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Stability indicating spectrophotometric methods for determination of phenazopyridine hydrochloride in presence of its oxidative degradation product

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ABSTRACT

Four simple, accurate and precise stability-indicating spectrophotometric methods processing spectra were developed and validated for determination of phenazopyridine hydrochloride (PAP) and its oxidative degradation product (2,3,6-Triaminopyridine), which is also the metabolite, without prior separation namely; first derivative (^{1}D), derivative ratio (^{1}DD), ratio difference spectrophotometric method (RDSM) and dual wavelength (DW). The accuracy, precision and linearity ranges of the proposed methods were determined. The methods were validated and the specificity was assessed by analyzing synthetic mixtures containing the drug and its degradate. The four methods were applied for the determination of the cited drug in tablets and the obtained results were statistically compared with those of a reported method. The comparison showed that there is no significant difference between the proposed methods and the reported method regarding both accuracy and precision.

KEYWORDS

Phenazopyridine hydrochloride;
Stability indicating methods;
First derivative;
Derivative ratio;
Ratio difference spectrophotometric method ;
Dual wavelength.

INTRODUCTION

Phenazopyridine HCL (PAP) [2, 6-diamino-3-(phenylazo) pyridine hydrochloride] Figure (1) exerts an analgesic effect on the mucosa of the urinary tract and is used to provide symptomatic relief of pain in conditions such as cystitis and urethritis. It is given in conjunction with an antibacterial agent for the treatment of urinary tract infections[1]. Several analytical methods were reported for the estimation of PAP either individually or in its combination with other drugs including colorimetry[2], UV spectrophotometry[3-7], HPLC[8,9], GC–MS[10,11], LC–MS[12], electrochemical[13-17] and imprinted polymer-electrospray ionization ion mobility spectrometry[18].

2,3,6-Triaminopyridine (TAP), a metabolite of phenazopyridine, is known to cause muscle necro-

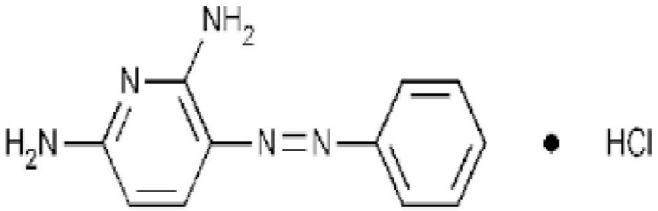

Figure 1 : Chemical structure of phenazopyridine HCL

sis and renal damage In susceptible individuals or after excessive doses of PAP. TAP is reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity in experimental animals[19]. TAP is able to generate "active oxygen" species under physiological conditions and to cause oxidative damage to cells in vitro[20]. Thus, study of stability indicating determination of PAP in the presence of its degradation product TAP, which is also the metabolite, would be of interest.

Reviewing the literature on the determination of PAP revealed the lack of any stability indicating assay methods for the determination of the intact drug in presence of its degradation product or its metabolite. Thus, there is acritical need for the development of simple, rapid, accurate and less time of analysis methods.

Objective of the current study was to develop four spectrophotometric methods namely; first derivative (^{1}D)[21-23], derivative ratio (^{1}DD)[24-26], ratio difference spectrophotometric method (RDSM)[27,28] and dual wavelength (DW)[29, 30].

EXPERIMENTAL

Instruments

SHIMADZU dual beam UV–visible spectrophotometer (Kyoto/Japan), model UV-1800 PC connected to IBM compatible and a HP P1102 laser jet printer. The bundled software, UV-Probe personal spectroscopy software version 2.43 (SHIMADZU) was used. The spectral band was 2 nm and scanning speed is 2800 nm/min and 1nm data interval.

Chemicals and reagents

(a) PAP was kindly supplied by Kahira Pharm., Egypt.
(b) Pharmaceutical Preparations: Urisept tablets, batch number (1310275). Each tablet is claimed to contain 100mg of PAP. They were purchased from a local pharmacy .
(c) Solvent: distilled water.

Standard solution

Stock solution of 100 ug/mL for PAP was prepared by dissolving 10 mg of PAP in 100 ml distilled water. Different sets of working solution at various concentrations were prepared by appropriate dilution of the stock solution.

Preparation of the forced degradation product of PAP

To accurately weighed 100 mg of PAP 10 ml of 3% H_2O_2 was added and the solution was then refluxed at 80°C for 1 h. An orange red precipitate was formed after cooling the reaction mixture for 2hr at 4°C, filtered, washed with water, and dried in a vacuum oven for 12 h at 60° C. The degradation process completeness was tested using TLC using chloroform–acetonitrile (80:20, v: v) as adeveloping system. The identity of the degradation product was checked using IR, NMR and mass spectral scans.

The obtained residue was dissolved in 100 ml distilled water to obtain a stock solution labeled to contain degradate derived from 1mg/ml of PAP. Aliquots of different concentrations of degradation product (TAP) were accurately transferred into series of 10 ml volumetric flasks and the volumes were completed to the mark with water.

PROCEDURE

Linearity and construction of calibration curves

First derivative method (^{1}D)

Aliquots from PAP stock standard solution were accurately measured, transferred into a set of 10 ml volumetric flasks and completed to volume with water to give (1-14 μg/ml). The zero order absorption spectrum of each solution was recorded versus water as a blank. The first derivative corresponding to each zero order spectrum was recorded, using $\Delta\lambda$ = 4 nm and scaling factor 10. The amplitude values at 370 nm were measured. The measured amplitude values versus the final concentrations in μg/ml were plotted to get the calibration graph. Alternatively, the regression equation was derived.

