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PAPER MICROFLUIDIC DEVICE AND SPECTROPHOTOMETRY METHODS FOR COLORIMETRIC DETECTION OF PHENYLEPHRINE HYDROCHLORIDE IN PURE AND PHARMACEUTICAL PREPARATIONS

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ABSTRACT. Two easy-to-use, rapid, and inexpensive colorimetric-based assays for the detection of active constituent in nasal decongestant phenylephrine hydrochloride (PHP), in solution and their respective pharmaceutical preparations were reported. The assay principle depends on the diazotized sulfadimidine (DSDM) coupling with PHP active pharmaceutical compound under alkaline conditions to produce a colored azo-dye product. A UV/VIS spectrophotometry method was utilized to monitor the color absorbance change at 460 nm, with a linear calibration graph in the concentration range of 1-20 mg L^{-1} and a limit of detection of 0.22 mg L^{-1} and limit of quantification of 0.72 mg L^{-1}. A comparison was done using a paper-based microfluidic analytical device (μPADs) and color intensity change was measured using a Samsung note 9 camera to capture digital images and Image J software to analyze the color intensity development. A linear trend was observed above approximately 10 mg L^{-1}. Thus, paper-based microfluidic analytical devices (μPADs) can be used as an alternative platform to conventional methods to perform a semi-quantitative PHP drug analysis. The proposed method was applied for the determination of PHP in commercial pharmaceutical preparations.

KEY WORDS: Phenylephrine hydrochloride, Spectrophotometry, Paper-based microfluidic analytical devices (μPADs), Pharmaceutical preparations, Colorimetric detection

INTRODUCTION

Most decongestants prescriptions are filled in outpatient settings and may be "falsified drugs" that have increased to satisfy the drugs-in-demand need by mimicking real drugs and are considered a serious problem in modern society, especially in low- and middle-income countries (LMIC) with weak Pharmacovigilance and drug regulatory systems [1-4]. Also, the increase in global access to the internet, the rise in e-commerce, and the globalization of pharmaceutical supply chains have led to rapid growth in online pharmacies especially in rich countries [5]. Therefore, there was a growing demand for low-cost, portable, quality assurance technologies that can assist distributors, pharmacists, and consumers in knowing the genuineness of their products.

Phenylephrine hydrochloride [(R)-1-(3-hydroxyphenyl)-2-(methylamino) ethanol hydrochloride], is a white crystalline powder, that belongs to the sympathomimetics group with predominantly alpha-adrenoceptor activity [6]. It has a vasoconstrictor, decongestant, and weak bronchodilator activities [7]. The therapeutic basis depends on the reduction of nasal congestion due to increased nasal blood flow associated with colds and influenza [8]. Various methods have been reported in the literature for the analysis of PHP as can be seen in Table 1.

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Table 1. Analytical methods used for PHP analysis.

Methods	Linear range	LOD	Remarks	Ref
Spectrophotometry	2–24 μg mL^{-1}	0.278 μg mL^{-1}	Coupling reaction with diazotized sulfacetamide sodium	[9]
Spectrophotometry	5–100 μg mL^{-1}		Oxidative coupling with the use of 4-aminoantipyrine	[10]
Spectrophotometry	10–200 μg 10 mL^{-1}		Di azo-coupling using 2,4-dinitroaniline	[11]
Spectrophotometry	0.5–9 μg mL^{-1}	0.006 μg mL^{-1}	Diazotization Reaction using clonazepam	[12]
Electrochemical sensor	5.0×10^{-6}–7.5×10^{-4} mol L^{-1}	3.7×10^{-7} mol L^{-1}	Multi-walled carbon nanotube-modified carbon paste electrode	[13]
voltammetry	$8 \times 10^{-6} – 1 \times 10^{-4}$ M	2.07×10^{-7} M	Use of ultra-trace graphite electrode (UTGE)	[14]
Potentiometric ion-selective electrodes	$1.0 \times 10^{-4} – 1.0 \times 10^{-2}$ M	6.31×10^{-5} M	The drug is subjected to oxidative degradation	[15]
HPLC	0.4–2.4 μg mL^{-1}		Use of toxic and expensive solvents(methanol and acetonitrile)	[16]
RP-HPLC	4–6 μg mL^{-1}		Dibasic phosphate buffer: acetonitrile (93: 07; v/v)	[17]

