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Whiter and Greener RP-HPLC Method for Simultaneous Determination of Dorzolamide, Brinzolamide, and Timolol Using Isopropanol as a Sustainable Organic Solvent in the Mobile Phase

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Abstract: A sustainable reversed-phase chromatographic method has been developed and validated for the simultaneous determination of three active pharmaceutical ingredients, dorzolamide, brinzolamide, and timolol, used to treat glaucoma. The eco-friendly solvent isopropanol has been used as an organic mobile phase constituent. According to the Hansen space green solvent selection tool, isopropanol has a G score of 6.5, comparable to ethanol, which has a G score of 6.6. The mobile phase consists of isopropanol: aqueous sodium acetate buffer (0.1 M, pH 4.25) in the ratio of 10:90 (*v*/*v*). The flow rate was maintained at 1 mL/min. Dorzolamide and brinzolamide were detected at 254 nm, and timolol was detected at 295 nm. A high-purity silica with a polymeric C18 modification column (150 × 4.6 mm, 5 µm particle size) was used for this separation. The three compounds were eluted within 8 min. The method was validated according to ICH guidelines. The calibration curves were linear in the range of 20–70 µg/mL, 40–140 µg/mL, and 20–70 µg/mL for dorzolamide, brinzolamide, and timolol, respectively. The LODs were found to be 1.61 µg/mL, 1.60 µg/mL, and 3.16 µg/mL for dorzolamide, brinzolamide, and timolol, respectively. Good accuracy and precision were obtained for the three compounds. The greenness and whiteness of the method were indicated using the AGREE, ChlorTox, and RGB12 tools.

Keywords: green analytical chemistry; white analytical chemistry; sustainability; sustainable solvent; greenness; isopropanol; RGB12; AGREE; ChlorTox; RP-HPLC; dorzolamide; brinzolamide; timolol

Citation: El Deeb, S.; Abdelsamad, K.; Parr, M.K. Whiter and Greener RP-HPLC Method for Simultaneous Determination of Dorzolamide, Brinzolamide, and Timolol Using Isopropanol as a Sustainable Organic Solvent in the Mobile Phase. *Separations* **2024**, *11*, 83. https://doi.org/10.3390/separations11030083

Academic Editor: Daniele Naviglio

Received: 1 February 2024
Revised: 26 February 2024
Accepted: 7 March 2024
Published: 12 March 2024

1. Introduction

Paul Anastas and John Warner introduced green analytical chemistry as a means to reduce adverse effects on the environment and individuals. The objective is to minimize the use of hazardous chemicals, solvents, and reagents or substitute them with more sustainable, greener, biodegradable, and environmentally friendlier solvents, and utilize energy-efficient resources with minimal waste generation [1].

Gluszka et al. developed the 12 principles of green chemistry to consider many factors that can contribute to a more environmentally friendly process [2]. The greenness of the analytical method is assessed by using five published assessment tools: Eco-Scale [3], National Environmental Method (NEMI) [4,5] Green Analytical Procedure Index (GAPI) [6], the Analytical Method Greenness Score (AMGS) [7], and Analytical GREEnness Metric Approach and Software v. 05 beta (AGREE) [8].

AGREE assesses the greenness of the analytical method according to the principles of green analytical chemistry; each of the 12 green analytical chemistry principles (GAC) is converted into a standardized scale ranging from 0 to 1. The cumulative assessment results for each principle determine the final evaluation. The outcome is depicted as a

clock-like graph, with the overall score and color representation situated at the center. The central pictogram displays the overall score, where values nearing one and a dark green color indicate a higher level of environmental friendliness in the reviewed analytical method. The color assigned to each section, corresponding to the number of each principle, illustrates the process involved in each assessment criterion. Among the five published assessment tools, AGREE stands out as a quantitative and more representative measure of how green analytical processes are, making it the most advantageous option. Furthermore, the software is free and considers the 12 GAC principles [8].

