Stamford Journal of Pharmaceutical Sciences

ORIGINAL RESEARCH ARTICLE

Qualitative and Quantitative Estimation of Water Insoluble Drugs from its Formulations Simultaneously: a Hydrotropic Approach

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Received – 09 September 2010 *Accepted for Publication - 31 October 2010*

ABSTRACT

Three accurate, precise, sensitive and economical procedures for simultaneous determination of ciprofloxacin hydrochloride and tinidazole in tablet dosage form have been developed. In the present investigation, 1.0M urea solution (hydrotropic solubilising agent) was employed to solubilise, ciprofloxacin, a poorly water-soluble drug, from fine powder of its tablets to carryout spectrophotometric analysis. The methods employed were derivative spectrophotometry method, area under curve method and multi-component method. The result showed that Beer,s-Lambert,s law was obeyed in concentration range of 5-50 µg/ml with good linearity, with a R^2 value >0.99, for both the drugs in all the methods. The recoveries were within 99.42 -101.27% for ciprofloxacin hydrochloride and 99.61-101.81% for tinidazole. 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 then 2.0%.

Key Words: Derivative spectrophotometry method, area under curve method, multi-component method, ciprofloxacin hydrochloride, tinidazole, hydrotropic agent.

INTRODUCTION

Hydrotropy is solubilising effect in water caused by materials that need not be surface active and that do not need to form micelles to effect their action. Hydrotropes are short chain organic compounds with polar groups that could serve as agents to dissolve poorly water soluble substances into water, if added in high concentrations (Swarbrick 2007; Coffman et al., 1996). Hydrotropy is used for solubility enhancement of different class of drugs such as anti-tumor, anti-viral, anti-inflammatory, antipyretic and analgesic drugs, xanthine derivatives etc. Hydrotropy is successfully applied for solubility enhancement of nimueslide, riboflavin, nifedipine, xanthine derivatives like theophylline and caffeine (Lee et al., 2005; Truelove et al., 1987; Jain et al., 1998; Etman et al., 1999). Various organic solvents have been employed for the solubilisation of poorly water soluble drugs for spectrophotometric estimations but due to some drawbacks of organic solvents such as higher cost, toxicity, pollution and error in analysis due to volatile nature of solvents, this approach can be conveniently applied to wide range of water insoluble compounds and thus the use of organic solvents can be precluded. Chemically ciprofloxacin hydrochloride (CPH) is 1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid which is a flouroquinolone derivative and antimicrobial agent with potent activity against a broad spectrum of bacteria (Indian Pharmacopoeia, 1996; British Pharmacopoeia, 1998; United State Pharmacopoeia, 2002). Literature review revealed that chromatographic method was reported for its estimation from tablet formulation (Garcia and Albero, 2001). Chemically tinidazole (TZ) is 1-(2-ethylsulfonylethyl)-2-methyl-5-nitro-imidazole which is an antiprotozoal and anti-bacterial drug (Current Index of Medical Specialities, 2006). These drugs are being used either alone or in combination for the treatment of diarrhoea and dysentery of amoebic, bacterial or mixed origin (Salomies and Salo, 2005). Literature review revealed that chromatographic method was reported for its estimation from tablet dosage form. Since no spectrophotometric methods have been reported for simultaneous estimation of CPH and TZ in combined dosage forms, an attempt has been made to develop simple, sensitive, economical, rapid, precise and accurate methods to analyze the drugs simultaneously.

MATERIALS AND METHODS

Instrumentation

UV-Visible double beam spectrophotometer, Shimadzu model-1700 having spectral bandwidth 3nm and of wavelength accuracy ±1nm, with 1cm quartz cells was used. All weighing were done on electronic balance (Shimadzu, model AY - 120).

Reagents and chemicals

Analytically pure samples of CPH and TZ were obtained as gift samples from Zest Pharma Pvt. Ltd, Indore (MP), India and were used as such without further purification. The tablet dosage form, Cipract TZ (containing CPH 500mg with TZ 600 mg) was procured from the local market, Indore, India. 1.0 M urea was selected as hydrotropic solubilising agent. All other materials used were of analytical reagent grade.

