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## Spectrophotometric determination of certain CNS stimulants in dosage forms and spiked human urine via derivatization with 2,4-dinitrofluorobenzene

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### ABSTRACT

A new spectrophotometric method is developed for the determination of phenylpropanolamine HCl (PPA), ephedrine HCl (EPH) and pseudoephedrine HCl (PSE) in pharmaceutical preparations and spiked human urine. The method involved heat-catalyzed derivatization of the three drugs with 2,4-dinitrofluorobenzene (DNFB) producing a yellow coloured product peaking at 370 nm for phenylpropanolamine HCl and 380 nm for ephedrine HCl and pseudoephedrine HCl, respectively. The absorbance concentration plots were rectilinear over the range of 2-20, 1-14 and 1-14  $\mu\text{g/mL}$ , for PPA, EPH and PSE, respectively. The limit of detection (LOD) values were 0.20, 0.13 and 0.20  $\mu\text{g/mL}$  for PPA, EPH and PSE, respectively and limit of quantitation (LOQ) values were 0.60 and 0.40 and 0.59,  $\mu\text{g/mL}$  for PPA, EPH and PSE, respectively. The analytical performance of the method was fully validated and the results were satisfactory. The proposed method was successfully applied to the determination of the three studied drugs in their commercial dosage forms including tablets, capsules and ampoules with good recoveries. The proposed method was further applied for the determination of PSE in spiked human urine with a mean percentage recovery of  $108.17 \pm 1.60\%$  for  $n = 3$ . Statistical comparison of the results with those of the comparison methods showed good agreement and proved that there was no significant difference in the accuracy and precision between the comparison and the proposed methods, respectively. The mechanism of the reaction pathway was postulated. © 2011 Trade Science Inc. - INDIA

### KEYWORDS

Spectrophotometry;  
Phenylpropanolamine HCl;  
Ephedrine HCl;  
Pseudoephedrine HCl;  
2,4-dinitrofluorobenzene;  
Tablets;  
Capsules;  
Ampoules;  
Spiked human urine.

### INTRODUCTION

Phenylpropanolamine hydrochloride, (PPA) is (1RS, 2SR)-2-amino-1-phenylpropanol hydrochloride<sup>[1]</sup> (Figure 1). It is a largely indirect acting sympathomimetic with an action similar to ephedrine, it is orally administered for the treatment of nasal congestion. It is

frequently used in mixture preparations for the relief of cough and cold symptoms. Other uses of phenylpropanolamine include the control of the urinary incontinence in some patients. It has also been used to suppress appetite in the management of obesity<sup>[1]</sup>. The United States Pharmacopoeia (USP)<sup>[2]</sup> and the British Pharmacopoeia (BP)<sup>[3]</sup> recommended non-aqueous tit-

rimetric method for the determination of PPA in the pure form in presence of mercuric acetate, using perchloric acid as a titrant and crystal violet as indicator. On the other hand, both USP<sup>[2]</sup> recommended HPLC method for its determination in dosage forms using a mixture of (1-hexanesulfonate, monobasic sodium phosphate and triethylammonium phosphate) and methanol as a mobile phase with UV detection at 210 nm.

Due to its clinical advantages, PPA received a great interest. A good guide to the work published is found as comprehensive monograph in analytical profiles for drugs<sup>[5]</sup>. Several analytical techniques have been reported for PPA determination either *persu* or in pharmaceutical preparations and biological fluids such as; titrimetry<sup>[6]</sup>, spectrophotometry<sup>[7]</sup>, fluorimetry<sup>[8]</sup>, HPLC<sup>[9]</sup>, capillary electrophoresis<sup>[10]</sup>, flow injection<sup>[11]</sup>, and gas chromatography<sup>[12]</sup>.

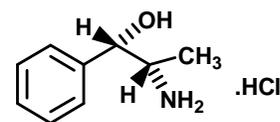
Ephedrine hydrochloride (EPH) is (1R,2S)-2-(Methylamino)-1-phenylpropan-1-ol hydrochloride<sup>[1]</sup> (Figure 1). It is a sympathomimetic drug with direct and indirect effects on adrenergic receptors. It has alpha and beta-adrenergic activity and has pronounced stimulating effects on the central nervous system<sup>[1]</sup>. It is reported to reduce the viscosity of tenacious sputum and is used as an expectorant. The USP<sup>[2]</sup> recommended a non aqueous titration method for its determination in pure form by addition of mercuric acetate and titration with 0.1N perchloric acid using crystal violet as indicator. The BP<sup>[4]</sup> favored a potentiometric titration method for its determination in pure form using 0.1M NaOH as a titrant. Both USP and BP recommended HPLC method with UV detection at 263 nm for its determination in dosage forms. Various reports have been described for the analysis of EPH a good guide to the work published for EPH is found as comprehensive monograph in analytical profiles for drugs<sup>[13]</sup>. Several techniques were reported including; titrimetry<sup>[14]</sup>, spectrophotometry<sup>[15]</sup>, fluorimetry<sup>[16]</sup>, flow injection<sup>[17]</sup>, capillary electrophoresis<sup>[18]</sup>, TLC<sup>[19]</sup>, HPLC<sup>[20]</sup>, and gas chromatography<sup>[21]</sup>.

