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A comparative study of two novel validated spectrophotometric techniques for resolution of four-component mixtures with severely overlapping spectra

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ABSTRACT

Two new smart spectrophotometric methods are developed and validated for the determination of the quatertnary mixture of paracetamol (acetaminophen), diphenhydramine, p-aminophenol, and N-oxide degradate of diphenhydramine. Method A is the novel triple divisor ratio difference method, where the triple divisor ratio spectrum of the component of interest shows a significant amplitude difference at two selected wavelengths where the three interfering substances are used as triple divisor and give constant amplitude all over the spectrum. The triple divisors are normalized spectra of tertiary mixtures containing 40 μg/mL of each of the 3 interfering components. The selected wavelengths are 256–290 nm, 220-230 nm, 230–245 nm and 275–260 nm for the 4 components, respectively. Method B is double divisor - ratio difference-dual wavelength, where the double divisor ratio spectrum of the component of interest shows a significant amplitude difference at two selected wavelengths where two interfering substances are used as double divisor and give constant amplitude all over the spectrum, while the third one shows zero amplitude difference at these two selected wavelengths. The double divisors used are normalized spectra of diphenhydramine /N-oxide degradate of diphenhydramine binary mixture for both paracetamol and p-aminophenol and paracetamol/p-aminophenol binary mixture for both diphenhydramine hydrochloride and its N-oxide degradate. The double divisors binary mixtures contain 40 μg/mL of each component. The selected wavelengths are 243–233 nm, 223.8–270.2 nm, 237–250 nm and 265–234.6 nm for the 4 compounds, respectively. Both methods were successfully applied for the quantification of the four components in laboratory prepared quaternary mixtures and for the quantification of paracetamol and diphenhydramine hydrochloride in panadol night® tablets. The results obtained by the developed methods were compared with those obtained by the United State Pharmacopeial method for the analysis of paracetamol and diphenhydramine, where no significant differences were found.

1. Introduction

UV-spectrophotometry is one of the easiest and most convenient methods of analysis. However the analysis suffers great difficulties when the analyzed mixtures contain a number of components with severely overlapping spectra.

This work aims to describe sensitive, precise and accurate spectrophotometric methods for the simultaneous quantification of quaternary mixtures with potentially overlapping components without interference or preliminary separation.

The suggested quaternary mixture consists of paracetamol (PC) or acetaminophen, diphenhydramine hydrochloride (DH), para aminophenol (PAP) and the N oxide degradation product of DH (NOD).

Paracetamol (PC) or acetaminophen is N-(4-hydroxy phenyl) acetamide [1,2]. It is widely used as a minor analgesic and is used as an alternative to aspirin without the side effects of salicylates on gastric mucosa [3].

Diphenhydramine hydrochloride (DH) is *N*,*N*-Dimethyl[2-(benzhydryloxy)ethyl]amine [4]. It is an antihistamine used for symptomatic relief of hypersensitivity reactions including allergic rhinitis [5].

Para aminophenol (PAP) is a potential impurity and a degradation product of PC which is well known by its nephrotoxicity [6]. The N oxide degradation product of DH (NOD) is diphenylmethoxy-*N*,*N*-dimethyl ethanamine N-oxide. Fig. 1 shows the chemical structures of the four components of the quaternary mixture.

PC and DH are co-formulated in Panadol night® tablets for temporary relief of pain associated with sleeping disorders.

Abbreviations: DD-RD-DW, double divisor - ratio difference-dual wavelength; DH, diphenhydramine; NOD, the N oxide degradation product of DH; PAP, para aminophenol; PC, paracetamol; TD-RD, triple divisor ratio difference.

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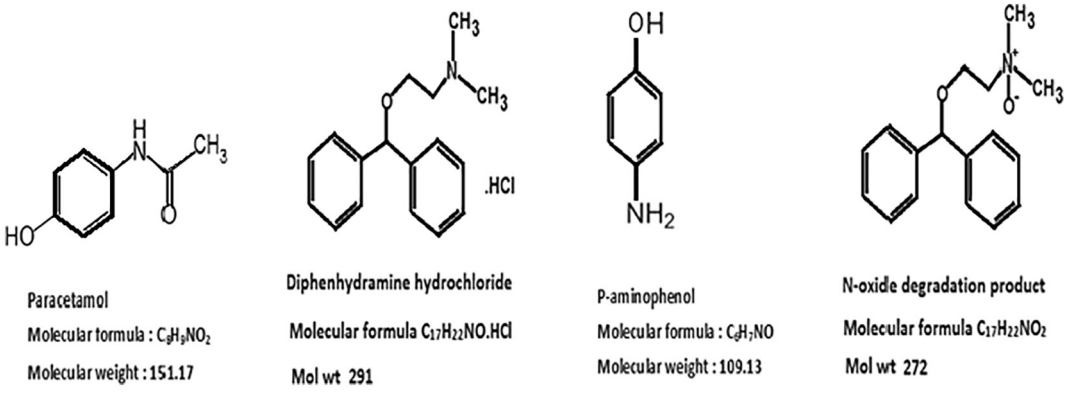

Fig. 1. The structural formula of the four studied components.

PC and DH were simultaneously determined in their binary mixture by few analytical methods including HPLC methods [1,7–9], NIR spectroscopy [10], multivariate spectrophotometry [11] and capillary gas chromatography [12].

Simultaneous quantification of PC and DH in presence of PAP was described in the literature using first derivative spectrophotometric, first derivative of ratio spectra, multivariate spectrophotometric and HPTLC-densitometric methods, in bulk material and pharmaceutical preparations [13].