Derivative ratio method (^{1}DD)

Ratio spectra obtained by dividing each zero order spectrum by the spectrum of the degradation product (9 μg /ml) used as a divisor concentration. The first derivative corresponding to each ratio spectrum was recorded, using $\Delta\lambda$ = 4 nm and scaling factor 10. The amplitude values at 360 nm were

measured. The measured amplitude values versus the final concentrations in μg/ml were plotted to get the calibration graph. Alternatively, the regression equation was derived.

Ratio difference spectrophotometric method (RDSM)

The amplitudes difference of the ratio spectra at 428 and 276 nm ($\Delta P_{428-276}$) were plotted against the corresponding concentrations in μg/ml of PAP to get the calibration graph. Alternatively, the regression equation was derived.

The dual wavelength method

In zero order spectra, the difference absorbance at 425 and 256 nm was found to be zero for degradate, the difference absorbance at 425 and 256 nm to the corresponding concentration of PAP were plotted to get the calibration graph. Alternatively, the regression equation was derived.

Application to laboratory prepared mixtures

Accurate aliquots of PAP and TAP were transferred from their working solutions into a series of 10 ml volumetric flasks to prepare mixtures containing different ratios of both. The volumes were completed with the solvent. The spectra of the prepared series from 200 to 550 nm were recorded and stored. The stored spectra were divided by the divisor as before. The concentrations of PAP were calculated as described under linearity for each proposed method.

Application to pharmaceutical preparation

Ten tablets were weighed and finely powdered. Appropriate weight of powder equivalent to the weight of one tablet was accurately weighed, transferred to 100 ml volumetric flask and the volume was made up to 75 ml with water. The solution was shaken vigorously for 15 min then sonicated for 30 min and filtered through Whatman filter paper no 41. The volume was completed to 100 ml with the same solvent. Necessary dilutions of the filtrate were made with water to obtain different concentrations of PAP. Samples were analyzed using the procedures stated under linearity. To assess the accuracy of the proposed methods, standard addition technique was applied.

RESULTS AND DISCUSSION

Degradation of PAP

Complete degradation of PAP was was checked by TLC using chloroform–acetonitrile (80:20, v: v) as adeveloping system. The proposed scheme for preparing the degradation product can be represented as Figure (2).

It has been confirmed that the main degradate is TAP which is also the major metabolite of the drug inside the human body. IR was performed for PAP and its degradation product as given in Figures 3 (a) and (b). Disappearance of aband at 1600 cm^{-1}, typical for free azo group, indicates the cleavage of azo group, also the appearance of group of bands between 3186,3302cm^{-1} due to the presence of more than two amino groups confirmed the oxidation which is followed by hydrolysis.

The ^{1}H NMR was performed for PAP and its degradation product as given in Figures 4 (a) and (b). Spectrum of degradation product showed that aromatic protons appear as douplet peaks at 5.18, 6.68 ppm and protons of aromatic amino groups appear as singlet peaks at 6.26, 7.78 ppm. Mass spectrometry was also performed for the degradation