Pseudoephedrine HCl (PSE) is (1S,2S)-2-(Methylamino)-1-phenylpropan-1-ol hydrochloride<sup>[1]</sup> (Figure 1) is a direct and indirect sympathomimetic. It is a stereoisomer of ephedrine and has a similar action, but has been stated to have less pressor activity and fewer CNS effects. It is given orally for the relief of nasal

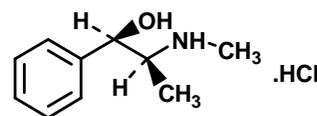
congestion. They are commonly combined with other ingredients for the relief of cough and cold symptoms<sup>[1]</sup>. The USP<sup>[2]</sup> recommended a non aqueous titration method for the determination of PSE in its pure form in presence of mercuric acetate and titration using 0.1M perchloric acid using crystal violet as indicator. The BP<sup>[4]</sup> preferred a potentiometric titration for its determination in pure form using 0.1M NaOH as a titrant. Both USP and BP recommended HPLC method with UV detection for its determination in dosage forms.

A good guide to the work published for PSE is found as comprehensive monograph in analytical profiles for drugs<sup>[22]</sup>. The literature revealed that the analysis of PSE was through techniques such as; spectrophotometry<sup>[23]</sup>, flow injection<sup>[24]</sup>, capillary electrophoresis<sup>[25]</sup>, HPTLC<sup>[26]</sup>, HPLC<sup>[27]</sup>.

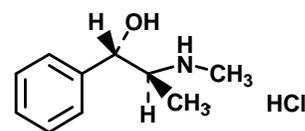
Sanger's reagent (DNFB) on the other hand has been applied as a chromogen for the spectrophotometric estimation of many compounds such as desloratadine<sup>[28]</sup>, enalapril<sup>[29]</sup>, lisinopril<sup>[30]</sup> and gabapentin<sup>[31]</sup>.



A) Phenylpropanolamine HCl



B) Ephedrine HCl



C) Pseudoephedrine HCl

Figure 1 : Structural formulae of the three drugs

## EXPERIMENTAL

### Instruments

- A shimadzu UV-Visible 1601 PC spectrophotometer (Kyoto, Japan) was used for spectrophotometric measurements (P/N 206-67001). The recording range was 0-1.2

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- A consort NV P901 digital pH Meter (Belgium) calibrated with standard buffers was used for checking the pH of the buffer solutions used.

### Materials and solutions

All the reagents used were of Analytical Grade and distilled water was used throughout the work.

- Phenylpropanolamine HCl was kindly supplied from Egyptian Pharmaceutical Industries CO. E.P.I.CO (10<sup>th</sup> of Ramadan – Egypt) (Batch #41204) its purity was 100.55 % as determined by the official method<sup>[4]</sup>.
- Ephedrine HCl (EPH) was kindly supplied from Egyptian INT. Pharmaceutical Industries CO. E.P.I.CO (10<sup>th</sup> of Ramadan - Egypt), (Batch #135705) its purity was 100.99 %, was checked by applying official method<sup>[4]</sup>.
- Pseudoephedrine HCl was kindly provided from Sigma company (Batch#050727) its purity was 99.65 % which was determined by applying official method<sup>[4]</sup>.
- 2,4 dinitrofluorobenzene (DNFB) (Fluka Chemie, Germany) was freshly prepared as 0.3 % v/v methanolic solution. -Borate buffer solution (0.2 M) was prepared by mixing appropriate volumes of 0.2 M boric acid and 0.2 M NaOH and adjusting the pH using a pH Meter. The buffer solution was kept in the refrigerator and left to reach the room temperature before use.
- Methanol and hydrochloric acid (32%) were purchased from (BDH, UK).
- Boric acid and tween-80 were purchased from (BDH, UK).Cetyltrimethylammonium bromide (cetrimide) was obtained from Merck (Darmstadt, Germany), 1% (w/v) aqueous solution was prepared.
- Sodium dodecyl sulphate (SDS) 95% was obtained from Winlab (Middlesex, England), 1% (w/v) aqueous solution was used.
- Urine samples were obtained by a healthy female volunteer around 30 years. The following dosage forms containing the drugs were purchased from local pharmacies:
- Allercet capsules, batch # 820304, each capsule labeled to contain 30 mg pseudoephedrine HCl and 10 mg cetirizine HCl, product of Global Napi Pharmaceuticals, 6<sup>th</sup> of October City-Giza-Egypt.
- Contaflu tablets, batch # 061342, each tablet labeled to contain 24 mg of phenylpropanolamine HCl, 3 mg of chlorpheniramine maleate and 200 mg of propyphenazone, product of Egyptian Int.Pharmaceutical Industries CO. E.P.I.CO,10<sup>th</sup> of Ramadan City, Egypt.
- Ephedrine ampoule:-batch # 11 each ampoule (1mL) labeled to contain 30 mg of ephedrine HCl, A product of Chemical industries Development(CID)-Giza-A.R.E.

Standard stock solutions of the studied drugs were prepared by dissolving 10.0 mg of the drugs in 100 mL of distilled water. Serial dilution with the same solvent was performed to obtain the appropriate concentration range. These solutions were stable for at least 10 days when stored in the refrigerator and protected from light.

