Assessment of greenness for the determination of voriconazole in reported analytical methods

Hemanth Kumar Chanduluru and Abimanyu Sugumaran*

Analytical research with adverse environmental impact has caused a severe rise in concern about the ecological consequences of its strategies, most notably the use and emission of harmful solvents/reagents into the atmosphere. Nowadays, industries are searching for the best reproducible methods. Voriconazole is a second-generation azole derivative used effectively in the treatment of Candida and Aspergillus species infections and oropharyngeal candidiasis in AIDS patients. Recently it has become the drug of choice in treating mucormycosis in several countries, which raises the need for production in large quantities. The present review deals with various recent important analytical techniques used to estimate voriconazole and its combination in pharmaceutical formulations and biological fluids. The methods show their own unique way of analyzing voriconazole in different matrices with excellent linearity, detection, and quantification limits. Additionally, this article deals with methods and solvents analyzed for their impact on the environment. This is followed by estimating the degree of greenness of the methods using various available assessment tools like analytical eco-scale, national environmental method index, green analytical procedure index, and AGREE metrics to confirm the environmental impact. The scores obtained with the evaluation tools depict the quantum of greenness for the reported methods and provide an ideal approach adopted for VOR estimation. Very few methods are eco-friendly, which shows that there is a need for the budding analyst to develop methods based on green analytical principles to protect the environment.

1. Introduction

Developing analytical methods to determine medicines in bulk or pharmaceutical dose form is never an easy endeavour due to chemical complexity and variety. Many attempts have been made by analytical scientists from a variety of sectors to produce an individual robust analytical technique that is also compliant.
with regulatory requirements. Regulators are increasingly seeking eco-friendly techniques to reduce or eliminate the formation of harmful effluents due to pollution created by analytes, solvents, and chemicals used in analytical departments. It is the purpose of this study to discuss different modern and essential analytical methods that have been developed to estimate voriconazole and its combination in pharmaceutical formulations and biological fluids, as well as the degree of greenness of the same.

1.1. Voriconazole

Voriconazole (VOR) is chemically \((2R,3S)-2-(2,4\text{-difuorophenyl})-3-(5\text{-fuiuoro}-4\text{-pyrimidinyl})-1-(1H-1,2,4\text{-triazol-1-yl})\)-2-butanol (Fig. 1). It works by inhibiting the fungal 14-alpha-lanosterol demethylation cytochrome P450-dependent 14-sterol demethylase, a vital enzyme in ergosterol biogenesis required to form fungal cell walls.\(^1\)-\(^4\)

VOR has become an essential drug for treating mucormycosis or black fungus in the initial stages of their existence as well as SARS-CoV-2. VOR is also used for people diagnosed with Candida and Aspergillus species infection, which have death rates of more than 60%. In addition, they are more likely to develop candidemia, leading to resistance to triazole antifungal drugs if not appropriately treated.\(^5\) While there are several treatment alternatives, currently available antifungal medicines do not meet the needs of many patients, especially those who take their medication by oral administration or through intravenous injection. Voriconazole (VOR) is the most critical triazole antifungal drug to enter the arsenal of antifungal agents. It has a structure similar to that of fluconazole and an activity spectrum comparable to that of itraconazole. In May 2002, the FDA approved VOR to treat Fusarium species refractory Scedosporium apiospermum and invasive aspergillosis infections. VOR has also been shown to be a promising drug for empiric treatment of febrile neutropenia in studies.\(^6\)

The Jing Wang et al. study revealed that VOR is effectively used as the best prophylaxis option for patients undergoing hematopoietic stem cell transplantation.\(^7\) Voriconazole has high bioavailability (96%) and has been shown to penetrate various eye areas, with adequate concentrations obtained to cover a wide variety of keratitis-causing fungi. Voriconazole eye drops, produced ad hoc and then used off-label, have been recommended successfully to treat keratitis. Voriconazole showed adequate penetration via the cornea into the aqueous humour after topical treatment without affecting intraocular tolerability.\(^8\)

VOR is available in different dosage forms for the treatment of many fungal infections. Analytical techniques are used throughout the drug development process, from pre-clinical to post-clinical testing, to understand the drug’s physical and chemical stability, impact on dosage form selection and design, and quantification of impurities. Various technological enhancements in separation science, modern sophisticated spectroscopic and liquid chromatographic techniques, and the use of bioanalytic tools for molecular recognition and testing have been of great advantage to pharmaceutical analysis in recent years. The whole dosage form development process requires effective, precise analytical procedures to support every step. Pharmaceutical companies are obliged to use the most accurate, prudent, and dependable quality control methods to quantify VOR. The reported best analytical methods for estimating VOR present in different pharmaceutical dosage forms are highlighted to illustrate the importance of VOR analyses (Fig. 2 and 3).

Analytical methods such as chromatographic techniques utilize toxic solvents and have a deleterious effect on the environment.
1.2. Effect of analytical methods on the environment

Liquid chromatography and its related methods are widely used and accepted for the estimation of pharmaceutical substances. The mobile phase commonly employs components such as water, buffers, additives to adjust pH, and organic modifiers like methanol (MeOH) and acetonitrile (ACN). These organic modifiers are mostly preferred for the LC method because of their ease of practical applicability as well as miscibility in water, low UV wavelength cut-off range (205 nm for MeOH and 190 nm for ACN), high purity, and low or no reactivity with most pharmaceutical substances.\(^9\)\(^{10}\) Despite the remarkable advantages of liquid chromatography, most reported solvents have a highly adverse environmental impact.\(^11\)\(^{12}\) ACN causes adverse health effects since it is a systemic irritant, is flammable, toxic, and volatile, and is categorized as an occupational hazard for analysts due to risk of inhalation and skin/eye contact. Methanol is also non-hazardous and volatile, and is categorized as an occupational hazard for laboratory workers due to its flammability, toxicity, and irritant effects since it is a systemic irritant, is flammable, and is categorized as an occupational hazard for analysts due to risk of inhalation and skin/eye contact. Methanol's vapor pressure is low; thus, it can easily volatilize into the surrounding air. Methanol degrades through interaction with airborne hydroxyl radicals after volatilization, and its half-life is about 18 days.\(^13\) Organic solvents like MeOH and ACN that are frequently used in chromatographic analysis affect animals, birds, and fish, cause death, and affect biota fertility. These two solvents are also included in the EPA's (Environmental Protection Act) TRI (Toxicity Reactivity Ignitability) list as hazardous solvents.

RP-HPLC consists of a stationary phase (column) and mobile phase (solvents) with a flow rate of 0.8–1.5 mL min\(^{-1}\). On average, usage of HPLC on a single day with a 50 : 50 ratio of organic phase and buffer with 1 mL min\(^{-1}\) flow rate generates 1.5 L/24 h (750 mL of organic waste and 750 mL of aqueous buffer waste containing toxic chemicals in a single working day). Developing an eco-friendly method by controlling the waste generated without affecting the method quality and performance for analyzing compounds in HPLC is a mammoth task. However, this problem can be reduced by applying green analytical principles in drug analysis. This particular review aims to summarize and examine various VOR estimation methodologies currently available using different instrumental methods along with the estimation of the degree of the greenness of the same.

Anastas\(^14\) portrayed 12 green analytical chemistry principles from the general green chemistry principles to help analysts in developing an environmentally fit method that can be used in the long term without affecting the environment.

1.3. Green analytical chemistry principles\(^15\)\(^{–}\)\(^20\)

Green analytical chemistry principles are derived from the modification of green chemistry principles. Each principle has its unique role in the development of an analytical method. However, it is impractical to apply all the principles, but the number of principles incorporated in the method development makes the output most eco-friendly. (1) The generation of hazardous waste in sampling can be reduced by eliminating large volume dilutions. (2) The direct sampling technique cannot be implemented to analyze all samples in liquid chromatography due to its limitation. Still, this principle can be satisfied by selecting other techniques like IR and FTIR to make the analysis eco-friendlier. (3) Green or biodegradable chemicals or solvents must be used instead of toxic chemicals in the environment, e.g., methanol; acetonitrile needs to be replaced by ethanol and propylene carbonate. (4) Renewable solvents like 1,1-diethoxymethane, isosorbide dimethyl ether, eucalyptol, rose oxide, \(\gamma\)-terpinene, and \(\alpha\)-pinene\(^21\) shall be replaced by the other toxic solvents for the analysis. (5) The waste generation in liquid chromatography (LC) techniques has a significant environmental impact and is inevitable. Instead, the generated waste can be recycled using different distillation processes. (6) Upgrading existing techniques by miniaturization, wherein a lab-on-a-chip miniature device is developed, is a sophisticated technique for analyzing compounds in a simple step. (7) The novel combination of molecular biology with microelectronics has resulted in the electronic detection of biomolecules through field-effect transistors (FETs) and lab-on-a-chip biosensors.\(^22\) (8) Multiple analytical techniques have to be applied for the new method development. (9) Energy consumption for analyzing a sample should be as less as possible to make the method greener; for example, the application of UPLC rather than HPLC uses less energy. (10) Derivatization is an extra step for analyzing a drug that should always be avoided in most cases but it is an inevitable step; then, the green reagents need to be utilized for this step e.g., nicotinic acid, hydridantin dihydrate, ferrocene carboxaldehyde, and \([+]-\) diacetyl-\(L\)-tartaric anhydride.

