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Research Article

DRUG FORMULATIONS

Status of Physicochemical and Microbiological Analytical Methods of Gatifloxacin: A Review

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Abstract

Background: Gatifloxacin (GAT), an antimicrobial of the fourth generation of fluoroquinolones, has a broad spectrum of action with activity against Gram-positive and Gram-negative, aerobic and anaerobic organisms, including mycobacteria.
Objective: The objective of this review is to discuss about (i) characteristics, (ii) properties, and (iii) analytical methods of gatifloxacin.
Results: Among the methods described in the literature for the evaluation of GAT, the most frequent was HPLC (50%) for both the analysis of pharmaceutical and biological matrixes. GAT has no monograph described in official compendia. Methods for evaluating GAT in pharmaceutical matrixes were the most found in the literature, 79%. Acetonitrile (42%), methanol (20%), and buffer solution (16%) were the most used diluents. GAT, being an antimicrobial, must be analyzed by physical-chemical and microbiological methods, since the evaluation of potency is essential. In this context, the literature is scarce (4%).
Conclusions: There is a gap in the literature for environmentally friendly methods for evaluating GAT. Faster, more optimized and dynamic microbiological methods, as well as physicochemical methods, use less aggressive solvents with fewer steps and less waste. Currently, pharmaceutical analyses require reliable analytical methods, but also safe for both the analyst and the environment.
Highlights: This review shows the status of analytical methods, both physicochemical and microbiological, for the analysis of GAT in pharmaceutical and biological matrixes, also addressing its context in green and sustainable analytical chemistry.

Gatifloxacin (GAT, Figure 1) is a fourth-generation fluoroquinolone antimicrobial. It has a broad spectrum of action with activity against Gram-positive and Gram-negative, aerobic and anaerobic organisms, including mycobacteria and *S. pneumoniae* resistant to other antibiotics (1–3).

GAT has indications for respiratory, genitourinary tract, and eye infections with better action on microorganisms than other older fluoroquinolones. The absorption of GAT is almost total in the gastrointestinal tract, and its oral bioavailability is 96% (4–6).

On the one hand, GAT does not present a monograph described in official compendia. On the other hand, the literature presents some analytical methods for its evaluation. Nonaqueous titration, thin-layer chromatography, spectrophotometry in the ultraviolet and visible regions, high-performance liquid chromatography, agar diffusion, and turbidimetry are some methods available in the literature for the evaluation of GAT in pharmaceutical and biological matrixes (1, 6–9).

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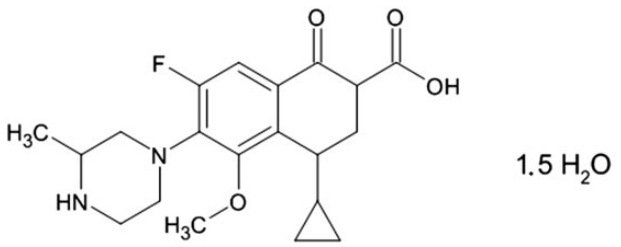

Figure 1. Chemical structure of gatifloxacin sesquihydrate (CAS180200-66–2).

Therefore, the objective of this review is to discuss (i) characteristics, (ii) properties, and (iii) analytical methods for the evaluation of the GAT.

Gatifloxacin

GAT, a fourth-generation fluoroquinolone antimicrobial, has a broad spectrum of action like the other members of this family. It was made commercially available in 1999 under the trade name Tequin® (Bristol-Myers Squibb Pharmaceuticals) in tablet form, which was used to treat respiratory tract infections. Currently, GAT is marketed mainly in the form of 0.3 and 0.5% eye drops under the trade name Zymar® (Allergan) for the treatment of eye infections, bacterial conjunctivitis, irritation, and bacterial inflammation (10).

Structural Formula

GAT, 1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7–(3-methyl-1-piperazinyl)-4-oxo-3-quinoline carboxylic acid sesquihydrate, has the chemical formula $C_{19}H_{22}FN_3O_4$. 1.5 H_2O, molecular weight 402.42 g/mol and appears in the form of a sesquihydrated, whitish, or slightly yellowish crystalline powder (2, 9, 10).