### General analytical procedures

#### Construction of the calibration curve

Aliquot volumes of PPA, EPH and PSE standered solution covering the working concentration range cited in (TABLE 1) were transferred in to a series of 10 mL volumetric flask; followed by specific volume of borate buffer of specific pH as shown in (TABLE 1), then the specific volume of 3% (v/v) of DNFB was added as abridged in (TABLE 1). The flasks were heated in a thermostatically controlled water bath at 70°C in case of PSE and at 80°C in case of EPH and PPA, respectively. Heating for 25 minutes in case of PSE, EPH and for 20 minutes in case of PPA was then performed. The reaction was stopped by cooling under tap water, and then 0.2 mL of concentrated HCl was added and the solutions were diluted to the volume with specific solvent and mixed well (TABLE 1). The absorbance was measured at 370 nm in case of PPA and at 380 nm for

**TABLE 1 : Assay parameters for the determination of the three drugs by the proposed method**

Item	PPA	EPH	PSE
Standard conc. (µg/mL)	100	100	100
Borate buffer pH	8.0 ± 0.5	8.0 ± 0.5	8.25 ± 0.5
Borate buffer volume (mL)	1 ± 0.2	1 ± 0.5	0.8 ± 0.2
DNFB volume (mL)	0.6 ± 0.1	0.8 ± 0.2	0.8 ± 0.2
Temperature (°C)	80	80	70
Time (min)	20 ± 5	25 ± 5	25 ± 5
Diluting solvent	water	methanol	water
λ <sub>max</sub> (nm)	370	380	380

EPH and PSE, respectively against a reagent blank. The absorbance was plotted *versus* the final concentration of the drug ( $\mu\text{g/mL}$ ) to obtain the calibration graph. Alternatively, the corresponding regression equations were derived.

### Application of the proposed method to the analysis of the studied drugs in their dosage forms

#### For tablets and capsules

An accurately weighed quantity of the mixed contents of ten capsules or powdered tablets equivalent to 10.0 mg of PSE or PPA were transferred into conical flask and extracted with 3 x 30 mL of distilled water in case of PSE and PPA, respectively by sonication for 30 min. The solution was filtered in to 100 mL volumetric flask and completed with the same solvent. Aliquots covering the working concentration ranges were analyzed as described under "Construction of the Calibration Curve". The concentrations of the drugs were determined using, either the calibration curves or the corresponding regression equations.

#### For ampoules

The contents of ten ampoules were emptied and mixed well. An accurately measured volume of the resulting solution equivalent to 10.0 mg of EPH was transferred into 100 mL volumetric flask, and diluted to volume with  $\text{H}_2\text{O}$ . Aliquots covering the working concentration ranges were analyzed as described under "Construction of the Calibration curve". The concentration of EPH was determined using, either the calibration curves or the corresponding regression equation.

### Application of the proposed method to the analysis of PSE in spiked urine

Five mL of urine were spiked with 20, 40, 60  $\mu\text{g}$  of PSE, basified with 0.5 mL of 5 M KOH to liberate the base, followed by 3g of NaCl as described by Avois *et al*<sup>[32]</sup>. The spiked urine is then extracted with three portions of 2 mL of *ter*-butyl methyl ether (TBME) centrifugated for 5 minutes at 2500g. After centrifugation the clear supernatant was collected, evaporated till dryness. The residue was then dissolved in 3 mL of distilled water and completed as described under "Construction of the Calibration Curve".

## RESULTS AND DISCUSSION

2,4-Dinitrofluorobenzene (DNFB) as an active aryl halide reacts with primary or secondary amine in aqueous alkaline medium to form a yellow colored product through a nucleophilic substitution reaction<sup>[33]</sup>. The presence of 1ry amine in PPA and 2ry amine in both of PSE and EPH which are highly susibtable for derivatization reaction with DNFB initiated the present study. In the present study, the studied drugs were found to react with DNFB in borate buffer producing a yellow color peaking at 380 nm for EPH and PSE and at 370 nm for PPA, respectively (Figure 2)

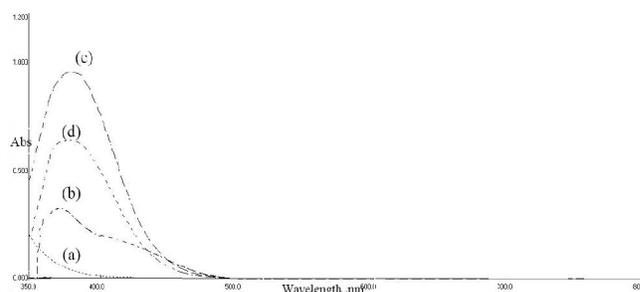


Figure 2 : Absorption spectra of the reactions products of:- (a) Reagent blank; (b) PPA (6  $\mu\text{g/mL}$ ) with DNFB at pH 8.25; (c) PSE (14  $\mu\text{g/mL}$ ) with DNFB at pH 8.25; (d) EPH (7  $\mu\text{g/mL}$ ) with DNFB at pH 8.0.

### Study of experimental parameters

Experimental parameters affecting color development and its stability were carefully studied and optimized each was changed while others were kept constant. Experimental parameters include; effect of pH, volume of buffer solution, concentration of DNFB solution, heating time, SAA temperature and effect of diluting solvent.

