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روش اسپکتروفتومتری فرابنفش برای ارزیابی همزمان کاریسوپرودول، پاراستامول و کافئین: کاربرد برای آنالیز قرص های تجاری

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UV-Spectrophotometric Method for the Simultaneous Estimation of Carisoprodol, Paracetamol and Caffeine: Validation and Application for Marketed Tablet Analysis

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چکیده

یک روش ساده، حساس، صحیح و دقیق بر پایه اسپکتروفتومتری فرابنفش برای اندازه گیری همزمان کاریسوپرودول، پاراستامول و کافئین در قرص های دارویی مورد مطالعه قرار گرفت. ماکزیمم جذب داروها در طول موج های ۲۲۱ و ۲۴۸ و ۲۷۳ نانومتر به ترتیب برای کاریسوپرودول، پاراستامول و کافئین به دست آمد. این سه دارو از قانون بیر در گستره غلظتی وسیعی پیروی می کنند. این گستره ها برای کاریسوپرودول، پاراستامول و کافئین به ترتیب عبارتند از ۲-۰/۴، ۱۰-۲ و ۱۰-۲ میکروگرم بر میلی لیتر و همچنین مقادیر ۰/۹۹۲۲، ۰/۹۹۱۷ و ۰/۹۹۴۳ برای ضریب همبستگی. روش به وسیله پارامترهای متعدد مطابق با کنفرانس های بین المللی دستورالعمل های هموژناسیون اعتبار سنجی شد. مقادیر انحراف استاندارد نسبی پایین نشان از دقت بالای روش است و درصدهای بازیابی خوب نیز نشان از صحت روش حاضر دارد.

واژه‌های کلیدی

کاریسوپرودول؛ پاراستامول؛ کافئین؛ روش اسپکتروفتومتری؛ روش معادلات هم زمان.

Abstract

A simple, sensitive, accurate and precise simultaneous UV spectrophotometric method has been developed for the estimation of Carisoprodol, Paracetamol and Caffeine in tablet dosage form. The absorption maxima of the drugs were found to be 221, 248 and 273 nm for Carisoprodol, Paracetamol and Caffeine respectively, in methanol, using a double beam UV/Visible Spectrophotometer. Carisoprodol, Paracetamol and Caffeine obeyed Beer's law in the concentration range of 0.4-2 µg/mL, 2-10 µg/mL and 2-10 µg/mL, respectively. The correlation coefficient was found to be 0.9922, 0.9917 and 0.9943 for Carisoprodol, Paracetamol and Caffeine, respectively. The method was validated for various parameters according to International Conference on Harmonization guidelines. The low relative standard deviation values indicate a good precision and high recovery values indicate the accuracy of the proposed method.

Keywords

Carisoprodol; Paracetamol; Caffeine; Spectrophoto-metric Method; Simultaneous Equation Method.

1. INTRODUCTION

Paracetamol (PAR) is a chemically N-acetyl-p-aminophenol (Fig. 1). PAR is a non steroidal anti-inflammatory and analgesic drug with an indistinct mechanism of action [1]. PAR is official in Indian Pharmacopoeia (IP), the British Pharmacopoeia (BP) and United State Pharmacopoeia (USP). Literature survey divulges that various UV and

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chromatographic methods are available for estimation of PAR in single and combined dosage forms. Literature survey also reveals LC-MS, GC-MS, IR and HPTLC methods are reported for estimation of PAR with other drugs in combination [2-14].

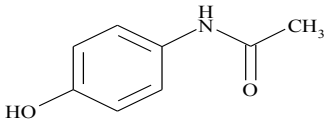

Fig. 1. Paracetamol

Caffeine (CAF) chemically is 1, 3, 7-Trimethyl-1H-purine-2, 6 (3H, 7H) -Dione (Fig. 2). It is a central nervous system stimulant. It acts by inhibition of cyclic nucleotide phosphodiesterases, antagonism of adenosine receptors, and modulation of intracellular calcium handling [15]. Caffeine is official in IP, BP and USP. Literature survey also reveals that various analytical methods are reported for estimation of CAF with other drugs in combination [16-21].

