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Review: Instrumental analytical techniques for evaluating some anti-infective drugs in pharmaceutical products and biological fluids

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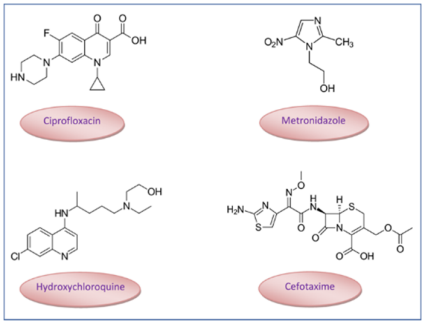
A B S T R A C T

Quality and safety of drugs are essential for effective therapeutic performance. Impurities can compromise the quality and safety of drugs, and they can arise during various stages of the development, production, storage and even transportation process. Therefore, detecting and measuring the number of impurities with high accuracy in drugs is necessary to ensure the quality and safety of drugs and to reduce the risks associated with taking them. Detecting and measuring impurities in drugs require advanced analytical techniques. The review highpoints a variety of analytical chemistry techniques include spectrophotometric and chromatographic methods in addition to some electrochemistry methods that have been applied for determination of certain drugs such as Ciprofloxacin, Metronidazole, Hydroxychloroquine and Cefotaxime in their pure form, combined form with other drugs, combined form with degradation products, and in biological fluids.



Graphical abstract

1. Introduction

Infectious diseases are caused by pathogenic microorganisms such as viruses, bacteria, fungi and parasites.[1] They're normally harmless or even helpful. But under certain conditions, some organisms may cause disease. These diseases can lead to a wide range of illness from the common cold to fatal illnesses like COVID-19 (WHO, 2022).[2-4] The burden of

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infectious diseases is immense, resulting in millions of deaths globally each year. Many infectious diseases are transmitted from person to person via direct contact, airborne respiratory droplets, contaminated surfaces or bodily fluids.[5-7] Basic hygiene interventions, including hand washing, can help reduce disease transmission.[8] Signs and symptoms vary depending on the micro-organism causing the infection, but often include fever and fatigue. Mild infections may respond to rest and home remedies, while some life-threatening infections may need hospitalization. Vaccines play a major role in prevention also by inducing an immune response against specific pathogens.[9]

Anti-infective drugs are a general term used to describe any drug that can inhibit the spread of an infectious agent or killing the infectious agent outright. Anti-infective drugs comprise antibiotics and antifungals, antibacterials, antivirals and antiprotozoals.[10] Currently, various categories of drugs are available in the market for the treatment of microbial infections. Common classes of drugs are β-lactums (Penicillins, Cephalosporins, Cefotaxime), quinolones (Ciprofloxacin, Levofloxacin), macrolides (Erythromycin, Clarithromycin),4-aminoquinoline (Hydroxychloroquine), tetracyclines (Doxycycline), lincosamides (Clindamycin), nitroimidazoles (Metronidazole, Tinidazole), polypeptides (Actinomycin, Bacitracin), oxazolidinones (Linezolid), glycopeptides (Vancomycin, Teicoplanin), and monobactams (Aztreonam) antibiotics.[11,12]

Heterocyclic compounds play a significant role in the discovery and design of effective drugs when dealing with infectious diseases and inflammations. Among these compounds, pyrazolopyrazines, pyridine, pyrimidine, and indole derivatives stand out as promising candidates for use in the development of anti-inflammatory pharmaceuticals. These compounds have been successfully utilized in research related to the design and development of anti-inflammatory drugs, demonstrating their powerful effectiveness against various inflammatory agent.[13-21]

Currently, an increasing number of drugs and numerous drug combinations are being introduced into the market at an alarming rate. Here comes the role of analytical chemistry in the pharmaceutical industries, as it contributes, through modern technologies, to playing a major role in ensuring the quality, safety and effectiveness of pharmaceutical products, detects impurities, and helps monitor the quality of raw materials used in the industry to ensure that pharmaceutical products comply with intended health standards.[22-26] The review highlights a variety of modern automated analytical techniques such as spectrophotometric and chromatographic methods in addition to some electrochemistry methods, have wide applications for determination of drugs such as Ciprofloxacin, Metronidazole, Hydroxychloroquine and Cefotaxime in different matrices.