The Chloroform-oriented Toxicity Estimation Scale (ChlorTox Scale) evaluates the chemical risk of the method on health and environment. The scale compares the chemical hazard of each used substance (CH_{sub}) with the chemical hazard of chloroform (CH_{CHCL3}), considering the mass of the substance needed for single analysis (m_{sub}) using the simple equation ChlorTox = $CH_{sub}/CH_{CHCL3} \cdot m_{sub}$. It can be used in a complementary manner with other green metrices such as AGREE for better comprehensive evaluation [9].

White analytical chemistry (WAC) emerged as a holistic approach to avoid prioritizing environmental friendliness at the expense of method performance. Consequently, it offers a more thorough assessment that considers various factors. Achieving a balance between the greenness and practical utility of an analytical method necessitates considerations of analytical efficiency, as well as practical and financial aspects. The adoption of white analytical chemistry serves as an illustration of this balance. Despite the ongoing challenges in many circumstances, striking this equilibrium remains challenging [10]. Analysts are tasked with preserving the utility of analytical methodologies for their intended purposes, ensuring a green approach without compromising quality.

The Blue Applicability Grade Index (BAGI) tool can be regarded as a step forward toward white analytical chemistry evaluation beyond the greenness of the method. It is concerned with evaluating the practicality of the analytical method. The metric scores methods between 25, the least practical, up to 100 for the best practical method, using 10 practical aspects including sample preparation, preconcentration, number of analytes that can be simultaneously treated, type of analysis, amount of sample, number of simultaneously determined analytes, analyzed samples per hour, type of reagents and materials, used instrumentation, and automation degree [11]. A free web application version of the metric is available [12].

An effective tool for assessing the analytical method's whiteness is the RGB12 tool.

This tool relies on three components: analytical efficiency (R), ecological efficiency (G), and practical economic efficiency (B). Notably, the assessment of analytical efficiency (R) and economic efficiency (B) is not covered by existing green assessment tools. Key validation parameters, such as the limit of detection (LOD), accuracy, and precision, serve as benchmarks for analytical efficiency; meanwhile, practical/economic efficiency (B) provides insights into productivity from both practical and economic standpoints. By combining the colors red, green, and blue, the tool generates a white score based on the saturation level of each color. A downloadable Excel file for calculating RGB12 is freely accessible [13].

The RGB model incorporates the 12 white analytical chemistry (WAC) principles, each comprising four concepts. In practice, prioritizing R and B over G is essential to develop a technique suitable for its intended purpose. The actual application of the 12 principles may involve varying weights and impacts. White analytical chemistry emerges from the harmonious integration of ecological and practical elements with analytical performance. Therefore, striving for sustainable advancements in analytical chemistry essentially means pursuing the white method.

Recent trends in enhancing method sustainability, influenced by the principles of both green analytical chemistry (GAC) and white analytical chemistry (WAC), include practices such as miniaturization, solvent-less sample preparation, and avoiding derivatization. Nonetheless, the substitution of hazardous organic solvents with greener and environmentally benign alternatives remains a crucial factor in promoting the sustainability of analytical methods.

In order to accomplish sustainability, suitable green solvents must be chosen when developing analytical methods. Several organizations and pharmaceutical companies, including Sanofi, Pfizer, and GlaxoSmithKline (GSK), have developed guidelines for solvent sustainability that enable them to determine favored solvents and evaluate them using safety data sheets (SDS) to compare their advantages and disadvantages.

Different solvent selection guidelines have been published. Ethanol and isopropanol are categorized within Class 3 of the International Council for Harmonization (ICH) Q3C (R8) guideline, which includes solvents less toxic and of lower risk to humans, while acetonitrile and methanol are categorized under Class 2, which includes solvents with inherent toxicity [14]. According to the CHEM21 solvent selection guide, ethanol and isopropanol also have the same safety, health, and environmental scores and the same ranking under recommended solvents [15]. According to the unified version of the general solvent selection guide for medicinal chemistry, which includes Pfizer, GlaxoSmithKline (GSK), and Sanofi solvent selection guides, ethanol and isopropanol have the same classification (preferred with green color as per Pfizer, some issues with yellow color as per GSK, and recommended with green color as per Sanofi). On the other hand, acetonitrile shows a worth profile with a classification (usable with yellow color as per Pfizer, major issues with green color as per GSK, and recommended with green color as per Sanofi) [16]. However, according to the GSK solvent guide, isopropanol shows better features than ethanol in terms of its status as a volatile organic compound (VOC) and air impact [17].

