Pharmaceutica Analytica Acta

Ali et al., Pharm Anal Acta 2011, 2:9
DOI: 10.4172/2153-2435.1000140

Review Article Open Access

Simultaneous Determination of Paracetamol and Diphenhydramine Hydrochloride in Presence of Paracetamol Degradation Product

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Abstract

Three sensitive , selective and precise stability indicating methods for simultaneous determination of Paracetamol and Diphenhydramine hydrochloride in their binary mixture and in presence of P-aminophenol; the potential impurity and degradation product of Paracetamol; were developed. In Method A, Paracetamol was determined in presence of Diphenhydramine Hydrochloride and P-aminophenol using the first derivative (^{1}D) spectrophotometric method by measuring the peak amplitude at 264.5 nm where linear relationship was obtained in the range of 2-12 µg mL^{-1} while Diphenhydramine Hydrochloride was determined in presence of Paracetamol and P-aminophenol using the first derivative of ratio spectra (^{1}DD) method at 224 nm. Method B utilized chemometric techniques [Principal Component Regression (PCR) and Partial Least Squares(PLS)] which successfully applied to quantify both drugs and degradation product using the information included in the absorption spectra of appropriate solutions of the three compounds in the range of 220-340nm. Method C used HPTLC-densitometric method in which the aforementioned components were separated on silica gel plates using chloroform-ethyl acetate-ammonia solution (4:6:0.2, by volume) as a developing system. This was followed by quantitative densitometric measurement at 220 nm .Linear relationship were obtained in the concentration ranges of 0.4-1.6 µg/band , 3-12 µg /band and 0.4-1.6 µg/band for Paracetamol, Diphenhydramine Hydrochloride and P-aminophenol respectively. The proposed methods have been successfully applied to the analysis of Paracetamol and Diphenhydramine Hydrochloride in their pharmaceutical formulation without interference from other dosage form additives and the results were statistically compared with official method .

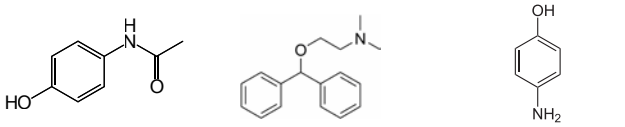
Keywords: Paracetamol; Diphenhydramine Hydrochloride; P-aminophenol; First derivative spectrophotometric; Derivative ratio spectrophotometric; Multivariate spectral analysis; HPTLC-densitometric method

Introduction

Multicomponent formulations have gained lot of importance nowadays due to greater patient acceptability, increased potency, multiple action and quick relief [1]. Market is flooded with combination of drugs in various dosage from one of such combination is PAR and DPH.

Paracetamol (PAR) is N-(4-hydroxy phenyl) acetamide. It is Para-aminophenol derivative, it has analgesic and anti pyretic properties [2].

Diphenhydramine Hydrochloride (DPH) is 2-Diphenyl methoxy-N,N dimethylethanamine, it has antihistaminic action and used for symptomatic relief of hypersensitivity reactions [3] (structure 1).



a-Paracetamol b-Diphenhydramine .HCl c- P-aminophenol

These two drugs are co-formulated in pharmaceutical product for temporary relief of pain when associated with sleeping difficulty.

Simultaneous estimation of drugs in combination is an important part in the field of pharmacy as it avoids time of extraction, separation and gets accurate and precise results. U.S. Pharmacopeia (USP 2007) has described a monograph to quantities the binary mixture of PAR and DPH using C18 column, phosphate buffer- acetonitrile (94:6 v/v) as a mobile phase with UV detection at 225 nm at 4.6 and 8.3 minutes for PAR and DPH respectively [4].

Few analytical methods have been described for simultaneous determination of PAR/DPH in their binary mixture including NIR spectroscopy [5], multivariate spectrophotometric method [6], and HPLC methods [7-9].

There is no published literature dealing with simultaneous quantification of PAR and DPH in presence of PAP; a potential impurity and major degradation product of PAR [10], in bulk material and pharmaceutical preparations.

The present work describes simple, sensitive, precise, accurate, less expensive and less time consuming methods compared with other HPLC method for the simultaneous determination of PAR and DPH to be used in stability studies and quality control applications associated with these drugs.