A single published literature described the simultaneous quantification of PC and DH in presence of PAP and NOD using double divisor second derivative of ratio spectrum spectrophotometry, principle component regression (PCR) and partial least squares (PLS) chemometric techniques [14].

Therefore, this work aims to describe new accurate selective and time saving spectrophotometric methods for the simultaneous quantification of the four components PC DH, PAP and NOD in their quaternary mixture without interference or preliminary separation, with no need for spectral derivatization steps.

The double divisor - ratio difference – dual wavelength method **DD-RD-DW** was used by Mohamed et al. for simultaneous determination of drotaverine/caffeine/paracetamol and p-amino phenol quaternary mixture [15].

The novel triple divisor- ratio difference method **TD-RD** described in this work is an extension of triple divisor ratio spectrophotometric method [16] coupling it with ratio difference dual wavelength method, where there is no need for zero amplitude difference for one of the interfering components, which is the main limitation in DD-RD-DW method.

These two methods were able to quantify PC, DH, PAP and NOD in pure forms and quaternary mixtures and PC/DH binary mixtures in pharmaceutical formulation without interference or prior separation.

2. Experimental

2.1. Materials

2.1.1. Pure standards

Paracetamol and Diphenhydramine Hydrochloride were kindly supplied by GlaxoSmithKline Company Cairo, Egypt. Their purities were found to be 100.111.197% and 99.960.743%, respectively according to the Pharmacopeial HPLC method [1].

P-aminophenol was purchased from Riedel-de Haen-AG-Germany; its purity was certified to be 99%.

N-oxide derivative of DH was prepared by oxidative degradation of Diphenhydramine hydrochloride using 30% H_2O_2 for 6 h after liberating the free base by sodium hydroxide as described by Nouruddin W. Ali et al. [14] .The degradation was followed by application to TLC plates and using ethyl acetate - acetone —20%methanolic sodium lauryl sulphate - acetic acid (5:5:1:0.25 by volumes) as a developing system. The complete oxidative degradation and the purity of the product were confirmed by the disappearance of diphenehydramine spot and the presence of only one spot for its N-oxide degradation product. The chemical identity was confirmed by mass and IR spectra (Fig. 2).

2.1.2. Pharmaceutical formulation

Panadol night® tablets (Batch No. 141118) labeled to contain 500 mg of Paracetamol and 25 mg of Diphenhydramine Hydrochloride, manufactured by GlaxoSmithKline company.

2.1.3. Chemicals and reagents

Methanol of HPLC grade was obtained from ChromasolvW, Sigma-Aldrich ChemieGmbh, Germany.

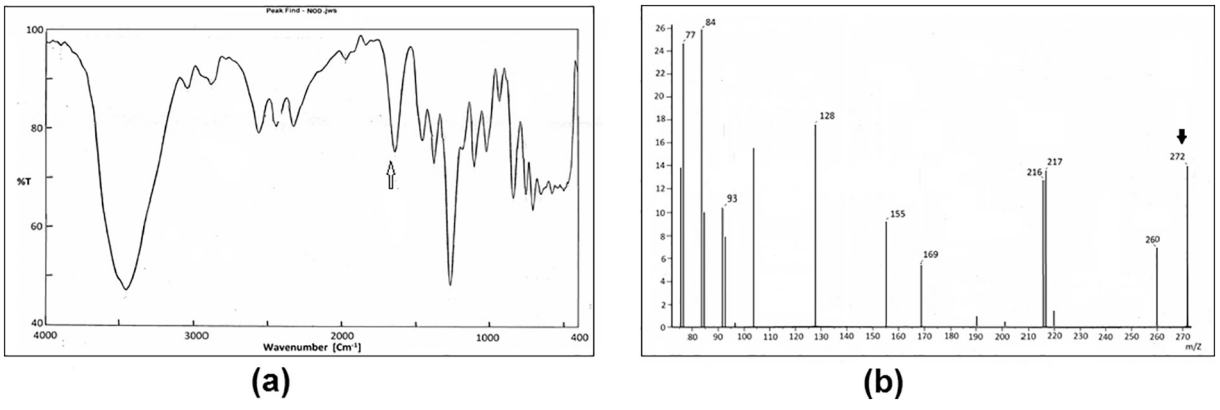

Fig. 2. The IR (a) and mass (b) spectra of *N,N*-Dimethyl [2-(benzhydryloxy)ethyl]amine N-oxide.

2.2. Instruments

A UV lamp with short wavelength 254 nm (USA) and a double beam, UV–visible spectrophotometer (SHIMADZU, Japan) model UV-1601 PC with quartz cells of 1 cm path length, connected to IBM compatible computer. The software was UVPC personal spectroscopy software version 3.7.

2.3. Prepared solutions

2.3.1. Standard solutions

For stock standard solutions containing 1 mg/mL; 0.1 g of each of the four components was accurately weighed into a separate 100 mL volumetric flask; dissolved in 50 mL of methanol and shaken to dissolve, and then the volume was completed with methanol. For working standard solutions containing 100 μg/mL, aliquots of 10 mL of each stock standard solution were transferred into four separate 100 mL volumetric flasks, and then the volume was completed with methanol.

2.3.2. Laboratory prepared mixtures

Mixtures containing different ratios of four components were prepared using their working solutions in methanol. The concentrations of the four components in different mixtures are shown in Table 1.

2.4. Methodology

2.4.1. Linearity and construction of calibration curves

For **TD-RD** spectrophotometric method, the ratio spectra of different concentrations of each drug were obtained by dividing the absorption spectra by the normalized spectrum of a ternary mixture containing equal concentrations (40 μg/mL) of the 3 interfering components. Two wavelengths were selected in the ratio spectrum of each drug to achieve a high amplitude difference. The selected wavelengths were (256–290 nm), (220–230 nm), (230–245 nm) and (275–260 nm) for PC, DH, PAP and NOD, respectively. The amplitude differences between the 2 selected wavelengths in the ratio spectra of each drug were plotted against the concentrations (μg/mL) to obtain linear calibration curves and the regression equations were computed.

