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Research Article

RP-HPLC METHOD FOR THE SIMULTANEOUS DETERMINATION OF OMEPRAZOLE, TINIDAZOLE AND DOXYCYCLINE MIXTURE IN THE PRESENCE OF OMEPRAZOLE AND TINIDAZOLE DEGRADATION PRODUCTS

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ABSTRACT

An isocratic RP-HPLC method has been developed and validated for rapid simultaneous separation and determination of the mixture of omeprazole, tinidazole and doxycycline in the presence of degradation products of omeprazole and tinidazole and in their pharmaceutical dosage forms. The separation was carried out on a Hypersil® RP- C_{18} column using a mobile phase of acetonitrile: methanol: phosphate buffer (pH 6.5) (20: 30: 50, v/v/v) in addition to 0.03 ml/ L triethylamine. The flow rate was 1.5 ml/min and the detection was set at 301 nm. The proposed method was successfully applied to the estimation of omeprazole, tinidazole and doxycycline in combined dosage forms.

Keywords: Omeprazole, Tinidazole, Doxycycline, RP-HPLC, Degradation products.

INTRODUCTION

Peptic ulcers are localized erosions of the mucous membranes of the stomach and duodenum. The pain associated with ulcers is caused by irritation of exposed surfaces by the stomach acids. The current approach for treating ulcers caused by *Helicobacter pylori* is to use combination of drugs, which includes a proton pump inhibitor and two antimicrobials, such as tinidazole and doxycycline.

Omeprazole

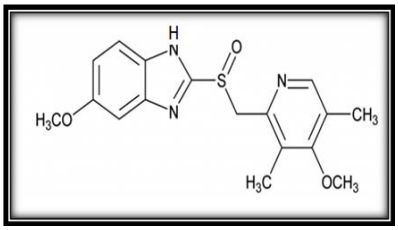

Fig. 1: Structure of omeprazole

Omeprazole is chemically known as 5-methoxy-2-[[(4-methoxy-3, 5-dimethyl-2-pyridinyl) methyl] sulfinyl] benzimidazole. It is officially listed in B.P.2011[1] and U.S.P.XXXII[2]. It is a proton pump inhibitor, used in treatment of peptic ulcer disease and NSAID-associated ulceration, in gastro-esophageal reflux disease and the Zollinger-Ellison syndrome [3]. A survey of the literature revealed that omeprazole has been estimated in pharmaceuticals by UV-spectrophotometry[4-6],spectrofluorimetry[7], HPLC [8-10], HPTLC [11], capillary electrophoresis [12]and electrochemical methods [13,14].

Tinidazole

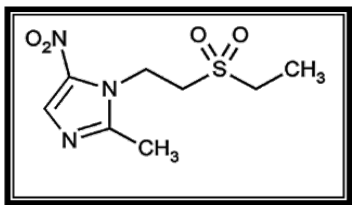

Fig. 2: Structure of tinidazole

Tinidazole is chemically known as 1-(2-ethyl-sulphonyl ethyl)-2-methyl-5-nitroimidazole. It is official in B.P. 2011 [1] and U.S.P. XXXII [2]·It has activity against anaerobic bacteria and protozoa. It is used to eradicate *H. pylori* in peptic ulcer disease with other antimicrobials and proton pump inhibitor [3]. It had been determined spectrophotometrically [15-17] and through HPLC [18-20].

Doxycycline hyclate

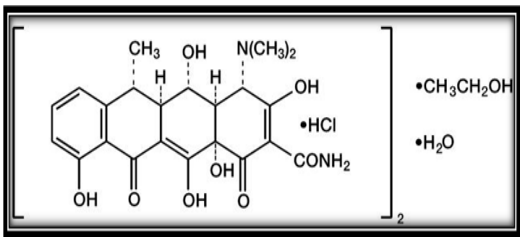

Fig. 3: Structure of doxycycline hyclate

Doxycyline hyclate is chemically known as 4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide monohydrochloride. It is official in B.P. 2011[1] and U.S.P. XXXII [2]. It is a tetracycline derivative which is bacteriostatic with a broad spectrum of antimicrobial activity including many aerobic and anaerobic Gram-positive and Gram-negative pathogenic bacteria and some protozoa. It is used in triple therapy along with tinidazole and proton pump inhibitor in the treatment of peptic ulcer [3]. A survey of the literature revealed that doxycyline has been estimated in pharmaceutical formulations by UV-spectrophotometry[21-23],spectrofluorimetry[24], HPLC [25-27] and electrochemical methods [28,29].

