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(Mini review)

Mini Review on different analytical approaches applied on some selected GIT acting combination

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ABSTRACT

In order to encourage and facilitate collecting literature review for other researchers who will have studies related to the presented compounds, the following article was introduced by presenting a mini-review of the different methods applied for quantitative determination of some drugs affecting GIT namely, Lansoprazole (LAN) co-formulated with Clarithromycin (CLR) and Tinidazole (TD), and Phloroglucinol (PHG) and Trimethylphloroglycinol (TMG) combination, in their drug mixture forms using different analytical techniques. *Helicobacter pylori* (H. Pylori) is a fairly prevalent infection that disrupts the stomach, causing several annoyances signs such as chronic gastritis, peptic ulcers, and gastric cancer. Lately, it has been shown that COVID-19 patients' gastrointestinal symptoms are negatively impacted by H. Pylori infection. An effective triple therapy regimen, which typically includes antibiotics and stomach protection, is required to eradicate this bacterial infection. This study discussed the pharmacopeia methods applied for the studied drugs in addition to different techniques which were used for simultaneous determination of the presented compounds in their mixture formulations; including spectrophotometric (UV and visible), electrochemical, in addition to chromatographic (TLC and HPLC) methods either in a biological fluid or in their pharmaceutical dosage forms. All the discussed approaches in this review were applied and

validated following the ICH guidelines. Some of these methods were accomplished according to the green chemistry and evaluated with the green assessment approaches.

Keywords: Clarithromycin; Tinidazole; Lansoprazole; Phloroglucinol; Trimethyl phloroglucinol

1. Introduction

The gastrointestinal tract (GIT) is considered a part of the digestive system which acts as a passageway for the digestive system. GIT is a vital system in the conservation of health in man and animals. It provides a protective barrier with consecutive functions for the uptake of nutrients. Diseases of the GIT, discomfort, and other symptoms related to GIT are among the most common patient complaints. Often, they are of no serious medical significance. Sometimes, however, they may be the first signs of serious illness. The GIT is a long tube surrounded by smooth muscle extending from the mouth to the anus. (1) Its main function is the digestion and absorption of nutrients, water, and electrolytes, as well as the elimination of waste (2). GIT diseases may occur at any point, and may be caused by infections, immunological diseases, cancer, or disturbances in motility (the regular muscle contraction of the small intestine), and may include symptoms such as constipation or diarrhea (3,4). Different diseases may affect GIT as Gastroesophageal reflux disease GERD, ulcers, abdominal pain, constipation, diarrhea, acidity, Irritable Bowel Syndrome (IBS), and spasm (5,6). Some viruses and bacteria may invade GIT like Helicobacter *Pylori*, salmonella, and other infections leading to various diseases (7–9).

GIT drugs are classified into several groups:

Antacids e.g. Aluminium hydroxide.

Antidiarrhoeals e.g. Diphenoxylate.

Antiemetics e.g. Metoclopramide.

Antisecretory drugs may generally be divided into (10–12) **:**

Histamine- H_2 receptor -antagonists (H2 – antagonists) e.g. Roxatidine.

Proton pump inhibitor e.g. lansoprazole and omeprazole.

Antiemetic e.g. Metoclopramide.

Prostaglandin analogs e.g. Misoprostol.

Antispasmodic e.g. Tiemonium, Phloroglucinol, and Trimethylphloroglucinol (13,14).

Laxatives e.g. Lactulose.

Mucosal protectants e.g. Sucralfate.

Prokinetic drugs e.g. Itopride.

Helicobacter Pylori eradication treatment includes **:**

- Antimicrobials e.g. Amoxicillin, Metronidazole, and Clarithromycin (15,16).
- Antiprotozoal e.g. Tinidazole and Metronidazole (17–19).
- Proton pump inhibitors (PPIs) as Lansoprazole, pantoprazole and omeprazole (20).

