Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2015, 7 (11):234-240
(http://scholarsresearchlibrary.com/archive.html)

Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Application of hydrotropic solubilization technique for simultaneous estimation and validation of ofloxacin and ornidazole in tablet dosage form

Rupali Joshi, Pankaj Bhandari and Dnyanshwar Sangle

Padmashri Dr.Vithalrao Vikhe Patil Foundation's College of Pharmacy, Vilad Ghat, Ahmednagar, (MS), India

---

ABSTRACT

Various organic solvents like methanol, chloroform, alcohol, dimethyl formamide; acetonitrile, hexane, acetone and carbon tetrachloride have been employed for solubilization of poorly water-soluble drugs for spectrophotometric estimations. Drawbacks of organic solvents include high cost, toxicity and error in analysis due to volatility. Attempting to minimize these drawbacks, three new, simple, accurate, environmental friendly, cost effective, safe, sensitive spectrophotometric methods have been developed for simultaneous estimation ofloxacin and ornidazole in tablet dosage form by using aqueous solution of 2.0 M sodium benzoate solution, as a hydrotropic agent. Aqueous solubility of these model drugs was enhanced to a great extent 5 and 11 fold for ofloxacin and ornidazole in 2.0 M sodium benzoate solution respectively. Sodium benzoate solution and additives of tablet did not interfere in analysis, as sodium benzoate did not show any absorbance above 300 nm. Quantitative estimation of these drugs in tablet dosage form was carried out by method-I, first order derivative spectrophotometric, method-II area under curve and method-III multi-component methods of spectrophotometric analysis. Both drugs obeyed Beer's law in the concentration ranges employed for theses methods. Results of analysis were tested and validated for various parameters according to ICH guidelines; hence the proposed methods can be adopted for the routine analysis of ofloxacin and ornidazole in tablet dosage form.

Keywords: Hydrotropic Solubilization, Ofloxacin, Ornidazole, Spectrophotometric Methods.

INTRODUCTION

Spectrophotometric estimation of poorly water soluble drugs generally requires the use of organic solvents, acid or base. Various organic solvents like methanol, 95% ethanol, cycloheaxne and 1,4-dioxane etc are generally used for solubilization of poorly water soluble drugs. High cost, toxicity and pollution are the drawbacks of organic solvents. These drawbacks can be overcome by the use of hydrotropic solubilizing agents such as sodium salicylate, sodium benzoate and sodium lauryl sulphate, sodium glycinate and sodium gentisate, urea, sodium acetate and sodium citrate and, niacinamide. The pH of 2.0M Sodium benzoate solution was 8.2 Therefore in order to check the influence of pH on solubilities of ofloxacin and ornidazole, buffer solution of 8.2 pH was made and the solubilities of the drugs were determined. There was nearly no difference in the solubility of ofloxacin and ornidazole in distilled water and buffer of pH 8.2. However aqueous solubility's of these model drugs were enhanced to a great extent 5 and 11 fold for ofloxacin and ornidazole in 2.0M sodium benzoate solution respectively. The study proved that increases in solubility of ofloxacin and ornidazole were not due to change in pH but was due to hydrotropic solubilization.

The said phenomenon has been successfully applied for the analysis of various drugs.[1-3]

Literature survey revealed that some methods are available for the determination of ofloxacin and ornidazole in combination by UV, HPLC.[4-6] None of these methods is without limitations so the need was felt to develop new, simple, accurate, cost effective, spectrophotometric methods for simultaneous estimation of ofloxacin and

ornidazole in tablet dosage form by using aqueous solution of 2.0 M sodium benzoate solution, as a hydrotropic agent.

MATERIALS AND METHODS

Materials

The commercially available tablets, Oflomac-OZ (Label claim: Ofloxacin-200mg, ornidazole- 500mg) was procured from local market. 2.0 M sodium benzoate solution was used as hydrotropic agent after considering the solubility factors of both the drugs as well as the interference due to excipient matrix present in tablet formulation.

