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Simultaneous determination of naproxen and diphenhydramine by reversed phase liquid chromatography and derivative spectrophotometry

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ABSTRACT

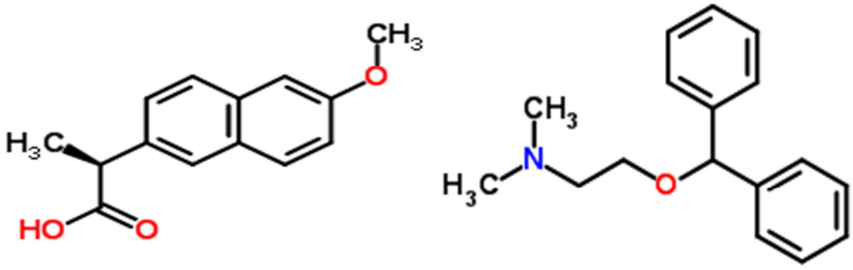
Highly sensitive, simple and accurate reversed phase liquid chromatographic and first derivative spectrophotometric methods were developed for determination of the non-steroidal anti-inflammatory drugnaproxen and the antihistaminic diphenhydramine in their binary mixtures. The HPLC method involves separation of naproxen and diphenhydramine on XBridge™C18 reversed phase (4.6×150 mm, particle size 5µm) column using a mobile phase consists of ethanol: phosphate buffer; pH 3in a ratio of 60:40 (v/v). The flow rate was 1 mL.min^{-1}with ultraviolet detection at 216 nm. The calibration graphs are linear from 0.5 to 100 µg.mL^{-1}for naproxen and from 2 to 100 µg.mL^{-1}for diphenhydramine .The mean % recoveries were found to be 100.910±0.358 and 99.863±1.14 for naproxen and diphenhydramine, respectively using this HPLC method. Thespectrophotometric method was based on measuring ^{1}D at 243.8 nm for determination of naproxen and ^{1}D at 230 nm for determination of diphenhydramine. Linearity ranges were found to be 0.5–3 µg.mL^{-1} and 2.5-25 µg.mL^{-1} for naproxen and diphenhydramine, respectively. The mean % recoveries were found to be 100.759±1.275 and 101.002±0.755 for naproxen and diphenhydramine, respectively using the proposed first derivative method.The developed methods were successfully applied for the determination of naproxen and diphenhydramine in laboratory prepared mixtures containing all possible excipients present in the tablet dosage form.

Keywords: Naproxen; Diphenhydramine; Derivative spectrophotometry; Reversed Phase liquid chromatography; Laboratory prepared mixtures.

INTRODUCTION

Naproxen ((+)-2-(6-Methoxy-2-naphthyl) propionic acid) [1, 2]is a non-steroidal anti-inflammatory drug with analgesic and antipyretic properties. Anti-inflammatory effects of naproxen are generally thought to be related to its inhibition of cyclooxygenase and consequent decrease in prostaglandin concentrations in various fluids and tissues[3].

Diphenhydramine (2-Diphenylmethoxy-N,N-dimethylethanamine)[4, 5] is a first-generationantihistaminicdrug possessing anticholinergic, antitussive, antiemetic, and sedative properties and is mainly used to treat allergies. Diphenhydramine is also used for the treatment of motion sickness and extra pyramidal symptoms[6]. Combination of naproxen and diphenhydramine is used for relieving occasional sleeplessness when associated with minor pains. The chemical structures of naproxen and diphenhydramine are shown in Figure 1.



Naproxen Diphenhydramine

Fig.1: Chemicalstructures of naproxen and diphenhydramine

Several methods for determination of naproxen individually or in combination with other drugs have been reported, these methods include: spectrophotometry [7-9],UHPLC [10], HPLC [11], fluorimetry [12], and differential pulse voltammetry at a platinum electrode [13]. Naproxen was also determined simultaneously with different drugs such as paracetamol [14], esomeprazole [15], ciprofloxacin [16], febuxostat [17], sumatriptansuccinate[18]and pseudoephedrine hydrochloride [19].

Diphenhydramine was determined by several methods including; spectrophotometry [20], HPLC [21]. Diphenhydramine was also determined simultaneously with different drugs such as; ondansteron [22], ibuprofen[23] and paracetamol [24]. To the best of our knowledge, there is no reported analytical method for simultaneous determination of naproxen and diphenhydramine in their mixture.

The aim of this work is to develop two simple, sensitive and validated methods for the determination of naproxen and diphenhydramine using HPLC and derivative spectrophotometry. Application of derivative spectrophotometry offers a powerful tool for quantitative analysis of multi-component mixtures. It shows greater selectivity than normal spectrophotometry as it decreases spectral overlap and allows better resolution [25, 26]. HPLC method allows greater sensitivity and rapid analysis time.

MATERIALS AND METHODS

1.1. MATERIALS

Naproxen (99.6%), diphenhydramine (99.9%), povidone, magnesium stearate, talc and microcrystalline cellulose (avicel) were kindly donated by Sigma Company for Pharmaceutical Industries (Quesna, Menofia, Egypt).Methanol (99.8%) analytical gradeand Ortho phosphoric acidwere purchased from Sigma-Aldrich (St. Louis, MO, USA). EthanolHPLC grade and Potassium dihydrogenorthophosphate were purchased from Fisher Scientific (Pittsburgh, PA, USA).

