International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol. 3, No.2, pp1169-1173, April-June 2011

Development and Validation of Densitometric Method for Metronidazole and Tetracycline hydrochloride in capsule Dosage form

S. Sharma[1], M. C. Sharma*

[1]Department of Chemistry Chodhary Dilip Singh Kanya Mahavidyalya, Bhind (M.P) India

***School of Pharmacy, Devi Ahilya Vishwavidyalaya, Indore (M.P) 452001, India**

Corres.author: mukeshcsharma@yahoo.com

Abstract: A new, simple, precise, and accurate HPTLC method for simultaneous quantitation of Metronidazole (MET) and and Tetracycline hydrochloride (TET) as the bulk drug and in capsule dosage forms have been developed. Chromatographic separation of the drugs was performed on aluminium plates precoated with silica gel 60 F254 as the stationary phase and the solvent system consisted of benzene: ethyl acetate: toluene: methanol: glacial acetic acid (9.5:2.0:5.0:1.5:0.5 v/v/v/v/v),Densitometric evaluation of the separated zones was performed at 254 nm. The two drugs were satisfactorily resolved with R_f values of 0.43 ± 0.02 and 0.74± 0.02 for MET and TET, and 0.29 Ofloxacin (OF) respectively. The accuracy and reliability of the method was assessed by evaluation of linearity (600-2400ng spot-1 for MET and 600-1800ng $spot^{-1}$ for TET).Ofloxacin (OF) was used as an internal standard. Linearity was observed over the concentration range of 600-2400 $ng.band^{-1}$. The linearity of the calibration plots was confirmed by the high value of the correlation coefficients (r2 = 0.9998 for MET and 0.9991 for TET). Hence it can be applied for routine quality control analysis of Metronidazole and Tetracycline hydrochloride in capsule Dosage form.
Keywords: Metronidazole, Tetracycline hydrochloride, Ofloxacin, Densitometric.

Introduction

Chemically Metronidazole (MET) is 2-(2-methyl-5-nitro-1H-imidazole-1y1) ethanol, acts by causing loss of helical structure of DNA, strand breakage and accompanying the impairment of DNA's function and used in the trichomoniasis, amoebiasis, girardiasis[1,2] while Tetracycline hydrochloride (TET) is (4s, 4as, 5as, 6s, 12as)-dimethylamino-1,4, 4as, 5a, 6, 11, 12a-octa hydro-10, 12, 12a-pentahydroxy-6-methyl-1,11-dioxo naphthacene-2-carboxamide hydrochloride which acts by inhibiting subsequent binding of amino acyl transfer RNA to ribosomes resulting in termination of peptide chain growth and used to S.T.D., U.T.I. infection, traveller's diarrhea[3,4]. Author of the article and his research team has developed a HPTLC Method development different pharmaceutical dosage form[5-9]. The proposed method overcomes many difficulties of tracing out lowest determination and quantification of related substances the products. Also the affirmative points are; less instrument set up time by mean of simple isocratic elution which results into a negligible noise as compare to gradient methods.

Materials and methods

Toluene, acetonitrile, methanol and formic acid used were of analytical grade. All dilutions were performed in standard volumetric flasks. The commercially available capsules; Meklin (Label claim: MET 400mg, TET 333mg) manufactured by Bennett Pharmaceuticals, Baroda, Gujarat, India was procured from local market. Acetonitrile and Phosphate buffer AR grade was obtained from Merck Limited, India.