(11) In situ measurements necessitate that equipment is placed directly at the site of analysis and in touch well with the subject of interest of the drug or sample. (12) Occupational hazards need to be nullified in consideration towards the analyst.

As it is mentioned earlier that application of all the principles in analytical methods is practically very difficult so some strategies have to be applied for developing the methods that should be environmentally safe.

1.4. Strategies for greening an analytical process – solvent reduction\(^23\)\(^{–}\)\(^33\)

Reduction of solvent consumption leads to the reduction of waste. There are numerous ways to reduce it. (i) Using RP-HPLC methods instead of the normal phase will allow for polar solvents that are non-hazardous. (ii) Short column usage will make the elution faster and reduce waste. (iii) Microflow and capillary HPLC columns will decrease the flow rates and reduce solvent consumption. (iv) Higher column temperature will decrease water viscosity and also enhances polar characteristic that reduces the use of organic modifiers. (v) Newer columns like fused core particle columns will have smaller particle sizes that makes the separation faster and better. (vi) Finally, miniaturization from HPLC to UPLC with short columns and high pressure makes the analysis faster and decreases the waste generated.

1.4.1. Solvent replacement\(^29\)\(^{–}\)\(^31\) Flammable and toxic solvents can be replaced by bio solvents, as follows: (i) toxic solvents such as ACN, MeOH, and ethyl acetate can be replaced by eco-friendly solvents such as propylene carbonate, ethanol, and ethyl lactate, respectively. These eco solvents have similar
properties to the other solvents and are a perfect replacement. (ii) Superheated water at 80–250 °C can be an alternative in specific cases as water is inexpensive, non-flammable, eco-friendly and has a low UV cut-off wavelength. However, this has drawbacks, such as the fact that hydrophobic samples cannot be analyzed by simple water and thermolabile drugs cannot be determined at these high temperatures. (iii) Superheated fluid can also be a better substitute as it provides higher separation and faster elution.

1.4.2. Sample preparation. Choosing a direct analytical method is not possible for analyzing all samples in LC. Instead, sample preparation can be done by (i) using eco-friendly solvents for preparation of samples by avoiding transportation and (ii) unavoidable extraction processes using toxic chemicals can be replaced by micro-wave assisted, ultrasound-assisted, and pressurized liquid extraction.

1.4.3. Analytical quality by design (AQBd). The QbD approach suggests looking into the quality of the analytical process during the development stage itself. It says that quality should be built into the process design rather than testing into results of the analytical process. When the QbD principles are applied in the method development of pharmaceutical substances, the strategy can be called analytical quality by design. The outcome of AQBd is well understood and fit for its intended purpose with sturdiness throughout the lifecycle.

2. Evaluation of greenness

A method should be carefully assessed before claiming the greenness of the technique. However, GAC’s lack of dedicated evaluation tools has been considered the main problem in greenness estimation. Although different evaluation tools are available for green chemistry, all have their limitations in GAC. A few green assessment tools that can be applied in GAC are as follows.

2.1. National environmental methods index (NEMI) NEMI is considered as the oldest evaluation tool used to assess green chemistry and represent a quadratic pictogram. Each quadra represents one factor: persistent bioaccumulative and toxic (PBT), hazardous, corrosive, and waste. If a chemical is used in the method listed in the EPA (Environmental Protection Act) TRI (Toxicity Reactivity Ignitability) list, the quadra must be left blank. If not listed, it should be coded with green color. Solvents/chemicals, if listed in Resource Conservation and Recovery Act (RCRA), were used in the method, then the next quadrant should be left blank; if the chemicals used in the process are non-corrosive, then the next quadrant should be coded green; finally, if the waste produced by the method is more than 50 g mL⁻¹ then the next quadrant has to be left blank.

2.2. Green analytical procedure index (GAPI) This evaluation system is also similar to NEMI, but it covers a few more aspects. The hazardous solvents need to be checked in the National Fire Protection Act (NFPA) instead of the EPA TRI list for GAPI. The pictogram contains three colors: green, yellow, and red instead of green or none. Colour should be coded based on the solvents used, like highly toxic coded as red, moderately toxic as yellow, and less or non-toxic as green. Different parameters that need to be checked for the GAPI are sample preparation, preservation, transport, storage, type of method, the scale of extraction, solvents used, additional treatments, and amount of reagents used. Although it covers most aspects required to evaluate GAC, the output based on color representation makes this a qualitative rather than quantitative tool.

Plotka developed a new tool in 2021 named complementary GAPI or complex GAPI, which is an advancement of GAPI. This complex GAPI is a combination of classical GAPI and E-Factor, where E-Factor mainly focused on the synthesis of the chemicals along with the product yield, purity, waste, etc. Because the study design is solely concerned with the development of analytical methods and their greenness estimate, and there is no published literature on synthesis data of analyte and solvent, the implementation of the complex GAPI is minimal.

2.3. Analytical eco-scale (AES) Although eco-scale was first introduced to evaluate GC, it shows promising results when applied to GAC. When eco-scale is used for analytical methods, then it can be called AES. This tool has an output of numerical values as the eco-scale score equals ‘100’. The deduction of the eco score is based on the penalty points (PP) scored by the method.

PP for reagents used:
- Less than 10 g or mL = 1 PP.
- 10–100 g or mL = 2 PP.
- More than 100 g or mL = 3 PP.

PP based on chemicals used:
- Pictogram with danger representation = 2 PP.
- Pictogram with warning representation = 1 PP.
- No Pictogram representation = 0 PP.

PP based on energy used per sample.
- Less than or equal to 0.1 kW h = 0 PP.
- Less than or equal to 1.5 kW h = 1 PP.
- More than or equal to 1.5 kW h = 2 PP.

PP based on waste generated.
- No waste = 0 PP.
- Less than 10 g or mL = 1 PP.
- 1–10 g or mL = 3 PP.
- More than 10 g or mL = 5 PP.

The results of greenness from the eco scale.
- Eco score ≥ 75 = worthy green method.
- Eco score ≥ 50 = optimal green method.
- Eco score < 50 = not a green method.

2.4. Analytical GREEEnness (AGREE) It is a software-based assessment tool covering all the 12 principles of GAC. It works based on graphical user interface (GUI) technology. It gives a result in numerical values with a high of 1 showing complete green, whereas the decrease in the number indicates a reduction in greenness. It contains 12 steps as the
sampling procedure, sample size, in situ measurement, steps in the process, miniaturization, derivatization, waste, number of analytes, energy, type of reagents, toxic reagents, and operator safety need to be filled in software for obtaining the green results. The score of 1 indicates a complete green method, and the result near 1 indicates that the process was obeying the eco-friendly conditions and vice versa.

2.5. Analytical method greenness score (AMGS)\textsuperscript{66–68}
AMGS is a spreadsheet calculator dedicated to the chromatographic method of analysis. It is an amalgamation of HPLC-environmental assessment tool cumulative energy demand (CED) for instrument and solvent selection, analytical mass volume intensity (AMVI) for assessing solvent wastage, and safety, health, and environmental assessment (SHE) for determining solvent safety through geometric mean. The present spreadsheet works by having the data of the method and executing it for the evaluation; the output value should be as low as possible to obtain the greenest of the results.

2.6. Carbon footprint
Carbon footprint is an important concept for calculating the emission of global warming gases into the atmosphere generated during any fossil fuel combustion. It is important to calculate the carbon footprint for an industry in a whole process to understand the overall emission of greenhouse gasses into the atmosphere. The present study deals with the analysis of voriconazole using different analytical instruments, as this equipment utilizes significantly fewer energies for the determination of single samples (HPLC consumes $\geq 1.5$ kW h, UPLC and UV-VIS spectrophotometry absorbs <1.5 kW h, and LCMS utilizes >1.5 kW h). This energy consumption generates significantly less CO$_2$, which produces negligible effects on the environment. This energy measurement was incorporated into a few greenness assessment metrics like GAPI, AES, and AGREE, which nullifies the complexity of the carbon footprint analysis regarding energy consumption.

The above assessment tools have been utilized to demonstrate the greenness in reported methods for the analysis of VOR to scrutinize and select the best approach in terms of eco-friendliness. Although each greenness assessment tool utilizes a different way of analyzing the greenness profile, the final results help to pictureize and identify the more environmentally benign method with its environmental impact. Here, four assessment tools were used for assessing the greenness of the reported methods.