1.2. Spectrophotometric equipment and conditions

Spectrophotometric measurements were carried out using Schimadzu (UV-1800) UV-VIS double beam spectrophotometer equipped with 1 cm quartz cells and connected to a personal computer loaded with UV-Probe 2.33 software. Absorption spectra were recorded on wavelength range 210-350 nm.

1.3. Chromatographic system and conditions

HPLC instrument: DionexUltiMate 3000 RS system was used, (Thermo Scientific™, Dionex™, Sunnyvale, CA, USA), equipped with Quaternary RS pump, RS auto-sampler injector, Thermostated RS Column Compartment and RS Diode array detector (DAD). The instrument was connected to a Dell compatible PC, bundled with Chromeleon® 7.1 Chromatography Data System software.

The Separation was carried out on XBridge^{TM}C18 reversed phase (4.6×150 mm, particle size 5µm) columnpurchased from (Waters Corporation, Milford, MA, USA). A mobile phase consisting of ethanol: potassium dihydrogen orthophosphate buffer; pH 3in a ratio of 60:40 (v/v) was prepared daily, filtered, sonicated and delivered isocratically at a flow rate of 1 mL.min^{-1}. The UV detector was programmed at a wavelength of 216 nm. The injection volume was 20µL.

The pH measurements were made with HANNA pH 211 Microprocessor pH-meter with double junction glass electrode.

1.4. Preparation of calibration curves

1.4.1. Derivative spectrophotometric method

2.4.1.1. Naproxen. A stock standard solution of naproxen (1 mg.mL^{-1}) was prepared by transferring accurately weighed 25 mg of naproxen powder into 25 mL volumetric flask, dissolved in methanol; and the volume was completed with the same solvent.

Working standard solution of naproxen (10 µg.mL^{-1}) was prepared by diluting 1 mL of naproxen stock standard solution to 100 mL with the same solvent.

A set of laboratory prepared solutions of naproxen were prepared by transferring different aliquots of naproxen working standard solution (10 µg.mL^{-1}) into 10 mL volumetric flask and diluting to volume with methanol to obtain solutions of naproxen ranging from 0.5 to 3 µg.mL^{-1}.

2.4.1.2. Diphenhydramine. A stock standard solution of diphenhydramine (1 mg.mL^{-1}) was prepared by transferring accurately weighed 25 mg of diphenhydramine powder into 25 mL volumetric flask, dissolved in methanol; and the volume was completed with the same solvent.

Working standard solution of diphenhydramine (100 µg.mL^{-1}) was prepared by diluting 1 mL of diphenhydramine stock standard solution to 10 mL with the same solvent.

Aset of laboratory prepared solutions of diphenhydramine were prepared by transferring different aliquots of diphenhydramine working standard solution (100 µg.mL^{-1}) into 10 mL volumetric flask and diluting to volume with methanol to obtain solutions of diphenhydramine ranging from 2.5 to 25 µg.mL^{-1}. These solutions were used to obtain different concentrations of both drugs covering their linearity range.

UV spectra were scanned for these solutions using methanol as blank. Then first order derivative spectra ^{1}D were calculated at $\Delta\lambda$ = 8 nm. The zero-crossing points of ^{1}D spectra of naproxen and diphenhydramine were assigned. Naproxen was determined by measuring ^{1}D amplitudes at 243.8 nm; which is the zero crossing for diphenhydramine. Similarly, diphenhydramine was determined by measuring ^{1}D amplitudes at 230 nm; which is the zero crossing for naproxen. Calibration curves were constructed by plotting ^{1}Damplitudes at 243.8 and 230 nm against corresponding concentrations for naproxen and diphenhydramine, respectively.

1.4.2. HPLC method

2.4.2.1. Naproxen. A stock standard solution of naproxen (1 mg.mL^{-1}) was prepared by transferring accurately weighed 25 mg of naproxen powder into 25 mL volumetric flask, dissolved in the mobile phase; and the volume was completed with the mobile phase.

Working standard solution of naproxen (100 µg.mL^{-1}) was prepared by diluting 5 mL of naproxen stock standard solution to 50 mL with the mobile phase.

A set of laboratory prepared solutions of naproxen were prepared by transferring different aliquots of naproxen working standard solution (100 µg.mL^{-1}) into 10 mL volumetric flask and diluting to volume with the mobile phase to obtain solutions of naproxen ranging from 0.5 to 100 µg.mL^{-1}.

2.4.2.2. Diphenhydramine. A stock standard solution of diphenhydramine (1 mg.mL^{-1}) was prepared by transferring accurately weighed 25 mg of diphenhydramine powder into 25 mL volumetric flask, dissolved in the mobile phase; and the volume was completed with the mobile phase.

Working standard solution of diphenhydramine (100 µg.mL^{-1}) was prepared by diluting 5 mL of diphenhydramine stock standard solution to 50 mL with the mobile phase.

Aset of laboratory prepared solutions of diphenhydramine were prepared by transferring different aliquots of diphenhydramine working standard solution (100 µg.mL^{-1}) into 10 mL volumetric flask and diluting to volume with the mobile phase to obtain solutions of diphenhydramine ranging from 2 to 100 µg.mL^{-1}.

