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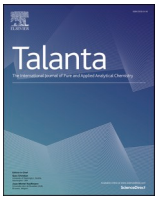

Scopolamine analysis in beverages: Bicolorimetric device vs portable nano liquid chromatography

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ABSTRACT

Scopolamine (SCP) is often involved in sexual assaults and robberies, particularly in recreational environments. Therefore, analytical tools are required for the analysis of this compound amenable for the field. In this work, a sensor for SCP is described based on the entrapment of $KMnO_4$ into polydimethylsiloxane (PDMS). The possibility of using $KMnO_4$ in combination with the reagent 1,2-naphtoquinone-4-sulfonate (NQS) giving a double sensor acting as a bicolorimetric device is also demonstrated. In contact with the sample, the PDMS composite delivers MnO_4^-, which reacts rapidly with SCP under basic conditions causing a change of the color of the solution that can be related to the concentration of drug using both, absorbances and color coordinates, while the NQS part of the sensor remains unchanged. After an exposure time to the sensors of 10 min, satisfactory linearity was obtained for concentrations of SCP up to 865 μg/mL, being the limit of detection (LOD) 108 μg/mL. A method using a portable nano liquid chromatograph with detection at 255 nm has been also developed; in this case the LOD was 100 μg/mL and the working linear interval was 250–2000 μg/mL. The precision, expressed as relative standard deviation (RDS), was ≤8% for both methods. Different beverages (cola, cola-whisky, tonic water-vodka, red wine and green tea) were assayed. The potential of the two proposed approaches for on-site tests is discussed.

1. Introduction

The consumption of drugs is an issue of growing concern all over the world, not only because of the potential serious and irreversible effects on consumers' health, but also because it is commonly associated with criminal activities. From an analytical perspective, different approaches are in current use to address this global problem, from highly sophisticated instrumental techniques such as liquid or gas chromatography coupled to mass spectrometry (MS), to simple color tests, each with different goals, advantages and limitations.

Because of their simplicity, rapidity and portability, color tests have been used for decades for the on-site presumptive analysis of suspected samples. Although portable instruments are nowadays available for many analytical techniques [1], advances in fields such as color digital analysis or in the development of solid colorimetric sensors and microfluidic devices have led to a growing interest in chemical spot tests [2].

There are several reagents that can be used for the on-site detection of the most used drugs, including amphetamines, cocaine or cannabinoids [2,3]. Unfortunately, only a few options are available for other relevant drugs. This is the case of SCP, a compound of increasing concern for law-enforcement authorities and health-care services. SCP, also known as a hyoscine, is a tropane alkaloid obtained from botanical species of the *Solanaceae* family, which is used for clinical purposes because of its strong parasympatolytic, anticholinergic and antiemetic actions [4]. This substance is also a psychoactive drug. In certain dose, it blocks memory and causes passivity, disorientation, fatigue and confusion. Because of such properties, SCP can be used to block free will of victims during robberies and sexual assaults, and several cases have been reported in which the victims consumed unconsciously SCP, often added to a drink [5–8]. However, only in few cases the presence of SCP in the body's victims could be confirmed in the laboratory, as this drug is rapidly metabolized and completely eliminated from the body a few hours after its intake [7,9]. Therefore, the rapid detection of SCP in evidence collected at crime scenes and at point-of-care places is a key factor to prove the offense and to provide the victims a proper treatment.

Several methods have been proposed for the identification and/or quantification of SCP in a variety of samples. In analytical laboratories,

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liquid chromatography (LC) coupled to MS is predominant for the analysis of complex samples such as biological fluids and plant extracts [10–16]. Recently, two studies reported the use of portable LC for on-site drug analysis [17,18] but none of them included SCP. So far, three techniques have been proposed in the literature for the rapid analysis of SCP in the context of drug analysis with portable devices, namely capillary electrophoresis with contactless conductivity detection [19], ion trap MS for direct analysis in real time (DART) [20], and square wave voltammetry [21], which demonstrates the lack of methods for in situ analysis of this compound. As stated earlier, no presumptive colorimetric tests are available for SCP suitable for field analysis. Moreover, no colorimetric methods have been described for the specific determination of SCP. A colorimetric assay based on the employment of ferric hydroxamate assay reported in 1970 [22]; this reaction was used for developing a sensor for esters by our group, although it cannot discriminate between SCP, cocaine and atropine [23]. On the other hand, colorimetric methods have been developed for hyoscine butylbromide (a derivative of SCP) based on the formation of ion-pair complexes with different bromocresol, bromothymol and bromophenol dyes [24], or on the oxidation of SCP with MnO_4^- under alkaline conditions [25,26]; those methods were only applied to the determination of the target compound in pharmaceutical formulations. Therefore, the development of methods rapid and simple methods applicable to establish not only the presence of SCP in drinks but also its concentration is still a need. The concentration of SCP in the sample can be used to estimate the total amount of drug ingested, and thus, to assess the real risk for the victim's health [21].

During the last years we have developed different sensors for drugs based on the entrapment of a colorimetric reagent into polydimethylsiloxane (PDMS). This approach was successfully applied to the identification and quantification of amphetamines [27,28], ketamine [29], ethanol [30] and ephedrines [31]. In the present work, a sensor has been developed for SCP based on the entrapment of the reagent $KMnO_4$ into PDMS which is intended to be used for the rapid analysis of SCP in beverages. A double PDMS composite sensor containing 1,2-naphtoquinone-4-sulfonate (NQS) and $KMnO_4$ has been synthetized for the first time for improving selectivity of the SCP assay. Recently developed portable nano LC has been applied for the first time to the analysis of SCP. The proposed approaches have been applied to the analysis of different alcoholic and non-alcoholic beverages.