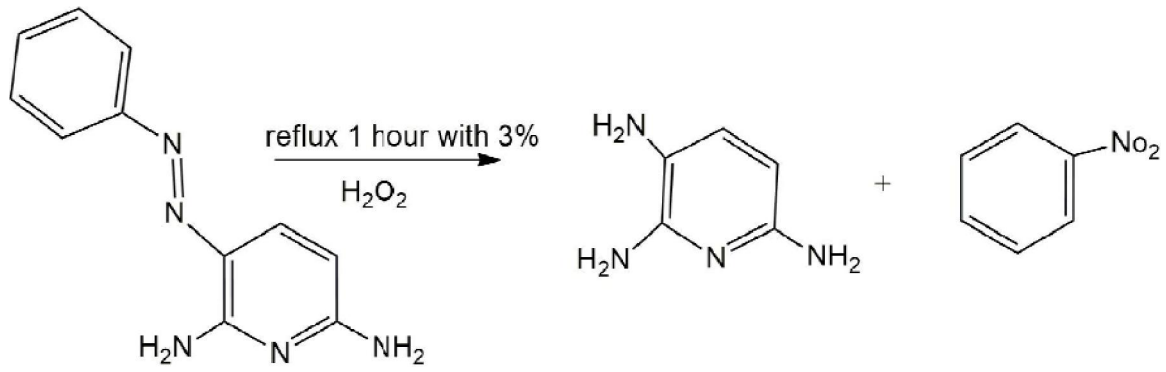

Figure 2 : proposed degradation pathway of phenazopyridine

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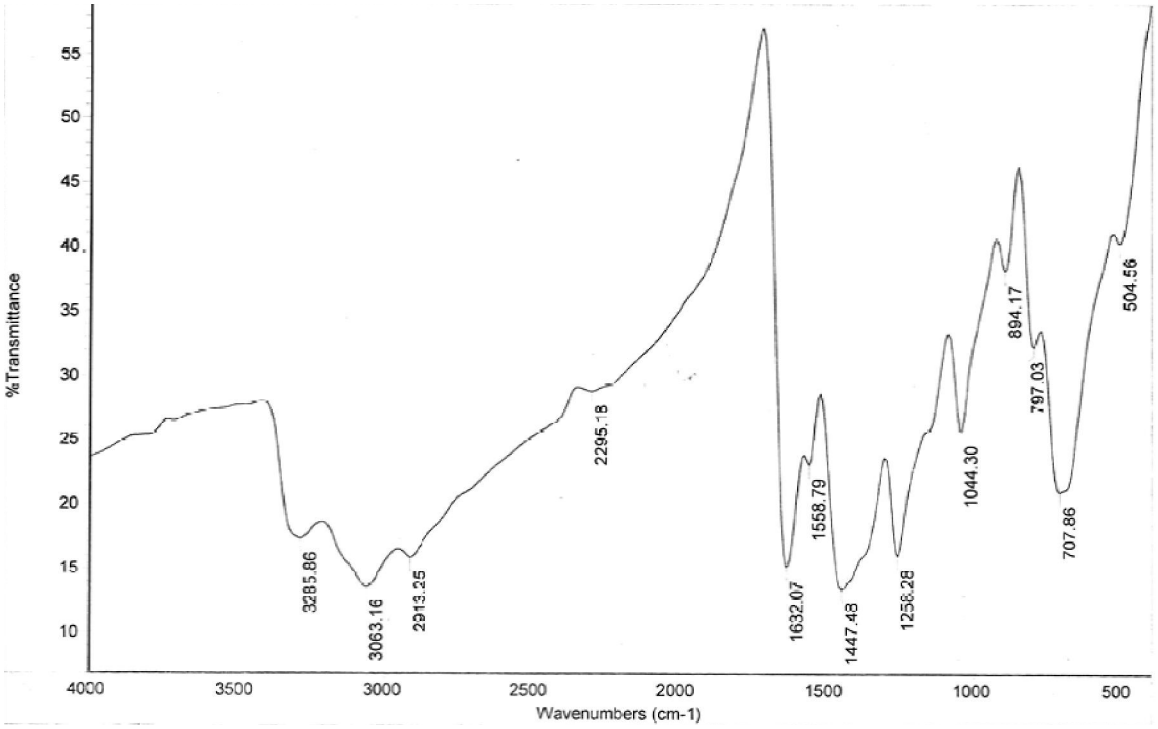

Figure 3 a : IR spectrum of PAP

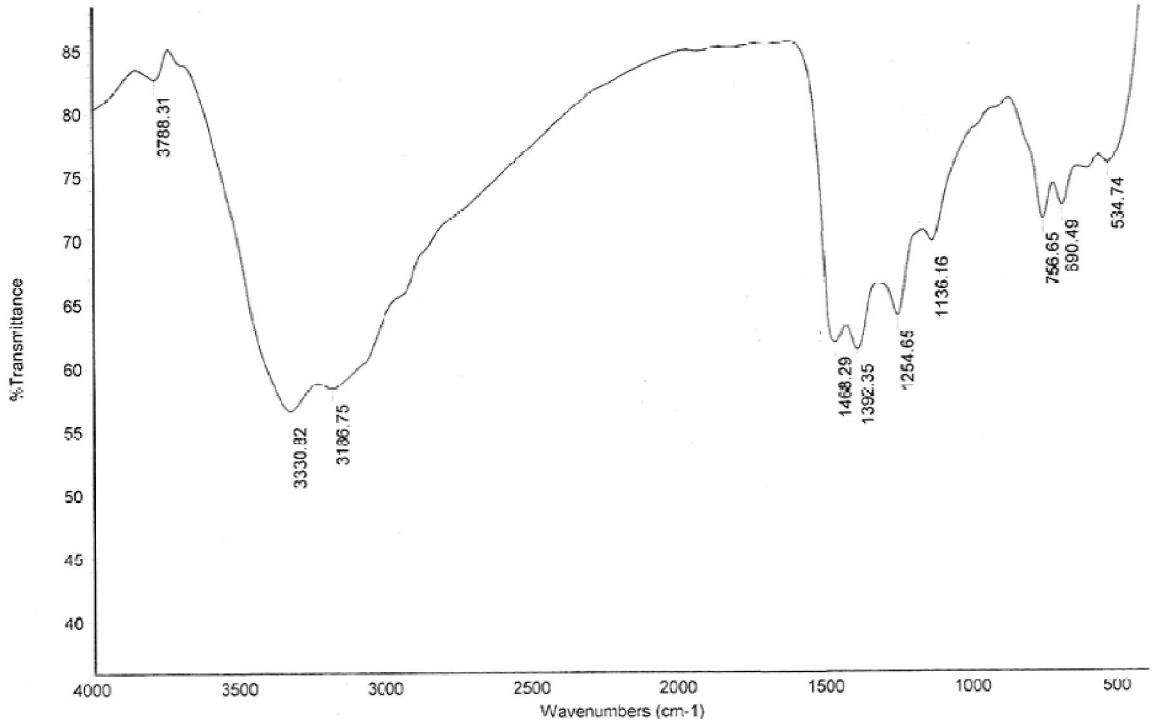

Figure 3b : IR spectrum of PAP degradation product

product and a parent peak was obtained at m/z = 125 indicating that TAP is formed as shown in Figure (5).

PAP and its degradation product spectra are severely overlapped as shown in Figure (6); direct determination of PAP is impossible, while upon the application of methods, the spectra can be resolved and determination of PAP in presence of its degradation product or its metabolite could be achieved.

First Derivative spectrophotometric method (^{1}D)

The zero-order absorption spectra of PAP and its oxidative degradate (TAP) are shown in Figure (6). The spectra displays overlapping in the region