Separately inject equal volumes (20 µl) of different solutions into the chromatograph, record the chromatograms and calculate the peak area of each solution andconstruct the calibration curves by plotting the peak area of each solution versus the corresponding working concentration. Then the regression equations were calculated.

To study the accuracy and precision of the proposed methods, recovery experiments were carried out on different laboratory prepared mixtures of diphenhydramine and naproxen at different ratios including the ratio (1:8.8) as present in the dosage form.

The proposed methods were applied for the analysis of laboratory prepared mixtures of each drug in presence of common tablet excipients as magnesium stearate, talc, povidone and microcrystalline cellulose (avicel) to assure the specificity of the method.

1.5. Preparation of Laboratory Prepared Mixtures

1.5.1.Derivative spectrophotometric method

The dosage form is not available in local market, so a laboratory prepared mixture simulated to this dosage form was preparedby mixing 220 mg naproxen, 25 mg diphenhydramine, and the following excipients: 7 mg povidone, 87.5 mg microcrystalline cellulose (avicel), 3.5 mg talc, and 7 mg magnesium stearate.

The Laboratory prepared mixture was transferred to a 100 mL volumetric flask, dissolved in 50 mL methanol, and sonicated for 15 minutes. Then the solution was made up to the required volume using methanol. The solution was filtered and the first 10 mL of the filtrate was discarded. An aliquot equivalent to 1 mL of the filtrate was transferred to a 100 mL volumetric flaskand made up to final volume with methanol. An aliquot equivalent to 1 mL of this solution was transferred to a 10 mL volumetric flask, spiked with 1 mL from standard solution of diphenhydramine (100 $\mu g.mL^{-1}$) and made up to final volume with methanol to obtain a solution containing; 2.2 $\mu g.mL^{-1}$ and 10.25 $\mu g.mL^{-1}$ of naproxen and diphenhydramine, respectively. The procedures were carried out as mentioned in the section (2.4.1.) and then concentrations of both drugs were calculated from the corresponding regression equations.

1.5.2. HPLC method

A laboratory prepared mixture was prepared by mixing 220 mg naproxen, 25 mg diphenhydramine, and the following excipients: 7 mg povidone, 87.5 mg microcrystalline cellulose (avicel), 3.5 mg talc, and 7 mg magnesium stearate.

The Laboratory prepared mixture was transferred to a 100 mL volumetric flask, dissolved in 50 mL of the mobile phase, and sonicated for 15 minutes. Then the solution was made up to the required volume using the mobile phase. The solution was filtered and the first 10 mL of the filtrate was discarded. An aliquot equivalent to 2 mL of the filtrate was transferred to a 100 mL volumetric flask and made up to final volume with the mobile phase to obtain a solution containing; 44$\mu g.mL^{-1}$ and 5$\mu g.mL^{-1}$ of naproxen and diphenhydramine, respectively. The procedures were carried out as mentioned in the section (2.4.2.) and then concentrations of both drugs were calculated from the corresponding regression equations.

RESULTS AND DISCUSSION

Analytical methods for the determination of binary mixture without previous separation are of interest to quality control (QC) labs and national regulatory authorities (NRA) around the world[26].

The present work describes the development of simple as well as highly sensitive techniques for the simultaneous determination of naproxen and diphenhydramine using the 1st derivative spectrophotometric technique and the HPLC technique. The common availability of the instrumentation, the simplicity of procedures, speed, precision and accuracy of the technique make spectrophotometric methods still attractive [27]. The high sensitivity and rapidness of HPLC technique make it suitable for quality control analysis.

3.1. Method Development

3.1.1.Derivative spectrophotometric method

The UV absorption spectra for naproxen and diphenhydramine show severe overlap (Figure 2). This difficulty can be solved using 1st derivative spectroscopy via studying of zero crossing point of both drugs. The first derivative spectrum of naproxen exhibits absorption minima at 243.8 nm while diphenhydramine reads zero. Diphenhydramine exhibits absorption minima at 230 nm while naproxen reads zero (Figure 3).

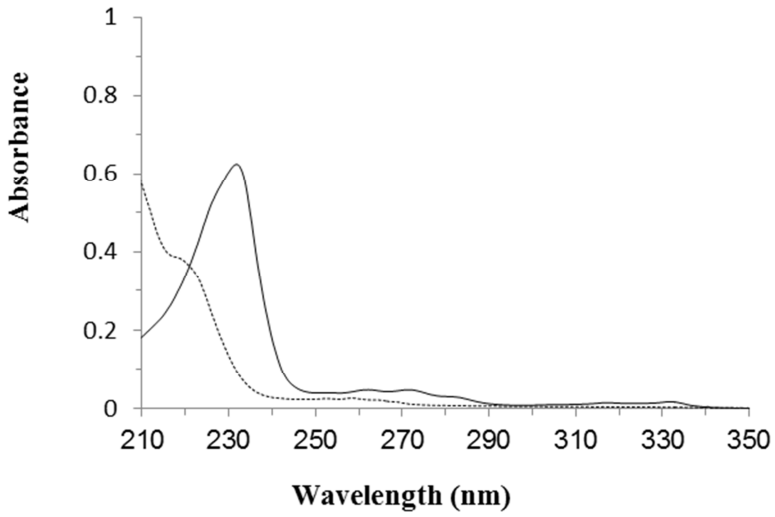


Fig.2: Zero-order UV spectra of 2 µg.mL^{-1} naproxen (____) and 10 µg.mL^{-1} diphenhydramine (……) in methanol