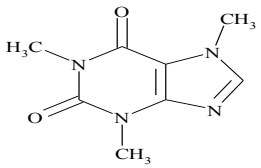

Fig. 2. Caffeine

Carisoprodol (CAR) chemically is (*RS*)-2-{[(aminocarbonyl) oxy] methyl}-2-methylpentyl isopropylcarbamate (Fig. 3). Carisoprodol is a CNS depressant which has sedative and skeletal muscle relaxant effects. The precise mechanism of action of the drug is not known. Carisoprodol does not appear to directly relax tense skeletal muscles in man. In animals, Carisoprodol produces muscle relaxation by blocking interneuronal activity in the descending reticular formation and spinal cord [22]. It is official in EP and USP. Literature survey also reveals UV, MS-MS, LC-MS-MS, GC-MS methods are reported for estimation of CAR with other drugs in combination [23-29].

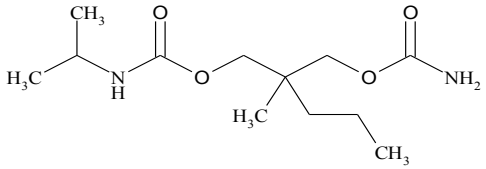

Fig. 3. Carisoprodol

The combination of Carisoprodol, Caffeine and Paracetamol (Carisoma compound tablet) is used for treatment of Low Back Pain, Post Traumatic Muscle Spasm, Sprains, Strains and Tenosynovitis. According to literature survey, there was not any developed analytical method which has been reported for simultaneous estimation of CAR, PAR and CAF in combined dosage form. So an attempt was being made to develop simple, accurate, precise, economical and reproducible spectrophotometric method for simultaneous estimation of CAR, PAR and CAF in tablet dosage form. The developed method was validated in accordance with ICH guideline [30-31] and successfully employed in the assay of CAR, PAR and CAF in combined tablet dosage form.

2. EXPERIMENTAL

2.1. Instruments, Reagents and Chemicals

The instrument used in the present study was JASCO double beam UV/Visible Spectrophotometer (Model V-630) with a spectral bandwidth of 1 mm and 10 mm a matched quartz cell was used. All weighing was done on an electronic balance (Model Shimadzu BL 320-H). Analytically pure working standards PAR and CAR were obtained from Wockhardt Ltd., Aurangabad, India Watson (Actavis) Pharmaceutical Pvt. Ltd, Goa, Maharashtra, India respectively. The analytical reagent grade methanol acquired from Loba Chemie, Mumbai, India and used as solvent. Marketed preparation of PAR, CAF and CAR was procured from local market.

2.2. Preparation of Stock Standard Solutions

Selection of solvent and wavelength: Solubility of CAR, PAR and CAF was checked in solvents like ethanol, water and methanol. UV spectrums of the three drugs in these solutions were recorded. The absorbance of the three drugs was found maximum in methanol solvent compared to other solvents and three wavelengths 221, 248 and 273 nm (Fig. 4-6) were selected which are the λ_{max} of CAR, PAR and CAF, respectively.

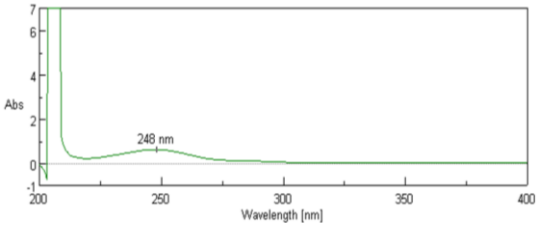

Fig. 4. UV spectra of Paracetamol

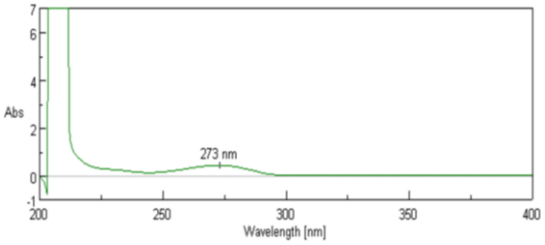

Fig. 5. UV spectra of Caffeine

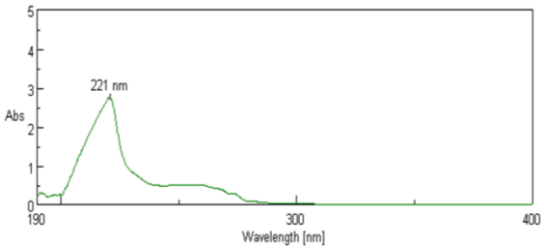

Fig. 6. UV spectra of Carisoprodol

2.3. Preparation Standard Stock Solutions

Standard stock solutions of PAR, CAF and CAR were prepared separately by dissolving 10 mg in 100 mL methanol to obtain concentration 100 µg/mL of each. From these stock solutions, working standard solutions having concentration 10 µg/mL of CAF, 10 µg/mL of PAR and 10 µg/mL of CAR were prepared by proper dilutions. They were scanned in the UV region, i.e. 400-200 nm. The overlain spectrum (Fig. 7) was obtained to determine the maximum absorbance (λ_{max}) and iso-absorptive point.