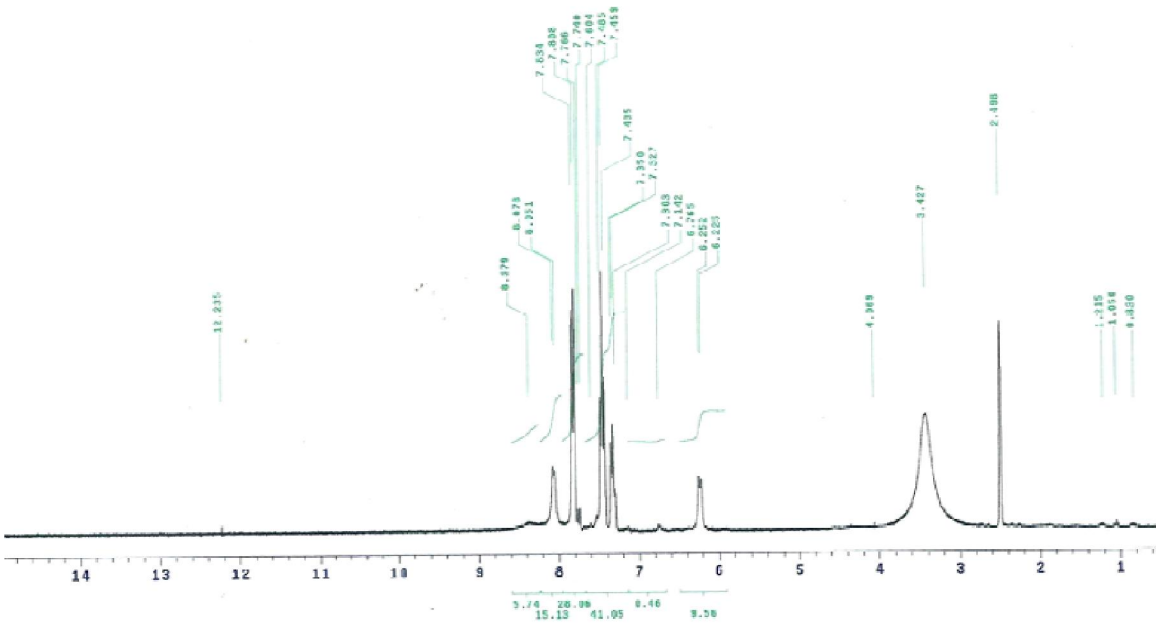

Figure 4a : H[1]NMR spectrum of PAP

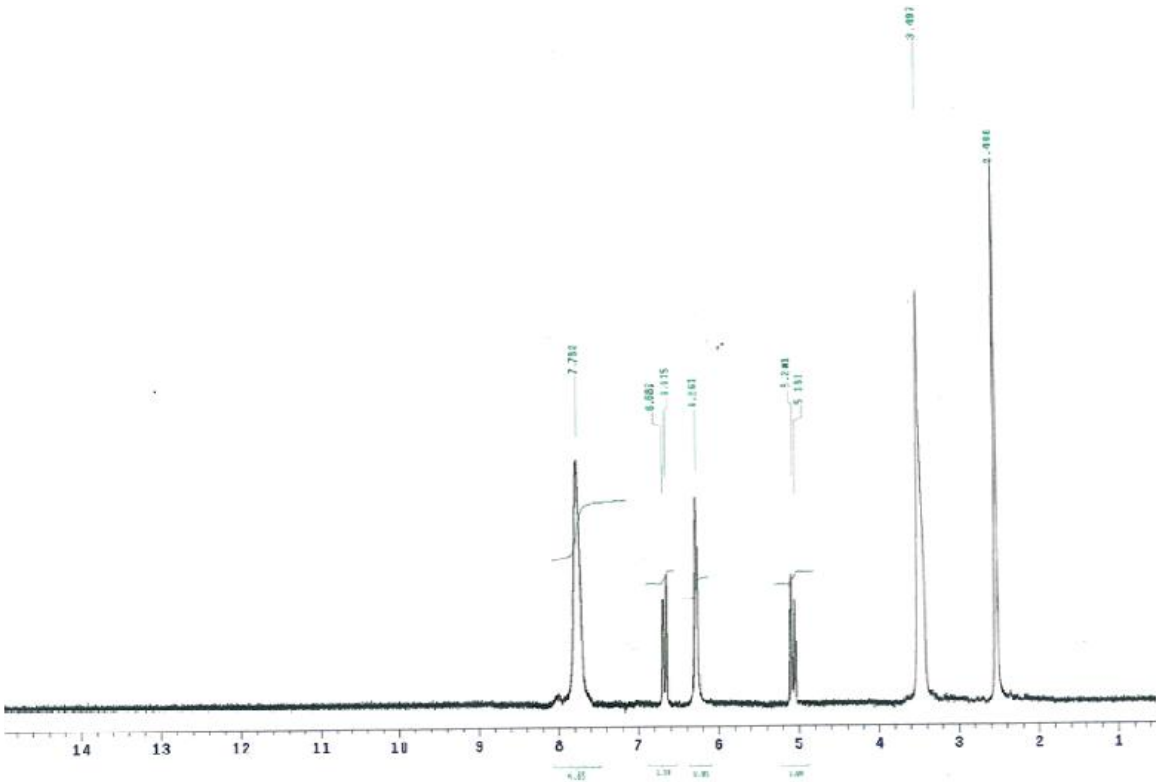

Figure 4b : H[1]NMR spectrum of PAP degradation product

of 330- 520 nm. This makes the determination of PAP in the presence of its degradation product by conventional UV spectrophotometer difficult. The derivative spectrophotometry technique was, therefore, chosen for the determination of the drug since it could remove broadband contributions from excipients and might also overcome the interference from peak overlapping. The experiments showed that the first derivative spectra of PAP was simple and give results with suitable precision at $\Delta\lambda$ =4 nm, scaling factor= 10 as shown in Figure (7). In this first derivative spectrum, the signals at 370 nm corresponding to zero crossing of the degradation product are proportional to PAP concentration. Linear correlation was obtained between the amplitude at 370 nm against the corresponding concentration of