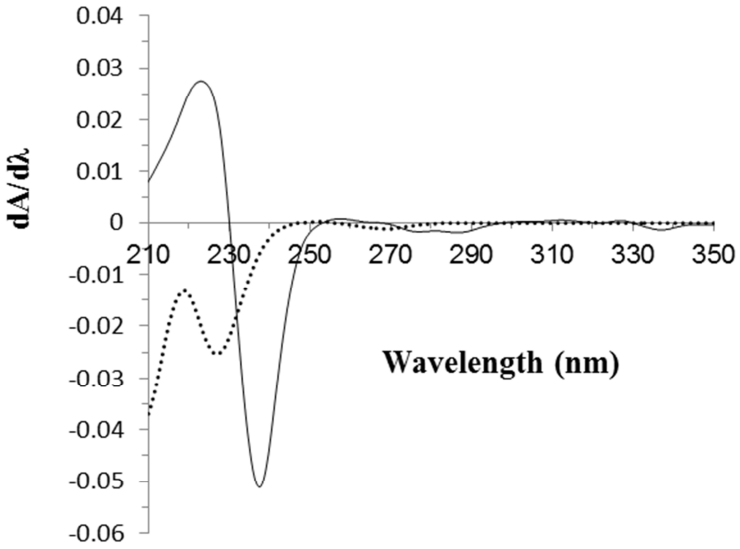


Figure 3: First-order derivative spectra of 2 µg.mL^{-1} naproxen (____) and 10 µg.mL^{-1} diphenhydramine (……) in methanol

3.1.2. HPLC method

Many factors affect theseparation and resolution of both drugs in theirmixtures. These factors include type of organic modifier, organic to aqueous ratio, pH,temperature, flow rate and injection volume.

These factors were studied separately to optimizethe chromatographic separation considering theresolution and the system suitability parameters. Different trials were carried out and optimum conditions were selected to give higher resolution and higher symmetry (Figure 4). All system suitability parameters are shown in Table 1. It involves the use of a mobile phase consists of ethanol: potassium dihydrogen orthophosphate buffer; pH 3in a ratio of 60:40 (v/v). The flow rate was 1 mL.min^{-1}with ultraviolet detection at 216 nm.

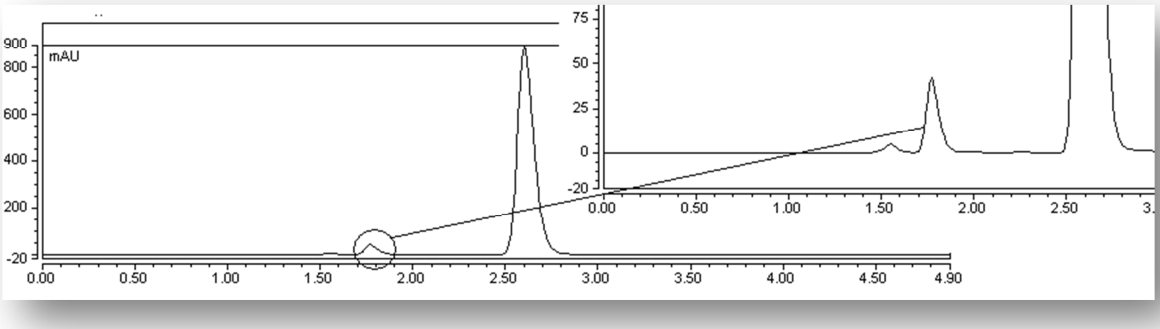


Figure 4: Chromatogram of diphenhydramine and naproxen at a ratio 1:9 as in dosage form using mobile phase consists of ethanol: potassium dihydrogen orthophosphate buffer; pH 3in a ratio of 60:40 (v/v). The flow rate was 1 mL.min^{-1}with UV detection at 216 nm, temp.= 25°C and inj. vol.= 20µL

Table 1: Results of system suitability tests for determination of naproxen and diphenhydramine by the proposed HPLC method

Parameters	Diphenhydramine	Naproxen
Retention Times (tR (min))	1.775±0.006	2.609±0.007
Capacity factor (k)	1.6	2.85
Resolution (Rs)	6.1	
Theoretical plates (N)	3423	4288
HETP (mm)*	0.0438	0.0349
Asymmetry factor	1.25	1.22

** HETP: height equivalent to a theoretical plate.*

3.2. Method Validation

The developed methods were validated according to the ICH guidelines [28, 29]. The following validation parameters were addressed:

3.2.1. Linearity

Calibration curves were obtained for naproxen and diphenhydramine in the linearity ranges 0.5–3 µg.mL^{-1} and 2.5-25 µg.mL^{-1} for naproxen and diphenhydramine, respectively using derivative spectrophotometric method (Figures 5-6). The HPLC method was found to be linear over a concentration range of 0.5 to 100 µg.mL^{-1}for naproxen and from 2 to 100 µg.mL^{-1}for diphenhydramine (Figures 7-8). The quantitative statistical parameters for the determination of naproxen and diphenhydramine are summarized in Table 2 for the derivative spectrophotometric method and in Table 3 for the HPLC method.The high values of correlation coefficients (r) with negligible intercepts indicate good linearity of the calibration curves.

