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A NOVEL APPLICATION OF HYDROTROPIC SOLUBILIZATION IN DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF PARACETAMOL AND DICLOFENAC SODIUM IN SOLID DOSAGE FORM

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ABSTRACT

Three simple, rapid, accurate and economical analytical methods are described for the determination of Paracetamol and Diclofenac sodium in combined tablet dosage form. In the present investigation, 1.0 M urea solution (hydrotropic solubilizing agent) was employed to solubilize, Paracetamol (a poorly water-soluble drug) from fine powder of its tablets to carryout spectrophotometric analysis. Simultaneous estimation was carried out by three method, Method –A derivative spectrophotometry method, Method-B area under curve method and Method-C multi-component method.The result showed that Beer's law was obeyed in concentration range of 2-40 µg/ml with good linearity (r^2>0.99) for both the drugs in all the three methods. The recoveries were within 98.93-101.01% for paracetamol and 99.6-101.4% for diclofenac sodium. Precision was good with acceptable limits of detection (LOD) and quantitation (LOQ) for both compounds. The average content of the compounds were 100.22 and 99.11% in method-A, 99.05 and 101.32% in method-B and 99.98 and 100.90% in method-C for paracetamol and diclofenac sodium respectively. The optimized methods showed good reproducibility and recovery with standard deviation of < 1.0% and percent relative standard deviation less then 2.0%.

KEYWORDS

Derivative spectrophotometry method, Area under curve method, Multi-component method, Paracetamol, Diclofenac Sodium, Hydrotropic agent

INTRODUCTION

Increasing the aqueous solubility of insoluble and slightly soluble drugs is of major importance. Various techniques have been employed to enhance the aqueous solubility of poorly water-soluble drugs. Hydrotropic solubilization is one of them. The term hydrotropy has been used to designate the increase in solubility of various substances in water due to the presence of large

amounts of additives[1],[2]. Various organic solvents have been employed for the solubilization of poorly water soluble drugs for spectrophotometric estimations. Drawbacks of organic solvents include higher cost, toxicity, pollution and error in analysis due to volatility. The primary objective of this study was to employ hydrotropic solubilizing agents for the selected drugs to preclude the use of organic solvents. Chemically Paracetamol(PA) is 4 – hydroxy acetanilide, has analgesic and antipyretic activity[3],[4]. Literature survey revealed that chromatographic method was reported for its estimation from tablet formulation [5],[6] and spectrophotometric methods for estimation in combined dosage forms[7].

Diclofenac Sodium(DS) is chemically Sodium salt of 2-[{2,6-dichlorophenyl}amino] benzene acetic acid. It is having anti-inflammatory and analgesic properties [3],[4]. Literature survey revealed that chromatographic method was reported for its estimation from tablet dosage form [8] and spectroscopic methods for estimation in combine dosage forms [9]. But so far no spectrophotometric methods has been reported for simultaneous estimation of PA and DS in combined dosage form, hence an attempt has been made to develop simple, sensitive, economical, rapid, precise and accurate methods to analyze the drugs simultaneously.

MATERIALS AND METHODS

UV-Visible double beam spectrophotometer, Shimadzu model-1700 having spectral bandwidth 3nm and of wavelength accuracy ±1nm, with 1cm quartz cells was used. Pure sample of PA and DS was obtained as gift sample from Zenith Pharma pvt. Ltd, Indore (MP), India. The tablet dosage form, Diclogesic (contain PA 500mg, DS 50Mg) was procured from the local market, Indore, India.1.0 M urea was selected as hydrotropic solubilizing agent. All other material used was of analytical reagent grade.

Method A: Derivative Spectrophotometry Method

In this method[10], [11] 20µg/ml solution for both the drugs were prepared and scanned in the range of 400nm to 200nm. The spectra obtained were derivatized in first order and overlain spectra of both drugs given in Figure1 which showed PA had zero crossing point at 247 nm while DS had zero crossing point at 276nm. At the zero crossing point of PA, DS showed a measurable dA/dλ where as at the zero crossing point of DS, PA showed appreciable dA/dλ. Hence both wavelengths 247 nm and 276 nm were selected as analytical wavelengths for estimation of PA and DS respectively. Calibration curves were plotted for PA (2-40µg/ml) at 247 nm and DS (2-40µg/ml) at 276 nm taking dA/dλ v/s concentration. The concentrations of both the drugs were obtained from the standard calibration curves by interpolation method.

