

# Simultaneous Determination of Omeprazole, Tinidazole and Clarithromycin in Bulk Powder and Helicure® Tablets by HPLC

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### Abstract

Sensitive and precise chromatographic method was developed and validated for simultaneous determination of omeprazole (OMP), tinidazole (TND) and clarithromycin (CLR) in bulk powder, laboratory prepared mixture and pharmaceutical preparation. The technique adopted for quantification is HPLC. A mixture of acetonitrile, methanol, phosphate buffer at pH 3.5 (33: 17: 50, v/v/v) was used as a mobile phase. The stationary phase used was (150 mm×4.6 mm, 10µm) C8 Lichrosorb™ analytical column. The method was linear in the range of 0.2-250 µg mL<sup>-1</sup>, 0.5-250 µg mL<sup>-1</sup> and 75-2000 µg mL<sup>-1</sup> for OMP, TND and CLR respectively. The selectivity of the proposed method was checked using laboratory prepared mixtures. The proposed method was successfully applied to the analysis of OMP, TND and CLR in their mixture and in pharmaceutical dosage form without interference from other additives.

**Keywords:** Omeprazole; Tinidazole; Clarithromycin; HPLC.

### Introduction

Omeprazole (OMP), is 6-methoxy-2-[[[4-methoxy-3,5-dimethyl-2-pyridinyl] methyl] sulphanyl]-1H-benzimidazole [1], (Figure 1). It is the first member of the “proton pump inhibitors” that are widely used for the prophylaxis and treatment of both gastro-duodenal ulcers and symptomatic gastro-esophageal reflux. It is highly effective in the treatment of Zollinger-Ellison syndrome [2]. Tinidazole (TND) is 1-[2-(ethyl sulphonyl) ethyl]-2-methyl-5-nitro-1H-imidazole, [1] (Figure 2). It is used as antiprotozoal agent. Clarithromycin (CLR), is (3R,4S,5S,6R,7R,9R,11R,12R,13S,14R)-4-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribohexopyranosyl) oxy]-14-ethyl-12,13-dihydroxy-7-methoxy-3,5,7,9,11,13-hexamethyl-6-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-xylo hexopyranosyl] oxy] oxacyclotetradecane-2,10-dione (6-O-methylerythromycin A), [1] (Figure 3). CLR is semi-synthetic macrolide antibacterial agent [1].

The literature survey reveals several analytical methods for quantitative estimation of OMP alone in body fluids and in

pharmaceutical formulations these methods include spectrophotometry [3-14], electrochemical methods [15], HPLC [16-21], liquid chromatography-electrospray ionization tandem mass spectrometry [22] and electrophoresis [23]. Tinidazole was estimated in body fluids and in pharmaceutical formulations by spectrophotometry [24-29], potentiometry [29], HPLC methods [29-31], polarography [32,33] and resonance light scattering technique [34]. Clarithromycin has been reported to be estimated in body fluids and in pharmaceutical formulations by spectrophotometry [35], HPLC methods [36-44]. Omeprazole, Tinidazole and Clarithromycin were simultaneously determined by spectrophotometry [45,46].

Up to our knowledge, there is no isocratic HPLC method was described for the simultaneous determination of the three studied drugs in their laboratory prepared mixtures and in the pharmaceutical dosage form without prior derivatisation. The present work aimed to develop an isocratic HPLC method for simultaneous determination of OMP, TND and CLR in laboratory prepared mixtures and pharmaceutical dosage form. The proposed method has advantage of being cheap, simple, rapid and time saving (one run in less than 7 minutes).

### Experimental

#### Instruments

A liquid chromatograph consisted of a quaternary pump (Agilent model G1316 A/G1316 B), a diode array multiple wavelength detector (model G1316 C/D and G1365C/D, Agilent 1200 Series), Standard and preparation autosamplers (Agilent 1200 series) equipped vacuum degasser, Agilent. Stationary phase (150 mm×4.6 mm, 10 µm)

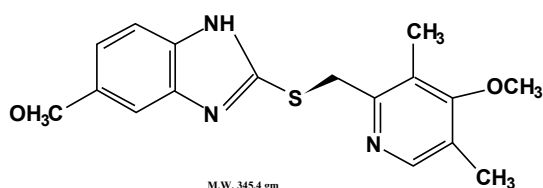


Figure 1: Chemical structure of Omeprazole (OMP).