2. Experimental

2.1. Chemicals and materials

All reagents used throughout the study were of analytical grade and purity ≥97%. SCP hydrobromide, atropine (ATR) sulfate, cocaine (COC), ephedrine (EPH) hydrochloride, amphetamine (AMP) sulfate, methamphetamine (MET) hydrochloride, morphine (MOR), ketamine (KET) hydrochloride, caffeine, sucrose, tetraethylsiloxane (TEOS), NQS, $KMnO_4$ and NaOH were purchased from Sigma-Aldrich (St. Louis, MO, USA). A PDMS kit consisting of Sylgard® 184 silicone elastomer base and Sylgard® 184 silicone elastomer curing agent was purchased from Dow Corning (Midland, MI, USA). Acetonitrile and methanol were purchased from VWR Chemical (Radnor, PA, USA).

Stock solutions of SCP and the other compounds assayed were prepared by dissolving the solid reagents in water. The stock solutions were stored at 4 °C until use. Working solutions of the target compounds were prepared by dissolving the stock solutions in water or in 0.1 M NaOH. Nanopure water was obtained from an Adrona system (Riga, Latvia).

2.2. Instrumentation and conditions

Spectrophotometric measurements of solutions were carried out in the 300–800 nm range using a UV–vis Agilent 8453 diode-array UV spectrophotometer (Agilent Technologies, Waldbronn, Germany). Spectrophotometric measurements of the sensors were carried out using a Cary 60 Fiber Optic UV–Vis spectrophotometer (Agilent Technologies), fitted with a remote fiber optic diffuse reflectance accessory from Harrick Scientific Products (Mulgrave, Victoria, Australia); the spectra of the sensors were registered in the 200–800 nm range. Data were recorded and processed using a Cary WinUV software (Agilent Technologies).

Images of the sensors were obtained with the cameras of different smart phones at an approximate distance of 10 cm and without flashlight. The images were then imported into a computer, and color analysis was carried out with the free image editor software GIMP (version 2.8). The color intensity was measured with the color picker tool of the program using an area of 100 × 100 pixels. The red-green-blue (RGB) and cyan-magenta-yellow-key (CYMK) color modes were applied to transform the images into numerical color coordinates.

For the chromatographic assays by nano LC (nanoLC), a portable Axcend Focus LCTM LC liquid chromatograph (Axcend Corp, Provo, UT, USA) was used. The system was equipped with a 100 mm × 150 μm i. d. column packed with an ODS stationary phase (1.7 μm particle size). On-capillary UV absorbance was measured at 255 nm using a LED. The volume of the internal injector loop was 40 nL; samples (10 μL) were loaded in the injection port by means of a 25 μL syringe. For data acquisition and calculation, the Axcend Focus v2.0 software was used. The mobile phase was a mixture of solvent A (water with 3% of acetonitrile) and solvent B (acetonitrile with 3% of water). Unless otherwise stated, the mobile phase contained A and B in a proportion 80:20 (all proportions expressed as v/v). Solvents A and B were filtered through 0.22 μm nylon membranes supplied by GVS (Dr. Sanford, ME, USA) before use. The mobile-phase flow rate was 2 μL/min.

2.3. Preparation of the sensors

For the preparation of the sensors, the $KMnO_4$ reagent was first ground in a glass mortar until a fine powder was obtained. Then, 10 mg of the reagent were mixed with 4 g of PDMS elastomer base. The mixture was stirred vigorously and then 0.4 g of the PDMS curing agent were added; the curing agent contained dimethyl methyl hydrogen siloxane which acted as a cross-linker during the formation of the polymer [32]. After 2–3 min of mixing, portions of 0.2 g of reagent/PDMS mixtures were placed in circular well polystyrene plates of 15 mm diameter. Finally, the composites were cured at room temperature for 2 days. The sensors were removed from the well plates and cut into four pieces of approximately the same size. The sensors were stored in polyethylene bags at room temperature until use.

Double sensors of $KMnO_4$ and NQS were prepared in two steps. Firstly, NQS/PDMS sensors were synthetized according to the procedure described in Ref. [27]. Briefly, 2 mg of the solid reagent were dispersed in 1 mL of TEOS, and the liquid was mixed with 2 g of PDMS elastomer base and 0.2 g of the curing agent. The resulting mixture was homogenized, and then portions of 0.2 g were placed into well plates (15 mm diameter) and cured at room temperature for 2 days. The NQS sensors were removed from the plates and then cut in two halves. Each half was introduced into a well, which was then filled with the mixture of $KMnO_4$, elastomer base and curing agent (in the same proportions used for preparation of the $KMnO_4$ sensors). The mixture was maintained at ambient temperature until solidification of the $KMnO_4$ part of the sensors. Finally, the resulting double sensors were removed from the plates and stored at room temperature until use.

2.4. Reaction conditions

For the reaction in solution studies, aliquots of the solutions of SCP 440 μL were mixed with 100 μL of a solution of NaOH 0.1 M and 60 μL of a solution of $KMnO_4$ of a concentration of 9.48 mg/L (60 μM) in glass vials. Then, the resulting solutions were transferred to a plastic cuvette using Pasteur pipettes, and the absorbance spectra were registered from

300 to 800 nm at a reaction time of 10 min (unless otherwise stated).

