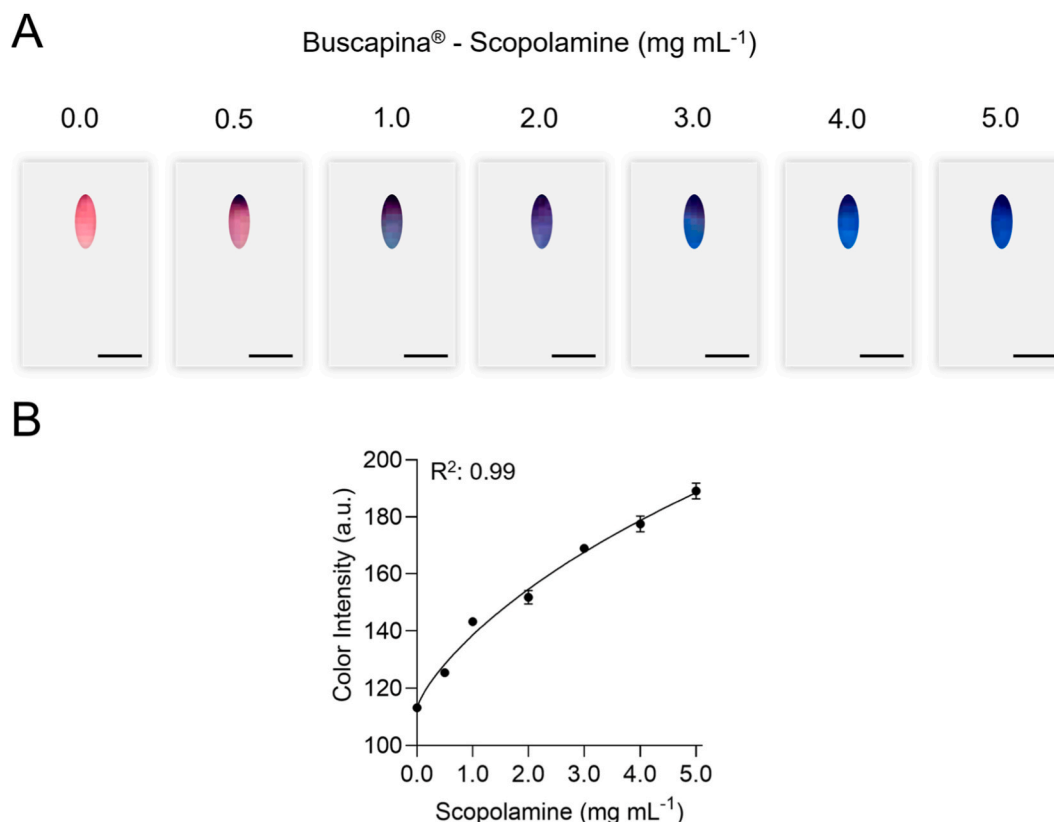


Fig. 3. Calibration curve for scopolamine detection in Buscapina® pharmaceutical formulation. A) Images of the microfluidic devices loaded with different concentrations of scopolamine in Buscapina® pharmaceutical formulation. Photographs were taken 10 min after the device performance. Scale bars represent 500 μ m. B) Graphical representation of Red values obtained for different concentrations of scopolamine in Buscapina® pharmaceutical formulation. Error bars represent the standard error of the mean (SEM) ($n = 3$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

concentration-dependent interaction between scopolamine and Co (SCN)₂, likely due to the increasing formation of coordination complexes [24,25]. In addition, the color gradient observed through the viewing window primarily originated from the flow of the sample across the cellulose paper. At the bottom of the disc, where the sample first enters the sensing area, the continuous flow causes partial washing of the retained reagents, leading to a noticeable decrease in color intensity. As the water-soluble sensing solution moves upward through the paper, it carries the analyte along the matrix. Although the reagents remain largely immobilized within the cellulose structure, they tend to accumulate at the upper edge of the disc, resulting in a higher local concentration and a corresponding increase in color intensity. This contrast between reagent washout at the bottom and accumulation at the top gives rise to the clear color gradient visible in the visualization window.

