Journal of Chromatography
Separation Techniques

Salem et al., J Chromatograph Separat Techniq 2014, 5:2
DOI: 10.4172/2157-7064.1000221

Research Article Open Access

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

Hesham Salem[1], Safa M Riad[2], Mamdouh R Rezk[2], Kholoud Ahmed[1]*

[1]*Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, October University for Modern Sciences and Arts, Egypt*
[2]*Analytical Chemistry Department, Faculty of Pharmacy-Cairo University, Kasr El-Aini Street, Egypt*

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 LichrosorbTM analytical column. The method was linear in the range of 0.2-250 µg mL^{-1}, 0.5-250 µg mL^{-1} and 75-2000 µg mL^{-1} 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] sulphinyl]-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-a-L-ribohexopyranosyl) oxy]-14-ethyl-12,13-dihydroxy-7-methoxy-3,5,7,9,11,13-hexamethyl-6-[[3,4,6-trideoxy-3-(dimethylamino)-b-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].

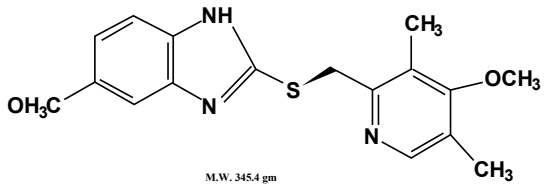


Figure 1: Chemical structure of Omeprazole (OMP).

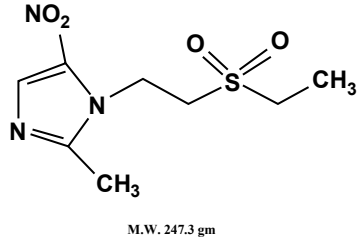


Figure 2: Chemical structure of Tinidazole (TND).

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

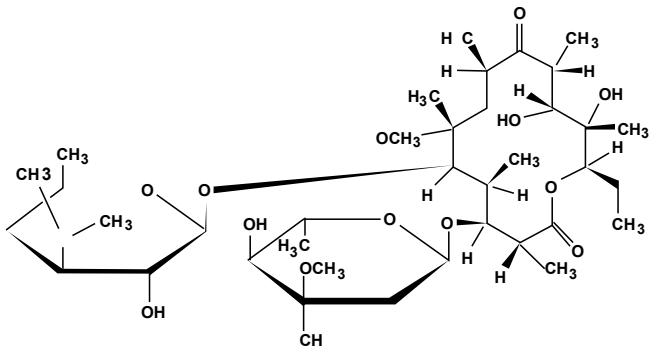
Instruments

A liquid chromatograph consisted of an 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)

***Corresponding author:** Kholoud Ahmed, Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, October University for Modern Sciences and Arts, Egypt; Tel.: +20 1226483727; E-mail: m.habashyyy@hotmail.com

Received February 21, 2014; **Accepted** April 22, 2014; **Published** April 24, 2014

Citation: Salem H, Riad SM, Rezk MR, Ahmed K (2014) Simultaneous Determination of Omeprazole, Tinidazole and Clarithromycin in Bulk Powder and Helicure® Tablets by HPLC. J Chromatograph Separat Techniq 5: 221. doi:10.4172/2157-7064.1000221



M.W. 748 gm

Figure 3: Chemical structure of Clarithromycin (CLR).

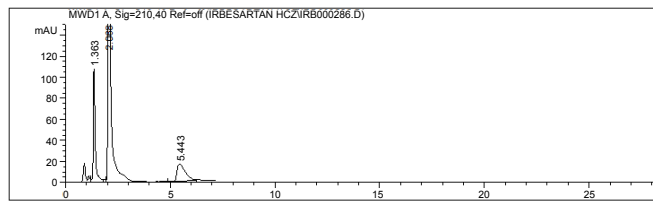


Figure 4: Separation of Omeprazole (tR value=2.06) from Tinidazole (tR value=1.36) from Clarithromycin (tR value=5.44) upon using acetonitrile, methanol and buffer pH 3.5 (33: 17 : 50, v/v/v).

