PHARMACIA

Pharmacia 70(3): 681–688
DOI 10.3897/pharmacia.70.e109211

Research Article

Development and validation of analytical procedure for analysis of Amoxiciline, Metronidazole and Omeprazole, used as anti-*Helicobacter pylori* agents alone and in mixture

Vania Maslarska[1], Lily Peikova[2], Maya Georgieva[2], Stefka Ivanova[3], Miglena Smerikarova[1], Stefan Balkanski[4], Stanislav Bozhanov[1]

1 *Department of Chemistry, Faculty of Pharmacy, Medical University – Sofia, Sofia, Bulgaria*
2 *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University – Sofia, Sofia, Bulgaria*
3 *Department of Pharmaceutical Chemistry and Pharmacognosy, Faculty of Pharmacy, Medical University – Pleven, Pleven, Bulgaria*
4 *Bulgarian Pharmaceutical Union, Sofia, Bulgaria*

Corresponding author: Lily Peikova (lily_peikova@yahoo.com)

Received 8 July 2023 ♦ **Accepted** 7 August 2023 ♦ **Published** 23 August 2023

Citation: Maslarska V, Peikova L, Georgieva M, Ivanova S, Smerikarova M, Balkanski S, Bozhanov S (2023) Development and validation of analytical procedure for analysis of Amoxiciline, Metronidazole and Omeprazole, used as anti-*Helicobacter pylori* agents alone and in mixture. Pharmacia 70(3): 681–688. https://doi.org/10.3897/pharmacia.70.e109211

Abstract

Background: The contemporary treatment of ulcerogenic diseases and gastroesophageal reflux disease is related usually to application of a combination of imidazole-based antibacterial, antibiotic and proton pump inhibitor. In the current study, the three most common representatives Amoxicilline (AMO), Metronidazole (MET) and Omeprazole (OME), respectively, are subjected to analysis through classical analytical procedure, providing high level accuracy, sensitivity and good separation abilities. As such a UV/VIS method was applied as a well known identification and quantitation technique for analyses in various samples. Furthermore, this technique is known to be a good detection method in combination with chromatographic systems.

Purpose: A simple, specific, accurate and precise reverse phase-high performance liquid chromatographic method has been developed for the simultaneous determination of Amoxicillin Trihydrate (AMO), Metronidazole (MET) and Omeprazole (OME) in synthetic mixture.

Materials and methods: Some important parameters like pH of the mobile phase, concentration of the acid or buffer solution, percentage and type of the organic modifier, etc. were tested for a good chromatographic separation. The sample was analyzed using a mobile phase of Acetonitrile: Phosphate buffer (pH=7.6±0.1) (40:60 v/v). The flow rate was 1.0 mL/ min with detection at 280 nm.

Results: The retention time for AMO, MET and OME was found to be 1.67, 2.86 and 5.99 min respectively, and the recoveries in the synthetic mixture were between 98 and 102%. The validated method was linear over the concentration range of 25 to 200 µg/mL for AMO, 12.5 to 100 µg/mL for MET and 5–40 µg/mL for OME, with a correlation coefficient > 0.999.

Conclusion: The developed method has been validated in accordance with the International Conference on Harmonization (ICH) guidelines and showed excellent linearity, accuracy, precision, specificity, robustness, as well as system suitability results within the acceptance criteria.

Keywords

Helicobacter pylori, simultaneous determination, HPLC-UV, Amoxicillin Trihydrate, Metronidazole, Omeprazole, analysis

Introduction

The drug forms of the present study are widely used in the treatment of ulcerative disease and gastroesophageal reflux disease (GERD), which are socially significant and widespread diseases. Between 10% and 15% of the European race suffers ulcerative defect at least once in life (Kusters et al. 2006). Stomach and duodenal ulceration is a localized defect in the gastric or duodenal mucosa that passes hrough its entire thickness and often affects the deeper layers of the wall. *Helicobacter pylori* (*H. pylori*) infection is one of the most common infections in humans in recent years (Puig et al. 2016; Graham et al. 2017; Kasiri et al. 2017). It affects 50% of humanity and is the cause of morbidity not only in the gastro-duodenal area but also in more distant organs and systems. *H. pylori* is a gram-negative bacterium discovered and cultivated in 1981 by Barry Marshall and Robert Warren. *H. pylori* infection is transmitted by oral-fecal or oral-oral route most often in the first years of life. Infection is more common in developing countries due to the presence of contaminated water and food (Kusters et al. 2006). The treatment of *H. pylori*, a cause for induced diseases includes the use of antisecretory agents, gastroduodenal mucoprotectors, and anti-helicobacter medications against the major agent. It has been found that the combination Amoxicillin-Metronidazole-Omeprazole is effective in treating *H. pylori* infection (Puig et al. 2016). However, the results indicate that primary resistance to Metronidazole may reduce its effectiveness, but increased daily dosin of Metronidazole may partially overcome this problem (Bayerdörffer et al. 1999).