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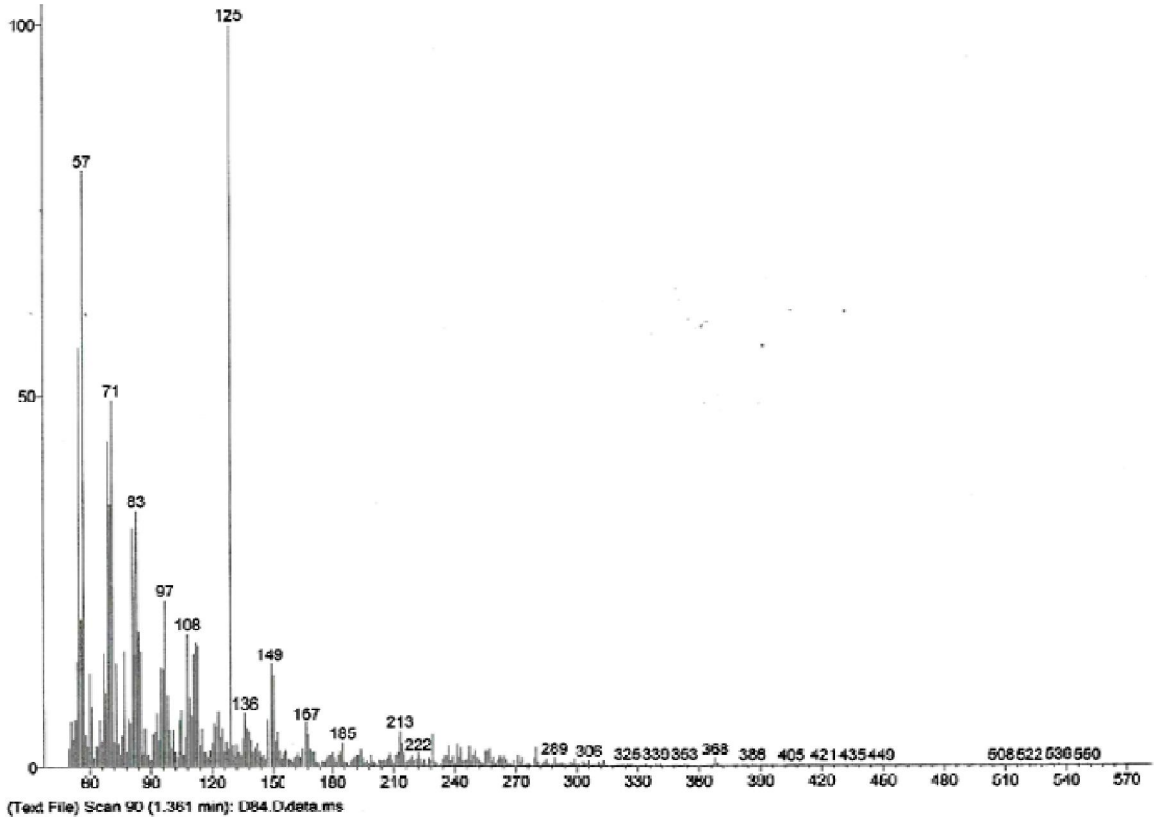

Figure 5 : Mass spectrum of PAP degradation product

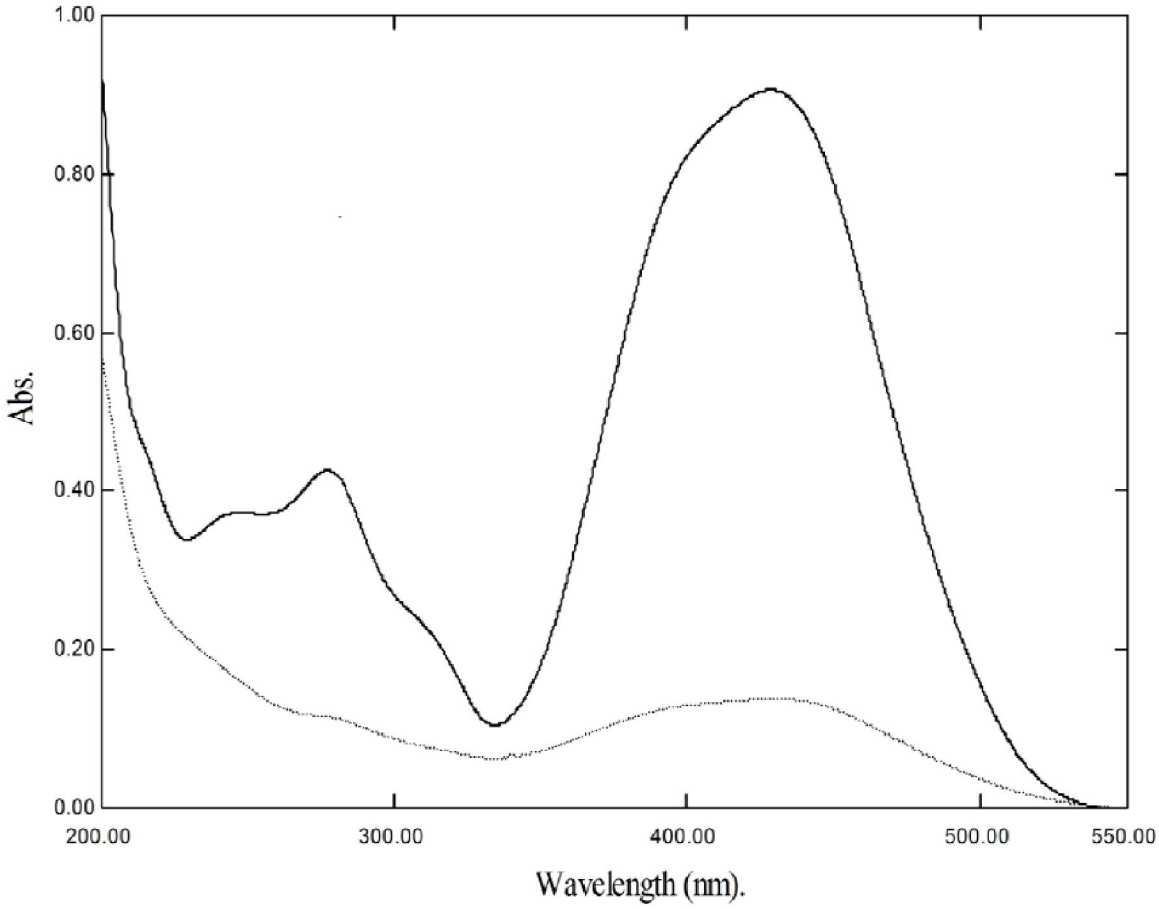

Figure 6 : Zero order spectra of (12 µg/mL) of PAP (−−−−−) and TAP (.....)