2. Ciprofloxacin (CIP):

Ciprofloxacin (**Fig. 1**) is a widely used fluoroquinolone with a wide range of medicinal applications and has broad-spectrum coverage against many gram-negative and gram-positive pathogens. The currently available clinical evidence points to this medication's potentially increased efficacy in the treatment of a variety of nosocomial and community-acquired diseases, including infections of the urinary tract, respiratory tract, and skin.[27] Additionally, CIP is utilized to treat anthrax, certain types of plague and sexually transmitted diseases.[28]

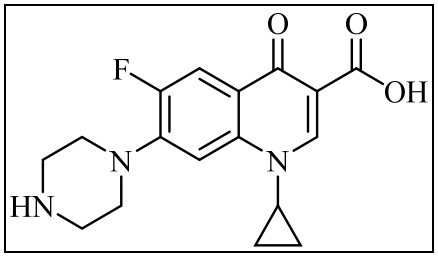

Fig. 1. Structure of Ciprofloxacin (CIP).

2.1. Chemical name (IUPAC name):

1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid.

2.2. Properties of Ciprofloxacin

Appearance at 25 °C	Faintly yellowish to light yellow crystalline powder.
Molecular Formula	$C_{17}H_{18}FN_3O_3$
Molecular Weight	331.34 g/mol
Solubility	Soluble in water: approx 36 mg/ml at 25 °C. Ciprofloxacin hydrochloride: Soluble in dilute (0.1N) hydrochloric acid. Practically insoluble in ethanol.[29]
Melting Point	225-257 °C, decomposes.

2.3. Methods of determination

2.3.1. Spectrophotometric methods

A spectrophotometric method was described for the determination of the antibacterial quinolone derivatives, ciprofloxacin through charge transfer complex formation with different acceptors. Chloranilic acid was utilized for their determination, forming charge transfer complex with λ_{max} 520 nm. The proposed method was applied for determination of Ciprocin tablets with mean percentage accuracy 99.58±1.25. Also, tetracyanoethylene was utilized in the determination of the concerned compounds forming charge transfer complexes with maximum absorbances at λ_{max} 335 nm for ciprofloxacin.[30]

A simple and inexpensive method for the determination of ciprofloxacin has been developed using solid-phase spectrophotometry. The intrinsic absorbance of ciprofloxacin fixed on a dextran-type cation-exchange resin, Sephadex SP C-25, was measured directly at 277 and 380 nm after packing the gel beads in a 1-mm cell. Using a sample volume of 10 mL, the calibration graph was linear over the range 0.05–0.3 μg mL^{-1} with a R.S.D. of 1.11% (n=8). The sensitivity obtained is 40 times higher than that of the corresponding solution method.[31]

2.3.2. Spectroflourimetric methods

Spectrofluorimetric method is presented for the determination of four fluoroquinolone drug, ciprofloxacin in pharmaceutical preparations. The proposed method is based on the derivatization of FQ with 4-chloro-7-nitrobenzofurazan in borate buffer of pH 9.0 to yield a yellow product.

The optimum experimental conditions have been studied carefully. Beer's law is obeyed over the concentration range of 23.5–500 ng mL^{-1} for ciprofloxacin using NBD-Cl reagent. The detection limits were found to be 7.0 ng mL^{-1} for ciprofloxacin.[32]

Spectrophotometric and spectrofluorimetric method for the determination of broad-spectrum fluoroquinolone antibacterial (ciprofloxacin), either in pure form or in tablets, are described. Method is based on the formation of a ternary complex between palladium (II), eosin and the fluoroquinolone in the presence of methyl cellulose, as surfactant. Spectrophotometrically, under the optimum conditions, the ternary complexes showed an absorption maximum at 545 nm, with apparent molar absorptivity of 3.4×104 mol^{-1} cm^{-1} and Sandell's sensitivity of 1.01×10^{-2} μg cm^{-2} for ciprofloxacin. The solution of the ternary complex obeyed Beer's law in the concentration range 3–10 μg mL^{-1} for quinolone. The proposed method was applied to the determination of the drug in pharmaceutical tablets.[33]