3. Methods for the quantification of VOR and its combinations

3.1. Official methods
VOR was added to the pharmacopeias such as the United States Pharmacopeia (USP), British Pharmacopeia (BP), and Indian Pharmacopoeia (IP) officially\textsuperscript{61–63} in 2018 after 16 years of approval from the FDA. All these methods have used liquid chromatography as a technique for the analysis of VOR.

3.2. United States Pharmacopeia, British Pharmacopoeia, and Indian Pharmacopoeia
The major official pharmacopoeias state that the method for the analysis of VOR was HPLC using an L1 packing column with end-capped octadecyl silyl silica gel (150 mm $\times$ 3.9 mm, 4 $\mu$m), with a mobile phase consisting of ACN : MeOH : ammonium formate (pH 4.0) in a ratio of 15 : 30 : 55 v/v/v at a flow rate of 1.0 mL min$^{-1}$ determined in a wavelength of 256 nm with a retention time of 8 min for VOR. The acceptance criterion according to USP, BP, and IP for VOR was 97.5–102.0%. The greenness assessment for the official method was performed using the NEMI, GAPI, AES, and AGREE assessment tools for the pharmacopeia data and is depicted in Fig. 4.

3.3. Reported methods
Articles were collected for VOR in pharmaceutical dosage forms from Science Direct, PubMed, Scopus, Taylor and Frances, Google, and different web sources from 1997 to 2021 and were organized and executed for the present review.

3.3.1. UV-VIS spectrophotometry. Spectroscopic methods like UV-VIS play a significant role in the quantification and qualification of most of the drugs. UV-VIS is essential in all quality control departments to make the analysis of drugs accurate and simple. It is also combined with modern analytical techniques like HPLC to give more accurate results. The determination of VOR by UV has used MeOH, water, HCL, and phosphate buffer in a wavelength range around 252–256. The visible range for the derivatization chemicals/solvents like tro-paetine ooo and azocarmine-G was used at a wavelength of 500 and 550 nm. The overall spectrophotometric methods reported for VOR analysis and the results of the assessment of greenness by applying four tools are depicted in Table 1.

3.3.2. High-pressure liquid chromatography. HPLC is a critical and widely used analytical method for quantifying the majority of pharmaceutical dosage forms. Additionally, HPLC is a very trustworthy technique for measurement due to its precision, robustness, and sensitivity. Estimating VOR has utilized a set of different mobile phase combinations with a common organic phase like MeOH and ACN and various phosphate buffers. Initial development results showed the longest retention time of 21.06, but technology improvisation leads to the shortest elution of analytes at 3.02 min.\textsuperscript{72} Although the linearity selected in the reported HPLC methods did not
| S. no. | Matrix of VOR | Reagents or solvents | Detection (nm) | Linearity (µg mL⁻¹) | NEMI | GAPI | AES | AGREE | Ref. |
|--------|---------------|----------------------|---------------|---------------------|------|------|-----|-------|------|
| 1      | Bulk and tablet | MeOH                | 252           | 5–80                |      |      |     |       | 0.77 | 66 |
| 2      | Bulk and tablet | Milli Q pore water  | 255           | 5–35                |      |      |     |       | 0.94 | 68 |
| 3      | Tablet         | MeOH                | 256           | 5–30                |      |      |     |       | 0.77 | 67 |
|        | (1) 0.1 M HCl and tropaeoline | (1) 500           | (1) 5.0–2     |                     |      |      |     |       | 0.86 | 71 |
| 4      | Tablets        |                     |               |                     |      |      |     |       | 0.75 | 69 |
|        | (2) pH 1.5 buffer and azo carmine-G | (2) 550           | (2) 10–50    |                     |      |      |     |       | 0.94 | 70 |
| 5      | Bulk powder and pharmaceutical dosage form 0.1 N HCl | | 256           | 10–60             |      |      |     |       | |
| 6      | Tablets        | Phosphate buffers (pH 2.0, 4.0, 6.8, and 7.0) 200–400 | 5–60 |                     |      |      |     |       | |
| S. no. | Drug substance | Stationary phase and mobile phase | Linearity (µg mL⁻¹) | Rₜ (min) | NEMI | GAPI | AES | AGRE | Ref. |
|-------|---------------|-----------------------------------|---------------------|---------|------|------|-----|------|------|
| 1     | VOR           | Merck LiChrospher 100 RP-8 (125 mm × 4.6 mm, 5 µm) 0.6% triethylamine (pH 6.0) : MeOH (50 : 50 v/v) | 20–100 NA |          |      |      |     | 36 + 1 + 3 + 5 = 45 | Eco score = 55 | 0.6 |
| 2     | VOR           | Chiral cel-OD (250 mm × 4.6 mm, 10 µm) n-Hexane : EtOH 9 : 1 (v/v) | 25–200 21.06 |          |      |      |     | 20 + 1 + 3 + 5 = 29 | ES = 71 | 0.64 |
| 3     | VOR           | Diamonsil C18 column (250 mm × 4.6 mm, 5 µm) ACN : water : CH₃COOH (40 : 60 : 0.25 v/v/v) | 1–100 14.002 |          |      |      |     | 12 + 1 + 3 + 5 = 21 | ES = 79 | 0.63 |
| 4     | VOR           | C-18 Hypersil BDS column (250 mm × 4.6 mm, 5 µm) Water : ACN : MeOH (50 : 25 : 25 v/v/v) | 20–400 12.98 |          |      |      |     | 15 + 1 + 3 + 5 = 24 | ES = 76 | 0.61 |
| 5     | VOR           | Hypersil C18 (250 mm × 4.6 mm, 5 µm) ACN : water (40 : 60 v/v) | 5–25 NA |          |      |      |     | 4 + 1 + 3 + 5 = 13 | ES = 87 | 0.62 |
| 6     | VOR           | Zorbax SB-C18 (250 mm × 4.6 mm, 5 µm) Ammonium phosphate dibasic buffer (pH 6.0 with; 50 mM ortho phosphoric acid)-ACN (52 : 48 v/v) | 10–100 6.7 |          |      |      |     | 7 + 1 + 3 + 3 = 14 | ES = 86 | 0.63 |
| 7     | VOR           | Hypersil, C18 (250 mm × 4.6 mm, 5 µm) Water : ACN (50 : 50 v/v) | 5–100 5.82 |          |      |      |     | 4 + 1 + 3 + 5 = 13 | ES = 87 | 0.63 |
| 8     | VOR           | Bondapak C18 (10 µm, 250 mm × 4.6 mm) ACN : 0.05 M disodium hydrogen phosphate buffer (pH 5.5) (1 : 1 v/v) | 6.0–60 7.3 |          |      |      |     | 4 + 1 + 3 + 3 = 11 | ES = 89 | 0.63 |
| 9     | VOR           | Intersil ODS C18 (150 × 4.6 mm, 5 µm) Phosphate buffer, ACN and MeOH (65 : 30 : 5 v/v/v) | 7.94–11.91 ppm 6.413 |          |      |      |     | 10 + 1 + 3 + 5 = 19 | ES = 81 | 0.63 |
Table 2 (Contd.)

| S. no. | Drug substance | Stationary phase and mobile phase | Linearity (µg mL⁻¹) | Rt (min) | NEMI | GAPI | AES | AGREE | Ref. |
|--------|----------------|-----------------------------------|---------------------|---------|------|------|-----|-------|------|
| 10     | VOR C18 G column (250 mm × 4.6 mm, 5 µm) | ACN and water (60 : 40 v/v) | 10–50 | 5.36 | 4 + 1 + 3 + 5 = 13 ES = 87 | 0.63 | 83 |
| 11     | VOR Develosil C18 column (100 mm × 4.6 mm, 3 µm) | MP A: phosphate buffer 0.05 M (pH 4.5) and ACN (800 : 200% v/v) MP B: CAN and water in the ratio 800 : 200% v/v A : B = 35 : 65 | 12–100 | 2.5 | 4 + 1 + 3 + 5 = 13 ES = 87 | 0.63 | 84 |
| 12     | VOR C18 column (150 mm × 4.6 mm, 5 µm) | ACN and ultrapure water (50 : 50 v/v) | 1 to 30 | 4.09 | 4 + 1 + 3 + 5 = 13 ES = 87 | 0.63 | 73 |
| 13     | VOR Prontosil C-18 (250 mm × 4.6 mm, 5 µm) | Isopropyl alcohol : water (80 : 20 v/v) | 5 to 25 | 7.92 | 4 + 1 + 3 + 5 = 13 ES = 87 | 0.75 | 85 |
| 14     | VOR Inertsil ODS 3V (150 mm × 4.6 mm, 5 µm) | MP A: 0.05 M KH₂PO₄ (pH 2.5 buffer) MP B: ACN : MeOH (90 : 10 v/v) | NA | 21.78 | 17 + 1 + 3 + 5 = 26 ES = 74 | 0.64 | 86 |
| 15     | VOR, UFLC C8 Luna column (250 mm × 4.6 mm, 5 µm) | ACN : 0.01% CH₃COOH (50 : 50 v/v) | 0.5–50 | 3.02 | 5 + 0 + 3 + 3 = 11 ES = 89 | 0.69 | 72 |

