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Review on analytical studies of some pharmaceutical compounds containing heterocyclic rings: brinzolamide, timolol maleate, flumethasone pivalate, and clioquinol

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Abstract

Background: The heterocyclic compounds are extremely important with wide array of synthetic, pharmaceutical, and industrial applications. Heterocyclic-containing compounds have been reported for their broad spectrum of biological activities including antibacterial, antifungal, antiviral, antiprotozoal, and anthelmintic activity.

Main text: Several techniques have been used for the quantitation of heterocyclic compounds in pharmaceutical samples such as high-performance liquid chromatography (HPLC) either equipped with UV-visible or fluorescence, in addition to liquid chromatography-mass spectroscopy, UV-visible spectrophotometry, and electrochemical techniques. This article reviewed several published methods that have been applied to detect and quantify some pharmaceutical drugs containing heterocyclic compounds focusing on four drugs: brinzolamide, timolol maleate, flumethasone pivalate, and clioquinol.

Conclusion: From literature reviews, HPLC is the most widely used analytical technique for the quantitative analysis of the four selected drugs.

Keyword: Heterocyclic, Brinzolamide, Timolol maleate, Flumethasone pivalate, Clioquinol, HPLC

Background

Analytical chemistry is the study of the separation, quantification, and chemical additives identification of herbal or synthetic materials containing one or more compounds. Recently, multi-component analysis has become one of the most attractive topics for chemists. Heterocyclic compounds are the cyclic organic compounds that contain at least one hetero atom; the commonest heteroatoms are the nitrogen, oxygen, and sulfur [1], while carbocyclic compound is a cyclic organic compound that contains the entire carbon atoms in ring formation. These compounds are vital and are widely utilized in several biological processes, owing to its efficacy in various diseases [2]. Biological molecules (DNA and RNA), chlorophyll, hemoglobin, and vitamins also contain heterocyclic ring [3]. There are numerous heterocyclic compounds applied in several diseases such as triazine derivatives, which are used as antimicrobial, herbicides, urinary antiseptics, and anti-inflammatory agents. Benzimidazole derivatives showed antibacterial, antifungal, antiviral, and anthelmintic activities [2, 4]. The analysis of bulk drug materials, intermediates, impurities, or even degradation products is essential. Recently, the assay techniques including titrimetry, chromatography, spectrometry, capillary electrophoresis, and electrochemical techniques have been developed. This article

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aimed to review the several published analytical techniques and their corresponding analytical methods, which have been applied for simultaneous separation of pharmaceutical drugs containing heterocyclic compounds. The forced degradation and its application for the development of stability indicating method are highlighted in this review. This presented review article focused on four heterocyclic drugs named brinzolamide, timolol maleate, flumethasone pivalate, and clioquinol.

Main Text: Heterocyclic compounds

Classification and general features of heterocyclic compounds

Though heterocyclic compounds might be inorganic or organic, the majority contains at least one carbon. While atoms, which are not carbon or hydrogen, are normally referred to as heteroatoms, this is often comparable to all-carbon skeleton [1]. Heterocyclic compounds could be categorized depending on its electronic structure. The saturated heterocycles behave similar to acyclic derivatives. Therefore, piperidine and tetrahydrofuran are conventional amines and ethers, with modified steric profiles. Thus, the heterocyclic chemistry emphasizes mainly on unsaturated derivatives with unstrained five- or six-membered rings including pyridine, thiophene, pyrrole, and furan [3]. Heterocycles as pyridine, thiophene, pyrrole, and furan being fused to benzene rings led to the development of quinoline, benzothiophene, indole, and benzofuran, respectively. However, fusion of two benzene rings developed a third large class of compounds, including acridine, dibenzothiophene, carbazole, and dibenzofuran. The unsaturated rings could be categorized based on the incorporation of the heteroatom in the conjugated system, pi system [3]. They are classified into three, four, five, and six-membered rings. The most common heterocycles are those having five- or six-membered rings and containing heteroatoms of nitrogen (N), oxygen (O), or sulfur (S).

Three-membered rings: like aziridine, oxirane, and thiirane, which contain a ring of three atoms composed of carbon atoms and one nitrogen atom, oxygen or sulfur, respectively. Four-membered rings: like azetidine, oxetane, and thietane, which contain a ring of four atoms composed of three carbon atoms and one nitrogen atom, oxygen or sulfur, respectively. Five-membered rings: like pyrrole, furan, and thiophene molecules, which contain a ring of five atoms composed of four carbons and one nitrogen, oxygen, or sulfur, respectively. Six-membered rings: like pyridine and pyrimidine, which contain a ring of six atoms composed of five carbons and one nitrogen or more, respectively.

Biological importance of heterocyclic compounds

Heterocyclic compounds have many applications: they are widely used as pharmaceuticals, as agrochemicals, and as veterinary products. They also exist in medical chemistry and as constituents of numerous biomolecules including enzymes, vitamins, and other natural products and biological compounds. Moreover, they are found in some pharmaceutical preparations including antifungals, anti-inflammatory, antibacterial, antioxidants, anticonvulsants, antiallergics, enzyme inhibitors, herbicides, anti-HIV, antidiabetics, anti-neoplastic, and insecticides.