Derivative spectrophotometry method (A)

In this method (Abdel-Aziz et al., 2002) 20 μgmL^{-1} solution for both the drugs was prepared and scanned in the range of 400 nm to 200 nm. 272 nm and 321 nm were selected as analytical wavelengths for estimation of CPH and TZ respectively as CPH had zero crossing point at 272 nm while TZ had zero crossing point at 321 nm (Figure 1). Calibration curves were plotted for CPH (5-50 μgmL^{-1}) at 272 nm and TZ (5-45 μgmL^{-1}) at 321 nm taking dA/dλ vs concentration. The concentrations of both the drugs were obtained from the standard calibration curves by interpolation method.

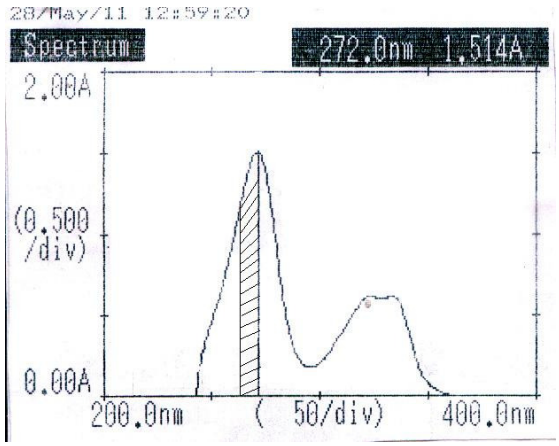

Figure 1: First order derivative overlain spectra of CPH and TZ.

Area under curve method (B)

This method (Zahran et al., 2007) involved 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 CPH in the regions of 272-270 nm and for TZ in the region of 320-317 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 CPH and TZ along with its area under curve region were reported in (Figure 2A and 2B) respectively.

$$\int_{270}^{272} A d\lambda = K_1 C_1 \text{ .....(Eqn.1)} \qquad \int_{317}^{320} A d\lambda = K_2 C_2 \text{ .....(Eqn.2)}$$

$$\int_{270}^{272} Ad\lambda = K_3C_1 \text{ ....(Eqn.3)} \qquad \int_{317}^{320} Ad\lambda = K_4C_2 \text{ .....(Eqn.4)}$$

Where C_1 and C_2 were concentration of CPH and TZ respectively in µg/ml and K_1, K2, K3 and K_4 were constant having values 0.2371, 0.4802, 0.2013 and 0.1008 respectively. Area of curve between 272-270 nm and 320-317 nm represented as $\int_{270}^{272} Ad\lambda$ and $\int_{317}^{320} Ad\lambda$ for CPH and TZ respectively. In view of that, following two final equations were developed for estimation of CPH and TZ.

$$\int_{270}^{272} Ad\lambda = K_1C_1 + K_2C_2 \text{ .....(Eqn.5)} \qquad \int_{317}^{320} Ad\lambda = K_3C_1 + K_4C_2 \text{ .....(Eqn.6)}$$

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

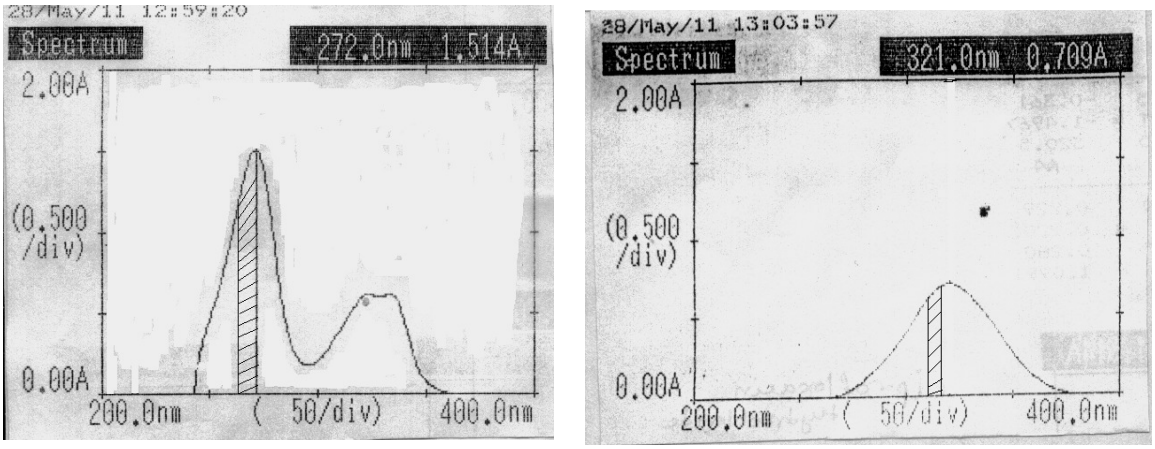

Figure 2: Left (A) UV spectra of CPH along with area under curve and right (B) UV spectra of TZ along with area under curve