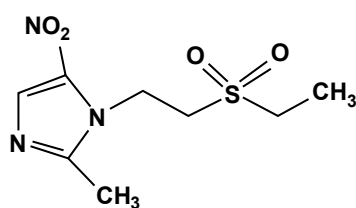


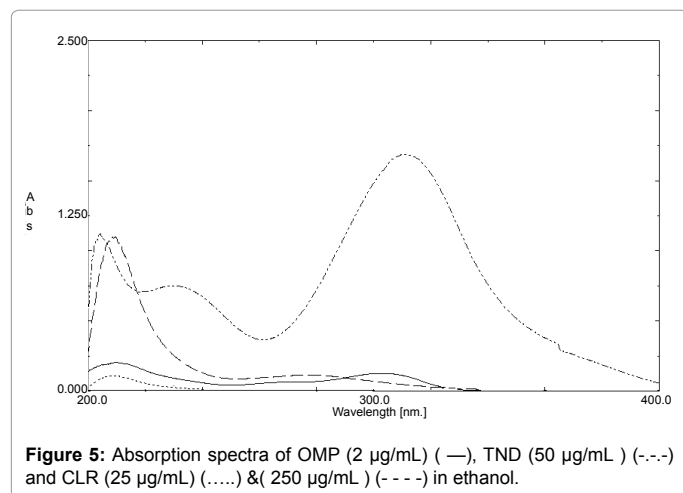
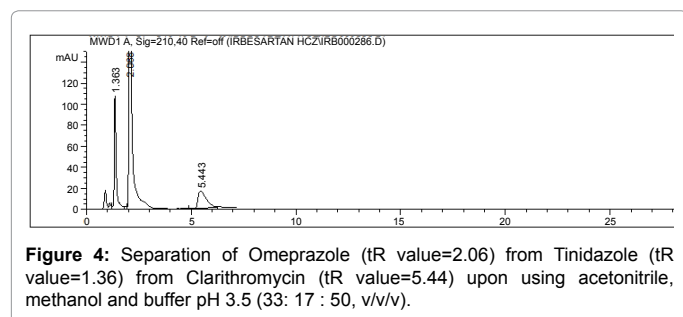
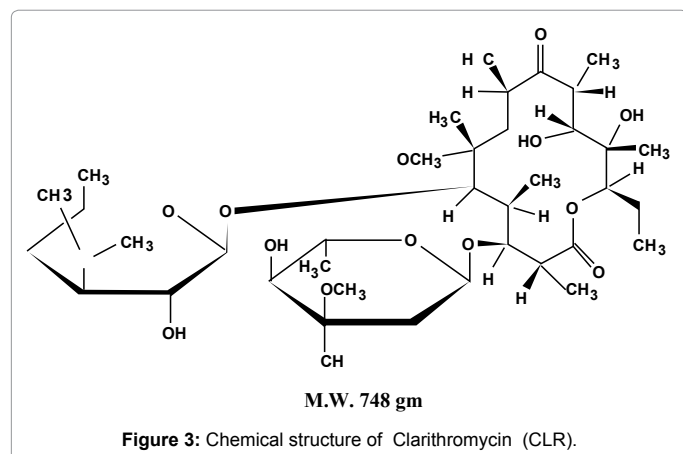
Figure 2: Chemical structure of Tinidazole (TND).

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C8 Lichrosorb™ analytical column. Mobile phase; acetonitrile, methanol, buffer at pH 3.5 (33: 17: 50, v/v/v). The mobile phase was filtered through a 0.45 µm Millipore membrane filter and was degassed for 15 min in an ultrasonic bath prior to use. UV-detection was done at 210 nm. The samples were filtered also through a 0.45 µm membrane filter.

### Standards, solvents, and pharmaceutical preparation

Reference omeprazole (OMP), reference tinidazole (TND) and reference clarithromycin (CLR) were kindly donated by EGYPHAR Pharmaceuticals Co. The potency was found to be 100.30%, 100.13% and 100.16% for OMP, TND and CLR, respectively. Pharmaceutical dosage form (Heli-cure tablets) were kindly supplied by EGYPHAR and were claimed to contain 20 mg of OMP, 500 mg TND and 250 mg of

CLR per tablet. Acetonitrile, methanol (HPLC grade) and phosphate buffer adjusted to pH 3.5.

### Standard solutions

OMP, TND standard solutions (each 0.5 mg mL<sup>-1</sup>) and CLR standard solution (2 mg mL<sup>-1</sup>) were prepared in mobile phase for the suggested HPLC method. The standards solutions were freshly prepared on the day of analysis and stored in a refrigerator to be used within 24 hr.