#### (a) Effect of pH

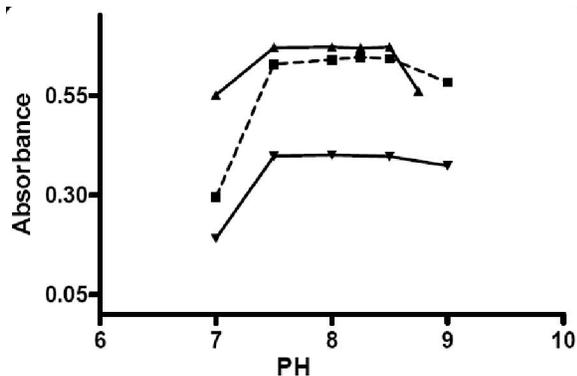
The reaction between the investigated drugs and DNFB was investigated over the pH range of 7.0- 9.0 using 0.2 M borate buffer. The reaction showed the highest absorption in buffer of pH  $8.0 \pm 0.5$  for EPH, PPA and  $8.25 \pm 0.5$  for PSE. Therefore, pH 8.0 was selected as the optimum pH value in case of EPH and PPA, where PH 8.25 was chosen for PSE throughout this study (Figure 3).

#### (b) Effect of volume of buffer solution

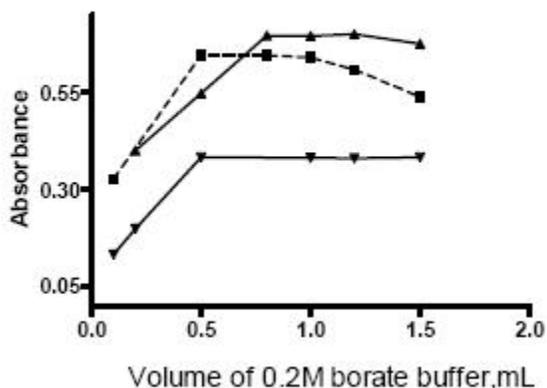
It was found that increasing the volume of the buffer produced a corresponding increase in the absorbance

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value of the reaction product up to 0.5 mL for PSE and EPH and 0.8 mL for PPA, and it remained constant up to 1 mL for PSE, 1.2 mL for PPA and 1.5 mL in case of EPH. (Figure 4). Therefore,  $0.8 \pm 0.2$  mL for PSE,  $1 \pm 0.2$  mL for PPA and  $1.0 \pm 0.5$  mL for EPH were chosen as the optimum buffer volume for the analysis.



**Figure 3 :** Effect of the pH of 0.2 M borate buffer on the reaction product of:- ■ PSE (10 $\mu$ g/mL) with DNFB; ▲ PPA (12 $\mu$ g/mL) with DNFB; ▼ EPH (7 $\mu$ g/mL) with DNFB

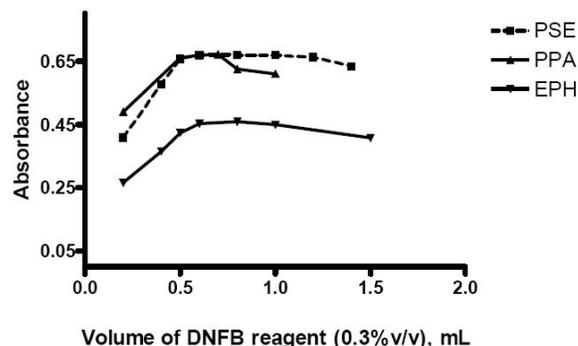


**Figure 4 :** Effect of the volume of 0.2 M borate buffer on the reaction product of:- ■ PSE (10 $\mu$ g/mL) with DNFB; ▲ PPA (12 $\mu$ g/mL) with DNFB; ▼ EPH (7 $\mu$ g/mL) with DNFB.

### (c) Effect of the concentration of DNFB solution

The influence of the concentration of DNFB was studied using different volumes of 0.3% v/v solution of the reagent. It was found that increasing volumes of the reagent produced a proportional increase in the absorbance value up to 0.6 mL for PSE, 0.5 mL for PPA. And 0.6 mL for EPH. However, no further increase in the absorbance value was observed upon increasing the volume of the reagent up to 1.2 mL for PSE, 0.7 mL for PPA and 1.0 mL for EPH after which further increase produced a gradual decrease in the absorbance value. Therefore,  $0.8 \pm 0.2$  mL for PSE,  $0.6 \pm 0.1$  mL for PPA and  $0.8 \pm 0.2$  mL for EPH of 0.3% v/v DNFB

solution were chosen as the optimal volume of the reagent (Figure 5).

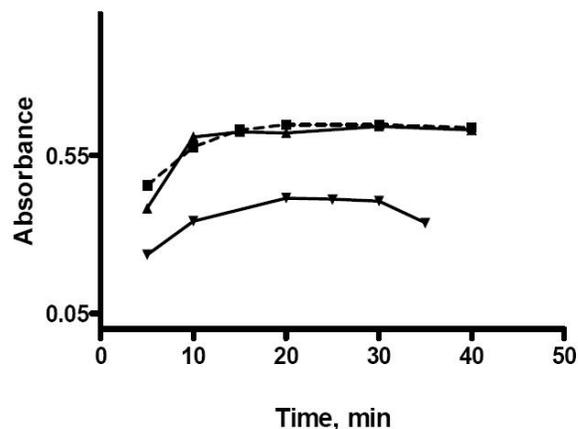


**Figure 5 :** Effect of volume of reagent on the reaction product of:- ■ PSE (10 $\mu$ g/mL) with DNFB; ▲ PPA (12 $\mu$ g/mL) with DNFB; ▼ EPH (7 $\mu$ g/mL) with DNFB.