Instrument

UV-visible double beam spectrophotometer, Shimadzu model 1700 with spectral bandwidth of 1 nm, wavelength accuracy of ± 0.3 nm and The absorption spectra of the reference and test solutions were recorded in 1 cm quartz cells over the range of 200– 400 nm.

Preliminary solubility studies of drugs

Solubility of both drugs was determined at 28±1°C. An excess amount of drug was added to three screw capped 30ml glass vials containing different aqueous systems viz. distilled water, buffer of pH 8.2 and 2.0 M sodium benzoate solution. The vials were shaken for 12 hrs in a mechanical shaker. These solutions were allowed to equilibrate for the next 24 hrs and then centrifuged for 5 minutes at 2000 rpm. The supernatant of each vial was filtered through Whatman filter paper No.41. The filtrates were diluted suitably and analyzed spectrophotometrically against corresponding solvent blank.

Preparation of standard stock and binary mixture solutions

The standard stock solutions of ofloxacin and ornidazole were prepared by dissolving 50 mg of each drug in 40 ml of 2.0 M sodium benzoate solutions and final volume was adjusted with distilled water in 100ml of volumetric flask. From the above solution 10 ml of solution was taken and diluted to 50 ml with distilled water to get a solution containing 100 µg/ml of each drug. Working standard solutions were scanned in the entire UV range of 400-200 nm. For method I and II six mixed standard solutions with concentration of ofloxacin and ornidazole in the ratio 30:5,25:10,20:15,15:20,10:25,5:30 and for method-III; seven mixed standards solutions with concentration of ofloxacin and ornidazole in the ratio 5:12.5,6:15,7:17.5,8:20,9:22.5,10:25 and 11:27.5 were prepared in distilled water by diluting appropriate volumes of the standard stock solutions.

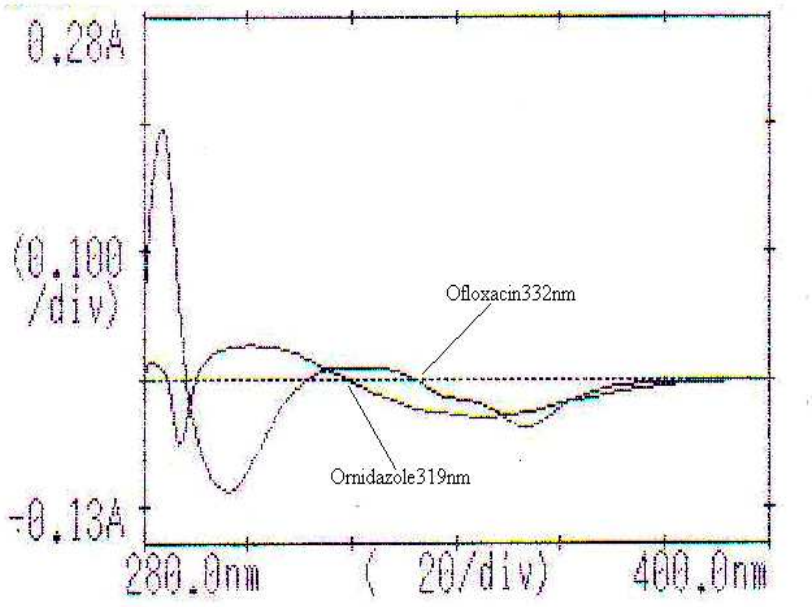

Fig.1 First order derivative overlain spectra of Ofloxacin and Ornidazole

Method-I: Derivative Spectrophotometry Method

In this method, Saudagar at al.(2006) 20 µg/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 then recorded, which showed ofloxacin had zero crossing point at 332 nm while ornidazole had zero crossing point at 319nm (Fig. 1). At the zero crossing

point of ofloxacin, ornidazole showed a measurable dA/dλ where as at the zero crossing point of ornidazole, ofloxacin showed appreciable dA/dλ. Hence both wavelengths 319 and 332 nm were selected as analytical wavelengths for estimation of ofloxacin and ornidazole respectively. Calibration curves were plotted for ofloxacin (5-35 µg/ml) at 319 nm and ornidazole (5-40 µg/ml) at 332nm as dA/dλ v/s concentration. The concentrations of both the drugs were obtained from the standard calibration curves by interpolation method.