Colorimetry-based technology has been widely used in routine analyses due to its simplicity, visual readout, straightforward operation, and feasibility for remote area applications [18-21]. In the last two decades, colorimetric techniques have witnessed intense use in building, and upgrading detection platforms due to the development of the miniaturization concept; especially paper-based microfluidic analytical devices (μPADs) that are made of paper or other porous membranes, which can wick 10^{-6} to 10^{-9} L volumes of fluids using capillary action [22]. The new promising generation of μPADs has paved the way for numerous microfluidic applications due to several advantages over the conventional microfluidic devices made from glass, polymer or silicon including simple fabrication with the eradicates the cleanroom facilities, inexpensive raw material, omit the use of pumps or other supporting equipment, environmentally being nature, eco-friendly platform disposal and in-situ analysis which are the fundamental factors that motivate the merger of colorimetric detection and μPADs [23, 24]. These characteristics implicit the benefits of paper as a substrate and colorimetric reaction as a detection platform to build an ideal and valuable point of need pharmaceutical preparation analysis.

The literature is still poor in analytical procedures based on colorimetric detection using paper microfluidic, especially for pharmaceuticals. Hence, it was planned to investigate the coupling reaction between PHP and DSDM in an alkaline medium to build a fast and simple method for PHP determination in pharmaceutical drugs to prevent their falsification using a spectrophotometric and paper-based microfluidic analytical devices (μPADs).

EXPERIMENTAL

Apparatus and materials

A digital single-beam spectrophotometer (Shimadzu UV-Visible 1240) was used to scan and measure absorbance with a 1cm path length quartz cell. All chemicals used were of analytical reagent grade. Pure PHP drug samples were kindly supplied from the State Company for Drug Industries and Medical Appliance (SDI-Samara/Iraq). Pharmaceutical application (Nasophrine®, phenylephrine HCl 0.25%, nasal drops-SDI/Iraq) was purchased from commercial sources.

Preparation of solutions

Phenylephrine hydrochloride (PHP): Stock standard solution 250 mg L^{-1} was prepared by dissolving 0.025 g of PHP pure drugs in 100 mL distilled water. Working standard solutions were prepared by suitable dilution of the stock standard solution with distilled water. Sodium hydroxide solution (2M): this solution was prepared by dissolving 20 g of NaOH (Merck) in 250 mL distilled water, and other diluted solutions were prepared by appropriate dilution with distilled water. Diazotized sulfadimidine (10 mM): was prepared by transferring 0.9 mL of standard sulfadimidine sodium solution (333 mg mL^{-1}) to a 100 mL volumetric flask, then 3 mL of 1M hydrochloric acid (36% w/w) was added in an ice–bath. After 5 min, 0.0690 g of sodium nitrite was added to the solution, shaken, and completed to mark with distilled water.

Commercial dosage preparation analysis

The contents of three bottles of Nasophrine® 0.25%, nasal drops of PHP were mixed. An aliquot corresponding to 25 mg of the drug (5 mL) was diluted to 50 mL with distilled water in a volumetric flask to obtain 250 mg L^{-1} of PHP. Different diluted concentrations were obtained by a simple dilution with distilled water.

Colorimetric detection using the spectrophotometric method

An increasing volume of PHP working solutions (250 mg L^{-1}) was transferred into a series of 10 mL volumetric flasks to prepare the calibration graph range (1-20 mg mL^{-1}), then 2 mL of DSDM (10 mM) and 0.5 mL of NaOH (0.5 M) were added. The solutions were made up to the mark with distilled water, mixed well, and left at room temperature (25 C°) for 10 min. The absorbance was measured at 460 nm versus the reagent blank. Calibration graph was drawn and regression equation was calculated. For the optimization of optimum condition experiments, 12.5 mg L^{-1} of PHP was utilized in triplicate to calculate the error bar. The same procedure was used for pharmaceutical preparations.