Photographs of the microfluidic device were analyzed using ImageJ, where RGB values were extracted (Fig. 2B). The Red channel values were used to construct the calibration curve, which was fitted using a four-parameter logistic (4 PL) equation: $y = 197.29 + (120.77 - 197.29)/(1 + (x/1.5)^{0.89})$, $R^2: 0.99$. From this model, a quantitative limit of detection (LOD) of 0.13 mg mL⁻¹ was determined using Equation (1).

$$y = \text{mean blank} + 3 * \text{SD}_{\text{blank}} \quad (\text{SD : standard deviation}) \quad \text{eq. 1}$$

Given the narrow therapeutic window of scopolamine (0.6 - 0.8 mg mL⁻¹), these results confirm that the microfluidic device is capable of detecting concentrations relevant to date-rape cases and potentially fatal overdoses. Notably, clear color changes were visually distinguishable to the naked eye between 0.5 and 1.0 mg mL⁻¹, making the device suitable for both qualitative and quantitative detection. Additionally, the calculated LOD falls well below the expected toxic

concentrations, further demonstrating its applicability in forensic and clinical scenarios. These results demonstrate that the microfluidic device is well-suited for rapid, on-site scopolamine detection.

The functionality of the microfluidic device was further evaluated using Buscapina®, a commercially available pharmaceutical formulation containing scopolamine butylbromide. To assess the device's sensitivity for this drug, Buscapina® was diluted to prepare seven solutions with final scopolamine concentrations of 0.0, 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 mg mL⁻¹ ($n = 3$). Fig. 3A shows images of the microfluidic devices taken 10 min after sample application, revealing color changes across all tested concentrations. As with the pure scopolamine solutions, a darkening of the pink coloration was observed at 0.5 mg mL⁻¹, while a bright blue hue appeared from 1.0 mg mL⁻¹ onwards, indicating a clear, concentration-dependent response.

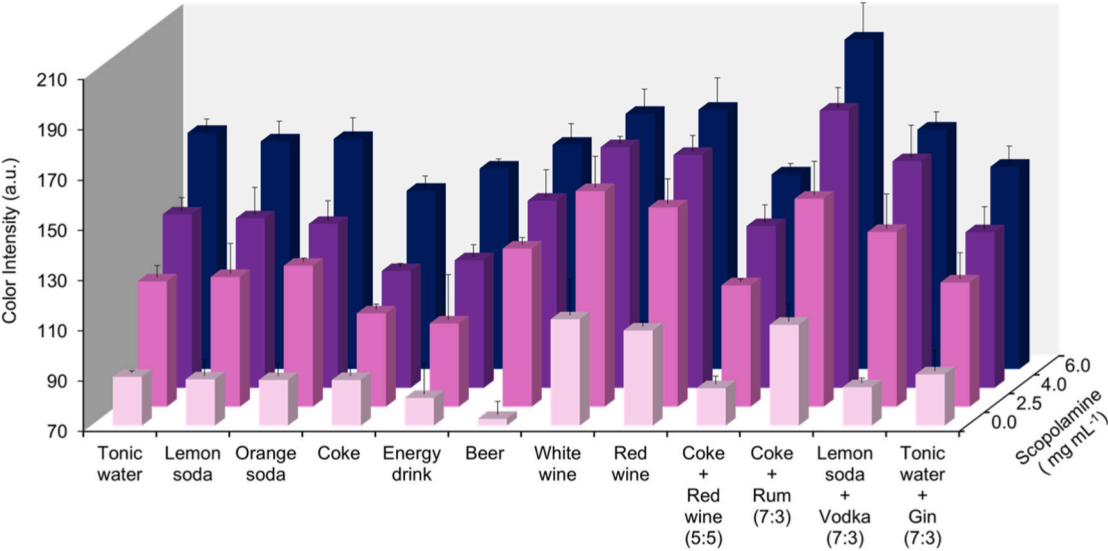
Photographs of the microfluidic device were analyzed using ImageJ, where RGB values were extracted (Fig. 3B). The Red channel values were used to generate the calibration curve, which was fitted using a four-parameter logistic (4 PL) equation: $y = 241.36 + (112.81 - 241.36)/(1 + (x/2.99)^{1.14})$, $R^2: 0.99$. From this model, a quantitative LOD of 0.11 mg mL⁻¹ was determined using Equation (1). These results confirm the suitability of the microfluidic device for detecting scopolamine in this commercial pharmaceutical preparation.