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Received August 05, 2011; **Accepted** November 15, 2011; **Published** November 17, 2011

Citation: Ali NW, Zaazaa HE, Abdelkawy M, Magdy MA (2011) Simultaneous Determination of Paracetamol and Diphenhydramine Hydrochloride in Presence of Paracetamol Degradation Product. Pharm Anal Acta 2:140. doi:10.4172/2153-2435.1000140

Material and Methods

Instruments

A double beam UV-visible spectrophotometer (SHIMADZU, Japan) model UV-1601 PC with quartz cell of 1 cm pathlength, connected to IBM compatible computer. The software was UVPC personal spectroscopy software version 3.7.The spectral band width was 2 nm and wavelength-scanning speed 2800 nm/min. All data analysis was performed using PLS-Toolbox 2.0 running under MATLAB®, version 6.5

- UV lamp with short wavelength 254 nm (USA).

- TLC scanner 3 densitometer (Camag, Muttenz, Switzerland).

The following requirements are taken into consideration:

- Slit dimensions:5mm ×0.2 mm.
- Scanning speed: 20 mm/S
- Sraying rate : 10 μL^{-1}
- Data resolution: 100 µm/step

- TLC plates (20 cm × 20 cm) coated with silica gel 60F254 (Fluka,Sigma-Aldrich Chemie Gmbh, Germany).

- Sample applicator for TLC Linomat IV with 100 µL syringe (Camag, Muttenz, Switzerland).

Materials

(a) Pure standard

Paracetamol and Diphenhydramine Hydrochloride were kindly supplied by GlaxoSmithKline Company Egypt. Their purity was found to be 100.11 ± 1.14 % and 99.96 ±1.02 %, respectively according to the official HPLC method [4].

(b) Pharmaceutical Dosage forms

Panadol night® tablets (Batch No. 117928) labeled to contain 500 mg of Paracetamol and 25 mg of Diphenhydramine Hydrochloride , manufactured by GlaxoSmithKline company .

(c) Chemicals and reagents

All reagents and chemicals used throughout this work were of analytical grade and were used without further purification

Methanol of HPLC grade (CHROMASOLV®,Sigma-Aldrich Chemie Gmbh, Germany). Chloroform, 33% ammonia solution and ethylacetate (El-Nasr Pharmaceutical Chemicals Co., Abu-Zabaal, Cairo, Egypt). P-aminophenol (Degradation product of PAR) was purchased from Riedel-dehaen-AG- Germany; its purity was certified to be 99%.

Standard solutions

(a) Stock standard solutions of PAR, DPH and PAP (1 mg mL^{-1} in methanol): 0.1 gm of PAR, DPH and PAP were accurately weighed into three separate 100 mL volumetric flasks, 50 mL of methanol was added to each flask, shaken to dissolve then the volume was completed to the mark with methanol.

(b) Working standard solutions of PAR and PAP (100 µg mL^{-1}), while working standard solution of DPH (25µg mL^{-1}). They were prepared by suitably diluting the stock standard solutions with methanol.

Laboratory prepared mixtures

Mixtures containing different ratios of PAR, DPH and PAP were prepared using their respective working solutions in methanol (for derivative, ^{1}DD methods and multivariate method) and from their stock standard solutions in methanol (for densitometric method).

Procedures

Derivative and first derivative of ratio spectra spectrophotometric methods

Spectral characteristics of Paracetamol, Diphenhydramine Hydrochloride and P-aminophenol: The absorption spectra of 10, 8 and 10 µg mL^{-1} of PAR, DPH and PAP solutions, respectively, were recorded using methanol as a blank.

Linearity and Construction of calibration curves: The ^{1}D spectra of PAR solutions in the range of 2 – 12 µg mL^{-1} were recorded using $\Delta\lambda$ = 4 and scaling factor =10, then the peak amplitude was measured at 264.5 nm. The ^{1}DD spectra of DPH solutions in the range of 5-18 µg mL^{-1} were recorded using10 µg mL^{-1} of PAP as a divisor, $\Delta\lambda$ = 4 and scaling factor =10, then the peak amplitude was measured at 224 nm.

The calibration curves were constructed relating the peak amplitudes against the corresponding drug concentrations and the regression equations were calculated.

Analysis of laboratory prepared mixtures of Paracetamol, Diphenhydramine Hydrochloride and P-aminophenol: Into a series of 10 mL volumetric flasks, mixtures containing 40-100 µg PAR and 5 µg DPH were prepared then each flask was spiked with 75 µg DPH, different aliquots of PAP in the range of 10-60 % PAR were added and finally the volume was completed with methanol. Into a separate volumetric flask solution (x) contain 7.5 µg mL^{-1} of DPH solution was prepared.

The peak amplitude of ^{1}D spectra of the mixture was then recorded and the measured peak at 264.5 nm was used to calculate concentration of PAR as mentioned under 3.1.2.