In order to help with the selection of environmentally friendly and sustainable solvents, Larsen, Christian, et al. [18] developed a tool that uses the GSK solvent sustainability guidelines to provide a quantitative evaluation. This assessment is based on an extensive array of aspects reflected by the composite score value (G), which is computed as the fourth root of the product of four important sustainability factors: waste disposal (W), environment (E), health (H), and safety (S), stated as $G = \sqrt[4]{(H \times S \times E \times W)}$. The composite score value (G) is a numerical value between 1 and 10, where lower values denote nonsustainable properties and higher levels indicate sustainable green solvents.

When studying the greenness of organic solvents, the following facts are to be considered. Any organic compound with a vapor pressure of 10 hPa at 20 °C or more (0.01 kPa at 293.15 K or more) is regarded as a VOC according to the European Union. Compounds with low vapor pressure to reduce the VOC losses into the atmosphere are favorable as green organic compounds. Isopropanol has a vapor pressure of 43 hPa at 20 °C; thus, considering this factor, it is regarded as a VOC against its greenness impact. Another greenness factor for organic compounds is the autoignition temperature and flash point. For safety considerations, the flash point threshold should be more than 60 °C. Isopropanol has a flash point of 12 °C. Thus, this factor also does not support the greenness of isopropanol as an organic solvent. A third important factor for classifying organic solvents as being green is the rat oral LD50 value as a health measure. A threshold value is 2000 mg/kg, below which the substance is recognized as harmful according to European Regulation 127272008, CLP. According to the isopropanol safety data sheet [19], its LD50 oral rat value is 5840 mg/kg, thus indicating a low risk to health and the good safety of isopropanol and supporting its greenness impact as an organic solvent. A fourth factor considering the greenness of organic solvents is the lipophilicity. In general, a low *n*-octanol/water partition coefficient with a log *p* value < 4 suggests a low potential for bioaccumulation. According to the safety data sheet, isopropanol has a log *p* *n*-octanol/water value of 0.05; therefore, bioaccumulation is not expected, which supports safety and greenness considerations.

Glaucoma represents a set of chronic degenerative eye disorders characterized by damage to the optic nerve, which can result in irreversible vision loss. Elevated intraocular pressure (IOP) is frequently linked to glaucoma [20,21]. It ranks among the primary causes of blindness globally [22,23]. Effective management strategies include intraocular pressure reduction through medications, laser therapy, or surgical intervention. The primary and effective treatment method is medication, which reduces intraocular pressure (IOP) to slow down further optic nerve damage. Several classes of medications, including beta-blockers,

prostanoid analogs, alpha-agonists, carbonic anhydrase inhibitors, and cholinergic agents, are frequently prescribed for the treatment of glaucoma [20].

Topical prostaglandin analogs or selective or nonselective beta-blockers are typically used as the initial line of treatment for glaucoma. The preferred second-line medications are topical carbonic anhydrase inhibitors and alpha-agonists. Pseudocarpine and other parasympathomimetic drugs are regarded as third-line therapeutic alternatives [24].

Topical carbonic-anhydrase inhibitors work by directly blocking carbonic anhydrase in ciliary processes, which lowers the generation of aqueous humor. Combination with the β blocker timolol causes an extra 17% drop in dorzolamide and brinzolamide [25].

Three drugs that are currently used in the treatment of glaucoma are targeted in this study. Dorzolamide ((4S,6S)-4-(ethylamino)-6-methyl-7,7-dioxo-5,6-dihydro-4H-thieno [2,3-b] thiopyran-2-sulfonamide) has the structure shown in Figure 1A and is the first topical carbonic anhydrase inhibitor approved for glaucoma therapy. Currently, it is extensively used as an adjunctive treatment for glaucoma in combination with other drug classes, including beta-adrenergic antagonists, alpha-adrenergic agonists, cholinergic, and synthetic prostaglandins [26–29].