Amoxicillin (AMO) is a semi-synthetic antibiotic from the group of Aminopenicillins with the general formula $C_{16}H_{19}N_3O_5S$. Its chemical name according to IUPAC is: (2S, 5R, 6R) -6 – [[(2R) -2-amino-2- (4hydroxyphenyl) acetyl] amino] -3,3-dimethyl-7- 1-azabicyclo [3.2.0] heptane-2-carboxylic acid. It belongs to the group of beta-lactam antibiotics derived from 6-aminopenicillanic acid (penicillins).

Metronidazole (MET) is a nitroimidazole derivative of the general formula $C_6H_9N_3O_3$. Its name according to IUPAC is 2- (2-methyl-5-nitro-1H-imidazol-1-yl) ethanol. Metronidazole and other nitroimidazole antibacterial agents disrupt the structure of DNA, believing that the cause of this is free radical formation. They initiate oxidative damage to DNA and thus kill microorganisms with low redox potential (Müller et al. 1983).

Omeprazole (OME) is a benzimidazole derivative of the general formula $C_{17}H_{19}N_3O_3S$. Its IUPAC chemical name is 5-methoxy-2 – [(RS) – [(4-methoxy-3,5-dimethylpyridin-2-yl) methyl] sulfinyl] -1H-benzimidazole. OME is a part of the proton pump inhibitors group also including esomeprazole, lansoprazole, pantoprazole and rabeprazole. They are prodrugs that pass into active products – sulphene acid and sulphenamid cation. Activation of prodrugs occurs in the parietal cells in the wall of the gastrointestinal tract with *H. pylori* considered one of the major causes of ulcer disease. The eradication of *H. pylori* with antimicrobial medicinal products and OME is associated with rapid relief of symptoms, high levels of mucosal lesion recovery, and long-term remission of peptic ulcer, resulting in reduced complications such as gastrointestinal haemorrhages and the need for prolonged antisecretory therapy. Double combinations were tested and found to be less effective than the triple combination (Müller et al. 1983). However, dual therapy may be preferred in cases where hypersensitivity excludes triple therapy.

The development of new anti – *H. pylori* therapies is of interest to clinical pharmacologists, not only in identifying new targets but also in delivering an adequate drug to *H. pylori* gastric mucosa. Animal models of *H. pylori* infection have been developed, but their clinical picture has not yet been established. Vaccination for the treatment of infection has been found in animal models. Applied human experiments are not appropriate. (Goddard et al. 2003)

In analytical determination of the above-mentioned drugs the most often used methods are Spectrophotometry and HPLC. Spectrophotometric methods are less applicable due to some limitations in the methods resolution, which corresponds to deterioration in the quality control of the dosage forms considered. The literature mainly contains data for quantitative analysis of investigational drugs, both alone (Belal et al. 2000; Al-Abachi et al. 2005; Siddappa et al. 2008; Bhandage et al. 2009; Anusha et al. 2014; Mishra et al. 2014; Naveed and Qamar 2014; Kathriarachchi et al. 2018) and in mixture (Daharwal and Saraf 2007; Baraka et al. 2014). A number of authors create HPLC techniques for independent analysis of AMO (Douša and Hsu 1992), MET (Chaudhry et al. 2012) and OME (Daniel et al. 2005; Chaudhry et al. 2012; Nataraj et al. 2012; Ghandour et al. 2014), as well as methods of analysis in combinations with other drug substances (Mainz et al. 2002; Maher et al. 2008; Bojaraju et al. 2012; Ghante et al. 2012; Baraka et al. 2014; Elkhoudary et al. 2016; Nidal et al. 2017). No effective method for the simultaneous determination of the combination of AMO, MET and OME has been developed. The aim of this study is to develop and validate a HPLC-UV method. It can be applied for quantitative and qualitative control and drug monitoring.

Materials and methods

List of abbreviations

AMO Amoxicillin Trihydrate;
CLR Clarithromycin;
DNA Deoxyribonucleic acid;
IUPAC International Union of Pure and Applied Chemistry;
OME Omeprazole;
LAN lansoprazole;
MET Metronidazole;
N acetylglucosamine residues (αGlcNAc);
TD tinidazole;
UV/Vis Ultraviolet-visible spectroscopy.

UV/VIS spectral analysis of Amoxicillin Trihydrate (AMO), Metronidazole (MET) and Omeprazole (OME)

For spectral evaluation of the target drugs UV-Vis Hewlett Packard 8452 Spectrophotometer was used. Accurately weighed amounts of the analyzed substances were dissolved in methanol and scanned in the wavelength range of 200–400 nm.