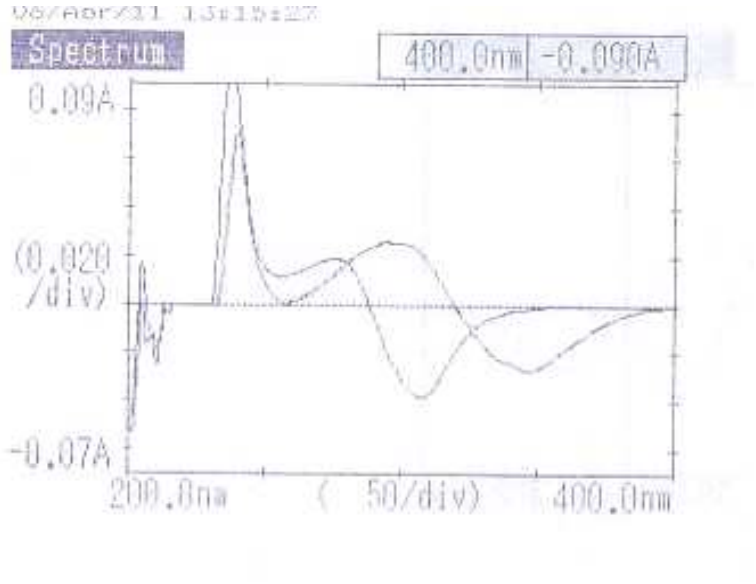

Figure 1.
First order derivative overlain spectra PA and DS.
Method B: Area Under Curve Method (AUC)

AUC method [12] involves the calculation of integrated value of absorbance with respect to wavelength. Area calculation processing item calculates the area of bounded by the curve and horizontal axis. Here horizontal axis represents baseline.

$$(\alpha+\beta)=\int_{\lambda_2}^{\lambda_1} A d\lambda$$

Where, α = area of portion bounded by curve data and a straight line connecting the start and end point, β= area of portion bounded by a straight line connecting the start and end point on curve data and horizontal axis, λ_1 and λ_2 are wavelengths representing start and end point of curve region.This method involved calculation of concentration for PA in the regions of 249-245 nm and for DS in the region of 280-276 nm, these regions were selected on the basis of repeated observation that plot area calculation of pure sample drug against the concentration. The UV spectra of PA and DS along with its AUC region are reported in (Fig. 2 A and 2 B) respectively.

$$\int_{245}^{249} A d\lambda = K_1 C_1 \ldots\ldots \textbf{(Eqn.1)}$$

$$\int_{276}^{280} A d\lambda = K_2 C_2 \ldots\ldots \textbf{(Eqn.2)}$$

$$\int_{245}^{249} A d\lambda = K_3 C_1 \ldots\ldots \textbf{(Eqn.3)}$$

$$\int_{276}^{280} A d\lambda = K_4 C_2 \ldots\ldots \textbf{(Eqn.4)}$$

Where C_1 and C_2 were concentration of PA and DS respectively in µg/ml and K_1, K2, K3 and K_4 were constant having values 0.2900, 0.3948, 0.1763 and 0.1308 respectively. Area of curve between 249-245 nm and 280-276 nm represented as $\int_{245}^{249} A d\lambda$ and $\int_{276}^{280} A d\lambda$ for PA and DS respectively. In view of that, following two final equations were developed for estimation of PA and DS .

$$\int_{245}^{249} A d\lambda = K_1 C_1 + K_2 C_2 \ldots\ldots \textbf{(Eqn.5)}$$

$$\int_{276}^{280} A d\lambda = K_3 C_1 + K_4 C_2 \ldots\ldots \textbf{(Eqn.6)}$$

Sample solutions were scanned and area was calculated within the indicated wavelength regions. Concentrations of both components were calculated using Eqn. 5 & 6.

