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PharmTech

International Journal of PharmTech Research
CODEN(USA): IJPRIF ISSN : 0974-4304
Vol.1, No.2, pp 121-124, April-June 2009

RP-HPLC Method Development and Validation for the Simultaneous Estimation of Ofloxacin and Tinidazole in Tablets

J.Dharuman[1*], M.Vasudevan[2], K.N.Somasekaran[3], B.Dhandapani[a] , Prashant D.Ghode[a] and M.Thiagarajan[a].

[1] Department of Pharmaceutical Analysis, K.M.C.H. College of Pharmacy, Kovai Estate, Kalapatti Road, Coimbatore-641035. Tamilnadu, India.

[2] Roxanne Research Laboratory Limited, Taramani, Chennai- 600013, India.

[3] Dean, School of Chemical and Biotechnology, SASTRA University, Thanjavur - 613402. India.

E-mail: dharumanj@yahoo.com.

Abstract: A simple Reverse phase liquid chromatographic method has been developed and subsequently validated for simultaneous determination of Ofloxacin and Tinidazole in combination. The separation was carried out using a mobile phase consisting of 0.5%v/v Triethylamine buffer of pH 3.0 and Acetonitrile in the ratio of 73: 27. The column used was Kromasil C_8, 5µ, 15 cm × 4.6 mm id with flow rate of 1.2 ml / min using PDA detection at 303 nm. The described method was linear over a concentration range of 10-50 µg/ml and 30-150 µg/ml for the assay of Ofloxacin and Tinidazole respectively. Ambroxol (50 µg/ml) was used as internal standard. The retention times of Ofloxacin, Tinidazole and Ambroxol were found to be 2.3, 4.1 and 5.1min respectively. Results of analysis were validated statistically and by recovery studies. The limit of quantification (LOQ) for Ofloxacin and Tinidazole were found to be 10 and 30 µg/ml respectively.

The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Ofloxacin and Tinidazole bulk drug and in its pharmaceutical dosage form..

Keywords: Ofloxacin, Tinidazole and Ambroxol.

Introduction

Ofloxacin is a broad spectrum, fluorinated quinolone antibacterial drug, chemically it is a 9-fluro-2, 3-dihydro - 3-methyl – 10 - (4-methyl – 1 - piperazinyl) - 7-oxo - 7H – pyrido [1, 2, 3–de]-1,4 benzoxacine-6-carboxylic acid [1]. Tinidazole (TNZ) is a 1-[2-(ethyl sulphonyl) ethyl] – 2- methyl – 5- nitro – 1H- imidazole, derivative used as antiprotozoal/antibiotic and antibacterial [2]. The literature survey revealed that few methods have been reported for the estimation of Ofloxacin and Tinidazole. So far, no method has been reported [3 - 8] for estimation of OFL and TNZ in combined dosage forms, hence we attempted to develop a simple, accurate, and economical analytical method. This paper describes validated RP-HPLC for simultaneous estimation of OFL and TNZ in combination using 0.5% triethylamine buffer of pH 3.0 and acetonitrile in the ratio of 73: 27. The column used was Kromasil C-8 with flow rate of 1.2 ml / min using PDA detection at 303 nm.

Materials and methods

Standard bulk drug sample Ofloxacin and Tinidazole and Ambroxal were provided by Micro Laboratories Ltd., Bangalore. Tablets of combined dosage form were procured from the local market. All other reagents used were of HPLC grade. HPLC (Shimadzu LC-20AT) method was developed using Kromasil C_8, 5μ, 15 cm × 4.6 mm id. Mobile phase selected for this method contained 73 parts of 0.4%v/v Triethylamine buffer (0.5ml /100ml) and 27 parts of Acetonitrile adjusted to pH 3 with 0.1% orthophosphoric acid that was filtered through 0.45-micron membrane filter. Flow rate employed was 1.2 ml/min. Detection of eluent was carried out at 303 nm using PDA detector. Method was developed using Ambroxal as internal standard. Standard stock solutions of pure drugs were made separately in mobile phase containing 10-50 µg /ml of Ofloxacin, 30-150 µg /ml of Tinidazole and 10 µg /ml of Ambroxal and filtered through a 0.45µ membrane filter. Each solution was injected and a chromatogram was recorded. Mean retention times Ofloxacin,

Tinidazole and Ambroxol were found to be 2.3, 4.1 and 5.1 min respectively.

Analysis of formulation:

Twenty tablets of the formulation were weighed and the average weight per tablet was calculated. Twenty tablets were crushed and ground to a fine powder. Powder equivalent to 150 mg of Tinidazole was weighed and transferred to a 100 ml volumetric flask .The tablet powder was dissolved in the mobile phase and filtered through a membrane filter (0.45µ). The sample solution was suitably diluted and used for the analysis. After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, the tablet sample solution was loaded in the 20 µl fixed **-** sample loop of the injection port. The solution was injected and a chromatogram was recorded. The injections were repeated six times and the peak areas were recorded. A representative chromatogram has been given in **Figure-1**. The peak area ratios of each of the drugs to the internal standard were calculated and the amount of each drug present per tablet was estimated from the respective calibration curves. The result of analysis reported in **[Table – 1].** The stability of the sample in mobile phase was analyzed after 24 hrs; it was found no change in analytical parameters [9].