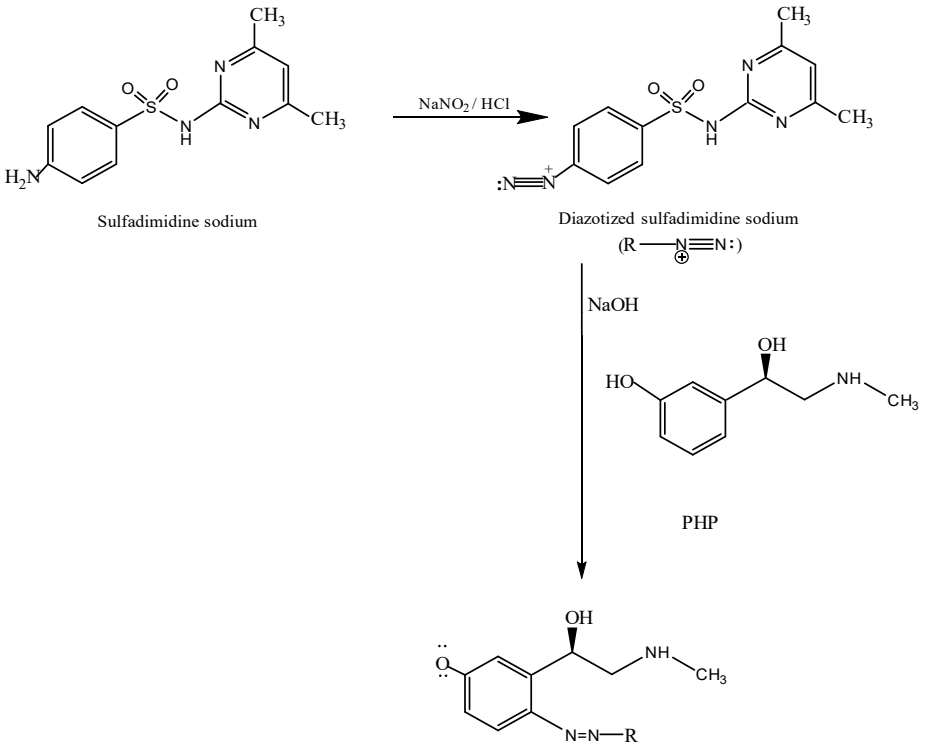
Colorimetric detection using the μPAD method

The paper-based microfluidic analytical devices (μPADs) was designed according to Carrilho *et al*. method [25]. The device had a total of eight circular reaction zone, 10 mm diameter, printed with 0.7 mm line width in green wax, and six colored squares as internal standard. This device would allow the measurement of eight separate standard concentrations of the drug on the same paper. The paper assay was done by spotting 5 μL of the different drug standards, separately in each detection zone located on a paper chip using an Eppendorf micropipette and a separated spot was used to calculate the blank (as reference). From that, 10 μL of the reagent DSDM and 5μL NaOH was pipetted into each detection zone merging into the dried drug area. Then, the mixture was left for a period of time until it completely dried before image processing and data acquisition for colorimetric analysis using a Samsung note 9 camera to photograph the platform and the data were analyzed with Image J software. The same procedure was repeated for the pharmaceutical preparations to measure their concentrations. Triplicate experiments were done for both pure drug and pharmaceutical preparations to calculate the error bar from the standard deviation of these repeats.

RESULTS AND DISCUSSION

PHP measurements via spectrophotometric analysis

An immediately orange azo dye was obtained as a result of coupling reaction between PHP and DSDM in alkaline conditions (Scheme 1). The colored product gave a maximum absorbance at 460 nm (Figure 1) and remained stable for at least 2 h.



Scheme 1. Proposed reaction scheme of the reaction between PHP with DSDM in an alkaline medium.