The peak amplitude of ^{1}DD spectra at 224 nm were then recorded for both mixture and solution (x) , concentration of DPH in the mixture could be obtained by calculating concentration of solution (x) from corresponding regression equation then subtract its concentration from the total concentration of DPH in the mixture.

Chemometric methods

Construction of the training set: Multilevel multifactor design was used for the construction of the calibration and validation sets [11]. A five-level, five-factor calibration design was used. Different mixtures of PAR, DPH and PAP in different ratios were prepared (Table 1). The absorption spectra of the prepared mixtures were recorded and transferred to Matlab for subsequent data manipulation. Thirteen mixtures were used for building the calibration model, while 8 mixtures were chosen to be used as an external validation set. Several multivariate calibration models (PCR and PLS) were constructed using the data obtained. Initial developed models were found to have high spectral residuals in the region below 220 and above 340 nm; as a result, this region was rejected.

Selection of the optimum number of factors to build the PCR and PLS models: The cross validation method was used, leaving out one sample at a time, to select the optimum number of factors [12].

Given a set of 13 calibration samples, the PCR and PLS calibrations were performed, and using this calibration, the concentration of the sample left out was predicted. The predicted concentrations were then compared with the actual concentrations and the root mean square error of cross validation (RMSECV) was calculated. The RMSECV was calculated in the same manner each time a new factor was added to the model. Visual inspection was used for selecting the optimum number of factors. Upon building the models mean centering the data gave better results for both PCR and PLS.

Construction of the validation set

Eight different mixtures of PAR, DPH and PAP were prepared by transferring different volumes of their working standard solutions as mentioned in Table 1. The developed models were applied to predict the concentration of PAR, DPH and PAP in each mixture.

HPTLC-densitometric method

Into a set of 10-mL volumetric flask, different aliquots of PAR, DPH and PAP were accurately transferred from their working solutions; the volume was then completed with methanol. 10 µL of each solution was spotted as bands of 6 mm width on TLC plates (20 × 10 cm with 250 µm thickness) using a Camag Linomat IV applicator. The bands were applied at 5 mm intervals and 10 mm from the bottom and sides. Linear ascending chromatogram developing to a distance of 8 cm was performed in a chromatographic tank previously saturated for 30 min with the developing mobile phase consisted of chloroform– ethyl acetate–ammonia solution (4: 6: 0.2 by volume) at room temperature.

The peak areas were recorded using scanning wavelength at 220 nm and the calibration curves were constructed by plotting the integrated peak area versus the corresponding concentrations of each drug and the regression equations were computed.

Analysis of laboratory prepared mixtures of PAR, DPH and PAP

Mixtures that contained different concentration of PAR, DPH and PAP were analyzed by applying the same procedure mentioned under 3.3.

Application to pharmaceutical formulation (Panadol night® tablets)

The contents of 10 Panadol night® tablets were powdered and mixed well. An accurately weighed portion of the powdered tablet equivalent to 500 mg of PAR and 25 mg of DPH was transferred into 100-mL volumetric flask; 75 mL methanol was added and sonicated for 30 min, filtered, and then completed to volume with methanol. Part of the above solution is diluted to obtain working solution for PAR (100 µgmL^{-1}).

Another part of the solution was diluted to obtain working solution of DPH (25 µg mL^{-1}) using methanol as a solvent. PAR, DPH and PAP were analyzed by applying the same procedure mentioned under 3.1.3 and 3.3 then the concentration of PAR and DPH was calculated from the corresponding regression equation.

Results and Discussion

Diphenhydramine Hydrochloride was formulated with PAR in a medicinally recommended ratio of 1:20. Analysis of such ratio is a challenging because the amount of the major component PAR is much greater than the amount of minor component DPH. Therefore the aim of this work was to develop simpler and less complicated method than HPLC method and more sensitive than NIR method for determination of minor component DPH in its binary mixture. Moreover; the modem quality control of pharmaceuticals concerns with the determination of the main active component and its purity and stability or quantification of impurities. The presence of impurities, even in small amounts, may influence the efficacy and safety of pharmaceutical products and, therefore control of pharmaceutical impurities has become a critical issue to the pharmaceutical industry [13]. P-Aminophenol (PAP) is the principal impurities of PAR and also the main degradation product which is known by its potent nephrotoxicity [14]. Thus accurate and precise quantification of active compound PAR together with its degradation product in the pharmaceutical formulations is necessary.