For **DD-RD-DW** spectrophotometric method, the ratio spectra of different concentrations of each drug were obtained by dividing the absorption spectra by the normalized spectrum of a binary mixture containing equal concentrations (40 μ/mL) of two of the interfering components. The used double divisors were DH/NOD mixture for PC and PAP, and PC/PAP mixture for DH and NOD. Two wavelengths were selected in the ratio spectra where the drug of interest gives considerable amplitude difference, while the third interfering component gives zero amplitude difference. The selected wavelengths were (243–233 nm), (223.8–270.2 nm), (237–250 nm) and (265–234.6 nm) for PC, DH, PAP and NOD, respectively. The amplitude differences between the 2 selected wavelengths in the ratio spectra of each drug were plotted against the concentrations (μg/mL) to obtain linear calibration curves and the regression equations were computed.

2.4.2. Analysis of laboratory prepared mixtures of PC, DH, PAP and NOD

Mixtures containing different concentrations of PC, DH, PAP and NOD were analyzed by applying the above described procedure "Table 1".

2.4.3. Application to pharmaceutical formulation; Panadol night ® tablets

Ten tablets were weighed, powdered and mixed well. An accurately weighed portion of the powdered tablets equivalent to 100 mg of PC and 5 mg of DH was transferred into 100-mL volumetric flask; dissolved by sonication for 30 min with 75 mL of methanol, completed to volume with the same solvent, and then filtered. Ten mL of the above solution is diluted with methanol in 100mL volumetric flask to obtain a working solution of the dosage form containing 100 μg/mL of PC and 5 μg/mL of DH. From the prepared working solution, a volume of 4 mL is accurately transferred into 10-mL volumetric flask and the volume is completed with methanol to obtain a solution of the tablets in which PC concentration is 40μg/mL and DH concentration is 2μg/mL. The same procedure for TD-RD and DD-RD-DW methods were applied, where the concentrations of PC and DH in the tablets were calculated from the corresponding regression equations.

N.B. The determination was done at room temperature and the spectrophotometric measurements were performed using scaling factor equals 1 and Δλ equals 2.

3. Results and discussion

3.1. Method development and optimization

The zero order absorption spectra of PC, DH, PAP and NOD show severe overlap which hinders the use of direct spectrophotometry for the simultaneous determination of the four compounds as shown in Fig. 3.

The aim of this work is to establish and validate spectrophotometric methods which can easily resolve this overlap.

3.1.1. Double divisor – ratio difference – dual wavelength (DD-RD-DW) spectrophotometric method

DD-RD-DW spectrophotometric method depends on dividing the absorption spectrum of the quatertnary mixture by a double divisor consisting of the normalized spectrum of a binary mixture of two of the interfering components. Thus, these two interfering compounds give constant amplitude all over the whole spectrum. In the ratio spectrum, two wavelengths are chosen at which the third interfering component give equal amplitudes, such that the difference between the amplitudes between these two wavelengths depends only on the concentration of one component, as these steps have been able to solve the interference of the other three components.

Table 1
The simultaneous determination of paracetamol (PC), diphenhydramine (DH), p-aminophenol (PAP) and N-oxide degradate of diphenhydramine (NOD) in laboratory prepared mixtures using DD-RD-DW and TD-RD.

Mixture	Ratio PC: DH	% of degradation products	Taken (μg/mL)				Recovery % obtained by DD-RD-DW				Recovery % obtained by TD-RD			
			PC	DH	PAP	NOD	PC	DH	PAP	NOD	PC	DH	PAP	NOD
1	1:2	2	25	50	0.5	1	99.323	100.822	–	98.286	98.983	100.786	–	98.153
2	1:2	2	100	200	2	4	–	–	98.947	99.929	–	–	98.851	99.876
3	1:1	5	20	20	1	1	99.935	101.833	–	100.286	99.875	101.687	–	100.154
4	2:1	10	40	20	4	2	100.641	99.796	100.263	98.857	100.562	99.951	100.103	98.754
5	2:1	20	40	20	8	4	100.542	100.389	101.250	99.214	100.653	100.451	101.390	99.456
6	20:1[a]	50	40	2	20	1	101.115	101.852	99.763	99.714	101.097	101.768	99.540	99.342
7	2:1	90	40	20	36	18	101.051	99.944	97.763	101.269	100.843	99.874	97.901	101.134
Mean ± SD							100.657 ±0.690	100.763 ±0.903	99.597 ±1.321	99.651 ±0.982	100.336 ±0.779	100.753 ±0.826	99.557 ±1.313	99.553 ±0.968

[a] The ratio present in Panadol night® tablets.