TABLE 1 : Linearity studies and regression equations of the proposed methods

Parameters	^{1}D	^{1}DD	RDSM	DW
Wavelength	370	360	428-276	425-256
Calibration range	(1-14 μg ml^{-1})	(1-14 μg ml^{-1})	(1-14 μg ml^{-1})	(1-14 μg ml^{-1})
Slope	0.0407	0.0685	0.1866	0.0450
Intercept	0.0074	0.0120	0.0469	0.0020
Regression coefficient	0.9998	0.9998	0.9999	0.9999
LOD	0.0938	0.0992	0.2789	0.2619
LOQ	0.2842	0.3007	0.8450	0.7935

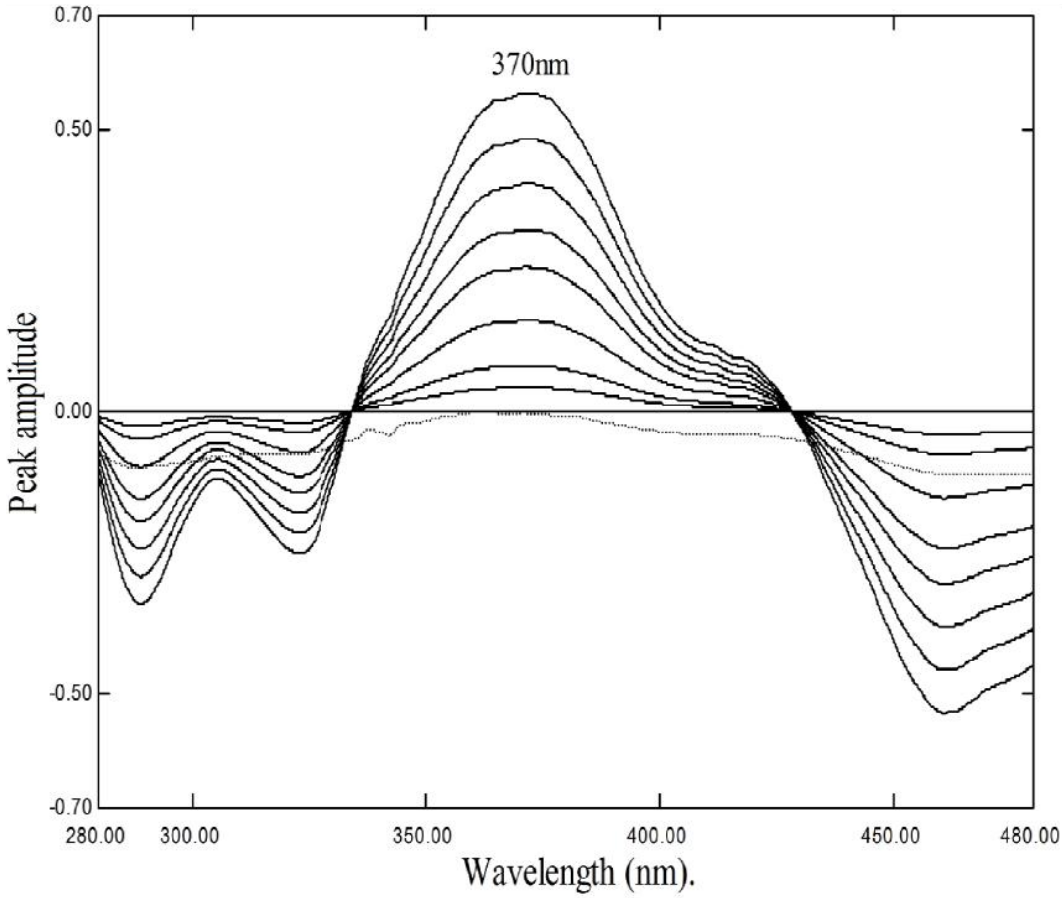

Figure 7 : First derivative spectra of (1-14 μg/mL) PAP (−−−−−) and (9 μg/ml) TAP (.....)

PAP. The yielded statistical results are summarized in TABLE (1).

Derivative ratio (^{1}DD)

In this method, the absorption spectra of the drug was divided by a suitable absorption spectrum of the degradate (divisor) to get the ratio spectra as shown in Figure 8 (a). By application of the first derivative to these ratio spectra, PAP can be quantitatively determined at 360 nm without any interference from its degradation product, as shown in Figure 8 (b). Careful choice of the divisor and the working wavelength were of great importance so different concentrations of degradation product were tried as a divisor (7, 9, 11 and 13 μg/ml); the best one was 9 μg/ml as it produced minimum noise and gave better results in accordance with selectivity. Linear correlation was obtained between the amplitude at 360 nm, against the corresponding concentration of PAP. The yielded statistical results are summarized in TABLE (1).