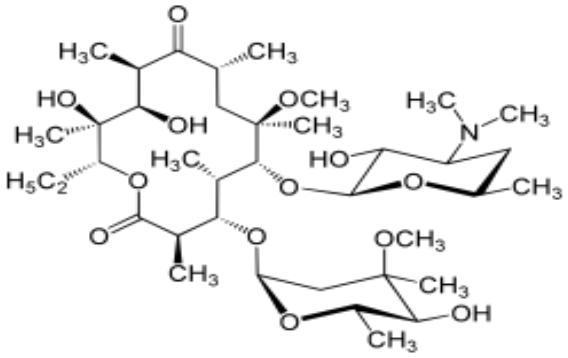
2. Selected drugs in this study

2.1. Mixture of Clarithromycin, Tinidazole, and Lansoprazole

2.1.1. Chemistry and physical properties

2.1.1. a. Clarithromycin (CLR)

Clarithromycin which is considered an active cure used to control most of the complications related to skin or tissue and respiratory system (21). Moreover, by inhibiting the process of protein synthesis, it also aids in the treatment of H. Pylori and stomach ulcer disease. (22).



Chemistry (21)

Chemical name: Ether of erythromycin A in 6-O-methyl.

Molecular formula: $C_{38}H_{69}NO_{13}$.

Molecular weight: 748 g/mol.

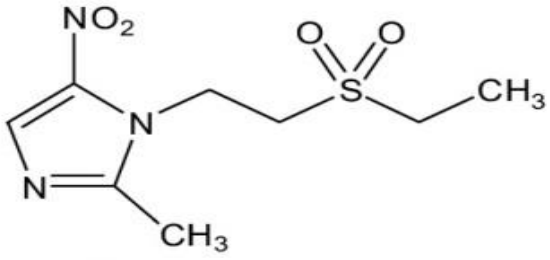
Physical properties (21)

Description**:** Crystalline powder that is white or almost white.

Solubility: In water, it is practically insoluble, acetone and methylene chloride are soluble, and methanol is slightly soluble.

2.1.1.b. Tinidazole (TD)

Tinidazole which is act as both antiprotozoal and antibacterial (21). It is usually used to treat trichomoniasis, vaginitis, giardiasis, amebiasis, and peptic ulcer associated with H. Pylori infection (23).



Chemistry (21)

Chemical name: 1-[2-ethylsulphonyl) ethyl]-2-methyl-5-nitroimidazole.

Molecular formula: $C_8H_{13}N_3O_4S$.

Molecular weight: 247.27 g/mol.

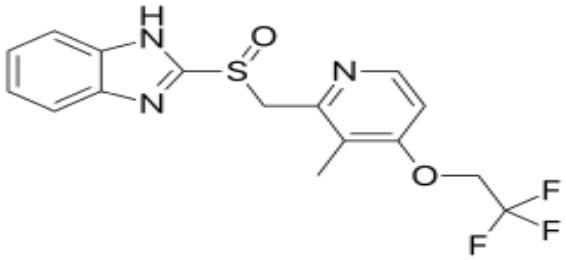
Physical properties (21)

Description: a crystalline powder that is white or whitish yellow.

Solubility: Sparingly soluble in methanol, acetone, and methylene chloride. Insoluble in water.

2.1.1. c. Lansoprazole (LAN)

Lansoprazole is a member of the pyridine family (24). It reduces stomach acidity by functioning as a proton pump inhibitor (PPI). Moreover, it is used to treat peptic ulcer disease and gastroesophageal reflux illness. (25,26).



Chemistry (21)

Chemical name: 2-([3-Methyl-4-(2,2,2 trifluoroethoxy)-2-pyridinyl]methylsulfinyl) -1H-benzimidazole.

Molecular formula: $C_{16}H_{14}F_3N_3O_2S$

Molecular weight: 368.37g/mol

Physical properties (21)

Description: Powder that is white or brownish in color.

Solubility: Water is practically insoluble, anhydrous ethanol is soluble, and acetonitrile is only somewhat soluble.

2.1.2. Pharmacological uses of the studied drugs in their mixture form.

Tinidazole (TD) is a kind of antibiotic used to treat specific types of vaginal infections (bacterial vaginosis, trichomoniasis). Moreover, several types of parasite infections are treated with it (giardiasis, amebiasis). It functions by preventing the development of specific bacteria and parasites. in addition to treating stomach ulcers appeared with H. Pylori infection (23). Clarithromycin (CLR) is an antibiotic used to treat many bacterial illnesses which includes strep throat, pneumonia, skin infections, and H. pylori infection [9,10]. Recently, CLR has had a clear role in the treatment of the CO VI d 19 protocol. (29,30). LAN (lansoprazole) is a drug that lowers stomach acid. It is used to treat peptic ulcer disease, gastroesophageal reflux disease leading to prevent stomach and intestinal ulcers (25,26).