Structural Modification

GAT was obtained from nalidixic acid through changes in structure such as: addition of a methyl-piperazine at carbon 7, a methoxy group ($AOCH_3$) at position 8, for greater activity in DNA gyrase and bacterial topoisomerase IV, and a group cyclopropyl in the N-1 ring, to increase Gram-positive and Gram-negative activity. The changes increased bactericidal activity and reduced problems related to microbial resistance against other quinolones (1, 6, 10, 11).

Mechanism of Action

The action of GAT occurs through the inhibition of topoisomerase II (DNA gyrase) and topoisomerase IV, enzymes essential for the processes of DNA replication, transcription, repair, and recombination. Thus, once these enzymes are inactivated, replication of bacterial genetic material does not occur (9, 12, 13).

Pharmacokinetics and Pharmacodynamics

GAT has a broad spectrum of antibacterial activity, being indicated for respiratory, genitourinary tract, and eye infections, with better action on Gram-positive organisms than other older fluoroquinolones. The absorption of GAT is almost total in the gastrointestinal tract, and its oral bioavailability is 96%; in addition, gatifloxacin has a long half-life, which allows it to be administered a few times a day (9).

For the ophthalmic pharmaceutical form, the action is local, not reaching considerable systemic concentrations. Recommended use is one drop every two hours for up to eight times a day. Hepatic drug metabolism is minimal and its excretion occurs mainly through urine, in which about 82 to 88% of the drug is eliminated unchanged (4, 5, 9).

Physicochemical Properties

Chemically, GAT consists of a racemate, so it does not exhibit any optical rotation. Solubility depends on pH, with the maximum aqueous solubility being 40–60 mg/mL in a pH range of 2–5 (9). Other studies have also demonstrated adequate solubility of GAT in methanol (12, 14), ethanol (15), and acetonitrile (15, 16).

Analytical Methods

Both physicochemical and microbiological analytical methods must be accurate, safe, and reliable for the evaluation of samples. Some methods for evaluating GAT in different types of samples, such as drugs and biological fluids, are described in the literature and shown in Table 1 (9).

Discussion

The literature shows evaluation of GAT in pharmaceutical matrixes (Figure 2A) such as tablets (1, 2, 6–8, 11, 17–26), capsules (27), eye drops (3, 11–14, 28–34), suspension (11), and standard (15) by Vis, UV, HPLC, HPTLC, SF, titration, and agar diffusion methods. The literature also shows evaluation of GAT in river water (35) and biological matrixes (Figure 2B) such as human plasma (36–39), mouse plasma (40), human serum (41, 42), urine (42), and aqueous humor (16, 43) by HPLC, HPLC-SF, and HPLC-MS methods.

The most identified method in the literature (Figure 3) was HPLC (1–3, 11, 13, 14, 16–19, 24, 26, 28, 30–32, 34–43) both for the analysis of biological matrixes as pharmaceuticals.

HPLC is a method widely used in the analysis of both pharmaceutical and biological matrixes because it presents advantages such as precision in the analysis, allowing the analysis of various types of samples, efficiency, and high resolution (44). One factor that must be considered in the HPLC method is the choice of detection system. It must be directly associated with the objective of the analysis, which sample is to be analyzed and what is the sensitivity required for analysis, aiming not only to reduce operating costs but also to ensure the conscious and necessary use of the detection system.

Spectrophotometry in the UV-Vis region is widely used for the analysis of pharmaceutical matrixes. Studies in the literature show UV-Vis methods with sensitivity and specificity necessary for the evaluation of GAT (2, 3, 6, 12, 15, 20, 21, 23, 27, 29, 33). The main advantages of this method when compared to HPLC are the lower cost and simpler equipment, in addition to using less solvents/diluents, which can be purified water, for example, as is the case of GAT, making the method green, ecologically correct, and low cost.

The titration method (7) was also described in this study for GAT quantification, being able to precisely and accurately quantify GAT in tablets. This method is a simple, fast, and inexpensive alternative, which can be easily handled and does not require high-cost equipment when compared to other physicochemical methods already described, thus being easily used in analytical routines.

