IJPSR (2015), Vol. 6, Issue 5 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 10 July, 2014; received in revised form, 13 November, 2014; accepted, 18 April, 2015; published 01 May, 2015

STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF DOXYCYCLINE MONOHYDRATE AND ORNIDAZOLE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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Keywords:

Doxycycline Monohydrate, Ornidazole, Method development, Validation, Stability studies

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ABSTRACT: A simple, rapid, precise, cost effective, stability indicating RP-HPLC method has been developed and validated for the simultaneous estimation of Doxycycline monohydrate (DOXM) and Ornidazole (ORN) in Bulk and pharmaceutical dosage form. The chromatographic separation was achieved on Hypersil BDS C_{18} column (250mm ×4.6mm, 5µm) using a mobile phase consisting of Buffer : Acetonitrile (55:45 v/v) pH 4 adjusted with ortho phosphoric acid at a flow rate of 1ml/min. Detection wavelength was found 260 nm. The retention time found for the drugs DOXM and ORN were 2.8 min and 4.3 min. respectively. The linearity of the method was over the range 12.5 – 75 µg/ml and 62.5– 375 µg/ml for DOXM and ORN, respectively. The validation of method was carried out utilizing ICH guidelines. The described RP-HPLC method was successfully employed for the analysis of pharmaceutical formulations containing combined dosage form. Developed HPLC method can resolve all degradant peaks of both the drugs, so this method is stability indicating in nature.

INTRODUCTION: Doxycycline (DOX) is a tetracyclic antibiotic and is commonly used to treat a variety of infections [1]. It is used in prophylaxis against malaria. Doxycycline monohydrate (DOXM) chemically is 2-naphthalene carboxamide -4-(dimethylamino)-1,4,4a,5, 5a, 6, 11, 12a - octahydro-3,5,10,12,12a–pentahydroxy-6-methyl-1,11-dioxo[4S(4α,4aα, 5α, 5aα, 6α, 12aα)] monohydrate. DOXM is a yellow crystalline powder with a molecular weight of 462.45 g/mol. Its empirical formula is $C_{22}H_{24}N_2O_8.H_2O$ [2]. It is an official drug in USP[3], IP[4], and BP[5].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.6(5).2228-39

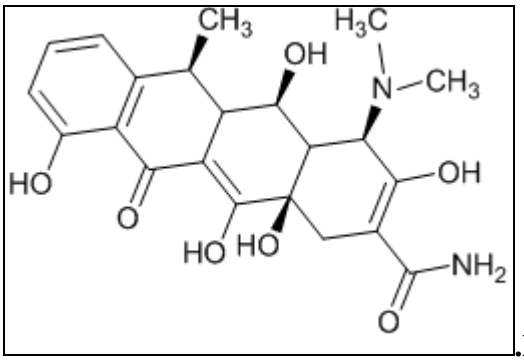
Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.6(5).2228-39

Several analytical techniques like bio analytical methods by HPLC using human plasma, human serum, RP-HPLC and spectrophotometric have been reported for estimation of doxycycline. Ornidazole (ORN) is antiprotozoal medication. ORN is chemically 1-(3-chloro-2-hydroxypropyl)-2-methyl-5-nitroimidazole, used as an anti-infective agent.

ORN is used in the treatment of susceptible protozoal infections and also in the treatment and prophylaxis of anaerobic bacterial infections. It is an official drug in IP[4]. ORN alone or in combination with other drugs, is reported to be estimated by spectrophotometry and HPLC in biological fluids or pharmaceutical formulations. DOXM [7-9] and ORN [10-17] combination is prescribed for amoebic dysentery. However, no HPLC method for the simultaneous estimation of DOXM and ORN in bulk and pharmaceutical dosage forms has

been reported so far. No method is available in the pharmacopoeias. No stability-indicating methods have been cited in the literatureconcerned with the determination of the intact drug in presence of its degradation product. This paper presents a study of acidic, alkaline, neutral, oxidative, thermal and photo degradation of DOXM and ORN. The scientific novelty of the present work is that the methods used are simple, rapid, and selective. The present work describes the development of simple, precise andaccurate isocratic reverse phase HPLC method for simultaneous estimation of DOXM and ORN in bulk and pharmaceutical dosage form.The chemical structures of the drugs DOXM and ORN are represented in **Fig. 1** and **2**, respectively



.H_2O

FIG. 1: STRUCTURE OF DOXYCYCLINE MONOHYDRATE

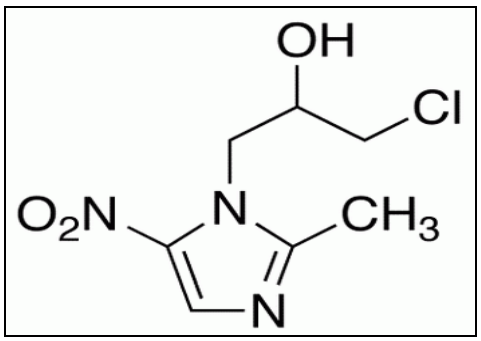


FIG. 2: STRUCTURE OF ORNIDAZOLE

MATERIALS & METHODS:

Chemicals and Reagents:

DOXM and ORN were provided by Dr. Reddy's Laboratories Ltd and ENDOC Pharma, respectively. Tablet formulation (DOBACT DOX), labelled amount 100 mg Doxycycline monohydrate and 500 mg Ornidazole manufactured by Tidal Labs, India was purchased from the local market. Acetonitrile and methanol of HPLC Grade were obtained from Rankem. Potassium dihydrogenortho phosphate and ortho phosphoric acid of analytical grade were obtained from SD Fine Chemicals (Hyderabad, India). Water [HPLC Grade] was purified by Milli Q purification system.0.45µm Nylon membrane filters were obtained from Spincotech Private Limited, Hyderabad.

Apparatus and Chromatographic Conditions:

Instrument:

HPLC analysis was performed on Waters 2695Alliance HPLC system connected with PDA Detector 2996. The drug analysis data were acquired and processed using Empower 2 software.