Chromatographic conditions

All chemicals and reagents used are of chromatographic purity. Reference standards were obtained from Sigma Aldrich. Tablet form containing omeprazole 20 mg, amoxicillin 500 mg and metronidazole 250 mg are commercially available. A Shimadzu HPLC system was used, consisting of the following components: LC –20AD pump, vacuum degasser DGU – 20A5 and UV/VIS variable detector SPD – 20A. The separation was carried out on a LiChrosorbRP-18 column (250 × 4.6 mm, particle size 5 µm) under reversed phase chromatographic distribution conditions. The mobile phase is 40:60% v/v mixture of Acetonitrile: Phosphate buffer (pH=7.6±0.1). The mobile phase was filtered through a 0.45 µm membrane filter and degassed using an ultrasonic bath for about 10 minutes. Sample solutions were also filtered using a 0.45 µm membrane filter. The working conditions are:

- flow rate of 1.0 mL/min
- Column temperature 30 °C
- The injection volume was 20 µL and the total retention time was 8 minutes
- The wavelength is 280 nm.

Preparation of stock and working standard solutions

Standard solutions

Accurately weighed quantityes of 50 mg AMO, 25 mg MET and 20 mg OME were transferred in 100 mL volumetric flasks, dissolved with 50 mL mobile phase and made up with mobile phase having the concentration of 200 µg/mL. From the stock solutions by diluting with mobile phase were prepared series of working standard solutions. Calibration curves at a concentration range of 5 to 40.0 µg/mL for OME, 12.5 to 100 µg/mL for MET and 25 to 200 µg/mL for AMO were prepared.

Sample Solutions

Twenty tablets were accurately weighed (to obtain the average mass of tablets) and finally powdered. Quantity equivalent to 500 mg AMO, 250 mg MET and 20 mg OME was taken and transferred into 50 mL volumetric flasks. Approximately 30 mL of the diluent (mobile phase) were added, and the mixture was sonicated for 5 minutes. The mixtures were then diluted to the volume with mobile phase and filtered off through a 0.45 µm filter discarding the first few mL of the filtrate. The working solutions were prepared from the filtrates at 20, 50 and 100 µg/mL for OME, MET and AMO, respectively.

Preparation of synthetic mixture

A bulk mixture of three drugs was prepared using 40 mg OME, 500 mg MET and 1000 mg AMO. Common excipients which are used in tablet formulation were added in this laboratory mixture, triturated well, and weighed. The powder equivalent to 20 mg OME, 250 mg MET and 500 mg AMO was weighed accurately and transferred into 50 mL volumetric flask. Then 30 mL of mobile phase was added and sonicated for 5 minutes to effect complete dissolution of the three substances. The suspension was made up to volume with mobile phase. The stock solution was filtered through a 0.45 µm Nylon syringe filter. The aliquot portion of the filtrate was further diluted to get final concentration of 20 µg/mL of OME, 50 µg/mL of MET and 100 µg/mL of AMO. 20 µL of the test solution were injected, chromatogram was recorded, and the amounts of the drugs were calculated.

Results and discussion

UV/VIS spectral analysis of Amoxicillin Trihydrate (AMO), Metronidazole (MET) and Omeprazole (OME)

UV/VIS spectroscopy is a well-known fast method, often applied for identification and regularly for quantitation of analytes in various samples. In addition, this technique is known as a good detection method in combinations with chromatographical systems.

In order to determine the appropriate wavelength for analysis and detection standard solutions of Amoxicillin Trihydrate (AMO), Metronidazole (MET) and Omeprazole (OME) were scanned at a wavelength range of 200–400 nm using UV-Vis Hewlett Packard 8452 Spectrophotometer. The determined wavelength (λmax) of 280 nm was found to be most appropriate. Thus, all further evaluations were performed at this wavelength.

It was of interest to determine whether this method is applicable for quantitation of Amoxicillin Trihydrate, Metronidazole and Omeprazole in drug products. The first step for this was to validate the used UV/VIS methodology.

Validation procedure

The applied UV/VIS method was tested with respect to the validation parameters enlisted in the ICH Q2 (R1) guidelines (ICH), in means of precision, linearity, accuracy and selectivity, following the below presented criteria:

Precision

One of the parameters of the validation precision was determined by analyzing six independent solutions of the tested at a wavelength of 205 nm. The final results are presented in Table 1.

Validation procedure

The applied UV/VIS method of analysis was tested against the validation parameters included in the guidelines (ICH). Accuracy was determined experimentally after analyzing six independent solutions of the combination of three medicinal products in a mixture, at a shaft length of 280 nm. The final results are presented in Table 1. Validation parameters for UV/VIS spectroscopy of amoxicillin Trihydrate, Metronidazole and Omeprazole.

At different concentration levels of 25, 50, 75, 100, 125 and 150 μg/mL are prepared two solutions for each evaluated drug (AMO, MET, OME). Three measurements were performed for each sample, assuring a total of six measurements for each drug. The corresponding relative standard deviation (RSD) is calculated for each sample. The obtained results are enlisted in Table 1.