The coloration observed in Buscapina® samples differed slightly from that of pure scopolamine, likely due to the presence of excipients in the formulation. However, the device successfully detected scopolamine at all concentrations, demonstrating its versatility and potential for both forensic screening and pharmaceutical analysis.

A

Drink	Negative	Scopolamine			
	0.0 mg mL ⁻¹	2.5 mg mL ⁻¹	4.0 mg mL ⁻¹	6.0 mg mL ⁻¹	
Coke					
Orange soda					
Lemon soda					
Tonic water					
Energy drink					
Gin-tonic water (3:7)					
Rum-coke (3:7)					
Vodka-lemon soda (3:7)					
Beer					
Red wine					
White wine					

B



(caption on next page)

Fig. 4. Detection of scopolamine in different beverages. A) Photographs of the sensing area of the scopolamine microfluidic device when immersed in non-alcoholic and alcoholic beverages such as tonic water, lemon soda, orange soda, coke, energy drink, beer, white wine, red wine, coke + red wine (5:5), coke + rum (7:3), lemon soda + vodka (7:3), tonic water + gin (7:3) (left column) according to different concentrations of scopolamine (0.0, 2.5, 4.0, 6.0 mg mL⁻¹). Photographs were taken 10 min after the operation of the device. B) Comparative diagram of the mean Red value according to different beverages (tonic water, lemon soda, orange soda, coke, energy drink, beer, white wine, red wine, coke + red wine (5:5), coke + rum (7:3), lemon soda + vodka (7:3), tonic water + gin (7:3)) and scopolamine concentrations (0.0, 2.5, 4.0, 6.0 mg mL⁻¹). Error bars mean standard deviation (SD) from n = 3. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.2. Detection of scopolamine in different common beverages

To evaluate the device's performance in real-world scenarios, a selection of commonly consumed beverages were selected as test matrices,

including tonic water, lemon soda, orange soda, coke, energy drink, beer, white wine, red wine, coke + red wine (5:5), coke + rum (7:3), lemon soda + vodka (7:3), and tonic water + gin (7:3). Each beverage was spiked with scopolamine at concentrations of 2.5, 4.0, and 6.0 mg