Antifungal activity: Fungi are heterotrophic microorganisms that lack photosynthetic ability. The antifungals kill fungal cells through binding the cell membrane leading to formation of pores in the membrane with leakage of proteins and cations and eventually cell death [5]. Molnar et al. detected a group of dipicolinic acid derivatives; some of them have antifungal properties against *Aspergillus flavus* [6]. Chitra et al. produced indole 3-acetic acid-based biopolymeric hydrogels, with antifungal activities [7]. Anti-inflammatory activity: The anti-inflammatory agents are substances used for management or reduction of inflammatory reactions or swellings. Anti-inflammatory agents are nearly half of analgesics since they relieve pain via decreasing inflammatory reaction without affecting the central nervous system, unlike opiates [8]. Pyrimidinone and triazine derivatives also have anti-inflammatory properties [9]. Antibacterial and anthelmintic activity: Aromatic heterocyclic derivatives are incorporated in many antibiotics including β-lactam derivatives, and some chemists have synthetized several compounds and evaluated them for antibacterial activity [10]. Benzimidazole, piperazine, quinolone, piperidine, and albendazole are different heterocyclics used as anthelmintics [10]. Antioxidant activity: Antioxidant is a molecule, which suppresses oxidation of another molecule. Such compounds include thiol or ascorbic acid that could inhibit oxidation, thus preventing cell damage [11]. Anticonvulsant and antidepressant activities: Paroxetine and reboxetine are considered as antidepressants and anticonvulsants containing heterocyclic moieties in their structure. Some piperidine and pyrimidine derivatives possess the same activities as well [12]. Antiallergic activity: There are many heterocyclic compounds with anti-allergic properties, new Bis-heteroaryl hydrazines have been synthesized as effective antiallergic compounds, and such compounds possess good potency [13]. Anticancer activity: Alkylating agents are compounds that show antineoplastic activity [14]. Antiulcer activity: A series of substituted benzimidazole compounds such as omeprazole, lansoprazole, and pantoprazole have gastric antisecretary and therefore anti-ulcer activity [15].

Methods of separation

Chromatographic methods

Chromatography is used to separate, identify, and purify components of a mixture both qualitatively and

quantitatively. Chromatographic techniques are classified into column, thin layer, and paper chromatography [16]. Separation is based on that some components of the mixture have affinity to the stationary phase higher than that to the mobile phase and vice versa. If the component has higher affinity to the mobile phase, it will pass rapidly with the mobile phase and leave the system to be eluted first. Stationary phase is composed of solid or layer of liquid adsorbed onto inert solid support. Mobile phase may be liquid or gas. If the mobile phase is liquid, it is termed as liquid chromatography, and if gas, it is known as gas chromatography [17]. There are different types of chromatography like column, paper, dye-ligand, affinity, gas, thin layer, and high-performance liquid chromatography [16, 17]. Thin layer chromatography (TLC): a liquid-solid adsorption technique, where stationary phase is solid being adsorbed onto glass plate like alumina and silica gel, while mobile phase is liquid that travels upwards through the stationary phase. The upward travelling rate is dependent on the polarity of substance, solid phase, and solvent [18]. In high-performance liquid chromatography (HPLC), the mobile phase is liquid, which passes through a column composed of a number of theoretical plates where separation is done. Paper chromatography: a liquid-liquid chromatography where the mobile phase is liquid present in a developing tank and the stationary phase is a layer of cellulose (support) saturated with water. Affinity chromatography is used for purification of proteins, enzymes, nucleic acids, hormones, and antibodies [19]. Gas chromatography: a gas-liquid chromatography where the mobile phase is inert gas like He or N_2 and the stationary phase is liquid adsorbed onto inert solid support. It is used in the separation and analysis of multicomponent mixtures such as essential oils, hydrocarbons, and solvents [20]. Column chromatography: a precursory technique used in the purification of compounds and proteins based on their hydrophobicity or polarity. The molecule mixture is separated depending on its differential partitioning between a stationary phase, which is column, and a mobile phase is a buffer or solvent passing through the column [16]. Compared to other chromatographic techniques, HPLC is more sensitive, extremely quick, qualitative, efficient, and largely automated with the ability to purify amino acids, proteins, nucleic acids, hydrocarbons, carbohydrate, and some medications such as antibiotics and corticosteroids [16]. The mobile phase passes through columns under 10–400 atmospheric pressure and with a high flow rate. HPLC separation ensures the presence of certain components including solvents, pressure pump, column, detector, and recorder. Duration of separation is controlled by a computer [21].

Spectroscopic methods

Spectroscopic methods are widely used for simultaneous determination of different mixtures of drugs without prior separation by some mathematical equations, and these were found to be simple, very rapid, and with low cost compared to LC-MS and LC-GC [22]. Absorption spectroscopy is a powerful tool for quantitative analysis of analyte since there is a relation between the concentration of analyte and amount of light absorbed [22]. Near-infrared and Raman spectroscopy have been increasingly used for real-time measurements of critical process during pharmaceutical processing, as these spectroscopic techniques allow rapid and nondestructive measurements without sample preparations [23]. They are used for quantitative analysis of multicomponent with aid of chemometric tool. Therefore, they are used as Process Analytical Technology (PAT) tool for pharmaceutical industry [24].