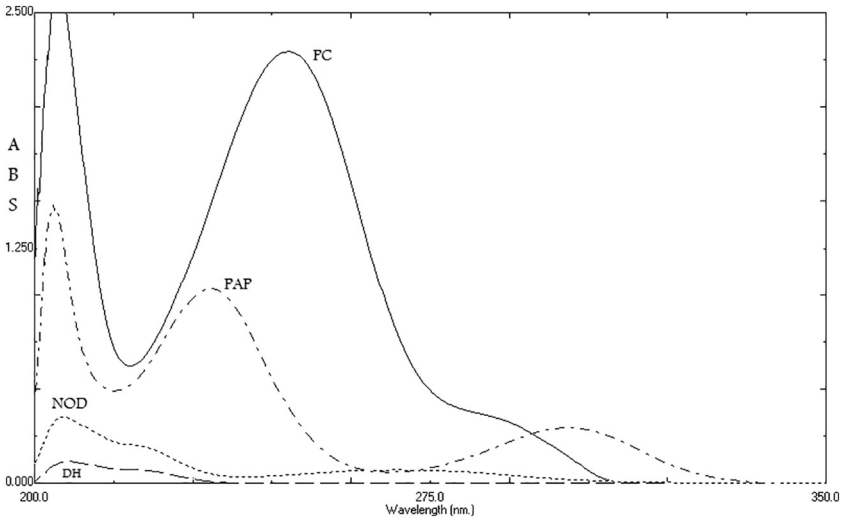

Fig. 3. The absorption spectra of 30 μg/mL Paracetamol (line), 5 μg/mL Diphenhydramine hydrochloride (dashes), 5 μg/mL p-aminophenol (dashes & dots) and 5 μg/mL N oxide degradate of diphenhydramine hydrochloride (dots).

3.1.1.1. Selection of the solvent. Several solvents were tried (e.g water, ethanol, methanol),where the best solvent was methanol regarding solubility, stability and sensitivity.

3.1.1.2. Selection of the divisor. Several binary mixtures selections from the components and different concentrations were tried as divisors. The chosen double divisors which showed the best sensitivity and selectivity were the normalized spectra of DH/NOD binary mixture for PC and PAP and PC/PAP binary mixture for DH and NOD. The binary mixtures used as divisors contain 40 μg/mL of each component.

3.1.1.3. Selection of the wavelengths. Different wavelength pairs in the ratio spectra were tried. The chosen wavelength pairs which achieved a zero amplitude difference for the third interfering component as well as a high amplitude difference for the component of interest are shown in "Figs. 4–7".

3.1.2. Triple divisor – ratio difference (TD-RD) spectrophotometric method

TD-RD spectrophotometric method depends on dividing the absorption spectrum of the quatertnary mixture by a triple divisor consisting of the normalized spectrum of a mixture of the three interfering components. All the interfering components give constant amplitude all over the whole spectrum. In the ratio spectrum of the component of interest, two wavelengths of maximum amplitude difference are chosen. There is no need to inspect the ratio spectrum for a zero amplitude difference for one of the interfering components, making the method simpler and less tedious.

3.1.2.1. Selection of the solvent. Several solvents were tried (e.g. water, ethanol, methanol), where the best solvent was methanol regarding solubility, stability and sensitivity.

3.1.2.2. Selection of the divisor concentration. Tertiary mixtures with different concentrations were tried as divisors. The normalized spectra of tertiary mixtures containing 40 μg/mL of each of the interfering components gave the best results regarding selectivity and sensitivity.

3.1.2.3. Selection of the wavelengths. Different wavelength pairs were tried. The chosen wavelengths for each of the four components are shown in "Figs. 8–11".

3.2. Method validation

The proposed methods were found to be linear in the ranges of 2–45 μg/mL, 2–50 μg/mL, 2–40 μg/mL and 1–50 μg/mL for PC, DH, PAP

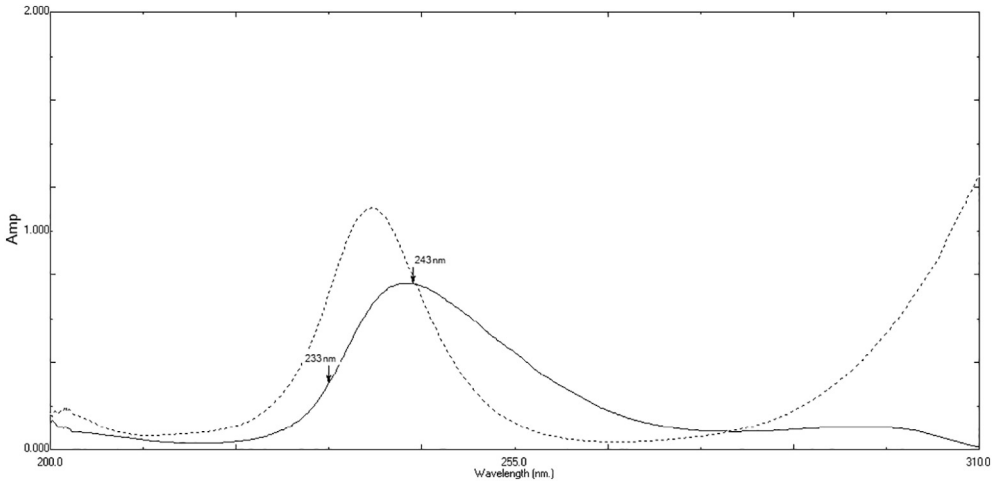

Fig. 4. The ratio spectra of paracetamol (line) and p-aminophenol (dots) using the normalized spectrum of the binary mixture containing 40 μg/mL of each of diphenhydramine HCL and its N-oxide degradate as a double divisor for the determination of paracetamol by DD-RD-DW spectrophotometric method.