Table 1. Analytical methods available in the literature for the evaluation of gatifloxacin

Method	Condition	Detection system	Matrix	Reference
Vis[b]	Diluent: Purified water with 3.0 mL of bromocresol green. Extraction with 5.0 mL of chloroform by mechanical stirring for 2 min, repeat 2 times. The organic phase should be read.	415 nm	Capsule	(26)
Vis	Diluent: Purified water with 3.0 mL of bromocresol purple. Extraction with 5.0 ml of chloroform by mechanical stirring for 2 min, repeat 2 times. The organic phase should be read.	412 nm	Capsule	(26)
Vis	Diluent: Purified water with 3.0 mL of bromophenol blue. Extraction with 5.0 ml of chloroform by mechanical stirring for 2 min, repeat 2 times. The organic phase should be read.	417 nm	Capsules	(26)
Vis	Diluent: Purified water with 3.0 mL of bromothymol blue. Extraction with 5.0 ml of chloroform by mechanical stirring for 2 min, repeat 2 times. The organic phase should be read.	414 nm	Capsules	(26)
Vis	Diluent: Purified water with 1.0 mL of ferric chloride, followed by 1 mL of BHT.[a] Keep the solution at room temperature for 10 min and proceed with the reading.	433 nm	Tablets	(51)
Vis	Diluent: Purified water with 0.5 mL of ferric nitrate	470 nm	Tablets	(50)
Vis	Diluent: Purified water and addition of eriochrome black	510 nm	Eye drops	(25)
UV[c]	Diluent: Purified water	287 nm	Tablets	(2, 6)
UV	Diluent: Methanol	295 nm	Tablets	(51)
UV	Diluent: Purified water and methanol (50:50, v/v)	286 nm	Tablets	(20)
UV	Diluent: Propylene glycol: sodium citrate (10%, w/v)	288 nm	Tablets	(21)
UV	Diluent: Methanol	268 nm	Eye drops	(12)
UV	Diluent: Purified water: acetonitrile (50:50, v/v)	279 nm	Eye drops	(3)
UV	Diluent: Purified water: acetonitrile (30:70, v/v)	348 nm	Standard	(15)
UV	Diluent: Ethanol	260 nm	Eye drops	(29)
HPLC[d]	Mobile phase: sodium dodecyl sulfate: tetrabutylammonium acetate: citric acid: acetonitrile (10:10:25:50, v/v/v/v). Column: Adsorbosphere HS C18 (250 × 4.6 mm, 5 μm). Flow 1.0 mL/min	293 nm	Human serum and urine	(54)
HPLC	Mobile phase: 0.025 M disodium hydrogen phosphate (pH 3.0): acetonitrile (80:20, v/v). Column: X Terra MS C 18 (50 × 3 mm, 5 μm). Flow 1.0 mL/min	293 nm	Human plasma	(31)
HPLC	Mobile phase: 5% acetic acid: methanol: acetonitrile (70:15:15, v/v/v). Column: Phenomenex Luna C18 (250 × 4.6 mm, 5 μm). Flow 1.0 mL/min	287 nm	Tablets	(1)
HPLC	Mobile phase: purified water: acetonitrile (80:20, v/v). Column: LiChrospher 100 RP-18 (125 × 4 mm, 5 μm). Flow 1.0 mL/min	293 nm	Tablets	(17)
HPLC	Mobile hase: acetonitrile: methanol: purified water (40:40:20, v/v/v). Column: Mediterranea C18 (250 × 4,6 mm, 5 μm). Flow 1.0 mL/min	286 nm	Tablets	(18)
HPLC	Mobile phase: purified water: acetonitrile (52:48, v/v). Column: Pher 100 RP-18e (250 × 4 mm, 5 μm). Flow 1.0 mL/min and injection volume 20 μL	292 nm	Eye drops	(24)
HPLC	Mobile phase: potassium dihydrogen phosphate: acetonitrile (70:30, v/v). Column: HiQSil C18 (250 × 4.6 mm, 5 μm). Flow 1.0 mL/min and injection volume 20 μL	247 nm	Tablets	(19)
HPLC	Mobile phase: 5% acetic acid: methanol: purified water (70:15:15, v/v/v). Column: Phenomenex Luna C18 (250 × 4.6 mm, 5 μm). Flow 1.0 mL/min	287 nm	Tablets	(2)