### Procedures

**Linearity:** Portions of OMP, TND standard solutions (each 0.5 mg mL<sup>-1</sup>) and CLR standard solution (2 mg mL<sup>-1</sup>) were transferred separately into a series of 10-mL volumetric flasks and completed with mobile phase. Several dilutions were done and the content of each flask was completed to volume with the mobile phase to get the concentrations of 0.2-250 µg mL<sup>-1</sup> OMP, 0.5-250 µg mL<sup>-1</sup> TND and 75-2000 µg mL<sup>-1</sup> CLR. The samples were then chromatographed using the following chromatographic condition. Stationary phase (150 mm×4.6 mm, 10 µm) C8 lichrosorb™. Many mobile phases such as methanol and acetonitrile (50:50, 60:40, 65:35, by volume), methanol, acetonitrile and phosphate buffer adjusted at pH 3.5 (30: 20:50, 40: 30: 30) by volume and different other ratios but the mobile phase which give the best separation and peaks shape was found to be a mixture of acetonitrile, methanol, buffer at pH 3.5 (33: 17: 50, v/v/v). The mobile phase was filtered through a 0.45 µm millipore membrane filter and was degassed for about 15 min in an ultrasonic bath prior to use, flow rate; 0.7 mL min<sup>-1</sup> [isocratically at temperature (35°C)], with UV-detection at 210 nm, the detection wavelength was set regarding the UV absorption spectra of the drugs (Figure 5) and their relative concentrations within the pharmaceutical formulation. Whereas TNZ is nominally 2 and 25 times more concentrated than CLR and OMP, respectively. The drugs have strong contributions in the overall UV region (200-375 nm). This is why an optimum detection wavelength was set at 210 nm during the chromatographic separation, favoring the quantification of both CLR and OMP, which represent the less concentrated components of this ternary mixture. In addition, this chosen detection wavelength can greatly improve the sensitivity of the proposed method for the CLR determination because it exhibits absorption maxima (at 210 nm). The samples were filtered also through a 0.45 µm membrane filter. To reach good equilibrium, the analysis was usually performed after passing 50-60 mL of the mobile phase, just for conditioning and pre-washing of the stationary phase. The relative peak area ratios were then plotted versus the corresponding concentrations of OMP, TND and CLR to get the calibration graphs and to compute the corresponding regression equations.

**Analysis of laboratory prepared mixtures containing different ratios of OMP, TND and CLR:** Aliquots of each standard solution were mixed to prepare different mixtures containing different ratios (3: 4:90, 1:0.2:26, 7:4:130, 2:0.2:23, 0.5:50:336, 30:12.5:168, 6:2.5:124, 0.5:1:144) of OMP, TND and CLR, respectively. The concentrations were calculated from the corresponding regression equations.

**Assay of pharmaceutical formulations (Heli-cure tablets):** Twenty tablets were powdered well and homogeneously mixed in a mortar. A mass of the powdered tablets equivalent to 20 mg of OMP, 250 mg of CLR and 500 mg of TND was weighed and transferred to a 100-ml volumetric flask. The powder was extracted by shaking with 3×30 mL mobile phase with vigorous shaking for 15 minutes then filtered. The volume was completed to the mark with the mobile phase. Several portions 0.5-2 mL of aliquot were transferred separately to 10-

mL volumetric flasks, the volumes were completed to the mark with mobile phase and chromatographed under the previous mentioned conditions.

## Results and Discussion

### High-performance liquid chromatography

A simple isocratic high-performance liquid chromatographic method was developed for the determination of OMP, TND and CLR in pure form and in pharmaceutical preparation using (150 mm×4.6 mm, 10 μm) C8 lichrosorb™ analytical column. The mobile phase consisted of acetonitrile, methanol, buffer at pH 3.5 (33: 17: 50, v/v/v). The mobile phase was chosen after several trials to reach the optimum stationary/mobile-phase matching. The average retention times under the conditions described are 2.06 min for OMP, 1.36 min for TND and 5.44 for CLR (Figure 4). One sample can be chromatographed in less than 6 min.