For the reaction with the sensors in aqueous media, aliquots of 600 μL the solutions of SCP (prepared in NaOH 0.01 M) were exposed to the sensors (quarters), and after a time of exposure of 10 min, the solutions were transferred to a cuvette and their absorbance spectra were registered. In the assays with the sensors in the presence of acetonitrile, 400 μL of the working solutions were mixed with 200 μL of acetonitrile; the mixtures were exposed to the sensors for 10 min, and then the spectra or the images of the resulting solutions were obtained.

2.5. Analysis of the samples

Different beverages were tested: cola drink, whisky mixed with cola drink in a proportion 1:3 (v/v) [17], vodka mixed with tonic water (1:3, v/v), red wine and green tea infusion. The infusion was prepared by treating 2 g of green tea with 200 mL of boiling water. Then, the infusion was cooled at room temperature, and a fraction of the supernatant was separated and used for the assays. All the samples were purchased from local markets.

For the SPE, first the C_{18} cartridges (100 mg, Phenomenex, CA, USA) were conditioned with 1 mL of methanol and then with 1 mL of 0.1 M NaOH. Next, 1 mL of the samples were passed through the cartridges, which were then washed with 1 mL of a mixture of water:acetonitrile (1:4, v/v) followed by 200 μL of acetonitrile (unless otherwise stated) in order to flush out the matrix components. The cartridges were then dried by passing air manually with a 1 mL syringe. Finally, the SCP was desorbed from the cartridges with 200 μL of acetonitrile. The collected extracts were either, mixed with 400 μL of NaOH 0.1 M and exposed to the sensors, or processed by nanoLC.

3. Results and discussion

3.1. Development of the bicolorimetric sensor

Different strategies to immobilize the $KMnO_4$ reagent into the PDMS were tested: the addition of a solution of the reagent prepared in acetone to the elastomer base, the dispersion of the reagent into TEOS and the subsequent addition of the resulting suspension to the elastomer, and the direct dispersion of the reagent into the elastomer. The first two approaches were unsuccessful because of the reduction of the MnO_4^- ion (the sensors obtained showed a brownish color). When the reagent was directly dispersed into the elastomer, the obtained sensors showed suitable homogeneity and stability (Fig. S1 in Supporting Information). The sensors with the dispersed reagent were divided in quarters and used in further assays.

The sensors were immersed into 0.6 mL of the working solutions. It was observed that the MnO_4^- was released from the sensors, so the solutions changed to magenta (color of MnO_4^- in water). According to previous works, a basic medium is required to for the reaction to take place [25,26]; no response or change of color was observed for acidic or neutral solutions. However, in presence of SCP and in basic media, the color of the working solution changed from magenta to green due to the reduction of the MnO_4^- ion to MnO_4^{2-} (see Fig. S2) [25]. A concentration of NaOH 0.1 M was selected because higher differences between the absorbances measured for a blank and for solutions of SCP exposed to the sensors were observed (see Fig. S3A).

The effect of the time of exposure of the solutions to the sensors was also evaluated. For this purpose, the absorbances were registered at different times up to 20 min. As a compromise between the analytical signal and the time of analysis, 10 min was selected for further assays (see Fig. S3B).

Finally, the sensors were tested under the selected conditions for solutions containing different concentrations of SCP in the 86.5–865 μg/mL range. After the time of exposure, the spectra of the solutions were recorded. The Results obtained were compared with those obtained by mixing solutions of SCP and the reagent (reaction in solution, see Fig. S4).

Fig. 1A shows the pictures of the solutions after being exposed to the sensors and the corresponding spectra. Best linearity was obtained by using as analytical signal the ratio of absorbances at 435 nm and 530 nm (maximum absorbance for the MnO_4^- ion) [33]. In Table 1 is shown the calibration equation obtained.

The addition of acetonitrile [30] to the samples in a proportion 1:2 (33.3% of acetonitrile in the reaction media) increased drastically the amount of reagent released from the sensor to the solution; as a result, linear responses were obtained at higher concentrations of SCP. The images and spectra of the solutions corresponding to different concentrations of SCP in acetonitrile:NaOH 0.1 M (1:2, v/v) are shown in Fig. 1B. A linear relationship was also observed between the absorbances measured at 435 nm and the concentration of SCP (see Table 1). For comparative purposes, the values obtained under the solution reaction approach are also listed in Table 1. The table also shows the LODs calculated as $3S_{y/x}/b$, where $S_{y/x}$ is the residual standard deviation and b is the slope of the calibration line. The precision, expressed as relative standard derivation (RSD) for three consecutive measurements of the absorbance of a solution of the analyte, is also given.

The possibility of using color images analysis was evaluated. The images taken from the solutions of SCP (containing 33.3% of acetonitrile) after being exposed to the sensors were used for color analysis. In this study the color coordinates obtained with the RGB and CYMK systems were used. Best linearity was observed with the blue and magenta colors in the RGB and CYMK systems, respectively (see Table 1). However, the sensitivity was better with the magenta coordinate. A negative slope of the calibration line is consistent with the decrement of the unreacted MnO_4^- (magenta) as the concentration of analyte increases; a negative slope was also found when using the blue coordinate.