(continued)

Table 1. (continued)

Method	Condition	Detection system	Matrix	Reference
HPLC	Mobile phase: sodium dihydrogen phosphate dihydrate: acetonitrile (80:20 v/v). Column: Kromasil C 18 column (250 × 4.6 mm, 5 μm). Flow 1.0 mL/min	293 nm	Human plasma	(56)
HPLC	Mobile phase: purified water: acetonitrile: triethylamine (75:25:0.35, v/v/v). Column: Lichrospher 100 C18 (250 × 4 mm, 5 μm). Flow 1.0 mL/min and injection volume 20 μL	320 nm	Tablets	(52)
HPLC	Mobile phase: methanol: buffer solution (55:45, v/v). Column: BDS Hypersil C8 C18 (250 × 4.6 mm, 5 μm). Flow 1.5 mL/min	270 nm	Eye drops	(55)
HPLC	Mobile phase: sodium dihydrogen phosphate dihydrate: acetonitrile (75:25, v/v). Column: BDS Hypersil C18 (250 × 4.6 mm, 5 μm). Flow 1.5 mL/min	260 nm	Human plasma	(32)
HPLC	Mobile phase: disodium hydrogen phosphate buffer: acetonitrile (75:25, v/v). Column: C18-DB, 50306-U (250 × 4.6 mm, 5 μm). Flow 1.0 mL/min and injection volume 20 μL	293 nm	Tablets, eye drops, suspension	(11)
HPLC	Mobile phase: purified water: acetonitrile (50:50, v/v). Column: Shiseido C18 (250 × 4.6 mm, 5 μm). Flow 1.0 mL/min and injection volume 20 μL	241 nm	Eye drops	(3)
HPLC	Mobile phase: phosphate buffer pH 3.0: methanol (42:58, v/v). Column: Hypersil C8, (250 × 4.6 mm, 5 μm). Flow 1.5 mL/min	270 nm	Eye drops	(14)
HPLC	Mobile phase: acetonitrile: 0.2% triethylamine (17:83, v/v). Column: Phenomenex C8 (250 × 4.6 mm, 5 μm) and PCX-BT pre-column. Flow 0.4 mL/min and injection volume 20 μL	330 nm	River water	(30)
HPLC	Mobile phase: 0.2M sodium dodecyl sulfate: 12.5% n-propanol: 0.3% triethylamine in 0.02M orthophosphoric acid (pH 7.0). Column: Shimadzu C18 (150 × 4.6 mm, 5 μm). Flow 1.0 mL/min	270 nm	Eye drops	(26)
HPLC	Mobile phase: acetonitrile: ethanol (90:10, v/v). Column: Phenomenex Lux cellulose-2 (250 × 4.6 mm, 3 μm). Flow 1.0 mL/min	290 nm	Eye drops	(27)
HPLC	Mobile phase: acetonitrile: methanol (70:30, v/v): ammonium acetate buffer (20:80, v/v). Column: C18 Zorbax Eclipse plus (100 × 4.6 mm, 5 μm). Flow 1.0 mL/min	288 nm	Eye drops	(13)
HPLC	Mobile phase: sodium dihydrogen phosphate buffer pH 3.0: acetonitrile (72:28, v/v). Column: Xbridge C18 (250 4.6 mm, 5 μm). Flow 1.0 mL/min	243 nm	Eye drops	(28)
HPLC-SF[g]	Mobile phase: phosphate buffer: acetonitrile (88:12, v/v). Column: Supelcosil ABZ + Plus (150 × 4.6 mm, 5 μm) and LiChroCART pre-column (4 × 4 mm, 5 μm). Flow 1.0 mL/min	296 nm (excitation) and 504 nm (emission)	Human serum	(35)
HPLC-SF	Mobile phase: phosphoric acid: methanol: acetonitrile: triethylamine (64.8:15:20:0.2, v/v/v/v). Column: C18 (250 × 4.6 mm, 5 μm). Flow 1.0 mL/min and injection volume 30 μL	295 nm (excitation) and 480 nm (emission)	Mouse plasma	(34)
HPLC-SF	Mobile phase: 0.1% aqueous formic acid (pH 3.0 with triethylamine): acetonitrile: methanol, 0 min (80:0:20, v/v/v), 3 min (82:2:16, v/v/v), 7 min (42:2:56, v/v/v), 13 min (80:0:20, v/v/v). Column: Purospher Star C18 (55 × 4 mm, 3 μm). Flow 1.0 mL/min	260 nm (excitation) and 455 nm (emission)	Human plasma	(33)
HPLC-MS[f]	Mobile phase: acetonitrile: water (20:80, v/v). Column: Xbridge C18 (50 × 2.1 mm, 3.5 μm). Flow 1.0 mL/min	*m/z* 376.2→358.2	Aqueous humor	(36)
HPLC-MS	Mobile phase: A (water containing 0.1% formic acid): B (methanol containing 0.1% formic acid); A 75–50% 3 min, 50–5% 0.2 min, 5% 0.2 min, 5–75% 0.1 min and 75% 0.5 min. Column: C18-Acquity	*m/z* 376.18→261.06	Aqueous humor	(16)