Spectrophotometric method is a multicomponent analysis technique where the spectra of drugs overlap, and some simultaneous equations can be done to resolve such overlapping where the concentration of each individual analyte can be determined. Spectrophotometric technique depends on the following: (a) The spectrum of the solution: since the spectroscopy is an additive technique, therefore the absorbance of the solution is the sum of the absorbance of its individual components, so the spectrum of the solution is the sum of the spectra of its separate components [25]. (b) Beer-Lambert's law must be obeyed, as [$A = abC$] where A is the absorbance, C is the concentration of analyte, a is the absorptivity constant, and b is the path length. There is a direct relation between A and C, so if we construct a calibration curve, it must pass through the origin. Beer-Lambert's law is only applied for the diluted solutions not more than 0.01 M, as the concentration increases, deviation from linearity will occur [26]. (c) The absorbance measured is always the difference between the absorbance of the solution of interest present in the sample cell and that of the solution present in reference cell (blank) [27].

Several spectrophotometric methods were applied on pharmaceutical compounds including the following: (1) Methods based on the zero order absorption spectra: Like dual wavelength, induced dual wavelength, dual wavelength resolution technique, absorption correction method, absorbance subtraction, advanced absorbance subtraction, absorptivity factor method, area under the curve correction, compensated area under the curve, and spectrum subtraction [22, 28]. (2) Methods based on derivative spectra: Like amplitude subtraction, modified amplitude subtraction, amplitude factor, amplitude summation method, simultaneous derivative ratio spectrophotometry,

modified graphical method via regression equation, differential dual wavelength, differential derivative ratio, successive derivative subtraction coupled with constant multiplication, and derivative transformation [22, 29]. (3) Methods based on subtraction of the amplitudes of ratio spectra: Ratio subtraction method, successive ratio subtraction, extended ratio subtraction method, and simultaneous ratio subtraction method [22, 28]. (4) Methods based on amplitude difference of ratio spectra: Ratio difference spectrophotometric method, constant center spectrophotometric method, constant center coupled with spectrum subtraction, constant value via amplitude difference, constant value and amplitude center method [22]. (5) Methods based on modulation of amplitudes of ratio spectra: amplitude modulation advanced amplitude modulation and induced amplitude modulation [22, 28]. (6) Methods based on computed geometrical representation: Geometrical amplitude modulation, geometrical induced amplitude modulation, and ratio H-point standard addition method [22]. (7) Methods based on mean centering and wavelet transformation: Mean centering using geometric mean, pure component contribution algorithm, and continuous wavelet transform [22].

Electrochemical methods

Electrochemical is a technique that provides quantitative or semi-quantitative information about the analyte. Electrochemical techniques are classified into voltammetric, impedimetric, conductometric, potentiometric, and field-effect transistor-based biosensors [30]. Voltammetric biosensors: voltammetric and amperometric biosensors apply a potential to a working electrode versus a reference electrode and measure the produced current. The current arises from electrolysis via electrochemical oxidation or reduction at the working electrode [31]. Conductometric biosensors: they measure alteration in electrical conductivity of a sample solution as the composition of the solution is altered during a chemical reaction [32]. Potentiometric biosensors: they measure the potential of an electrochemical cell while drawing negligible current. They often contain an electrochemical cell with two reference electrodes able to measure the potential across an ion-selective membrane, which reacts with the charged ion of interest [31]. Field-effect transistor-based biosensors: they detect a change in the source-drain channel conductivity originating from the electric field of its environment. The electrical conductance of the channel is proportional to its carrier density, and this is readily sensed via the change in the source-drain voltage–current [33].

The studied drugs (brinzolamide, timolol maleate, flumethasone pivalate, and clioquinol)

Chemistry and drug indications

First: Brinzolamide (BRZ)

Brinzolamide is (4R)-4-(ethylamino)-2-(3-methoxypropyl)-1,1-dioxo-3,4-dihydrothieno[3,2-e] thiazine-6-sulfonamide (Fig. 1a) [34]. Its molecular formula is $C_{12}H_{21}N_3O_5S_3$, and the molecular weight is 383.5 g/mol. BRZ is whitish to off-white powder or crystals. It is insoluble in water but slightly soluble in alcohol and methanol. The melting point is 131 °C [35]. BRZ is a carbonic anhydrase inhibitor used for lowering the elevated intraocular pressure as in patients with ocular hypertension or open-angle glaucoma [36].

