Ibn Al-Haitham Journal for Pure and Applied Sciences
Journal homepage: jih.uobaghdad.edu.iq
PISSN: 1609-4042, EISSN: 2521-3407
IHJPAS. 2025, 38(2)

Spectrophotometric Investigations for Simultaneous Analysis of Certain Antibacterial: A Brief Review

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Received: 5 April 2023 Accepted: 25 July 2023 Published: 20 April 2025
doi.org/10.30526/38.2.3385

Abstract

Antibacterial substances belong to a group of compounds that attack dangerous microorganisms. Therefore, killing bacteria or reducing their metabolic activity will lessen their adverse effects on a biological system. They originated from either synthetic materials, microbes, or mold. Many of these medications treat the gram-negative bacteria from the critical precedence group, such as pseudomonas, carbapenem-resistant acinetobacter, and enterobacterales. This study aims to investigate the simultaneous analysis of specific antibacterial spectrophotometrically. The WHO maintains this list of priority infections with antibiotic resistance. Drug combinations in single dosage forms are becoming increasingly popular in the pharmaceutical industry. This has created a significant issue for pharmaceutical administrators, particularly in combating fake medications and pharmaceutical analysts and developing reliable and accurate methodologies with minimal overlapping effects on quantification. The basics of several spectrophotometric methods utilized to conduct multi-component analysis are collected in the current work, and the validation criteria that are an essential component of the approaches are also described. Numerous analytical techniques, including high-performance liquid chromatography, electrochemical methods, flow injection techniques, gas chromatography, spectrofluorometric techniques, capillary electrophoresis, and spectrophotometric techniques, have been reported to determine antibacterials. This study conducts a concise narrative evaluation of the many spectrophotometric methods that have been published for the simultaneous investigation of Levofloxacin, Sulfamethoxazole, Metronidazole, and Rifampicin in their pure forms, pharmaceutical dosage forms, and biological samples due to their sensitivity, simplicity, and cost-effectiveness.

Keywords: Antibacterial agents, Levofloxacin, Metronidazole, Rifampicin, Spectrophotometric techniques, Sulfamethoxazole.

1. Introduction

Streptomycin was discovered in 1943 by Selman A. Waksman and Albert Schatz. Waksman introduced the term "antibiotic," which refers to a substance created by an organism to eradicate or stop the growth of other species (1). Pathogenic bacteria are those that cause bacterial infections and sickness. When they enter the body, start reproducing, displace beneficial bacteria, or develop into typically sterile tissues, they cause illnesses and ailments. Antibacterial medications, considered the most promising chemotherapeutic agents, have been discovered by researchers to treat infectious diseases (2). Although antibacterials are life-saving medications, improper use can result in antibiotic resistance, a significant public health risk. The rise of antibiotic-resistant bacterial infections is one of the major factors contributing to increased morbidity and mortality, especially in the elderly and individuals with medical conditions (3,4).

Antibacterials, a subclass of antibiotics, have previously been categorized in several ways; however, to make it more straightforward, antibacterial agents can be divided into five categories (5). Based on their mode of action, antibacterials can usually be divided into bacteriostatic (like Sulfamethoxazole) and bactericidal (i.e., Levofloxacin, Metronidazole, and Rifampicin). While bactericidal antibacterials aim to kill germs by attacking their cell wall or membranes, bacteriostatic antibacterials work to slow or prevent bacterial growth (6).