Peak purity was confirmed for the HPLC peaks of OMP, TND and CLR by a pilot run using a photodiode array detector. Calibration graph was obtained by plotting the relative peak area ratios against concentrations. Linearity range was found to be 0.2-250 μg mL<sup>-1</sup> for OMP, 0.5-250 μg mL<sup>-1</sup> TND and 75-2000 μg mL<sup>-1</sup> CLR. The regression equation for OMP: A=0.1832C+0.1946 (r=0.9999), for TND: A=0.0241C+0.0513 (r=0.9999) and for CLR: A=0.0021C+0.0280 (r=0.9999) where A is the relative peak area ratio, C is the concentration in μg mL<sup>-1</sup> and r is the correlation coefficient. The mean percentage recovery was found to be 100.08 ± 0.454 for OMP, 100.40 ± 0.535 for TND and 100.65 ± 0.862 for CLR (Tables 1 and 2).

### Analysis of laboratory prepared mixtures containing different ratios of OMP, TND and CLR

The suggested HPLC method was successfully applied for the determination of the studied drugs in their laboratory prepared mixtures. The precision of the proposed method was checked by the analysis of different concentrations (Table 2).

The mean percentage recovery was found to be:

$$99.96 \pm 0.407 \quad \text{for OMP}$$

$$100.14 \pm 0.332 \quad \text{for TND}$$

$$99.97 \pm 0.216 \quad \text{for CLR.}$$

### Analysis of dosage form (Heli-cure tablets)

The suggested HPLC method was successfully applied for the

Parameter	OMP	TND	CLR
Range (μg mL <sup>-1</sup> )	0.2-250	0.5-250	2000-75
Slope	0.183	0.024	0.002
Intercept	0.195	0.051	0.028
Variance	0.206	0.286	0.743
Coefficient of variation	0.454	0.535	0.862
Correlation coefficient (r)	1	1	1
Accuracy mean	100.08	100.40	100.65
RSD%	0.454	0.535	0.862
Precision (RSD%)	0.201	0.184	0.237
Repeatability	0.332	0.409	0.294
Intermediate precision			
Specificity mean	99.96	100.14	99.97
RSD%	0.407	0.332	0.216

Table 1: Validation and regression parameters for the determination of OMP, TND & CLR by the proposed HPLC method.

OMP : TND : CLR	OMP			TND			CLR		
	Taken ug mL <sup>-1</sup>	Found ug mL <sup>-1</sup>	R (%)	Taken ug mL <sup>-1</sup>	Found ug mL <sup>-1</sup>	R (%)	Taken ug mL <sup>-1</sup>	Found ug mL <sup>-1</sup>	R (%)
3 : 4 : 90	30	29.75	99.17	40	40.15	100.38	900	900.14	100.02
0.5 : 1 : 144	2.5	2.503	100.12	5	4.99	99.80	720	721.33	100.18
1 : 0.2 : 26	50	49.92	99.84	10	10.02	100.20	1300	1299.05	99.93
7 : 4 : 130	7	6.98	99.71	4	3.98	99.50	130	130.1	100.08
0.5 : 50 : 336	0.5	0.502	100.40	50	50.11	100.22	336	334.82	99.65
30 : 12.5 : 168	60	60.23	100.38	25	25.09	100.36	336	336.71	100.21
6 : 2.5 : 124	30	29.96	99.87	12.5	12.52	100.16	620	620.14	100.02
2 : 0.2 : 23	20	20.04	100.20	2	2.01	100.50	230	229.19	99.65
Mean ± SD	99.96 ± 0.407			100.14 ± 0.332			99.968 ± 0.216		

Table 2: Determination of OMP, TND and CLR in laboratory prepared mixtures containing different ratios (3: 4: 90, 0.5: 1: 144, 1: 0.2: 26, 7: 4: 130, 0.5: 50: 336, 30: 12.5: 168, 6: 2.5: 124, 2:0.2:23 ) of OMP, TND and CLR, respectively by the proposed method.

OMP			TND			CLR		
Taken ug mL <sup>-1</sup>	Found ug mL <sup>-1</sup>	R (%)	Taken ug mL <sup>-1</sup>	Found ug mL <sup>-1</sup>	R (%)	Taken ug mL <sup>-1</sup>	Found ug mL <sup>-1</sup>	R (%)
10	10.03	100.30	4	4.01	100.25	125	124.67	99.74
15	14.99	99.93	5	4.98	99.60	250	250.71	100.28
20	20.09	100.45	30	30.05	100.17	500	501.33	100.27
100.227 ± 0.268			100.01 ± 0.354			100.097 ± 0.216		

Table 3: Determination of OMP, TND and CLR in Helicure® tablets by the proposed method