According to the literature, the correlation between the absorbance at the working wavelength selected and the color coordinate relies on the sensitivity of the measurement [34]. In the present instance, a good correlation was observed between the absorbance at 435 and both, the magenta and blue coordinates (see Fig. S5), which confirmed that the performance of the color analysis approach was also suitable (see Table 1). The main advantage of the sensors is that the procedure is simplified with respect the solution reaction approach. For on-site purposes, the procedure can be further simplified by using color images analysis. In this sense, a simple smart phone could be used in replacement of a portable colorimeter, although the sensitivity and precision achieved with the latter approach are usually better [27,29].

Different studies were carried out to assess the stability of the sensors. The $KMnO_4$/PDMS sensors were stable at ambient temperature at least for at least 3 months; the differences in the absorbances measured for solutions of SCP with freshly prepared sensors and with sensors prepared 3 months before were <10%, thus comparable to the RSD values of Table 1.

The selectivity of the sensor was studied against different compounds that are typically found in illicit drug samples. The drugs tested were amphetamine (AMP), methamphetamine (MET), morphine (MOR), ketamine (KET), ephedrine (EPH) and cocaine (COC). The Results can be observed in Fig. 2A. No color changes were observed for EPH and COC. In contrast, MOR and KET resulted rapidly oxidized, so that after only 2 min the green color was observed, while the solutions of SCP were still violet. For a reaction time of 10 min both, MOR and KET lead to yellow-brown solutions. Therefore, SCP could be differentiated from MOR and KET by controlling the observation/measurement time, as in many other chemical spot tests [2]. As regards the results observed for the amphetamines, only MET produced a positive response. In an attempt to eliminate this interference the double sensor was developed. The sensors were also tested for atropine (ATR). This compound can be present in Solanaceae plants, and it is used in many pharmaceutical preparations [13]. According to the literature, ATR is reactive to MnO_4^- ions under acidic conditions [35]. However, in an acetonitrile-0.1 M NaOH medium ATR produced orange-colored solutions and could be differentiated

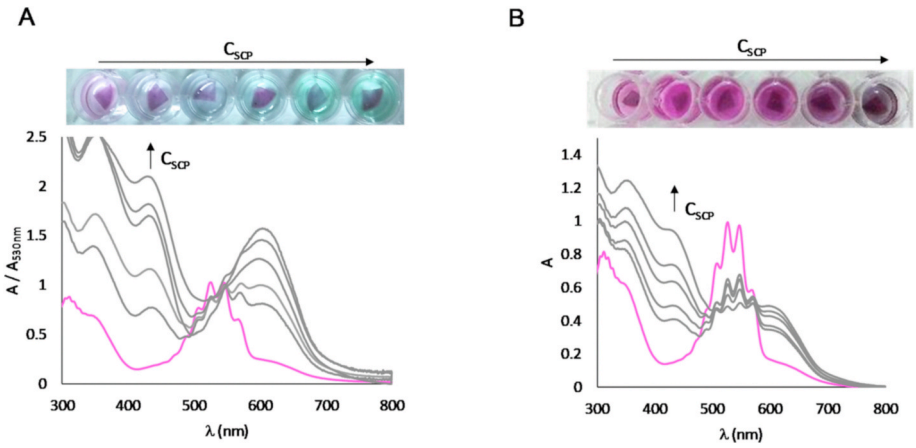

Fig. 1. Images obtained for a blank and solutions of SPC (86.5, 173.3, 346.7, 520 and 865 μg/mL) exposed to the sensors, and their respective spectra: (A) reaction in NaOH 0.1 M, and (B) reaction in NaOH 0.1 M:acetonitrile (2:1, v/v). For other experimental details, see the text.

Table 1
Linearity, LOD and precision obtained for SCP with the different approaches tested.

Conditions	Tested concentration range (μg/mL)	Analytical signal	Linearity[a] (y = a+bx), n = 10			LOD (μg/mL)	RSD[b], (n = 3) (%)
			$a \pm S_a$	$b \pm S_b$	R^2		
Reaction in NaOH 0.1 M	86.5–865	A_{435}	0.03 ± 0.02	$(0.83 \pm 0.04)\ 10^{-3}$	0.996	108	6
Reaction with sensors in NaOH 0.1 M	86.5–345	A_{435}/A_{530}	0.23 ± 0.06	$(5.5 \pm 0.3)\ 10^{-3}$	0.996	63	8
Reaction with sensors in NaOH 0.1/ acetonitrile (2:1 v/v)	86.5–865	A_{435}	0.07 ± 0.02	$(0.88 \pm 0.04)\ 10^{-3}$	0.998	108	7
		$I_{magenta}$	59 ± 1	−0.123 ± 0.0370	0.997	170	8
		I_{blue}	156 ± 2	−0.093 ± 0.005	0.990	209	6
NanoLC	250–2000	Peak area at 255 nm	- 0.07 ± 0.02	0.056 ± 0.002	0.990	100	6

[a] Concentrations expressed in μg/mL.
[b] Calculated at concentration of 400 μg/mL and 500 μg/mL in the colorimetric and nanoLC methods, respectively.