Table 1. Validation parameters for UV/VIS spectroscopy of Amoxicillin Trihydrate, Metronidazole and Omeprazole in a mixture.

	Mixture of AMO, MET, OME	Criterion
Repeatability (% RSD)*	0.46	$X < 1\%$
Precision (% RSD)#	3.0	$X < 5\%$
Linearity (correlation coefficient)§	0.9899	R > 0.9898
Accuracy (%)#	100.5	$X = 100 \pm 5\%$
Selectivity	No interference	No interference

*Six measurements.
Two samples, three measurements of each solution.
§ At 25, 50, 75, 100, 125 and 150 μg/mL concentration level.
% RSD: Relative standard deviation in %.

Linearity was determined over a range of 25 to 150 μg/mL for a standard mixture of amoxicillin Trihydrate, Metronidazole and Omeprazole in a mixture.

The calibration curve was created using 6 points covering 6 different concentrations of the test compounds in the specified concentration range. Linear regression was used to process the calibration data. At the next accuracy metric. Solutions were prepared using a placebo solution and a stock solution of the test drugs. Each solution was measured on a UV/VIS spectrometer three times at 280 nm. Next validation parameter is Precision. It is presented in Table 1.

From the experiments performed, the method was found to be accurate with a reproducibility of 99.95%–100.5%. The selectivity was determined by comparing the UV/VIS spectra of the solution of standard substances from investigated medicinal products with solvent solutions used alone and in a mixture. No influence of the solvent present was observed, indicating that the method is selective and can be used for further analysis. The validated UV/VIS method was applied for the preliminary identification of a mixture of Amoxicillin Trihydrate, Metronidazole and Omeprazole.

Selectivity: The selectivity is determined by comparing the UV/VIS spectra of the solution of standard substances from the investigated medicinal products with the solutions of the solvent used alone and in a mixture. No influence of the present solvent was observed, indicating that the method is selective and can be used for further analysis.

The results from the evaluations were not conclusive enough, which determined the necessity for development of more appropriate method for analysis. It is a well-known fact that chromatography is preferred in this situation. Thus, this defined our next analytical processing.

All of the analytical validation parameters for this proposed method were determined according to ICH guidelines (Goddard et al. 2003) as follows.

Method development

The literature discussed shows that most of the applied analytical methods for the analysis of Amoxicillin Trihydrate, Metronidazole and Omeprazole in a mixture require either specific stationary phase conditions or the use of gradient eluent flows, and that no information is available for analysis in a mixture (Hsu et al. 1992; Daniel et al. 2005; Baraka et al. 2014; Kathriarachchi et al. 2018). This directed our attention to simplifying and improving the applicability of the chromatographic system by developing a new and rapid PR-HPLC detection method for the identification and quantification of Amoxicillin Trihydrate, Metronidazole and Omeprazole in mixture. Various combinations of methanol, acetonitrile, deionized water, pH of the mobile phase, concentration of the acid or buffer solution, percentage, and type of organic modifier, etc., were tested to optimize the mobile phase for good chromatographic separation.

The interference of the available solvents in the UV-Vis evaluations was investigated by changes in their ratio in the corresponding applied mobile phases. The initial combination of mobile phase components containing methanol and deionized water was determined to be unsuitable due to the influence of this reagent in the spectra at the most suitable wavelength of 280 nm. Therefore, based on the experiments, the most suitable mobile phase was established, which is acetonitrile: phosphate buffer (pH=7.6±0.1) (40:60 v/v). The flow rate was 1.0 mL/min with detection at 280 nm.

Furthermore, the selection of column, temperature and flow rate was performed based on literature data (Mainz et al. 2002; Ghante et al. 2012; Elkhoudary et al. 2016) and some additional experimental results obtained by us as the most suitable column is LiChrosorbRP-18 (250 × 4.6 mm, particle size 5 μm) under reversed-phase partition chromatography conditions. The mobile phase was a 40:60% v/v mixture of acetonitrile: phosphate buffer (pH=7.6±0.1).

The mobile phase was filtered through a 0.45 µm membrane filter and degassed using an ultrasonic bath for about 10 min before use. Sample solutions were also filtered using 0.45 µm membrane filters. Operating conditions are at flow rate 1.0 mL/min, column temperature 30 °C, shaft length at 280 nm.

A standard solution was injected into the introduced HPLC system with a concentration of 100 µg/mL. The modified method was subjected to validation as required by the ICH Q2 (R1) guidelines (ICH 2005).

System suitability

From the experiments performed, a complete separation of AMO, MET and OME was found as shown in Fig. 1. The peaks obtained for AMO, MET and OME are sharp and have a clear baseline separation. The retention time is good for drug separation and there is no overlap between peaks obtained from resolution data indicating an accurate system.