UV/VIS spectrophotometer: T60PG Instruments

P^H meter: Mettler Toledo S 220

Weighing balance: Shimadzu AY 220

Sonicator: Wensar MUC 6L

Temperature: 30°C

Column: Hypersil BDS-C_{18} 250mm x 4.6 mm ID, 5 µm column was used.

Mobile phase: Phosphate Buffer: Acetonitrile (55: 45v/v)

Flow rate: 1 ml per min

Wavelength: 260 nm

Injection volume: 10 µl

Run time: 8 min

Preparation of buffer:

The buffer solution was prepared by dissolving accurately weighed 1.36 grams of potassium dihydrogenortho phosphate (KH_2PO_4) and transferred into a clean and dry 1000 ml volumetric flask, about 900 ml of water was added and degassed in an ultrasonic water bath for 15 min,and finally made up with water [HPLC Grade] to get 10mM buffer strength. The final pH of the buffer was adjusted to 4 by using orthophosporic acid. Later the buffer was filtered through 0.45µm nylon membrane filter.

Preparation of mobile phase: The mobile phase was prepared by mixing 550ml (55%) of the above buffer and 450ml of acetonitrile (45%) and degassed in an ultrasonic water bath for 15min prior to use. Then the resultant solution was filtered through 0.45 µ filter under vacuum filtration.

Preparation of stock and working standard solution:
The stock solution was prepared by weighing accurately 5 mg of DOXM and 25 mg ORN and transferred into a clean and dry 10 ml volumetric flask. About 7 ml of diluent was added and sonicated for 30 minand made up to the final volume with diluent. From the above stock solution, 1ml of solution was transferred into a 10ml volumetric flask to that the diluent was added upto the mark to get final concentration of (50µg/ml DOXMand 250µg/ml ORN).

Preparation of sample solution:
Twenty tablets were weighed and powdered. Tablet powder equivalent to 100 mg of DOXM and 500 mg of ORN was accurately weighed and transferred into 100ml volumetric flask to that70ml of diluent was added and sonicated for 30 min. Further, the volume was made up to the mark using diluent and filtered. From the filtered solution 0.1ml was pipetted out into a 10 ml volumetric flask and made upto 10ml with diluents. This is then filtered through 0.45µ membrane filter to obtain clear solution.

Method Validation: The developed method of analysis was validated as per the ICH for the parameters like system suitability, specificity, linearity, precision, accuracy, robustness, limit of detection (LOD) and limit of quantitation (LOQ).

System suitability:
The chromatographic systems used for analysis must pass the system suitability limits before sample analysis can commence. Set up the chromatographic system, allow the HPLC system to stabilize for 40 min. Inject blank preparation (single injection) and standard preparation (six replicates) and record the chromatograms to evaluate the system suitability parameters like resolution (NLT 2.0), tailing factor (NMT 1.5), theoretical plate count (NLT 3000) and % RSD for peak area of six replicate injections of LMS standard (%RSD NMT 2.0).

Specificity:
The evaluation of the specificity of the method was determined against a placebo, blank and sample. The interference of the excipients of the claimed placebo present in pharmaceutical dosage form was derived from a placebo solution. Furthermore, the specificity of the method toward the drug was established by checking the interference of the degradation products in the drug quantification for assay during the forced degradation study. The chromatogram for placebo, blank, standard and sample solution indicating the specificity of developed method.

Linearity:
Linearity was established by least squares linear regression analysis of calibration curve. Linearity was determined in the range of 0 - 75 ppm of DOX and 0 - 375 ppm of OZ.

Limit of detection:
The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantities as an exact value

$$\text{Limit of detection (LOD)} = \sigma / S \times 3.3$$

where
S – slope of the calibration curve
σ – residual standard deviation

Limit of quantification:
It is defined as lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy and reliability by a given method under stated experimental conditions
It can be determined from linearity curve by applying the following formula

$$\text{Limit of quantification (LOQ)} = \sigma / S \times 10$$

where
S – slope of the calibration curve
σ – residual standard deviation

Precision:
System precision: Six replicate injections of the mixture of standard solution at working concentration showed % RSD (% Relative Standard Deviation) less than 2 concerning peak areas for both the drugs, which indicates the acceptable reproducibility and thereby the precision of the system.

Method precision: Method precision was determined by performing assay of sample under

the tests of (i) repeatability (Intraday precision) and (ii) Intermediate precision (Inter day precision).

Repeatability (Intraday precision):
Six consecutive injections of the sample at working concentration showed % RSD less than 2 concerning % assay for all the drugs which indicate the method developed is precise by the test of repeatability and hence can be understood that the method gives consistently reproducible results.

Ruggedness (Intermediate Precision / Inter day precision):
Six consecutive injections of the sample solution at working concentration on different days, showed % RSD less than 2 for % assay for all the drugs within and between days, which indicate the method developed is inter day precise / rugged.

Robustness:
The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It is concluded that the method is robust as it is found that the % RSD is less than 1 concerning % assay despite deliberate variations done concerning flow rate (± 0.1 ml), composition of mobile phase (±10 ml) and temperature (±5ºc).

Stability studies:
In order to establish whether the developed method is stability indicating, both the drugs were stressed under various conditions (acid, base, oxidation and thermal) to perform stability (forced degradation studies).

Acid degradation studies:
To 1 ml of stock solution DOXM and ORN, 3 ml of 2N HCl wasadded and refluxed for 30min at 60°C. The resultant solution was diluted to obtain 50µg/ml and 250µg/ml and 10µl solutions were injected in to the system and the chromatograms were recorded to assess the stability of sample.