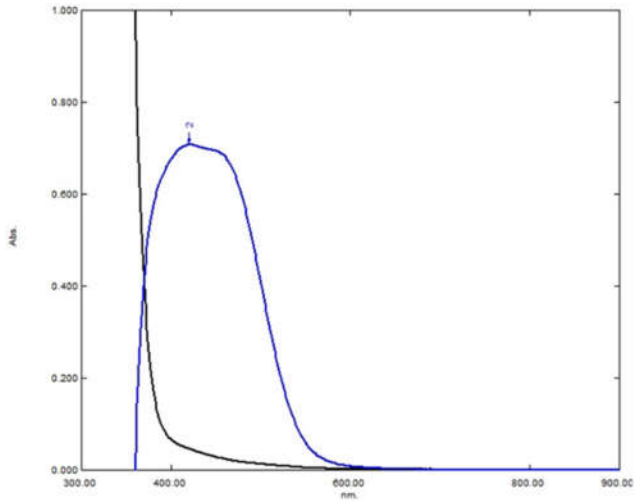


Figure 1. Absorption spectra of complex formed by PHP (12.5 mg L^{-1}) reacted with DSDM in alkaline medium (blue curve) versus reagent blank (0 mg L^{-1}) (black curve).

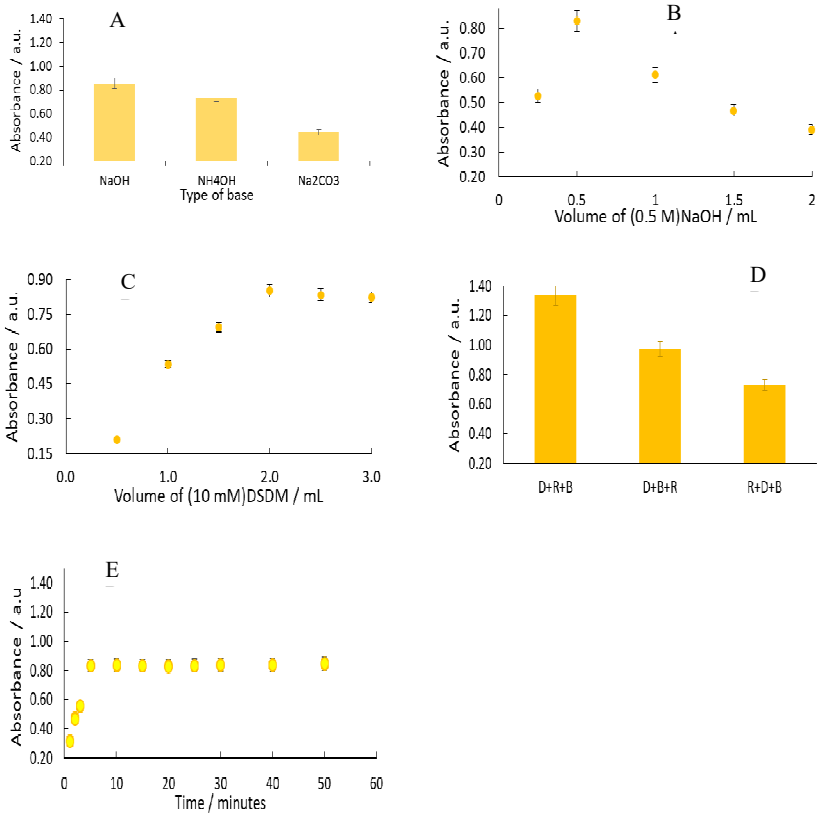


Figure 2. Optimization of parameters for detection of PHP via the formation of azo-dye complex, all optimization experiments were carried out using 12.5 mg L^{-1} PHP solution, n = 3. (A) Type of base (NaOH, NH_4OH, and Na_2CO_3), (B) Volume of NaOH (0.5 M) varied between 0.25 mL and 2 mL, (C) Volume DSDM (10 mM) varied between 0.5 mL and 3.0 mL, (D) Optimization of reagent addition order, where D = PHP (12.5 mg L^{-1}), R = DSDM (10 mM), B = NaOH (0.5 M), and (E) Development of azo-dye product over time, absorbance recorded between 1 min and 60 min.