Recovery studies:

To study the accuracy, reproducibility and precision of the above methods, were carried out by addition of standard drug solution to pre-analyzed sample at different levels. Results of recovery studies were found to be satisfactory and are reported in **[Table – 1].**

Table 1: Analysis of formulation and Recovery studies

Drugs	Labelled Amount (mg)	Amount taken for assay (µg/ml)	*Amount found(mg)	% label claim	*%Recovery	*Precision# (%RSD)	
						Inter day	Intra day
Ofloxacin	15	15	14.98±0.679	99.86	100.85±1.256	0.348	0.684
Tinidazole	45	45	44.86±1.140	99.62	99.58±0.896	0.289	0.782

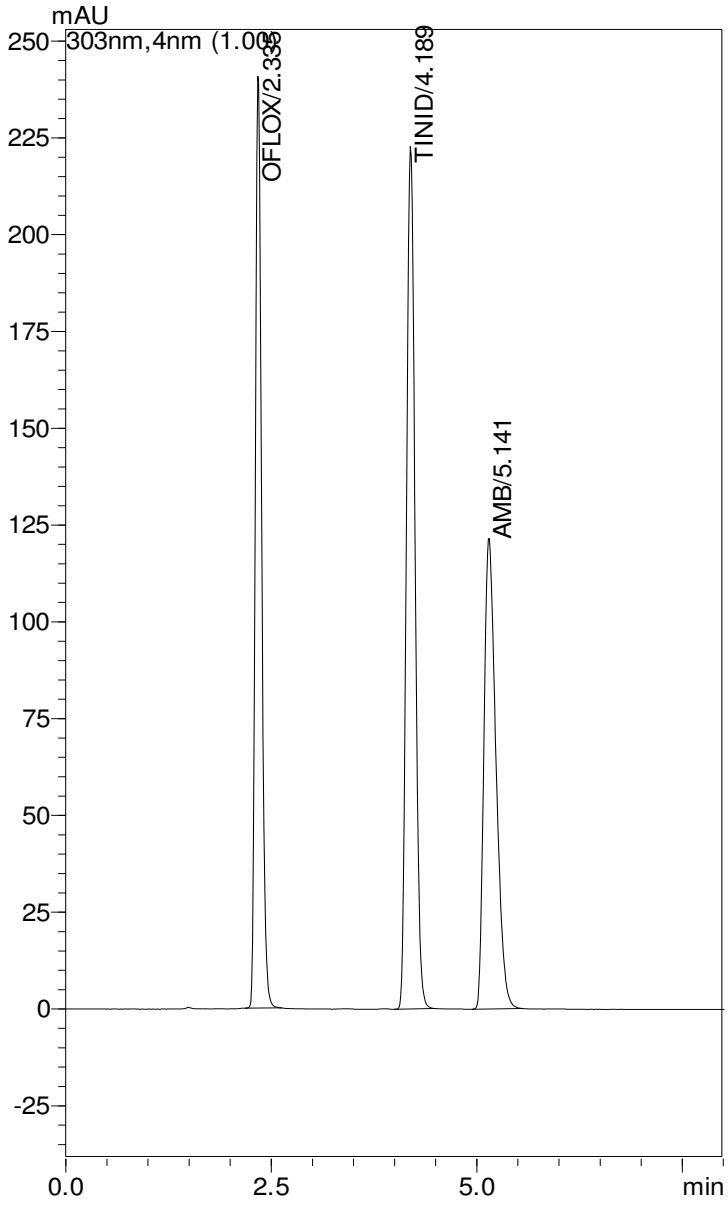
*Each value is a mean of six observations.

Table 2:

Validation Parameters	**OFL**	**TNZ**
Linearity range (µg / ml)	10-50	30-150
r	0.9982	0.9987
LOD (ng /ml)	5	10
LOQ (ng /ml)	10	30
Intra day (% RSD)*	0.684	0.782
Inter day (% RSD)*	0.384	0.289
Repeatability (% RSD)*	0.3251	0.4250
Accuracy	100.85±1.256	99.58±0.896
Peak purity index	1.0000	1.0000
Resolution factor (R_s)	-	6.218
Asymmetry factor (A_s)	0.95	
No.of theoritical plates (N)	6452	6957
Capacity factor ($K^{'}$)	-	0.330
High equivalent to theoritical plates (HETP)	21.075	23.475
Tailing factor	1.320	1.443
Seletivity factor (α)	3.959	

* Each value is a mean of six observations.

Figure – 1 Chromatogram for formulation



Results and Discussion

The developed RP-HPLC method for simultaneous estimation of Ofloxacin and Tinidazole from combined dosage form utilizing C_8 column and 0.5 % Triethylamine and Acetonitrile as mobile phase. Detection of eluent was carried out using PDA detector at 303 nm. The method was developed using Ambroxal as internal standard. The run time per sample is just 6 min. The excipients in the formulation did not interfere in the accurate estimation of Ofloxacin and Tinidazole. The method was validated as per ICH guidelines in terms of linearity, accuracy, specificity, intraday and interday precision, repeatability of measurement of peak area as well as repeatability of sample application and the results are shown in **Table -2**. Since none of the methods is reported for simultaneous estimation of Ofloxacin and Tinidazole from combined dosage form, this developed method can be used for routine analysis of two components in formulation.

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