First Derivative (^{1}D) and first derivative of ratio spectra (^{1}DD) spectrophotometric methods

As shown in Figure 1, the spectra of PAR, DPH and PAP showed extensive overlap; therefore congenital UV-spectra cannot be used for their determination. Sharp band was produced for PAR in this combination when ^{1}D spectra were recorded (DPH and PAP showed zero-crossing at 264.5 nm), Figure 2. Different factors affecting resolution including type of solvent, Δλ and scaling factor were studied to optimize resolution of PAR. The best resolution was obtained using Δλ= 4nm, scaling factor=10 and methanol as solvent.

While selective determination of DPH could be achieved by ^{1}DD spectrophotometric method where the trough at 224nm (zero crossing of PAR) can be adopted for determination of DPH in presence of PAR and PAP, Figure 3. Different factors affecting resolution including divisor concentration, type of solvent, Δλ and scaling factors were studied to optimize resolution of drugs. The best resolution was obtained using 10 µg mL^{-1} of PAP as a divisor, Δλ=4nm, scaling

Mixture No.	Paracetamol (µg mL^{-1})	Diphenhydramine Hydrochloride (µg mL^{-1})	Para-aminophenol (µg mL^{-1})
1	6	12	6
2	10	15	8
3	8	18	6
4	6	15	10
5	10	12	10
6	10	18	2
7	2	18	8
8	2	15	6
9	6	6	8
10	8	12	8
11	8	15	4
12	4	15	2
13	2	9	4
14	4	6	6
15	2	12	2
16	4	18	10
17	6	18	4
18	4	12	4
19	8	9	10
20	2	12	4
21	10	6	10

- The concentrations of mixtures used in the validation set are highlighted.

Table 1: The concentration of mixtures of Paracetamol, Diphenhydramine Hydrochloride and Para-aminophenol used in the training and validation sets.

factor=10 and methanol as solvent in terms of signal to noise ratio, sensitivity and selectivity.

However the quantitative determination of DPH was failed and deviation from Beer's law occur. This may be due to the fact that; solution mixtures and tablets contain two drugs, one in low concentration and the second in high concentration, the molar absorbitivity of the former is alerted due to the electrostatic attraction between both ions and deviation from Beer's law occurs [15,16]. Therefore the use of derivative ratio technique can't cope with the level of interference of PAR in the absorption spectrum of DPH. Thus, in tablets formulation DPH should be detected in a very low concentration where the interference of PAR is eliminated and this could be achieved by adding a fixed amount of standard DPH to each experiment then subtract its concentration before calculating the claimed concentration of the drug [17,18].

Linear correlations were obtained between peak amplitudes at 264.5nm for ^{1}D spectra of PAR in the concentration range 2–12 µg mL^{-1} and peak amplitudes at 224 nm for ^{1}DD spectra of DPH in concentration range of 5–18 µg mL^{-1} from which the regression equations were calculated and found to be:-

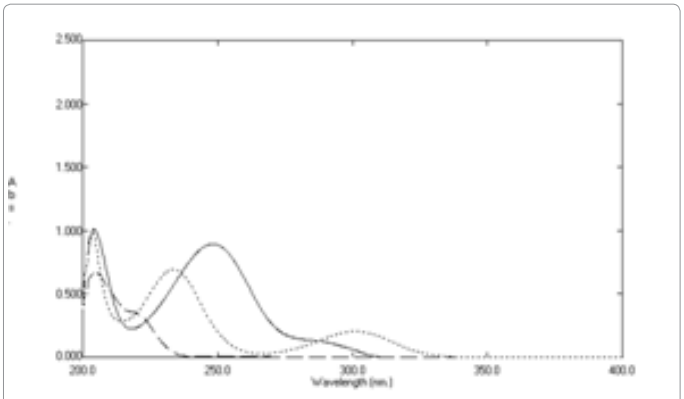

Figure 1: Zero order absorption spectra of 10 µg mL^{-1} of Paracetamol (—), 8 µg mL^{-1} of Diphenhydramine Hydrochloride (- - -) and 10 µg mL-1 P-aminophenol (.....) using methanol as a solvent.