Helicobacter pylori (H. Pylori) is a fairly prevalent infection that disrupts the stomach, causing several annoyances signs such as chronic gastritis, peptic ulcers, and gastric cancer. (7). Lately, it has been shown that COVID-19 patients' gastrointestinal symptoms are negatively impacted by H. Pylori infection. (31–33). An effective triple therapy regimen, which typically includes antibiotics and stomach protection, is required to eradicate this bacterial infection. (34). This combined therapy lessens symptoms while allowing inflammatory tissue to recover(20).

Antibiotic resistance is a problem with this bacteria that can be resolved by using triple therapy (20,34). To avoid some side effects, such as osteoporosis, after numerous trials with triple therapy, replacing metronidazole with Tinidazole and omeprazole with lansoprazole proved much preferable (26). As a result, utilizing TD, CLR, and LAN in combination produces effective H. Pylori eradication results. The formula is offered as kits, each of which includes a dosage form for each drug separately. The three medications are administered twice daily for a one-week regimen in accordance with European standards.

Patients may experience toxicity or even therapeutic inefficacy as a result of multi- and add-on medicines' complex and unanticipated interactions. As a result, therapeutic drug monitoring (TDM) is frequently necessary to decrease toxicity, boost clinical efficacy, and prevent the development of antimicrobial resistance. For the greatest conceivable patient benefit, this

monitoring tries to maintain medication levels in plasma or blood within a specific therapeutic range(35,36).

2.1.3. Methods of analysis

2.1.3.a. Pharmacopeial methods

Clarithromycin

British Pharmacopoeia describes a liquid chromatographic technique for the assay of CLR in pure form using Nucleosil C_{18} (15 cm, 4.6 mm), and potassium dihydrogen orthophosphate (pH=3.5)-methanol (40:60, v/v) as mobile phase. UV detection was achieved at 210 nm (21).

United States Pharmacopeia describes an HPLC technique for the assay of CLR in pure form using a mixture of methanol and monobasic potassium phosphate in a ratio of 650:350, v/v, pH 4.0 using C_{18} column (15 cm, 4.6 mm) and detection at 210 nm (37).

Tinidazole

British Pharmacopoeia introduces a titrimetric way for assay TD in its pure form was established, using anhydrous acetic acid as a solvent, 0.1 M perchloric acid as a titrant, and the end-point is assayed potentiometrically (21).

United States Pharmacopeia presents a titrimetric method for the determination of TD in its pure form *via* employing 0.1 N perchloric acid as a titrant and glacial acetic acid as the solvent. The endpoint is determined potentiometrically with a suitable electrode (37).

Lansoprazole

British Pharmacopoeia describes a titrimetric method for the determination of LAN in its pure form by dissolving the powder in ethanol (96%) and diluted with water, then titrating it with 0.1 M sodium hydroxide to determine the end-point with potentiometric method (21).

United States Pharmacopeia, presents a chromatographic HPLC method using water-acetonitrile- trimethylamine (60:40:1, by volume), pH 7.0, and C_{18} column (150 cm, 4.6 mm) with UV determination at 285.0 nm (37).

2.1.3. b. Reported methods

Chromatographic methods

Thin layer chromatographic method

High-performance thin-layer chromatography silica gel 60 F254 plates were used as the stationary phase in an effective chromatographic separation, and the developing system was

composed of ethanol, acetone, and ammonia (10.0:4.0:0.1, by volume). At 210 nm, the bands' scanning was completed. The proposed approach has been extensively verified and is appropriate to use in routine quality control of the tested pharmaceuticals in their dosage form, with average percent recoveries of 99.97± 0.62, 100.89± 0.51, and 99.78± 0.56% for Clarithromycin, Tinidazole, and Lansoprazole, correspondingly (38).

HPLC chromatographic methods

There are some chromatographic methods which are found for simultaneous determination of TD, CLR and LAN in their therapeutic formulation with different detection modes, these methods are summarized in **Table 1.**

Table 1: Summary of separation HPLC methods described in the literature for determination of Clarithromycin, Tinidazole, and Lansoprazole.