From the reaction of any of the three drugs with DNFB a yellow colored product was formed along with the alkaline hydrolysis product of DNFB (2,4-dinitrophenolate), which exhibits an intense yellow color. The spectra of the two products are overlapped rendering impossible measurement of the analyte derivative. However, upon acidification with conc HCl after the completion of the reaction, the yellow 2,4-dinitrophenolate turns to the colorless 2,4-dinitrophenol, allowing the quantitative measurement of the Drug-DNFB derivative which remains stable.

### (d) Effect of heating time

The effect of the heating time of the reaction between the investigated drugs and DNFB was studied. Increasing the heating time resulted in gradual increase in the absorbance of the reaction product up to 20 min



**Figure 6 :** Effect of heating time on the reaction product of:- ■ PSE (10 $\mu$ g/mL) with DNFB; ▲ PPA (12 $\mu$ g/mL) with DNFB; ▼ EPH (7 $\mu$ g/mL) with DNFB.

for PSE, 15 min for PPA and 20 min for EPH after which no further increase in the absorption intensities occur, therefore  $25 \pm 5$  minutes for PSE,  $20 \pm 5$  minutes for PPA and  $25 \pm 5$  min for EPH were chosen as the optimum buffer volume for the analysis (Figure 6).

#### (e) Effect of heating temperature

In order to obtain the highest and most stable absorbance, the effect of the reaction time and heating temperature was investigated (Figure 7). It was found that the reaction proceeds very slowly at room temperature. A gradual increase in the heating temperature produced a significant increase in the absorbance of the reaction product up to  $70^\circ\text{C}$  for PSE,  $80^\circ\text{C}$  for PPA and EPH.

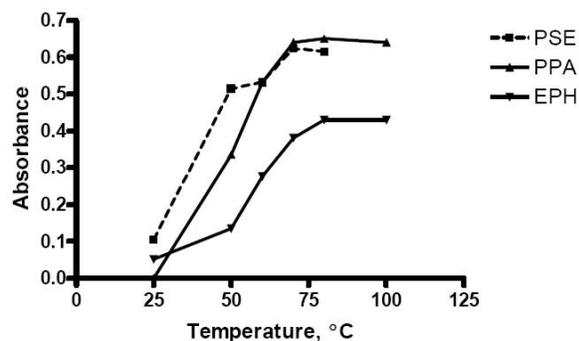


Figure 7 : Effect of heating temperature on the reaction product of:- ■ PSE ( $10\mu\text{g/mL}$ ) with DNFB; ▲ PPA ( $12\mu\text{g/mL}$ ) with DNFB; ▼EPH ( $7\mu\text{g/mL}$ ) with DNFB.

#### (f) Effect of diluting solvent

The effect of diluting solvent was tested using different solvents *viz* water, methanol, acetone, acetoni-

trile, dimethylformamide, dimethylsulfoxide and isopropanol. Using water as diluting solvent gave the highest absorbance value in case of PSE and PPA while in case of EPH, the maximum absorbance intensity was produced by using methanol as diluting solvent. On the other hand, dilution with dimethylformamide and dimethylsulfoxide resulted in high back ground absorbance of the blank.

#### (g) Effect of surface active agent

Each of 1% (w/v) SDS, 1% (w/v) Cetrimide and 1% (w/v) tween were tested, no marked increase in the absorbance intensity was observed. Therefore, the reaction was carried out without using SAA.

#### (h) Effect of time on the stability of the formed adduct

The reaction product was found to be stable for at least 60 min at room temperature.

A summary for the optimization of the variables affecting the reaction of DNFB is given in (TABLE 1).

#### Validation of the proposed method

The validity of the proposed methods was tested regarding linearity, specificity, accuracy, repeatability and precision according to ICH Q2(R1) recommendations<sup>[34]</sup>.

#### (a) Linearity

The absorbance concentration plots were rectilinear over the ranges 2-20, 1-14 and 1-14  $\mu\text{g/mL}$  for PPA, EPH and PSE respectively, cited in TABLE 1. The pro-

TABLE 2 : Analytical performance data for the determination of the studied drugs by the proposed spectrophotometric method.

Parameter	PPA	EPH	PSE
Linearity range ( $\mu\text{g/mL}$ )	2-20	1-14	1-14
Limit of detection, LOD, ( $\mu\text{g/mL}$ )	0.20	0.13	0.20
Limit of quantification, LOQ ( $\mu\text{g/mL}$ )	0.60	0.40	0.59
Correlation coefficient (r)	0.9998	0.9999	0.9999
Slope	0.050	0.059	0.063
Intercept	0.044	0.005	0.011
S.D. of residuals, $S_{y/x}$	$5.04 \times 10^{-3}$	$3.00 \times 10^{-3}$	$5.63 \times 10^{-3}$
S.D. of intercept, $S_a$	$2.99 \times 10^{-3}$	$2.36 \times 10^{-3}$	$3.76 \times 10^{-3}$
S.D. of slope, $S_b$	$3.00 \times 10^{-4}$	$2.75 \times 10^{-4}$	$4.71 \times 10^{-4}$
Percentage relative standard deviation, % RSD	1.08	0.96	0.99
Percentage error, % Error	0.41	0.34	0.40
Molar absorptivity, $\epsilon \text{ L.mol}^{-1}.\text{cm}^{-1}$	$9.3 \times 10^3$	$1.43 \times 10^4$	$1.55 \times 10^4$

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posed method was evaluated for the accuracy as percent relative error (% Er) and the precision as percent relative standard deviation (% RSD) (TABLE 2).