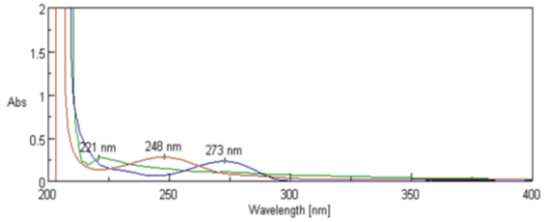

Fig.7. Overlain spectra of Carisoprodol, Paracetamol and Caffeine showing selected wavelength.

2.4. Application of Simultaneous equation method

In quantitative estimation of three components by Simultaneous equation method, three wavelengths i.e., 221 nm of CAR, 248 nm of PAR and 273 nm of CAF were selected as their respective λ_{max} from the overlain spectrum, at which three drugs have maximum absorbance. The concentrations of three drugs in the mixture can be calculated using the following equations:

$$C_{CAR} = \frac{(A1\ (ay2az3-az2ay3) - ay1\ (A2az3-az2A3) + az1\ (A2ay3-ay2A3)}{ax1(ay2az3-az2ay3) - ay1(ax2az3-az2ax3) + az1(ax2ay3-ay2ax3)}$$

$$C_{PAR} = \frac{(ax1(A2az3-az2A3) - A1(ax2az3-az2ax3) + az1(ax2A3-A2ax3)}{ax1\ (ay2az3-az2ay3) - ay1\ (ax2az3-az2ax3) + az1\ (ax2ay3-ay2ax3)}$$

$$C_{CAF} = \frac{(ax1(ay2A3-A2ay3) - ay1(ax2A3-A2ax3) + A1(ax2ay3-ay2ax3)}{ax1\ (ay2az3-az2ay3) - ay1\ (ax2az3-az2ax3) + az1(ax2ay3-ay2ax3)}$$

where, C_{CAR}, C_{PAR} and C_{CAF} are the concentrations of CAR, PAR and CAF respectively in mixture and in sample solutions. A1, A2 and A3 are the absorbances of sample at 221, 248 and 273 nm, respectively, ax1, ax2 and ax3 are the absorptivity of CAR at 221, 248 and 273 nm respectively, ay1, ay2 and ay3 are the absorptivity of PAR at 221, 248 and 273 nm, respectively, az1, az2 and az3 are the absorptivity of CAF at 221, 248 and 273 nm, respectively.

2.5. Analysis of marketed formulation

For the analysis, 20 tablets were weighed and their average weight was determined. The tablets were then crushed to fine powder and powder equivalent to weight of one tablet was transferred to 100 mL volumetric flask and dissolved in 50 mL of methanol for 10 min with vigorous shaking. Finally, the volume was made up to the mark with methanol. The solution was then filtered through whatmann filter paper. From this solution, 1 mL was pipetted out into a 10 mL volumetric flask and diluted with methanol up to the mark. From this solution, 0.4 mL was transferred into a 10 mL volumetric flask and diluted with methanol up to the mark. The absorbance of the above solution was measured at 221, 248 and 273 nm (Fig. 8). The concentration of each analyte was determined using the simultaneous equation.

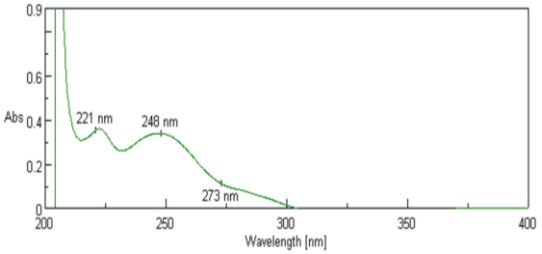

Fig. 8. Spectrum of formulation containing Carisoprodol, Paracetamol and Caffeine.

2.6. Analytical Method Validation

Validation of the proposed spectrophotometric method was carried out as per ICH guidelines [30-31] by means of the following parameters. The analytical method was validated with respect to parameters such as linearity, precision, limit of detection (LOD), limit of quantitation (LOQ) and accuracy.