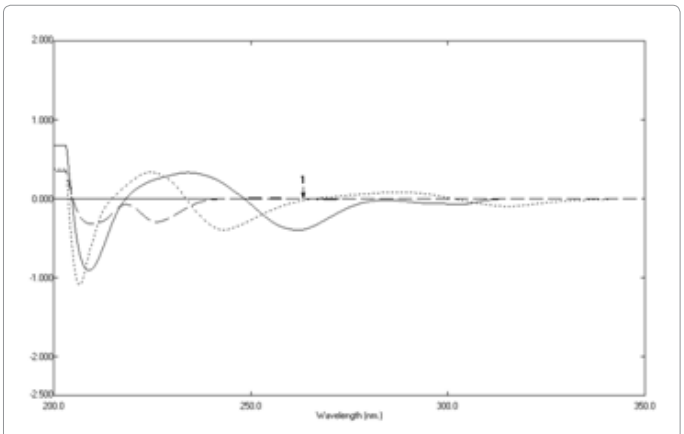

Figure 2: First derivative absorption spectra of 10 µg mL^{-1} of Paracetamol (—), 8 µg mL^{-1} of Diphenhydramine Hydrochloride (- - -) and 10 µg mL-1 P-aminophenol (.....) using methanol as a solvent.

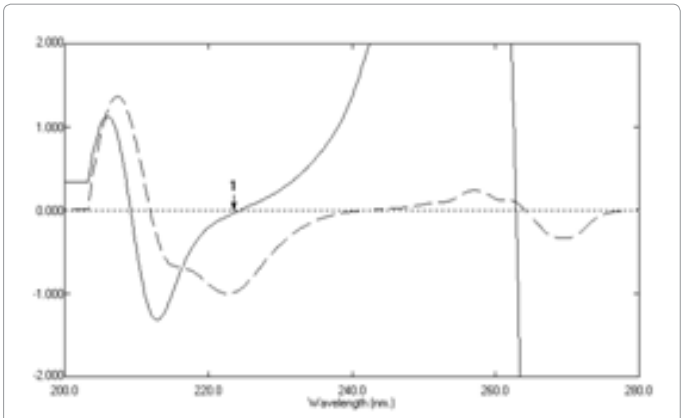

Figure 3: First derivative of ratio spectra of 25 µg mL^{-1} of 10 µg mL^{-1} of Paracetamol (—), 8 µg mL^{-1} of Diphenhydramine Hydrochloride (- - -) and 10 µg mL^{-1} P-aminophenol (.....) using 10 µg mL^{-1} of Para-aminophenol as a divisor and methanol as a solvent.

$P.A_1 = 0.037386C_1 + 0.007467$ $r_1 = 0.9997$ at 264.5 nm for PAR

$P.A_2 = 0.110529\ C_2 + 0.09632$ $r_2 = 0.9998$ at 224 nm for DPH

Where $P.A_1$ and $P.A_2$ are the peak amplitudes of PAR and DPH using ^{1}D and ^{1}DD methods respectively, C_1 and C_2 are the concentration of PAR and DPH in µg mL^{-1}, respectively, and r_1, r_2 is the correlation coefficients.

Results described in Table 2 showed that this method is selective, valid and applicable for the determination of PAR and DPH in presence of up to 60 % of PAP in different laboratory prepared mixtures.

Chemometric methods

In this method, different chemometric approaches were applied for the determination of PAR, DPH and PAP, including PCR and PLS. These multivariate calibrations were useful in spectral analysis because the simultaneous inclusion of many spectral wavelengths instead of a single wavelength greatly improved the precision and predictive ability [19].

The first step in the simultaneous determination of the components by multivariate calibration methods involves constructing the calibration matrix for ternary mixture. The calibration set was obtained by using the absorption spectra of a set of 13 mixtures of PAR, DPH and PAP with different ratios of each component as given in Table 1. Better results were obtained upon rejecting the spectral region above 220 nm and below 340 nm.

In this study, the „leave one out cross validation method was used and the RMSECV values of different developed models were compared. Nine factors were found suitable for both PCR and PLS models. To validate the prediction ability of the suggested models, the validation set given in Table 1 was used to predict the concentration of PAR, DPH and PAP, where satisfactory results were obtained as shown in Table 3.

The predicted concentrations of the validation samples were plotted against the true concentration values. This was used to determine whether the model accounted for the concentration variation in the validation set. All plots had a slope of nearly one and an intercept close to zero.

The RMSEP was another diagnostic tool for examining the errors

Deg (PAP) %	Ratio DHP: PAR	Concentration (µg ML^{-1})			^{1}D method (PAR) Recovery %	^{1}DD method (DPH) Recovery %
		DPH	PAR	PAP		
0.00	*1:20	8	10	0.00	100.00	98.75
10	*1:20	8	9	1	101.11	98.50
20	*1:20	8	8	2	100.48	98.37
30	*1:20	8	7	3	98.42	98.62
40	*1:20	8	6	4	98.33	98.75
50	*1:20	8	5	5	100.80	98.50
60	*1:20	8	4	6	102.50	98.87
Mean ± SD					100.48±1.486	98.62±0.176

* The ratio present in Panadol night® tablets prepared by spiking technique where 8µg/mL of DPH is (7.5 µg/mL + 0.5 µg/mL) [17].