2.3.3. Chromatographic methods

Chromatographic methods have been widely applied for determination of Ciprofloxacin in pure form, in pharmaceutical formulations or in biological fluids. These methods include HPLC method for the determination of Ciprofloxacin in commercial product, Areversed phase high performance liquid chromatographic method was validated for the determination of the content of ciprofloxacin in three pharmaceuticals forms: generic, similar and compounded. The results of the validation showed that the method was highly efficient for quantification of ciprofloxacin in the matrices evaluated. The recovery rates were between 97.4 to 104.3 %, and the relative standard deviations were lower than 5 % for repeatability, and lower than 5.15 % for intermediate precision.[34]

A simple and sensitive high-performance liquid chromatographic method is described for the quantitative analysis of ciprofloxacin in pharmaceuticals and human plasma. The method employs reversed phase chromatography using an RP-C18 column with an isocratic mobile phase of acetonitrile-2% acetic acid aqueous solution (16:84, v/v), umbelliferone as an internal standard, and a flow rate of 1.0 mL/min. The UV detector is set at 280 nm. The limit of detection is 0.25 μM (S/N = 3, injection volume = 10 μL). The regression equations are linear ($r > 0.9999$) over a range between 0.51~130 μM for the pharmaceutical analysis of ciprofloxacin and 0.51~64.8 μM for the biological analysis of ciprofloxacin in human plasma.[35]

2.3.4. Miscellaneous method

Ciprofloxacin was determined by potentiometric titration through determination of acid dissociation constants (pKa). After validation of analysis method using phosphoric acid as a model compound, a second-derivative method was primarily applied to determining pKa's from titration curve for most antibiotic due to its convenience and accuracy. Results indicate that the pKa value is approximately 10–11 for fluoroquinolone (Ciprofloxacin).[36]

Ciprofloxacin was also determined in milk by new magnetic molecular imprinting-high performance liquid chromatography. The new magnetic molecular imprinting material was synthesized with $CoFe_2O_4$-graphene as the carrier, dopamine as the functional monomer and ciprofloxacin as the template molecule. After the milk sample was extracted with acetonitrile, the new magnetic molecular imprinting material was used to adsorb the ciprofloxacin. The solution was eluted

by methanol-acetic acid (9:1, V:V), filtered through membrane, and quantitative determination was carried out by high performance liquid chromatograph. Results: The new magnetic molecular imprinting material could reach the adsorption equilibrium rapidly within 15 minutes and had good identification. Under the best experimental conditions, ciprofloxacin showed a good relationship between peak areas and concentrations.[37]

3. Metronidazole (MET)

Metronidazole (**Fig. 2**) is an antibiotic that is employed in the treatment of bacterial infections of the vaginal tract, liver, stomach, skin, joints, heart, brain and spinal cord, lungs, and bloodstream. MET is also used to treat trichomoniasis, a parasite-based sexually transmitted illness. Even if one sexual partner has no symptoms, it is typical to treat both at once.[28]

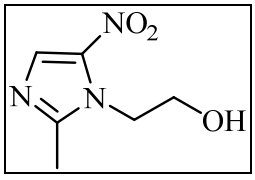

Fig. 2. Structure of Metronidazole (MET).

3.1. Chemical name (IUPAC name)

2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethanol

3.2. Properties of Metronidazole

Appearance at 25 °C	White to pale-yellow crystalline powder with a slight odor. Bitter and saline taste. pH (saturated aqueous solution) about 6.5.[38]
Molecular Formula	$C_6H_9N_3O_3$
Molecular Weight	171.15 g/mol
Solubility	Solubility (g/100 mL at 25 °C): Slightly soluble: 1.0 in water, 0.5 in ethanol. Very slightly soluble: less than 0.05 in ether, chloroform. Soluble in dilute acids.
Melting Point	158-160 °C.[37]

3.3. Methods of determination

3.3.1. Spectrophotometric methods

Sensitive quantitative estimation of MET in each of its pharmaceutical preparations and its level in human blood with one color reaction and one simple spectrophotometric technique. MET the antibiotic and antiprotozoal medication is determined by the reduction reaction of MZOL to 2-(2-Methyl-5-amino-1H-imidazole-1-yl) ethanol, followed by coupling with diazotized p-amino benzophenone (PABPh) reagent. The produced color complex is measured at 431 nm. The proposed method is successfully applied for the determination of MET in different dosage forms (tablet, suspension, and intravenous injection) with high precision (RSD% from ±0.011 to ±2.3).[39,40]

The UV-Visible instrument has shown a well-defined protocol to be followed and applied for MET quantification procedures. Three of the widely available pharmaceutical dosage forms of MET were chosen and analyzed. These pharmaceutical dosage forms are tablets, vials, and oral suspensions. The samples were handled with modified USP guidance and reasonably diluted and inoculated with the MET standard solutions. The UV readings were observed and the standard curves were plotted.