*VOR – voriconazole; MeOH – methanol; ACN – acetonitrile; FeCl₃ – ferric chloride; KH₂PO₄ – potassium dihydrogen phosphate; NaOH – sodium hydroxide; HCl – hydrochloric acid; N – normal; NA – not available; LOQ – limit of quantification; LOD – limit of detection; mg mL⁻¹ – microgram per millilitre; MP – mobile phase.
broadly vary from the corresponding procedures, the chosen narrow range among the methods was 1 to 30.74 Detectors like UV and photo diode array (PDA) were considered as another important factor for more accurate analysis of drugs with the reported methods of RP-HPLC. Among the two detectors PDA was selected by most of the methods due to their advantages like fast and more sensitive detection at multiple wavelengths. Table 2 summarizes the RP-HPLC techniques reported for the VOR study in single and combination dosage forms along with the application of four green assessment tools.

3.3.3. High-performance thin layer chromatography (HPTLC). HPTLC is a quick, dependable, and accurate qualitative and quantitative drug analysis; it is a viable alternative method for drug testing. Unfortunately, there are just a few HPTLC techniques available for determining VOR alone or in combination.

Khetre et al.87 utilized HPTLC to develop a technique for detecting VOR in human plasma in API and therapeutic dosage forms. This technique used silica-gel 60 F254 precoated on aluminum sheets as the stationary phase and mobile phase comprising MeOH : toluene (7 : 3 v/v), and VOR is quantified at 255 nm using densitometric analysis. VOR’s Rf values were determined to be 0.58 ± 0.02. The linear connection between the 200–1000 ng per spot concentration range showed an excellent linear regression. The detection and quantification limits were 12.05 and 36.55 ng per spot, correspondingly. This method helps to identify the VOR in the cream formulation and makes the analysis simplified.

Also, Santosh V. et al.89 established a method for estimating VOR in raw materials and cream formulations using the stationary phase of aluminum plates coated using silica-gel 60 RP-18F-254S and mobile phase composed of MeOH : water (60 : 40 v/v). Under a 200 to 1200 ng per band concentration, the absorbance of 257 nm was calculated with an Rf of 0.48 ± 0.02. The R2 value is 0.999, indicating a strong linear correlation. The levels of detection and quantification are 19.99 ng and 60.60 ng, correspondingly. This method helps to identify the VOR in the cream formulation and makes the analysis simplified.

Similarly, Dewani et al.88 developed an HPTLC method to determine VOR in human plasma using the mobile phase combination of triethylamine : MeOH : toluene in the proportion of 0.1 : 4 : 6 v/v/v, in silica gel 60 F254 as a stationary phase. The sample was prepared by dissolving plasma protein precipitation using ACN solvent. The analysis of the VOR has been performed at a wavelength of 254 nm in the concentration range between 50 and 400 ng per band, which exhibits a good range of linearity. The mean rate of drug recovery was determined to be 98.82% for VOR using the reported method.

In another study, Jain et al.88 quantified VOR in raw materials and cream formulations using the stationary phase of aluminum plates coated using silica-gel 60 RP-18F-254S and mobile phase with a mix of ACN : water (60 : 40 v/v). Under a 200 to 1200 ng per band concentration, the absorbance of 257 nm was calculated with an Rf of 0.48 ± 0.02. The R2 value is 0.999, indicating a strong linear correlation. The levels of detection and quantification are 19.99 ng and 60.60 ng, correspondingly. This method helps to identify the VOR in the cream formulation and makes the analysis simplified.

3.3.4. Bioanalytical methods. Bioanalysis is a critical component of the pharmacokinetic/pharmacodynamic evaluation of a new drug entity, beginning with its discovery and continuing through various stages of drug development and approval. This compilation discusses critical bioanalytical characteristics and their implementation to drug discovery methodologies, which will aid in the production of safer and more effective medications with less time and expense. It is

| S. no. | Mobile phase | NEMI | GAPI | AES | AGREE | Ref. |
|-------|--------------|------|------|-----|-------|------|
| 1     | MeOH : toluene (7 : 3 v/v) | ![Diagram](image1.png) | ![Diagram](image2.png) | ![Diagram](image3.png) | ![Diagram](image4.png) | ![Diagram](image5.png) | 87 |
| 2     | Triethylamine : MeOH : toluene (0.1 : 4 : 6 v/v/v) | ![Diagram](image6.png) | ![Diagram](image7.png) | ![Diagram](image8.png) | ![Diagram](image9.png) | ![Diagram](image10.png) | 88 |
| 3     | ACN : water (60 : 40 v/v) | ![Diagram](image11.png) | ![Diagram](image12.png) | ![Diagram](image13.png) | ![Diagram](image14.png) | ![Diagram](image15.png) | 89 |
| 4     | MeOH : toluene (2 : 8 v/v) | ![Diagram](image16.png) | ![Diagram](image17.png) | ![Diagram](image18.png) | ![Diagram](image19.png) | ![Diagram](image20.png) | 90 |
| Drug and instrument                                | Sample          | Extraction                               | Column                                                                 | Linearity                     | NEMI | GAPI | AES | AGREE | Ref. |
|--------------------------------------------------|-----------------|------------------------------------------|------------------------------------------------------------------------|-------------------------------|------|------|-----|-------|------|
| VOR, multidimensional HPLC with size-exclusion   | Plasma          | Sephadex G-25 superfine column of (100 x 10 mm ID) | Spherisorb ODS IMX (250 mm x 4.6 mm, 2.5 μm) | 10 to 3000 ng mL\(^{-1}\)   | 15+1+ | 3+5+ |     |       | 93   |
| chromatography                                    |                 |                                          | ACN : 0.1 M TEMED phosphate (pH 7.0) (42 : 58 v/v)                    |                               |      |      | 24  |       |      |
|                                                  |                 |                                          | Kromasil C18 (250 mm x 4.6 mm, 5 μm)                                  |                               | 5+1+3 | +3 = 12 | ES  | 88    | 94   |
| VOR, HPLC                                        | Human plasma    | With can                                 | 0.04 M ammonium phosphate (pH 6.0) 0.2–10 μg mL\(^{-1}\)              |                               |      |      | 4+2+3 | +3 = 12 | ES   | 88   |      |
| VOR, HPLC-ESI-MS                                 | Human plasma    | Protein precipitation                     | C18 column (50 mm x 2.1 mm, 3.5 μm)                                   |                               | 6+1+3 | +3 = 13 | ES  | 87    |      | 91   |
|                                                  |                 |                                          | ACN : water (0.1% HCOOH) (40 : 60 v/v) 2.49–293 ng L\(^{-1}\)       |                               |      |      |     |       |      |
| VOR, HPLC                                        | Plasma          | SPE through Bond Elute columns C18, 100 ng mL\(^{-1}\) | Luna 5 m C18 column (250 mm x 4.6 mm, 5 μm) | 0.2–10 μg mL\(^{-1}\)   | 6+1+3 | +3 = 13 | ES  | 87    |      | 95   |
|                                                  |                 |                                          | ACN : TEMED (pH 7.4 using phosphoric acid) (45 : 55 v/v)              |                               |      |      | 5+1+3 | +3 = 11 | ES   | 88   |      |
| VOR, HPLC                                        | Human plasma    | Direct injection                          | Silica particles, bonded with a GFFP (150 mm x 4.6 mm, 5 μm)        | 0.5–10 μg mL\(^{-1}\)       | 5+1+3 | +3 = 11 | ES  | 88    |      | 96   |
|                                                  |                 |                                          | ACN : KH2PO4 (pH 6.0) (17 : 83 v/v)                                   |                               |      |      |     |       |      |
| VOR, LCMS                                        | Plasma          | Online solid-phase extraction             | Waters Oasis HLB (25 mm x 2.0 mm, 2.1 μm)                             | 78–5000 mg L\(^{-1}\)      | 11+1+3 | +3 = 14 | ES  | 86    |      | 92   |
|                                                  |                 |                                          | Water : MeOH (95 : 5 v/v)                                             |                               |      |      |     |       |      |
| VOR, HPLC                                        | Plasma          | LLE                                      | Chromolithic RP 18 monolithic silica rod (100 mm x 4.6 mm)           | 0.05–10 μg mL\(^{-1}\)   | 6+2+3 | +3 = 14 | ES  | 86    |      | 97   |
|                                                  |                 |                                          | Tetrahydrofuran : ACN : NH4HCO\(_3\) (pH 5.8): (3 : 25 : 72 v/v/v) |                               |      |      | 11+1+3 | +3 = 18 | ES   | 82   |      |
| VOR, HPLC-MS                                     | Human plasma    | Protein precipitation, automated SPE      | LiCrospher 100 RP-18 (125 mm x 4 mm, 5 μm)                             | 78–5000 ng L\(^{-1}\)      | 6+2+3 | +3 = 14 | ES  | 86    |      | 98   |
|                                                  |                 |                                          | Water : ACN (50 : 50 v/v)                                             |                               |      |      |     |       |      |
| VOR, LC-MS/MS                                    | Rat plasma      | With MeOH                                 |                                                                          | 50–2500 ng mL\(^{-1}\)     | 50–2500 ng mL\(^{-1}\)     | 50–2500 ng mL\(^{-1}\)     |      |      | 99   |
| Drug and instrument | Sample | Extraction | Column | Linearity | NEMI | GAPI | AES | AGREE | Ref. |
|---------------------|--------|------------|--------|-----------|------|------|-----|-------|------|
| VOR, HPLC           | Rat and beagle dog plasma | With MeOH : ACN (1 : 2) | Shim-pack HPLC column (150 mm × 4.6 mm, 5 μm) ACN : HCOOH : water (60 : 0.05 : 40 v/v) | 14 + 2 + 3 + 3 = 22 | ES = 78 | 0.6  |
| Water : ACN : CH₃COOH (pH 4.0) (45 : 55 : 0.25 v/v) | ES = 78 | | Diamonsil C18 (250 mm × 4.6 mm, 5 μm) | 0.10–50.0 μg mL⁻¹ | | |
| VOR, HPLC           | Serum | Vortexed with mobile phase | Nova-Pak CN-HP (100 mm × 3.9 mm, 4 μm) 15% ACN (0.1% n-butyl amine, 0.2% H₃PO₄) | 0.4–10.0 mg L⁻¹ | 15 + 1 + 3 + 3 = 24 | ES = 76 | 0.63 | 101 |
| VOR, HPLC           | Plasma | With can | C18 column (150 mm × 4.6 mm) 50% MeOH : 0.01 M CH₃COONa (pH 5.0 v/v) | 0.25–16 mg L⁻¹ | 10 + 1 + 3 + 3 = 17 | ES = 83 | 0.6  | 102 |
| VOR, HPLC           | Serum and plasma | LLE | Supelcosil LC-18-DB (25 mm × 4.6 mm, 5 μm) MP A: 10% ACN : 90% 0.01 M K₃PO₄ pH 3.0 (v/v) MP B: 100% ACN | 7 + 1 + 3 + 3 = 14 | ES = 86 | 0.6  | 103 |
| VOR, HPLC-FLD       | Human plasma and saliva | With n-hexane : ethyl acetate (3 : 1 v/v) | LUNA C18 (250 mm × 3.0 mm, 5 μm) ACN : 0.01 M KH₂PO₄ buffer (0.01 M TEMED pH 6.8) (45 : 55 v/v) | 0.1–10 μg mL⁻¹ | 17 + 1 + 3 + 3 = 24 | ES = 76 | 0.6  | 104 |
| VOR, HPLC           | Plasma | With can | LiChrospher-100 RP-18 (125 × 4 mm, 5 μm) 0.04 M ammonium phosphate (pH 6.0) : ACN (60 : 40 v/v) | 1.5–10 μg mL⁻¹ | 7 + 1 + 3 + 3 = 14 | ES = 86 | 0.6  | 105 |
| VOR, HPLC           | Serum | With MeOH | Hibar, LiChrospher C8 RP column (100 mm × 5 mm, 5 μm) | 0.26 to 10.1 μg mL⁻¹ | 19 + 1 + 3 + 3 = 26 | | |