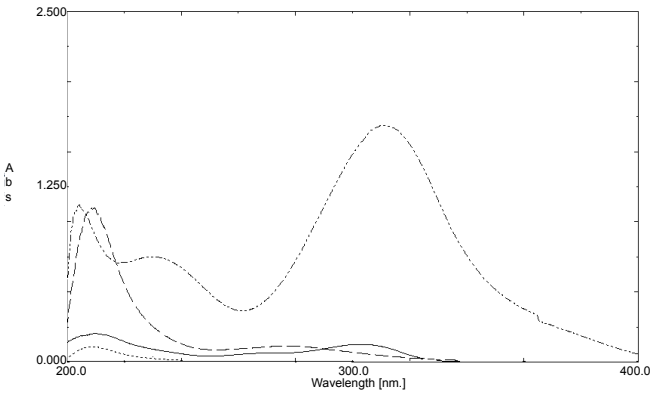


Figure 5: Absorption spectra of OMP (2 µg/mL) (—), TND (50 µg/mL) (-.-.-) and CLR (25 µg/mL) (.....) &(250 µg/mL) (- - - -) in ethanol.

C8 Lichrosorb TM 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^{-1}) and CLR standard solution (2 mg mL^{-1}) 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^{-1}) and CLR standard solution (2 mg mL^{-1}) 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^{-1} OMP, 0.5-250 µg mL^{-1} TND and 75-2000 µg mL^{-1} 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^{-1} [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 morter. 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^{-1} for OMP, 0.5-250 µg mL^{-1} TND and 75-2000 µg mL^{-1} 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^{-1} 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 ± 0.407 for OMP

100.14 ± 0.332 for TND

99.97 ± 0.216 for CLR.

Analysis of dosage form (Heli-cure tablets)

The suggested HPLC method was successfully applied for the 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 ± 0.268 for OMP

100.01 ± 0.354 for TND

100.097 ± 0.216 for CLR.

Parameter	OMP	TND	CLR
Range (µg mL^{-1})	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^{-1}**	**Found ug mL^{-1}**	**R (%)**	**Taken ug mL^{-1}**	**Found ug mL^{-1}**	**R (%)**	**Taken ug mL^{-1}**	**Found ug mL^{-1}**	**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	9980	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^{-1}	**Found ug mL^{-1}**	**R (%)**	**Taken ug mL^{-1}**	**Found ug mL^{-1}**	**R (%)**	**Taken ug mL^{-1}**	**Found ug mL^{-1}**	**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^{-1})	**Authentic added (ug mL^{-1})**	**Authentic found (ug mL^{-1})**	**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^{-1})	**Authentic added (ug mL^{-1})**	**Authentic found (ug mL^{-1})**	**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^{-1})	**Authentic added (ug mL^{-1})**	**Authentic found (ug mL^{-1})**	**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.

Conclusion

Validation of the accuracy of the proposed HPLC method was

Parameters	HPLC			B.P official method[2] OMP	B.P official method[2] TND	B.P official method[2] 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[48] OMP	Reported method[48] TND	Reported method[48] 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 [49,50]
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 (α)	3.27			α>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.