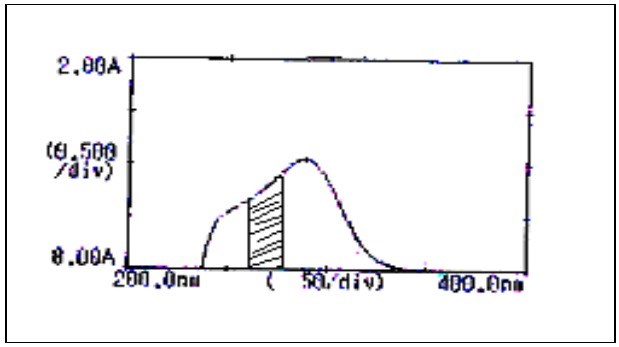

Figure 2. A.*UV Spectra of PA along with area under curve.*

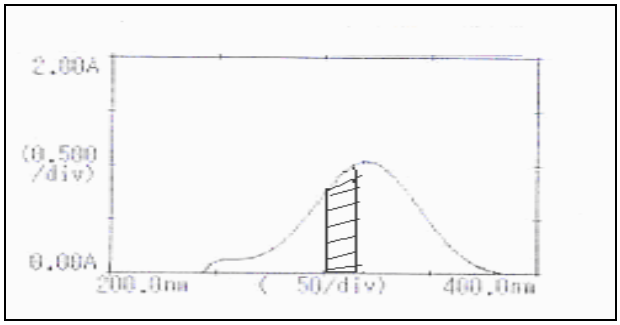

Figure 2B.*UV Spectra of DS along with area under curve.*

Method C: Multi-component Method

In this method[13]five mixed standards of PA and DS in the ratio of 1:10 having concentrations in µg/ml of 0.5:5, 1:10, 1.5:15, 2:20 and 2.5:25 were prepared by appropriate dilution of the standard stock solutions and scanned in the region of 400 nm to 200 nm. Sampling wavelengths (247 nm and 276 nm) were selected on the trial and error basis. The concentration of individual drug was feed to the multi-component mode of the instrument. The instrument collects and compiles the spectral data from mixed standards and concentration of each component were obtained by spectral data of sample solution with reference to that of five mixed standards. Overlain spectra of mixed standards are given in Figure 3.

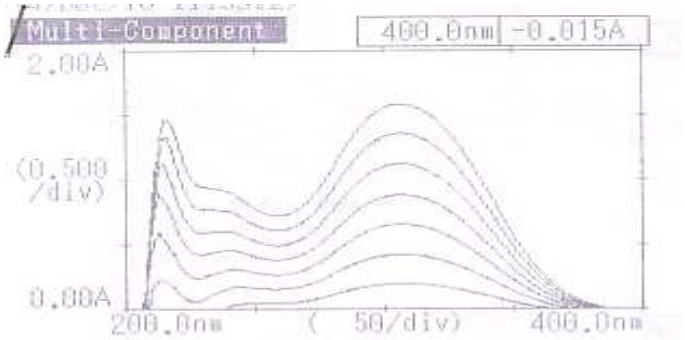

Figure 3.*Overlain spectra of mixed standards of PA and DS.*

Preliminary solubility studies of drugs[14]
Solubility of both drugs was determined at 28 ± 2 ^{0}C. An excess amount of drug was added to two screw capped 30ml glass vials containing different aqueous systems viz distilled water, buffer of pH 6.4, buffer of pH 8.2, and 1.0 M urea. The vials were shaken mechanically for 12 h at 28+1˚ in a mechanical shaker. These solutions were allowed to equilibrate for next 24 h and then centrifuged for 5 min at 2000 rpm. The supernatant liquid was taken for appropriate dilution after filtered through whatmann filter paper # 41 and analyzed spectrophotometrically against corresponding solvent blank. After analysis, it was found that the enhancement in the solubility of PA and DS was found to be more than 50 and 10 folds respectively in 1.0 M urea as compared to solubility studies in other solvents.

Preparation of standard stock solution and calibration curves of PA and DS
About 50mg each of PA and DS were accurately weighted and transferred to 50ml of volumetric flask separately. 40 ml, 1.0 M urea was used to solubilize after shaking for 10 to 15 minutes. Rest of the volume was made up with distilled water to get solution of 1000µg/ml. Stock solutions of 100µg/ml of each drugs were prepared by further dilution and scanned over the range of 400nm-200nm in the spectrum mode to get the overlain spectra of both drugs. The spectra exhibit major absorbance maxima at 247 nm and 276 nm for PA and DS respectively. Beers-Lambert law obeyed in the range of 2-40 µg/ml and 2-40 µg/ml for PA and DS respectively. Five mixed standards 5,10,15,20,25,30 for PA; and 30,25,20,15,10,5 for DS were prepared from stock solutions of PA and DS for further study.