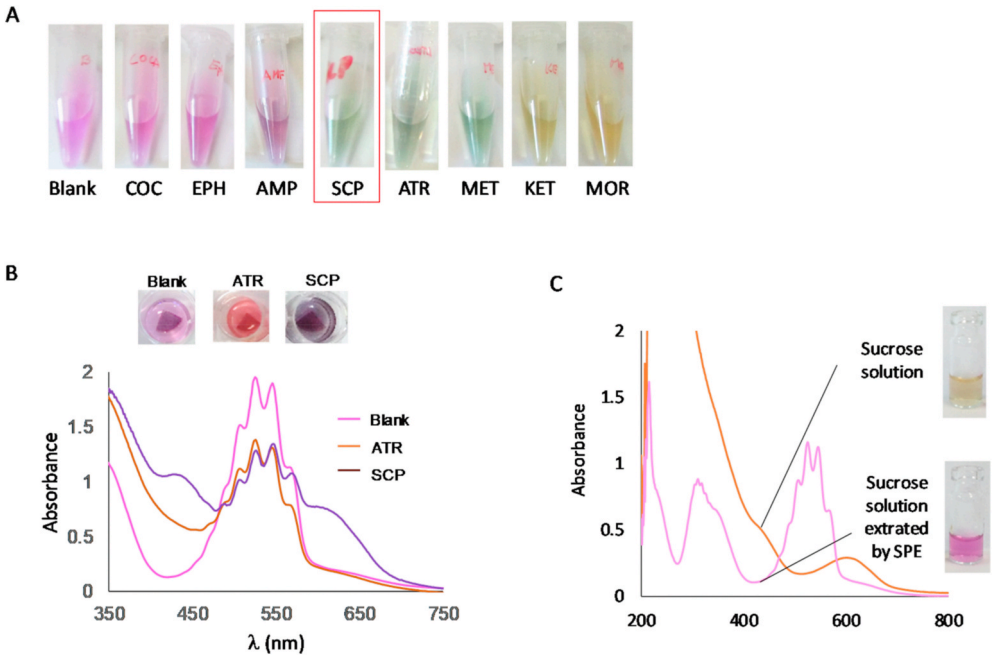

Fig. 2. Selectivity of the sensors: (A) images of the solutions obtained for other drugs (500 μg/mL, each compound); (B) images and spectra of the solutions obtained for a blak, ATR (520 μg/mL) and SCP (520 μg/mL) in NaOH 0.1 M:acetonitrile (2:1, v/v); (C) images and spectra obtained for a solution of sucrose (100 mg/mL) directly and after SPE. For other experimental details, see the text.

from the color and spectra obtained for SCP (see Fig. 2B). This means that in an acetonitrile-0.1 M NaOH, the discrimination of, at least, pure solutions of ATR and SCP would be possible (see Fig. S6). Finally, common diluents such as caffeine, sucrose, paracetamol and acetylsalicylic acid were tested at concentration of 100 mg/mL. None of them produced positive response except sucrose. For this compound, a green

color was visible after 10 min of exposure, with is consistent with the reductive character of sugars. Sugars are expected to be present in some of the samples of interest in a context of illicit consumption of SCP. Therefore, a proper sample treatment needs to be applied in order to prevent this interference (see 3.3 Section).

As attempt to reduce false positive due to the presence of MET, the reagent NQS was selected, as a PDMS sensor with embedded NQS had been developed previously for amphetamines [27]. The reaction between the amphetamines and the entrapped NQS takes place inside the sensors due to the diffusion of the analytes from the sample to the PDMS matrix. Details on the preparation of the double sensors are given in section 2.3 and in Fig. S7.

Fig. 3 shows the images and spectra obtained with the double sensors after being exposed to a blank and to solutions of SCP and MET. Fig. 3A shows the UV spectra of the solutions after being exposed to the sensors, whereas Fig. 3B shows the spectra of sensors obtained by diffuse reflectance. As expected, when exposed to the sensors, the $KMnO_4$ is released from the polymeric matrix into the solution and then the solutions of SCP turned green color, but no significant difference in the color in the area of the sensor with the immobilized NQS was observed (negative response for MET). This was further confirmed by the UV spectra of the sensor in this area by reflectance diffuse (Fig. 3B). In contrast, when the sensor was immersed in a solution of MET, both the color of the solution and the color of the NQS part of the sensor changed. Therefore, it was concluded that, if required, the proposed double sensor could be used for discriminating between samples containing SCP and MET (or other amphetamines reactive towards NQS).

3.2. Portable nanoLC

Portable miniaturized liquid chromatography is gaining attention recently, because of its inherent advantages for on-site analysis such as the potential to provide a rapid response, the reduction of the risks associated with the alteration of the sample until analysis in the lab, and the fully portability, low size and high analytical performance of modern equipments. The possibility of generating a nearly real-time response is of particular interest for drug analysis, as a rapid result may be critical to assists the victims. Liquid chromatography, which is a reference technique for drug analysis in analytical laboratories, has been used only very recently for the analysis of illicit drugs [17,18] but, to the best of our knowledge, portable nanoLC has never been applied to SCP. In the present study, a procedure has been developed for the first time for the chromatographic analysis of SCP using a portable nanoLC chromatograph.

Initially, different mobile phase compositions were assayed in order to obtain satisfactory a peak profile for SCP and a complete separation between this compound and ATR (see section 3.1). Different acetonitrile:water mixtures were tested using isocratic and gradient elution (Table S1). In all instances the mobile-phase flow rate was 2 μL/min. Examples of the chromatograms obtained along the study are depicted in Fig. 4A. Although the separation of SCP and ATR was possible with the four elution conditions assayed, best Results were obtained when using isocratic elution with a mixture of solvent A (water with 3% acetonitrile) and solvent B (acetonitrile with 3% of water) in a proportion 80:20 (v/v). Under such conditions, the time required for the separation was minimum, as SCP eluted at a retention time of 1.80 min and ATR eluted at 2.21 min. In addition, because of using isocratic elution, the time between consecutive analysis could be also minimized.