High performance liquid Chromatography (HPLC) is probably the most powerful tool for quantitative determination of many individual components in a mixture in one single procedure [30]. Safety and efficacy of pharmaceuticals are important in drug therapy. The impurities in drugs often have undesired pharmacological or toxicological effects. So, the products intended for human consumption must be characterized as completely as possible. Among the quality and safety of a drug is generally assured by monitoring and controlling the impurities effectively. Thus, the analytical activities concerning impurities in drugs are among the most important issues in modern pharmaceutical analysis [31].

MATERIALS AND METHODS

Apparatus

ISCO 2350®HPLC instrument (U.S.A.).

HPLC system consists of reversed phase Hypersil C_{18} column (250 x 4.6 mm, i.d., 5 µ p.s.), a manually driven 10 µl loop injector, two pumps and UV-Vis detector.

Digital pH-meter (Cosort P400) ® for pH adjustment.

Materials

Omeprazole (Sigma Pharmaceutical Industries, Quesna City, Egypt).

Tinidazole (Sigma Pharmaceutical Industries, Quesna City, Egypt).

Doxycycline (Pharco Pharmaceutical Industries, Alexandria, Egypt).

Pharmaceutical preparations

Trio® Capsules (Hikma Pharmaceutical Co., 6th of October City, Egypt), labeled to contain 20 mg of omeprazole, 50 mg of tinidazole and 500 mg of doxycyline.

Reagents

All solvents and reagents were of HPLC analytical grade.

Acetonitrile, Sodium hydroxide, Triethylamine and methanol (Aldrich Chemical Co. Ltd., Dorset, England).

Orthophosphoric acid and potassium dihydrogen phosphate (EL-Nasr Pharm. Chem. Co., Egypt).

Buffer prepararion

0.03 M phosphate buffer was prepared by dissolving 4.08 gm of potassium dihydrogen phosphate in 1 liter of double distilled water. The value of pH for the buffer was adjusted using orthophosphoric acid. The mobile phase was filtered before analysis, through 0.45 µm nylon membrane and degassed before use.

Preparation of standard solutions

Stock solutions of omeprazole, tinidazole and doxcycline (500 µg/ml) were prepared by dissolving 50 mg tinidazole and doxcycline in 100 ml methanol in volumetric flasks, whereas 50 mg omeprazole was dissolved in few drops 0.1 M NaOH then completing to 100 ml with methanol in a volumetric flask.

Construction of calibration curves

Working solutions were prepared immediately before use by further dilutions of the stock solutions with methanol, to cover the concentration ranges of 10-110 µg/ml, 5-125 µg/ml and 5-500 µg/ml for omeprazole, tinidazole and doxycyline, respectively. Detection was performed at wavelength 301 nm. The calibration graph was constructed by plotting the peak areas obtained versus the corresponding injected concentrations.

Preparation of the Acid-Degradation Products for omeprazole

Twenty milligrams of omeprazole were transferred into a 50-ml conical flask; 25 ml of 0.1 M hydrochloric acid were added. The solution was set aside at room temperature (25±2 °C) for not less than 1.5 hours, neutralized with 0.1 M sodium hydroxide solution, quantitatively transferred into 100-ml volumetric flask and completed to 100 ml with methanol. [32]

Preparation of the Alkali-Degradation Products for tinidazole

Twenty milligrams of tinidazole were transferred into a 50-ml conical flask; 25 ml of 0.1 M sodium hydroxide were added. The solution was heated at of 80°C. The solution was quantitatively transferred into 100-ml volumetric flask and completed to 100 ml with methanol. [33]

Preparation and assay of laboratory prepared mixtures in the presence of omeprazole and tinidazole degradation products

Five mixtures were laboratory prepared by transferring aliquots of each drug standard solution of omeprazole, tinidazole and doxycycline to a series of 10-ml volumetric flasks, beside to adding of 20 µg/ml of degradation products for both omeprazole and tinidazole and completing to 10 ml with the mobile phase. Prepared mixtures were chromatographed under the specified conditions and the concentration of each drug was calculated from the corresponding regression equations. Results are listed in table 1.