Column	Mobile Phase	UV Detection at	Application
C_{18} column (250×4.0 mm, 5 µm)	Acetonitrile-0.001M phosphate buffer (35:65, v/v), pH 7.8.	**275 nm.**	**synthetic mixture (39).**
C_{18} column, (150 ×4.6 mm, 5 µm)	Orthophosphoric acid buffer-acetonitrile in gradient elution.	**210 nm.**	**Pharmaceutical formulation (40).**
Ultisil XB-CN, column (250× 4.6 mm, 5 µm)	Potassium dihydrogen phosphate-acetonitrile in gradient elution.	**210 nm.**	**Pharmaceutical formulation (41).**
CN column (250 × 4.6 mm, 5 µm)	acetonitrile and 10.0 mM phosphate buffer, pH 7.5 ± 0.1	**210.0 nm for CLR and 290.0 nm for TD and LAN.**	**Pharmaceutical formulation, dissolution testing and biological applications(42)**

Electrochemical method

For the determination of TD, CLR, and LAN depending on various pH effects, a sensitive and economical voltammetric approach was created, optimized, and implemented. Prussian blue analog nanoparticles and multi-walled carbon nanotubes were used to enhance the electrode of carbon paste for the investigation (PbA-NPs/MWCNT/CP). Concerning the chosen electrode, X-ray diffraction (XRD) patterns, scanning electron microscopes (SEM), and transmission electron

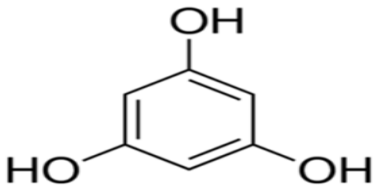
microscopes were used to study the morphology and characterization of the nanoparticle composite (TEM). On the voltammetric performance of the three medicines, the impact of various electrodes and PbA-NP content was examined. After estimating differential pulse voltammetry (DPV), linear correlations were found for LAN, CLR, and TD in the ranges of 0.01-0.9, 1.0-10.0, and 0.5-12.0 g/mL, respectively. In accordance with ICH recommendations, the optimized approach has been verified and determined to be suitable for routine quality control of the investigated pharmaceuticals in their pharmaceutical formulation in addition to spiking human plasma. (43).

2.2. Mixture of Phloroglucinol and Trimethyl phloroglucinol

2.2.1. Chemistry and physical properties

2.2.1.a. Phloroglucinol (PHG)

Phloroglucinol is a spasmolytic agent that helps in the management and relief of GIT spasms and irritable bowel syndrome in addition to other disorders accompanying the spasm of smooth muscle. In addition to its employment in plant culture media, it also serves as a coupling agent in printing, the manufacture of explosives, and pharmaceuticals. (21).



Chemistry (21)

Chemical name: 1, 3, 5- trihydroxy benzene

Molecular formula: $C_6H_3(OH)_3$

Molecular weight: 126.11 g/mol

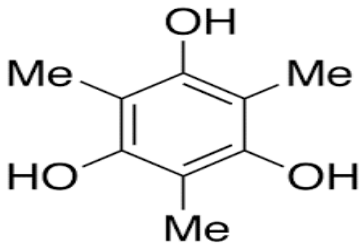
Physical properties (21)

Description: White or nearly white powder.

Solubility: Sparingly soluble in water, freely soluble in ethanol (96 %), almost insoluble in methylene chloride.

2.2.1.b. Trimethyl phloroglucinol (TMG)

Trimethyl phloroglucinol is used to reduce and treat pain strength in patients suffering from irritable bowel syndrome. (13).



Chemistry (44).

Chemical name: 1,3,5-Trimethoxybenzene

Molecular formula: $C_9H_{12}O_3$

Molecular weight: 168.19 g/mol

Physical properties (44)

*Description***:** White or nearly white powder.

*Solubility**:*** Partially soluble in water

2.2.2. Pharmacological uses of the studied drugs in their mixture forms.

Phloroglucinol (PHG), has been used in trials studying the diagnostic of Colonoscopy as a spasmolytic agent to treat colic as well as spastic pain of the digestive and biliary tracts. (21). Following consultation with their general practitioners, trimethyl phloroglucinol (TMG) is co-formulated with PHG for the reduction and treatment of pain intensity in patients with irritable bowel syndrome (13). Irritable Bowel Syndrome (IBS) is a nervous disease that causes unpleasant pain. PHG and TMG are spasmolytic drugs that can help with pain relief and IBS treatment.