Alkali degradation studies:
To 1 ml of stock solution DOXM and ORN, 3 ml of 2N NaOH was added and refluxed for 30mins at 60°C. The resultant solution was diluted to obtain 50 µg/ml and 250 µg/ml and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Thermal degradation studies:
The standard drug solutions were placed in oven at 105 °C for 6 h to study thermal degradation. For HPLC study, the resultant solution was diluted to 50µg/ml (DOXM) and 250 µg/ml (ORN) solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

Photo stability studies:
The photochemical stability of the drug was also studied by exposing the solution by keeping them in UV chamber for 7 days or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 50 µg/ml and 250 µg/ml solutions and 10µl was injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral degradation studies:
Stress testing under neutral conditions was studied by refluxing the drug in water for 6 h at a temperature of 60ºC. For HPLC study, the resultant solution was diluted to 50 µg/ml and 250 µg/ml solution and 10µl was injected in to the system and the chromatograms were recorded to assess the stability of the sample**.**

Oxidative degradation:
To 1 ml of stock solution, 1 ml of 20 % hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 60°C. For HPLC study, the resultant solution was diluted to obtain 50 µg/ml and 250 µg/ml solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of sample

RESULTS AND DISCUSSION:

Selection of Wavelength:
Suitable wavelength for the HPLC analysis was determined by recording UV spectra in the range 200-400 nm for individual drug solutions of DOXM and ORN and it was observed that both drugs showed considerable absorbance at 260 nm,

so thesuitable wavelength was selected as 260 nm (**Fig. 3**).

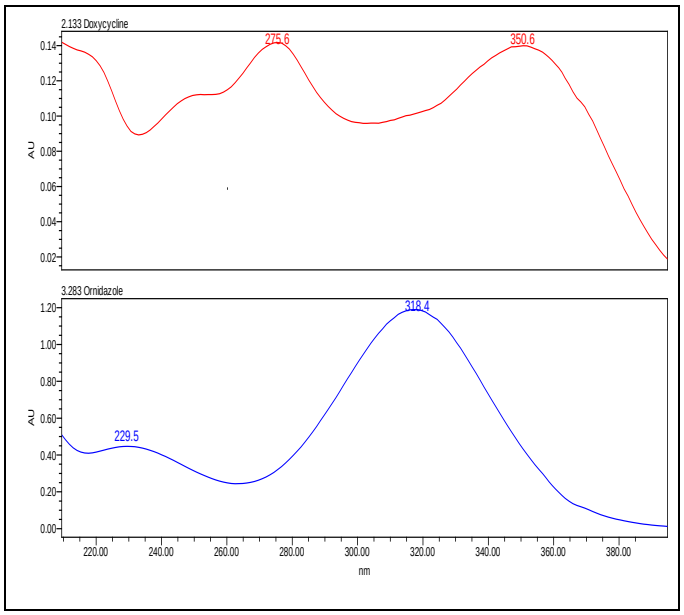


FIG.3: UV SPECTRA OF DOXYCYCLINE MONOHYDRATE AND ORNIDAZOLE

Method development:

Initially, wavelength was selected for the method development and different compositions, pH and flow rate of the mobile phase were tried during method development. Wavelength of 260 nm was selected for the current method since at this wavelength DOXM and ORN show high sensitivity. In the course of optimizing the composition of mobile phase, acetonitrile in combination with various buffers like phosphate and acetate with varying pH values were tried. After a series of preliminary experiments it was concluded that potassium dihydrogenortho phosphate buffer resulted in better peak shape. Peak with good shape and symmetry was observed by the mobile phase consisting of potassium dihydrogenorthophosphate: acetonitrile (pH 4, 10mM) (55:45, v/v) set at a flow rate of 1ml/min.

A reverse phase HPLC method was developed keeping in mind the system suitability parameters i.e. tailing factor (T), number of theoretical plates (N), runtime and the cost effectiveness. The optimized method developed resulted in the elution of DOXM at 2.8 min and ORN at 4.3 min. **Fig. 4** and **5** represent chromatograms of blank solution and mixture of standard solutions, respectively. The total run time is 8 min with all system suitability parameters as ideal for the mixture of standard solutions.

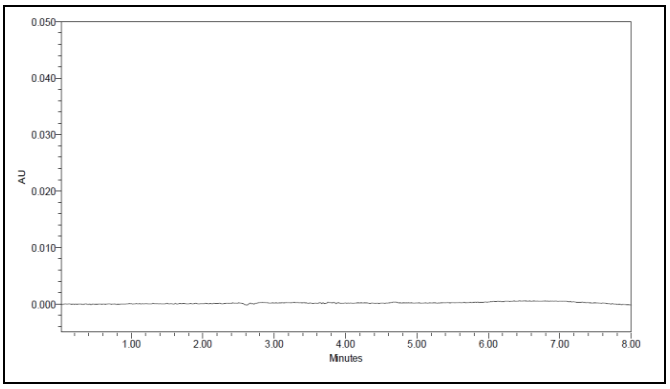


FIG.4: TYPICAL CHROMATOGRAM FOR BLANK SOLUTION

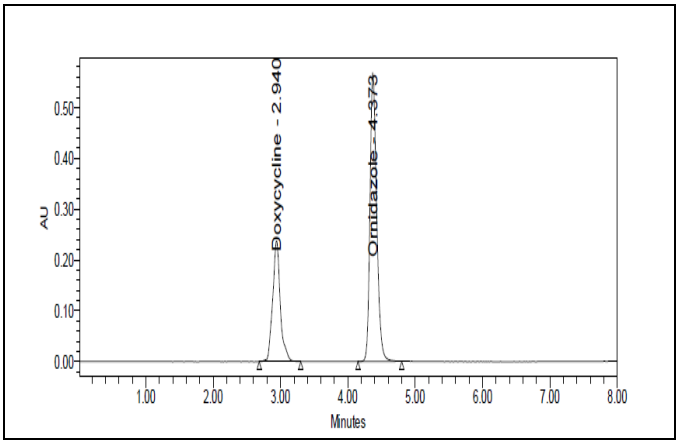


FIG.5: CHROMATOGRAM SHOWING SIMULTANEOUS ESTIMATION OF DOXM AND ORN

System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (R_t), number of theoretical plates (N), and peak tailing factor (T) were evaluated for six replicate injections of the standards at working concentration. The system suitability parameters for DOXM and ORN by the proposed method are tabulated in **Table 1** and it is found that they arewithin acceptable limits

TABLE 1: SYSTEM SUITABILITY PARAMETERS FOR DOXM AND ORN

Parameters*	DOXM	ORN
Retention time (min)	2.884	4.319
Theoretical plates (N)	3288	9456
Tailing factor (T)	1.23	1.22

Method validation:

RP-HPLC method developed was validated according to International Conference on

Harmonization (ICH) guidelines [20] for validation of analytical procedures. The method was validated for the parameters like system suitability, specificity, linearity, accuracy, precision, ruggedness, robustness, limit of detection (LOD) and limit of quantitation (LOQ) and stability studies.