© 2022 The Author(s). Published by the Royal Society of Chemistry RSC Adv., 2022, 12, 6683–6703 | 6693

Review RSC Advances
| Drug and instrument | Sample | Extraction | Column | Linearity | NEMI | GAPI | AES | AGREE | Ref. |
|---------------------|--------|------------|--------|-----------|------|------|-----|-------|------|
| VOR, LCEIMS         | Aqueous humour | NA | C18 column (300 mm x 5 mm, 15 µm) | 70% ACN : 30% water : 0.01% TFA | 0.02-30 µg mL\(^{-1}\) | 15 + 2 + 3 + 3 = 23 | ES = 77 | 0.6 | 107 |
| VOR, HPLC-ESI-MS    | Plasma | Protein precipitation | C18 column (50 mm x 2.1 mm, 3.5 µm) | ACN : water (0.1% HCOOH) 40 : 60 v/v | 2.49-293 ng mL\(^{-1}\) | 10 + 2 + 3 + 3 = 18 | ES = 82 | 0.57 | 108 |
| VOR, LC-MS/MS       | Serum | Vortexed and then centrifuged | C18 column (100 mm x 3.0 mm, 2.6 µm) | MP A: 0.1% HCOOH : 2 mM CH\(_3\)COONH\(_4\) | 0.1 and 10.0 µg mL\(^{-1}\) | 7 + 2 + 3 + 3 = 15 | ES = 85 | 0.57 | 109 |
| VOR, HPLC-FLD       | Human serum | With ice-cold ACN | SunFire C18 (150 mm x 4.6 mm, 5 µm) | ACN : ultrapure water (70 : 30 v/v) | 0.25-16.0 µg mL\(^{-1}\) | 6 + 1 + 3 + 3 = 13 | ES = 87 | 0.6 | 111 |
| VOR, HPLC-FLD       | Human plasma and serum | Protein precipitation and ACN extraction | ODS HYPER SIL column (250 mm x 4.6 mm, 5 µm) | 0.1 M CH\(_3\)COONH\(_4\) solution, ACN and TFA (409 : 590 : 1 v/v/v) | 0.05-10 µg mL\(^{-1}\) | 5 + 1 + 3 + 3 = 12 | ES = 88 | 0.63 | 112 |