(continued)

Table 1. (continued)

Method	Condition	Detection system	Matrix	Reference
	UPLCBEH (100 × 2.1 mm, 1.7 μm) Flow 0.3 mL/min and injection volume 5 μL at 45 °C			
HPTLC[e]	Mobile phase: n-butanol: methanol: ammonia (5:1:2, v/v/v). Stationary phase: silica gel 60F254 (20 × 10 cm). Chamber saturation time 30 min, temperature 29 ± 4 °C, migration distance 40 mm, spray rate 10 sec/μL	292 nm	Tablets	(53)
SF	Diluent: Purified water	290 nm (excitation) and 487 nm (emission)	Tablets	(22)
Titration	Diluent: Glacial acetic acid. Titrator: 0.1 M perchloric acid. Indicator: 0.1% crystal violet (5 drops)	Color change	Tablets	(7)
Agar diffusion	Stock solution diluent: Purified water. Diluent for dilutions: Phosphate buffer pH 6.0. Concentrations 4, 8, 16 μg/mL (3 × 3 design), *Bacillus subtilis* ATCC 9372 on Grove Randall 11 agar (1:50, v/v), 37 °C for 18 hours.	Inhibition halos	Tablets	(8)
Agar diffusion	Stock solution diluent: Purified water. Diluent for dilutions: Phosphate buffer pH 6.0. Concentrations 4, 8, 16 μg/mL (3 × 3 design), *Bacillus subtilis* ATCC 9372 in nutrient broth (1:50, v/v), 37°C overnight	Inhibition halos	Tablets	(2)

[a] BHT = Butylated hydroxytoluene.
[b] Vis = Spectrophotometry in the visible region.
[c] UV = Spectrophotometry in the ultraviolet region.
[d] HPLC = High-performance liquid chromatography.
[e] TLC = Thin-layer chromatography.
[f] HPLC-MS = Liquid chromatography of high efficiency coupled to mass spectrometry.
[g] SF = Spectrofluorimetry.