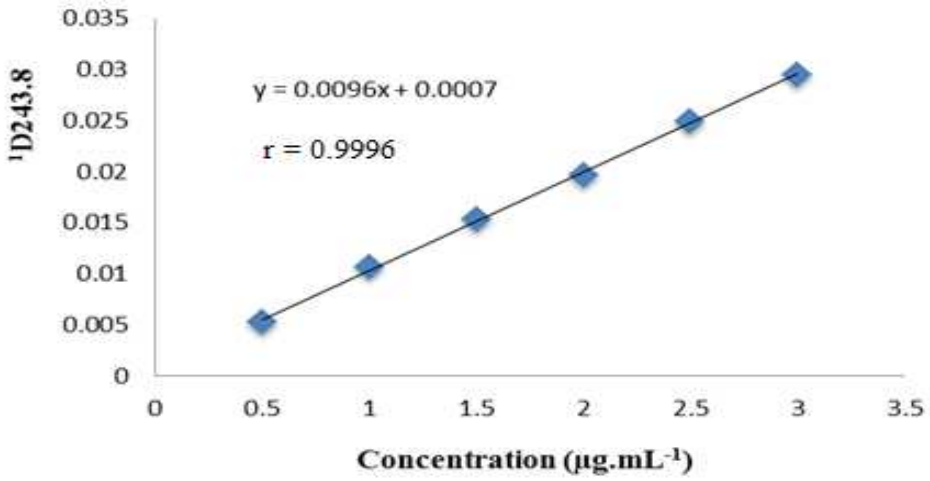


Fig.5: Calibration curve of naproxen by the proposed first derivative spectrophotometric method at 243.8 nm

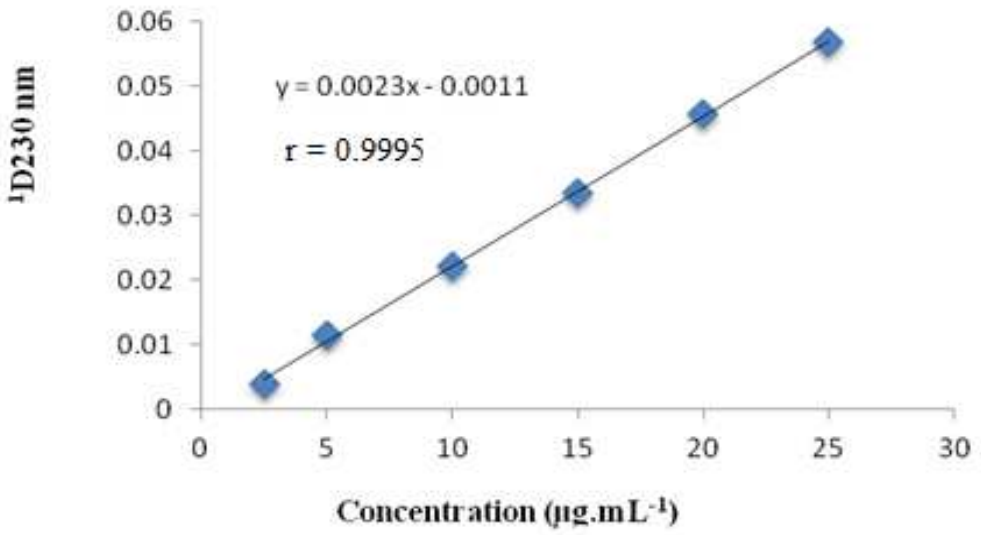


Fig.6: Calibration curve of diphenhydramine by the proposed first derivative spectrophotometric method at 230 nm

Table 2: Quantitative parameters for the determination of naproxen and diphenhydramine by the proposed spectrophotometric method

Drug	Linearity µg. mL^{-1}	λ(nm)	r	a (10^{-3})	b (10^{-3})	$S_{y/x}$ (10^{-4})	S_a (10^{-4})	S_b (10^{-5})	DL µg.mL^{-1}	QL µg.mL^{-1}
Naproxen	0.5-3	243.8	0.9996	0.713	9.611	2.71	2.52	12.9	0.053	0.159
Diphenhydramine	2.5-25	230	0.9995	-1.09	2.321	6.94	5.4	3.56	0.025	0.753

***N.B.**: r: correlation coefficient, a: intercept, b: slope, Sy/x: residual standard deviation of the regression line, Sa: standard error of intercept, Sb: standard error of slope, DL: detection limit (calculated), QL: quantitation limit (calculated).*