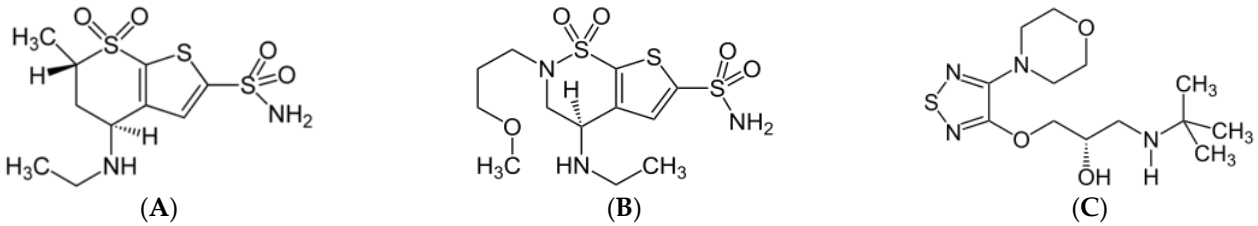


Figure 1. Structural formula of (**A**) dorzolamide, (**B**) brinzolamide, and (**C**) timolol.

Brinzolamide((4R)-4-(ethylamino)-2-(3-methoxypropyl)-1,1-dioxo-3,4-dihydrothieno [3,2-e] thiazine-6-sulfonamide) has the structure shown in Figure 1B and is the second topical carbonic anhydrase inhibitor to receive approval from the Food and Drug Administration (FDA) for the treatment of glaucoma [30,31].

Timolol((Z)-but-2-enedioicaid;(2S)-1-(tert-butylamino)-3-[(4-morpholin-4-yl-1,2,5-thiadiazol -3-yl) oxy] propan-2-ol) has the structure shown in Figure 1C and is a non-selective β-adrenergic antagonist. For more than 30 years, it has been applied topically to treat glaucoma and increased intraocular pressure (IOP) [26,30,32].

The literature reports a number of RP-HPLC methods for the individual determination of dorzolamide [33,34], brinzolamide [35], and timolol [36].

Furthermore, the simultaneous determination of either dorzolamide with timolol [37–39], brinzolamide with timolol [35,40], or the determination of any of the three drugs with other related drugs [41,42] has also been reported in the literature.

The above-reported methods used either acetonitrile or methanol or both together as organic constituents in the mobile phase. To our knowledge, only two HPLC methods have been published reporting the simultaneous separation and determination of the three targeted drugs; in both of them, acetonitrile [43,44], the nongreen solvent, was used in the mobile phase, no reported method used isopropanol as a mobile phase constituent.

Ibrahim et al. [42] developed the only reported RP-HPLC method coupled with a UV detector for the simultaneous separation and quantification of the three targeted drugs using acetonitrile as an organic constituent in the mobile phase. This HPLC method was carried out under the following chromatographic conditions. The isocratic single mobile phase containing acetonitrile and 0.05 M sodium dihydrogen phosphate buffer in the ratio (30:70, v/v), respectively, was used at pH 3.5. The analytical column was the Promosil C18 column (250 mm × 4.6 mm, 5 µm particle size), Agela Technologies, Wilmington, DE, USA. The wavelength was adjusted at 220 nm at a flow rate 1 of mL/min. This method was regarded as the reference method for subsequent comparisons.

This study aimed to develop a greener and whiter RP-HPLC method for simultaneously determining dorzolamide, brinzolamide, and timolol. Additionally, assessment of the

developed method of determining greenness using the AGREE metric and the whiteness using the RGB12 tool was also performed.

2. Materials and Methods

2.1. Chemicals and Reagents

The reference materials of dorzolamide hydrochloride, brinzolamide, and timolol maleate were obtained from Sigma-Aldrich Chemie GmbH (Darmstadt, Germany). Glacial acetic acid for HPLC and sodium acetate were obtained from Applichem (Darmstadt, Germany), and hydrochloric acid analytical grade was obtained from Fisher Scientific (Loughborough, UK). Isopropanol HPLC grade was obtained from Sigma-Aldrich Chemie GmbH (Darmstadt).