Multi-component method (C)

In this method (Zahran et al., 2007) six mixed standards of CPH and TZ in the ratio of 5:6 having concentrations in µg/ml of 5:6, 10:12, 15:18, 20:24, 25:30 and 30:36 were prepared by appropriate dilution of the standard stock solutions and scanned in the region of 400 nm to 200 nm. Sampling wavelengths (272 nm and 321 nm) were selected on trial and error basis. The concentration of individual drug was fed to the multi-component mode of the instrument. The instrument was collected and was compiled the spectral data from mixed standards and concentration of each component were obtained by spectral data of sample solution with reference to that of six mixed standards. Overlain spectra of mixed standards are given in Figure 3.

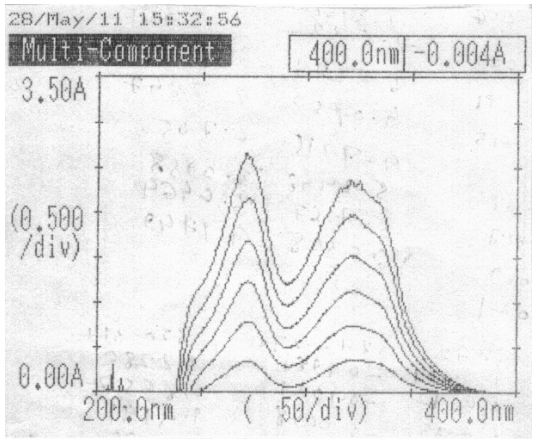

Figure 3: Overlain spectra of mixed standards of CPH and TZ.

Preliminary solubility studies of drugs

Solubility of both drugs was determined at 28 ± 2^0C. An excess amount of drug was added to two screw capped 30 ml glass vials containing different aqueous systems viz distilled water, buffer at pH 6.4, buffer at pH 8.2, and 1.0 M urea. The vials were shaken mechanically for 12 hrs. at 28 ± 1^0C in a mechanical shaker. These solutions were allowed to equilibrate for next 24 hrs. and then centrifuged for 5 mins. at 2000 rpm. The supernatant liquid was taken for appropriate dilution after filtering through Whitman filter paper #41 and analyzed spectrophotometrically against corresponding solvent blank. After analysis, it was found that the enhancement in the solubility of CPH and TZ was found to be more than 30 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 CPH and TZ

About 50 mg each of CPZ and TZ were accurately weighed and transferred to 50 ml of volumetric flask separately. 40 ml, 1.0 M urea was used to solubilise after shaking for 10 to 15 minutes. Rest of the volume was made up with distilled water to get solution of 1000 μgmL^{-1}. Stock solutions of 100μgmL^{-1} of each drugs were prepared by further dilution and scanned over the range of 400 nm - 200 nm in the spectrum mode to get the overlain spectra of both drugs. The spectra exhibited pick absorbance at 272 nm and 321 nm for CPH and TZ respectively. Beer's-Lambert's law obeyed in the range of 5-50 μgmL^{-1} and 5-45 μgmL^{-1} for CPH and TZ respectively. Six mixed standards 5ml, 10ml, 15ml, 20ml, 25ml, 30ml for both CPH and TZ were prepared from stock solutions of CPH and TZ for further study.

Analysis of tablet formulation

Twenty tablets of TZ were taken and their average weight was determined, they were crushed to fine powder. Then powder equivalent to 50 mg (101.91 mg) of TZ was taken in 50 ml volumetric flask and 40 ml, 1.0 M urea was used to solubilise after shaking for 10 to 15 minutes. Rest of the volume was made up with distilled water to get solution of 1000 μgmL^{-1}. Stock solutions of 100 $\mu g mL^{-1}$ of each drugs were prepared by further dilution. The supernatant liquid was transferred to 50 ml of volumetric flask through a Whitman filter paper #41. 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 12 μgmL^{-1} of TZ. Similarly dilution for CPZ was made containing 10 μgmL^{-1} of CPH. The above binary mixture was analyzed at appropriate wavelengths and values of the absorbance were substituted in the respective formulas (Eqn.1, 2, 3, 4) to obtain the content of CPH and TZ. CPH and TZ were determined from their calibration curve plotted between absorbance difference and concentration.