Table 2. Linearity of Amoxicillin, Metronidazole and Omeprazole.

Compounds	AMO	MET	OME
Concentration range, (µg/mL)	25–200	12.5–100	5–40
Slope	3684.0	20016.6	29016.3
Intercept	21117.1	21609.7	19821.5
Correlation coefficient, (r)	0.999_5	0.999_5	0.998_4

*Average of three determinations.

Linearity

Five different concentrations of AMO, (25, 50, 100, 150 and 200 µg/mL), MET (12.5, 25, 50, 75 and 100 µg/mL) and OME (5, 10, 20, 30 and 40 µg/mL) to evaluate the linearity and range of the method were prepared. Three injections from each concentration were analyzed under the same conditions. Table 2 presents the regression equations, correlation coefficients (r^2), values of the slope and intercept for each compound between the peak areas and concentrations of 25–200 µg/mL with $r^2=0.999_5$ for AMO, 12.5–

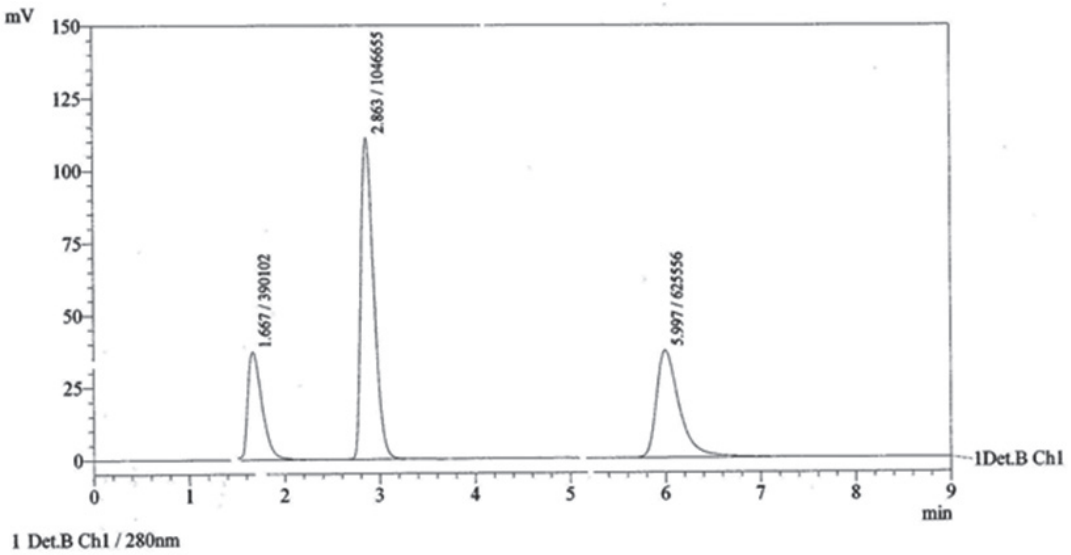

Figure 1. Chromatogram of Amoxicillin, Metronidazole and Omeprazole.

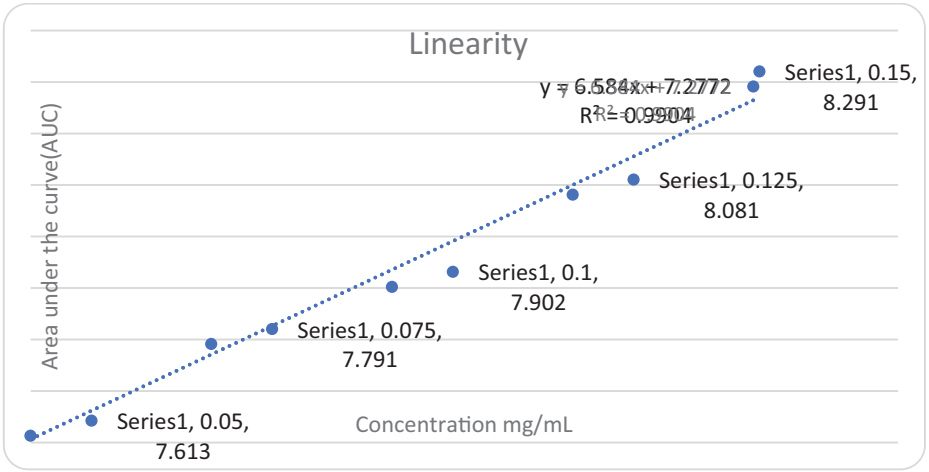

Figure 2. Linearity of Amoxicillin, Metronidazole and Omeprazole, in Acetonitrile: Phosphate buffer (pH=7.6±0.1) (40:60 v/v).

100 μg/mL with r^2=0.999$_5$ for MET and 5–40 μg/mL with r^2=0.998$_4$ for OME. The experiment showed good linearity of the method for all three analyzed substances (Fig. 2).