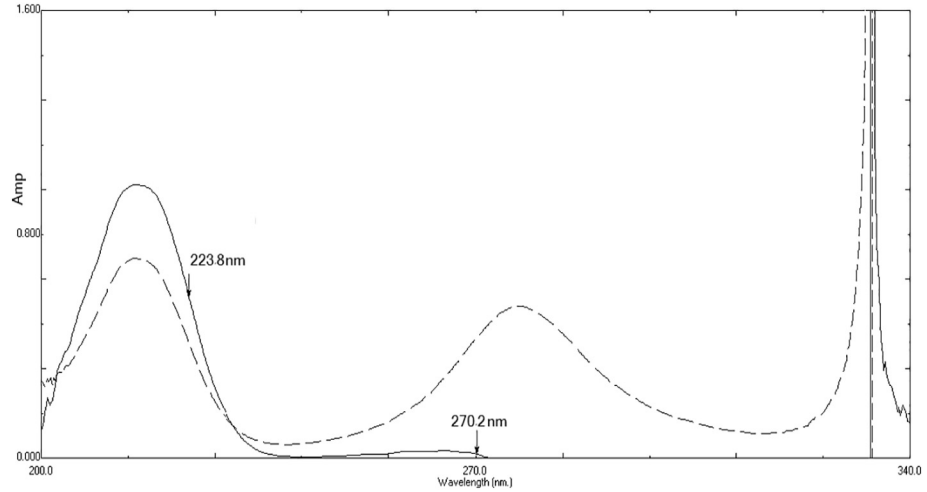

Fig. 5. The ratio spectra of diphenhydramine HCl (line) and its N-oxide degradate (dots) using the normalized spectrum of the binary mixture containing 40 μg/mL of each of paracetamol and p-aminophenol as a double divisor for the determination of diphenhydramine HCl by DD-RD-DW spectrophotometric method.

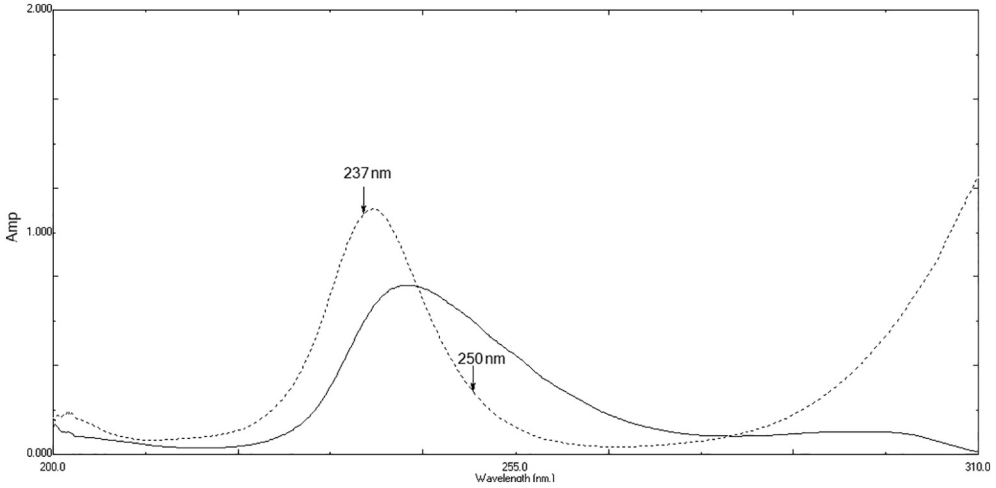

Fig. 6. The ratio spectra of paracetamol (line) and p-aminophenol (dots) using the normalized spectrum of the binary mixture containing 40 μg/mL of each of diphenhydramine HCL and its N-oxide degradate as a double divisor for the determination of p-aminophenol by DD-RD-DW spectrophotometric method.

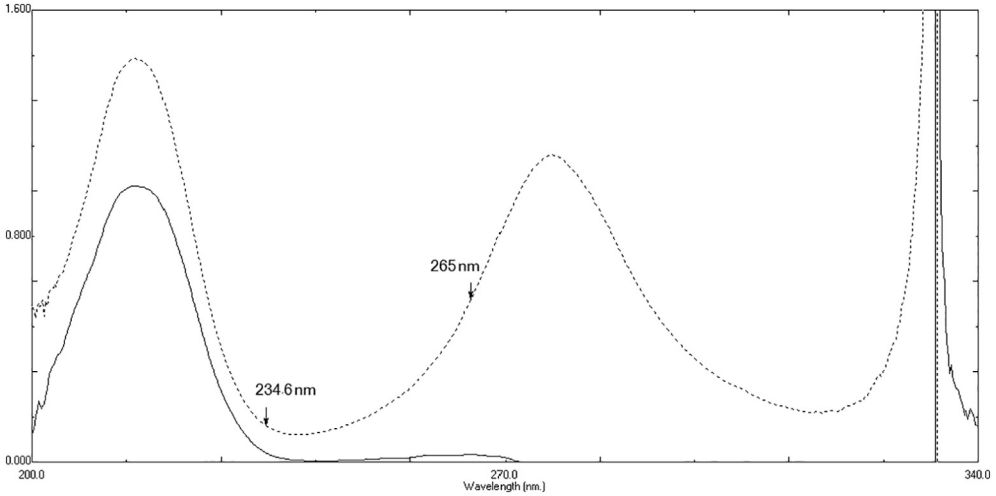

Fig. 7. The ratio spectra of diphenhydramine HCl (line) and its N-oxide degradate (dots) using the normalized spectrum of the binary mixture containing 40 μg/mL of each of paracetamol and p-aminophenol as a double divisor for the determination of the N-oxide degradate of diphenhydramine HCl by DD-RD-DW spectrophotometric method.