Second: Timolol maleate (TM)

Timolol maleate is (-)-1-(tert-Butylamino)-3-[(4-morpholino-1,2,5-thiadiazol-3-yl) oxy]-2-propanol maleate (1:1) (salt) (Fig. 1b) [37]. Its molecular formula is $C_{13}H_{24}N_4O_3S{\cdot}C_4H_4O_4$, and the molecular weight is 432.49 g/mol. It is whitish or almost whitish, odorless crystalline powder. It is soluble in water, alcohol, and methanol, sparingly soluble in chloroform and propylene glycol but insoluble in ether and cyclohexane. Its melting point is 202–203 °C [38]. TM is a non-selective beta-adrenergic blocker used as eye drops to decrease intraocular pressure for treatment of open-angle glaucoma. It is also utilized as tablets to reduce elevated blood pressure [39].

Third: Flumethasone pivalate (FP)

Flumethasone pivalate is 6a,9-difluoro-11b,17a-dihydroxy-16a-methyl-21-trimethylacetoxy1,4-pregnadiene-3, 20-dione (Fig. 1c) [40]. Its molecular formula is $C_{27}H_{36}F_2O_6$, and molecular weight is 494.6 g/mol. FP is virtually whitish, odorless, fine crystalline powder. It is slightly soluble in methanol as well as ethanol, very slightly soluble in chloroform as well as methylene chloride, but insoluble in carbon tetrachloride and isooctane [41]. FP is a moderately potent difluorinated corticosteroid ester with anti-inflammatory, antipruritic, and vasoconstrictive activities. Its anti-inflammatory effect is concentrated at application site. Such local action causes reduction in inflammatory reaction, exudation, and pruritis [41].

Fourth: Clioquinol (CL)

Clioquinol is 5-chloro-7-iodo-8-quinolinol (Fig. 1d) [42]. The molecular formula of CL is C_9H_5ClINO, and its molecular weight is 305.5. CL is freely soluble in pyridine, dimethylformamide, and hot ethyl acetate; sparingly soluble in dioxane; and slightly soluble in ethanol, while water insoluble [43]. It belongs to hydroxyquinolines that suppress particular enzymes associated with DNA

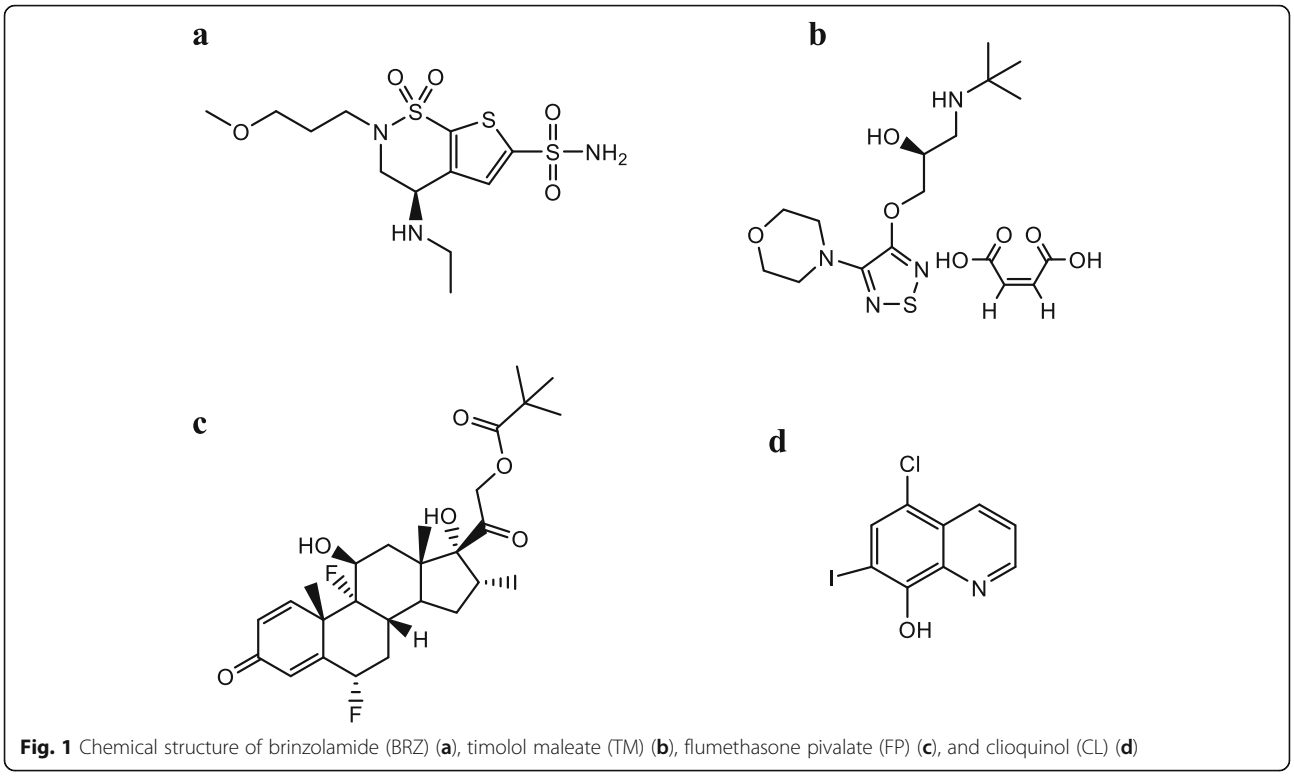

Fig. 1 Chemical structure of brinzolamide (BRZ) (**a**), timolol maleate (TM) (**b**), flumethasone pivalate (FP) (**c**), and clioquinol (CL) (**d**)

replication. It has antifungal and antiprotozoal activities [43].