The selected conditions were applied to the analysis of solutions of SCP and ATR at concentrations up to 2000 μg/mL. As summarized in Table 1, linear responses were obtained by plotting the peak areas at 255 nm against the concentration of drug within the 250–2000 μg/mL concentration interval. The LOD (established as the concentration of SCP that resulted in signal-to-noise ratio of 3) was 100 μg/mL. The chromatograms obtained for a solution containing SCP at a concentration corresponding to the LOD and at half of the highest concentration in the tested concentration range are depicted in Fig. 4B. A good linearity was also observed for ATR ($y = 0.0061\,x + 0.145$, $R^2 = 0.990$); thus, the proposed nanoLC approach could be applied if required to the simultaneous detection and determination of SCP and ATR. This would be the option of choice when sample are suspected to contain ATR among their ingredients, as well as for totally unknown samples.

The analysis by nanoLC is faster, as the whole chromatographic analysis can be carried out in less than 5 min. The method with the sensors is clearly superior in terms of instrumental requirements and cost.

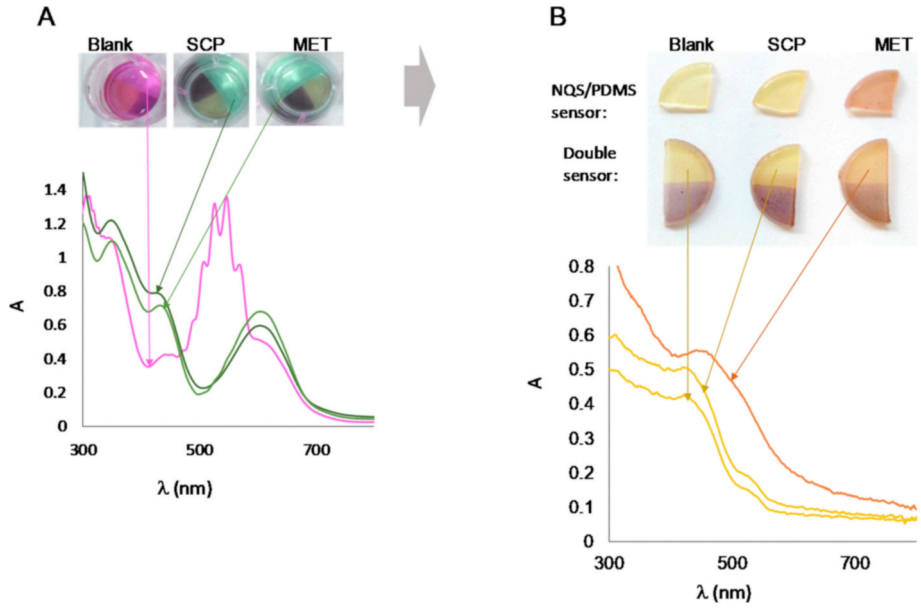

Fig. 3. Responses obtained with the double sensors. (A) Images and UV spectra obtained of the solutions obtained for a blank, a solution of SCP (1000 μg/mL) and a solution of MET (1000 μg/mL). (B) Images of the sensors exposed to the same solutions and diffuse reflectance spectra of the NQS part of the sensors. For other experimental details, see the text.

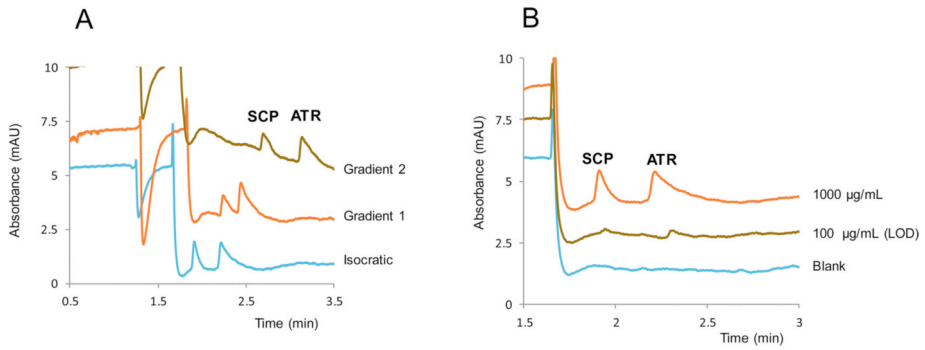

Fig. 4. Chromatograms obtained with the portable nanoLC equipment for: (A) a mixture of SCP and ATR (1000 µg/mL each) using different mobile-phases (Table S1); (B) a blank and mixtures of SCP and ATR (100 µg/mL and 1000 µL each compound) under the selected isocratic conditions. For other experimental details, see the text.

3.3. Application to the analysis of beverages

Beverages may have sugars and/or ethanol among their ingredients, as well as intense colors. Therefore, a proper sample treatment is required before applying the sensors; a previous treatment of the samples is also needed if the samples are going to be analysed by the portable nanoLC method. In this case, SPE with C_{18} sorbents was the option selected for sample conditioning because it can be easily adapted to on-site tests [36]. In most SPE protocols an organic solvent (i.e. acetonitrile) is used to desorb the analytes from the cartridges once they have been isolated from the sample matrix. If the extracts are going to be processed with the $KMnO_4$/PDMS sensors, the reaction conditions could be adjusted by evaporating the solvent followed by the redissolution the residue of SCP in 0.1 M NaOH. However, solvent evaporation operations are undesirable in on-site analysis. In the present instance and taking into account the compatibility between acetonitrile and the sensors (see section 3.1), acetonitrile extracts was directly used for analyzing SCP; for this purpose, the extracts were mixed with NaOH 0.1 M and then exposed to the sensors. In the nanoLC method, the extracts were filtered and injected into the chromatograph.