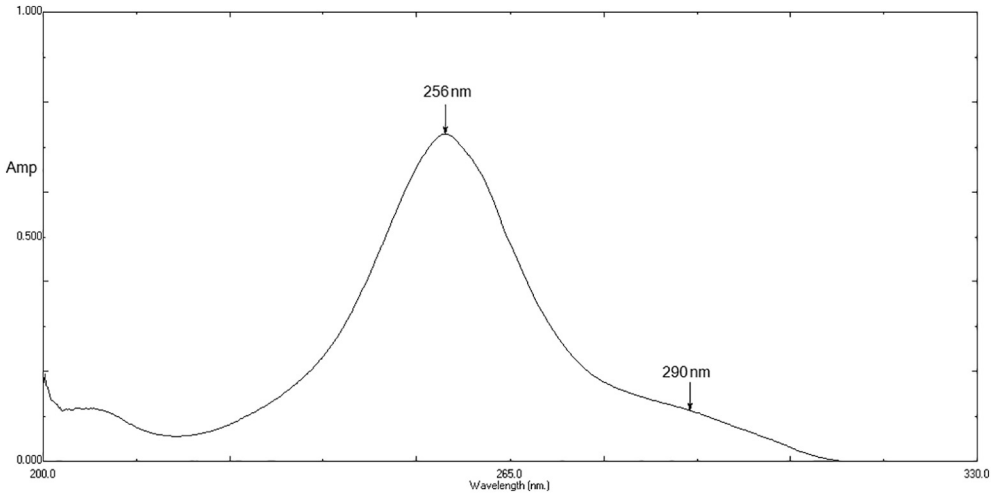

Fig. 8. The ratio spectrum of paracetamol using the normalized spectrum of the ternary mixture containing 40 μg/mL of each of p-aminophenol, diphenhydramine HCl and its N-oxide degradate as a triple divisor for the determination of paracetamol by TD-RD spectrophotometric method.

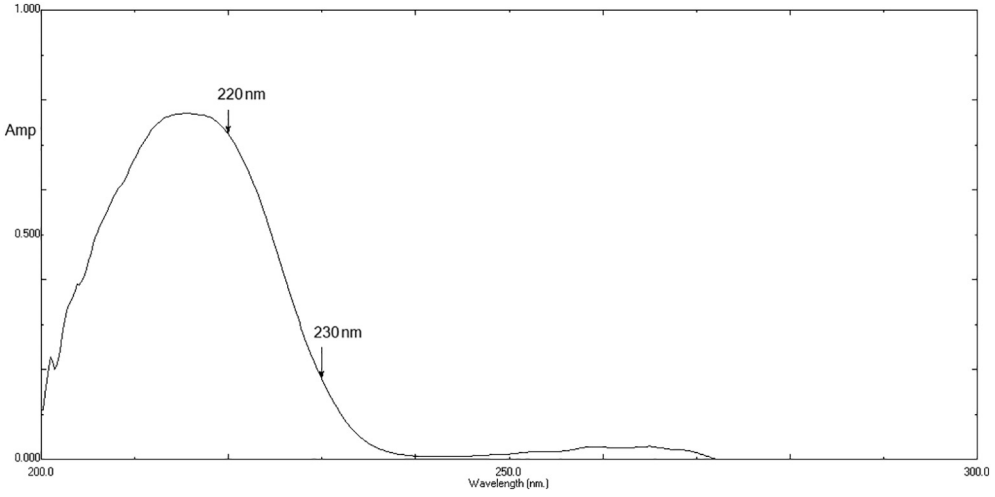

Fig. 9. The ratio spectrum of diphenhydramine HCl using the normalized spectrum of the ternary mixture containing 40 μg/mL of each of its N-oxide degradate, paracetamol and p-aminophenol as a triple divisor for the determination of diphenhydramine HCl by TD-RD spectrophotometric method.

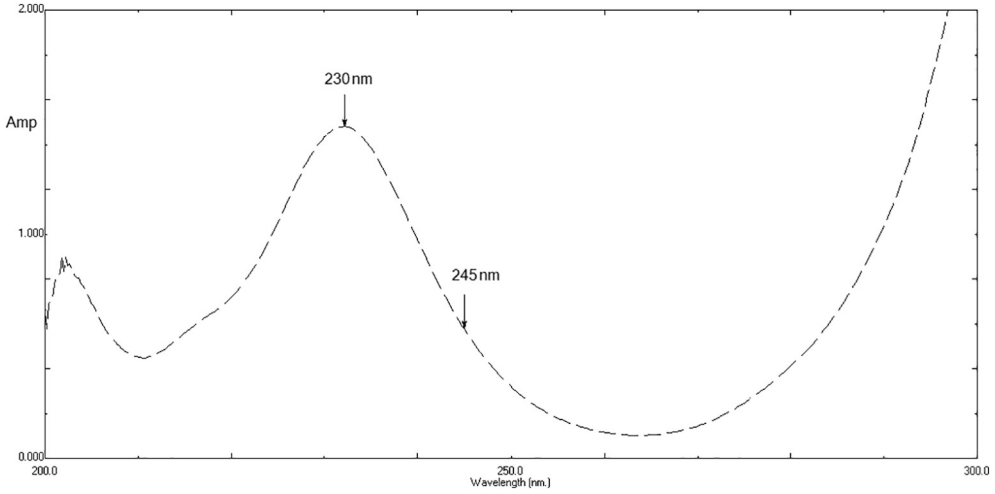

Fig. 10. The ratio spectrum of p-aminophenol using the normalized spectrum of the ternary mixture containing 40 μg/mL of each of paracetamol, diphenhydramine HCl and its N-oxide degradate as a triple divisor for the determination of p-aminophenol by TD-RD spectrophotometric method.