Analysis of the data gave the following regression equations:

$$\text{For PPA } A = 0.0439 + 0.0496 C \quad (r = 0.9998)$$

$$\text{For EPHA } A = 0.0049 + 0.0588 C \quad (r = 0.9999)$$

$$\text{For PSE } A = 0.0109 + 0.0633 C \quad (r = 0.9999)$$

### (b) Limit of quantitation and limit of detection

The limits of quantitation (LOQ) and (LOD) were calculated according to ICH Q2(R1) recommendation<sup>[34]</sup>. The results are shown in (TABLE 2).

LOQ and LOD were calculated according to the following equations<sup>[34]</sup>:

$$\text{LOQ} = 10 S_a / b$$

$$\text{LOD} = 3.3 S_a / b$$

Where  $S_a$  is the standard deviation of the intercept of regression line, and  $b$  is the slope of the regression line.

### (c) Accuracy

To test the validity of the proposed methods they are applied to the determination of pure sample of PPA, EPH and PSE over the concentration ranges cited in (TABLE 3). The results obtained were in good agreement with those obtained using the comparison method<sup>[35-37]</sup>. Statistical analysis of the results using Student  $t$ -test and the variance ratio  $F$ -test<sup>[38]</sup> revealed no significance differences between the performance of the proposed and comparison methods regarding the accuracy and precision, respectively (TABLE 3). The spectrophotometric comparison method<sup>[35]</sup> for PPA depends on its determination via reaction with NBD-Cl and measuring the absorbance of the reaction product at 455 nm. The comparison method for EPH<sup>[36]</sup> was through Charge transfer reaction using  $p$ -chloranil and acetaldehyde measuring the absorbance of the reaction product at 680 nm. On the other hand, the comparison method for PSE was based on first derivative ratio derivative spectrophotometric method in 0.1 N HCl<sup>[37]</sup>.

The validity of the methods were proved by statistical evaluation of the regression line, using the standard deviation of the residuals ( $S_{y/x}$ ), the standard deviation of the intercept ( $S_a$ ) and standard deviation of the slope ( $S_b$ ). The results are abridged in TABLE 2. The small values of the figures indicate low scattering

**TABLE 3 : Application of the proposed and comparison methods to the determination of the studied drugs in pure forms.**

Parameter	Proposed method			Comparison method <sup>[35]</sup>
	Conc. taken ( $\mu\text{g/ml}$ )	Conc. found ( $\mu\text{g/ml}$ )	% Found <sup>a</sup>	% Found <sup>a</sup>
PPA	2.0	1.96	98.00	
	4.0	4.01	100.25	99.53
	6.0	5.97	99.50	102.01
	10.0	10.07	100.70	100.11
	12.0	12.13	101.08	
	18.0	17.81	98.94	
	20.0	20.07	100.35	
$\bar{X} \pm \text{S.D.}$		99.83 $\pm$ 1.08		100.55 $\pm$ 1.29
T		0.339 (2.306)		
F		1.426 (5.14)		

Parameter	proposed method			Comparison Method <sup>[36]</sup>
	Conc. taken ( $\mu\text{g/ml}$ )	Conc. found ( $\mu\text{g/ml}$ )	% Found <sup>a</sup>	% Found <sup>a</sup>
EPH	1.0	1.02	102.00	
	3.0	2.98	99.33	
	5.0	5.02	100.40	102.00
	7.0	6.94	99.14	99.98
	8.0	7.98	99.75	101.05
	9.0	9.10	101.11	
	10.0	9.98	99.80	
14.0	13.99	99.93		
$\bar{X} \pm \text{S.D.}$		100.18 $\pm$ 0.96		100.01 $\pm$ 1.01
t		1.264 (2.262)		
F		1.106 (4.74)		

Parameter	proposed method			Comparison Method <sup>[37]</sup>
	Conc. taken ( $\mu\text{g/ml}$ )	Conc. found ( $\mu\text{g/ml}$ )	% Found <sup>a</sup>	% Found <sup>a</sup>
PSE	1.0	1.01	101.00	102.00
	3.0	3.00	100.00	98.33
	4.0	3.94	98.50	98.01
	10.0	10.06	100.60	
	12.0	12.10	100.83	
	14.0	13.89	99.21	
$\bar{X} \pm \text{S.D.}$		100.02 $\pm$ 0.99		99.45 $\pm$ 2.22
t		0.597 (2.365)		
F		5.028 (5.79)		

<sup>a</sup> Each result is the average of three separate determinations.  
\*Values between parentheses are the tabulated  $t$  and  $F$  values, at  $p$  0.05<sup>[38]</sup>.

of the calibration points around the calibration line and high precision.

#### (d) Precision

##### a) Repeatability

The repeatability was performed through replicate analysis of three concentrations of the three drugs in pure form on three successive occasions. The results are presented in (TABLE 4).