2.2.3. Methods of analysis

2.2.3.a. Pharmacopeial method

Phloroglucinol

In British pharmacopoeia, a pure powder is dissolved in 50 mL of purified water, titrate with 1 M sodium hydroxide, and the end-point is determined potentiometrically (21).

2.2.3.b. Reported methods

Spectrophotometric methods

Application to content uniformity testing of advanced spectrophotometric resolution methods for spectrally overlapping spasmolytic binary mixture with 3,5-Dichloroaniline as a hazardous contaminant. To resolve the substantial overlap between the spectra of PHG and TMG in their pharmaceutical and pure forms, as well as to estimate them in the presence of DCL as a PHG hazardous impurity without the necessity for initial separation, many innovative approaches were put forth as a challenge. The univariate methods used in the presented work, including derivative ratio (DR), ratio difference (RD), mean centering (MC), and deconvoluted Fourier method (DF), allowed for the simultaneous determination of PHG and TMG in their binary mixture after DCL was assayed in the zero order, wherever the two drugs give zero absorption at 247.0 nm, and its contribution was removed by using the ratio subtraction method. To assess PHG and TMG simultaneously in the presence of DCL impurity, multivariate chemometric PLS and PCR models were also used. For PHG, TMG, and DCL, respectively, univariate approaches were used in the range of 5.0-30.0, 2.5-25.0, and 1.0-12.0 g/mL. For PHG, TMG, and DCL, respectively, the suggested chemometric models were applied in the ranges of 6.0-14.0, 5.0-25.0, and 2.0-10.0 g/mL. The presented techniques were successful in determining the pharmaceutical dosage forms of the specified medications and determining the consistency of dosage units' contents (45).

Chromatographic methods

Thin layer chromatographic method

The chromatographic HPTLC-densitometric method was applied for the estimation and separation of two spasmolytic compounds; Phloroglucinol (PHG) and Trimethyl phloroglucinol (TMG) in their pure or pharmaceutical formulation and in the presence of toxic impurity; 3,5-Dichloroaniline. For HPTLC, efficient separation was achieved by utilizing HPTLC silica gel 60 F_{254} plates as a stationary phase with a developing system consisting of ethyl acetate: butanol: ammonia in a ratio (8.0:2.0:0.2) v/v/v. Band scanning was developed at 210.0 nm (46)**.**

HPLC chromatographic method

There are some chromatographic techniques were described to estimate PHG and TMG in its formulations and/or biological samples with different detection methods, these methods are summarized in **Table 2.**

Table 2: Summary of separation methods described in the literature for determination of Phloroglucinol and Trimethylphloroglucinol.

Column	Mobile Phase	U.V Detection at	Application
C_{18} column (300×3.9 mm; 10µm)	Acetonitrile-water (1:1, v/v), pH was adjusted to 3 with phosphoric acid	242 nm	**Raw materials, in bulk drugs & formulation (47)**
C_{18} column, (250×4.6 mm; 5 µm)	Methanol:-Heptane sulfonate Buffer-Sulfuric acid(0.1 mol/L) (60:40:0.3, by volume)	234 nm.	**Bulk, pharmaceutical products, and urine (48)**
C_{18} column (250×4.6 mm; 10 µm)	Acetonitrile-Heptane sulfonate Buffer (0.005 mol/L)-sulfuric acid 0.1 M (50:50:0.3, by volume)	Dual wavelength at 266 nm and 205 nm for PHG and TMG,	**Pharmaceutical formulation and human Serum (49)**
XTerra HPLC RP-C_{18} (4.6 × 250 mm, 5 µm)	methanol-10.0 mM phosphate buffer, pH 3.7 ± 0.1 as mobile phase in the ratio of 75.0:25.0, v/v	220.0 nm	**Raw materials, in bulk drugs and formulation(46)**

3. Conclusion

Simple and precise high points for the collected data about the pharmacopeia and the reported analytical methods used for the determination of some selected with each other. This conclusion includes the HPLC technique, spectroscopy, and electrochemical methods which were applied for some GIT-acting compounds that have great effect in some vital diseases such as H.pylori infection. The presented methods were validated according to ICH guidelines in addition to some of them were assessed using various green tools.

- **Conflict of Interest**

The Authors declare no conflict of interest

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