Table 2: Determination of Paracetamol and Diphenhydramine Hydrochloride in presence of P-aminophenol in laboratory prepared mixtures by the proposed derivative and first derivative of ratio spectra spectrophotometric methods.

Validation parameters	PAR		DPH		PAP	
	PLS	PCR	PLS	PCR	PLS	PCR
Mean ± SD	101.00± 1.20	100.61± 1.42	100.09± 0.75	100.40± 1.10	99.41± 1.16	99.51± 1.35
RMSEP	0.1025	0.1184	0.0106	0.1166	0.0836	0.00707
Predicted versus actual concentration plot						
a-Slope b-Intercept c-Correlation coefficient (r)	1.0050 0.0100 0.9996	1.0106 -0.0139 0.9998	1.0011 -0.0187 0.9996	1.0029 0.0042 0.9994	0.9837 0.0637 0.9996	1.0189 -0.1065 0.9994

Table 3: Results of assay validation parameters of the proposed multivariate method for determination of Paracetamol, Diphenhydramine Hydrochloride and P-aminophenol.

Mix NO	Deg (PAP) %	Concentration (µg/band)			Recovery %		
		DPH	PAR	PAP	DPH	PAR	PAP
1	20	5	1.6	0.4	98.20	98.12	100.25
2	30	5	1.4	0.6	102.00	99.00	98.33
3	40	5	1.2	0.8	100.00	100.00	100.62
4	50	5	1	1	99.20	101.20	101.00
5	60	5	0.8	1.2	99.40	98.00	100.83
6	70	5	0.6	1.4	101.60	102.00	99.28
7	80	5	0.4	1.6	98.00	99.00	99.37
8*	0.00	5*	100*	ZERO	99.20	-------	Zero
9*	0.00	0.05*	1*	ZERO	-------	101.30	Zero
Mean ± SD					99.30± 1.45	99.50± 1.53	100.25± 0.98

*The ratio present in Panadol night® tablets
N.B: DPH concentration was determined in mixture No 8, while in mix No 9 PAR concentration was determined after dilution of mix No 8

Table 4: Determination of Paracetamol and Diphenhydramine Hydrochloride in presence of Para-aminopheno in laboratory prepared mixtures by the proposed densitometric method.

in the predicted concentrations; it indicates both the precision and accuracy [12].

Results of assay validation parameters of the proposed models are shown in table 3.

HPTLC-densitometric method

TLC-densitometry is a useful technique for the resolution and, in turn, for the determination of drug mixtures. This technique offers a simple way to quantify directly on TLC plate by measuring the optical density of the separated bands. The amounts of compounds are determined by comparing to a standard curve from reference materials chromatographed simultaneously under the same condition [20].

To improve separation of bands, it was necessary to investigate the effect of different variables. Studying the optimum parameters for maximum separation was carried out as following:

Mobile phase

Different developing systems of different composition and ratios were tried for separation, e.g., chloroform–methanol (9:1, v/v), chloroform–ethyl acetate (8:2, v/v), chloroform–acetone (7:3, v/v) and chloroform–methanol–acetic acid (8:2:0.2, by volume). The best mobile phase was chloroform–ethyl acetate–ammonia solution (4:6:0.2, by volume). This selected mobile phase allows good separation between the ternary mixtures with good R_f values without tailing of the separated bands (Figure 4).

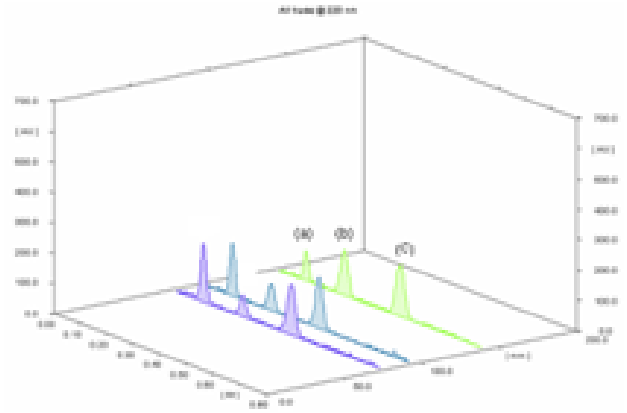

Figure 4: Thin layer chromatogram of separated peaks of (a) Paracetamol, (b) P-aminophenol and (c) Diphenhydramine Hydrochloride using chloroform:ethylacetate:ammonia solution (4:6:0.2, by volume) as developing system

Band dimensions

Different band dimensions were tested in order to obtain sharp and symmetrical separated peaks. The optimum band width chosen was 6 mm and the inter-space between bands was 5 mm.