For the extraction, after the activation of the cartridges (C_{18}, 100 mg) with 1 mL of methanol, aliquots of 1 mL of the working solutions (standard solutions of SCP or drinks spiked with SCP) were passed through the cartridges; the concentration of SCP in the working solutions was 1730 µg/mL. Then, the cartridges were washed with 1 mL acetonitrile-water 1:4 (v/v). After drying the cartridges with air, the analyte was desorbed with acetonitrile and collected for further analysis. In previous experiments aimed at optimizing the SPE procedure, three 200 µL portions of acetonitrile were successively passed through the cartridges and collected into 2 mL glass vials. The collected extracts were mixed with 400 µL of NaOH 0.1 M and then exposed to the MnO_4^- sensors. The absorbances measured for the collected extracts showed that most part of SCP was desorbed from the cartridges with the two first 200 µL portions of acetonitrile. However, assays with some of the beverages included in our study, especially for cola drinks, showed that significant amounts of colored matrix components were not eliminated from the cartridges with the first portion of washing solvent, and thus they were found in the first 200 µL-extract. In order to facilitate the visual identification of SCP, the first extract was discarded, so that the assays with the sensors were carried out with the second 200 µL-portion of acetonitrile collected. Although the percentage of SCP in the collected extracts was 20.0 ± 0.1% (n = 3), the absorbances measured were comparable to those untreated solutions. This is because the concentration of drug in the extracts was:

$$C_e = R \times C_0 \times \frac{V0}{Ve}$$

where *Ce* and C_0 denote the concentrations of SCP in the extract and sample, respectively, *R* the recovery, V_0 the volume sample subjected to SPE, and V_e the volume of the collected extract. In the present instance *R* was 0.2, and V_0 and V_e were 1.0 mL and 0.2 mL, respectively, so that concentration of the SCP in the sample and in the extracts were the same within experimental variations (1730 µg/mL). In other words, the poor recovery of the SPE was compensated by the reduction of the volume of the extracts with respect to the volume of samples.

It has to be remarked that with the proposed treatment sucrose is eliminated during the washing operations. This is illustrated in Fig. 2C, which shows the images and the spectra obtained for a solution of sucrose (100 mg/mL) processed with the sensors directly and after SPE. As it can be seen, the extracts showed the magenta color, which confirms that sucrose was removed with the SPE treatment.

The proposed procedure was applied to the analysis of different drinks (cola, cola mixed with whisky, tonic water mixed with vodka, red wine and a tea infusion), and the same samples after being spiked with SCP at a concentration of 1730 µg/mL (equivalent to a final concentration in the extracts of 577 µg/mL) [21]. In Fig. 5 are shown the images and spectra obtained for a blank, for the samples assayed, and for the same samples spiked with SCP. As it can be observed, positive responses were observed for the spiked samples. A green-brownish color was observed for the tea infusion, probably due to the high content of antioxidants in tea leaves. In Table 2 are shown the recoveries found for SCP in the samples assayed. These values were calculated from the differences of absorbances at 435 nm measured for the spiked and unspiked drinks (Fig. 5), and the values measured for standard solutions of the analyte prepared in NaOH 0.1 M, all subjected to the whole extraction and reaction protocol. In all instances values close to 100% were obtained, which indicated that the signals obtained for the different samples assayed were rather similar to those obtained for standard solutions of SCP; in other words, the absorbances measured with the sensors were not dependent on the type of sample.

Finally, the extracts collected for drinks after SPE were processed by the portable nanoLC based method. As illustrative example, in Fig. 6 are depicted the chromatograms obtained for the cola drink, and for the same sample spiked with SCP (two replicates). As it can be seen in this figure, no significant peaks were observed for the extracts collected for the samples, which confirmed that the SPE was effective in removing the matrix components. It has to be noted that the proposed SPE procedure is well-suited for on-site tests. A kit with the sensor, solvents, extraction cartridge and syringe with a connector would facilitate its application in the field, even by non-specialists. In this respect, SPE is a simple technique that can be applied manually without extra instrumentation [36]. In drug analysis the utility of C18 packed cartridges for was demonstrated by Scheel et al., who developed a method for the on-site analysis of the synthetic cathinone ethylone in seized street drug samples before analyzing them by voltammetry [37]. There are other recent examples of methods that involve some forms of SPE (or microextraction) for the