References

1. The British pharmacopoeia I & II (2014) Bp commission (7th edn) Great Britain, her Majesty's stationery office, Dublin.
2. Souney PF, Matthews SJ (1994) Comprehensive Pharmacy Review, (2nd edn) Harwal, 765-777.
3. Mahmoud AM (2009) New sensitive kinetic spectrophotometric methods for determination of omeprazole in dosage forms. Int J Anal Chem 2009: 307045.
4. Bhandage A, Bhosale A, Kasture A, Godse VP (2009) Extractive Spectrophotometric determination of omeprazole in pharmaceutical preparations. Trop J Pharm Res 8: 449-454.
5. Wahbi AM, Abdel-Razak O, Gazy AA, Mahgoub H, Moneeb MS (2009) Spectrophotometric determination of omeprazole, lansoprazole and pantoprazole in pharmaceutical formulations. J Pharm Dev technol 14: 516-523.
6. Dhumal SN, Dikshit P M, Ubharay II, Mascarenhas BM, Gaitonde CD (1991) Individual UV-spectrophotometric assays of trazodone hydrochloride and omeprazole from separate pharmacetical dosages. J Indian Drugs 28: 565-567.
7. Ozaltin N, Koçer A (1997) Determination of omeprazole in pharmaceuticals by derivative spectroscopy. J Pharm Biomed Anal 16: 337-342.
8. Castro D, Moreno MA, Torrado S, Lastres JL (1999) Comparison of derivative spectrophotometric and liquid chromatograpic methods for the determination of omeprazole in aqueous solutions during stability studies. J Pharm Biomed Anal 21: 291-298.
9. El-Kousy NM, Bebawy LI (1999) Stability-indicating methods for determining omeprazole and octylonium bromide in the presence of their degradation products. J AOAC Int 82: 599-606.
10. Karljikovic-Rajic K1, Novovic D, Marinkovic V, Agbaba D (2003) First-order UV-derivative spectrophotometry in the analysis of omeprazole and pantoprazole sodium salt and corresponding impurities. J Pharm Biomed Anal 32: 1019-1027.
11. Salama F1, El-Abasawy N, Abdel Razeq SA, Ismail MM, Fouad MM (2003) Validation of the spectrophotometric determination of omeprazole and pantoprazole sodium via their metal chelates. J Pharm Biomed Anal 33: 411-421.
12. Wahbi AA, Abdel-Razak O, Gazy AA, Mahgoub H, Moneeb MS (2002) Spectrophotometric determination of omeprazole, lansoprazole and pantoprazole in pharmaceutical formulations. J Pharm Biomed Anal 30: 1133-1142.
13. el-Kousy NM, Bebawy LI (1999) Stability-indicating methods for determining omeprazole and octylonium bromide in the presence of their degradation products. J AOAC Int 82: 599-606.
14. Li HK, Zhou WB, Li GSY (2004) Spectrophotometric Determination of Omeprazole Based on the Charge Transfer Reaction between Omeprazole and Chloranilic Acid. Chin J spectroscop Lab 21: 646-649.
15. Qaisi AM, Tutunji MF, Tutunji LF (2006) Acid decomposition of omeprazole in the absence of thiol: a differential pulse polarographic study at the static mercury drop electrode (SMDE). J Pharm Sci 95: 384-391.
16. Rezk NL, Brown KC, Kashuba ADM (2006) A Simple and sensitive bioanalytical assay for simultaneous determination of omeprazole and its three major metabolites in human blood plasma using RP-HPLC after a simple liquid-liquid extraction procedure. J Chromatogr B 844: 314-321.
17. Hofmann U, Schwab M, Treiber G, Klotz U (2006) Sensitive quantification of omeprazole and its metabolites in human plasma by liquid chromatography-mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 831: 85-90.
18. Linden R, Ziulkoskil AL, Wingert M, Tonello P, Souto AA (2007) Simultneous determination of omeprazole, hydroxyl omeprazole and omeprazole sulphone in human plasma by isocratic HPLC-DAD: application to the phenotyping of CYP2C19 and CYP3A4 in Brazilian volunteers. J Braz Chem Soc 18: 733-740.
19. Sivasubramanian L, Anilkumar V (2007) Simultaneous HPLC estimation of omeprazole and domperidone from tablets. Ind J Pharm Sci 69: 674-676.
20. Yeung PKF, Little R, Jiang Y, Buckley SJ, Pollak PT, et al. (1998) A simple high performance liquid chromatography assay for simultaneous determination of omeprazole and metronidazole in human plasma and gastric fluid. J Pharm Biomed Anal 17: 1393-1398.
21. Schubert A, Werle AL, Schmidt CA, Codevilla C, Bajerski L, et al. (2003) Determination of omeprazole in bulk and injectable preparations by liquid chromatography. J AOAC Int 86: 501-504.
22. Vittal S, Ganneboina R, Layek B, Trivedi RK, Hotha KK, Bharathi DV, et al. (2009) Highly sensitive method for the determination of omeprazole in human plasma by liquid chromatograpy-electrospray ionization tandem mass spectrometry: application to a clinical pharmacokinetics study. J Biomed Chromatogr 23: 390-396.
23. Perez-Ruiz T, Martínez-Lozano C, Sanz, E Bravo A, Galera R (2006) Determination of omeprazole, hydroxyl omeprazole and omeprazole sulfone

using automated solid phase extraction and micellar electrokinetic capillary chromatography. J Pharm Biomed Anal 42: 100-106.