The observed curves show well-fitted straight lines for the all three pharmaceutical preparations (i.e. tablets, vials, and suspensions). The coefficients of regressions were found 0.9982, 0.9993, 0.9996, for tablets, vials, and suspensions, respectively. Consequently, the contents percentages for the MET tablets, vial, suspensions were recorded as 100.6%, 102.4%, and 99.5%, respectively.[41] It is better to mention here that the individuality of many different type nitroazoles were earlier performed using HPLC/UV technique.[42-51]

3.3.2. Spectrofluorometric methods

The g-C_3N_4 nanosheet was used as a switch-off fluorescence sensor for rapid and sensitive sensing of MET in biological fluids. These nanosheets were characterized by X-ray diffraction (XRD), transmission electron microscopy (TEM), and

Fourier transform infrared (FTIR) spectroscopy. The fluorescence of the solution of the g-C_3N_4nanosheets was quenched effectively by MET through two mechanisms: fluorescence resonance energy transfer and the formation of a donor−acceptor charge-transfer complex between π-electron rich donors. Under optimal conditions, the detection linear range for MET was found to be from 0.01 to 0.10 μg mL^{-1}, with a limit of detection (LOD) of 0.008 μg mL^{-1} which can cover standard range of MET in real samples.[52]

G-C_3N_4nanosheets were facilely fabricated by thermal polymerization and then exfoliated into ultrathin nanosheets through ultrasonication in water media. Low-cost C-N nanosheets prepared by melamine possessed a highly π-conjugated structure and fluorescence property[52].

An optical sensor has been suggested to measure a trace amount of MET (MTZ). In the first step, an eco-friendly method was used to synthesize carbon dots (CDs) using Eucalyptus leaves for the first time. The obtained CDs showed high solubility in water and high fluorescence intensity. In the next step, the CDs was used as a fluorescence probe for the determination of MTZ. This probe was modified with sensitive MTZ silica, which was sensitized using imprinting technology. The sensitive composite called CDs@MIPs demonstrated a linear range from 0.4 to 10.0 μg L^{-1} with a detection limit to 0.2 μg L^{-1}. High sensitivity, excellent selectivity, ease of fabrication, cheapness, and use of an environment-friendly method to synthesize the fluorescence probe are the advantages of this method.[53]

Non-conjugated polymer carbon dots (PCDs) with a 9% fluorescence quantum yield were synthesized by a pyrolytic method using polyethyleneimine as the sole precursor. The PCDs have an average size about 2.1 nm and a blue fluorescence, with excitation/emission maxima at 380/457 nm, that is quenched by the drug MET. The method has a linear response in the 0.06–15 μg mL^{-1} MET concentration range and a 20 ng mL^{-1} detection limit. Milk samples were spiked at two levels (0.6 and 5.0 μg mL^{-1}), and the recoveries of MET are in the range of 96.7–102.2%.[54]

3.3.3. Chromatographic methods

An analytical method based on AQbD and GAC for the simultaneous determination of MET in oval dosage form using RP-HPLC. The separation was achieved on the stationary phase Zorbax C18 150 mm × 4.6 mm (i.d); 5 μm, using a gradient mobile phase containing Ethanol and Phosphate buffers. Linearity has been obtained for MET 240-390 μg/mL, with LOD and LOQ as 31.25, 94.705 μg/mL. Finally, the method assessed for greenness using the three tools showed that the developed method was eco-friendly.[55]

A comparative evaluation of High-Performance Liquid Chromatography (HPLC) and potentiometric titration methods for the content determination of six MET API samples gathered from six pharmaceutical companies in Algeria. For the content determination by HPLC, Thermo Scientific Dionex UltiMate 3000 Rapid Separation LC system was used as a liquid chromatography apparatus, and the chromatographic parameters were used: temperature: 25 °C, flow rate: 1 mL/min, injection volume: 10 μL, Column: C18 (5 μm x 4.6 mm x 250 mm), and wavelength at 315 nm. For the content determination by potentiometric titration, a METTLER TOLEDO DL50 potentiometer, potassium phthalate acid standard solution 0.1 M, and a titrant solution of perchloric acid 0.1M were employed. All samples had a content meeting the required standard. The tested techniques proved to be effective in determining the amount of MET in the pharmaceutical raw material. In contrast to the HPLC method, the potentiometric titration did not require the use of a reference substance, but the manipulation was quick and the margin of error was significant.[56]