Linearity:

Standard solutions of DOXM and ORN of different concentrations (25, 50, 75, 100, 125, and 150%) were prepared. Calibration curves were constructed by plotting the concentration of drug versus corresponding mean peak area.

The results show that an excellent correlation exists between mean peak area and concentration level for both the drugs and the results are given in **Table 2** and **3** and **Fig.7** and **8**. The method is said to be linear in the range of 12.5-75 µg/ml for DOXM and 62.5 - 375 µg/ml for ORN.

TABLE 2: ANALYTICAL PERFORMANCE PARAMETERS FOR THE DRUGSDOXM AND ORN

Drugs	Linearity range (µg/ml)	R^2	Slope	Intercept
DOXM	12.5 - 75	0.9998	39771.6	421.6071
ORN	62.5 - 375	0.9993	16146	5221.1

TABLE 3: CALIBRATION DATA FOR DOXM AND ORN

	Doxycycline monohydrate		Ornidazole	
%Level	Concentration (µg/ml)	Peak Area	Concentration (µg/ml)	Peak Area
25	12.5	500775	62..5	965052
50	25	1000266	125	2088227
75	37.5	1503206	187.5	3102312
100	50	1959961	250	3958001
125	62.5	2474476	312.5	5028096
150	75	3004328	375	6086595

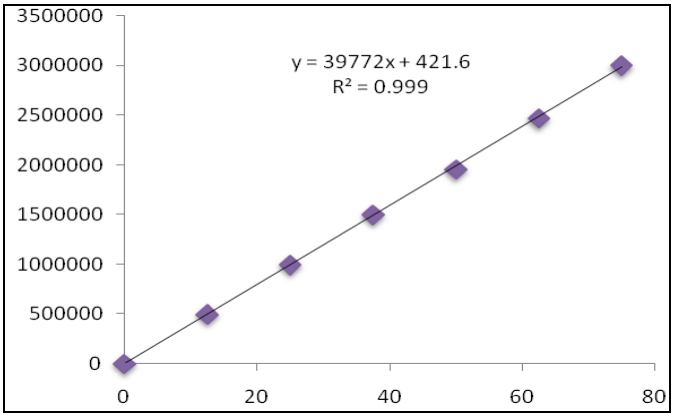


FIG. 6: CALIBRATION CURVE FOR DOXYCYCLINE MONOHYDRATE

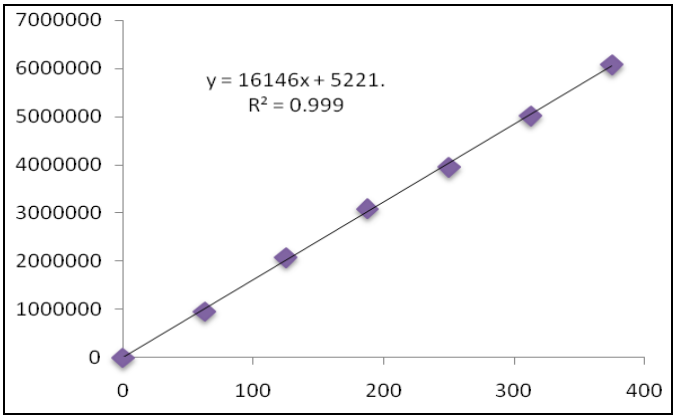


FIG.7: CALIBRATION CURVE FOR ORNIDAZOLE

Sensitivity (LOD and LOQ):

The sensitivity of measurement of DOXM and ORN by use of the proposed method was estimated in terms of the limit of quantitation (LOQ) and the limit of detection (LOD). To determine the LOD and LOQ, sample was dissolved in mobile phase and injected until peak disappeared and the results are shown in **Table 4.** LOD was obtained as 0.03µg/ml for DOXM and 1.07µg/ml for ORN and LOQ as 0.11µg/ml for DOXM and 3.23µg/ml for ORN.

TABLE 4: LOD AND LOQ VALUES FOR DOXYCYCLINE MONOHYDRATE AND ORNIDAZOLE

Drug name	Standard deviation	Slope	LOD	LOQ
DOXM	421.6	39772	0.03µg/ml	0.11µg/ml
ORN	5221	16146	1.07µg/ml	3.23µg/ml

Precision:

System precision:

Six replicate injections of the mixture of standard solution at working concentration showed % RSD (% Relative Standard Deviation) less than 2 concerning peak areas for both the drugs, which indicates the acceptable reproducibility and thereby

the precision of the system. System precision results are tabulated in **Table 5**.

TABLE 5: SYSTEM PRECISION RESULTS FOR DOXYCYCLINE MONOHYDRATE AND ORNIDAZOLE

Injection no.	Doxycycline Monohydrate	Ornidazole
1	1845742	3873964
2	1847242	3862207
3	1862737	3889841
4	1864737	3878161
5	1864194	3894646
Mean	1856930	3879764
Standard deviation (SD)	9571.636	12917.80
%RSD	0.5	0.33

Method precision:

Method precision was determined by performing assay of sample under the tests of (i) repeatability (Intraday precision) and (ii) Intermediate precision (Inter day precision) performed at working concentration by three different analysts on three consecutive days.

Repeatability (Intraday precision):

Six consecutive injections of the sample at working concentration showed % RSD less than 2 concerning % assay for all the drugs which indicate the method developed is precise by the test of repeatability and hence can be understood that the method gives consistently reproducible results (**Table 6**).

TABLE 6: REPEATABILITY RESULTS

	Area at 260 nm		% Assay	
S No	DOXM	OZ	DOXM	OZ
1	1844557	3914116	99.23433	100.7845
2	1850113	3900558	99.53323	100.4354
3	1860289	3854870	100.0807	99.25901
4	1874876	3862028	100.8654	99.44332
5	1865578	3863536	100.3652	99.48215
6	1847532	3887854	99.39438	100.1083
Mean	1857158	3880494	99.91	99.919
SD	11795.797	23968.02	0.6346	0.617
%RSD	0.64	0.617654	0.64	0.618

Intermediate Precision / Inter day precision:

Six consecutive injections of the sample solution at working concentration on three different days, showed % RSD less than 2 for % assay for all the drugs within and between days, indicating that the method developed is inter day precise / rugged (**Table 7**).