Analysis of Tablet Formulation
Twenty tablets (brand name- Diclogesic) were taken and their average weight was determined, they were crushed to fine powder. Then powder equivalent to 50 mg (74.96 mg) of PA was taken in 50ml volumetric flask and 40 ml, 1.0 M urea was used to solubilize after shaking for 10 to 15 minutes. Rest of the volume was made up with distilled water to get solution of 1000µg/ml. Stock solutions of 100µg/ml of each drugs were prepared by further dilution. The supernatant liquid was transferred to 50ml of volumetric flask through a whatman No-41 filter paper. The residue was washed twice with water and the combined filtrate was made up to 50ml mark with water. The above solution was further diluted to get a solution containing 20 µg/ml of PA and 2 µg/ml of DS. The above binary mixture was analyzed at appropriate wavelengths and values of the absorptions were substituted in the respective formulas (Eqn.1,2,3,4) to obtain the content of PA and DS. PA and DS was determined from their calibration curve plotted between absorption difference and concentration. The results of analysis were given in Table 1.

Table 1.

Result of pharmaceutical formulation analysis

Parameters	Method-A		Method-B		Method-C	
	PA	DS	PA	DS	PA	DS
Label claim (mg/Tab)	500	50	500	50	500	50
Found (mg/Tab)	500.11	49.73	500.14	51.78	498.36	50.77
Drug content[a]	100.22	99.11	100.05	101.32	99.98	100.90

±S.D	0.684	0.421	0.163	0.714	0.321	0.696
%COV	0.201	0.394	0.301	0.498	0.469	0.301
SE	0.433	0.303	0.538	0.473	0.298	0.438

[a]Value for drug content (%) are the mean of five estimation, Method-A: Derivative spectrophotometry method, Method-B: Area under curve method, Method-C: Multi-component method S.D: Standard deviation , COV : Coefficient of variance and S.E : Standard error.

Recovery studies

To check the accuracy of the developed methods and to study the interference of formulation additives, analytical recovery experiments was carried out by standard addition method. From that total amount of drug found and percentage recovery was calculated. The results were reported in Table 2.

Table 2.
Result of Recovery studies

Method	Drug	Labelclaim (mg/tab)	Amount (mg/ml) taken	Amount (mg/ml) added	% Recovery±S.D COV%	
Method-A	PA	500	20	5		
			60	10	100.21±0.312	0.404
			80	15	100.01±0.610	0.301
					98.93±0.211	0.415
	DS	50	20	5		
			60	10	101.1±0.451	0.119
			80	15	99.91±0.213	0.475
					100.00±0.61	0.621
Method-B	PA	500	20	5	101.01±0.112	0.293
			60	10	100.11±0.307	0.218
			80	15	99.89±0.212	0.292
	DS	50	20	5	99.6±0.106	0.209
			60	10	101.4±0.21	0.412
			80	15	100.6±0.123	0.211
Method-C	PA	500	20	5	100.34±0.230	0.337
			60	10	99.21±0.090	0.311
			80	15	100.97±0.146	0.176
	DS	50	20	5	100.3±0.440	0.271
			60	10	100.11±0.142	0.601
			80	15	101.00±0.266	0.365

%Recovery is mean of three estimation, Method-A: Derivative spectrophotometry method, Method-B: Area under curve method, Method-C: Multi-component method, S.D is standard deviation and COV is coefficient of variance.

Validation of the developed methods [15], [16]

The developed methods for simultaneous estimation of PA and DS were validated as per ICH guidelines.

Accuracy: To check the accuracy of the developed methods and to study the interference of formulation additives, analytical recovery experiments was carried out by standard addition method. Total amount of drug found and percentage recovery was calculated and results were reported in Table 2.

Precision: Precision of the method was verified by repeatability and intermediate precision studies.