To study the influence of various operating conditions (summarized in Figure 2) affecting the azo dye formation, a 12.5 mg L^{-1} solution of PHP was used and measured at 460 nm. Different alkaline solutions (0.5 M) including sodium hydroxide, ammonium hydroxide and sodium carbonate were investigated. Preliminary results showed that sodium hydroxide gave the highest absorbance as can be seen in Figure 2 A, therefore, it was used in consequent experiments. The absorbance of the orange product affected by different sodium hydroxide (0.5 M) volumes ranging from (0.25-2) mL was studied and measured at λ_{max} = 460 nm whilst the other parameters were fixed. The strongest color intensity was found at 0.5 mL and a further increase in base addition resulted in a decrease in product absorbance (Figure 2 B). Therefore, 0.5 mL of 0.5 M of the base was used in further experiments. Next, different volumes (0.5 mL to 3 mL) of DSDM (10 mM)

reagent were studied. It was found that 2 mL was the optimum volume that gave the best absorbance (Figure 2 C). The effect of varied orders for reagents addition including drug (D), NaOH base (B) and DSDM reagent (R) on maximum absorbance were examined (Figure 2 D). The three combinations gave a similar absorbance with a slightly higher absorbance for the drug, followed by reagent and base which were chosen for the subsequent experiments. As a final optimization parameter, the effect of reaction time was investigated over the time interval from (0-60) min (Figure 2 E). The color was visible immediately, however, the absorbance increased with time and gave a plateau at 10 min and this time was adequate for the product best development time.

Using the optimized reaction parameters, the calibration curve was obtained by plotting a range of PHP concentrations against colored product absorbance (Figure 3). A linear trend was observed over the range (1-20 mg L^{-1}) with limits of detection and limits of quantification values of 0.22 mg L^{-1} and 0.72 mg L^{-1}.

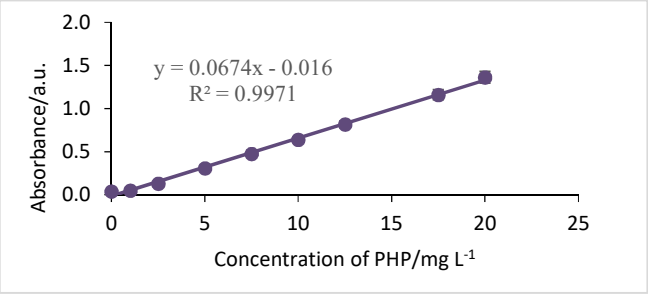


Figure 3. Linear calibration obtained for PHP (1-20 mg L^{-1}), n = 3.

PHP measurements via µPADs

The concept of lab-on-a-paper was used as a cheap, simple, and easy-to-use alternative to the spectrophotometry method. This was achieved by patterning two region including hydrophobic barriers and hydrophilic microchannel that enable the azo dye complex storage (Figure 4).

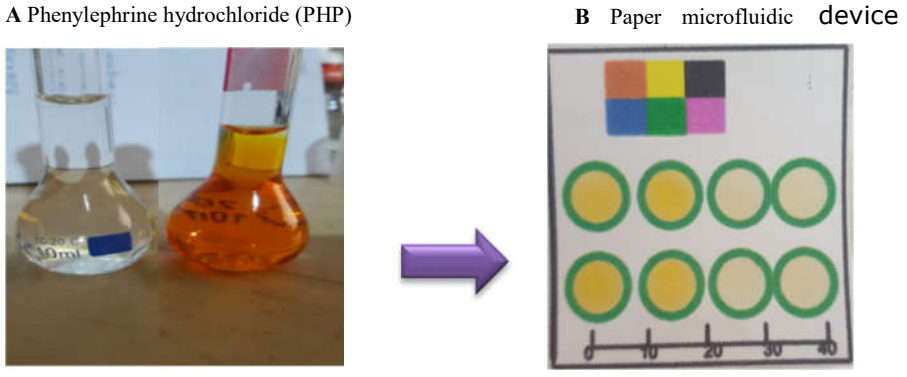


Figure 4. From traditional laboratory bench to lab-on-a-paper device. Reaction of PHP (0 and 12.5 mg L^{-1}) with DSDM in alkaline conditions to produce an orange azo-dye done on paper-based microfluidic analytical devices (µPADs).