3.3.4. Miscellaneous methods

Various analytical papers that identify MET in pharmaceutical preparations and clinical samples have been reviewed. The reviewed literature included spectrophotometric, chromatography, and ion selective electrodes, Photo-Fenton Oxidation Technology, Charge-Transfer Complexes Formation, Glassy Carbon Electrode Modified with Gold-Copper Nanoparticles as Novel Electrochemical Sensor for Determination of MET, and Cerium doped magnetite nanoparticles highly sensitive detection of MET via chemiluminescence.[57]

3.4. Analysis of Ciprofloxacin and MET

Several methods for analyzing Ciprofloxacin and Metronidazole in their mixture form or alone were discovered in the literature. Ciprofloxacin and MET were determined by spectrophotometric methods,[58-71] Reversed-phase ion-pair HPLC, TLC-densitometric methods,[72] RP-UPLC Technique,[73] LC methods,[74, 75] UPLC-mass,[76] HPLC[77-80] and potentiometric and electrochemical determination.[80-83]

4. Hydroxychloroquine (HCQ)

Hydroxychloroquine (**Fig. 3**) raises lysosomal pH impairing parasite/microbe growth. It exhibits activity against malaria and autoimmune conditions like rheumatoid arthritis. Recently data on efficacy against COVID-19 and chronic hepatitis C has been inconsistent. Among potential drugs to treat COVID-19, repositioning of old drugs such as Chloroquine and

Hydroxychloroquine for use as antiviral treatment is an interesting strategy because knowledge on their mode of action, safety profile, side effects, dosage and their interactions with other biological molecules are well known.[84-86]

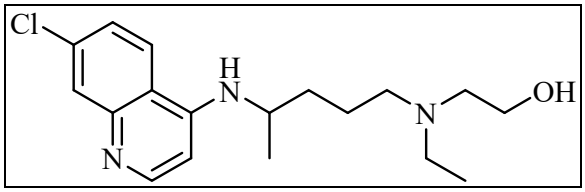

Fig. 3. Structure of Hydroxychloroquine (HCQ).

4.1. Chemical name (IUPAC name)

2-[4-[(7-Chloroquinolin-4-yl)amino]pentyl-ethylamino]ethanol

4.2. Properties of Hydroxychloroquine

Appearance at 25 °C	Solid
Molecular Formula	$C_{18}H_{26}ClN_3O$
Molecular Weight	335.9 g/mol
Solubility	Slightly soluble in water 0.0417 mg/ml.
Melting Point	89-91 °C.[84]

4.3. Methods of determination

4.3.1. Spectrophotometric methods

Five spectrophotometric methods were found from various sources available in internet.[76-78] Feraz *et al.* used USP method and performed quality by design in the determination of hydroxychloroquine in tablet formulations.[76,78] The limit of quantitation and limit of quantitation found are 1.27 and 0.38 µg/mL, respectively.

In another method 0.1 N HCl was used as diluent and determination performed at same wavelength as in the previously described method. Pharmacopeia method found cited here is referred from United States Pharmacopoeia.[77] Two methods in single paper in which one involves simple spectrophotometry method and another first derivative method developed by Mehta and Patel. In this method water is used as diluent for the preparation of sample in contrast to other methods using acidic medium.[78]

4.3.2. Spectrofluorometric methods

Synchronous spectrofluorometric measurement provides sensitive tool for resolving the overlapped spectra of multicomponent drugs through converting the wider spectra to narrower sharp spectra. This work introduces the first fluorescence spectroscopic method for quantitative analysis of favipiravir, remdesivir and hydroxychloroquine in spiked human plasma. Testing the fluorescence spectra of favipiravir, remdesivir and hydroxychloroquine shows severe overlap, which hinders the direct quantification of the cited drugs. To overcome the overlapping issue, the drugs under the study have been measured in the synchronous mode at $\Delta\lambda = 60$ nm. Favipiravir could be measured directly at 423 nm without interference of remdesivir or hydroxychloroquine. Synchronous measuring the cited drugs at $\Delta\lambda = 130$ nm with mathematical transforming to the first order derivative spectra allowing remdesivir and hydroxychloroquine at 384 nm and 394 nm, respectively without interference from favipiravir. Different factors affecting the spectrofluorometric measurement process have been verified. The drugs under the study have been successfully quantitatively analyzed in the spiked plasma using the proposed method.[87]