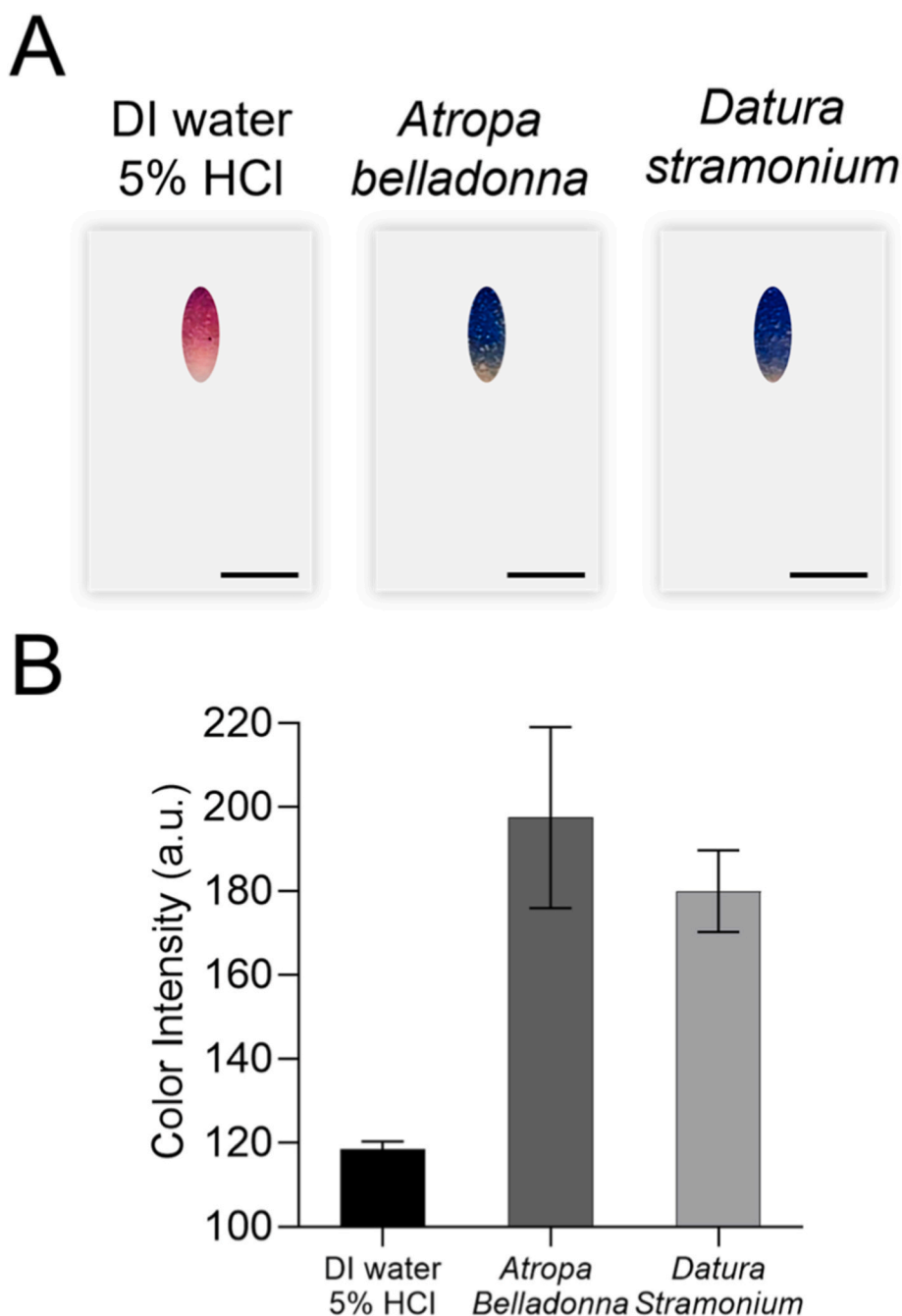


Fig. 5. Analysis of *Atropa belladonna* and *Datura stramonium* extracts using the scopolamine microfluidic device. A) Images of the microfluidic devices loaded with extraction solvent (DI water 5 % HCl, left), and *Atropa belladonna* (middle) and *Datura stramonium* (right) extracts diluted 1:1 in DI water. Photographs were taken 10 min after the device test. Scale bars represent 500 μ m. B) Graphical representation of Red values obtained for the different extracts. Error bars represent the standard error of the mean (SEM) (n = 3). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

mL^{-1} . The beverage in its natural state, containing only its original ingredients and free from adulteration, was considered the ideal baseline matrix. Since the sensor was not intended to detect a specific drug, any substance capable of producing a cobalt(II) thiocyanate response that was not naturally present in the beverage was considered evidence of contamination and, consequently, part of the adulteration itself rather than a limitation of the test's selectivity. Therefore, selectivity was defined as the ability of the device to distinguish between adulterated and non-adulterated samples across a wide range of matrices. Fig. 4A presents cropped images of the sensing areas of the microfluidic devices, illustrating both negative and positive responses for each beverage type.

In non-alcoholic beverages adulterated with scopolamine, the sensing area exhibited a distinct blue color change at all tested concentrations. However, the exact blue hue varied depending on the beverage, suggesting that different ingredients and compositions influence the colorimetric response. This highlights the importance of matrix effects when interpreting results from different drink types.