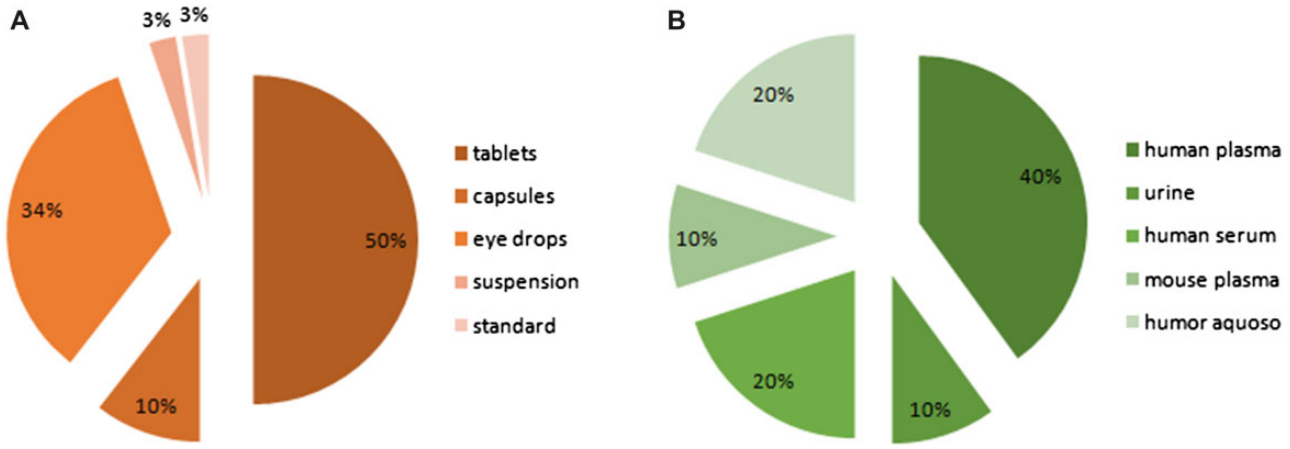

Figure 2. Distribution of gatifloxacin samples analyzed in (A) pharmaceutical matrixes and (B) biological matrixes.

Two microbiological studies by agar diffusion were found in the literature (2, 8) and none by turbidimetry to assess the potency of GAT. The turbidimetric method is an excellent alternative for green analytical chemistry, as it reduces analysis time and optimizes materials, equipment, and analysts. Turbidimetric methods are already described in the literature for the analysis of other fluoroquinolones and they have been shown to be as sensitive and specific as conventional methods (45–51). Furthermore, turbidimetric methods can be further miniaturized, employing smaller amounts of products, thus having the added advantage of reducing waste generated and costs.

Microbiological analyzes are fundamental and essential when it comes to antimicrobial products. A physicochemical method is not always able to reveal the real potency of the active; only agar diffusion or turbidimetric methods are capable of this—hence, the importance of their association. An antimicrobial product on the market with potency below the specifications, because it has been analyzed only by physicochemical methods, can cause microbial resistance, decrease in the