Preliminary investigations on various parameters were done to give the optimized color intensity on the paper device. Optimization was carried out by changing one parameter and keeping the others fixed and so on. To ensure a sufficient basic medium for azo complex formation, varied volumes of NaOH (0.5 M) between (1-18 µL) were studied and results showed in Figure 5 A revealed that 3 µL gave the best color intensity and further addition lead to a slight decrease in color intensity. Different volumes of reagent DSDM (1-18 µL) were spotted into detection zone, individually. The color intensity increased with the increase of DSDM volume up to 12 µL which was adequate for color development (Figure 5 B) without the reagent leakage over the sensing zone. Due to the significant effect of reagent, base, and PHP reagents addition order, a study was carried out with diverse addition of these solutions (Figure 5 C). Optimum color intensity was obtained from the addition of PHP, DSDM, and then NaOH solutions. Finally, time interval from (0-60) min to obtain the highest color intensity was studied. Figure 5 D showed a graduated increase within 25 min and therefore, was recommended for azo complex optimum reaction time. Then, the color intensity decreased after 25 min indicating instability of the azo complex in contrast to the solution based method.

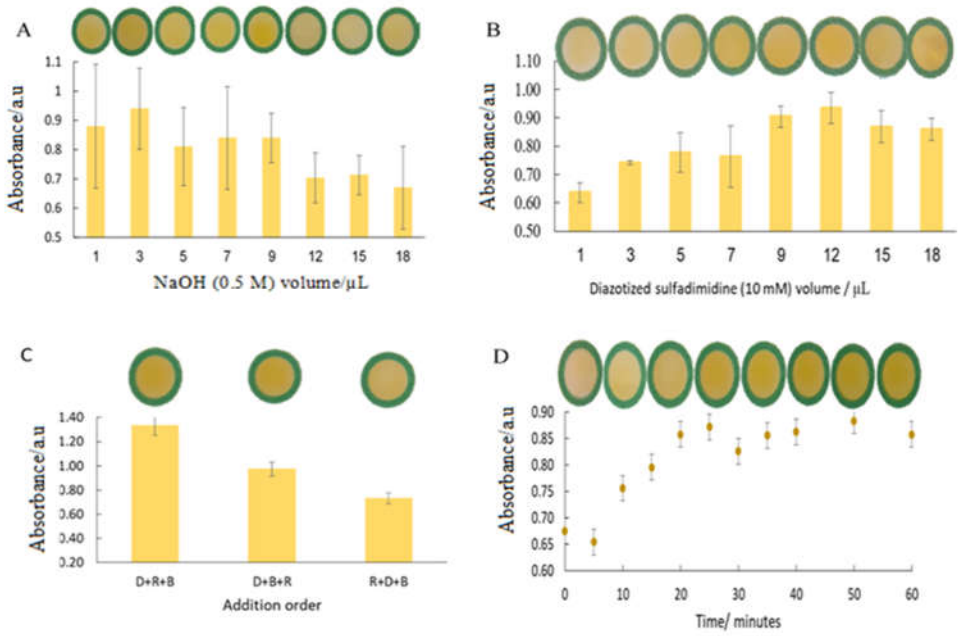


Figure 5. Optimization of reaction parameters for detection of PHP via the formation of an orange azo-dye on the paper device, all optimization experiments were carried out using 12.5 mg L^{-1} PHP solution, n = 3. (A) Volume of NaOH (0.5 M) varied between 1 µL and 18 µL, (B) Volume of DSDM reagent (10 mM) varied between 1 µL and 18 µL, (C) Effect of order of reagent addition order with D = PHP (12.5 mg L^{-1}), R = DSDM (10 mM) and B = NaOH (0.5M), (D) Development of orange azo-dye product over time, absorbance recorded between 0 and 60 min.