A subclass of antibiotics known as antibacterials can be produced synthetically, chemically altered from naturally existing substances, or spontaneously from fungal sources (7). Classification by activity spectrum is another way of classifying antibiotics or antimicrobials based on target specification. In this category, antibiotics are either narrow-spectrum (Metronidazole) or broad-spectrum (Levofloxacin, Rifampicin, Sulfamethoxazole). Generally, a narrow-spectrum antibiotic is favored over a broad-spectrum antibiotic, which is ideal. This is because narrow-spectrum antibiotics are less likely to result in superinfections than broad-spectrum antibiotics. After all, they do not entirely eradicate the body's usual microbes. Bacteria become less immune as a result (8). A variety of skeletal antibiotics display various curative behaviors. As a result, it is crucial to categorize antibiotics according to their molecular structure. This classification is also vital because comparable structural units share similar toxicity, potency, and other related properties. Antibiotics are typically split structurally into β-lactams (group A) and aminoglycosides (group B). However, β-lactam/β-lactamase, β-lactams inhibitor mixtures, amine glycosides, quinolones, macrolides, and fluoroquinolones are more specific antibiotic categories (5). Function refers to a substance's mode of operation or mode of activity. One of the most crucial aspects of antimicrobial agents is this. Cell membrane function, cell wall synthesis, nucleic acid synthesis, protein synthesis, etc., are the primary processes or activities in bacterial growth. Antibiotics can be used to treat any one of these mechanisms. Antibiotics can be divided into four categories based on how they interfere with or disrupt these processes: inhibitors of cell wall synthesis, protein synthesis inhibitors (Metronidazole, etc.), membrane function inhibitors, and nucleic acid synthesis inhibitors (Levofloxacin, Rifampicin, Sulfamethoxazole, etc.) (9). This study aims to investigate the simultaneous analysis of specific antibacterial spectrophotometrically.

2. Materials and Methods

2.1. Levofloxacin

Levofloxacin (**Figure 1a**), chemically is 7H-Pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid, 9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-hydrate (2:1), (S)-; (−)-(S)-9-Fluoro-2,3-dihydro-3 methyl-10- (4-methyl-1-piperazinyl)- 7-oxo-7H-pyrido [1,2,3-de]-1,4-benzoxazine-6-carboxylic acid, hemihydrate (10). It is a third-generation broad-spectrum fluoroquinolone antibiotic used to treat bacterial infections such as acute bacterial sinusitis, acute bacterial exacerbation of chronic bronchitis, acute bacterial prostatitis, and acute pyelonephritis. Levofloxacin was patented in 1987 and subsequently in the United States in 1996 for treating hospital-acquired pneumonia, community-acquired pneumonia, urinary tract infections, and skin or skin structure infections. Also, it is used to prevent and treat plaque caused by Yersinia pasties. Levofloxacin was FDA-approved to control the progression of inhaled anthrax (11,12).

2.2. Metronidazole

As an antiprotozoal, antiamebic, and antibacterial medication, Metronidazole with chemical formula (2-(2-Methyl-5-nitro-1H-imidazol-1-yl) ethanol) (13) **Figure 1b**, is a bactericidal synthetic derivative of azomycin. Due to its effectiveness against anaerobic bacteria and protozoa, it has a wide range of applications. In the human liver, Metronidazole is converted into more polar metabolites. Metronidazole is the best option for colitis brought on by Clostridium difficile (14–16).

2.3. Rifampicin

Rifampicin (**Figure 1c**) is a semi-synthetic antibiotic derived from rifamycin SV and is chemically ((2S,12Z,14E,16S,17S,18R,19R,20R,21S,22R,23S,24E)- 5,6,9,17,19-pentahydroxy-23-methoxy-2,4,12,16,18,20,22- heptamethyl-8-[[(4-methylpiperazin-1-yl) imino] methyl]-1,11-dioxo-1,2-dihydro-2,7-(epoxypentadeca {1,11,13} trieneimino) naphtho[2,1-b] furan-21-yl acetate)) (13) active *in vitro* against microbacteria and gram-positive micro-organisms. Since its development, it has been used in veterinary and human medicine to treat tuberculosis and other mycobacterial infections (17,18).

2.4. Sulfamethoxazole

Sulfamethoxazole is a sulfonamide antibiotic (**Figure 1d**) with the IUPAC name (4-amino-N- (5-methylisoxazol-3-yl)-benzenesulfonamide) (13). Sulfamethoxazole inhibits bacterial dihydro-folate synthesis due to its structural similarity to para-aminobenzoic acid, an endogenous substrate that interferes with folate synthesis in susceptible bacteria (19). The emergence of resistant strains has limited its use, and it is now mainly used as a mixture with trimethoprim (20).