##### b) Intermediate precision

Intermediate precision was tested by replicate analysis of the three drugs in pure form using the concentrations shown in (TABLE 4) for a period of 3 successive days. The results are summarized in (TABLE 4).

**TABLE 4 : Accuracy and precision data for the determination of the studied drugs by the proposed spectrophotometric method**

Parameter	Intra - day precision			Inter - day precision		
	Conc. taken ( $\mu\text{g/mL}$ )	Conc. found ( $\mu\text{g/mL}$ )	% Found	Conc. taken ( $\mu\text{g/mL}$ )	Conc. found ( $\mu\text{g/mL}$ )	% Found
PPA	4.00	4.03	100.75	4.00	3.97	99.25
	6.00	5.98	99.67	6.00	5.88	98.00
	10.00	10.00	100.00	10.00	9.80	98.00
$\bar{x} \pm \text{SD}$	100.14 $\pm$ 0.55			98.42 $\pm$ 0.72		
%RSD	0.52			0.73		
%Er	0.32			0.42		
EPH	7.00	6.98	99.71	7.00	7.07	101.00
	8.00	8.12	101.50	8.00	8.09	101.12
	10.00	10.02	100.20	10.00	9.15	101.67
$\bar{x} \pm \text{SD}$	100.47 $\pm$ 0.92			101.26 $\pm$ 0.36		
%RSD	0.91			0.35		
%Er	0.52			0.20		
PSE	6.00	6.12	102.00*	6.00	5.96	99.33
	10.00	10.23	102.30	10.00	10.07	100.70
	12.00	12.29	102.42	12.00	12.09	100.75
$\bar{x} \pm \text{SD}$	102.24 $\pm$ 0.22			100.26 $\pm$ 0.80		
%RSD	0.21			0.80		
%Er	0.12			0.46		

\* Each result is the average of three separate determinations.

##### (e) Robustness of the method

The robustness of the proposed method was examined by evaluating the influence of small, deliberate variations in the method variables on the absorbance of the reaction product. The tested variables included the pH  $8.0 \pm 0.5$  for EPH and PPA, and  $8.25 \pm 0.5$  for PSE,

the change in the volume of the buffer solution  $0.8 \pm 0.2$  mL for PSE,  $1 \pm 0.2$  mL and  $1 \pm 0.5$  mL for PPA. The change in the volume of DNFB (0.3% v/v),  $0.8 \pm 0.2$  mL for PSE,  $0.6 \pm 0.1$  mL for PPA and  $0.8 \pm 0.2$  for EPH, the change in the heating time,  $25 \pm 5$  min for PSE and EPH and  $20 \pm 5$  min for PPA. These minor changes that may take place during the experimental operation didn't affect the absorbance of the reaction product.

##### (f) Specificity

The specificity of the methods was investigated by observing any interference encountered from the common tablet excipients such as talc, starch, magnesium stearate and avicil didn't interfere with proposed method.

#### Pharmaceutical applications

The proposed method was successfully applied for determination of the studied drugs in their pharmaceutical preparations. The results obtained were statistically compared to those of comparison methods<sup>[35-37]</sup> using student's *t*-test for accuracy and the variance ratio *F*-test for precision as recorded in (TABLE 5). The experimental values of *t* and *F* did not exceed the theoretical one, indicating no significance difference in the performance of the two methods between the compared methods.

#### Biological analysis

Upon oral administration of 30 mg of PSE. It was found that up to about 90% of the dose is excreted unchanged in the urine in 24 hours with less than 1% as norpseudoephedrine (cathine)<sup>[39]</sup>. This initiated the present study. Such concentration lies within the working concentration range of the present study.

Therefore, the proposed method was applied for the determination of PSE in spiked human urine. The extraction adopted by Avois *et al*<sup>[32]</sup> was applied here. (TABLE 6).

#### Molar ratio and mechanism of the reaction

The stoichiometry of the reaction was studied adopting the limiting logarithmic method<sup>[40]</sup>. Plots of log absorbance versus log [DNFB] and log [Drug] gave two straight lines, the slopes of which were 0.57/0.78 for DNFB/PPA, 0.41/0.37 for DNFB/EPH and 0.72/0.94 for DNFB/PSE (Figure 8). Hence, It was concluded that the reaction proceeds in the ratio of 1:1,

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**TABLE 5 :** Application of the proposed and comparison methods to the determination of the studied drugs in dosage form