Preparation and analysis of pharmaceutical preparations

The contents of twenty capsules of each drug were emptied and pulverized. A portion of finely powdered drug equivalent to 20 mg of omeprazole, 500 mg of tinidazole and 50 mg of doxycycline in Trio® capsules was accurately weighed and transferred to a 100 ml volumetric flask. Fifty milliters of the mobile phase was added to the mixture, which was dissolved through ultrasonication for 30 minutes, finally the solution was completed to 100 ml with the mobile phase. The solution was filtered using 0.45 µm nylon membrane filter disc before use. Further dilution was performed with the mobile phase.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

Chromatographic parameters including wavelength detection, mobile phase composition and proportions, pH and flow rate were carefully studied in order to recognize the most suitable chromatographic system. The choice was based on the best resolution in a reasonable time.

Detection wavelength

Spectroscopic analysis of the drugs showed that omeprazole, tinidazole and doxycycline have maximum absorbance at 290 nm, 310 nm and 305 nm, respectively. Therefore, the chromatographic detection was performed at 301 nm using UV-Vis. detector.

Buffer pH

Choosing suitable mobile phase pH was an important factor. Where omeprazole decomposes in acidic medium (after protonation) and the molecule possesses a half life of only 1.4 hours at pH 5.1, increasing to 38.5 hours at pH 7.4 [34], reaching its maximum stability at pH 11 without any significant difference from pH 9 [35].

So, basic medium was preferred. However, an analysis above pH 7 was avoided for the following reasons; mobile phase with pH > 7 could damage the silica- based column. Also doxycyline exhibited severe peak tailing and band broadening due to its increased hydrophobicity. After experimental study, buffer pH 6.5 was optimum one giving good baseline separation. Several types of buffer (phosphate, acetate and citrate buffers) were examined. It was found that phosphate buffer gave better peak symmetry than other types of buffers.

Mobile phase composition and proportions

Mixture of methanol and phosphate buffer (20: 80, v/v) was tried. However, doxycyline peak tailing was obtained and retention time for omeprazole was delayed (t_R =17.8 minutes). When methanol was replaced with acetonitrile at the same ratio, omeprazole peak tailing was obtained. Therefore, mixture of acetonitrile: methanol: phosphate buffer was used for separation. Addition of triethylamine (TEA; as ion-pair reagent) prevents tailing and band broadening. After experimental trials, it was found that good separation was achieved upon using a mobile phase consisting of acetonitrile: methanol: phosphate buffer (pH 6.5) (20: 30: 50, v/v/v) in addition to 0.03ml/ L TEA.

Flow rate

The effect of flow rate was studied to optimize the chromatographic efficiency of the proposed method and improve the resolution of the eluted peaks. When flow rate was set at 2 ml/min., tinidazole was eluted at 1.2 minutes near the peak of the solvent. So; the flow rate was decreased to be 1.5 ml/min. to separate tinidazole at 3.4 minutes away from the peak of the solvent.

So, the optimum chromatographic performances were achieved when using isocratic mobile phase composed of acetonitrile: methanol: phosphate buffer (pH 6.5) (20: 30: 50, v/v/v) in addition to 0.03 ml/ L TEA., injection volume 10 µl, detection wavelength 301 nm and flow rate 1.5 ml/min.

Chromatographic Conditions for the proposed HPLC method are listed in table 2. Under the optimized conditions; tinidazole, doxycycline and omeprazole were separated within 3.4, 5.07 and 8.02 minutes, respectively in the presence of degradation products of omeprazole and tinidazole. Fig. 4 and 5

Method validation

The developed methods were validated according to international conference on harmonization guidelines ICH [36].

Linearity and range

The calibration graphs obtained by plotting the values of the peak areas versus the drug concentrations (µg/ml) were found to be rectilinear over the concentration ranges cited in the table 3.

Correlation coefficient, intercept and slope for the calibration data are summarized in table 3.

