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Novel UV spectrophotometric methods for simultaneous estimation of alprazolam and paracetamol in bulk and pharmaceutical formulations

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Abstract

To develop two validated UV spectroscopic method for simultaneous estimation of Alprazolam (ALP) and Paracetamol (PAR) in bulk and pharmaceutical formulation Area under curve (Method 1) and simultaneous equation (Method 2) methods were developed for the determination of ALP and PAR in their combined tablet formulations without prior separation. The solutions of standard and sample were prepared in methanol for both the methods. Quantitative determination of the drugs was performed at the wavelength ranges of 211-231nm and 238-258nm (method 1) and at 221 and 248 nm (method 2) for ALP and PAR, respectively. Proposed methods were evaluated for the different validation parameters like precision, reproducibility, linearity and accuracy as per ICH guidelines. The linearity was observed in the range of 2-10 µg/mL for both drugs with correlation coefficient of 0.9998 and 0.999 for ALP and PAR respectively. These methods are simple, precise, sensitive and applicable for the simultaneous determination of these drugs in pure powder and combined formulation.

Keywords: Alprazolam, Paracetamol, Area under curve technique, Simultaneous equation method, UV Spectroscopy.

1. INTRODUCTION

Alprazolam (ALP) is chemically 8-chloro-1-methyl-6-phenyl-4H-[1, 2, 4] triazolo [4,3,-α]-[1,4] benzodiazepine in figure 1 derived from 1,4-benzodiazepines of new generation. It is a benzodiazepine mainly used as anxiolytic in humans, and may be effective in the treatment of depression and panic disorder. Besides this, ALP is also used to treat panic disturbances with or without agarophobia [1,2]. Paracetamol (PAR) chemically is N-(4- hydroxylphenyl) acetamide in **figure-2**. It is an analgesic and antipyretic agent. It act primarily in the CNS, increasing the pain threshold by inhibiting both isoforms of cyclooxygenase, COX-1, COX-2, and COX-3 enzymes involved in prostaglandin synthesis. The antipyreitic properties of PAR are likely due to direct effects on the heat-regulating centres of the hypothalamus resulting in peripheral vasodilation, sweating and hence heat dissipation [3].

ALP and PAR are official in IP [4], USP [5], and BP [6]. Literature survey reveals various UV spectrophotometry[7,8] and HPLC[9,10] methods for the estimation of ALP in pharmaceutical dosage form or biological fluids in isolation or in combination with other drugs. For estimation of PAR various UV spectrophotometry[11-14] and HPLC[15,16] methods have been reported. However, to the best of our knowledge, no method has been reported for the simultaneous estimation of ALP and PAR in combination dosage form. Therefore, an attempt was made to develop a simple, precise, accurate UV method for the simultaneous determination of ALP and PAR in pure powder and formulation. The developed method validated as per ICH guidelines [17].

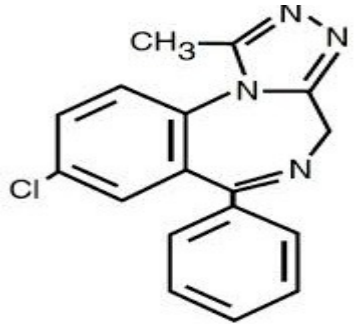

Figure - 1: Structure of alprazolam

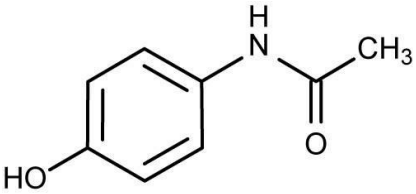

Figure - 2: Structure of paracetamol

2. EXPERIMENTAL DETAILS

2.1. Instrument and reagents

Pure ALP and PAR were obtained as gift sample from Darwin labs pvt Ltd and their marketed combination (RESTA) was purchased from the local pharmacy. Methanol (analytical grade) was used as the solvent. A Shimadzu UV-1800 spectrophotometer, with a pair of 1 cm matched quartz cells were used for the spectral measurements.