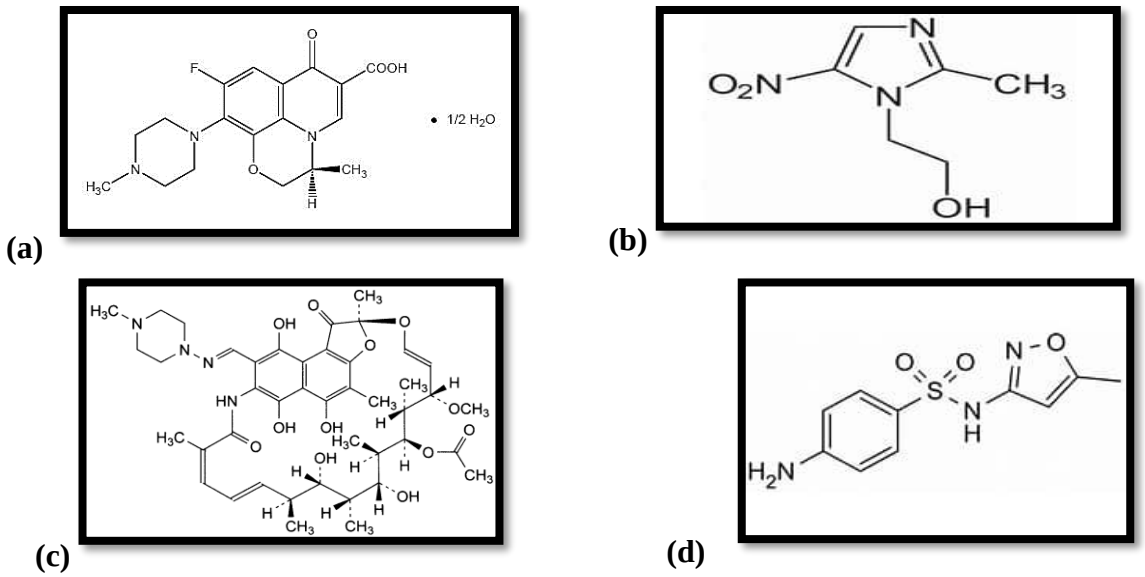

Figure 1. Structures formula of (a) Levofloxacin, (b) Sulfamethoxazole, (c) Metronidazole and (d) Rifampicin.

3. Results and Discussion

Several methods have been introduced to measure Levofloxacin, Sulfamethoxazole, Metronidazole, and Rifampicin separately or simultaneously. High-performance liquid chromatography, spectrophotometry, capillary electrophoresis, titration, spectrofluorometry, and voltammetry are a few examples. Simultaneous spectrophotometry is the most widely used due to its simplicity, sensitivity, and cost-effectiveness (21–23).

The primary application of UV spectrophotometry techniques is multicomponent analysis, which minimizes the laborious process of isolating interferences, shortens analysis time and expenses, and enables the measurement of more analytes (24). The foundation of multicomponent UV spectrophotometry is the documentation of standard absorption spectra and its mathematical analysis. The benefits of spectrophotometry are as follows: It is broadly applicable to both organic and inorganic systems while avoiding traditional separation methods, such as extraction, occupant enrichment, and potentially essential purification steps; spectral data acquisition is easy to acquire; detection limits typically of 10^{-4}-10^{-5} M; reasonable to high selectivity and good accuracy (25).

Diverse spectrophotometry, Vierodt's method, derivative spectrophotometry, dual wavelength method, absorbance ratio spectral method, differential ratio spectral method, absorption coefficient method, continuous ratio differential spectral method, Q-absorption ratio method, isosbestic points method, and other techniques are all examples of multicomponent spectrophotometry. In addition, chemometric methods have been used for multicomponent analysis, including multivariate regression models, principal component analysis, partial least squares, parallel factor analysis, and artificial neural networks (26).

Table 1 illustrates a brief discussion of each method, including the combined drug(s), method(s), analytical results, and application remark.

Table 1. Spectrophotometric methods for simultaneous determination of Levofloxacin, Sulfamethoxazole, Metronidazole and Rifampicin.