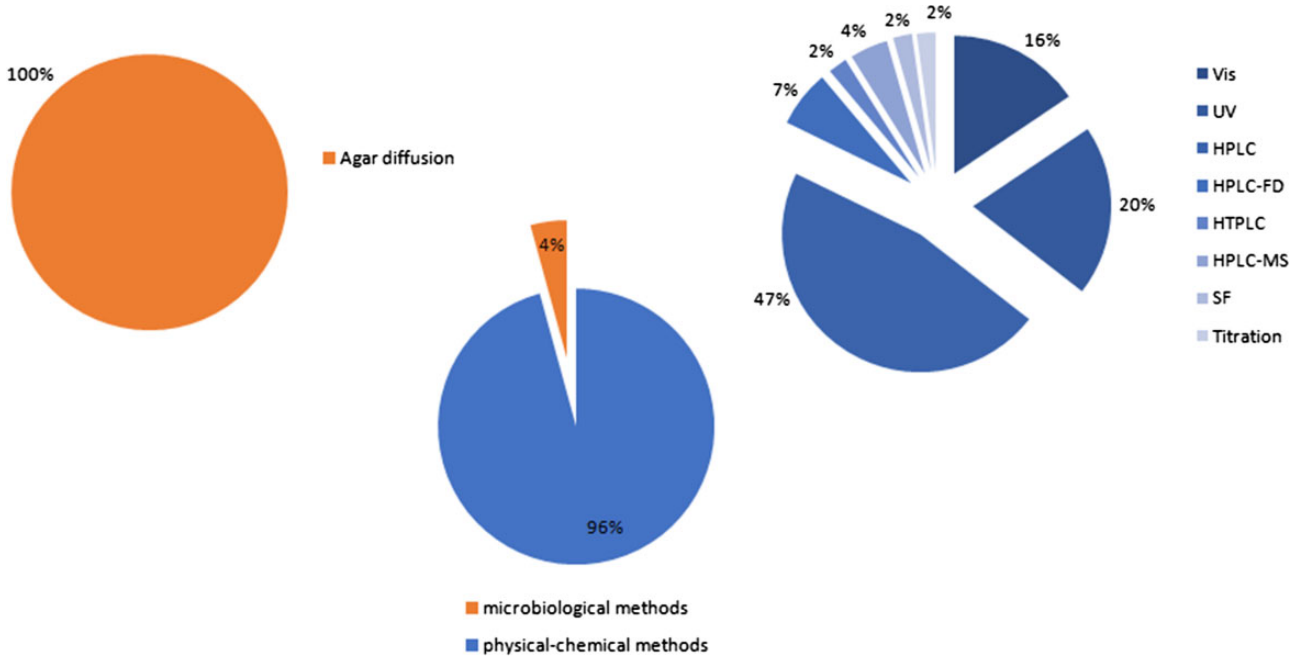

Figure 3. Distribution of physicochemical (blue) and microbiological (orange) analytical methods available in the literature for the evaluation of gatifloxacin.

patient's quality of life, overload the health system, spread of resistant microorganisms, costs for the patient . . . (52, 53).

The most used reagents in the analyzes were acetonitrile (1, 3, 13, 15, 17–19, 24, 28, 31, 37–39, 41–43), methanol (1, 2, 12–14, 16, 18, 20, 25, 34, 35, 38), and buffer (2, 8, 11, 13, 14, 19, 32, 34, 41).

The use of methanol, acetonitrile, and buffer in analytical methods such as HPLC are very common, being first choices in many cases, without trying to use other less aggressive solvents, such as ethanol, for example. Most solvents have characteristics that contribute to air pollution, such as being volatile, flammable, and toxic, not only for the analyst, but also for the environment. On the other hand, buffer solutions, in addition to demanding preparation time, have a short half-life, which requires frequent preparations, contributing to an increase in the costs and time of the methods (54–56).

Acetonitrile (42% of the methods chose to use) is easily absorbed by the body and, when metabolized, produces cyanide, which is capable of affecting respiratory functions. Furthermore, even though acetonitrile is neutralized, the disposal process takes place by incineration, which generates residues that contribute to acid rain. Acid rain damages structures and affects rivers and vegetation. Therefore, the disposal process will cause damage to human health and the environment, which is why the use of certain solvents must be rethought during the analytical processes (56).

Since GAT is soluble in water and methods that employ it are already available in the literature (2, 3, 6, 15–18, 20, 22–24, 27–29, 43, 44), the use of solvents such as methanol, acetonitrile, and buffer solutions for the analysis of GAT in pharmaceutical and biological matrixes should be rethought, either for the complete replacement of these solvents or for a reduction in their use. Thus, when developing a method, it is interesting and intelligent for the analyst to consider the specifications of the analysis, the intended objective as well as the multidimensional impacts of the analytical choices.

Conclusions

GAT is a fourth-generation fluoroquinolone antimicrobial used to treat respiratory, genitourinary, and eye infections caused by Gram-positive and Gram-negative bacteria. Among the physicochemical analytical methods described for quantification of GAT in pharmaceutical and biological matrixes are UV-Vis, HPLC, HPTLC, spectrofluorimetry, and titration. As for microbiological methods, few studies were found and only one method used, by diffusion in agar. In all the methods described there are opportunities for improvements in time, cost, dynamics, generation of less waste, and/or use of less toxic agents. Thus, this review shows the status of analytical methods, both physicochemical and microbiological, for the analysis of GAT in pharmaceutical and biological matrixes, also addressing its context in green and sustainable analytical chemistry.

Conflict of Interest

The authors have no financial or other potential conflict of interest.

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