Accuracy and precision

The accuracy and precision of the method were performed at three concentrations of the sample solutions (50%, 100% and 150%) by nine determinations (three replicates of each concentration). The percentage recovery and relative standard deviation (RSD) were calculated for each of the replicate samples. The results of accuracy and precision testing showed that the method is accurate and precise within the acceptable limits. The percentage recovery and RSD were calculated for the three active ingredients AMO, MET and OME, and all the results were within the limits. The acceptable accuracy was within the range of 98.0% to 102.0% recovery, and the precision of the RSD% was not more than 2.0%, as demonstrated in Tables 3, 4, 5.

Table 3. Chromatographic system suitability test for Amoxicillin, Metronidazole and Omeprazole.

Parameter	AMO	MET	OME
Retention time, (min)	1.67	2.86	5.99
Tailing factor	1.20	1.22	2.01
Theoretical plates	2779	5249	4194
Selectivity factor	0.00	4.55	9.23
Resolution factor	1.68	1.42	1.57
Retention factor	0.44	1.57	1.85

Limit of detection and limit of quantification

The limit of detection is the lowest amount of analyte in a sample that can be detected, but not necessarily quantitated. It may be expressed as a concentration that gives a signal-to-noise ratio of approximately 3:1. The limit of quantification is the lowest amount of analyte in a sample that can be quantitatively determined with suitable accuracy and precision, with a signal-to-noise ratio of approximately 10:1. The method showed LOQs of 0.005, 0.003 and 0.004 μg/mL for AMO, MET and OME respectively; and LODs of 0.05, 0.03 and 0.042 μg/mL for AMO, MET and OME respectively.

Robustness

Some modifications were made to the analysis by slightly varying the mobile phase flow rate, column temperature and mobile phase composition. A mixture of AMO, MET, and OME at a concentration of 100 μg/mL, 20 μg/mL, and 50 μg/mL, respectively, were assayed. The results showed that there were no obvious changes in the chromatograms, indicating that the developed method was stable.

Discussion

Some preliminary analysis on the influence of a number of important parameters like pH of the mobile phase, the concentration of the acid or buffer solution, the percentage content and type of organic modifier, etc. were tested for good chromatographic separation in our laboratory. Analysis has shown this gives symmetrical and sharp peaks. To optimize the RP-HPLC parameters, several mobile phase combinations were studied. Resolution is the most important criteria for the method and is imperative to achieve good resolution among the both compounds. As per the value of K_a and solubility of both the compounds, various compositions of mobile phase with different pH ranges (2.75 to 8.0) were studied and the best resolution was obtained with mobile phase consisting of Acetonitrile:Phosphate buffer (pH=7.6±0.1) solution in the proportion of (40:60 v/v). The flow rate was also studied at 0.7; 1.0 and 1.5 mL/min. The flow rate at 1mL/min proved to be better than the other two in terms of resolution and peak shape. The optimum wavelength for detection was set at 280 nm at which much better detector responses for the three drugs were obtained. As shown in Fig. 1, the retention times were 1.67 min for AMO, 2.86 min for MET and 5.99 min for OME. The optimal regression characteristics and validation parameters are showed in Table 3. The method was simple and had short run time of 7 min, which makes the method rapid. The result of the study indicates that the proposed HPLC method is simple, precise, accurate and less time consuming. The method was validated in terms of specificity, linearity, accuracy, precision and limits of detection and quantitation according to ICH guidelines (ICH Guideline 2005).

Table 4. Accuracy results of Amoxicillin, Metronidazole and Omeprazole.

Omeprazole		Metronidazole		Amoxicillin	
Amount taken (mg/tablet)	Amount found (mg/tablet)	Amount taken (mg/tablet)	Amount found (mg/tablet)	Amount taken (mg/tablet)	Amount found (mg/tablet)
20.00	20.40	250.0	249.3	500.0	500.9
	20.60		249.4		499.8
	19.80		250.1		499.6
	20.10		250.4		500.4
	19.70		249.5		498.9
	19.90		249.2		499.3
Mean	20.08	Mean	249.6	Mean	499.1
Standard deviation (±SD)	0.354	Standard deviation (±SD)	0.485	Standard deviation (±SD)	0.731
Relative Standard deviation (±RSD%)	1.765	Relative Standard deviation (±RSD%)	0.194	Relative Standard deviation (±RSD%)	0.146

Table 5. Precision results of Amoxicillin, Metronidazole and Omeprazole.

Sample	%, Taken	Amount taken, mg	Amount found, mg	Assay, % found	±SD	±RSD, (%)
Omeprazole*	50%	10.0	9.96 20.2	99.60	0.452	1.612
	100%	20.0	29.8	101.0	0.324	1.332
	150%	30.0		99.33	0.303	1.224
Metronidazole*	50%	125.0	124.6	99.68	0.395	0.159
	100%	250.0	249.3	99.72	0.482	0.181
	150%	375.0	374.8	99.95	0.352	0.194
Amoxicillin*	50%	250.0	249.6	99.84	0.724	0.131
	100%	500.0	449.3	89.86	0.743	0.145
	150%	750.0	750.6	100.1	0.781	0.154

*Average of three determinations.