2.2. Buffer and Sample Preparation

Sodium acetate buffer 0.1 M pH 4.25 was prepared by adding 5.772 g of sodium acetate and 1.778 g of acetic acid to 800 mL Milli Q water. The pH was adjusted to 4.25 by adding 10 N HCl, and the volume was raised to 1 L with Milli Q water. Stock solutions of 2000 µg/mL of dorzolamide, 500 µg/mL of brinzolamide, and 500 µg/mL of timolol were made to prepare calibrants and quality control samples.

2.3. Method Validation

Stock solutions were prepared at concentrations of 2000 µg/mL for dorzolamide, 500 µg/mL for brinzolamide, and 500 µg/mL for timolol to prepare calibrants and quality control samples. These solutions were utilized in the preparation of standards for the validation study. For linearity assessment, calibration curves were generated by plotting analyte peak areas against their respective concentrations, incorporating six different standard concentrations (80, 120, 160, 200, 240, and 280 µg/mL) for dorzolamide, (40, 60, 80, 100, 120, and 140 µg/mL) for brinzolamide, and (20, 30, 40, 50, 60, and 70 µg/mL) for timolol.

For accuracy testing, three quality control (QC) standards at low (LQC), medium (MQC), and high (HQC) concentration levels within the linear range were examined, using 20, 40, and 60 µg/mL for dorzolamide, 40, 80, and 120 µg/mL for brinzolamide, and 20, 40, and 60 µg/mL for timolol. These quality control (QC) standards were then employed to determine the precision of the proposed methodology. The limits of quantification (LOQ) were calculated based on the standard deviation of the response and the slope, using the equation LOQ = 10 σ/S, where σ represents the standard deviation of the response, and S is the slope of the calibration curve. The limits of detection (LOD) were calculated using the standard deviation of the responses and the slope, employing the equation LOD = 3.3 σ/S.

2.4. HPLC Analysis

An Agilent 1260 (Agilent Technologies GmbH, Waldbronn, Germany) instrument with a quaternary pump (G1311B), autosampler (G1329B), and diode array detector (G1315D) was used. The Purospher STAR RP-18 endcapped (150 × 4.6 mm, 5 µm particle size) column (Merck, Darmstadt, Germany) was used.

3. Results

3.1. Method Development

A greener method was developed to simultaneously determine dorzolamide, brinzolamide, and timolol using isopropanol as a mobile phase constituent. Isopropanol was tested as a mobile phase organic solvent constituent for replacement of acetonitrile in the reported reference method, as shown in Figure 2. Based on the Hansen space green solvent selection tool, isopropanol had a G score of 6.5, close to ethanol, which had a G score of 6.6, much better than that of acetonitrile, which had a G score of 5.8 [45], as shown in Figure 3.

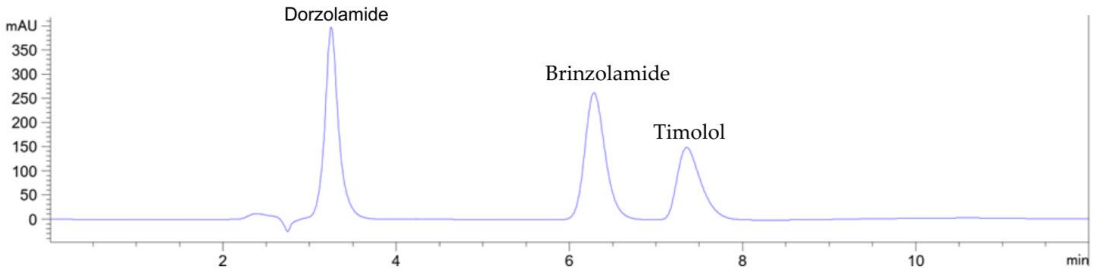


Figure 2. Chromatogram showing the separation of dorzolamide, brinzolamide, and timolol on high-purity silica with polymeric C18 column (150 × 4.6 mm, 5 µm particle size) at a flow rate of 1 mL/min. The three substances were eluted using isopropanol: 0.1 M sodium acetate buffer pH 4.25 in the ratio of 10:90 (*v*/*v*).

