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RESEARCH ARTICLE

Simultaneous Estimation and Validation of Acelofenac and Paracetamol in Bulk and Tablets Using Mixed Hydrotropic Agents

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Abstract: Two accurate, precise, sensitive and economical procedures for simultaneous determination of aceclofenac and paracetamol in tablet dosage form have been developed. In the present investigation, mixture of 20 mL (2 M) urea, 30 mL of (5 M) sodium acetate (hydrotropic solubilizing agent) was used to solubilize aceclofenac and paracetamol and carried out spectrophotometric analysis. The methods employed were absorbance ratio method (method I), derivative method (method II).The result showed that Beer's law was obeyed in concentration range of 5-50 µg/mL with good linearity (r^2>0.99) for both the drugs in both methods. The recoveries were within 99.67-101.33% for aceclofenac and 99.46-101.92% for paracetamol. Precision was good with acceptable limits of detection (LOD) and quantitation (LOQ) for both compounds. The optimized methods showed good reproducibility and recovery with standard deviation of < 1.0% and percent relative standard deviation less than 2.0%.

Keywords: Absorbance ratio method, Derivative method, Acelofenac, Paracetamol, Mixed hydrotropy.

Introduction

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 aceclofenac (ACF) is 2-[2-[2-[(2,6-dichlorophenyl)amino]phenyl]-acetyl]oxyacetic acid is a potent inhibitor of the enzyme cyclo-oxygenase, which is involved in the production of prostaglandins.[3,4]. Chemically paracetamol (PCM) is *N*-(4-hydroxyphenyl) acetamide a good and promptly acting antipyretic and anti-inflammatory action[3-,5]. These drugs are being used either alone or in combination for the treatment of pain, fever and

arthritis[5]. Literature survey revealed that only few methods have been reported for determination of ACF[6-8] and PCM[9-11] individually and few methods simultaneously[12,13]. But so far no spectrophotometric methods has been reported for simultaneous estimation of ACF and PCM in combined dosage form using hydrotropic agents, hence an attempt has been made to develop simple, sensitive, economical, rapid, precise and accurate methods to analyze the drugs simultaneously.

Experimental

UV-Visible double beam spectrophotometer, SIMADZU UV_1800 UV-VIS having spectral bandwidth 3 nm and of wavelength accuracy ±1 nm, with 1 cm quartz cells was used. All weighing were done on electronic balance (Shimadzu, Model AY - 120).

Reagents and Chemicals

Analytically pure samples of ACF and PCM were obtained as gift sample from Ranbaxy Pvt. Ltd. Dewas (M.P), India and were used as such without further purification. The tablet dosage form, Aclospar (containing ACF 100 mg with PCM 500 mg) was procured from the local market, Ujjain, India. Mixture of 20 mL (2 M) urea, 30 mL of (5 M) sodium acetate was selected as hydrotropic solubilizing agent.

Graphical absorbance ratio Q-Analysis method (Method I)

It was based on the absorption at two selected wavelength, one of which is an iso-absorptive point and the other being the wavelength of maximum absorption of one of the two components. From overlain spectra (Figure 1) 268 nm (Iso-bestic point) and 238 nm (λ_{max} of PCM) were selected for formation of Q-absorbance equation. The concentration of the individual components were calculated by using the following equations; Cx = Qm-Qy/Qx-Qy)×A_1 /ax_1, Cy = Qm-Qy/Qy-Qx)×A_1 /ax_1 where Qm = A_2 /A_1, A_1 is absorbance of sample at isoabsorptive point, A_2 is absorbance of sample at λ_{max} of one of the two components, Qx = ax_2 /ax_1, Qy = ay_2 /ay_1, ax_1 and ax_2 represent absorptivities of ACF at λ_1 and λ_2 and ay_1 and ay_2 denote absorptivities of PCM at λ_1 and λ_2 respectively; Cx and Cy be the concentration of ACF and PCM respectively.

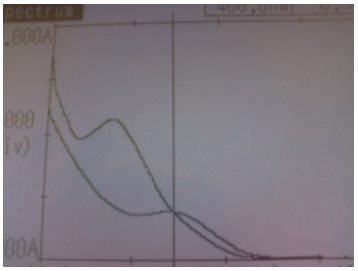

Figure 1. Overlain spectra of tablet formulation

Derivative spectrophotometry method (Method II)

In this method 20 µg/mL solution for both drugs were prepared and scanned in the range of 200-400 nm. The spectra obtained were derivatized in the first order and then recorded, which showed ACF had zero crossing point at 238 nm while PCM zero crossing point at 268 nm. At zero crossing point of ACE, PCM showed a measurable dA/dλ where as at the zero crossing point of PCM, ACE showed appreciable dA/dλ. Hence both wavelengths 268 nm and 238 nm were selected as analysis wavelengths for estimation of ACF and PCM respectively.

Calibration curves were plotted for ACF at 238 nm and PCM at 268 nm as dA/dλ concentration. The concentrations of both the drugs were obtained from the standard calibration curves by interpolation method.

Preliminary solubility studies of drugs

Solubility of both drugs was determined at 28±2 ^{0}C. An excess amount of drug was added to two screw capped 30 mL glass vials containing different aqueous systems *viz* distilled water, buffer of pH 6.4, buffer of pH 8.2, 2.0 M urea and mixture of 20 mL (2 M) urea, 30 mL of (5 M) sodium acetate. The vials were shaken mechanically for 12 h at 28±1 ^{o}C 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 Whatman filter paper # 41 and analyzed spectrophotometrically against corresponding solvent blank. After analysis, it was found that the enhancement in the solubility of ACF and PCM was found satisfactory in mixture of 20 mL (2 M) urea, 30 mL of (5 M) sodium acetate than the other combination of hydrotropic agents.