Scanning wavelength

Different scanning wavelengths were tried, where 220 nm was the best wavelength for all drugs at which peaks were sharper and symmetrical and minimum noise was obtained; at this wavelength maximum sensitivity for all drugs was obtained.

Slit dimensions of scanning light beam

The slit dimensions of the scanning light beam should ensure complete coverage of band dimensions on the scanned track without interference of adjacent bands. Different slit dimensions were tried, where 5 mm ×0.2 mm proved to be the slit dimension of choice which provides highest sensitivity.

System suitability

System suitability testing of TLC-densitometric method gave good resolution (RS= 3.25, 2.11), selectivity factor (=1.93, 1.7), capacity factor (for PAR=0.4, for PAP=1.7, for DPH=3.6) and symmetry factor of 1.1 for PAR, 1.04 for PAP and of 1.4 for DPH.

This method is based on the difference in the R_f values of PAR (R_f= 0.13), of PAP (R_f= 0.3) and of DPH (R_f= 0.5)

The calibration curves were constructed by plotting the integrated peak area versus the corresponding concentrations in the range of 0.4–1.6 µg /band for PAR, 3–12 µg/ band for DPH and 0.4-1.6 µg/ band for PAP

The concentration of PAR, DPH and PAP were calculated from the following regression equations.

For PAR $Y_1 = 0.2675C_1 + 0.0782$ $r_1 = 0.9997$

	Technique	component	Taken (µg mL-1)		Found % ± SD	
Panadol night® tablets claimed to contain 25mg DPH and 500 mg PAR/ tablet (Batch No 117928)	**First derivative spectrophotometric method (D^1)**	PAR	4		98.50 ± 0.76	
	First derivative of ratio spectra specrophotometric method (DD^1)	DPH	8*(7.5pure + 0.5 dosage form)		102.00 ± 0.72	
	Multivariate method	PAR	PLS	4	99.25 ± 0.39	100.99 ± 1.47
			PCR	4	98.35 ± 0.85	99.37 ± 1.40
		DPG	PLS	7*	101.40 ± 0. 31	99.58 ± 1.63
			PCR	7*	98.20 ± 0.25	99.70 ± 1.35
	Densitometric method	PAR	0.4		99.00 ± 0.25	100.00 ± 1.31
		DPH	5		101.60 ± 1.70	101.70 ± 1.62

* The ratio present in Panadol night® tablets prepared by spiking technique [17], where 7µg/mL of DPH is (6.5 µg/mL of pure drug + 0.5 µg/mL of dosage form)

Table 5: Determination of Paracetamol and Diphenhydramine Hydrochloride in their pharmaceutical formulation by the proposed methods and application of standard addition technique.

Parameters	**Derivative method (PAR)**	**^{1}DD method (DPH)**	**Densitometric method**		
			PAR	**DPH**	**PAP**
Range (µg mL-1)	2-12	5-18	0.4-1.6	3-12	0.4-1.6
Linearity Slope Intercept Correlation coefficient (r)	0.0373 0.0074 0.9997	0.1105 0.0963 0.9998	0.2675 0.0782 0.9997	0.0651 0.1561 0.9999	0.3151 0.1206 0.9998
Accuracy (mean ± SD)	99.94 ± 1.33	99.70 ± 0.99	100.00 ± 0.93	100.35 ± 0.86	100.10 ± 0.91
Selectivity	100.48 ± 1.48	98.62 ± 0.17	99.50 ± 1.53	99.30 ± 1.45	100.25 ± 0.98
Precision (RSD%) Repeatability* Intermediate precision*	0.72 0.82	0.25 0.40	0.44 0.52	0.57 0.73	0.57 0.65

* The intraday precision (n=3), average of three different concentrations repeated three times within day. The interday precision (n=3), average of three different concentrations repeated three times in three successive days.

Table 6: Results of assay validation parameters of the proposed methods for determination of Paracetamol, Diphenhydramine Hydrochloride and P-aminophenol.