Ratio difference spectrophotometric method (RDSM)

In this method, the absorption spectra of the drug was divided by a suitable absorption spectrum of

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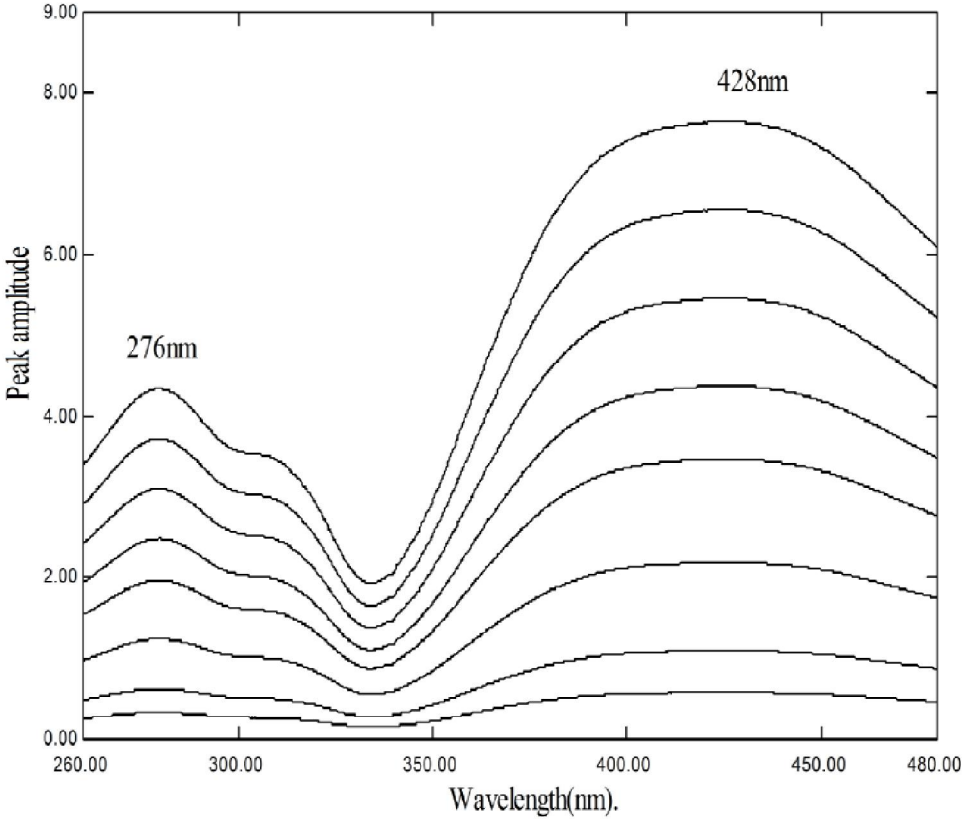

Figure 8 : (a) Ratio spectra of PAP (1–14 µg/mL) using TAP (9µg/mL) as a divisor

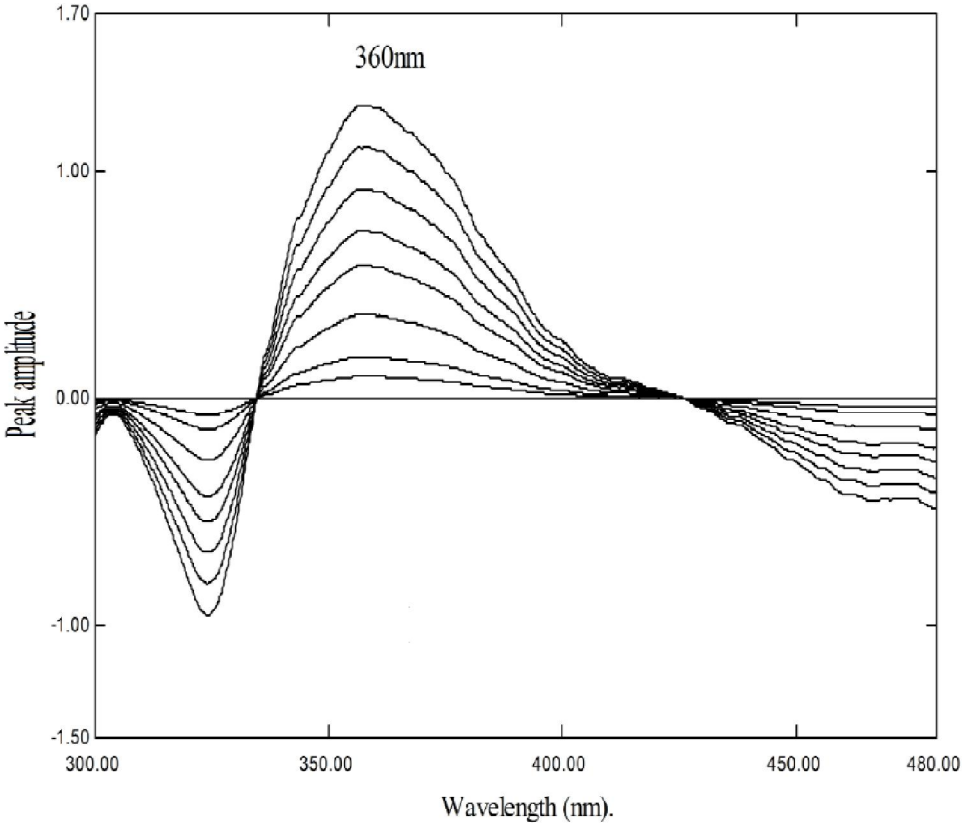

Figure 8(b) : First derivative of ratio spectra of PAP (1-14 µg/mL)

the degradate (divisor) to get the ratio spectra. The difference in peak amplitudes between two selected