Preparation of standard stock solution and calibration curves of ACF and PCM

About 50 mg each of ACF and PCM were accurately weighted and transferred to 50 mL of volumetric flask separately. 40 mL, 2.0 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 1000 µg/mL. Stock solutions of 100 µg/mL of each drugs were prepared by further dilution and scanned over the range of 400nm-200 nm in the spectrum mode to get the overlain spectra of both drugs. The spectra exhibit major absorbance maxima at 238 nm and 268 nm for ACF and PCM respectively. Beers-Lambert law obeyed in the range of 5-50 µg/mL and 5-50 µg/mL for ACF and PCM respectively. Six mixed standards 5, 10, 15, 20, 25, 30 for ACF and 30, 25, 20, 15, 10, 5 for PCM were prepared from stock solutions of ACF and PCM for further study. An absorbance spectrum of ACF and PCM was shown in the Figure 2 and Figure 3 respectively.

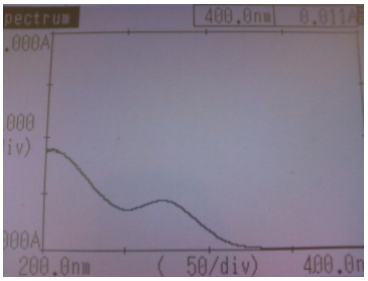

Figure 2. Absorbance graph of aceclofenac

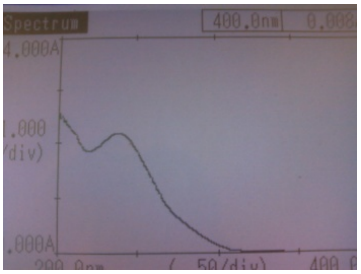

Figure 3. Absorbance graph of paracetamol

Analysis of tablet formulation

Twenty tablets Aclospar (containing ACF 100 mg with PCM 500 mg) were taken and their average weight was determined, they were crushed to fine powder. Then powder equivalent to 50 mg of PCM was taken in 50 mL volumetric flask and mixture of 20 mL (2 M) urea, 30 mL of (5 M) sodium acetate 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 1000 µg/mL. Stock solutions of 100 µg/mL of each drugs were prepared by further dilution. The supernatant liquid was transferred to 50 mL 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 50 mL mark with water. The above solution was further diluted to get a solution containing 5 µg/mL of ACF and

10 µg/mL of PCM. The above binary mixture was analyzed at appropriate wavelengths and values of the absorptions were substituted in the respective formulas to obtain the content of ACF and PCM. ACF and PCM 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	
	ACF	PCM	ACF	PCM
Label claim (mg/Tab)	100	500	100	500
Found (mg/Tab)	100.28	500.09	100.43	501.20
Drug content[a]	99.13	500.82	99.55	501.05
±S.D	0.384	0.100	0.240	0.304
%COV	0.341	0.210	0.220	0.238
SE	0.331	0.248	0.281	0.354

[a]Value for drug content (%) are the mean of six estimation, Method-A: Absorbance ratio method, Method-B: Derivative 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.

Validation of the developed methods[15]

The developed methods for simultaneous estimation of ACF and PCM 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.

Table 2. Result of recovery studies

Method	Drug	Labelclaim, mg/tab	Amount, mg/mL taken	added	% Recovery±S.D COV%	
Method-A	ACF	100	30	5	99.42±0.442	0.321
			60	10	101.18±0.820	0.521
			90	15	99.93±0.001	0.119
	PCM	500	30	5	101.81±0.420	0.219
			60	10	99.95±0.103	0.104
			90	15	100.27±0.711	0.471
Method-B	ACF	100	30	5	100.01±0.182	0.413
			60	10	101.18±0.307	0.108
			90	15	99.99±0.182	0.222
	PCM	500	30	5	100.26±0.326	0.209
			60	10	101.32±0.621	0.491
			90	15	99.61±0.536	0.203

%Recovery is mean of three estimation, Method-A: Absorbance ratio method, Method-B: Derivative method, S.D is standard deviation and COV is coefficient of variance

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.

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	ACF	0.241	0.204	0.341	0.249	0.417	0.118
	PCM	0.256	0.590	0.121	0.219	0.410	0.521
Method B	ACF	0.128	0.410	0.412	0.425	0.241	0.513
	PCM	0.129	0.719	0.103	0.241	0.242	0.542

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

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.

Results and Discussion

All UV spectrophotometric methods were found to be simple, accurate, economic and rapid for simultaneous estimation of ACF and PCM in tablet dosage form. By performing these methods it was found that both drugs shown good regression value at their respective wavelengths and the recoveries were within 99.67-101.33% for ACF and 99.46-101.92% for PCM. Precision was good with acceptable limits of detection (LOD) and quantitation (LOQ) for both compounds. The average content of the compounds were 100.03 and 100.81% in method-A, 99.55 and 101.05% in method-B for ACF and PCM respectively. The optimized methods showed good reproducibility and recovery with standard deviation of < 1.0% and percent relative standard deviation less than 2.0%. Hence, the proposed methods could be successfully applied to the determination of ACF and PCM 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 mixture of 20 mL (2 M) urea, 30 mL of

(5 M) sodium acetate 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 ACF and PCM containing dosage forms.

Conclusion

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 mixture of 20 mL (2 M) urea, 30 mL of (5 M) sodium acetate 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 ACF and PCM containing dosage forms.

The proposed method is new, simple, cost effective, accurate, safe, free from pollution and precise and can be successfully useful in the routine qualitative and quantitative analysis of poorly water soluble drugs in pharmaceutical dosage forms.

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