Table 4 (Contd.)
| Drug and instrument | Sample | Extraction Method | Column | Linearity | NEMI | GAPI | AES | AGREE | Ref. |
|---------------------|--------|-------------------|--------|-----------|------|------|-----|-------|------|
| VOR, HPLC           | Human blood | With hexane and ethyl acetate | Eclipse X DB C18 (4.5 mm × 5 μm) | 1.0 to 8.0 μg mL⁻¹ | 16 + 1+ | 3 + 3 = 23 | ES = 77 | 0.6 | 114 |
| VOR, HPLC-FLD       | Human serum | Protein precipitation with ACN | LiChrospher RP-18e column (125 mm × 4 mm, 5 μm) | VOR 0.2–20.0 μg mL⁻¹ | 13 + 1+ | 3 + 3 = 20 | ES = 80 | 0.6 | 115 |
| VOR, HPLC           | Plasma   | Ultrafiltration method with tween 80 | LiChrospher (125 mm × 4 mm, 5 μm) | ACN : 10 mM KH₂PO₄ (10 mM of TEMED pH 6.5) : ACN 65 : 35 (v/v) | 15 + 1+ | 3 + 5 = 22 | ES = 78 | 0.6 | 115 |
| VOR, LCMS           | Human plasma | Vortexed and centrifuged by using MeOH | C18 column (100 mm × 2.1 mm × 3.5 μm) | MeOH : 0.1% HCOOH (70 : 30 v/v) | 15 + 1+ | 3 + 5 = 24 | ES = 76 | 0.63 | 116 |
| VOR, surface-enhanced Raman spectroscopy | Plasma | Centrifugation | Diamonsil C18 | ACN : 0.1% HCOOH (43 : 57 v/v) | 4 + 1+3 | + 3 = 11 | ES = 89 | 0.6 | 117 |
| VOR, HPLC           | Humans | Centrifugation | C18 column (250 mm × 4.6 mm, 3.5 μm) | ACN : 0.05 M CH₃COONH₄ : MeOH (20 : 40 : 40 v/v) | 15 + 1+ | 3 + 3 = 12 | ES = 88 | 0.63 | 118 |
| VOR, HPLC           | Beagle plasma | Vortexed and centrifuged by using MeOH | Venusil XBP C18 (250 mm × 4.6 mm, 5 μm) | ACN : 20 mM KH₂PO₄ (65 : 35 v/v) | 15 + 1+ | 3 + 3 = 22 | ES = 78 | 0.6 | 119 |
| VOR, ITC, HPLC      | Human serum | Heptane-isoamyl alcohol (90 : 10 v/v) | Zorbax SB-C18 (250 mm × 4.6 mm, 5 μm) | 50 mM phosphate buffer [pH 6.0 with 1 M KOH] : MeOH : ACN (35 : 20 : 45) (v/v/v) | 19 + 1+ | 3 + 3 = 26 | ES = 74 | 0.6 | 120 |
| Drug and instrument | Sample | Extraction | Column | Linearity | NEMI | GAPI | AES | AGREE | Ref. |
|---------------------|--------|------------|--------|-----------|------|------|-----|-------|-----|
| VOR, PSC, HPLC      | Human plasma | Hexane–methylene chloride (70 : 30 v/v) | C8 plus (250 mm × 3 mm, 5 μm) | VOR 0.2–10.0 mg L⁻¹ | 15 + 1 + 3 + 3 = 22 | 0.6 | 121 |
|                     |        |            |        |           |      |      |     |       |      |
| VOR, PSC, HPLC      | Human plasma | LLE with diethyl ether | ACN : 0.09 M monobasic ammonium phosphate (pH 5.3) (50 : 50 v/v) | 1.0–20.0 μg mL⁻¹ | 7 + 1 + 3 + 3 = 14 | 0.6 | 122 |
| VOR, ITG, PSC, HPLC-MS | Human plasma | Protein precipitation extraction with ACN | C18 Atlantis T-3 (150 mm × 4.6 mm, 5 μm) | ITC 0.04–1.18 μg mL⁻¹ | 6 + 1 + 3 + 3 = 13 | 0.56 | 123 |
| VOR, PSC, FLZ, ITZ, LC-MS/MS | Human serum | Protein precipitation | Phenomenex Luna C8 (50 mm × 2 mm, 3 μm) | VOR 0.01–10 μg mL⁻¹ | 5 + 1 + 3 + 3 = 11 | 0.56 | 124 |
| VOR, TFL, HPLC      | Plasma | Without extraction | 50 mM phosphate buffer (pH 3.0) / propylene carbonate : ethanol (10 : 90 v/v) | 0.1 μg mL⁻¹ and 0.5 μg mL⁻¹ | 4 + 1 + 0 + 1 = 6 | 0.87 | 125 |
| VOR, ITG, PSC, UPLC-MS/MS | Human plasma | Centrifugation | BEH C18 column (50 mm × 2.1 mm, 1.7 μm) | VOR 0.13 and 6.54 mg L⁻¹ | 7 + 1 + 3 + 3 = 14 | 0.56 | 126 |
| VOR, IMB, UPLC-MS/MS | Rat plasma | Protein precipitation by can | Acquity UPLC BEH C18 column (2.1 mm × 50 mm, 1.7 μm) | Inmatinib, \( y = \frac{(2.70755x + 166.688, r^2 = 0.99818)}{} \) | 6 + 1 + 3 + 3 = 13 | 0.56 | 127 |

*VOR* – voriconazole; HPLC – high-performance liquid chromatography; Rₜ – retention time; SPE – solid-phase extraction; HCOOH – formic acid; KH₂PO₄ – potassium dihydrogen phosphate; NaH₂PO₄ – sodium dihydrogen phosphate; DAP – daptomycin; MeOH – methanol; ACN – acetonitrile; ITC – itraconazole; PSC – posaconazole; TED – tedizolid; LLE – liquid–liquid extraction; FLZ – fluconazole; IMB – imatinib; TFL – tadalafil; LC-MS/MS – liquid chromatography-tandem mass spectrometry; TEMED – N,N,N',N’-tetramethyl ethylene diamine; K₂PO₄ – potassium phosphate; HPLC-FLD high-performance liquid chromatography fluorescence detection; GPPP – glycine phenylalanine–phenylalanine peptide.
aimed to provide some broad views in this field that will serve as the foundation for a general framework for approaching bioanalysis from the start (i.e., identification of a pioneer chemical) to the different stages of the development process. VOR estimated in various biological matrices like blood (human), plasma (human, rat, dog, and beagle), serum (human and rat), and aqueous humour (human). Solvent systems like ACN, MeOH, hexane, ethyl acetate, heptane, isooamyl alcohol, hexane, methylene chloride, and diethyl ether were used in different reported methods for the efficient extraction of VOR from various biological matrices. The minimum linearity concentration reported was 2.49 ng mL\(^{-1}\) (ref. 91) to the maximum of 5000 mg L\(^{-1}\) (ref. 92). The detection of VOR in biological matrices used different detectors like PDA, UV, fluorescence detection, surface-enhanced Raman spectroscopy, mass spectrometry (MS) and MS/MS. Although each detector has its own advantages, mass spectrometry was the predominantly utilized detector, which has a great potential of more accurate detection even at very low concentration. This also forces the methods to select volatile buffers like formic acid and acetic acid for better compatibility with the organic phase of MeOH and ACN. The bioanalytical techniques for measuring VOR alone and in combination with other medications, along with their greenness assessment, are summarized in Table 4.

3.3.5. Miscellaneous methods. A chromatographic method was proposed by Babu et al.\(^\text{128}\) for analyzing VOR in pharmaceutical formulations using a quality by design approach. The stationary phase utilized for the method was the C18 column (250 mm × 4.6 mm, 5 μm), and a 50 : 50 v/v blend of ACN and water as the mobile phase with a 1.0 mL min\(^{-1}\) flow rate. Three variables were taken into account while determining robustness, which were the proportion of ACN in the mobile phase, the pH, and the flow rate; a rising inflow leads to a reduction in the concentration of the drug detection, while the proportion of ACN and the pH had significantly less impact on the response. A correlation coefficient of 0.9999 was determined to prove the noteworthiness of the developed method. The RSD result (0.45%, n = 24) showed that the analytical technique is precise and accurate and shall be used for long-term use due to applying the analytical quality by design concept.

In another study, Lin et al.\(^\text{129}\) established a technique for determining VOR concentrations in a patient’s plasma sample using sweeping-micellar electrokinetic chromatography on a fused silica capillary of 75 cm × 50 μm ID column. The solution included 110 mM sodium dodecyl sulfate, 20% ACN, and 40 mM phosphoric acid. The voltage applied was −23 kV, and the wavelength of detection was 254 nm. VOR was isolated from endogenous materials within 10.5 min under optimum analytical conditions, limiting the detection at 0.075 g mL\(^{-1}\). Plasma VOR levels were quantified in 16 individuals; the findings were consistent with those acquired by the HPLC method. This method may be recognized as a new technique by applying a new concept called sweeping-micellar electrokinetic chromatography. Still, this technique was eventually used to develop a green analytical method. In this technique, the authors used ACN as an organic modifier, making this method vulnerable towards eco-friendly usage. However, these types of methods are encouraged and need to be optimized by applying some biodegradable solvents.

Similarly, Corbini et al.\(^\text{130}\) devised a new technique for quantifying VOR using differential pulse polarography (DPP) in pharmaceuticals. A distinct peak (−1.01 V versus Ag/AgCl) was produced using a 0.01 M KH\(_2\)PO\(_4\) buffer (pH 4.5) supporting electrolyte. Accordingly, the concentration stood linear in the series of 0.5 to 5.0 μg mL\(^{-1}\), through a LOD and LOQ of 0.03 and 0.10 μg mL\(^{-1}\). This resultant method consumes fewer toxic substances and may be used for sustainable development.

Smith et al.\(^\text{131}\) developed a GCMS method to determine VOR in serum. The sample extraction was performed with the help of cold methanol and ethyl acetate by adding the internal standard THC-deuterium 9 (THC-d9) and derivatized using N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA). The run time used for every run was about 11 min with a linearity range of 0.4 to 10 μg mL\(^{-1}\). This method showed a better result with no interaction with the other drugs. It was the best adaptable one for VOR analysis in the serum without interference from the other substances.

Recently, Lerch et al.\(^\text{132}\) developed a rapid and efficient analytical technique called paper spray mass spectrometry (PSMS), used for the first time to quantify VOR in the complex biological matrix without using chromatographic or traditional sample separation. An innovative PSMS technique for quantitating VOR in equine tears has been determined and corroborated over a series of 10 to 1000 ng mL\(^{-1}\). The method demonstrates excellent accuracy, linearity (\(R^2 > 0.990\)), inter and intra-day precision, and selectivity for the quantitation limit in equine tears. VOR was computed using three products compared to an internal standard with an isotope label, voriconazole-d3, with a 250 ng mL\(^{-1}\) standard concentration in samples. The authors further applied this technique to the analysis of 126 test samples, and acquired the sample dilution’s integrity, and carryover impact was further examined and determined within acceptable limits.