Method II: Area Under Curve Method (AUC)

AUC method, Thomas at al. (2007) 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 the calculation of concentration of ofloxacin in the region of 350-347 nm and for ornidazole in the region 331-328nm and 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 ofloxacin and ornidazole along with their AUC region are given in Fig. 2 and 3 respectively.

$$\int_{328}^{331} A d\lambda = K_1 C_1 \text{ .....Eqn.1} \qquad \int_{347}^{350} A d\lambda = K_2 C_1 \text{ .....Eqn.2}$$

$$\int_{328}^{331} A d\lambda = K_3 C_2 \text{ .....Eqn.3} \qquad \int_{347}^{350} A d\lambda = K_4 C_2 \text{ .....Eqn.4}$$

Where C_1 and C_2 are the concentration of ornidazole and ofloxacin in µg/ml respectively and K_1, K_2, K_3 and K_4 were constant having values 0.1122, 0.0603, 0.0963 and 0.0691 respectively. Area of curve between 350-347nm and 331-328nm represented as $\int_{347}^{350} A d\lambda$ and $\int_{328}^{331} A d\lambda$ for ofloxacin and ornidazole respectively. In view of that, following two final equations were developed for the estimation of ofloxacin and ornidazole.

$$\int_{347}^{350} A d\lambda = 0.0603 x C_1 + 0.0691 x C_2 \text{ .....Eqn.5} \qquad \int_{328}^{331} A d\lambda = 0.1122 x C_1 + 0.0963 x C_2 \text{ .....Eqn.6}$$

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

Method III: Multi-component Method

In this method, Gangwal and Trivedi (1999) seven mixed standards of ofloxacin and ornidazole in the ratio of 1:2.5 having concentrations in µg/ml 5:12.5,6:15,7:17.5,8:20,9:22.5,10:25 and 11:27.5 were prepared in distilled water by diluting appropriate volumes of the standard stock solutions and scanned in the region of 400 nm to 200 nm. Sampling wavelengths (320 and 332 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 seven mixed standards. Overlain spectra of mixed standards are given in Fig. 4.