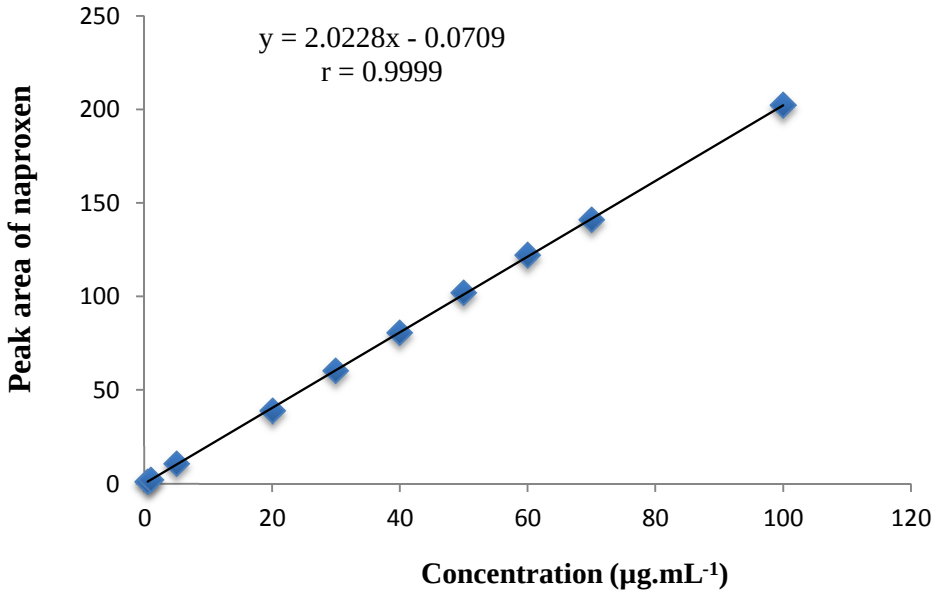


Fig.7: Calibration curve of naproxen by the proposed HPLC method

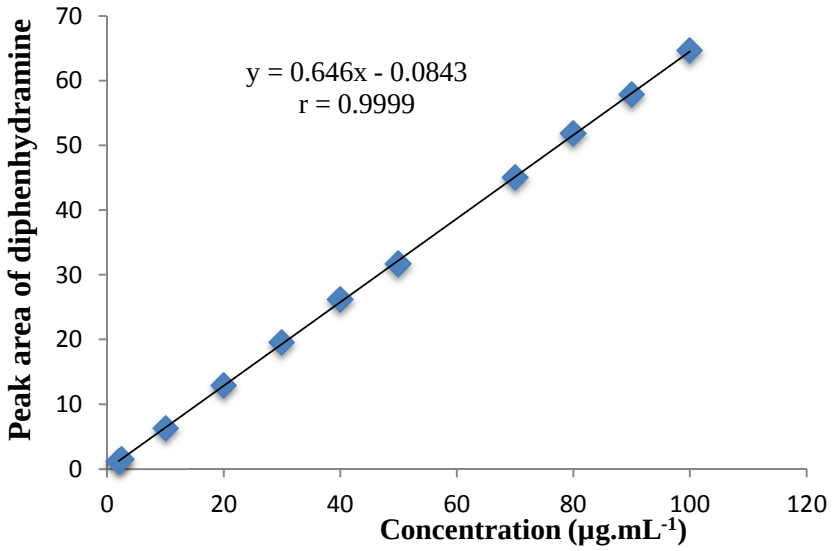


Fig.8: Calibration curve of diphenhydramine by the proposed HPLC method

Table 3: Quantitative parameters for the determination of naproxen and diphenhydramine by the proposed HPLC method

Drug	Linearity µg. mL^{-1}	r	A (10^{-2})	b	$S_{y/x}$	S_a	S_b (10^{-3})	DL µg.mL^{-1} (10^{-2})	QL µg.mL^{-1} (10^{-2})
Naproxen	0.5-100	0.9999	-7.092	2.023	0.715	0.354	7.237	4.996	15.139
Diphenhydramine	2-100	0.9999	-8.4	0.646	0.276	0.138	2.448	1.303	3.949

***N.B.**: r: correlation coefficient, a: intercept, b: slope, Sy/x: residual standard deviation of the regression line, Sa: standard error of intercept, Sb: standard error of slope, DL: detection limit (calculated), QL: quantitation limit (calculated).*

3.2.2. Detection and Quantitation Limits

Detection (DL) and quantitation (QL) limits were calculated depending on standard deviation ofthe blank response and the slope. They may be expressed as: DL = 3.3 σ / S and QL = 10 σ / S where; "σ" is the standard deviation of blank response and "S" is the slope of the calibration curve [28, 29]. Calculated DL and QL are shown in Table 2 for the proposed derivative spectrophotometric method and in Table 3 for the proposed HPLC method.

3.2.3. Accuracy

The accuracy of the proposed methods was evaluated by analyzing three different laboratory prepared mixtures of naproxen and diphenhydramine within the linearity range three times. Accuracy was expressed as the % recovery ±S.D as shown in Table 4 for the derivative spectrophotometric method and in Table 5 for the HPLC method.