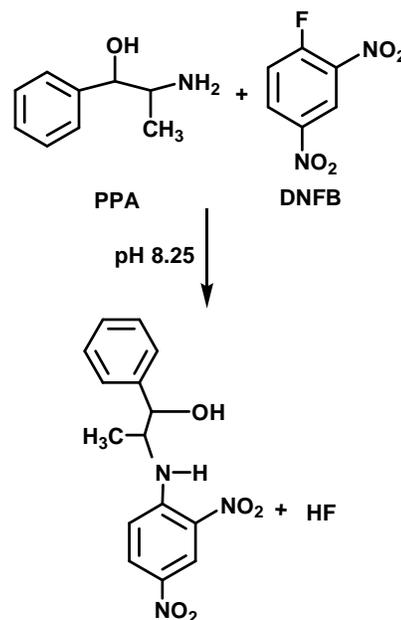
Parameter	The proposed method		Comparison method <sup>[35]</sup>
	Conc. taken (µg/ml)	% Found <sup>a</sup>	% Found
Contafly tablets <sup>c</sup>	8.00	99.92	99.77 <sup>[35]</sup>
(24 mg of PPA, 3 mg of chlorpheniramine maleate and 200 mg of propyphenazone/ tablet)	12.00	99.47	99.36
	14.00	99.87	100.60
$\bar{x} \pm S.D.$		99.75 <sup>d</sup> ± 0.25	99.91 ± 0.63
T		0.588 (2.776)*	
F		6.350 (19.00)*	
Ephedrine Ampoule <sup>d</sup>	7.00	100.57	100.73 <sup>[36]</sup>
(30 mg of EPH / Ampoule)	8.00	100.87	101.44
	10.00	101.91	102.36
$\bar{x} \pm S.D.$		101.12 <sup>e</sup> ± 0.70	100.51 ± 0.82
T		0.647 (2.776)*	
F		1.372 (19.00)*	
Allercet <sup>®</sup> capsule <sup>b</sup>	6.00	100.18	100.98 <sup>[37]</sup>
(30 mg PSE and 10 mg cetirizine HCl/ capsule)	10.00	101.40	99.56
	12.00	102.04	102.15
$\bar{x} \pm S.D.$		101.21 <sup>f</sup> ± 0.94	100.90 ± 1.30
T		0.334 (2.776)*	
F		1.913 (19.00)*	

<sup>a</sup>Each result is the average of three separate determinations; <sup>\*</sup>Values between parentheses are the tabulated *t* and *F* values, at *p* 0.05<sup>[38]</sup>; <sup>c</sup>Contafly tablets, batch # 061342, each tablet labeled to contain 24 mg of phenylpropranolamine HCl, 3 mg of chlorpheniramine maleate and 200 mg of propyphenazone, product of Egyptian Int..Pharmaceutical Industries CO. E.P.I.CO, 10<sup>th</sup> of Ramadan City, Egypt; <sup>b</sup>Ephedrine ampoule:-batch # 11 each ampoule (1mL) labeled to contain 30 mg of ephedrine HCl, A product of Chemical industries Development(CID)-Giza-A.R.E; <sup>c</sup>Allercet capsule, batch # 820304, each capsule labeled to contain 30 mg pseudoephedrine HCl and 10 mg cetirizine HCl, product of Global Napi Pharmaceuticals, 6<sup>th</sup> of October City-Giza-Egypt; <sup>d</sup>PPA content found in Contafly tablet was found to be 23.94 mg/ tablet; <sup>e</sup>EPH content found in Ephedrine ampoule was found to be 30.34 mg/ ampoule; <sup>f</sup>PSE content found in Allercet<sup>®</sup> capsule was found to be 30.36 mg/ capsule.

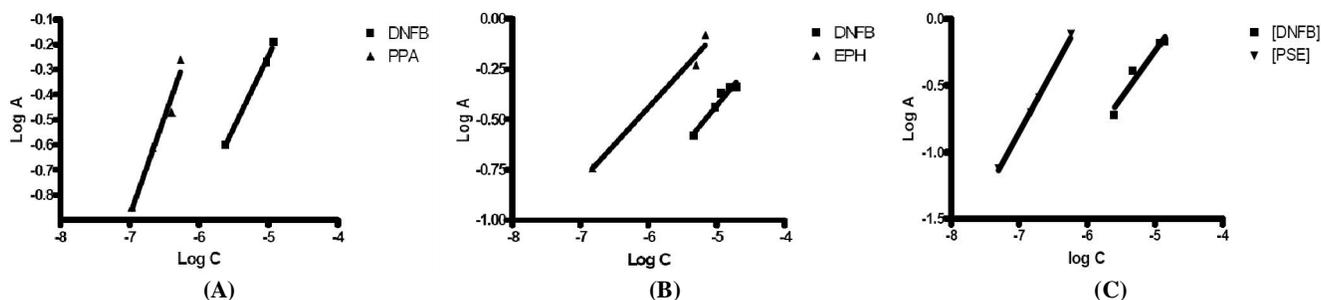
**TABLE 6 :** Application of the proposed method to the determination of PSE in spiked urine

Parameter	Amount added (µg/mL)	Amount found (µg/mL)	Recovery %
Spiked urine sample	2.00	2.15	107.50
	4.00	4.28	107.00
	6.00	6.60	110.00
Mean found, $\bar{x}$		108.17	
± S.D		1.60	
% RSD		1.48	
% Error		0.85	

confirming that one molecule of the drug condenses with one molecule of DNFB. Based on the observed molar ratio and by analogy to previous reports<sup>[28]</sup>, the mechanism of the reaction is postulated to proceed as shown in the following Scheme 1.



**Scheme 1 :** Proposed reaction pathway between DNFB and PPA as a model example of the three drugs under the described reaction conditions.



**Figure 8 :** Limiting logarithmic plots for the molar reactivity of: (A) PPA with DNFB; (B) EPH with DNFB; (C) PSE with DNFB.

## CONCLUSION

The proposed spectrophotometric method provided simple, reproducible and accurate method for determination of PSE, PPA and EPH. The established method was validated and applied to determination of the studied drugs in their dosage forms without interference from common excipients. The developed method showed to be a simple and suitable technique to quantify these drugs and might be employed for quality control analysis.

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