The recovery studies, Validation of the developed methods as per ICH guidelines (International Conference on Harmonization, 1995), accuracy, precision**,** repeatability, limit of detection (LOD) and limit of quantitation (LOQ), intermediate precision (inter-day and intra-day precision) were studied for the data obtained.

RESULTS AND DISCUSSION

Table 1: Result of pharmaceutical formulation analysis

Parameters	Method A		Method B		Method C	
	CPH	TZ	CPH	TZ	CPH	TZ
Label claim (mg/Tab)	500	600	500	600	500	600
Found (mg/Tab)	499.87	600.09	500.43	601.24	501.56	600.11
Drug content[a]	100.03	100.82	99.55	101.05	99.70	100.31
±S.D	0.384	0.530	0.120	0.764	0.281	0.296
%COV	0.331	0.284	0.421	0.108	0.529	0.111
SE	0.403	0.203	0.681	0.303	0.298	0.550

[a]Value for drug content (%) are the mean of six 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.

Table 2: Result of recovery studies

Method	Drug	Lebel claim (Mg/tab)	Amount (mg/ml)		%Recovery ± S.D.	COV%
			Taken	Added		
Method A	CPH	500	30	5	99.42±0.442	0.321
			60	10	101.18±0.820	0.521
			90	15	99.93±0.001	0.119
	TZ	600	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	CPH	500	30	5	100.01±0.182	0.413
			60	10	101.18±0.307	0.108
			90	15	99.99±0.182	0.222
	TZ	600	30	5	100.26±0.326	0.209
			60	10	101.32±0.621	0.491
			90	15	99.61±0.536	0.203
Method C	CPH	500	30	5	100.21±0.110	0.720
			60	10	99.92±0.320	0.331
			90	15	101.27±0.296	0.228
	TZ	600	30	5	101.31±0.200	0.120
			60	10	100.01±0.142	0.311
			90	15	101.58±0.216	0.295

% Recovery is mean of three estimations, 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.

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

Method	Drug	Intraday recision %COV(n =3)	Interday precision %COV			LOD (µg/ml)	LOQ (µg/ml)
			Day 1[a]	Day 2[a]	Day 3[a]		
Method A	CPH	0.331	0.215	0.300	0.309	0.127	0.718
	TZ	0.282	0.490	0.118	0.119	0.180	0.821
Method B	CPH	0.167	0.210	0.337	0.715	0.101	1.013
	TZ	0.329	0.119	0.213	0.111	0.212	0.991
Method C	CPH	0.510	0.110	0.329	0.613	0.180	1.917
	TZ	0.122	0.391	0.110	0.912	0.441	2.011

[a]Mean of six determinations, COV is Coefficient of Variance, LOD is Least of Detection, and LOQ is Least of Quantitation.

All the described UV spectrophotometric methods were found to be simple, accurate, economic and rapid for simultaneous estimation of CPH and TZ in tablet dosage forms. By performing these methods it was found that both drugs showed good regression value at their respective wavelengths and the average content of the compounds were 100.03 and 100.81% in method A, 99.55 and 101.05% in method B and 99.70 and 100.31% in method C for CPH and TZ respectively. The results were reported in Table 1. 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. The recoveries were within 99.42 -101.27% for CPH and 99.61-101.81% for TZ. The results were reported in Table 2. Precision was good with acceptable limits of detection (LOD) and quantitation (LOQ) for both compounds. The results were reported in Table 3. The optimized methods showed good reproducibility and recovery with standard deviation of <1.0% and percent relative standard deviation less then 2.0%. Since urea do not interfere above 245 nm other poorly water-soluble drugs can also be estimated above 245 nm by hydrotropy avoiding the use of

organic solvents.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 CPH and TZ with different composition ratio. Hence, the proposed methods could be successfully applied to the determination of CPH and TZ in the commercially available bulk and tablet dosage forms.

CONCLUSION

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 using 1.0 M urea solution as solubilising 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 CPH and TZ containing dosage forms.

ACKNOWLEDGEMENT

The authors express their gratitude to the Zest Pharma Pvt. Ltd, Indore (MP), India for providing gift samples of pure drug and one of the author Geetam Pathodiya is thankful to AICTE for providing financial assistance.

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