Methods and techniques of analysis

Chromatographic analysis

Brinzolamide and/or timolol maleate

Surveying literature 2000–2019 revealed that many chromatographic analysis methods have been applied for the determination of brinzolamide as well as timolol maleate. HPLC is the most commonly applied chromatographic technique for the determination of brinzolamide and timolol maleate [44–57].

1- *High performance liquid chromatography technique (HPLC)*

Ibrahim et al. [44] introduced an HPLC method for simultaneous determination of dorzolamide, brinzolamide, and brimonidine combined with timolol. The mobile phase was acetonitrile to 0.05 M phosphate buffer (30:70 v/v) at pH 3.5 and wavelength of 220 nm using Promosil C18 column. The linearity was 1.25–25 μg/mL for timolol, 4–80 μg/mL for dorzolamide, 5–50 μg/mL for brinzolamide, and 2–20 μg/mL for brimonidine [44]. Yet, a simple RP-HPLC method was developed by Patel et al. [45], for the simultaneous determination of brinzolamide and brimonidine in their combined dosage form. The separation was carried out by Zorbax SB C18 (250 mm × 4.6 mm × 2.6 μm) column and buffer (potassium phosphate, pH 3.0 to acetonitrile (60:40 v/v) as mobile phase. The flow rate was 1 mL/min, and the detection wavelength was 225 nm. The linearity was 2–6 μg/mL for brimonidine and 10–30 μg/mL for brinzolamide [45]. Previously, Anusha et al. [46] showed that chromatographic separation of brinzolamide and timolol maleate was achieved by Inertsil ODS C18 column (250 × 4.6 mm, 5-μm particle size) with mobile phase consisting of sodium dihydrogen phosphate buffer (0.2 M) to methanol (70:30 v/v) pH 7.5 with sodium hydroxide solution at a flow rate of 1.0 mL/min and injection volume of 10 μL. The analytes were detected at 279 nm using by UV detector. The linearity was 0.0001–0.0018 mg/mL for brinzolamide and 0.0001–0.0023 mg/mL for timolol maleate [46]. Moreover, Shankar and Venkateshwarlu [47] developed a method for the determination of brinzolamide and timolol maleate by utilizing C18 Column (150 mm × 4.6 mm, 5 μm). The flow rate was 1 mL/min while mobile phase was methanol to phosphate buffer pH 4.0 (70:30 v/v). The detection was performed at 260 nm. The linearity range of brinzolamide was 5–25 μg/mL, while for timolol maleate was 20–100 μg/mL [47]. Hassib et al. [48] published a unique research for a LC-MS/MS method for the simultaneous determination of timolol maleate with other co-administered drugs as dorzolamide hydrochloride, brinzolamide, and brimonidine tartrate in rabbit aqueous humor utilizing eslicarbazepine as internal standard. The separation was performed with a mobile phase of 10 mM ammonium format pH 7 to