TABLE 7: INTERMEDIATE (DAY-DAY) PRECISION RESULT

S.NO	Injections	Area of DOXM	Area of ORN
1.	Injection-1	1717916	3759290
2.	Injection-2	1737862	3760545
3.	Injection-3	1739300	3772657
4.	Injection-4	1714770	3754320
5.	Injection-5	1717806	3765018
Mean		1725531	3762366
S.D		11990.64	6900.3
%RSD		1	0.20

Accuracy:

Accuracy was determined by means of recovery experiments, by the addition of active drug to pre-analyzed sample at different spiked levels (50-150%). At each level, three determinations were performed and results obtained. The accuracy was calculated from the test results as the percentage of the analyte recovered by the assay. The amounts recovered values of percent mean recovery were calculated as shown in **Tables 8** and **9.** The accepted limits of recovery are 98%-102% and all observed data are within the required range indicating good recovery values and hence the accuracy of the method developed.

TABLE 8: RESULTS OF ACCURACY STUDIES FOR DOXM

Recovery level	Amount added (mg)	Amount recovered (mg)	% recovery	Mean % Recovery ± SD	%RSD
	2.5	2.48	99.32	99.7±0.90	0.91
50%	2.5	2.51	100.76		
	2.5	2.47	99.10		
	5	4.95	99.03	99.53±0.5	0.551
100%	5	4.97	99.54	4	
	5	5	100.13		
	7.5	7.51	100.21	99.83±0.6	0.601
150%	7.5	7.51	100.20		
	7.5	7.43	99.16		

TABLE 9: RESULTS OF ACCURACY STUDIES FOR ORN

Recovery level	Amount added (mg)	Amount recovered (mg)	% recovery	Mean % Recovery ± SD	%RSD
	12.5	12.58	100.66	99.97±0.715	0.715
50%	12.5	12.5	100.03		
	12.5	12.4	99.23		
	25	25.07	100.3	99.66±0.57	0.57
100%	25	24.81	99.27		
	25	24.81	99.38		
	37.5	37.66	100.4	99.95±0.44	0.44
150%	37.5	37.34	99.58		
	37.5	37.44	99.84		

Assay:

The validated method was applied to the determination of DOXM and ORN in commercially available DOBACT DOX tablets. The percentage assay was found to be 99.56% for DOXM and 99.6 % for ORN, respectively. The results of assay indicate that the developed method is selective without interference from excipients of tablet.

Robustness:

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It is concluded that the method is robust as it is found that the % RSD is less than 1 concerning % assay despite deliberate variations done concerning flow rate (± 0.1), % organic phase (± 10%), and temperature (±10ºC) (**Table 10**)

TABLE 10: ROBUSTNESS STUDIES FOR DOXM AND ORN

Para-meters	Optimized conditions	Used	Doxycycline monohydrate (R_t) min	Area	Mean ±%RSD	Ornidazole (R_t) min	Area	Mean ± % RSD
Flow rate (± 0.1)		0.9	3.179	2067353	2075251± 0.5	4.581	4309113	4326042 ± 0.6
			3.220	2083148		4.667	4342971	
	1.0 mL/min	1.1	2.546	1566048	1606893± 0.6	3.689	3392544	3415886 ± 1.0
			2.577	1647738		3.740	3439227	
Mobile phase composition		45:65	2.851	1735105	1740156 ± 0.4	4.041	3761358	3760024 ± 0.1
			2.857	1745208		4.059	3758689	
[Buffer : ACN]		65:35	2.860	1685645	1681809 ± 0.3	4.159	3636629	3636131 ± 0.0
	55:45v/v		2.862	1677973		4.338	3635633	
Temperature (±10°C)		20	2.866	1671750	1684818 ± 1.1	4.186	3596441	3620519 ± 0.9
			2.873	1697885		4.191	3644597	
	30	40	2.873	1718274	1704849 ± 1.1	4.168	3749099	3728169 ± 0.8
			2.874	1691424		4.206	3707240	

Specificity:

The specificity of the method was determined against a blank, placebo and a mixture of sample drug solutions. Chromatograms reveal that the peaks generated in mixture of sample solution are only because of the drugs as blank (**Fig. 4**) as well as placebo (**Fig. 8**) did not show any peaks at the retention times of DOXM and ORN as was seen in **Fig.9** establishing the specificity of the method developed (**Table 11**).