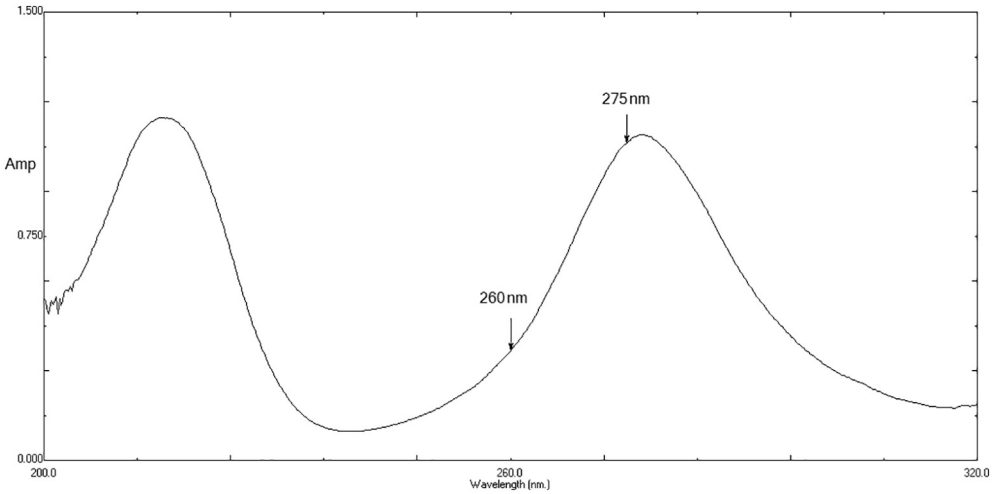

Fig. 11. The ratio spectrum of N-oxide degradate of diphenhydramine HCl using the normalized spectrum of the ternary mixture containing 40 μg/mL of each of paracetamol, diphenhydramine HCl and p-aminophenol as a triple divisor for the determination of N-oxide degradate of diphenhydramine HCl by TD-RD spectrophotometric method.

and NOD, respectively. The regression equations were calculated and found to be:

$A = 0.091\ C - 0.012$ r = 0.999 for PC by DD-RD-DW

$A = 0.028\ C - 0.010$ r = 0.999 for DH by DD-RD-DW
$A = 0.081\ C + 0.007$ r = 0.999 for PAP by DD-RD-DW
$A = 0.043\ C + 0.006$ r = 0.999 for NOD by DD-RD-DW
$A = 0.078\ C - 0.037$ r = 0.999 for PC by TD-RD
$A = 0.027\ C$ r = 0.999 for DH by TD-RD
$A = 0.057\ C + 0.004$ r = 1 for PAP by TD-RD and
$A = 0.035\ C + 0.001$ r = 0.9998 for NOD by TD-RD

Where A is the amplitude difference between the two chosen wavelengths, C is the concentrations in μg/mL and r is the correlation coefficient.

Good linearities are evident from the closeness of the correlation coefficient values to 1 and low values of intercepts as shown in Table 2.

The precision of the results obtained by the proposed methods was tested and the results are shown in Table 2.

The accuracy of the methods was verified by applying the methods for determination of pure samples of the four components, where the concentrations were obtained by substitution in the corresponding regression equations and the results are shown in Table 2.

The accuracy was further confirmed by applying the standard addition technique to Panadol night® tablets through the addition of pure PC and DH separately to the tablet solutions, where good recoveries were obtained as shown in Table 3.

Selectivity of the proposed methods is ascertained through their application for simultaneous determination of the four components in laboratory prepared mixtures of different concentration ratios as well as simultaneous determination of PC and DH in Panadol night® tablets, as the good recoveries in Tables 1 and 3 show no interference from other drugs, impurities, excipients or inactive ingredients.

The limits of detection and limits of quantification of the four studied compounds by the two proposed methods were calculated and presented in Table 2.

The robustness of the two methods was studied by testing their ability to remain unchanged upon making some small intended changes in the method conditions such as the concentrations of the divisors, solvent suppliers (two different suppliers) and temperature (4 °C, 35 °C & 45 °C). The results shown in Table 4 confirmed good robustness.

Comparing our methods with the double divisor second derivative spectrophotometric method described by Nouruddin W. Ali et al. [14] for determination of PC and DH in presence of PAP and NOD, the double divisor second derivative method couldn't quantify PC or NOD. DD-RD-DW and TD-RD are simpler than the double divisor second derivative method as they need no derivatization steps after division. DD-RD-

Table 2
Results of assay validation parameters of the proposed methods for the determination of paracetamol (PC), diphenhydramine (DH), p-aminophenol (PAP) and N-oxide degradate of diphenhydramine (NOD).

Parameters	Double divisor ratio difference dual wavelength DD-RD-DW				Triple divisor ratio difference TD-RD			
	PC	DH	PAP	NOD	PC	DH	PAP	NOD
Range (μg/mL)	2–45	2–50	2–40	1–50	2–45	2–50	2–40	1–50
Linearity								
Slope	0.091	0.028	0.081	0.043	0.078	0.027	0.057	0.035
Intercept	−0.012	−0.010	0.007	0.006	−0.037	0.000	0.004	0.001
Correlation coefficient	0.999	0.999	0.999	0.999	0.999	0.999	1	0.999
Accuracy (mean ± SD)	99.636±1.168	100.488±1.327	100.094±0.740	100.660±1.141	100.465±0.675	100.612±0.913	100.084±0.921	99.792±0.728
Precision (RSD%)								
Repeatability[a]	0.429	0.457	0.595	0.512	0.353	0.391	0.516	0.498
Intermediate precision[b]	0.542	0.596	0.630	0.591	0.456	0.468	0.612	0.551
LOD (μg/mL)[c]	0.577	0.625	0.621	0.267	0.521	0.580	0.634	0.301
LOQ (μg/mL)[d]	1.731	1.875	1.863	0.800	1.564	1.741	1.903	0.903

[a] and [b]: the intra-day and inter-day relative standard deviations of concentrations (5, 25 and 45 μg/mL).
[c]: LOD is calculated using the formula (LOD = 3.3 × SD/slope).
[d]: LOQ is calculated using the formula (LOQ = 10 × SD/slope).