methanol to acetonitrile (5:50:45 by volume). The flow rate was 0.8 mL/min on an INERTSIL(®) C18 ODS-3 column (150 mm × 4.6 mm, 3.5 μm). The method was carried out utilizing electrospray ionization source in a positive ionization mode, and the detection was performed via multiple reaction monitoring at the succeeding transitions: m/z 317.2 → 261.0 for timolol maleate, m/z 325.1 → 199.0 for dorzolamide hydrochloride, m/z 384.2 → 281.0 for brinzolamide, m/z 292.1 → 212.0 for brimonidine tartrate, and m/z 255.0 → 237.0 for internal standard. The linearity range was 50–5000 ng/mL for the entire medications [48]. Agrawal et al. [49] introduced a RP-HPLC method for the simultaneous determination of brimonidine tartrate and brinzolamide. The separation was carried out on C18 column (250 × 4.6 mm, 5 μm), and the mobile phase was composed of methanol to 0.01 M ammonium acetate buffer (49.5:50.5 by volume), pH adjusted to 3.8. The flow rate was 1.1 mL/min, and the detection wavelength was 260 nm. The linearity range was 0.2–1.4 μg/mL for brimonidine tartrate and 1–7 μg/mL for brinzolamide [49]. Christian et al. [50] suggested a HPLC method for the detection of brinzolamide as well as brimonidine tartrate. Using an isocratic RP-HPLC Phenomenex C18 (5 μm, 250 × 4.6 mm) column and a mobile phase of phosphate buffer (pH 6.6) to acetonitrile to methanol (45:15:40 by volume) flowed at a rate of 1.0 mL/min, and detection was achieved at 254 nm. The linearity of the two drugs was 50–1600 ng/mL [50]. Kumari et al. [51] introduced a stability indicating RP-HPLC method for the simultaneous detection of travopost and timolol in bulk and pharmaceutical dosage forms. The separation was performed on Intersil ODS (150 × 4.6 mm, 5 μm) column, and the mobile phase was acetonitrile to buffer in the ratio of 10:90. The flow rate was 1 ml/min, and the detection wavelength was 213 nm. The linearity ranged from 12.5 to 75 μg/mL for timolol and 10 to 60 μg/mL for travopost [51]. Rizk et al. [52] developed a stability-indicating micellar liquid chromatographic (MLC) method for detecting timolol maleate in presence of its degradation products. An isocratic, rapid, and mobile phase saving the micellar LC method was developed using a Bio Basic Phenyl column (150 × 1.0 mm, 5-μm particle size), and a micellar mobile phase of 0.1 M sodium dodecyl sulfate, 10% of 1-propanol, and 0.1% of triethylamine in 0.035 M ortho-phosphoric acid flowed at a rate of 0.1 mL/min. The detection wavelength was 298 nm. The linearity was 50–2500 mg/mL for timolol [52]. Hafez et al. [53] suggested an UPLC method for the determination of brimonidine tartrate, timolol maleate, and benzalkonium chloride in eye drops. The method was carried out on Phenomenex Kinetex C18 (50 × 4.6 mm, 2.6 μm), and the mobile phase was methanol and 1-Decane sulphonic acid sodium (pH 3.0). The flow rate was 0.6 mL/min at 50 °C in gradient manner. Detection was done at wavelength 320 nm. The linearity was 1–100 μg/mL for brimonidine and 2.25–225 μg/mL for timolol [53]. Also, Khatun and Islam [54] described a reversed phase-HPLC (RP-HPLC) method for the simultaneous detection of brinzolamide and timolol maleate in ophthalmic formulations. The separation was carried out via Zorbax Eclipse Plus, Agilent Technology (150 mm × 4.6 mm, 5 μm) column with a mobile phase of triethylamine phosphate buffer to acetonitrile to methanol (70:20:10 by volume). The flow rate was 1.0 mL/min, and PDA detection was at 274 nm. The linearity was 40–140 μg/mL for brinzolamide and 20–70 μg/mL for timolol maleate [54]. Laddha et al. [55] developed a stability indicating method for timolol maleate where the separation was performed on C18 column of Kromasil (250 mm × 4.6 mm, 5 μm), and the mobile phase was phosphate buffer to methanol (60:40 v/v). The pH of buffer was maintained to 3.5 by O-phosphoric acid. The detection wavelength was 295.3 nm. The linearity for timolol was 10–50 μg/m. The flow rate was 1 ml/min. Timolol maleate was observed to be stable in the entire conditions except in alkaline one [55]. Elshanawane et al. [56] established a HPLC method for the determination of brimonidine tartrate and timolol maleate using BDS HYPERSIL Cyano column (250 × 4.6 mm, 5 μm) and a mobile phase formed of ammonium acetate (pH 5.0) to methanol (40:60 v/v). The flow rate was 1.5 mL/min. The detection was performed at 254.0 nm for brimonidine tartrate and 300.0 nm for timolol maleate. The linearity range was 4–24 μg/mL and 10–60 μg/mL for brimonidine tartrate and timolol maleate, respectively [56]. Sharma et al. [57] developed a RP-UPLC method for the detection of dorzolamide hydrochloride as well as timolol maleate in the existence of their impurities, degradation products, and placebo. Such method utilized Waters UPLC BEH C18, 100 × 2.1 mm, 1.7 μm column, and a mixture of solvents A and B as mobile phase. Phosphate buffer (0.04 M), pH 2.6 was utilized as buffer, where buffer pH 2.6 represented solvent A, while Milli-Q water, methanol, and acetonitrile (2:3:6 by volume) represented solvent B. The gradient protocol was 0/5, 8/8, 10/15, 16/45, 20/55, 24/80, 25/5, and 30/5. Dorzolamide hydrochloride along with its impurities were detected at 254 nm, while timolol maleate along with its impurities at 295 nm. The run time was half an hour [57].

2- *TLC densitometry*

Salem et al. [58] established a thin layer chromatography (TLC)-densitometric method for simultaneous determination of a binary mixture of timolol and travoprost without prior separation in their bulk or dosage forms. The separation was carried out on Merck

HPTLC aluminum sheets of silica gel 60 F254 using ethyl acetate-methanol-ammonia (5:3:0.5 by volume) as mobile phase. Detection was done at 274 nm, and linearity was 0.4–50 μg/band for both drugs [58]. Eissa et al. [59] suggested a TLC densitometric method for the simultaneous detection of brinzolamide and timolol maleate in pharmaceutical products. Separation was carried out with a mobile phase of chloroform to methanol to ammonia (9:0.5:0.1 by volume) on silica gel 60 F254 plates; densitometric analysis was performed at 254 nm for brinzolamide and 298 nm for timolol. The linear range was 3–16 μg/spot for brinzolamide and 4–14 μg/spot for timolol maleate [59]. Another HPTLC method was developed by Kulkarni and Amin for analysis of timolol maleate using ethyl acetate to methanol to isopropyl alcohol to ammonia (25%) (80:20:2:1 by volume) as mobile phase. The calibration curve was linear over a range of 100–600 ng, and the spectro densitometric detection was achieved at 294 nm [60].