4.3.3. Chromatographic methods

The first paper related to the chromatography method was found to be published in 1985. HCQ and its metabolites are basic compounds and fluoresce at high pH, facilitating sensitive detection using chromatographic methods. This may be the reason that most of the methods utilizing spectrometry detection are based on fluorescence technique. First published paper is assay for HCQ and three major metabolites, using fluorescence detection in blood and plasma of RA patients. In this method, chloroquine was used as an internal standard. HCQ were found not interfere with the method.[78] Liquid chromatographic methods developed in the last 10 years were summarized focusing on sample preparation and detection methods for HCQ and CQ determination in biological fluids and pharmaceutical preparations.[88]

4.3.4. Miscellaneous methods

Various analytical techniques have been reported for asynchronous and simultaneous estimation of Hydroxychloroquine and their metabolites in pharmaceuticals and biological samples like (serum, whole blood, and urine). The analytical techniques are Square-wave voltammetry employed with the cathodically pretreated boron-doped diamond electrode, fast UHPLC–fluorescent method, UV spectrophotometry, UHPLC-UV analysis, RP-HPLC, mass spectrometry, NMR, and CE.[89]

5. Cefotaxime (CEF)

Cefotaxime (**Fig. 4**) belongs to the third generation of Cephalosporin antibiotics. It is widely utilized in the formulation of recommended antibiotic medications as an effective treatment against both gram-positive and gram-negative pathogens. Most viral infections can be managed with over-the-counter medications for symptoms until the patient feels better. Certain viral infections have special medications to treat them, like antiretroviral therapy for HIV.

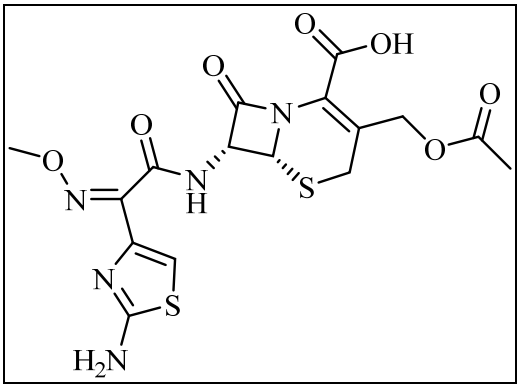

Fig. 4. Structure of Cefotaxime (CEF).

5.1. Chemical name (IUPAC name)

(6R,7R)-3-(acetyloxymethyl)-7-[[(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2-methoxyiminoacetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

5.2. Properties of Cefotaxime

Appearance at 25 °C	Solid
Molecular Formula	$C_{16}H_{17}N_5O_7S_2$
Molecular Weight	455.5 g/mol
Solubility	Cefotaxime (sodium salt) feerly soluble in water, slightly soluble in ethanol, insoluble in chloroform.
Melting Point	89-91 °C.[90]

5.3. Methods of Determination

5.3.1. Spectrophotometric methods

Two sensitive spectrophotometric and atomic absorption spectrometric procedures are developed for the determination of cephalosporins (cefotaxime sodium and cefuroxime sodium). The spectrophotometric methods are based on the charge-transfer complex formation between these drugs as n-donors and 7,7,8,8-tetracyano-quinodimethane (TCNQ) or p-chloranilic acid (p-CA) as π-acceptors to give highly coloured complex species. The coloured products are measured spectrophotometrically at 838 and 529 nm for TCNQ and p-CA, respectively. Beer's law is obeyed in a concentration range of 7.6–15.2 and 7.1–20.0 μg mL^{-1} with TCNQ, 95.0-427.5 and 89.0-400.5 μg mL^{-1} with p-CA for cefotaxime sodium and cefuroxime sodium, respectively.