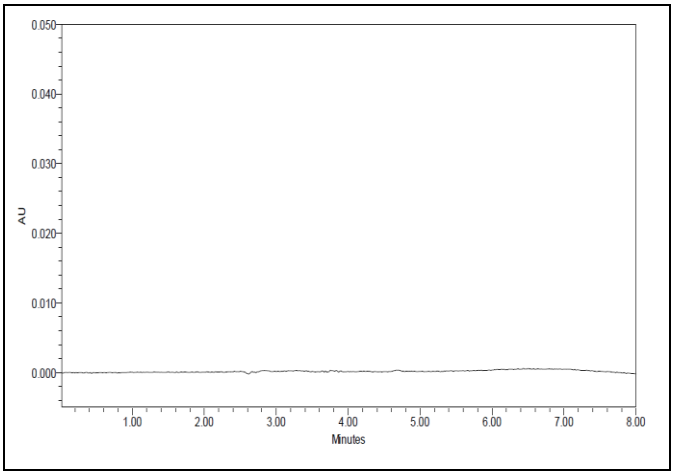


FIG. 8: TYPICALCHROMATOGRAM FOR PLACEBO SOLUTION

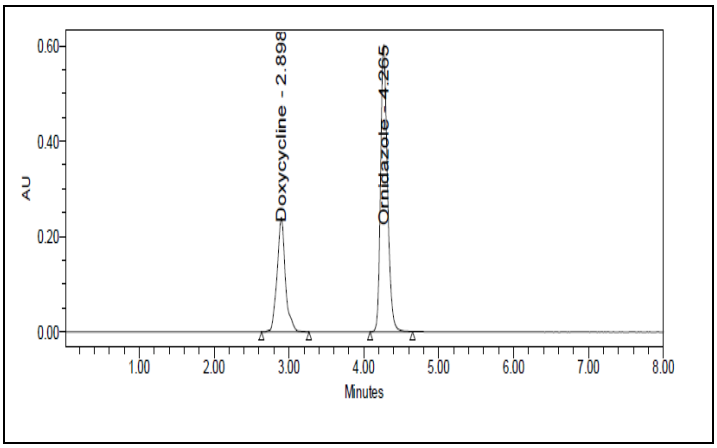


FIG.9: TYPICAL CHROMATOGRAM FOR SAMPLE SOLUTION

TABLE 11: SPECIFICITY RESULTS

Name of the solution	Retention time, (Rt) min
Blank	No peak
Placebo	No peak
Sample	DOXM , 2.8 ORN, 4.2

Furthermore, the specificity of the method towards the drug was established by checking the interference of the degradation products in the drug quantification for assay during the forced degradation study.

Stability studies:

In order to establish whether the developed method is stability indicating both the drugs were stressed under various conditions (acid, base, neutral, oxidation and photo stability, thermal) to perform forced degradation studies. The peaks of degraded products were well separated from the analyte peak with good resolution The peak purity of DOXM and ORN was found to satisfactory under different stress conditions as shown in the chromatograms in **Fig. 10 (a, b, c, d, e)** which indicates that the developed method is stability indicating. **Table 11** and **12** illustrate the percentage degradation, purity angle and purity threshold for DOXM and ORN, respectively.