Items	Derivative method (PAR)	DD[1] method (DPH)	Multivariate method				Densitometric method		Official method [4]*	
			PAR		DPH					
			PLS	PCR	PLS	PCR	PAR	DPH	PAR	DPH
Mean	99.95	99.7	101.00	100.61	100.09	100.40	100.00	100.35	100.11	99.96
SD	1.33	0.99	1.20	1.42	0.75	1.10	0.93	0.86	1.14	1.02
RSD %	1.33	0.99	1.18	1.41	0.74	1.09	0.93	0.85	1.13	1.02
n	6	7	8	8	8	8	7	8	6	7
Variance	1.76	0.98	1.44	2.01	0.56	1.21	0.86	0.73	1.29	1.04
Student's t-test	0.071 (2.22)	0.798 (2.17)	0.943 (2.17)	0.684 (2.17)	0.734 (2.16)	0.108 (2.16)	0.251 (2.20)	0.218 (2.16)	-	-
F-value	1.365 (5.05)	1.059 (4.28)	1.107 (4.87)	1.550 (4.87)	1.857 (3.86)	1.928 (4.20)	1.489 (4.38)	1.424 (3.86)	-	-

* HPLC method using C18 column , phosphate buffer- acetonitrile (94 :6 by volume) as a mobile phase with UV detection at 225 nm at 4.6 and 8.3 min for PAR and DPH respectively at a flow rate of 1 ml min^{-1}

Table 7: Statistical comparison of the results obtained by the proposed methods and the official method for the determination of pure Paracetamol and Diphenhydramine Hydrochloride.

Items	Derivative method (PAR)	DD[1] method (DPH)	Multivariate method				Densitometric method		Official method [4]*	
			PAR		DPH					
			PLS	PCR	PLS	PCR	PAR	DPH	PAR	DPH
Mean	98.50	102.00	99.25	98.35	101.4	98.20	100.0	101.7	98.69	99.90
SD	0.76	0.72	0.39	0.85	0.31	0.25	1.31	1.62	0.64	1.54
RSD%	0.77	0.70	0.39	0.86	0.30	0.25	1.31	1.59	0.65	1.54
n	6	6	6	6	6	6	6	6	6	6
Variance	0.57	0.51	0.15	0.72	0.09	0.06	1.71	2.62	0.40	2.371
Student's t-test	0.256 (2.30)	2.260 (2.73)	1.697 (2.26)	1.036 (2.30)	2.095 (2.26)	1.810 (2.36)	0.788 (2.36)	0.321 (2.30)	-	-
F-value	1.276 (5.40)	3.002 (6.25)	2.640 (6.25)	1.751 (5.40)	0.042 (0.15)	0.026 (0.05)	6.601 (19.2)	1.225 (5.40)	-	-

Table 8: Statistical comparison of the results obtained by the proposed methods and the official method for the determination of dosage form Panadol night® tablets.

For DPH $Y_2 = 0.0651C_2 + 0.1561$ $r_2 = 0.9999$

For PAP $Y_3 = 0.3151C_3 + 0.1206$ $r_3 = 0.9998$

Where Y_1, Y_2 and Y_3 are the integrated peak area of PAR, DPH and PAP respectively, C1, C2 and C3 are the concentration of PAR, DPH and PAP in µg/band and r_1,r_2 and r_3 are the correlation coefficient of PAR, DPH and PAP respectively.

The proposed methods were successfully applied for the determination of PAR and DPH in their pharmaceutical formulation (Panadol night® tablets). Results obtained are shown in table 5 which reveals that there is no interference by excipients and additives that was further assessed by applying the standard addition technique.

Method validation according to ICH guidelines was performed and results of accuracy, repeatability and intermediate precision of the proposed methods are shown in table 6.

Other regression equation parameters in table 6 show good linear relationship for the method as revealed by the correlation coefficient.

Tables 7 and 8 shows statistical comparison of the results obtained by the proposed methods and the official HPLC method for the determination of PAR and DPH. The calculated t and F-values are less than the theoretical ones indicating that there is no significant difference between the proposed methods and the official method with respect to accuracy and precision.

Conclusion

The proposed methods are efficient for providing sensitive, accurate and selective analysis for determination of PAR and DPH in their binary mixture and in presence of PAP either in bulk powder or in pharmaceutical formulation.

Spectrophotometric methods are well known for their simplicity and easy application when used for determination of pharmaceutical drugs. The development of chemometric techniques has solved many of the problems of the simultaneous analysis of multi component mixtures with the advantage of no pretreatment steps. Spectrophotometric methods can be regarded as a useful alternative to chromatographic techniques in the routine quality control analysis of pharmaceutical formulations allowing rapid determination at relatively low cost.

The advantage of HPTLC densitometric method over spectrophotmetric one is that several samples can be run simultaneously using a small quantity of mobile phase, thus lowering analysis time and cost per analysis and providing high sensitivity and selectivity. The disadvantage of HPTLC densitometric method is that it requires a complicated instrument.

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