2.2. Preparation of standard stock solutions

Accurately weighed 10 mg of ALP and PAR were taken separately in 10mL volumetric flasks, dissolved in small amount of methanol and sonicated for 3 minutes. The final volume was adjusted up to the mark with methanol to get a solution of 1mg/mL.

2.3. Preparation of sample solutions

Twenty tablets of RESTA (ALP 0.25mg and PAR 500mg) were procured from the local pharmacy. Tablets were accurately weighed and finely powdered. The amount of powder equivalent to 10mg of PAR was transferred into a 10 mL volumetric flask, dissolved in methanol and sonicated for 3 minutes. The volume was made up after filtration through nylon disc filter (0.22µ).

2.4. Method -1

For the simultaneous determination using the area under curve (AUC) method, suitable dilutions of the standard stock solutions (1000 µg/mL) of ALP and PAR were prepared separately in methanol. The solutions of drugs were scanned in the range of 400-200 nm, the wavelength of 221 and 248 were selected as λmax of ALP and PAR respectively. , the 'X' values of each of the two drugs were determined at the selected wavelengths ranges i.e., 211 to 231 nm and 238 to 258 nm (±10 nm of λmax of the two drugs). The 'X' values was determined as, X= Area under curve of component(from 211 to 231 nm or 238 to 258 nm) /concentration of the component in g/100 mL. The 'X' values are reported are the mean of six independent determinations. Applying equations (1) and (2), concentrations C_{ALP} and C_{PAR} can be obtained. Series of mixed standard were prepared from the stock (1000µg/mL) of different concentration in the range (2-10) µg/mL for both the drugs. Linear response with increasing concentration, hence the same wavelength range were used for estimation of tablet formulations. Sample spectra were shown in figure 3.

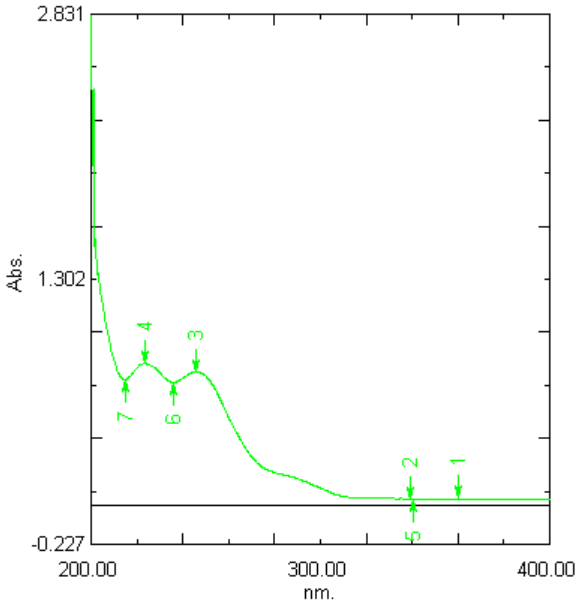

Figure – 3: Assay of sample spectra.

$$C_{ALP} = \frac{AUC(238-258)\times X_A(211-231) - AUC(211-231)\times X_A(238-258)}{X_D(238-258)\times X_A(211-231) - X_D(211-231)\times X_A(238-258)}$$

$$C_{PAR} = \frac{AUC(211-231)\times X_D(238-258) - AUC(238-258)\times X_D(211-231)}{X_D(238-258)\times X_A(211-231) - X_D(211-231)\times X_A(238-258)}$$

Where,

C_{ALP} (Eq 1), C_{PAR} (Eq 2) are the concentrations of the ALP and PAR,

AUC (211-231), AUC (238-258) are the area of the mixture,

X_A (211-231), X_A (238-258) are the absorptivities of ALP in the given ranges and

X_D (211-231), X_D (238-258) are the absorptivities of PAR in the specified ranges.