Table 3
Determination of paracetamol (PC) and diphenhydramine hydrochloride (DH) in Panadol night® tablets (batch no. 141118) by the proposed double divisor ratio difference dual wavelength (DD-RD-DW) and triple divisor ratio difference (TD-RD) methods and application of standard addition technique.

Panadol night® tablets (batch no. 141118)	Double divisor ratio difference dual wavelength (DD-RD-DW)		Triple divisor ratio difference (TD-RD)	
	Recovery% ± SD	Standard addition technique[a] (recovery% ± SD)	Recovery% ± SD	Standard addition technique[a] (recovery% ± SD)
PC (taken concentration 40 μg/mL)	98.325 ± 0.206	100.479 ± 0.592	98.337 ± 0.221	100.457 ± 0.575
DH (taken concentration 2 μg/mL)	103.743 ± 0.479	100.507 ± 0.392	103.557 ± 0.458	100.497 ± 0.416

[a] Standard addition technique was performed by adding pure PC (2, 3 & 4 μg/mL) and DH (5, 20, 40 μg/mL) separately to tablet solution.

Table 4
Robustness study of the proposed double divisor ratio difference dual wavelength (DD-RD-DW) and triple divisor ratio difference (TD-RD) methods in terms of RSD%.

Robustness parameters	Double divisor ratio difference dual wavelength DD-RD-DW				Triple divisor ratio difference TD-RD			
	PC	DH	PAP	NOD	PC	DH	PAP	NOD
Two different methanol suppliers[a]	0.578	0.682	0.609	0.593	0.525	0.614	0.673	0.632
Different temperatures[b] (4 °C, 35 °C & 45 °C)	0.891	0.725	0.654	0.652	0.825	0.689	0.696	0.678
Divisor concentration (40 ± 5 μg/mL)	0.312	0.341	0.590	0.456	0.303	0.314	0.610	0.415

[a] ChromasolvW, Sigma-Aldrich ChemieGmbh, Germany & Al-Nasr pharmaceutical chemicals company, Abu Zaabal, Cairo, Egypt.
[b] The samples have been kept at the mentioned temperatures for 3 h before analysis.

Table 5
Statistical comparison of the results obtained by the proposed methods and the Pharmacopoeal HPLC method when applied to pure Paracetamol (PC) and Diphenehydramine hydrochloride (DH).

Items	Double divisor ratio difference dual wavelength DD-RD-DW		Triple divisor ratio difference TD-RD		Pharmacopoeal HPLC method [1]	
	PC	DH	PC	DH	PC	DH
Mean	99.967	100.207	100.225	100.375	99.997	99.897
SD	1.076	1.131	0.668	0.888	0.833	0.756
N	10	10	10	10	10	10
Variance	1.158	1.279	0.446	0.789	0.694	0.572
Student's *t*-test (2.262)[a]	0.945	0.482	0.508	0.211		
F-value (3.18)[b]	0.457	0.246	0.521	0.639		

[a] Figures in parentheses represent the corresponding tabulated values of t at P = 0.05.
[b] Figures in parentheses represent the corresponding tabulated values of F at P = 0.05.

DW and TD-Rd are somewhat less accurate and have nearly the same linearity ranges as the double divisor second derivative method.

Statistical comparison of the results obtained by the proposed methods to those obtained by the pharmacopeial HPLC method [1], when applied for determination of PC and DH in pure samples and pharmaceutical formulation are shown in Tables 5 and 6, respectively. No significant differences were found with respect to accuracy and precision, as revealed by the calculated t and F values.

Table 6
Statistical comparison of the results obtained by the proposed methods and the Pharmacopoeal HPLC method when applied to Panadol night® tablets (batch no. 141118).

Items	Double divisor ratio difference dual wavelength DD-RD-DW		Triple divisor ratio difference TD-RD		Pharmacopoeal HPLC method [1]	
	PC	DH	PC	DH	PC	DH
Mean	98.325	103.743	98.337	103.557	98.360	103.665
SD	0.206	0.479	0.221	0.458	0.218	0.472
N	6	6	6	6	6	6
Variance	0.042	0.229	0.049	0.210	0.048	0.223
Student's *t*-test (2.23)[a]	0.780	0.782	0.859	0.696		
F-value (4.28)[b]	0.901	0.974	0.981	0.948		

[a] Figures in parentheses represent the corresponding tabulated values of t at P = 0.05.
[b] Figures in parentheses represent the corresponding tabulated values of F at P = 0.05.

3.3. The comparison of the method validation results of the two proposed methods

The comparison between the method validation results of the two proposed methods showed that; TD-RD is more accurate than DD-RD-DW for the determination of PC, DH and NOD, while DD-RD-DW is more accurate for the determination of PAP.

TD-RD was found to be more precise for the determination of the four components.

The results of the robustness studies of the two methods showed that TD-RD is more robust than DD-RD-DW for the determination of PC and DH, while DD-RD-DW is more robust for the determination of PAP and NOD.

The future research plan is to develop and validate a successful HPLC method for separation and quantification of the four components and to study the factors or parameters affecting the stability of the drugs and the presence of any other related compounds.

4. Conclusion

The present work provides new sensitive, accurate and selective spectrophotometric techniques for the simultaneous determination of PC, DH, PAP and NOD in pure forms, laboratory prepared quaternary mixture and in pharmaceutical formulation. The novel method TD-RD is simpler and less tedious. Both methods need no spectral derivatization, which recommends the use of the proposed methods for routine quality control analysis.

CRediT authorship contribution statement

Nessreen S. Abdelhamid: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Writing - original draft, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests [13–16] or personal relationships that could have appeared to influence the work reported in this paper. [3]

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