Drug		Method(s)	Analytical Results	Application remark	Year	Ref
Target	**Combined(s)**					
Levofloxacin	Azithromycin	Area under curve method using DDQ and p-CA	λ= (390-650) nm and (400-700) nm, Linearity range: 14.0-140.0 µg/mL and 30-300.0µg/mL, R^2 0.999 for both, Recovery 99.71-102.64% and 99.38-101.76%, LOD 1.40 µg/mL and 3.00 µg/mL	Pure mixture, Pharmaceutical binary dosage forms	2019	(27)
	Azithromycin	Direct measurement of Abs in perchloric acid methanolic solution	λ= 224nm, Linearity range: 2.5-20.0 µg/mL, R^2 0.9991, Recovery100.95%, LOD 0.80 µg/mL	Bulk powder, Laboratory-prepared tablets	2020	(28)
		First derivative peak-to-peak amplitudes	λ= 280–253nm, Linearity range: 5.0-40.0 µg/mL, R^2 0.9992, Recovery100.53%, LOD 1.50 µg/mL			
	Atenolol, Paracetamol, and Hydrochlorothiazide	Extended derivative ratio	λ= 282.8nm, Linearity range: 1.0-15.0 µg/mL, R^2 0.9996, Recovery 100.15% LOD 0.25 µg/mL	Dosage forms, Human urine	2020	(29)
		Multivariate curve resolution - alternating least squares	λ= (220.0-290.0) nm, Linearity range: 1.0-7.0 µg/mL, R^2 0.9999, Recovery 99.56%			
	Ambroxol	Absorbance ratio method	λ= (244.6, 218,6)nm, Linearity range: 3.0-7.0 µg/mL, R^2 0.9999, Recovery 99.0-100.0%, LOD 0.18 µg/mL	Tablets	2021	(30)
	Doxycycline	Absorbance ratio method, Simultaneous equation method	λ= 287nm, Linearity range: 2-20 µg/mL, R^2 0.9998, Recovery 99.66%, LOD 0.63 µg/mL	Synthetic mixture, Combined pharmaceutical dosage forms	2022	(31)
	Ornidazole	Area under curve method using DDQ and p-CA	λ=(390-690)nm and (400-700) nm, Linearity range: 10.0-200.0 µg/mL and 30-600.0 µg/mL, R^2 0.998 and 0.999, Recovery 98.81-101.55% and 98.8-100.86%, LOD 3.3 µg/mL and 9.9µg/mL	Pure mixture, Pharmaceutical binary dosage forms	2022	(32)
Metronidazole	Spiramycin	Direct UV-spectrophotometry	λmax= 311 nm., Linearity range: 5–25 µg/mL, R^2 0.9997, Recovery 100.16%, LOD 0.3938µg/mL	Pure form, Tablets	2010	(33)

Drug		Method(s)	Analytical Results	Application remark	Year	Ref
Target	Combined(s)					
		Ratio derivative spectrophotometry (^{1}DD)	λmax= 311nm, Linearity range: 5–25 µg/mL, R^2 0.9997, Recovery 100.16%, LOD 0.3938µg/mL			
		Mean centring of the ratio spectra (MCR)	λ= (200-400)nm, Linearity range: 5–25 µg/mL, R^2 1.0000, Recovery 101.1%, LOD 0.4967µg/mL			
	Ciprofloxacin hydrochloride	Second derivative ratio spectrophotometry technique (^{2}DD)	λ= 301nm, Linearity range: 4–16 µg/mL, R^2 0.9992, Recovery 100.21%, LOD 0.4900µg/mL	Pure form, Tablets	2012	(34)
	Moxifloxacin	Partial least squares regression (PLS), Principal component regression (PCR)	λ= 200-500nm, Standard error of prediction (SEP): 0.036 (PLS), 0.043 (PCR) Prediction residual error sum-of squares (PRESS): 0.052 (PLS), 0.064 (PCR), Recovery:99.4%(PLS), 99.43% (PCR)	Pharmaceutical tablets	2017	(35)
	Metronidazole benzoate	1st and 2nd derivative spectrophotometry	λ= 200-350nm, Linearity range: 1–25 µg/mL, R^2 0.9999, Recovery 99.75%-101.25%	Pharmaceutical preparations	2017	(36)
	Amoxicillin Trihydrate, Pantoprazole	Artificial Neural Networks	λ= 200-400 nm, 3 layers feed-forward neural networks with the lower backward-spread set of rules, RMSE: 0.34	Tablets	2022	(37)
Rifampicin	Piperine	Q-Absorbance method	λ= 387 nm, 337 nm, Linearity range: 5–40 µg/mL, R^2 0.999, Recovery 98.36-99.53%, LOD 1.51µg/mL	Combined capsule	2012	(38)
	Isoniazid	Partial least squares regression (PLSR)	λ= 449 nm, Linearity range: 8 -57 µg/mL, Recovery 92-119%, $RMSEP_{3LVs}$1.64 µg/mL	Urine, Pharmaceutical formulations	2013	(39)
	Isoniazid	Simultaneous equation	λ= 338 nm, Linearity range: 5 -50 µg/mL, R^2 0.992, Recovery 98.12-98.32%, LOD 3.50 µg/mL	Co-formulated tablets	2017	(40)
		First-derivative spectrophotometry	λ= 263 nm, Linearity range: 5-50 µg/mL, R^2 0.996, Recovery 98.80-99.15%,LOD2.30 µg/mL			