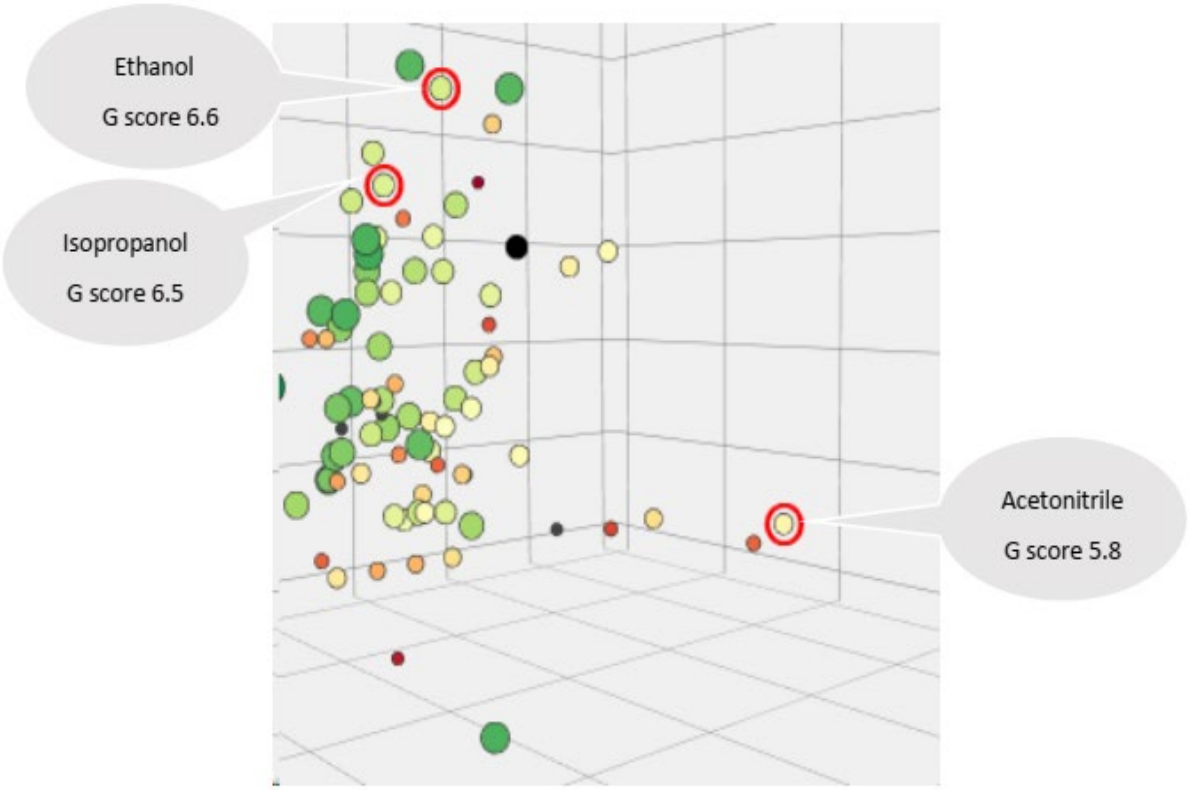


Figure 3. Isopropanol, ethanol, and acetonitrile solvent sustainability levels are shown by the size and color of the spheres generated by the Hansen space green solvent selection tool [44]; a larger and greener sphere indicates better solvent sustainability.

The UV cutoff value of isopropanol (205 nm) was the same as that of methanol and higher than that of acetonitrile. However, it was still below the maximum absorbance wavelength of most active pharmaceutical ingredients. Isopropanol is miscible with water in any proportion, making it compatible with aqueous buffer systems.

Isopropanol exhibited a lower Kamlet–Taft π^* solvent parameter value compared to the other solvents listed in Table 1; this suggests lower polarity and, consequently, enhanced elution power in RP-HPLC. As a result, a smaller proportion of isopropanol in the mobile phase was needed to replace acetonitrile. This reduction contributes to a decrease in the overall quantity of organic solvents in the mobile phase and, consequently, minimizes generated waste. Collectively, these aspects make the analytical method more environmentally friendly and sustainable.