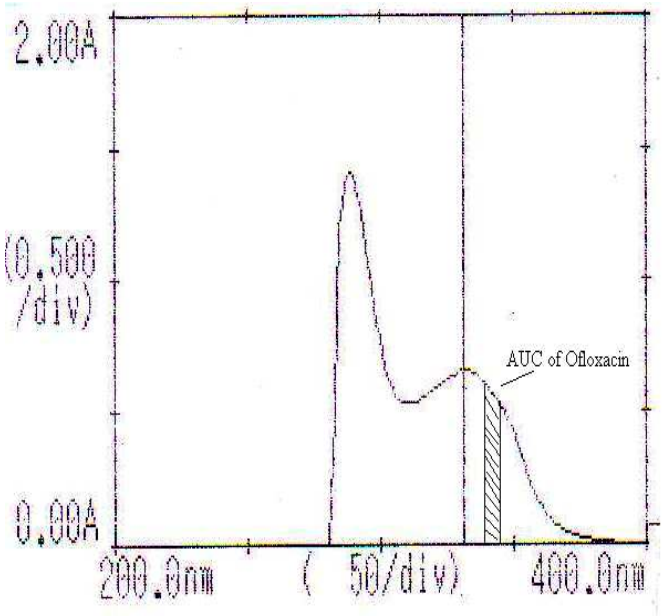

Fig.2. The UV spectra of ofloxacin along with it's AUC

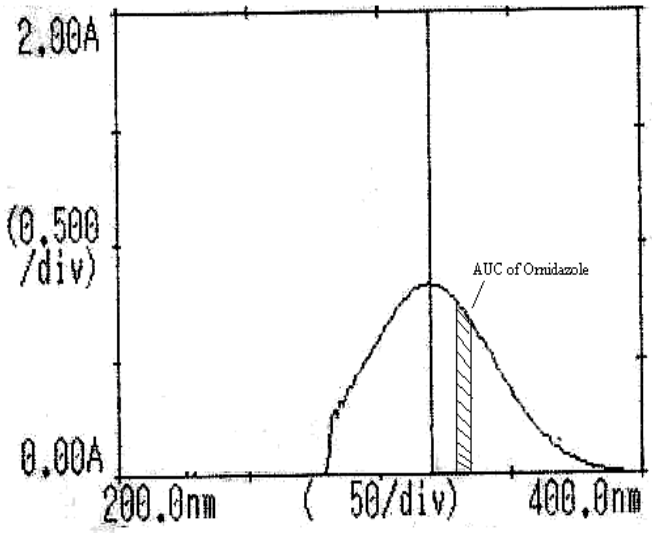

Fig.3 The UV spectra of ornidazole along with it's AUC

Analysis of the tablet formulations

Twenty tablets of marketed formulation were accurately weighed and powdered. A quantity of powder equivalent to 50 mg of ornidazole was transferred to 100 ml volumetric flask and dissolved in 40 ml of 2.0M sodium benzoate with frequent shaking for 15minutes and final volume was made up with distilled water. The sample solution was then filtered through Whatman filter paper No.41 and first few ml were rejected. From the above solution 10ml of solution was taken and diluted to 50ml with distilled water to get a solution containing 100 µg/ml of ornidazole and corresponding concentration of ofloxacin. This solution contains ofloxacin and ornidazole in the proportions of 1:2.5 For three methods- 2.0 ml of solution was transferred in 10ml volumetric flask and diluted with distilled water to obtain final concentration of 8µg/ml of ofloxacin and 20 µg/ml of ornidazole Then these sample solutions were scanned using proposed three methods and the results were obtained. Analysis procedure was repeated six times with tablet formulation. The result of analysis of tablet formulation was reported in Table 1.

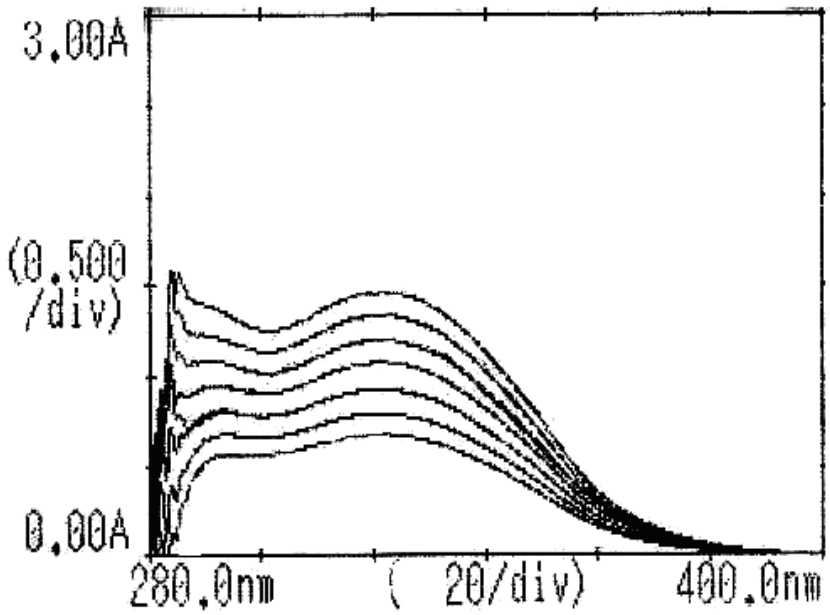

Fig-4 Overlain spectra of mixed standard of ofloxacin and ornidazole

VALIDATION OF THE DEVELOPED METHODS

The developed methods for simultaneous estimation of ofloxacin and ornidazole were validated as per ICH guidelines. [7, 8]

Accuracy

To check the accuracy of the proposed methods, recovery studies were carried out at 80,100, and 120% of the pure drug concentration as per ICH guidelines. The recovery study was performed three times at each level. The results of the recovery studies are given in Table 2.