OMP			
Claimed amount taken (ug mL <sup>-1</sup> )	Authentic added (ug mL <sup>-1</sup> )	Authentic found (ug mL <sup>-1</sup> )	Recovery (%)
40.00	20.00	20.11	100.55
	40.00	40.11	100.28
	60	60.14	100.23
Mean ± SD			100.35 ± 0.172
TND			
Claimed amount taken (ug mL <sup>-1</sup> )	Authentic added (ug mL <sup>-1</sup> )	Authentic found (ug mL <sup>-1</sup> )	Recovery (%)
25.00	10.00	10.04	100.40
	25.00	24.94	99.76
	50.00	49.95	99.90
Mean ± SD			100.02 ± 0.336
CLR			
Claimed amount taken (ug mL <sup>-1</sup> )	Authentic added (ug mL <sup>-1</sup> )	Authentic found (ug mL <sup>-1</sup> )	Recovery (%)
1000.00	2000.00	1987.62	99.38
	1500.00	1499.23	99.95
	4000.00	3998.60	99.97
Mean ± SD			99.78 ± 0.335

Table 4: Application of the standard addition technique to the proposed HPLC method of OMP, TND & CLR in their pharmaceutical formulation.

determination of the studied drugs in their pharmaceutical formulation which is Heli-cure tablets. The precision of the proposed method was checked by the analysis of different concentrations (Table 3).The mean percentage recovery was found to be:

$$100.23 \pm 0.268 \quad \text{for OMP}$$

$$100.01 \pm 0.354 \quad \text{for TND}$$

$$100.097 \pm 0.216 \quad \text{for CLR.}$$

## Conclusion

Validation of the accuracy of the proposed HPLC method was

Parameters	HPLC			B.P official method <sup>2</sup> OMP	B.P official method <sup>2</sup> TND	B.P official method <sup>2</sup> CLR
	OMP	TND	CLR			
Mean	100.076	100.403	100.647	100.30	100.13	100.16
± S.D	0.454	0.535	0.862	0.401	0.722	0.531
Variance	0.206	0.286	0.743	0.161	0.521	0.282
F-test	1.28 (4.95)a	1.82 (4.95)a	2.63 (5.05)a			
Student,s t-test	0.723 (2.201)a	0.763 (2.201)a	1.176 (2.23)a			
N	7	7	6	6	6	6

Table 5: Statistical comparison for the results obtained by the proposed method and the official method for analysis of OMP, TND and CLR.

Parameters	HPLC			Reported method <sup>48</sup> OMP	Reported method <sup>48</sup> TND	Reported method <sup>48</sup> CLR
	OMP	TND	CLR			
Mean	100.227	100.01	100.097	100.20	100.12	100.12
± S.D	0.268	0.354	0.216	0.283	0.167	0.281
Variance	0.072	0.125	0.047	0.080	0.028	0.079
F-test	1.14 (5.79)a	4.46 (5.79)a	1.68 (5.79)a			
Student,s t-test	0.13 (2.57)a	0.497 (2.57)a	0.123 (2.57)a			
N	3	3	3	4	4	4

aThe values in the parenthesis are corresponding theoretical t- and F-values at P=0.05 [44]

Table 6: Statistical comparison for the results obtained by the proposed HPLC method and the reported TLC method for analysis of OMP, TND and CLR in dosage form.

Parameter	OMP	TND	CLR	Limit <sup>(49,50)</sup>
Retention time ( $t_R$ )	2.063	1.353	5.443	
Resolution ( $R_s$ )		3.95		$R_s > 2$
Tailing factor (T)	0.80	0.88	0.40	T=1 for a typical symmetric peak
Capacity factor ( $K'$ )	1.58	1.48	5.06	1-10 acceptable
Selectivity factor ( $\alpha$ )		3.27		$\alpha > 1$
Column efficiency (N)	9811	6991	2451	N>2000
Height equivalent to theoretical plate (HETP)	0.003	0.004	0.010	The smaller the value, the higher the column efficiency

Table 7: System suitability parameters of the proposed HPLC method.

confirmed using standard addition technique (Table 4). Statistical comparison with the official and reported methods showed that the proposed HPLC is sensitive and precise (Table 5 and 6). Application of the proposed methods to the analysis of OMP, TND and CLR in their pharmaceutical formulation (Table 3) shows that excipients do not interfere with the determination. The system suitability parameters of the proposed HPLC method (Table 7). The proposed method has advantage of being sensitive and applicable over wide range. The proposed method can be used for routine analysis of omeprazole, tinidazole and clarithromycin in quality control laboratories.

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