2.6.1. Linearity

As per ICH guidelines the linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. Linearity study for the proposed method was established by least square linear regression analysis. An appropriate volume of PAR, CAF and CAR in the range of 2-10, 2-10 and 0.4-2 µg/mL respectively, were transferred into a series of separate 10 mL volumetric flasks and volume was made up to mark with methanol to get concentrations.

2.6.2. Accuracy (Recovery study)

The (recovery) accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the

value found. The accuracy of the proposed methods was checked by recovery studies (standard addition method), by the addition of the standard drug solution to pre analyzed sample solution at three different concentration levels within the range of linearity for all the drugs. The recovery data as mentioned in Table 1 indicates RSD of three different level is less than 2.

2.6.3. Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The precision of the analytical method was checked by repeated scanning and measurement of absorbance of solutions (n=3) for PAR, CAF and CAR (5-15 µg/mL for all three drugs) without changing the parameter of the proposed spectrophotometric method.

2.6.4. Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. The proposed method found specific to the analyte concern.

2.6.5. Limit of Detection

According to ICH guidelines the detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantities as an exact value. Limit of detection can be calculated using the following equation as per ICH guidelines.

$$LOD = 3.3 \times N/S$$

Where, N is the standard deviation and S is the slope of the corresponding calibration curve.

LOD was found to be 0.1973 µg/mL for CAR, 0.07342 µg/mL for PAR, 0.2225 µg/mL for CAF, respectively.

2.6.6. Limit of Quantification

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices and is used particularly for the determination of impurities and/or degradation products. Limit of quantification can be calculated using the following equation as per ICH guidelines.

$$LOQ = 10 \times N/S$$

Where, N is the standard deviation and S is the slope of the corresponding calibration curve.

LOQ was found to be 0.5980 µg/mL for CAR, 0.1736 µg/mL for PAR, 0.5265 µg/mL for CAF, respectively.

3. RESULTS AND DISCUSSIONS

3.1. Results

The absorbance of the PAR, CAF and CAR was measured at 248, 273 nm and 221 nm, respectively and calibration curves were plotted as concentrations versus absorbance.

The Relative Standard Deviation (RSD) for intra-day analysis of PAR, CAF and CAR was found in the range of 0.0059- 0.1716 % (248 nm), 0.0089-0.008O % (273 nm) and 0.0073-0.0068 % (221), respectively. RSD for Inter-day analysis of PAR, CAF and CAR was found to be 0.0063-0.0466 % (248 nm), 0.0081-0.0131 % (273 nm) and 0.0078-0.0120 % (221), respectively. The accuracy and reproducibility is evident from the data as results are close to 100 % and the value of standard deviation and % R.S.D. were found to be < 2 %; shows the high precision of the method. LOD was found to be 0.1973 µg/mL for CAR, 0.07342 µg/mL for PAR, 0.2225 µg/mL for CAF respectively. LOQ was found to be 0.5980 µg/mL for CAR, 0.1736 µg/mL for PAR, 0.5265 µg/mL for CAF, respectively. The proposed method is

Table 1. Recovery studies of PAR, CAF and CAR

Drug	Level (%)	Amount taken (µg/mL)	Amount added (µg/mL)	Total amount (ug/mL)	Amount found (µg/mL)	Percent recovery ± SD*	% RSD*
PAR	50	5.0	2.5	7.5	7.410	98.8±0.02	0.0202
	100	5.0	5.0	10	10.005	100.05±0.001	0.0010
	150	5.0	7.5	12.5	12.390	99.12±0.02	0.0218
CAF	50	5.0	2.5	7.5	7.440	99.02±0.026	0.0262
	100	5.0	5.0	10	9.890	98.90±0.05	0.0505
	150	5.0	7.5	12.5	12.430	99.44±0.035	0.0352
CAR	50	5.0	2.5	7.5	7.475	99.92±0.26	0.2678
	100	5.0	5.0	10	9.879	99.69±0.19	0.1846
	150	5.0	7.5	12.5	12.561	100.48±0.85	0.8513

* Indicates determination of three replicate

simple, economical, rapid, precise and accurate. Hence it can be used for routine analysis of PAR, CAF and CAR in pharmaceutical formulation. The proposed method was found to be specific as there is no interference from other excipients. Marketed brand of tablet (SPASMOL Compound) was analyzed, the amounts of PAR, CAF and CAR determined by proposed method were found to be 99.57%, 98.75% and 99.49%, respectively (see Table 2).