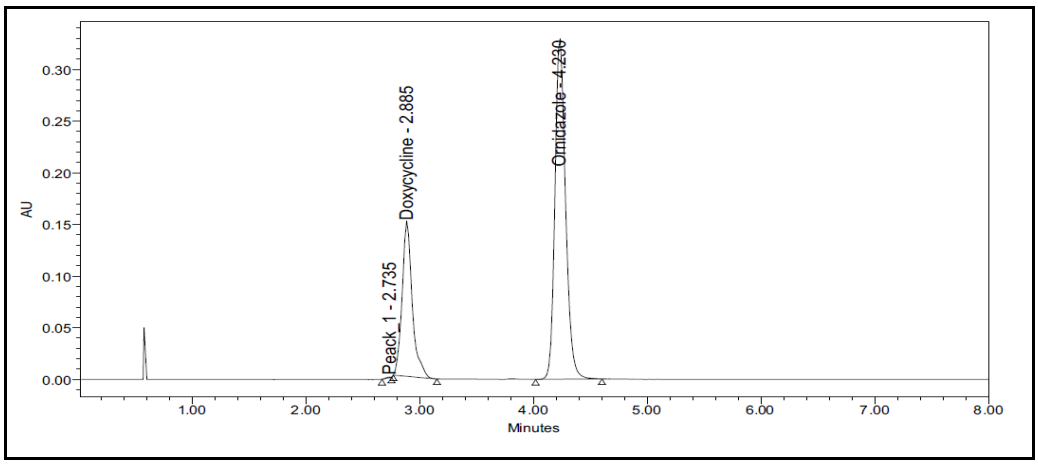


FIG.10a: CHROMATOGRAM SHOWING ACID DEGRADATION OF DOXM AND ORN

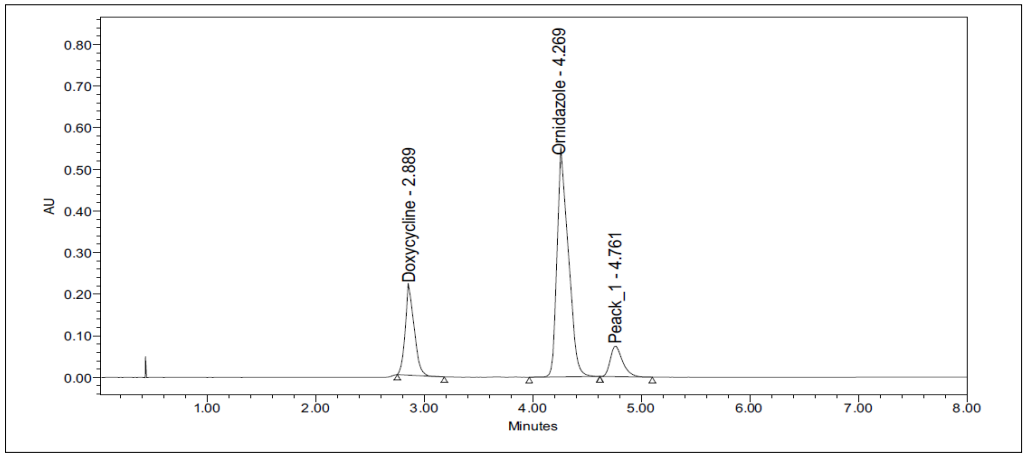


FIG. 10b: CHROMATOGRAM SHOWING BASIC DEGRADATION OF DOXM AND ORN

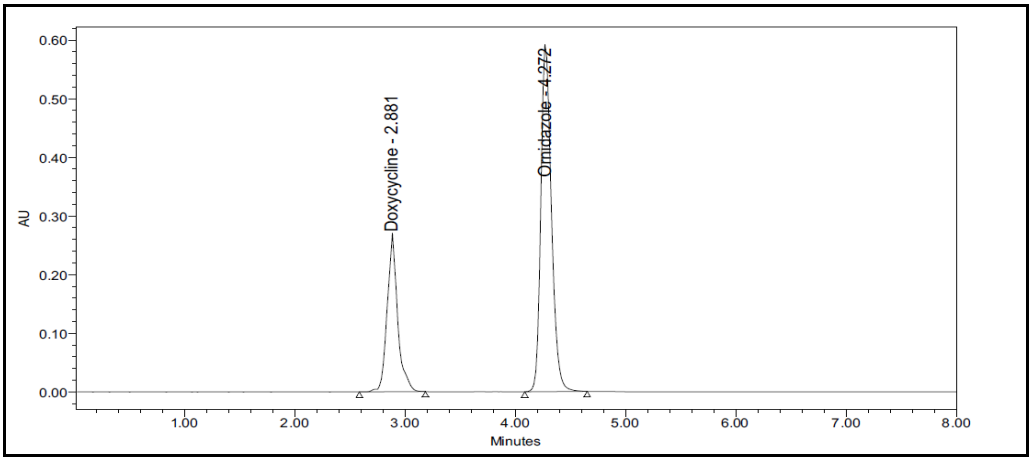


FIG.10c: CHROMATOGRAM SHOWING NEUTRAL DEGRADATION OF DOXM AND ORN

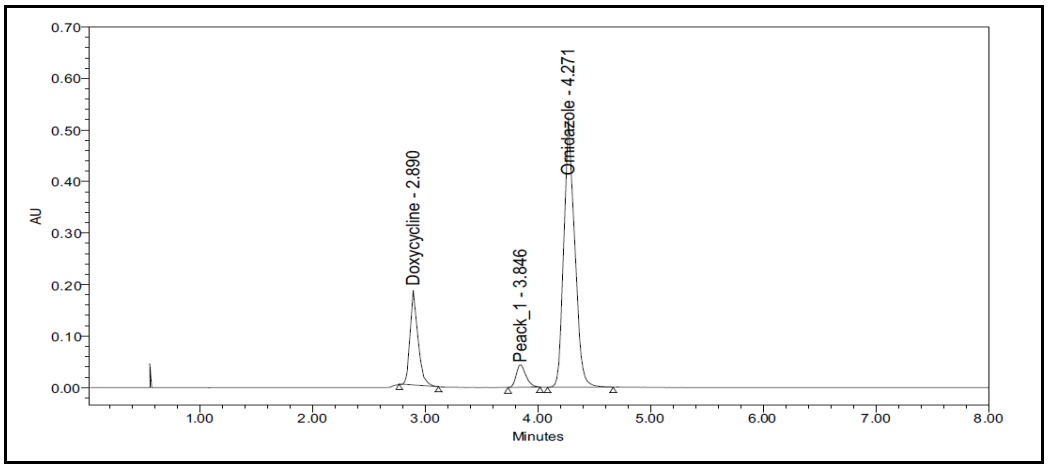


FIG.10d: CHROMATOGRAM SHOWING OXIDATIVE DEGRADATION OF DOXM AND ORN

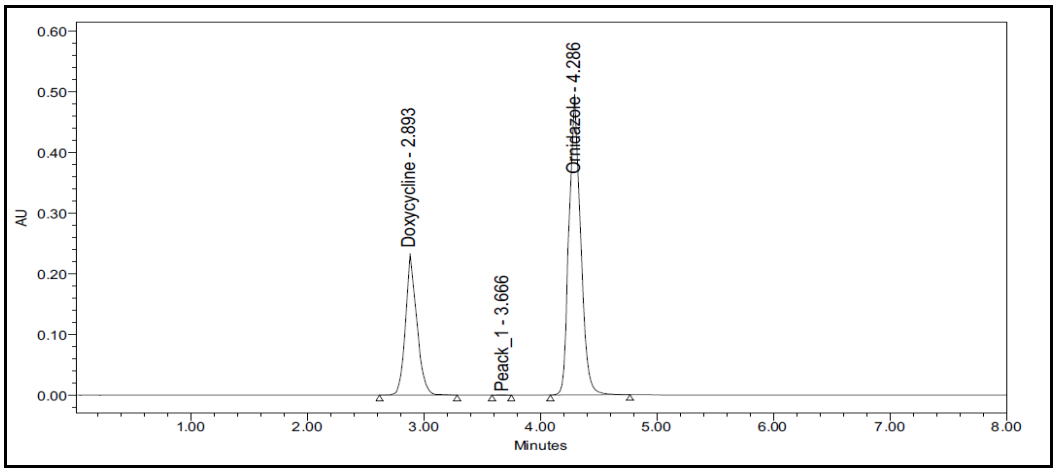


FIG.10e: CHROMATOGRAM SHOWING THERMAL DEGRADATION OF DOXM AND ORN

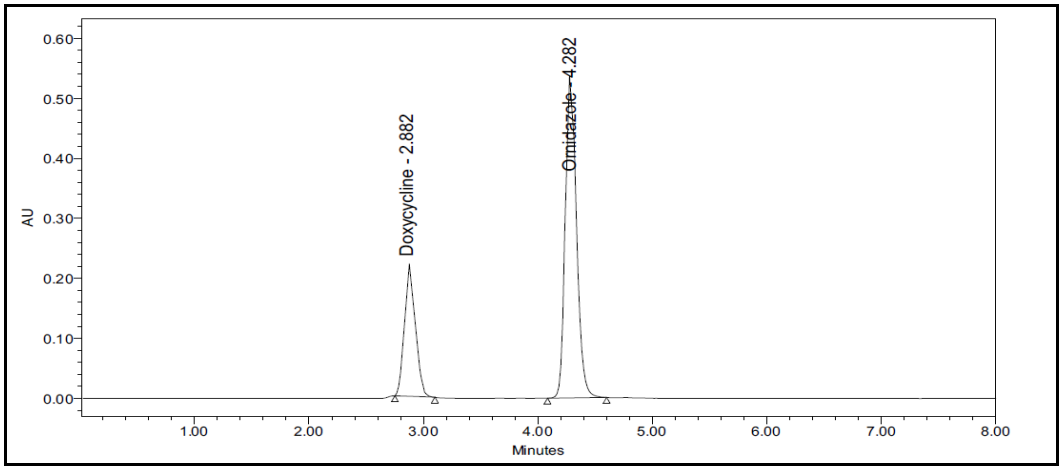


FIG.10f: CHROMATOGRAM SHOWING PHOTO-STABILITY STUDIES OF DOXM AND ORN

TABLE 11: DEGRADATION STUDIES FOR DOXYCYCLINE MONOHYDRATE

Degradation studies	Area	% Assay	% Degradation	Purity angle	Purity threshold
Acid	1730235	92.77	-7.68	0.077	0.289
Base	1751032	93.88	-6.4	0.995	1.394
Oxidation	1764873	94.63	-5.56	0.391	0.567
Thermal	1772234	95.02	-5.12	0.246	0.284.
Photo stability	1832819	98.27	-1.65	0.978	2.272
Neutral	1852915	99.35	-0.55	0.454	0.680