Repeatability: To check the degree of repeatability of the methods, suitable statistical evaluation was carried out. Five samples of the tablet formulations were analyzed for the repeatability study. The standard deviation, coefficient of variance and standard error was calculated. The results were reported in Table 1.

Intermediate precision (inter-day and intra-day precision)

Intermediate precision of the method was checked by assay the sample solution on same day at an interval of one hour (intraday precision) for three hours and on three different days (interday precision) the result was reported in Table 3. This study indicates that the solutions can be analyzed within 48-72 h without having any bad effect on chemical stability of the drug in presence of urea.

Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD and LOQ were separately determined based on the standard deviation of response of the calibration curve. The standard deviation of Y-intercept and slope of the calibration curves were used to calculate the LOD and LOQ by using the equations 3.3σ/s for LOD and 10σ/s for LOQ, where σ stands for standard deviation of Y-intercept and S stands for slope of the calibration curve. The results of the same were given in Table 3.

Table 3.
Intraday , Interdays , LOD and LOQ data of tablet formulation.

Method	Drug	Intraday precision %COV(n =3)	Interday precision %COV			LOD (µg/ml)	LOQ (µg/ml)
			Day 1[a]	Day 2[a]	Day 3[a]		
Method A	PA	0.101	0.225	0.398	0.259	0.607	0.928
	DS	0.237	0.230	0.148	0.100	0.194	1.021
Method B	PA	0.100	0.190	0.287	0.315	0.301	1.133
	DS	0.492	0.329	0.183	0.621	0.502	0.981
Method C	PA	0.622	0.290	0.404	0.213	0.189	1.577
	DS	0.179	0.281	0.129	0.502	0.341	1.753

[a]*Mean of five determinations, COV is coefficient of variance, LOD is least of detection, and LOQ is least of quantitation.*

RESULTS

All three UV spectrophotometric methods were found to be simple, accurate, economic and rapid for simultaneous estimation of PA and DS in tablet dosage form. By performing these methods it was found that both drugs shown good regression value at their respective wavelengths and recoveries were within 98.93-101.01% for PA and 99.6-101.4% for DS. Precision was good with acceptable limits of detection (LOD) and quantitation (LOQ) for both compounds. The average content of the compounds were 100.22 and 99.11% in method-A, 99.05 and 101.32% in method-B and 99.98 and 100.90% in method-C for PA and DS respectively. The optimized methods showed good reproducibility and recovery with standard deviation of < 1.0% and percent relative standard deviation less then 2.0%.

DISCUSSION

In the present work, three methods namely derivative spectrophotometry (method A), area under curve (method B) and multicomponent (method C) were developed for the simultaneous estimation PA and DS in tablet dosage form using 1.0 M urea as solubilising agent. Urea is a well known hydrotropic agent and it has been demonstrated for its ability to solubilize a wide variety of drugs [1] . Most of the organic solvents like ethanol, methanol, acetonitrile, hexane , cyclohexane, diethyl ether, chloroform and toluene find wide use in spectrophotometic analysis of poorly water-soluble drugs. Most of these organic solvents are toxic in nature, costlier and responsible for pollution morever inaccuracy in spectrophotometric estimation due to volatility is another drawback of organic solvents. Since urea do not interfere above 245 nm, therefore other poorly water-soluble drugs can also be estimated above 245 nm by hydrotropy avoiding the use of organic solvents [13], [14].There was no interference of urea and commonly used additives present in tablet formulations. A critical evaluation of the proposed methods was performed by statistical analysis of the experimental data. In order to demonstrate the validity and applicability of the proposed methods, recovery studies were performed by analyzing synthetic mixture of PA and DS with different composition ratio.

Hence, the proposed methods could be successfully applied to the determination of PA and DS in the commercially available bulk and tablet dosage form. Thus, it may be concluded that the proposed methods of analysis are new, simple, cost-effective, environmentally friendly, safe, accurate and reproducible. Definitely, there is further scope of 1.0 M urea solution as solubilizing agent for other poorly water-soluble drugs. There was no interference of urea in the estimation. The proposed method can be successfully employed in the routine analysis of PA and DS containing dosage forms.

It is thus concluded that the proposed method is new, simple, cost effective, accurate, safe, free from pollution and precise and can be successfully employed in the routine analysis of Poorly water soluble drugs in pharmaceutical dosage forms.

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