Table 1. Comparison of the physical properties of isopropanol with a range of environmentally friendly and conventional RP-HPLC solvents *.

Solvent	UV Cut-Off Value (nm)	Water Solubility	Density (g/cm^3) at 20 °C	Polarity Parameter Kamlet—Taft π *	Partition Coefficient *n*—Octanol/Water (Log Value)	Boiling Point °C	Flash Point °C at 1.013 hPa (c.c.)	G Score
Acetonitrile	190	miscible in any proportion	0.78	0.75	−0.54	82	2	5.8
Methanol	205	1000 g/L at 20 °C—completely miscible	0.791	0.61	−0.77	64.7	9.7	5.8
Ethanol	210	≥1000 g/L at 20 °C	0.81	0.54	−0.31	78	9.7	6.6
Isopropanol	205	miscible in any proportion	0.786	0.48	0.05	82.4	12	6.5

* Data were gathered from safety data sheets of solvents and the references [44].

Isopropanol has one main drawback when used in liquid chromatography with analytical columns. This drawback is the high density, which is even higher than that of methanol. It might generate higher backpressure depending on the percentage used, the flow rate, and the total composition of the mobile phase. However, the developed method generated an acceptable backpressure below 160 bar using the Puroshper RP-18 endcapped column.

3.2. Method Validation

The validation of the method was conducted in accordance with the ICH guidelines [45] and key validation parameters are outlined in Table 2. Good linearity was found for the three analytes, with correlation coefficients (R^2) higher than 0.9979 across the investigated ranges, demonstrating good accuracy and precision. Detection and quantitation limits were higher compared to those reported in the reference method, yet they still fitted the intended purpose of the method. A representative chromatogram for dorzolamide, brinzolamide, and timolol elution using isopropanol as an organic mobile phase constituent is shown in Figure 2.

Table 2. Key validation parameters for the developed Isopropanol-based method.

Parameter	Dorzolamide	Brinzolamide	Timolol
Linearity (R^2)	0.9995	0.9999	0.9979
Equation	y = 3947.2x + 10691	y = 1624.1x − 1048.1	y = 117.05x − 213.15
Linearity Range (µg/mL)	20–70	40–140	20–70
LOD (µg/mL)	1.61	1.60	3.16
LOQ (µg/mL)	4.87	4.86	9.59
Accuracy (µg/mL)	99.1–101.0%	99.3–100.1%	95.3–101.8%
Precision RSD%			
LQC	0.08%	0.04%	0.03%
MQC	0.02%	0.01%	0.02%
HQC	0.02%	0.07%	0.04%

3.3. Greenness and Whiteness Assessments of the Methods

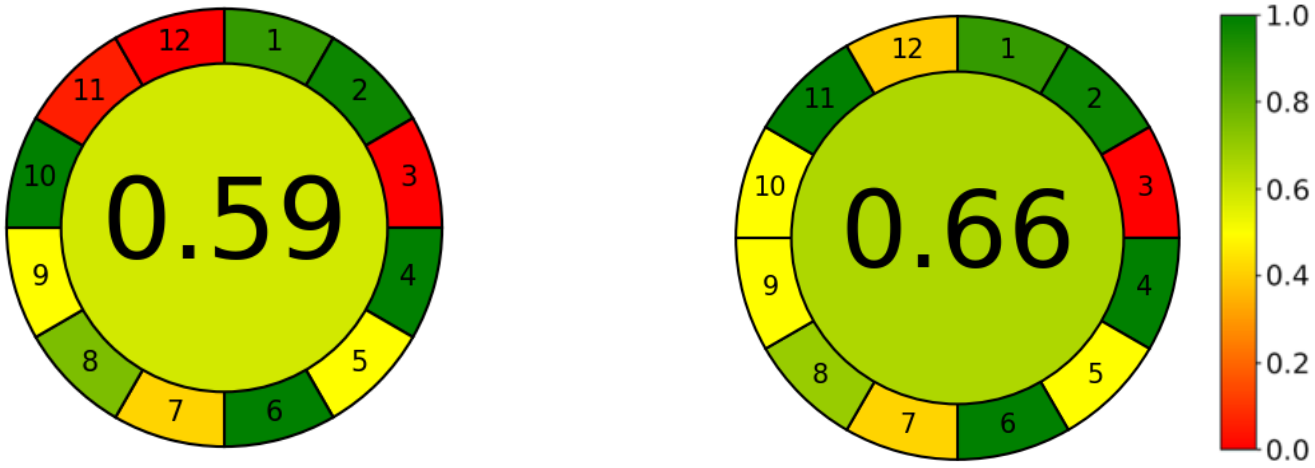
The environmental impact (greenness) of the two methods, employing the elution conditions specified in Table 3, was assessed and compared using the quantitative greenness assessment tool AGREE. The greenness profiles are depicted in Figure 4. The findings indicated that the method based on isopropanol exhibited a higher greenness score in comparison to the acetonitrile (ACN)-based method.