Instrument

Chromatographic separation of drug was performed on Merck TLC plate pre-coated with silica gel 60 F_{254} (10 cm ×10 cm with 250 mm layer thickness) from E. Merck, Germany. The samples were applied onto the plates as a band with 6 mm width using Camag 100 µl sample syringe with a Linomat 5 applicator. Linear ascending development was carried out in a twin trough glass chamber (for 10 x 10 cm). Densitometric scanning was performed using Camag TLC scanner 3 in the range of 600-3000 ng/spot and operated by win CATS software (V 1.4.2, Camag). The plates were developed in a twin through chamber previously saturated for 30 min with mobile phase, benzene: ethyl acetate: toluene: methanol: glacial acetic acid (9.5:2.0:5.0:1.5:0.5 v/v/v/v/v), for a distance of 8 cm. The spots on the air dried plate were scanned with a scanner III at 283 nm using the deuterium source. The plates were prewashed by methanol and activated at 120^{o}C for 5 min prior to chromatography. A constant application rate of 0.1 was employed and space between two bands was 5 mm. The slit dimension was kept at 5mm, 0.45mm and 10 mm/s scanning speed was employed. The monochromatic bandwidth was set at 20 nm, each track was scanned thrice and baseline correction was used. The optimized chamber saturation time for mobile phase was 20 min at room temperature (35 ^{o}C) at relative humidity of 60% . The length of chromatogram run was 8 cm. Subsequent to the development; TLC plates were dried in current of air with the help of air dryer in wooden chamber with adequate ventilation. The flow rate in laboratory was maintained unidirectional (laminar flow, towards exhaust). The solution was sonicated for 15min. the extracts were filled through Whatmann filter paper No. 41 and residue washed thoroughly with methanol. The extracts and washings were transferred to 25ml volumetric flask and volume was made up to 25ml with methanol. Required dilutions were made to get 100µg/ml.

Preparation of Standard Stock Solutions

50 mg of each drug MET and TET were weighed separately and dissolved in 20 ml of methanol and then volume was made up to 50 ml so as to get the concentration 1 mg mL^{-1}. From each of these solutions 1ml of solution was pipette out and transferred to 10 ml volumetric flasks and volume was made up to the mark using methanol so as to get the concentration 100 µg mL-1. The stock solution was stored at 2–8 ^{0}C protected from light.

Preparation of working solution

Further, the mixture of working solution was prepared by diluting 50 mL of MET and 12 mL of TET with 1.0 mL of Ofloxacin (OF), internal standard stock solution in 50.0 mL volumetric flask.

Preparation of sample solution

Twenty tablets were Meklin (Label claim: MET 400mg, TET 333mg) weighed and average weight was calculated. These tablets were crushed, powdered and taken in a 10 mL volumetric flask weight equivalent to one tablet and dissolved in minimum amount acetonitrile. To this flask 1.0 mL of stock\ solution of internal standard was added and diluted up to the mark with methanol and sonicated for 30min. This solution was then filter through Whatman no. 41.The filtrate was collected in the flask and used as sample solution.

Selection of Detection Wavelength

After chromatographic development bands were scanned over the range of 200-400 nm. It was observed that the drug showed considerable absorbance at 283 nm.

Method Validation[10-12]

Linearity

Seven different concentrations of mixture of MET and TET were prepared from stock solution of MET and TET in the range of 600 to 2400.50 µg/mL and 600to 1800 µg/mL respectively, in methanol to obtain desire linearity range. 10 µL of each solution was applied to a plate (*i.e.* 0.5-2.5 µg/spot for MET and 0.75-3.75 µg/spot for TET) by sample applicator and the plate was developed. The detector response to the different concentrations was measured.

Limit of detection (LOD) and Limit of quantification (LOQ):

The LOD and LOQ were calculated using following equations as per International conference on Harmonization (ICH) guideline.

LOD = 3.3 x σ/S and LOQ = 10 x σ/S

Where σ = standard deviation of the response and S = the standard deviation of y intercept of regression lines.

Precision

Precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analysis of three different concentrations (600- 2400 ng.band-1) of the drug, each concentration injected six times on the same day. Intermediate precision of the method was checked by repeating studies on three different days.

Robustness of the method

Small changes in the mobile phase composition (± 0.1 mL for each component) were made and the effects on

the results were examined. Time from spotting to chromatography and from chromatography to scanning was varied by ± 15 min.

Accuracy

Accuracy was determined by recovery studies. It was carried out by spiking 80%, 100% and 120% of the standard drugs to the pre-analysed marketed sample of MET and TET. Three determinations were performed at each level.