Conclusion

A rapid, simple, inexpensive and validated UV/VIS spectrometric method was developed for the evaluation of Amoxicillin, Metronidazole and Omeprazole in a mixture. An HPLC method with UV detection was developed and validated for the routine quality control analysis of three medicinal products, alone and in mixture, used in the therapy of *H. pylori* and its co-morbidities AMO, MET and OME. The proposed method is very fast, cheap, and easily accessible. The method showed stability, at different analytical conditions. And it meets all validation criteria.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

References

Al-Abachi Q, Hind H, Anas M (2005) Spectrophotometric determination of amoxicillin by reaction with N,N-dimethyl-p-phenylenediamine and potassium hexacyanoferrate(III). Analytica Chimica Acta 554(1–2): 184–189. https://doi.org/10.1016/j.aca.2005.08.030

Anusha N, Kamath UB (2014) Spectrophotometric determination of Amoxycillin in different formulations. Asian Journal of Scientific Research 7(3): 335–340. https://doi.org/10.3923/ajsr.2014.335.340

Baraka MM, El-Sadek ME, Lobna M, Aziz A, El-Bermawi SS (2014) Spectrophotometric determination of Omeprazole sodium and Ranitidine hydrochloride using 2,4-dinitrofluorobenzene and 4-chloro-7-nitrobenzen-2-oxa-1, 3-diazole accompanied with a kinetic study. American Journal of Analytical Chemistry 1(1): 1–23.

Baraka M, Elsadek M, Ibrahim A (2014) HPLC method for the simultaneous determination of Secnidazole, Omeprazole and Amoxicillin mixture in pure forms and pharmaceutical formulations. Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry 2(4): 197–207.

Bayerdörffer E, Lind T, Díte P, Bardhan KD, O'Morain C, Delchier JC, Spiller R, Veldhuyzen van Zanten S, Sipponen P, Mégraud F, Zeijlon L (1999) Omeprazole, Amoxycillin and Metronidazole for the cure of helicobacter pylori infection. European Journal of Gastroenterology & Hepatology 2: 19–22. https://doi.org/10.1097/00042737-199908002-00004

Belal F, Mel-Kerdawy M, El-Ashry SM, El-Wasseef DR (2000) Kinetic spectrophotometric determination of ampicillin and amoxicillin in dosage forms. Il Farmaco 55(11–12): 680–686. https://doi.org/10.1016/S0014-827X(00)00080-X

Bhandage A, Ashok B, Ashok K, Godse VP (2009) Extractive spectrophotometric determination of Omeprazole in pharmaceutical preparations. Tropical Journal of Pharmaceutical Research 8(5): 449–454. https://doi.org/10.4314/tjpr.v8i5.48089

Bojaraju V, Sireesha D, Prasad VVLN, Diwan PV (2012) Reverse phase high performance liquid chromatography method for the simultaneous estimation of Amoxicillin trihydrate and tinidazole in the tablet dosage form. World Journal of Chemistry 7(2): 47–52.

Chaudhry A, Malik T, Ashfaq K, Shafiq M, Aslam M, Akhtar M (2012) A simple HPLC method for the determination of Omeprazole *in vitro*. International Journal of Pharmacy and Chemistry 2(4): 126–128. https://doi.org/10.7439/ijpc.v2i4.605

Daharwal SJ, Saraf S (2007) Spectrophotometric methods for the simultaneous estimation of amoxycillin and tinidazole in tablet dosage form. Indian Journal of Pharmaceutical Education and Research 41(1): 35–41.

Daniel K, Bempong G, Manning RG, Mirza T, Bhattacharyya L (2005) A stability-indicating HPLC assay for Metronidazole benzoate. Journal of Pharmaceutical and Biomedical Analysis 38: 776–780. https://doi.org/10.1016/j.jpba.2005.02.019

Douša M, Hosmanová R (2005) Rapid determination of amoxicillin in premixes by HPLC. Journal of Pharmaceutical and Biomedical Analysis 37(2): 373–377. https://doi.org/10.1016/j.jpba.2004.10.010

Elkhoudary MM, Abdel Salam RA, Hadad GM (2016) Development and optimization of HPLC analysis of Metronidazole, Diloxanide, Spiramycin and Cliquinol in pharmaceutical dosage forms using experimental design. Journal of Chromatographic Science 54(10): 1701–1712. https://doi.org/10.1093/chromsci/bmw126

Ghante H, Pannu K, Amruta L, Tejashree S (2012) Development and validation of a RP- HPLC method for simultaneous estimation of Metronidazole and Norfloxacin in bulk and tablet dosage form. International Journal of Pharmacy and Pharmaceutical Sciences 4(4): 241–245.