Table 4: Evaluation of the accuracy for the determination of naproxen and diphenhydramine by the proposed spectrophotometric method according to ICH guidelines

Drug	Concentration taken µg.mL^{-1}	Concentration found µg.mL^{-1}			Mean concentration found* µg.mL^{-1}	Recovery %	Mean Recovery % ± S.D.
Naproxen	**0.8**	0.813	0.823	0.813	0.816	102	100.759±1.275
	1.4	1.385	1.417	1.375	1.392	99.452	
	2.6	2.635	2.625	2.621	2.621	100.826	
Diphenhydramine	**6**	6.174	5.996	6.087	6.086	101.428	101.002±0.755
	12	12.217	12	12.304	12.174	101.447	
	22	22	21.869	22.217	22.029	100.13	

***N.B.**: * n = 3.*

Table 5: Evaluation of the accuracy for the determination of naproxen and diphenhydramine by the proposed HPLC method according to ICH guidelines

Drug	Concentration taken µg.mL^{-1}	Concentration found µg.mL^{-1}			Mean concentration found* µg.mL^{-1}	Recovery %	Mean Recovery % ± S.D.
Naproxen	**10**	10.030	10.075	10.060	10.055	100.550	100.910±0.358
	45	45.460	45.490	45.760	45.570	101.267	
	80	80.520	80.810	80.860	80.730	100.913	
Diphenhydramine	**5**	4.990	4.940	5.040	4.990	99.800	99.863±1.14
	15	14.930	14.770	14.740	14.813	98.756	
	60	60.660	60.420	60.780	60.620	101.033	

***N.B.**: * n = 3.*

3.2.4. Precision

Precision was carried out by analyzing three different laboratory prepared mixture "three replicates" of naproxen and diphenhydramine within the linearity range on the same day (intraday precision) and on three consecutive days (inter-day precision). Standard deviation (S.D.) and relative standard deviation (R.S.D. %) values of the results obtained were calculated as shown in Table 6for the derivative spectrophotometric method and in Table 7 for the HPLC method.

Table 6: Evaluation of the intra-day and inter-day precision for the determination of naproxen and diphenhydramine by the proposed spectrophotometric method according to ICH guidelines

	Concentration taken µg.mL^{-1}	Intra-day			Inter-day		
		Mean concentration found* µg.mL^{-1}	S.D	%R.S.D.	Mean concentration found* µg.mL^{-1}	S.D	%R.S.D.
Naproxen	**0.8**	0.816	0.006	0.743	0.815	0.002	0.213
	1.4	1.392	0.022	1.555	1.403	0.012	0.891
	2.6	2.621	0.016	0.610	2.619	0.002	0.066
Diphenhydramine	**6**	6.086	0.089	1.463	6.076	0.088	1.447
	12	12.174	0.067	0.548	12.087	0.138	1.146
	22	22.029	0.176	0.798	22.231	0.181	0.812

***N.B.**: * n = 3.*

Table 7: Evaluation of the intra-day and inter-day precision for the determination of naproxen and diphenhydramine by the proposed HPLC method according to ICH guidelines

Drug	Concentration taken µg.mL^{-1}	Intra-day			Inter-day		
		Mean concentration found* µg.mL^{-1}	S.D	%R.S.D.	Mean concentration found* µg.mL^{-1}	S.D	%R.S.D.
Naproxen	**10**	10.055	0.023	0.228	10.027	0.045	0.452
	45	45.570	0.165	0.363	45.736	0.152	0.333
	80	80.730	0.184	0.227	80.508	0.292	0.363
Diphenhydramine	**5**	4.990	0.050	1.002	5.016	0.025	0.499
	15	14.813	0.102	0.690	14.807	0.034	0.229
	60	60.620	0.183	0.302	60.957	0.292	0.478

***N.B.**: * n = 3.*

3.2.5. Specificity

According to ICH guidelines [28, 29] specificity is “the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.” For testing specificity; the % recovery of naproxen and diphenhydramine was determined in mixtures containing both drugs with the possible excipients present in dosage form by the proposed spectrophotometric method as shown in Table 8 and in Table 9 for the proposed HPLC method. The Results indicate that there is no interference from dosage form excipients as shown in Figure 9 for the proposed spectrophotometric method and in Figure 10 for the proposed HPLC method.

Table 8: Recovery data of naproxen and diphenhydramine from laboratory prepared mixture by the proposed spectrophotometric method

Drug	Concentration taken µg.mL^{-1}	Concentration found µg.mL^{-1}						Mean concentration found* µg.mL^{-1}	Mean Recovery % ± S.D.
Naproxen	2.2	2.208	2.167	2.229	2.250	2.333	2.239	2.238	101.712± 0.050
Diphenhydramine	10.25	10.261	10.521	10.174	10.217	10.304	10.130	10.268	100.174± 0.126