Flumethasone pivalate and/or clioquinol

1- *High-performance liquid chromatography technique (HPLC)*

Hashem [61] developed a HPLC method for the simultaneous quantification of flumethasone pivalate and salicylic acid in bulk and in an ointment dosage form. Separation was achieved on Calixarene stationary phase. The mobile phase was acetonitrile and water (70:30 v/v) at a flow rate of 1 mL/min at 40 °C. Detection wavelength was 240 nm. Linearity range was 0.5-50 μg/mL [61]. Sayed et al. [62] established an HPLC method for the simultaneous determination of flumethasone pivalate, flumethasone pivalate-related substance and impurity and clioquinol. Separation was achieved within 11 min using ODS column using acetonitrile–water (70:30 v/v) as the mobile phase at a flow rate of 1 mL/min with ultraviolet detection was achieved at 235 nm. The calibration plots were linear over the concentration range of 5–50, 2–35, and 10–70 mg/mL, respectively [62]. Chhalotiya et al. [63] introduced a stability indicating RP-HPLC method for the determination of clioquinol in bulk and in pharmaceutical dosage forms. A Sunfire Clgs 4.5 μm column with mobile phase of acetonitrile-water pH 3 (90:10 v/v) was utilized. The flow rate was 1.0 L/min, and eluent was monitored at 254 mn. Linearity range was 0.1–30 mg/mL [63]. Lotfy et al. [64] suggested a RP-HPLC method for the simultaneous detection of betamethasone valerate, clioquinol along with their potential interferents including their degradation products, methyl paraben and propyl paraben (preservatives) as well as gentamycin and tolnaftate. Separation was performed on a Zorbax C18 column using water to methanol to acetonitrile to glacial acetic acid (394:50:550:6, by volume) as a solvent, and the effluents was monitored at 275 nm. The method was linear over the concentration range of 12–240 mg/mL, 30–3000 mg/mL, 7–140 mg/mL, and 3.5–70 mg/ mL for betamethasone valerate, clioquinol, methyl paraben, and propyl paraben, respectively [64]. Bondiolotti et al. [65] described an HPLC method for determining clioquinol levels in hamster plasma and tissue. Clioquinol underwent separation on a Nucleosil C18 300 mm × 3.9 mm i.d. 7-μm column at 1 mL/min utilizing a phosphate/citrate buffer 0.1 M (400 ml) with 600 mL of a methanol to acetonitrile (1:1 v/v) as solvent. The method was linear for clioquinol at 5–2000 ng/mL in plasma and at 10–1000 ng/g in tissues [65].

2- *TLC densitometry*

Sayed et al. [62] developed a TLC-densitometric method for the simultaneous determination of flumethasone pivalate, flumethasone pivalate-related substance and impurity and clioquinol. The proposed TLC-densitometric was performed using silica gel plates 60 F254 as a stationary phase with benzene–hexane–acetone–formic acid (5:4:2:0.13, by volume) as a developing system followed by densitometric measurements at 235 nm. The studied components were quantified in the range of 0.3–4, 0.3–3, and 1.5–5 μg/band, respectively [62].

Spectroscopic analysis

Brinzolamide and/or timolol maleate

Salem et al. [58] suggested spectrophotometric and chemometric methods for the simultaneous determination of binary mixture of timolol and travoprost without prior separation in their bulk drugs and dosage forms. The spectrophotometric methods used were derivative spectrophotometry and isosbestic point methods. Multivariate methods involved the application of three chemometric techniques: classical least square (CLS) principal component regression (PCR), and partial least-squares regression (PLS) [58]. Jadhav et al. [66] utilized the spectrophotometric technique for the simultaneous determination of brimonidine tartrate and brinzolamide in bulk and ophthalmic formulation. The absorbance was measured at two wavelengths: 252.40 nm (λ max of brinzolamide) and 246 nm (λmax of brimonidine tartrate) in methyl alcohol. Linearity was 5–35 μg/mL and 3–18 μg/mL, respectively [66]. Annapurna et al. [67] suggested two spectrophotometric methods for the simultaneous detection of brimonidine and timolol in ophthalmic solutions using borate buffer. Simultaneous equation method and Q-analysis were used. Linearity was 1–60 μg/mL for timolol and 1–40 μg/mL for brimonidine [67]. Annapurna et al. [67] performed ratio derivative and multicomponent spectrophotometric techniques for the simultaneous detection of brimonidine tartrate and timolol maleate in