Accuracy and Precision

The accuracy of the proposed method was checked by performing recovery experiments through standard addition technique. The results are shown in table 4.

Intraday precision was evaluated by calculating standard deviation (SD) of three independent concentrations for each drug and for the mixture of drugs with the degradation products. The SD values revealed the high precision of the method (values vary from 0.792 to 0.992). For inter - day reproducibility, a series was run, the SD values were in the range of 0.923 - 1.21.

Table 1: Results of the analysis of omeprazole, tinidazole and doxycyline in presence of the degradation products of omeprazole and tinidazole laboratory –prepared mixture using the proposed HPLC method

Mixture	Conc. added form omeprazole µg ml^{-1}	Conc. added form tinidazole µg ml^{-1}	Conc. added form doxycyline µg ml^{-1}	Recovery*% for omeprazole	Recovery*% for tinidazole	Recovery* % for doxycycline
I	10	30	100	99.82	101.45	99.35
II	30	50	200	101.2	101.2	100.3
III	50	70	300	100.98	100.58	99.57
IV	70	90	400	100.21	100.76	100.54
V	90	120	500	101.12	101.29	99.85
Mean*±SD				100.66±0.615	101.056±0.369	99.92±0.495

* Average of three independent procedures.

Table 2: Chromatographic Conditions for the proposed HPLC method

Parameters	Conditions
Column	Reversed phase Hypersil C_{18} column (250 x 4.6 mm, i.d., 5µ, p.s.)
Mobile phase	Isocratic mobile phase of acetonitrile: methanol: phosphate buffer (pH 6.5) (20: 30: 50 v/v/v) in addition to 0.03ml/ L TEA., filtered and degassed using 0.45µm membrane filter
UV detection, nm	301 nm
Flow rate, ml/min	1.5 ml/min.
Injected volume, µl	10 µl
Temperature	ambient
Retention time, min	
Tinidazole	3.4 min.
Doxycycline	5.07 min.
Omeprazole	8.02 min.
Degradation product of tinidazole	1.2 min.
Degradation products of omeprazole	2.4 min. and 7.01 min.

Table 3: Results and characteristic parameters for the simultaneous determination of omeprazole,tinidazole and doxycycline by the proposed method.

parameters		Omeprazole			Tinidazole			Doxycycline		
		Conc. taken µg/ml	Conc. found µg/ml	%Recovery	Conc. taken µg/ml	Conc. found µg/ml	%Recovery	Conc. taken µg/ml	Conc. found µg/ml	%Recovery
		10	9.71	98.95	5	5.07	101.56	5	5.02	100.36
		30	29.93	99.77	30	30.59	101.99	100	102.18	102.18
		50	50.13	100.27	55	54.74	99.53	200	203.44	101.72
		70	70.36	100.51	80	80.73	100.91	300	299.72	99.91
		90	90.71	100.78	110	108.98	99.07	400	401.37	100.34
		110	109.22	99.28	125	126.35	101.08	500	509.26	101.85
Mean recovery*				99.92			100.54			101.06
N				6			6			6
±SD				0.720			0.999			0.965
±RSD				0.719			0.993			0.955
Regression Equation**	Slope (b)			540.3			38.49			24.85
	Intercept (a)			87781			10791			6358
LOD µg ml^{-1}				0.0082			0.11			0.128
LOQ µg ml^{-1}				0.024			0.32			0.388
Correlation coeffiecient				0.999			0.999			0.999

* Average of three independent procedures. **Y = a + bC where Y is peak area, C is the concentration of the drug in µg ml^{-1}

Sensitivity

The limit of detection (LOD) for the proposed HPLC method was calculating using the following equation: LOD = 3.3S/K and The limit of quantification, LOQ is defined as: LOQ = 10S/K

Where S is the standard deviation of the three replicate determination values under the same conditions as for the drug analysis and K is the slope of calibration graph. According to these equations, the limits of detection and the limits of quantification were calculated and are listed in table 3.