TABLE 2 : Method validation obtained by applying the proposed methods

Method	Conc. (µg ml^{-1})	Intraday		Interday	
		Accuracy (R%) ±SD	Precision (RSD %)	Accuracy (R%) ±SD	Precision (RSD %)
^{1}D	3	101.03 ± 0.603	0.597	99.18 ± 0.882	0.889
	5	99.78 ± 1.69	1.701	99.45 ± 1.08	1.09
	8	98.85 ± 0.789	0.798	101.43 ± 0.178	0.175
^{1}DD	3	100.90 ± 1.43	1.42	100.58 ± 0.548	0.545
	5	100.24 ± 1.755	1.759	98.92 ± 0.76	0.775
	8	101.14 ± 0.75	0.74	99.89 ± 1.348	1.349
RDSM	3	100.65 ± 0.854	0.845	100.73 ± 1.12	1.11
	5	99.75 ± 0.334	0.335	99.13 ± 0.903	0.911
	8	99.92±1.754	1.755	100.56 ± 0.826	0.821
DW	3	100.17±1.541	1.539	99.36±1.026	1.032
	5	100.87±0.641	0.635	100.25±0.227	0.277
	8	99.86±1.74	1.750	100.38±1.06	1.064

TABLE 3 : Determination of intact PAP in laboratory prepared mixtures with its degradation product by the proposed methods

Conc. of PAP (µg ml^{-1})	Conc. of DLBT (µg ml^{-1})	% of TAP	^{1}D[a]	^{1}DD[a]	RDSM[a]	DW[a]
12	2	14.2	99.88	99.46	101.62	98.16
9	4	28.5	99.56	98.05	98.35	100.71
7	7	50	101.84	100.46	101.75	99.96
5	9	64.2	101.72	98.54	99.47	98.17
3	11	78.5	98.86	119.22[b]	101.63	116.96[b]
	Mean		100.37	99.12	100.56	99.25
	SD(RSD)		1.339(1.334)	1.625	1.499	0.852

[a] % Recovery, [b] Underlined values are out of accepted range and not considered in the calculation of mean or SD.

wavelengths in the ratio spectra is proportional to the concentration of the drug without interference from its degradate (divisor), as shown in Figure 8 (a). The method comprises two critical steps, the first is the choice of the divisor, and the selected divisor should compromise between minimal noise and maximum sensitivity. The divisor concentrations of 9 ¼g /ml gave the best results. The second critical step is the choice of the wavelengths at which measurements are recorded. Any two wavelengths can be chosen provided that they exhibit different amplitudes in the ratio spectrum and good linearity is present at each wavelength individually. The selected wavelengths are 428and 276 nm (ΔP 428-276 nm) which gave the best results. Linear correlation was obtained between the differences in amplitude at 300-240nm, against the corresponding concentration of PAP. The yielded statistical results are summarized in TABLE (1).

Dual wavelength

The most striking feature of the dual wavelength method is its simplicity and rapidity. From the overlay spectra of PAP and its degradation product shown in Figure (6), two wavelengths 425 nm and 256 nm were selected for the estimation of intact PAP in presence of its degradation product TAP as the degradation product shows the same absorbance at these wavelengths. Linear correlation was obtained between the difference absorbance at 425nm and 256nm, against the corresponding concentration of PAP. The yielded statistical results are summarized in TABLE (1).

METHODS VALIDATION

The proposed methods were validated in compliance with the ICH guidelines[31]. TABLE (2) shows the accuracy and precision of the proposed

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TABLE 4 : Application of standard addition technique to the analysis of urisept® tablets by applying the proposed methods

Taken (μg ml^{-1})	Added standard (μg ml^{-1})	^{1}D[a]	^{1}DD[a]	RDSM[a]	DW[a]
5	3	98.68	100.48	98.34	99.92
	6	98.23	98.41	101.42	99.95
	8	101.25	101.73	100.06	100.70
	9	99.91	101.62	98.08	101.87
Mean ± RSD%		99.52 ± 1.36	100.56± 1.52	99.48 ± 1.57	100.61 ± 0.907

[a]% Recovery

TABLE 5 : Statistical comparison between the results obtained by applying the proposed spectrophotometric methods and reported methods for determination of PAP in urisept® tablets

Parameter	^{1}D	^{1}DD	RDSM	DW	Reported Method
Mean	99.117	100.21	100.24	100.648	100.59
S.D.	0.946	1.386	0.999	1.246	1.541
N	5	5	5	5	5
Variance	0.895	1.92	0.998	1.553	2.374
t-test	1.743 (2.306)	0.332 (2.306)	0.283 (2.306)	0.135 (2.306)	
F-value	2.654 (6.388)	1.236 (6.388)	2.380 (6.388)	1.529 (6.388)	

The values in the parenthesis are the corresponding theoretical values of *t* and *F* at (*P* = 0.05).

methods while TABLE(3) shows the specificity ; recovery of the laboratory prepared mixture of the drug with its oxidative degradation product. LOD, LOQ, linearity and range were shown earlier in TABLE (1).

The validity of the proposed procedures is further assessed by applying the standard addition technique showing no excipients interference. The results obtained were shown in TABLE (4).

STATISTICALANALYSIS

The validity of the proposed methods was tested applying both Student's t- and F-tests (at 95% confidence level)[32]. The results show that the calculated t- and F-values did not exceed the theoretical values as shown in TABLE (5). The assay results were in good agreement with values obtained by applying the reference method[14].

CONCLUSION

Unlike the mostly recommended HPLC-procedures, the proposed spectrophotometric methods are simple and not expensive. The reagents used in the proposed methods are cheap and readily available. The procedures applied in each method do not involve any critical reactions or tedious preprations. This aspect of spectrophotometric analysis is of major interest in analytical pharmacy since it offers distinct possibility of assaying PAP in its pharmaceutical formulation without interference due to the excipient or the degradation product. The suggested methods are found to be accurate, selective and equally sensitive with no significant difference of the precision compared with the reference method. They could be applied for routine analysis of pure drug or in its pharmaceutical formulation.

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