Drug		Method(s)	Analytical Results	Application remark	Year	Ref
Target	Combined(s)					
	Isoniazid, Pyrazinamide	First order derivative spectrophotometry	λ= 322 nm, Linearity range: 1.5-7.5 µg/mL, R^2 0.9927, Recovery 100.80%, LOD 0.03µg/mL	Bulk form, Pharmaceutical dosage form	2020	(41)
	Isoniazid	Vierordt's method	λ= 335 nm, Linearity range: 6-36 µg/mL	Simulated Lungs Alveolar Macrophages Fluid	2022	(42)
Sulfamethoxazole	Trimethoprim	First derivative method	λ= 288 nm, Linearity range: 4-20 µg/mL, R^2 0.9999, Recovery 108.64%	Tablets, Syrups	1997	(43)
		Classical least squares (CLS) method	λ= 200-350 nm, Linearity range: 4-20 µg/mL, Recovery 100.00%			
	Sulfacetamide	1st derivative: Peak to baseline, Peak to peak, and Peak area	λ= 219.75 nm, (219.0, 253.0) nm, and (270.2-322.5)nm, Linearity range: 1–8µg/mL, Recovery 98.9-101.4%	Synthetic Sample and Urine	2014	(44)
		2nd derivative: Peak to baseline, Peak to peak, and Peak area	λ= 275.0nm, (232.0, 275.0) nm, and (215.2-227.4)nm, Linearity range: 1–8µg/mL, Recovery 98.1-102.3%			
		PLS1, PLS2	λ=190-350 nm, Linearity range:1–8µg/mL, Recovery100.72%, 01.16%			
	Trimethoprim	Partial Least Square (PLS) regression	λ= 200-400 nm, Linearity range: 1–16µg/mL, R^2 0.99 RMSEC 0.167%, Recovery 100.72%, 101.16%	Combined Tablets	2015	(45)
	Trimethoprim	Mean Centering of Ratio Spectra (MCR)	λ= 200-400 nm, Linearity range:3.5–9.5µg/mL, Recovery 103.31±0.34%	Tablets	2018	(46)
	Trimethoprim	Aea under peak of SIA	λ= 540 nm and 264 nm, Linearity range: 0.1-10 µg/mL and 0.03-5 µg/mL, R^2 0.9797 and 0.9928, LOD 0.01 and 0.03 µg/mL	Tablets	2020	(47)

4. Conclusion

Due to numerous benefits such patient compliance, timely dose administration, and fewer doses, multi-drug therapies and formulations are becoming increasingly important. Analytical chemists must pursue new directions and develop new, accurate, precise, cost-effective, time-effective, and simple methodologies to estimate pharmaceuticals in a multi-component system.

Due to their inherent advantages, simultaneous analysis procedures are used more frequently for drug estimation in multi-component pharmaceutical formulations. These advantages include avoiding time-consuming extraction and separation, being economical by using fewer expensive regents, and being equally accurate and precise. According to the International Conference on Harmonization recommendations, techniques must be validated using the same parameters.

Acknowledgment

This work was partially supported by the Department of Chemistry/ College of Education for Pure Science (Ibn Al- Haitham)/ University of Baghdad.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Funding

No financial support was found.

Ethical Clearance

The study has been approved by the Committee of the University of Baghdad/ College of Education for Pure Science (Ibn Al-Haitham).

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