Specificity

The specificity studies revealed the absence of any other excipient interference (such as talc, starch, microcrystalline cellulose and magnesium stearate), since none of the peaks appeared at the same retention time of omeprazole, tinidazole and doxycyline. Fig. 6

Robustness

The robustness of the method was evaluated by making small changes in the flow rate (1.9, 2, 2.1), pH of mobile phase within a range of ± 0.2 of the optimized pH and mobile phase ratio keeping the other chromatographic conditions constant where the effect of the changes was studied on the percent recovery of drugs. The changes had negligible influence on the results as revealed by small SD values (=0.95).

System Suitability

The system suitability test was applied to a representative chromatogram to check the various parameters such as retention factor, number of theoretical plates, resolution and selectivity factors according to U.S.P. XXXII [2]. The results obtained are shown in table 5.

Analysis of pharmaceutical formulations

The validated HPLC method was applied for the simultaneous determination of omeprazole, tinidazole and doxycycline in combined pharmaceutical mixtures in Trio® capsules. Results obtained were compared to those obtained by applying reference methods [2,17], where Student's t-test and F-test were performed for comparison. Results are shown in tables 6. The calculated *t* and F values were less than tabulated values for the cited drugs which in turn indicate that there is no significant difference between proposed method and reference ones.

Table 4: Application of standard addition technique for the determination of omeprazole, tinidazole and doxycycline in Trio® capsules

Items	Omeprazole			Tinidazole			Doxycycline		
	Conc. added form pure drug (µg/ml)	Conc. taken from capsules (µg/ml)	Recovery*%	Conc. added form pure drug (µg/ml)	Conc. taken from capsules (µg/ml)	Recovery*%	Conc. added form pure drug (µg/ml)	Conc. taken from capsules (µg/ml)	Recovery*%
	10	0	101	20	0	101.76	100	0	101
	10	20	100.66	20	10	100.78	100	100	101.5
	10	30	100	20	20	100.15	100	200	100.33
	10	40	101	20	30	101.88	100	300	101.5
	10	50	101.33	20	40	101.17	100	400	101..2
Mean*			100.80			101.00			101.13
N			5			5			5
S.D.			0.505			0.716			0.481
R.S.D.			0.501			0.709			0.476
V			0.255			0.528			0.304
S.E.			0.226			0.358			0.241

*Mean of three different experiments.

Table 5: System suitability parameters for the proposed HPLC method according to U.S.P. XXXII [2]

Parameter	Omeprazole	Tinidazole	Doxycycline	Recommended values
Retention time (t_R min.)	8.02	3.4	5.07	-
Retention factor (k`)	9.02	3.25	5.34	0.5<k`>10
Number of theoretical plates (N)	6432	739.8	1142	The more plates, the better.
HETP	0.039	0.338	0.219	The smaller values, the better.
Parameter	**OMP-TNZ**	**DOX-TNZ**	**OMP-DOX**	**Recommended values**
Resolution factor (R_s)	8.40	3.03	4.91	>1.5
Selectivity factor(α)	2.77	1.64	1.69	>1

Table 6: Statistical analysis of results obtained by the proposed HPLC method applied on Trio® capsules compared with the reference methods:

Statistics	Omeprazole		Tinidazole		Doxycycline	
	Official method[2]	Proposed method	Reference method[17]	Proposed method	Official method[2]	Proposed method
Mean recovery*±	100.24±	100.80±	100.71±	101.00±	101.28±	101.13±
SD	0.635	0.505	0.881	0.716	0.862	0.481
N	5	5	5	5	5	5
Variance	0.404	0.255	0.777	0.528	0.743	0.304
*t-test***		1.543		0.995		0.592
F-test**		1.582		1.514		3.212

* Average of three experiments. **Theoretical *t* and F values are 2.306 and 5.05, respectively at p=0.05.