2.4. Method - 2

For the determination of ALP and PAR using the Simultaneous equation method, standard stock solutions of ALP and PAR (1000 µg/mL) were diluted with methanol to get the concentration of 10 µg/mL and the solutions were scanned in the wavelength range of 400–200 nm. From the overlain spectrum of ALP and PAR, two wavelengths i.e., 221 nm and 248 nm were selected for ALP and PAR respectively. The calibration curves were constructed in the concentration range of 2-10 µg/mL at each of the wavelengths. The absorptivity coefficients were determined for both the drugs at the selected

wavelengths and calculated by using the following formula. ALP and PAR overlay spectra was shown in figure 4.

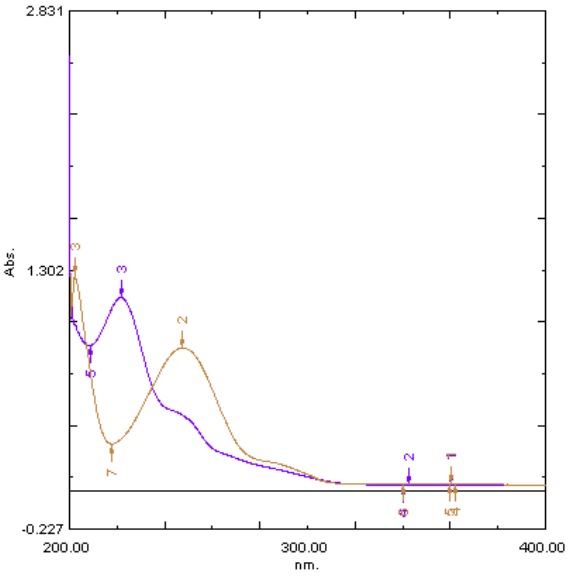

Figure – 5: overlaying of ALP and PAR

$$C_X = \frac{A_1 ay_2 - A_2 ay_1}{ax_1 ay_2 - ax_2 ay_1}$$

$$C_Y = \frac{A_1 ax_2 - A_2 ax_1}{ax_1 ay_2 - ax_2 ay_1}$$

Where,

A_1 and A_2 are absorbance of sample at 221 nm and 248 nm, respectively.

ax_1 and ax_2 are absorptivities of ALP at 221 nm and 248 nm respectively.

ay_1 and ay_2 are absorptivities of PAR at 221 nm and 248 nm respectively.

Cx and Cy are concentrations of ALP and PAR respectively.

2.5. Validation

Validation of the proposed methods was carried out for its accuracy, precision, specificity and linearity according to ICH guidelines

2.5.1. Accuracy

Recovery studies were carried out at three different levels by adding the pure drug to previously analyzed tablet powder sample. Accurately weighed quantities of tablet powder equivalent to 80%, 100%and 120% of label claim of ALP were taken in a series of 100 mL volumetric flasks and dilutions were made as described in the methods above. From the amount of the total drug found, percentage recovery was calculated by proposed two methods and results are shown in table 2.

2.5.2. Precision

2.5.2.1. Inter-Day precision

It was done by analyzing the solution by same analyst on the alternate days till 5th day. Results indicate that the solution is stable up to three days. Thereafter degradation may have taken place leading lower percent label claim.

2.5.2.2. Intra-day precision

It was done by analyzing the solution by same analyst within a day. Results indicate that the solution is stable.

2.5.3. Linearity

Linearity was checked by diluting standards stock solution at five different concentration. Standard solution of PAR and ALP were prepared separately in the concentration range of 2-10 µg/mL. Linearity plots were constructed at the 238nm-258nm and 211nm-231nm respectively for method 1. calibration curves (n=5) were plotted between concentration and area of drugs and optical parameters were calculated.

Linearity of simultaneous equation method was checked by diluting standards stock solution at five different concentration. standard solution of PAR and ALP were prepared separately in the concentration range of 2-10 µg/mL at 248nm and 221nm respectively. calibration curve (n=5) were plotted between concentration and absorbance.