TABLE 12: DEGRADATION STUDIES FOR ORNIDAZOLE

Degradation studies	Area	%Assay	%Degradation	Purity angle	Purity threshold
Acid	3722340	92.30	-4.54	0.237	0.369
Base	3631839	93.23	-7.15	0.126	0.317
Oxidation	3667322	94.14	-6.11	0.078	0.289
Thermal	3722340	95.55	-4.5	0.086	0.291
Photo stability	3823045	98.14	-1.79	0.090	0.286
Neutral	3880019	99.6	-0.29	0.087	0.285

CONCLUSION: A simple, sensitive, specific, accurate and precise stability indicating RP-HPLC isocratic method was developed and validated for the routine analysis of bulk and tablet dosage form of doxycycline monohydrate and ornidazole. The method is sensitive enough for the detection of analyte in pharmaceutical formulation when compared to the research works found in the literature. A good linear relationship was observed for both the drugs between concentration ranges of 12.5 to 75µg/ml and 62.5 to 375µg/ml of doxycycline monohydrate and ornidazole respectively. The correlation coefficients were greater than 0.999 for both the drugs. The inter day and intraday precision results were good enough to say that the method developed is precise and reproducible. Accuracy studies revealed that mean recoveries after experiments were between 98 and 102%, an indicative of accurate method. The results of forced degradation studies reveal that the method is stability indicating.

The peak purity of doxycycline monohydrate and ornidazole was found to satisfactory under different stress conditions. There was no interference of any peaks of degradation product with drug peaks. Hence, results of forced degradation studies reveal that the method is stability indicating. Simplicity, stability and economical nature make the method superior to the other reported HPLC methods.Accordingly it can be concluded that the developed reverse phase HPLC method is accurate, precise, linear and robust and therefore the method can be used for the routine analysis of doxycycline monohydrate and ornidazole.

ACKNOWLEDGEMENT: We would like to thank the Director, CSIR-Indian Institute of Chemical Technology, Hyderabad for giving us an opportunity to carry out our dissertation work in the institute.

REFERENCES:

1. O'Neil M J. The Merck Index- An encyclopedia of chemicals, Drugs and Biological, New Jersey, Merck and co., doxycycline monohydrate; 3474:606 and ornidazole 1229:6938
2. Martindale, The Complete references; Pharmaceutical Press Lambeth High Street, London UK; Thirty sixth edition:1:267,843
3. United States Pharmacopoeia NF. 23; 2004: 667.
4. Indian Pharmacopoeia 2010; 2(3):1257, 1823.
5. British Pharmacopoeia 2003; 3: 676.
6. Uday A. Deokate, Rajesh B.Nawale, Monals.Salvi. Development and validation of RP-HPLC method for simultaneous estimation of Doxycycline hyclate and Tinidazole in bulk and tablet dosage form. International Journal of Pharmacy and Pharmaceutical Science. 2013; July 5(4): 400-5.
7. Chaitanya Krishna A, sathiyaraj M, saravanan R.S, chelladurai R and vignesh R. A Novel and Rapid Method to Determine Doxycycline in Human Plasma by Liquid Chromatography Tandem Mass Spectrometry. Indian journal of pharmaceutical science. 2012
8. Ana Carolina Kogawa, Herida Regina Nunes Salgado. Quantification of Doxycycline hyclate in tablets by HPLC–UV Method. Journal of chromatographic science. 2012: 1-7
9. Liberato Brum Junior, Flávia de Toni Uchoa, Sílvia Stani sçuaski Guterres & Teresa Dalla Costa· Development and Validation of LC-MS/MS Method for the Simultaneous Determination of Quinine and Doxycycline in Pharmaceutical Formulations. Journal of Liquid Chromatography & Related Technologies. 2009; 32(18) : 2699-2711
10. Gandhi. V.M, Nair. S.B, Menezesand. C, Narayan. R. Development of UV-spectrophotometric method for the quantitative estimation of ofloxacin and ornidazole in combined liquid oral dosage form by simultaneous equation method. International Journal of Research in Pharmacy and Chemistry. 2013; 3(1): p.6-10
11. Suhagia BN, Shah SA, Rathod IS, Patel HM, Shah DR, et al. Determination of gatifloxacin and ornidazole in tablet dosage forms by high-performance thin-layer chromatography. Journal of Analytical Sciences. 2006 ;22: 743-745
12. Manisha Puranik, DV Bhawsar, Prachi Rathi, PG Yeole; Simultaneous determination of Ofloxacin and Ornidazole in solid dosage form by RP-HPLC and HPTLC techniques, Indian Journal of pharmaceutical science;72(4),513-517
13. Maheshwari R.K, V. K. Srivastav, and S. Sahu, New spectrophotometric estimation of ornidazole tablets employing urea as a hydrotropic solubilizing additive, Indian Journal of pharmaceutical science;72(2):258-281.
14. Róna K, Gachálvi B. Simple liquid chromatographic method for the determination of ornidazole and

metronidazole in human serum. Journal of Chromatographic sciences. 420: 228-230.
15. International Conference on Harmonization, guideline Q1A (R2), Stability testing of new drug substances and products. Federal Register 2003, 3, 29103.
16. International Conference on Harmonization, guideline Q2A, Text on validation of analytical procedures. Federal Register 1995; 60:11260.
17. International Conference on Harmonization, guideline Q2B, Validation of analytical procedures: methodology. Federal Register 1997; 62:27463.

How to cite this article:
Srilekha A, Pavani P and Sreedhar B: Stability Indicating RP-HPLC Method Development and Validation for Simultaneous Estimation of Doxycycline Monohydrate and Ornidazole in Bulk and Pharmaceutical Dosage Form. Int J Pharm Sci Res 2015; 6(5): 2228-39.doi: 10.13040/IJPSR.0975-8232.6(5).2228-39.