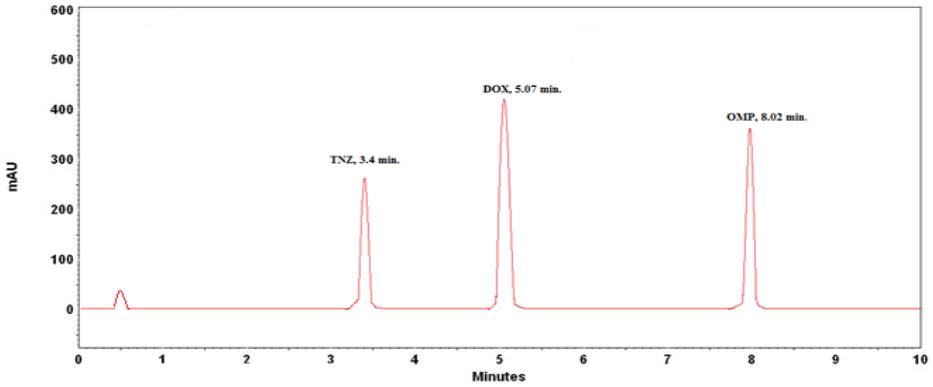

Fig. 4: HPLC chromatogram of synthetic mixture of 50 µg ml^{-1} omeprazole, 30 µg ml^{-1} tinidazole and 100 µg ml^{-1} doxycycline

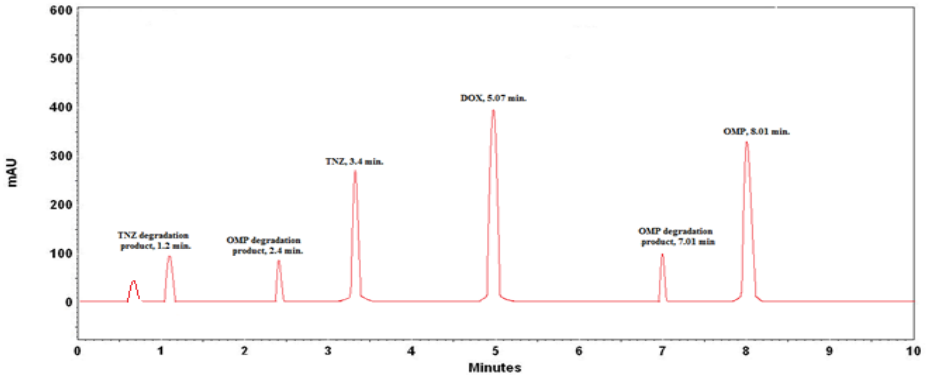

Fig. 5: HPLC chromatogram for the laboratory prepared mixture of 50 µg ml^{-1}omeprazole, 30 µg ml^{-1} tinidazole and 100 µg ml^{-1} doxycycline and 20 µg ml^{-1} degradation products of omeprazole and tinidazole.

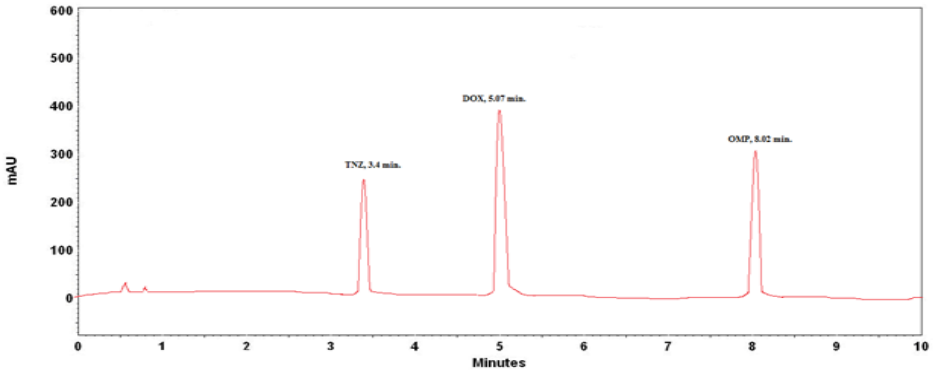

Fig. 6: HPLC chromatogram for the mixture of 50 µg ml^{-1} omeprazole, 30 µg ml^{-1} tinidazole and 100 µg ml^{-1} doxycycline in Trio® capsules

CONCLUSION

An isocratic RP- HPLC method has been developed for the simultaneous estimation of mixture of omeprazole, tinidazole and doxycycline in bulk, in combined capsules and in the presence of omeprazole and tinidazole degradation products. The developed method was validated and it was found to be simple, precise, accurate, and sensitive. Excipients present in the capsules show no interference in the determination. The proposed method can be used in quality control laboratories for routine analysis of omeprazole, tinidazole and doxycycline in combined dosage form.

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