24. Singh L (2010) Validated method for determination of tinidazole using direct colorimetry, UV-visible spectrophotometry and difference spectrophotometry in pure and tablet dosage form. J Pharmacy Research 3: 1211.
25. Okunrobo LO (2007) Titrimetric and spectrophotometric determination of tinidazole tablets. World J Chem 2: 63-66.
26. Prasad CV, Sripriya V, Saha RN, Parimoo P (1999) Simultaneous determination of tinidazole, furazolidone and diloxanide furoate in a combined tablet preparation by second-derivative spectrophotometry. J Pharm Biomed Anal 21: 961-968.
27. Adegoke OA, Umoh OE (2009) A new approach to the spectrophotometric determination of metronidazole and tinidazole using p-dimethylaminobenzaldehyde. Acta Pharm 59: 407-419.
28. Prasad CV, Parihar C, Sunil K, Parimoo P (1997) Simultaneous determination of tinidazole-clotrimazole and tinidazole-norfloxacin in combined tablet preparations by derivative spectroscopy. J Pharm Pharmacol Commun 3: 337-341.
29. Basavaiah K, Nagegowda P, Chandrashekar U (2005) Determination of tinidazole by potentiometry, spectrophotometry and high performance liquid chromatography. Indian J Chemical Technology 12: 273-280.
30. Feng MX, Gao H, Yu FY (1997) Determination of tinidazole and its degradation products in tinidazole injections by reverse-phase HPLC. Fenxi Zazhi 17: 247-249.
31. Pai PN, Rao GK, Srinivas B, Puranik S (2008) RPLC Determination of Tinidazole and Diloxanide Furoate in Tablets. Indian J Pharm Sci 70: 670-672.
32. Abu Zuhri AZ, Al-Khalil S, Shubietah RM, El-Hroub I (1999) Electrochemical study on the determination of tinidazole in tablets. J Pharm Biomed Anal 21: 881-886.
33. Rege PV, Sathe PA, Salvi VS, Trivedi ST (2011) Simultaneous determination of norfloxacin and tinidazole in combined drug formulation by a simple electroanalytical technique. Int J Pharm Research Development 3.
34. Jiang XY, Chen XQ, Dong Z, Xu M (2007) The application of resonance light scattering technique for the determination of tinidazole in drugs. J Autom Methods Manag Chem 2007: 86857.
35. Shah J, Rasul JM, Suraya M (2001) Extractive Spectrophotometric methods for determination of clarithromycin in pharmaceutical formulations using bromothymol blue and cresol red. J Biomed Chromatography 15: 507-508.
36. Niopas I, Daftsios AC (2001) Determination of clarithromycin in human plasma by HPLC with electrochemical detection: validation and application in pharmacokinetic study. Biomed Chromatogr 15: 507-508.
37. De Velde F, Alffenaar JW, Wessels AM, Greijdanus B, Uges DR () Simultaneous determination of clarithromycin, rifampicin and their main metabolites in human plasma by liquid chromatography-tandem mass spectrometry, J Chromatogr B 877: 1771-1777.
38. Amini H, Ahmadiani A (2005) Sensitive determination of clarithromycin in human plasma by high-performance liquid chromatography with spectrophotometric detection. J Chromatogr B Analyt Technol Biomed Life Sci 817: 193-197.
39. Jiang Y, Wang J, Li H, Wang Y, Gu J (2007) Determination of clarithromycin in human plasma by liquid chromatography-electrospray ionization tandem mass spectrometry. J Pharm Biomed Anal 43: 1460-1464.
40. Gurule S, Verma PRP, Monif T, Khuroo A, Partani P (2008) Sensitive liquid chromatographic determination of clarithromycin and 14-hydroxy clarithromycin in human plasma with tandem mass spectrometry. J Liquid Chromatogr & Related Technol 31: 2955-2973.
41. Oswald S, Peters J, Venner M, Siegmund W (2011) LC-MS/ MS method for the simultaneous determination of clarithromycin, rifampicin and their main metabolites in horse plasma, epithelial lining fluid and broncho-alveolar cells, J Pharm Biomed Anal 55: 194-201.
42. Elkhoudary MM , Abdel Salam RA, Hadad GM (2014) Current Pharm Anal 10: 58-70.
43. Darwish KM, Salama I, Mostafa S, El-Sadek M (2013) RP-HPLC/pre-column derivatization for analysis of omeprazole, tinidazole, doxycycline and clarithromycin. J Chromatogr Sci 51: 566-576.
44. Kasnia V, Kumar M S, Mahadevan N (2012) International Journal of Recent Advances in Pharmaceutical Research 2: 78-83
45. Lotfy HM (2006) Simultaneous determination of omeprazole, tinidazole and clarithromycin in combination. Bull Fac Pharm Cairo Univ 44: 27-39.
46. Lotfy HM, Abdel-Monem Hagazy M (2012) Comparative study of novel spectrophotometric methods manipulating ratio spectra: An application on pharmaceutical ternary mixture of omeprazole, tinidazole and clarithromycin. J Spectrochim Acta A Mol Biomol Spectrosc 96: 259-270.
47. Spiege MR, Stephns LJ (1999) Schaumoutline of theory and problems of statistics, Schaum Outline Series.
48. Salem H, Riad SM, Rezk MR, Ahmed K (2013) Simultaneous determination of omeprazole, tinidazole and clarithromycin in bulk powders and Helicure tablets by TLC densitometric technique. J Pharm Educ Res 4: 34.
49. Andrea W, Phyllis R (1997) HPLC and CE principles and practice, London: Academic press. 7-15.
50. Adamovics AJ (1997) Chromatographic analysis of pharmaceuticals, New York: Marcel Dekker Inc.