3.2. Discussion

In Simultaneous equation method, the primary requirement for developing a method for analysis is that the entire spectra should follow the Beer's law at all the wavelength, which was fulfilled in the case of all these three drugs. The three wavelengths were used for the analysis of the drugs were 248 nm (λ-max of PAR), 273 (λ-max of CAF) and 221 (λ-max of CAR), at which the calibration curves were prepared for all three drugs. In methanol, PAR, CAF and CAR obeyed linearity in the concentration range of 2-10 µg/mL, 2-10 µg/mL and 0.4-2 µg/mL, respectively at their respective λ_{max} with a correlation coefficient ($r^2 > 0.99$) in the all three drugs cases. In proposed method precision was studied as repeatability (%RSD<2) and inter and intra-day variations (%RSD<2) for all three drugs; shows the high precision of the method (Table 3-5). The accuracy of the method was determined by calculating mean percentage recovery. It was determined at 50, 100 and 150 % level and data are presented in Table 1. The ruggedness of the methods was studied by two different analysts using the same operational and environmental conditions. For projected method we used easily available and cheap solvent like methanol (AR grade).

4. CONCLUSIONS

Optical, regression characteristics and validation parameters of Simultaneous equation method for analysis of PAR, CAF and CAR were shown in Table 6. The developed and validated UV estimation method reported here is rapid, simple, accurate, sensitive and specific. The method was also successfully used for quantitative estimation and analysis of PAR, CAF and CAR in combined dosage form. By observing validation parameter and statistical data, the proposed method was found to be satisfactory over other reported chromatographic methods. The developed spectrophotometric method is more accurate, precise, reliable and specific for estimation of PAR, CAF and CAR as compared to other reported chromatographic method.

Table 2. Analysis of Marketed formulation

Brand Name of Formulation	Drug	Amount Present (mg)	Amount Found (mg)	% Assay	SD*	RSD*
SPASMOL (Compound)	Paracetamol	350	348.5	99.57%	0.031	0.0039
	Caffeine	32	31.6	98.75%	0.0781	0.04661
	Carisoprodol	175	174.12	99.49%	0.0217	0.0149

***Indicates estimation of three replicates**

Table 3. Precision data for PAR

Conc. (µg/mL)	Intraday Mean ± SD*(n= 3)	% RSD*	Interday Mean ± SD*(n = 3)	% RSD*
5	0.6513±0.0039	0.0059	0.6414±0.0041	0.0063
10	1.1541±0.0030	0.0026	1.1566±0.0037	0.0032
15	1.6849±0.2892	0.1716	1.682±0.0784	0.0466

* Indicates determination of three replicates

Table 4. Precision data for CAF

Conc. (µg/mL)	Intraday Mean ± SD* (n= 3)	% RSD*	InterdayMean ± SD* (n = 3)	% RSD*
5	0.7721±0.0069	0.0089	0.7710±0.0063	0.0081
10	1.2943±0.0056	0.0043	1.2664±0.0076	0.006
15	2.0849±0.0168	0.0080	2.0779±0.0274	0.0131

* Indicates determination of three replicates

Table 5. Precision data for CAR

Conc. (µg/mL)	Intraday Mean ± SD*(n= 3)	% RSD*	Interday Mean ± SD*(n = 3)	% RSD*
5	0.6821±0.0059	0.0073	0.6710±0.0052	0.0078
10	1.1943±0.0052	0.0052	1.1964±0.0066	0.004
15	1.5390±0.0172	0.0068	1.5379±0.0374	0.0120

* Indicates determination of three replicates

Table 6. Optical, Regression characteristics and validation parameters of Simultaneous equation method for analysis of PAR, CAF and CAR.

Parameters		PAR	CAF	CAR
λ max		248 nm	273 nm	221 nm
Beer's Law Limit (µg/mL)		2-10	2-10	0.4-2
Correlation coefficient (r^2)		0.9917	0.9943	0.9922
Slope (s)		0.1290	0.1180	0.01015
Intercept (c)		0.158	0.144	0.0106
Standard Deviation (S.D)		0.031	0.0781	0.0217
Relative Standard Deviation (RSD)		0.0039	0.04661	0.0149
LOD (µg/mL)		0.07342	0.2225	0.1973
LOQ (µg/mL)		0.1736	0.5265	0.5980
Precision (%RSD) (n=3)	Intraday	0.0059-0.1716	0.0089-0.0080	0.0073-0.0068
	Interday	0.0063-0.0466	0.0081-0.0131	0.0078-0.0120

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