pharmaceutical solutions in borate buffer pH 9.0. Linearity range was 1–60 μg/mL for timolol maleate and 1–40 μg/mL for brimonidine tartrate [68]. Eissa et al. [69] developed four spectrophotometric methods for the simultaneous detection of brinzolamide and timolol maleate in pharmaceutical formulations. Method A is ratio difference spectrophotometric method (RDSM) that can measure difference in amplitudes between 251 and 265 nm of ratio spectrum for brinzolamide and between 285 and 306 nm of ratio spectrum for timolol. Method B is mean centering of ratio spectra method (MCR) by measuring peak amplitude at 252 nm for brinzolamide and 304 nm for timolol. Method C is area under the curve in which the area under the curves for brinzolamide and timolol were selected over the ranges of 260–265 nm and 282–288 nm. Method D is the bivariate method (BVM), which depends on quantification of absorbances at 265 and 285 nm, respectively. Beer-Lambert's law for the adopted methods were obeyed over the concentration range of 6–36 μg/mL for brinzolamide and 6–42 μg/mL for timolol [69]. Agrawal et al. [70] suggested a UV spectrophotometric method for the simultaneous determination of brimonidine tartrate and brinzolamide using methanol as a solvent. The λmax for brimonidine tartrate and brinzolamide were found to be 244 nm and 253 nm, respectively. The linearity was found in the range of 1–7 μg/mL and 5–35 μg/mL for brimonidine tartrate and brinzolamide, respectively [70]. Shah et al. [71] introduced three UV spectroscopic methods named simultaneous equation (method 1), Q-absorbance ratio (method 2), and ratio first derivative (method 3) for simultaneous detection of brinzolamide and timolol maleate in ophthalmic formulation. Vijya et al. [72] established a spectrophotometric method for the simultaneous detection of brimonidine tartrate as well as brinzolamide in bulk and in pharmaceutical preparations. Such technique involves first-order derivative spectroscopy utilizing 232 nm and 219.40 nm as zero crossing points for brimonidine tartrate and brinzolamide, respectively. Methanol was utilized as a solvent. The linearity was 1–7 μg/mL and 5–35 μg/mL for brimonidine tartrate and brinzolamide, respectively [72]. Lotfy et al. [73] developed two spectrophotometric methods (absorbance subtraction as well as amplitude modulation) in order to determine a binary mixture of timolol maleate as well as dorzolamide hydrochloride in the existence of benzalkonium chloride.

Flumethasone pivalate and/or clioquinol

Abdel-Aleem et al. [74] developed three spectrophotometric methods in order to determine flumethasone pivalate and clioquinol in their binary mixture as well as ear drop solutions. Method A is a ratio subtraction spectrophotometric method (RSM). Method B is a ratio difference spectrophotometric one (RDSM), whereas method C is the mean center spectrophotometric one (MCR). The linearity range was 3–45 μg/mL for flumethasone pivalate and 2–25 μg/mL for clioquinol [74]. Nief and Ayad [75] developed spectrophotometric methods for the determination of clioquinol in pharmaceutical formulations and industrial waste water samples. This technique depends on the drug chelation using Fe (III) to produce bluish-green-colored metal chelate at room temperature that absorbs maximally at 639 nm. Beer's law is obeyed over a range of concentration of 2.00–20.00 μg/mL (6.5×10^{-6} to 6.5×10^{-5} M) [75].

Electrochemical analysis

Electrochemical methods have the advantages of being simple, fast, and inexpensive with easy sample preparation. Fast thin-layer electrolysis was developed for monitoring the oxidative intermediates of clioquinol in acidic and alkaline media. Two thin-layer electrochemical cells were fabricated and coupled with a UV-visible spectrometer as well as an electrophoresis apparatus, respectively [76]. The voltammetric decrease of flumethasone pivalate was studied using fullerene-C60-modified edge plane pyrolytic graphite electrode (PGE), and the study showed that two well-defined peaks were obtained with a peak potential of ~ − 1220 mV and ~ − 1351 mV, respectively, and the linearity was obtained with sensitivity of 0.685 μA μM^{-1} and 0.570 μA μM^{-1} [77].

Conclusion

This review investigated the pharmaceutical analysis of four drugs containing heterocyclic ring from 2000 to 2019. These drugs are brinzolamide, timolol maleate, flumethasone pivalate, and clioquinol. The literature review demonstrated various analytical techniques such as chromatographic, spectroscopic, and electrochemical methods that have been employed for the analysis of the aforementioned drugs. The HPLC is the most widely used analytical technique for the analysis of the four selected drugs. Also, the analysis of brinzolamide and timolol maleate is more frequently studied in literature review than flumethasone pivalate and clioquinol (17 studies versus 6 studies, respectively, in chromatographic assay and 8 studies versus 2 studies, respectively, in spectroscopic assay).

Abbreviations

TLC: Thin-layer chromatography; HPLC: High-performance liquid chromatography; RP-HPLC: Reversed phase-HPLC; BRZ: Brizolamide; TM: Timolol maleate; FP: Flumethasone pivalate; CL: Clioquinol; LC-MS: Liquid chromatography-mass spectroscopy; LC-GC: Liquid chromatography-gas chromatography; MLC: Micellar liquid chromatographic; UPLC: Ultra performance liquid chromatography; UV: Ultraviolet; CLS: Classical least square; PCR: Principal component regression; PLS: Partial least-squares; RDSM: Ratio difference spectrophotometric method; MCR: Mean centering of ratio spectra method; BVM: Bivariate method; RSM: Ratio subtraction spectrophotometric method; PGE: Pyrolytic graphite electrode

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