In another study, Sahitya et al.\(^\text{133}\) used Candida albicans as the test microorganism to develop a novel microbiological technique for examining VOR tablets. It was necessary to experiment with different mediums, species, and circumstances to optimize the diffusion test. During a prospective validation, the method showed excellent linearity (0.995), accuracy less than 2% RSD, and consistency (mean recovery = 101.77%). VOR was evaluated using HPLC, which was used as a comparative method for the study. The results of both the microbiological and HPLC techniques have been compared using the Student’s t-test. The VOR content measured from both ways has demonstrated a high degree of consistency. When employed in dosage forms for regular quality control analysis of VOR, the newly developed microbiological analytical technique gives a genuine indicator of biological activity. It may be utilized to detect actual biological activity.
Surface design is used for optimization studies to identify critical constraints such as organic phase mix in the mobile phase and flow rate that affect variables such as peak tailing, theoretical plates, peak area, and retention time by a central composite design. The optimum operating conditions for the technique were determined using graphical refinement and then verified by Monte Carlo simulations. The optimum mobile phase condition was ACN and 0.05% acetic acid (pH 4) (50:50 v/v). The flow rate of 1 mL min⁻¹, at 256 nm detection, demonstrated linearity within 0.1–50 μg mL⁻¹ in Hanks balanced salt solution and methanol. Corroboration data showed the proposed analytical method’s efficacy and sensitivity in quantifying VOR. Quantification of VOR in pharmaceutical nano-formulations was effectively accomplished using the established analytical technique. The above-mentioned miscellaneous methods were assessed using the four green assessment tools and are depicted in Table 5.

4. Discussion

The present review compiles the analytical methods available for VOR estimation by spectroscopic, chromatographic, bio-analytical, and other techniques. The UV spectroscopic method mostly uses solvents like MeOH, water, HCl, and NaOH with a wavelength range of 252 to 256 nm, which shows that the method is effortless to use and accurate. The most eco-friendly approach was the one reported by Roy S. et al.² This method utilized only water as a solvent for the analysis, making the technique more environmentally benign. Derivatization or photometric methods use different chemicals like tropaeoline ooo. Azo carmine-G has no serious toxic indication reported by any manufacturers in their data safety sheet, which shows that these chemicals shall be considered eco-friendly and can be used to determine other drugs. Assessment results also show that the method was eco-friendly related to the chemicals, but
the number of steps involved in this method reduced the score towards its environmental friendliness.

The HPLC methods utilized different solvents for the analysis of VOR in pharmaceutical substances. The eco-friendly method among the reported methods was Singh et al. in this, isopropyl alcohol and water was used as the mobile phase, which makes the process justifiable from an environmental standpoint. An approach can be picked based on the $R_t$ as well. A method was developed by Lingamaneni K. et al. with an $R_t$ of 3.02, which utilized less solvent compared to the other methods. The solvents used by the various reported methods in performing HPLC are depicted in Fig. 5. This indicates that most reported studies have utilized ACN and MeOH as solvents by changing the buffers at various pH levels. Among the fifteen reported methods four authors have employed the same mobile phase components, which showed the same green assessment results by changing the ACN : water composition. Adams et al. have used a mobile phase containing MeOH and triethylamine (TEA), in which TEA is considered toxic to human health and the environment as this reagent contains three pictograms indicating danger and has an NFPA score with three in both health and flammability, which leads to a decrease in the eco-score when applying the assessment tools. Nagarjuna et al. reported a method using ethanol and $n$-hexane as a mobile phase. Despite ethanol being considered an eco-friendly solvent, incorporating $n$-hexane made the method lose its environmental friendliness, as $n$-hexane contains four pictograms indicating danger. Both Huang et al. and Lingamaneni et al. have used a similar mobile phase composed of ACN and acetic acid. Still, variation in the assessment results was observed due to the run time and elution of the compound. As discussed earlier, the Lingamaneni et al. method had a better greenness profile than the other methods due to their run time. The remaining methods have utilized a common organic solvent such as ACN and MeOH along with a solid buffer. The different buffers used for the reported methods were phosphate with the corresponding salts of ammonium, sodium, and potassium, which produced similar results in the greenness assessment.

Analysis of VOR and its combinations in biological fluids shows the importance of the drug and the need to develop an eco-friendly method without compromising the method quality as a better alternative to the reported approaches. The reported methods used several extraction techniques to separate VOR and its combinations such as biological matrix-like liquid-liquid extraction (LLE), protein precipitation, centrifugation, vortexing, simple mixing with the solvent, and direct injection. Among these extraction methods, direct injection and green extraction may help the technique be more environmentally sound and eco-friendlier. In the analysis of bioanalytical samples, the most preferred organic phase was again MeOH, ACN, and an appropriate buffer. The selection of the organic phase and the corresponding number of times it appears in the reported methods are shown in Fig. 6. According to this, ACN is considered as the predominant solvent used in most of the available methods, followed by MeOH. The present assessment tools showed that most methods had a similar greenness because the mobile phase selection was very similar with very slight variation.

Apart from the spectroscopy and chromatography methods, few reported methods explore the analysis of the drugs by applying the AQBD method for producing a long-term method, few other methods like sweeping-micellar electrokinetic chromatography, differential pulse polarography, paper spray mass spectrometry, and microbiological technique for the analysis of the VOR. The minimum linearity concentration reported was $2.49 \text{ ng mL}^{-1}$ to the maximum of $100 \text{ μg mL}^{-1}$.

The greenness was further analyzed using NEMI, GAPI, analytical eco-scale, and AGREE metrics for all reported methods, and the results are shown in Tables 1–5. As explained earlier, each tool used in assessing greenness follows a different method for performing the greenness assessment. Considering the reported methods, ACN is used to a greater extent than MeOH. Even though MeOH is less toxic when compared to ACN, only a few LC methods have been reported with MeOH and buffer as the mobile phase. Most methods mentioned using ACN as an organic modifier and other buffers show a repetitive technique that could be avoided. A green LC method has been reported to estimate VOR with the aid of eco-friendly solvents like propylene carbonate and ethanol and buffer with suitable method performance characteristics. There are very few green
production, as the quantity of production increases that directly
and easily applicable for industrial purposes.

developing new green methods, which should be eco-friendly
and utilized propylene carbonate as a mobile phase. NEMI
concludes that miscellaneous methods like sweeping-micellar
electrokinetic chromatography, DPP, PSMS, and microbiolog-
ical methods are safe and supportable for the environment.

The GAPI tool results strengthen NEMI’s assumption in
spectrophotometric, HPTLC, and bioanalytical methods, but
picked a UFLC method as the best eco-friendly one among the
reported ones; GAPI also indicates that the PSMS and micro-
biological methods are safest compared to the other methods
concluded by the NEMI.

Pictographic assessment of AES is a numerical assessment
tool that gives conclusive evidence with the help of a specific
value. AES results support the inference given by GAPI for
spectrophotometric, HPTLC, HPLC, and reported bio-analytical
methods and strengthen it with a numerical value. In miscel-
naneous methods, AES showed a very slight edge toward the
microbial method and showed a score of 100.

The final evaluation technique, AGREE, a very conclusive
tool for green assessment, has confirmed the green-collar of the
reported methods for greenness. AGREE has confirmed the
method’s greenness and supports AES’s interpretation for
spectrophotometric, HPTLC, HPLC, and reported bio-analytical
methods but supported NEMI in the HPLC reported practices.
Unlike AES, AGREE also showed a very slight edge toward the
microbial process and showed a score of 0.95.

But prior consideration by four assessment tools states that
the microbial examination method has the most eco-friendly
results followed by the PSMS. The only drawback of these
methods was that one consumes time. The other utilized only
tears as a sample, nullifying the sampling procedure concept as
it is not recognized as a transferable method. Only one bio-
analytical method\(^{25}\) was eco-friendly, which utilized propylene
carbonate as a solvent. The results indicate the importance of
developing new green methods, which should be eco-friendly
and easily applicable for industrial purposes.

Time is a crucial aspect for any industry in terms of
production, as the quantity of production increases that directly
enhances the effect of analysis time. So, it is always essential
from an industrial point of view to consider an analytical
method that can give the best results in less time by consuming
less energy. Among the present methods for determination of
VOR, the UV method consumes significantly less energy and
and time for analysis but has some flaws like reliability or repro-
ducibility, whereas chromatographic methods are well adopted
due to their several advantages. Among the chromatographic
techniques, HPTLC is the most time and energy-consuming
technique. LCMS and GCMS are the highly accurate methods
for determinations but have a disadvantage like high energy
consumption, which negatively affects the environment. The

RP-HPLC is the most affordable and reliable in most industries
for the analysis of pharmaceuticals, but this also has a disad-

avantage like energy consumption. Finally, the UPLC method is
the most advantageous due to its highly reliable results in less
time. Here, the application of time indicates an added advan-
tage to the industries and reduces the generation of harmful
 greenhouse substances into the environment.