Precision

Repeatability

To check the degree of repeatability of the methods, suitable statistical evaluation was carried out. Repeatability was performed for six times with tablets formulation. The standard deviation, coefficient of variation and standard error was calculated. The result of statistical evaluation are given in Table 1.

Intermediate Precision- (Inter-day and Intra-day precision)

The intra-day and inter-day precision was determined by assay of the sample solution on the same day at different time intervals and on different days respectively. The result of the same are presented in Table 3.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD & LOQ for ofloxacin and ornidazole by proposed methods were determined using calibration standards. LOD and LOQ were calculated as $3.3\sigma/S$ and $10\sigma/S$, respectively, where S is the slope of the calibration curve and σ is the standard deviation of response. The results of the same are shown in Table 3.

RESULTS AND DISCUSSION

Solubility studies indicated that aqueous solubility of ofloxacin and tinidazole were enhanced more than 5 and 11 folds in 2.0 M sodium benzoate solution as compared to solubility in distilled water and buffer of pH 8.2 respectively.

For each drug, appropriate dilutions of standard stock solutions were assayed as per the developed methods. For method-I,II and III, the Beer- Lambert's concentration range was found to be 5-35 µg/ml for ofloxacin and 5-40 µg/ml for ornidazole at respective selected wavelengths and coefficient of correlation were found 0.9999, 0.9998, 0.9998 for ofloxacin at 319nm, 350-347nm, 332nm and 0.9987, 0.9995, 0.9997 for ornidazole at 319 nm, 331-328nm 320nm respectively.

Percentage estimation of both drugs was found in tablet dosage form were 99.96 and 99.98 in method I, 99.70 and 98.98 in method II and 99.49 and 99.25 for ofloxacin and ornidazole respectively with standard deviation <1 (Table 1).

Table 1: Analysis Data of Tablet Formulation

Method	Drug	Label claim mg/tab	Amount found* mg/tab	Label claim (%)	S.D.*	% COV	S.E*.
I	OF	200	198.54	99.96	0.0212	0.0212	0.0086
	OR	500	496.615	99.98	0.0126	0.0126	0.0051
II	OF	200	198.049	99.70	0.3874	0.3885	0.1581
	OR	500	492.534	98.98	0.2695	0.2722	0.1100
III	OF	200	197.253	99.49	0.7746	0.7785	0.3162
	OR	500	493.879	99.25	0.4959	0.4996	0.2024

*OF: Ofloxacin, OR:Ornidazole, S.D.: Standard deviation, COV: Coefficient of variation, S.E.: Standard error,*Average of six estimation of tablet formulation.*

The validity and reliability of proposed methods were assessed by recovery studies. Sample recovery for both the methods are in good agreement with their respective label claims, which suggested non interference of formulation additives and hydrotropic solubilizing agent sodium benzoate in estimation. (Table-2)

Table 2 Result of Recovery studies

Replicate	Amount taken (µg/ml) Tablet		Amount added at	(µg/ml)		% Recovery Method I		% Recovery Method II		% Recovery Method III	
	OF	OR	%	OF	OR	OF	OR	OF	OR	OF	OR
1	4.8	12		3.8	9.6	100.00	101.00	99.12	100.00	99.890	101.25
2	4.8	12	80%	3.8	9.6	98.60	100.22	101.77	101.22	99.250	99.980
3	4.8	12		3.8	9.6	99.97	99.45	100.13	101.45	102.06	99.450
1	4.8	12		4.8	12	98.55	101.00	99.55	99.10	98.970	99.780
2	4.8	12	100%	4.8	12	99.10	100.55	101.10	99.55	100.20	100.08
3	4.8	12		4.8	12	100.00	99.54	99.00	98.45	101.50	102.40
1	4.8	12		5.7	14.4	99.85	98.12	98.00	99.99	99.980	101.05
2	4.8	12	120%	5.7	14.4	98.63	100.77	99.60	100.50	100.09	99.470
3	4.8	12		5.7	14.4	100.54	99.13	100.97	99.00	99.120	99.160

OF-ofloxacin, OR-ornidazole

Precision was determined by studying the repeatability and intermediate precision. Repeatability result indicates the precision under the same operating conditions over a short interval time and inter-assay precision. The standard deviation, coefficient of variance and standard error were calculated for ofloxacin and ornidazole. The results were mentioned in Table 1. Intermediate precision study expresses within laboratory variation in different days. In both intra and inter-day precision study for both the methods %COV were not more than 2.0% indicates good repeatability and intermediate precision (Table 3).