Tablet formulation Assay

50 µL of sample solution was spotted along with same concentration of working solution (.5-2.5 µg/spot for MET and 0.75-3.75 µg/spot for TET) on to the plate under the optimized chromatographic conditions. The procedure was repeated seven times. The peak area ratio values of MET and TET to the internal standard were calculated. The densitometric responses from the standard and sample were used to calculate the amounts of the drug in the tablet.

Recovery studies

Recovery experiments were carried out to check for the presence of positive or negative interferences from excipients present in the formulation, and to study the accuracy and precision of the method. Recovery experiment was performed by the standard addition method. The recovery of the added standard was studied at three different levels *viz* 110%, 120% and 130% of the estimated amount of the drug.

Method precision (repeatability) –

The precision of the instrument was checked by repeatedly injecting (n= 6) mixed standard solution of MET and TET.

Intermediate precision (reproducibility) –

The intraday and interday precision of the proposed method was determined by analyzing mixed standard solution of MET and TET at concentration 600, 1800, 2400 ng/spot; 700, 1400, 1800 ng/spot three times on the same day and on three different days. The results are reported in terms of relative standard deviation.

Table 1 Regression Analysis of Calibration Graph for MET and TET

Parameter	MET	TET
R_f(SD)	0.43	0.74
Linearity and range (ng\spot)	2400	1800
Linearity detection (ng\spot)	81	97
Limit of quantification (ng\spot)	215	331
Repeatability of application(%RSD)	0.32	0.74
Repeatability of measurement (%RSD)	0.18	0.29
Intraday (%RSD)	0.95	1.13
Inter day (%RSD)	0.54	0.83
LOD[a]	0.88	0.91
LOQ[b]	1.93	1.27

[$] SD = Standard Deviation

Table 2 -Recovery Study

MET				TET			
Label claimed	**%Amount added**	**Found in(µg/ml)**	**%recovery**	**Label claimed**	**%Amount added**	**Found in(µg/ml)**	**%recovery**
400	80	401.28	100.09	333	80	334.01	101.43
	100	399.04	99.95		100	333.87	101.04
	120	400.15	100.10		120	332.99	99.92

Table 3 Result of Assay of Tablet Formulation

MET		TET	
Amount claimed (mg/tablet)	**Amount found (mg/tablet)**	**Amount claimed (mg/tablet)**	**Amount found (mg/tablet)**
400	400.83	333	333.17
	401.03		332.98
	399.90		334.10
Mean	0.98	Mean	1.176
±SD	0.265	±SD	0.218