5. Conclusion

According to the current review, the methodological approaches
accessible for VOR determination comprise spectrophotometry,
chromatography, and biological test methods. Each method has
its own set of advantages and disadvantages when compared to
others. As methods have been continuously developed to assess
VOR, this drug’s importance shows a need for a non-polluting
methodology for drug analysis. Among the methods reported,
only one approach has utilized more environmentally support-
able practices, an introductory note for future research
regarding this compound. Analysts and expert formulators
must create more environmentally friendly techniques for
estimating VOR that use less hazardous solvents. Additional LC-
MS/MS-based methods may quantify the medication in biolog-
ical matrixes, which might be more critical for VOR therapeutic
monitoring. Analytical quality by design, which was performed
using Box–Behnken design, shows some supremacy among the
methods due to long-term sustainability. AQbD deals with
developing strategies for the future, but the drawback of the
methods used to determine VOR is that they have not applied
bio-degradable solvents. Therefore, incorporation of quality
(AQbD) and eco-friendly (GAC) principles in developing tech-
niques is highly recommended for the estimation of VOR in
various matrixes, and more methods need to be developed to
analyze VOR, which should be based on clean green analytical
chemistry and make the environment and environment analyst
safe.

6. Future aspects

The pharmaceutical and chemical industries are concerned
about environmental safety and green analytical method
development as a value in the vision of pharma industries 4.0.
There is an opportunity to develop environmentally benign
methods more positively by applying greener analytical tech-
niques in analytical research and development via regular
quality control activities. The future development of a stable
green HPLC method with the aid of quality by design to esti-

mate VOR without the need for revalidation would be more
beneficial. There were no green HPLC methods for estimating
VOR and its known and unknown impurities, and the same
should be developed. The new transformation of extractions
included using lower organic solvents, sorbents, better extraction
and clean-up, fewer pre-treatment steps for a sample, and
improved selectivity adoptions into the method development of
bioanalytical matrixes more eco-friendly.

The new transformation of extractions included the usage of
lower organic solvents, sorbents, better extraction and clean-up,
fewer pre-treatment steps for a sample, and improved selectivity. Green microextraction technology is a user-friendly platform for analysts and far less environmentally damaging and provides even fewer toxic solvents, miniaturization, greater automation, and online coupling power with analysis techniques. Adopting these technologies for pharmaceutical analysis makes the method more stable, environmentally benign, and lasts longer.

Furthermore, no quantitative IR or NIR method was reported to estimate VOR, which should be developed and shall be the greenest method over LC and other methods and delivers the scope of developing quantitative IR methods to assess VOR.

Conflicts of interest

There are no conflicts to declare.

References

1 J. K. B. T. Aronson, *Mealy’s Side Effects of Drugs*, Elsevier, Oxford, 16th edn, 2016, pp. 504–521.
2 A. L. Marano, J. Hooten and S. A. Myers, in *Kidney Transplantation - Principles and Practice*, Elsevier, 2019, pp. 578–590.
3 M. A. Keller and E. R. Stiehm, *Clin. Microbiol. Rev.*, 2000, 13, 602–614.
4 N. D. Greer, *Baylor University Medical Center Proceedings*, 2003, 16, 241–248.
5 C.-Y. Low and C. Rotstein, *F1000 Medicine Reports*, 2011, 3, 14.
6 R. Olum, J. B. Baluku, A. Kazibwe, L. Russell and F. Bongomin, *PLoS One*, 2020, 15, e0240374.
7 J. Wang, M. Zhou, J.-Y. Xu, R.-F. Zhou, B. Chen and Y. Wan, *JAMA Network Open*, 2020, 3, e2017652.
8 D. Al-Badriyeh, C. F. Neoh, K. Stewart and D. C. M. Kong, *Clin. Ophthalmol.*, 2010, 4, 391–405.
9 L. R. Snyder, J. J. Kirkland and J. W. Dolan, *Introduction to modern liquid chromatography*, John Wiley & Sons, New Jersey, USA, 2011.
10 R. Hartman, R. Helmy, M. Al-Sayah and C. J. Welch, *Green Chem.*, 2011, 13, 934–939.
11 US EPA, O. US EPA, defining hazardous waste: listed, characteristic and mixed radiological wastes, US EPA, https://www.epa.gov/hw/defining-hazardous-waste-listed-characteristic-and-mixed-radiological-wastes, accessed 5 April 2021.
12 M. M. Kirchhoff, *Resour., Conserv. Recycl.*, 2005, 44, 237–243.
13 G. Shepherd, in *Encyclopedia of Toxicology*, Elsevier, 2005, pp. 54–56.
14 P. T. Anastas, *Crit. Rev. Anal. Chem.*, 1999, 29, 167–175.
15 L. H. Keith, L. U. Gron and J. L. Young, *Chem. Rev.*, 2007, 107, 2695–2708.
16 P. T. Anastas and J. C. Warner, *Green chemistry: theory and practice*, Oxford University Press, Oxford [England], New York, 1998.
17 M. Koel and M. Kaljurand, *Pure Appl. Chem.*, 2006, 78, 1993–2002.
18 A. Galuszka, Z. Migaszewski and J. Namieśnik, *TrAC, Trends Anal. Chem.*, 2013, 50, 78–84.
19 M. Tobiszewski, M. Marć, A. Galuszka and J. Namieśnik, *Molecules*, 2015, 20, 10928–10946.
20 H. K. Chanduluru and A. Sugumaran, *Acta Chromatogr.*, 2021, DOI: 10.1556/1326.2021.00949.
21 A. Ismael, A. Gevorgyan, T. Skrydstrup and A. Bayer, *Org. Process Res. Dev.*, 2020, 24, 2665–2675.
22 S. N. Sawant, in *Biopolymer Composites in Electronics*, Elsevier, 2017, pp. 353–387.
23 B. A. Jones, *J. Liq. Chromatogr. Relat. Technol.*, 2004, 27, 1331–1352.
24 M. Novotny, *Clin. Chem.*, 1980, 26, 1474–1479.
25 J. M. Cunliffe and T. D. Maloney, *J. Sep. Sci.*, 2007, 30, 3104–3109.
26 S. Chen and A. Kord, *J. Chromatogr. A*, 2009, 1216, 6204–6209.
27 M. Kaljurand and M. Koel, *Crit. Rev. Anal. Chem.*, 2011, 41, 2–20.
28 S. Dulaurent, S. El Balkhi, L. Poncelet, J.-M. Gaulier, P. Marquet and F. Saint-Marcou, *Anal. Bioanal. Chem.*, 2016, 408, 1467–1474.
29 L. T. Taylor, *J. Supercrit. Fluids*, 2009, 47, 566–573.
30 R. M. Smith, *J. Chromatogr. A*, 2008, 1184, 441–455.
31 Y. Yang, *J. Sep. Sci.*, 2007, 30, 1131–1140.
32 R. M. Smith, *Anal. Bioanal. Chem.*, 2006, 385, 419–421.
33 J. Plotka-Wasyłka, M. Rutkowska, K. Owczarek, M. Tobiszewski and J. Namieśnik, *TrAC, Trends Anal. Chem.*, 2017, 91, 12–25.
34 Y. A. Olcer, M. Tascon, A. E. Eroglu and E. Boyaci, *TrAC, Trends Anal. Chem.*, 2019, 113, 93–101.
35 F. Tache, S. Udrescu, F. Albu, F. Micăle and A. Medvedovici, *J. Pharm. Biomed. Anal.*, 2013, 75, 230–238.
36 K. D. Clark, C. Zhang and J. L. Anderson, *Anal. Chem.*, 2016, 88, 11262–11270.
37 K. P. Kannaiah and A. Sugumaran, *Curr. Chem. Lett.*, 2022, 11, 11–22.
38 H.-A. H. Abd-ELSalam, M. Gamal, I. A. Naguib, M. A. Al-Ghobashy, H. E. Zaazaa and M. Abdelkawy, *Separations*, 2021, 8, 137.
39 S. Beg, J. Haneef, M. Rahman, R. Peraman, M. Taleuzzaman and W. H. Almalki, in *Handbook of Analytical Quality by Design*, Elsevier, 2021, pp. 1–14.
40 F. G. Vogt and A. S. Kord, *J. Pharm. Sci.*, 2011, 100, 797–812.
41 T. Sharma, A. Jain, S. Saini, O. Kataro and B. Singh, *Sep. Sci. plus*, 2020, 3, 384–398.
42 K. P. Kannaiah and A. Sugumaran, *Journal of Taibah University for Science*, 2021, 15, 1156–1167.
43 K. P. Kannaiah and A. Sugumaran, *Microchem. J.*, 2022, 172, 106968.
44 H. Shaaban and A. Mostafa, *J. AOAC Int.*, 2018, 101, 1781–1787.
45 Z. Li, K. H. Smith and G. W. Stevens, *Chin. J. Chem. Eng.*, 2016, 24, 215–220.