Graham DY, Hong L, Shiotani A (2017) Failure of optimized dual proton pump inhibitor amoxicillin therapy: What now? Saudi Journal of Gastroenterology 23(5): 265–267. https://doi.org/10.4103/sjg.SJG_292_17

Ghandour MA, Hassan A, Ali HM (2014) Electrochemical behaviour of Omeprazole interacting with mycin derivatives and doxycycline. International Journal of Pharmaceutical Science and Research 5(12): 5183–5190.

Goddard AF, Logan RPH (1992) Diagnostic methods for *Helicobacter pylori* detection and eradication. British Journal of Clinical Pharmacology 56(3): 273–283. https://doi.org/10.1046/j.1365-2125.2003.01941.x

Hsu MC, Hsu PW (1992) High-performance liquid chromatographic method for potency determination of amoxicillin in commercial preparations and for stability studies. Antimicrob Agents Chemother 36(6): 1276–1279. https://doi.org/10.1128/AAC.36.6.1276

ICH Guideline (2005) Harmonised Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2(R1).

Kasiri KA, Khoshdel A, Karimi A, Sedehi M, Kasiri N (2017) Comparison of amoxicillin and metronidazole effect on three-drug regimen for the treatment of *Helicobacter pylori* infection in children. Journal of Advanced Pharmaceutical Technology & Research 8(2): 63–66. https://doi.org/10.4103/jphi.JPHI_5_19

Kathriarachchi UL, Vidhate SS, Al-Tannak N, Thomson AH, da Silva Neto MJJ, Watson DG (2018) Development of a LC-MS method for simultaneous determination of amoxicillin and metronidazole in human serum using hydrophilic interaction chromatography (HILIC). Journal of Chromatography B 1089: 78–83. https://doi.org/10.1016/j.jchromb.2018.05.012

Kusters JG, van Vliet AH, Kuipers EJ (2006) Pathogenesis of *Helicobacter pylori* infection. Clinical Microbiology Reviews 19(3): 449–490. https://doi.org/10.1128/CMR.00054-05

Maher HM, Youssef RM, Khalil RH (2008) Simultaneous multiresidue determination of Metronidazole and Spiramycin in fish muscle using high performance liquid chromatography with UV detection. Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences 876(2): 175–181. https://doi.org/10.1016/j.jchromb.2008.10.033

Mainz D, Borner K, Koeppe P, Kotwas J, Lode H (2002) Pharmacokinetics of lansoprazole, amoxicillin and clarithromycin after simultaneous and single administration. Journal of Antimicrobial Chemotherapy 50: 699–706. https://doi.org/10.1093/jac/dkf172

Mishra AK, Kumar A, Mishra A, Mishra HV (2014) Development of ultraviolet spectroscopic method for the estimation of metronidazole benzoate from pharmaceutical formulation. Journal of Natural Science, Biology and Medicine 5(2): 261–264. https://doi.org/10.4103/0976-9668.136154

Müller M (1983) Mode of action of metronidazole on anaerobic bacteria and protozoa. Surgery 93(1): 165–171.

Naveed S, Qamar F (2014) Simple UV spectrophotometric assay of Metronidazole. Open Access Library Journal 1(6): 1–4. https://doi.org/10.4236/oalib.1100615

Nataraj K, Duza M, Pragallapati K, Kumar D (2012) Development and validation of RP-HPLC method for the estimation of omeprazole in bulk and capsule dosage forms. International Journal of Current Pharmaceutical Research 1(11): 366–369. https://doi.org/10.3329/icpj.v1i11.12062

Nidal B, Shorouq W, Al-Rimaw FA (2017) A validated stability-indicating HPLC method for simultaneous determination of Amoxicillin and enrofloxacin combination in an injectable suspension. Scientia Pharmaceutica 85(1): 6–12. https://doi.org/10.3390/scipharm85010006

Puig I, Baylina M, Sánchez-Delgado J, López-Gongora S, Suarez D, García-Iglesias P, Muñoz N, Gisbert JP, Dacoll C, Cohen H, Calvet X (2016) Systematic review and meta-analysis: triple therapy combining a proton-pump inhibitor, amoxicillin and metronidazole for *Helicobacter pylori* first-line treatment. Journal of Antimicrobial Chemotherapy 71: 2740–2753. https://doi.org/10.1093/jac/dkw220

Siddappa K, Mallikarjun M, Reddy PT, Tambe M (2008) Spectrophotometric determination of metronidazole through Schiff's base system using vanillin and PDAB reagents in pharmaceutical preparations. Ecletica Quimica 33(4): 41–46. https://doi.org/10.26850/1678-4618eqj.v33.4.2008.p41-46