2.5.4. Limit of detection

The Limit of detection (LOD) is the smallest concentration of the analyte that gives the measurable response.LOD was calculated using the following formula and shown in **table**-1.

$$LOD=3.3(\sigma /S)$$

Where, S= slope of calibration curve, σ =standard deviation of the response.

2.5.5. Limit of quantification

The limit of quantification (LOQ) is the smallest concentration of the analyte, which gives a response that can be accurately quantified. LOQ was calculated using the following formula and shown in table 1.

$$LOQ=10(\sigma /S)$$

Where, S= slope of calibration curve, σ=standard deviation of the response.

3. RESULTS

For the two methods linearity was observed in the concentration range of 2-10 µg/mL for both the drugs. Commercial formulations containing ALP and PAR were

analyzed by the proposed methods. Three replicate analysis of formulation were carried out and the mean assay values were found in the range of 98.4 to 99.5 % shown in table 3. Validation of the proposed methods was performed as per the ICH guidelines and results shown in table 1. The accuracy of the proposed method was determined by recovery studies. Pure ALP and PAR was added to the preanalysed tablet powder at three levels viz 80, 100, 120 %. Three replicate analyses were carried out at each level. The mean percent recovery was found in the range of 99.4 to 100.3 % for all the methods shown in table 2. Precision is calculated as interday and intraday variations for both the drugs. Percent relative standard deviations for estimation of ALP and PAR under intraday and interday variations were found to be less than 1.

Table – 1: Results of validation parameters

Parameters	Method 1		Method 2	
	ALP	**PAR**	**ALP**	**PAR**
Area range (λ)	211-231	238-258	221	248
Beer's-Lambert's range (µg/mL)	2-10	2-10	2-10	2-10
Regression equation y =mx + c	y =0.4844x-1.1266	y =0.0801x-0.0203	y = 0.115x - 0.011	y = 0.080x - 0.001
Slope (m)	0.4844	0.0801	0.115	0.080
Intercept (c)	1.1266	0.0203	0.011	0.001
Correlation coefficient (r2)	0.9999	0.9998	0.998	0.999
Recovery + S. D. (n = 3)	99.5	99.4	99.31	99.63
Repeatability (% RSD, n = 6)	0.768	0.987	0.831	0.927
Intermediate precision (% RSD)				
Interday (n = 3)	0.514	0.426	0.431	0.520
Intraday (n = 3)	0.615	0.715	0.632	0.691
LOD (µg/mL)	1.418	13.2	34.5	0.721
LOQ (µg/mL)	4.29	40	104.5	1.76

S. D. = Standard deviation. RSD = Relative standard deviation. LOD = Limit of detection. LOQ = Limit of quantification. n is number of determinations.

Table – 2: Accuracy data for Alpraolam and Paracetamol

Level of recovery (%)	Drug	Amount of drug taken	Amount of std drug added	Recovery	% RSD
80	ALP	0.25	0.2	99.5	**0.51**
	PAR	500	400	99.4	**0.44**
100	ALP	0.25	0.25	99.6	**0.643**
	PRA	500	500	99.3	**0.72**
120	ALP	0.25	0.3	100.1	**0.518**
	PAR	500	600	100.3	**0.641**

Table – 3: Assay data

Sample solution concentration (µg/mL)	Amount Found (%)	Mean Amount Found (%)	% RSD*
10	98.4		
10	99.3	99	0.58
10	99.5		

n=3, % RSD = % Relative Standard Deviation

4. DISCUSSION

The new UV spectrophotometric method developed and validated for simultaneous determination of ALP and PAR in combined pharmaceutical dosage form was satisfactory with good precision and accuracy. The method was found to be simple, accurate, economical, rapid and can be applied for routine quality control of ALP and PAR in bulk and their combined formulations.

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