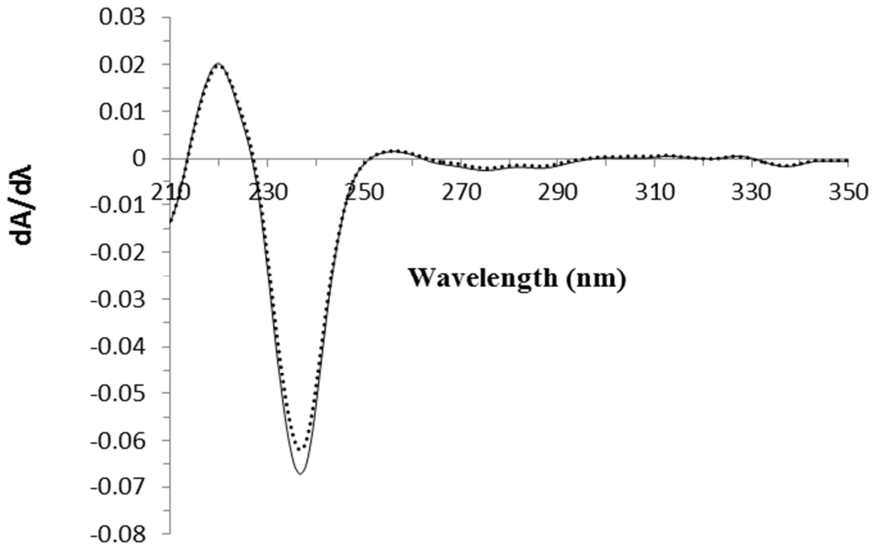


Fig.9: First-order derivative spectra of laboratory prepared mixture containing excipients (____), and a mixture of naproxen and diphenhydramine without excipients (……) in methanol

Table 9: Recovery data of naproxen and diphenhydramine from laboratory prepared mixture by the proposed HPLC method

Drug	Concentration taken µg.mL^{-1}	Concentration found µg.mL^{-1}						Mean concentration found* µg.mL^{-1}	Mean Recovery % ± S.D.
Naproxen	44	44.528	44.033	44.379	44.755	44.676	44.429	44.470	101.068± 0.592
Diphenhydramine	5	4.944	4.960	5.053	5.078	5.084	5.022	5.037	100.509± 0.051

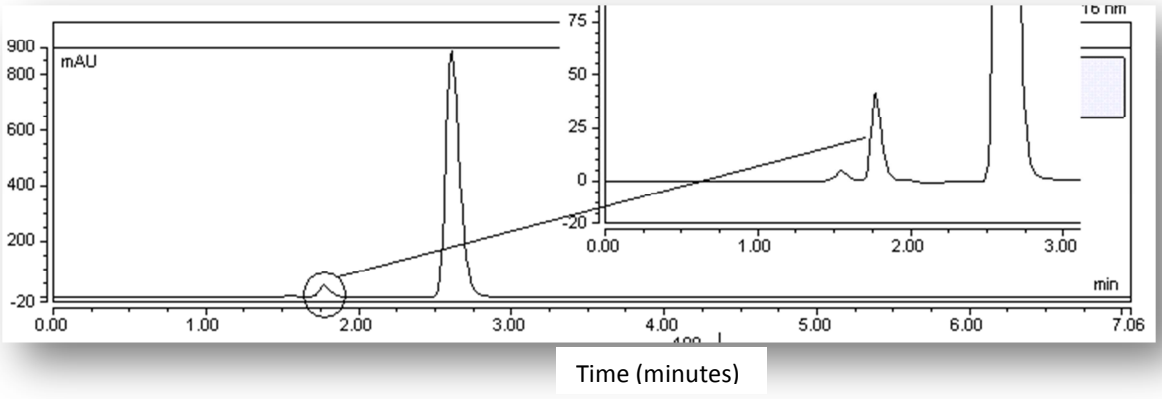


Fig.10: Chromatogram of laboratory prepared mixture containing excipients using mobile phase consists of ethanol: potassium dihydrogen orthophosphate buffer; pH 3in a ratio of 60:40 (v/v). The flow rate was 1 mL.min^{-1}with UV detection at 216 nm, temp.= 25°C and inj. vol.= 20µL

3.2.6. Robustness

Robustness of the proposed HPLC method was evaluated by analyzing laboratory prepared mixture with intentional slight variation of the selectedparameters. The RSD % of the recovery obtained by analyzing the same sample after introducing small deliberate changes in the method parameters was calculated. The intentional slight variation that were applied to the method parameters include: pH of the aqueous component of the mobile phase (3 ± 0.2), the % organic modifier (60 ± 2 %), the temperature(25 ± 2 °C).the low value of the RSD % of the recoveries % indicated the robustness of the method. The results were shown in Table 10.

Table 10: Robustness results for the proposed HPLC method

Drug	Parameters	Modification	Recovery %	Mean Recovery %	S.D	%R.S.D.
Naproxen	**pH**	2.8	101.744	101.092	0.583	0.577
		3	100.910			
		3.2	100.621			
	Temperature (°C)	23	101.690	101.179	0.443	0.438
		25	100.910			
		27	100.937			
	Mobile phase Ratio	58:42	101.670	101.024	0.597	0.591
		60:40	100.910			
		62:38	100.492			
Diphenhydramine	**pH**	2.8	100.693	100.703	0.846	0.840
		3	99.863			
		3.2	101.555			
	Temperature (°C)	23	101.287	100.613	0.715	0.711
		25	99.863			
		27	100.688			
	Mobile phase Ratio	58:42	101.309	100.769	0.789	0.784
		60:40	99.863			
		62:38	101.137			

***N.B.**: * n = 3.*

CONCLUSION

This work describes two simple methods for simultaneous determination of both drugs without prior separation with the advantage of highsensitivity of derivative spectrophotometry and the speed of HPLC method; the analysis time is short which about 3 minutes. Results demonstrated the lack of interference from dosage form excipients and the usefulness of the methods. The methods are simple, sensitive, precise, accurate, inexpensive and ecofriendly. These methods are suitable for routine quality control analysis of naproxen and diphenhydramine in pharmaceutical preparations.

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