The atomic absorption spectrometric methods are based on the reaction of the above cited drugs after their alkali-hydrolysis with silver nitrate or lead acetate in neutral aqueous medium. The formed precipitates are quantitatively determined directly or indirectly through the silver or lead content of the precipitate formed or the residual unreacted metal in the filtrate by atomic absorption spectroscopy. The optimum conditions for hydrolysis and precipitation have been

carefully studied. Beer's law is obeyed in a concentration range of 1.9–11.4 and 1.78–8.90 μg mL^{-1} with Ag(I), 14.2–57.0 and 13.3–53.4 μg mL^{-1} with Pb(II) for cefotaxime sodium and cefuroxime sodium, respectively (for both direct and indirect procedures).[91]

5.3.2. Chromatographic methods

A reversed-phase high-performance liquid chromatographic assay for the simultaneous determination of cefotaxime and its metabolite desacetylcefotaxime in plasma and urine was developed. Plasma was deproteinized with small amounts of acetonitrile. After separation of the proteins the supernatant was extracted with a mixture of chloroform and 1-butanol which are chemical compounds useful in a lot of applications.[92-95] A phase separation was obtained leaving the cephalosporin and its metabolite in the aqueous part and extracting most of the interfering endogenous material. Calibration curves were set up and were linear up to 25 μg/mL for desacetylcefotaxime and 250 μg/mL for cefotaxime.[96]

An analytical method for detecting and quantifying cefotaxime in plasma and several tissues is described. The method was developed and validated using plasma and tissues of rats. The samples were analyzed by reversed phase liquid chromatography (HPLC) with UV detection (254 nm). Calibration graphs showed a linear correlation ($r > 0.999$) over the concentration ranges of 0.5–200 μg/mL and 1.25–25 μg/g for plasma and tissues, respectively. The recovery of cefotaxime from plasma standards prepared at the concentrations of 25 μg/mL and 100 μg/mL was 98.5 ± 3.5% and 101.8 ± 2.2%, respectively.[97]

5.3.3. Miscellaneous methods

The literature review revealed that several analytical approaches have been utilized in the determination of cefotaxime Such as using poly(l-cysteine) and graphene composite modified glassy carbon electrode, potientiometry, electrophoresis and hydrophilic interaction chromatography.[98-105]

6. Conclusion

In conclusion, a variety of analytical methods have been developed and validated for the quantitative determination of metronidazole, ciprofloxacin, hydroxychloroquine, and cefotaxime in pharmaceutical formulations and biological matrices. High performance liquid chromatography (HPLC) coupled with UV detection remains the predominant technique used for analysis of these anti-infective agents. HPLC methods enable simultaneous determination of multiple analytes in a single run while providing adequate sensitivity and selectivity for therapeutic drug monitoring. For research and clinical testing purposes, other emerging techniques are gaining increasing utility for quantitative and qualitative detection of these drugs. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) facilitates multiplex assays with enhanced specificity and faster run times compared to traditional HPLC-UV methods. Capillary electrophoresis also offers rapid, affordable separation for determination of these analytes. Electroanalytical techniques including voltammetry and biosensor-based methods allow miniaturized testing platforms ideally suited for point-of-care analysis. However, traditional HPLC-UV assays remain the reference standard techniques for therapeutic drug monitoring and pharmacokinetic testing. Moving forward, development of chiral separation processes will be essential for isomer-specific drug quantitation particularly with levofloxacin, the S-enantiomer component of racemic ciprofloxacin. Additionally, LC-MS/MS assays offer promise for future targeted metabolomics research enabling better elucidation of metabolic pathways and pharmacodynamic compound interactions important for optimizing combination chemotherapy regimens against resistant infections. This literature review represents an up-to-date survey about all reported methods that have been developed for determination of certain drugs such as ciprofloxacin, metronidazole, hydroxychloroquine, and cefotaxime, in their pure form, combined form with other drugs, combined form with degradation products, and in biological samples such as liquid chromatography, spectrophotometry, and voltammetry.

Authors' contributions

Mahmoud M. Sebaiy, Sobhy M. El-Adl, Alaa Nafie, and Amr A. Mattar: supervision, conceptualization, designed the study, paper preparation, writing original draft, visualization, and revised the manuscript. Samar S. Elbaramawi: performed the searches, extracted the data, paper preparation, and writing original draft. Mokhtar A. Abdul-Malik and Shaban A. A. Abdel-Raheem: revising and adjusting the paper linguistically and spelling and adjusting the paper according to the style of the journal.

Conflict of interest

The authors declare that there is no conflict of interest in the manuscript.

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