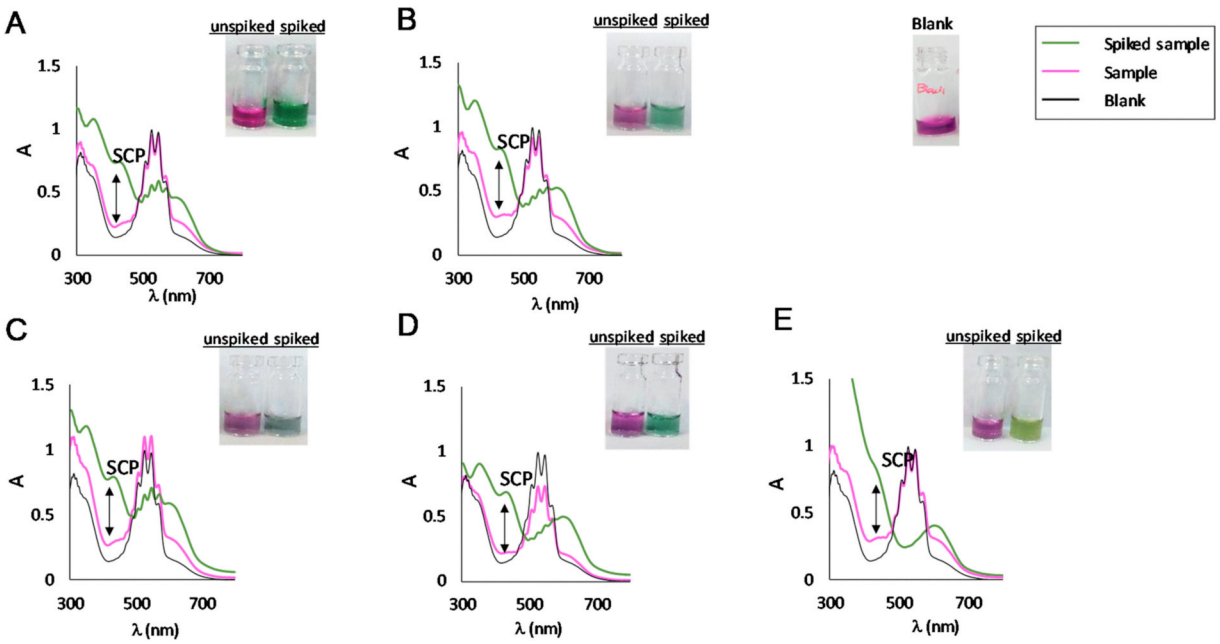

Fig. 5. Images and spectra of the solutions obtained with the SPE/reaction with the sensors proposed approach for different drinks: (A) cola, (B) mixture whisky-cola, (C) mixture tonic water:vodka, (D) red wine, and (E) tea infusion (top right, image obtained for a blank). For other experimental details, see the text. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Recoveries of SCP obtained in the spiked drink samples with the proposed SPE; these values were calculated from the absorbances measured for the spiked samples with respect to those obtained for aqueous solutions of SPC, both processed with the whole analytical procedure; concentration of SCP in the spiked samples and standard solutions, 1730 μg/mL μg/mL.

Beverage	Recovery, (n = 3) (%)
Cola-whisky	83 ± 4
Tonic-vodka	100 ± 3
Red wine	82 ± 2
Cola	104 ± 4
Tea infusion	91 ± 4

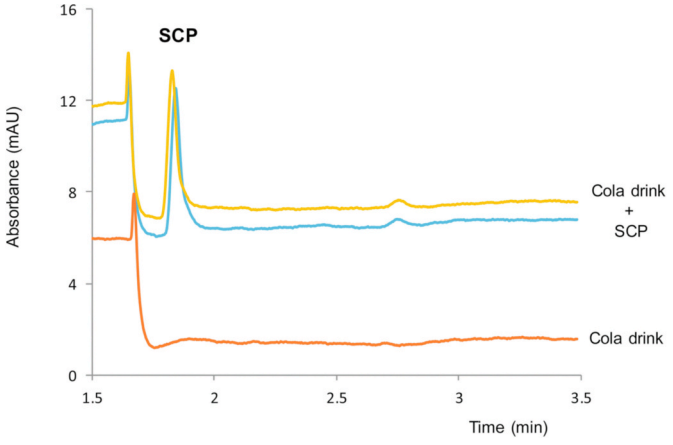

Fig. 6. Chromatograms obtained with the portable nanoLC for the extracts obtained by SPE for a cola drink, and for two replicates of the same sample spiked with SCP (1000 μg/mL). For other experimental details, see the text.

analysis of drugs other than SCP prior to their analysis with portable instruments, such as those developed for cocaine, amphetamine, oxycodone and other drugs by DART-MS [38], amphetamines by capillary electrophoresis [39] in oral fluid, and for benzodiazepines in dietary supplements by ion mobility spectrometry [40].

4. Conclusions

For the first time, a bicolorimetric sensor for the identification and quantification of SCP has been proposed based on the immobilization of reagent $KMnO_4$ and NQS into PDMS. A method for the analysis of SCP using a portable nanoLC instrument has been also developed, which allows the detection and quantification of SCP free from the interference of ATR in less than 5 min. For the analysis of SCP in drinks, a simple SPE procedure has been developed for the effective isolation of the analyte. The extracts collected after SPE are compatible with the sensors, so no intermediate steps such as solvent evaporation are required, whereas for the portable nanoLC approach a simple filtration of the extracts is required.

The analytical performance of the two approaches tested was comparable. In terms of selectivity, the chromatographic approach is superior. This would be the method of choice if the samples are suspected to contain ATR. However, the sensors based method can be considered a simple, rapid and chip alternative for the analysis of this drug in drinks.

The Results of our study can be considered as a contribution in the area of drug analysis, taking into account the scarcity of rapid tests for SCP (no colorimetric assays have been reported for this drug), and the fact it is increasingly involved in sexual crimes and robberies.

Credit author statement

N. Jornet-Martínez: Conceptualization, Methodology, Data curation, Investigation, Writing – original draft preparation, **R. Herráez-Hernández**: Conceptualization, Visualization, Writing-Review and Editing, **P. Campins-Falcó**: Conceptualization, Supervision

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.2021.122406.

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