In alcoholic beverages, scopolamine was detected at all concentrations except for the coke + rum (7:3) mixture at 2.5 mg mL^{-1} , where the color change was less distinguishable. In some cases, the response was muted but still discernible, likely due to the baseline bluish tint observed in the negative controls. The presence of ethanol appears to interfere with the colorimetric reaction, altering the baseline color and reducing contrast, which can make differentiation between positive and negative results more challenging.

To support these visual observations, quantitative analysis of Red intensity values confirmed that scopolamine was detected at all tested concentrations in all beverage matrices. As shown in Fig. 4B, a strong correlation was observed between increasing scopolamine concentrations and changes in Red channel intensity values, providing an objective and quantifiable detection method. This confirms that the device enables both qualitative detection via direct visual inspection and quantitative analysis through image processing, enabling accurate and reliable scopolamine detection in a wide range of beverage types.

3.3. Detection of alkaloids from *Atropa belladonna* and *Datura stramonium* extracts

To further evaluate the applicability of the microfluidic device, the detection of total tropane alkaloids was performed following a solid-liquid extraction from *Datura stramonium* and *Atropa belladonna* dried plants. These plants are well known for their high content of tropane alkaloids, including scopolamine, making them relevant sources for forensic and toxicological studies [28,29].

When the microfluidic device was introduced into non-adulterated samples such as distilled water, the sensing area turned pink as expected, confirming a negative result. In contrast, when tested with both the original and 1:1 diluted plant extracts, the sensing area exhibited a clear bright blue coloration, indicating the presence of tropane alkaloids. This response mirrors the device's reaction to scopolamine in aqueous solutions, reinforcing its effectiveness for detecting this class of compounds (Fig. 5).

These results demonstrate that the microfluidic device is capable of detecting total tropane alkaloids extracted from plant material, further validating its potential for forensic, pharmaceutical, and toxicological applications.

4. Conclusions

We developed a paper-based microfluidic device that uses cobalt(II) thiocyanate in an acidic medium as a colorimetric sensor for the rapid on-site detection of scopolamine in pharmaceuticals, plant extracts and conventional beverages. This research optimizes, for the first time, the Co(SCN)_2 reaction on a solid support for the detection of scopolamine, establishing a robust and stable sensing method for practical use.

Designed as a discrete, autonomous, and portable platform, the

device integrates a paper-based sensor within a multilayer polymeric structure, enabling self-powered operation and simple interpretation of results. It achieved a LOD as low as 0.13 mg mL^{-1} for scopolamine and 0.11 mg mL^{-1} for the pharmaceutical formulation, Buscapina®, confirming its practical utility. The device provides a fully functional platform for on-site colorimetric detection of scopolamine, supporting both, visual qualitative detection and smartphone photograph-based quantitative analysis, making it adaptable to different detection scenarios.

This microfluidic device represents a significant advance in drug detection, providing a rapid and reliable method with a direct visual readout for detecting scopolamine and related compounds at the point-of-need. This work also brings us closer to the objective of developing a self-powered paper-based microfluidic platform that detects multiple drugs with a single colorimetric reaction. Future developments could include the integration of positive controls on the support to enhance the usability and accuracy of the device, and the addition of a color reference label on the package to facilitate proper result interpretation.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work, the authors used ChatGPT solely to improve the grammar, clarity and readability of text originally written by the authors. The tool was not used to generate scientific ideas, experimental data, results, analyses, figures, interpretations or conclusions. All suggested changes were carefully reviewed and edited by the authors, who take full responsibility for the content of the manuscript.

CRediT authorship contribution statement

Isabel Poves-Ruiz: Conceptualization, Formal analysis, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing. **Enrique Azuaje-Hualde:** Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing. **Laura Lartitegui Elorza:** Investigation. **Oihana Diaz-Cantos:** Investigation. **Lourdes Basabe-Desmonts:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. **Fernando Benito-Lopez:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.talanta.2026.130319>.

Data availability

Data will be made available on request.

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