Under the optimized reaction parameters of 12 µL DSDM (10 mM), and 3 µL NaOH (0.5 M); the calibration curve was obtained by plotting a range of PHP concentrations (1-20 mg L^{-1}) against

colored product absorbance (Figure 6). Images were taken 25 min after the addition of PHP and the analyzed data showed a linear trend above 10 mg L^{-1}. On other hand, at lower concentrations, data have the tendency to vary due to fairly weak color intensity, therefore, this range was omitted for quantitative analysis.

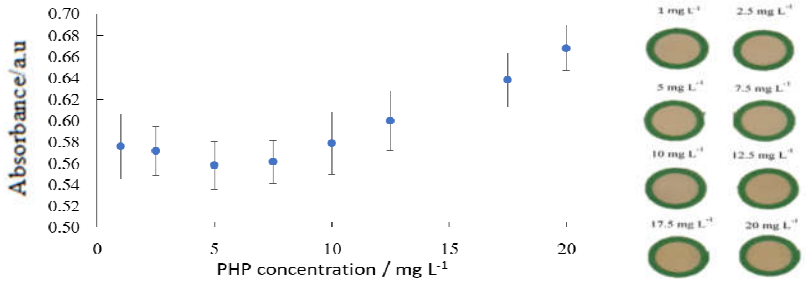


Figure 6. Calibration graph dispelling a linear trend above 10 mg L^{-1} for PHP (1-20 mg L^{-1}), n = 3.

Pharmaceutical dosage form application

To assess the validity of the proposed method, two different concentrations of PHP nasal drops solution (5 and 10 mg L^{-1}) were analyzed using Nasophrine® (0.25%) nasal drops solution. The results tabulate in Table 2 indicate the good accuracy and precision of the proposed method and omit potential interference from the matrix of the sample.

Table 2. Analysis via UV/Vis absorption of Nasophrine® (0.25%) nasal drops diluted to 5 and 10 mg L^{-1}.

UV/Vis method	Concentration of prepared solution (mg L^{-1})	Concentration determined after analysis (mg L^{-1}), n = 3	%R	RSD
Nasophrine® Nasal drops	5.00	4.8	95.00	1.00
	10.0	9.7	97.00	1.00

Real pharmaceutical samples from Nasophrine® (0.25%) nasal drops solution were also investigated using the paper microfluidic device. A 10 mg L^{-1} concentration was utilized and compared to the blank, there was a significant difference (Figure 7). Therefore, paper-based microfluidic analytical devices (μPADs) can be considered a suitable semi-quantitative device for PHP pharmaceutical preparations determination as well as for pure form.

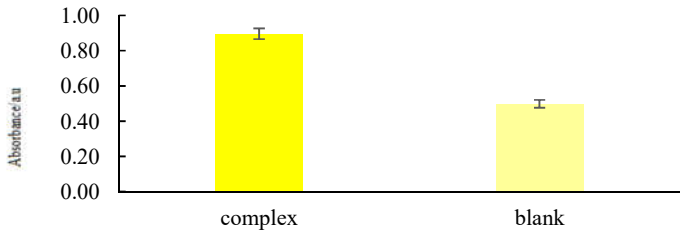


Figure 7. Comparison of a real sample (10 mg L^{-1}) from Nasophrine® (0.25%) nasal drop solution with a blank (reagents only). A significant difference was found between pharmaceutical formulation and blank color intensity, n = 3.

CONCLUSION

We have proposed a diazotization reaction for PHP quantitative analysis using spectrophotometric method and compared with paper-based microfluidic analytical devices (μPADs). The methods are rapid, easy, omit sophisticated instrumentation or training, and the materials used are environmentally friendly. The unique features of the paper microfluidic chip (lab-on-a-paper) showed advantages for real-word routine drug analysis as well as in research with semi-quantitative PHP drug analysis.

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