Table 3: Validation Parameters

Method	Drug	LOD* µg/ml	LOQ* µg/ml	Precision (% COV) Intraday *	Interday n=3
I	OF	0.0528	0.1601	0.7989	0.6132
	OR	0.0824	0.2499	0.6863	0.6254
II	OF	0.0235	0.0712	0.8137	0.6546
	OR	0.1059	0.3210	0.5089	0.7102
III	OF	0.1576	0.4778	0.4532	0.6880
	OR	0.1255	0.3805	0.3442	0.7743

*OF: Ofloxacin, OR: Ornidazole, COV: Coefficient of variation, * Average of six determination*

The value of LOD and LOQ were 0.0528µg/ml, 0.1601 µg/ml for ofloxacin and 0.0824 µg/ml, 0.2499for ornidazole in method I, 0.0235 µg/ml, 0.0712 µg/ml for ofloxacin and 0.1059 µg/ml, 0.3210µg/ml for ornidazole in method II, 0.1576 µg/ml, 0.4778 µg/ml for ofloxacin and 0.1255µg/ml,0.3805 µg/ml for ornidazole respectively.(Table-3)

CONCLUSION

The present paper describes application of hydrotropic solubilization phenomenon for the simultaneous estimation of ofloxacin and ornidazole in tablet dosage form by first order derivative spectrophotometric, area under curve and multi-component methods of spectrophotometric analysis. Both drugs showed good regression values at their respective wavelengths and the results of recovery study reveled that any small change in the drug concentration in

the solution could be accurately determined by the proposed methods and low values of LOD and LOQ indicated good sensitivity of proposed methods. Hence proposed methods are new, simple, accurate, sensitive, free from pollution and precise and can be adopted for routine analysis of ofloxacin and ornidazole in tablet dosage form. Further, as sodium benzoate does not absorb above 300 nm, a large number of drugs having λmax above 300 nm can be used for estimation by proposed methods.

REFERENCES

[1]Revathi Gunji, Rama Rao Nadendla, Venkata Suresh Ponnuru, *Int. J. Drug Dev. & Res.*, 4 (1) **2012** 316-324.
[2]S. R. Kadam, Minakshi V. Janjale, S. B. Akole, S. S. Bhosale, *International Journal of Pharmaceutical & Biological Archives*, 3(2) **2012** 383-390.
[3]A. P. Sherje and K. J. Desai, *Indian J Pharm Sci.* 73(5)**2011** 579–582. doi: 10.4103/0250-474X.99021.
[4]V. M Gandhi, S. B. Nair, C. Menezes and R. Narayan, *International Journal of Research In Pharmacy And Chemistry*, , 3(1) **2013** 6-11.
[5]B.Dhandapani, N.Thirumoorthy, Shaik Harun Rasheed, M.Rama kotaiah and N.Anjaneyalu, *International Journal of Pharma Sciences and Research* Vol.1(1) **2010** 78-83.
[6]Soumya Jyoti Ghosh, Soumendra Darbar, Partha Pratim Chowdhury, Shyama Prasad Chattopadhyay and Matish Ranjan Chakraborty, *International Journal of PharmTech Research* 2(1) **2010** 367-374.
[7]S. Rupali Joshi, S. Nilima Pawar, S. Sameer Katiyar, B. Devendra Zope, T. Amol Shinde, *Der Pharmacia Sinica*, **2011**, 2(3), 164-171
[8]Text On Validation Of Analytical Procedures, **1994**, International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Steering Committee.