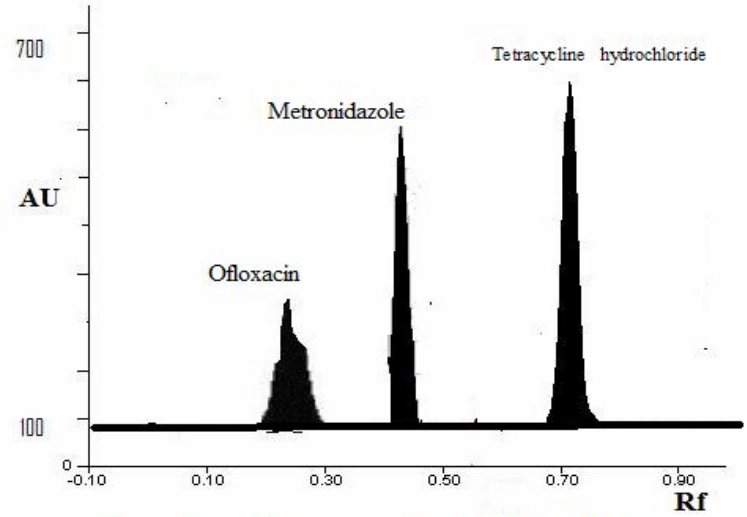

Figure- Typical chromatogram Peak MET and TET

Results and Discussion

To optimize the HPTLC parameters, several mobile phase compositions were tried. Satisfactory separations for AT, RA and AS were obtained with mobile phase consisting of benzene: ethyl acetate: toluene: methanol: glacial acetic acid (9.5:2.0:5.0:1.5:0.5 v/v/v/v/v), Quantification was achieved with UV detection at 283 nm based on peak area. Better resolution of the peaks with clear baseline separation was found. Small changes in the mobile phase composition, the effects on the results were examined. Mobile phases having different composition like Chloroform: Methanol: Toluene: Acetic acid (8.1: 1:1:0.1 v/v/v/v), were tried and chromatograms were run. The plates were prewashed by methanol and activated at 110°C for 5, 10, 15 min respectively prior to chromatography. Time from spotting to chromatography and from chromatography to scanning was varied from 0, 20, 40 and 60 minutes. Robustness of the method was done at three different concentration levels 600, 1200, 2400ng $spot^{-1}$and 600, 1400, 1800ng $spot^{-1}$ for MET and TET, respectively. The method was a normal phase HPTLC method. It makes use of a silica gel $60F_{254}$ stationary phase precoated on aluminium sheet. The mobile phase comprises benzene: ethyl acetate: toluene: methanol: glacial acetic acid (9.5:2.0:5.0:1.5:0.5 v/v/v/v/v), which gives good separation between Ofloxacin (R_f =0.29), MET (R_f=0.43) and TET (R_f=0.74). Linearity was observed over the concentration range of 600-2400 $ng.band^{-1}$. The linearity of the calibration plots was confirmed by the high value of the correlation coefficients (r2 = 0.9998 for MET and 0.9991 for TET). The LOD and LOQ for a signal to noise ratio of 8:1 and 12:1 was found to be 81 and 97 $ng.band^{-1}$ for MET and TET which indicates the method has sufficient sensitivity. There was no indication of degradation in solutions of MET and TET as revealed by peak purity data and from the value of RSD (< 2%) for peak areas of bands of solution stored at different times. In Conclusion proposed HPTLC method was validated as per ICH guidelines. The standard deviation, %RSD and standard error calculated for the method are low, indicating high degree of precision of the methods. The results of the recovery studies performed show the high degree of accuracy of the proposed methods. The proposed method is highly accurate, selective and precise hence can be used for a routine quality-control analysis and quantitative

simultaneous determination of MET and TET in pharmaceutical preparations.

Acknowledgement
We are grateful to referee for given valuable suggestions.

References

1. Nagulwar, V.; Upadhye, P.A.; Mehta, N.K.; Upadhye, K.;Bakhe, S.;Deshpandey, S.;Dixit, G. Indian J. Pharma . Sci. 2005, 67(2), 258-260.
2. Patel, P.U.; Suhajia, B.N.; Patel, M.M. Indian Drugs. 2005, 42(3), 155-157.
3. Indian Pharmacopoeia, Vol. 2, Ministry of Health and Family Welfare, Government of India, New Delhi, 1996, 748.
4. Mitscher, L.A. Antibiotics and antimicrobial agents, In; Foye's Principle of Medicinal Chemistry, 5th Edn., Lippincott Williams and Wilkins, Philadelphia P.A., 2003, p.p.857.
5. Sharma, M. C.; Sharma, S.; Kohli, D. V.; Sharma, A. D. Archives of Applied Science Research. 2010, 2 (1) 1-7
6. Sharma, M. C.; Sharma, S.; Kohli, D. V.Annals of Biological Research. 2010, 1 (1): 24-129
7. Sharma, M. C.; Sharma, S.; Kohli, D. V.; Sharma, A. D. Der Pharma Chemica. 2010, 2(1), 121-126
8. Sharma, M. C.; Sharma, S.; Kohli, D. V.; Sharma, A. D. Der Pharmacia Lettre. 2010, 2 (1) 489-494.
9. Sharma,S.;Sharma,M.C. Indian Drugs .47(11),68-72(2010).
10. ICH, Q2A Validation of Analytical Procedure: Methodology International Conference on Harmonization, Geneva, October 1994.
11. ICH, Q2B Validation of Analytical Procedure: Methodology International Conference on Harmonization, Geneva, March 1996.
12. ICH Guidance on Analytical Method Validation, in: Proceedings of the International Convention on Quality for the Pharmaceutical Industry, Toronto, Canada, September 2002.

*****