Table 3. The elution conditions of the reported and developed methods for the simultaneous analysis of dorzolamide, brinzolamide, and timolol.

	Method	Elution Conditions	Reference
A	Reported acetonitrile (ACN)-based nongreen method.	HPLC-DAD using RP-C18 column. Isocratic elution using ACN and phosphate buffer (30:70, *v*/*v*) as mobile phase	[43]
B	Newly developed green isopropanol-based reference method	HPLC-DAD using the C18 column. Isocratic elution using isopropanol and 0.1 M sodium acetate buffer pH 4.25 (10:90, *v*/*v*) as mobile phase	This work

Both the developed greener isopropanol-based method and the reference acetonitrile-based method had the same run time of less than 8 min. The developed greener method showed higher limits of detection and quantification but still fitted the intended purpose.

The developed method showed a better number of theoretical plates, as mentioned in Table 4.



(**A**) (**B**)

Figure 4. Assessment of environmental impact (greenness) comparing (**A**) the reported acetonitrile-based nongreen reference method and (**B**) the newly developed green isopropanol (IPA)-based method, conducted using the AGREE tool. Higher numerical values indicate enhanced greenness.

Table 4. Comparing the performance of the developed isopropanol-based method (IPA) and the reference acetonitrile-based method (ACN) *.

Parameter	Dorzolamide		Brinzolamide		Timolol	
	IPA Method	ACN Method	IPA Method	ACN Method	IPA Method	ACN Method
t_R	3.223	3.7	6.138	4.8	7.369	2.3
N	4888	1025	8535	2100	9340	1390
L	150	250	150	250	150	250
HETP	0.03	0.24	0.02	0.12	0.02	0.18

* Where t_R: retention time in minutes, N: number of theoretical plates calculated as N = 16 (t_R/wb) 2, L: the column length in mm, HETP: height equivalent to a theoretical plate calculated as HETP = L/N.

The relative hazard of isopropanol in respect to chloroform (CH_{IPA}/CH_{CHCl3}) according to the WHN (weighted hazards number) model is 0.35; when multiplied by the mass of the used isopropanol in a single run of 6280 mg, the resulting total ChlorTox value was 0.219 g, assuming a 0 relative hazardous of sodium dihydrogen phosphate as the other component used in the mobile phase. On the other hand, the relative hazard of acetonitrile in respect to chloroform ($CH_{acetonitrile}/CH_{CHCl3}$) according to the WHN model is 0.39; when multiplied by the mass of the used acetonitrile in a single run of 1876 mg, the resulting total ChlorTox value was 0.733 g, giving a 0 relative hazardous of sodium acetate as the other component used in the mobile phase. This also indicates the lower chemical hazardous of the developed isopropanol method compared to the reported acetonitrile method.

Moreover, an assessment of the whiteness of the isopropanol-based method was conducted and compared with the reference acetonitrile method using the RGB12 tool. The results, as depicted in Figure 5, indicated that the isopropanol-based method is a whiter analytical method, primarily due to superior green and blue components, without markedly compromising the red component in the evaluation matrix.

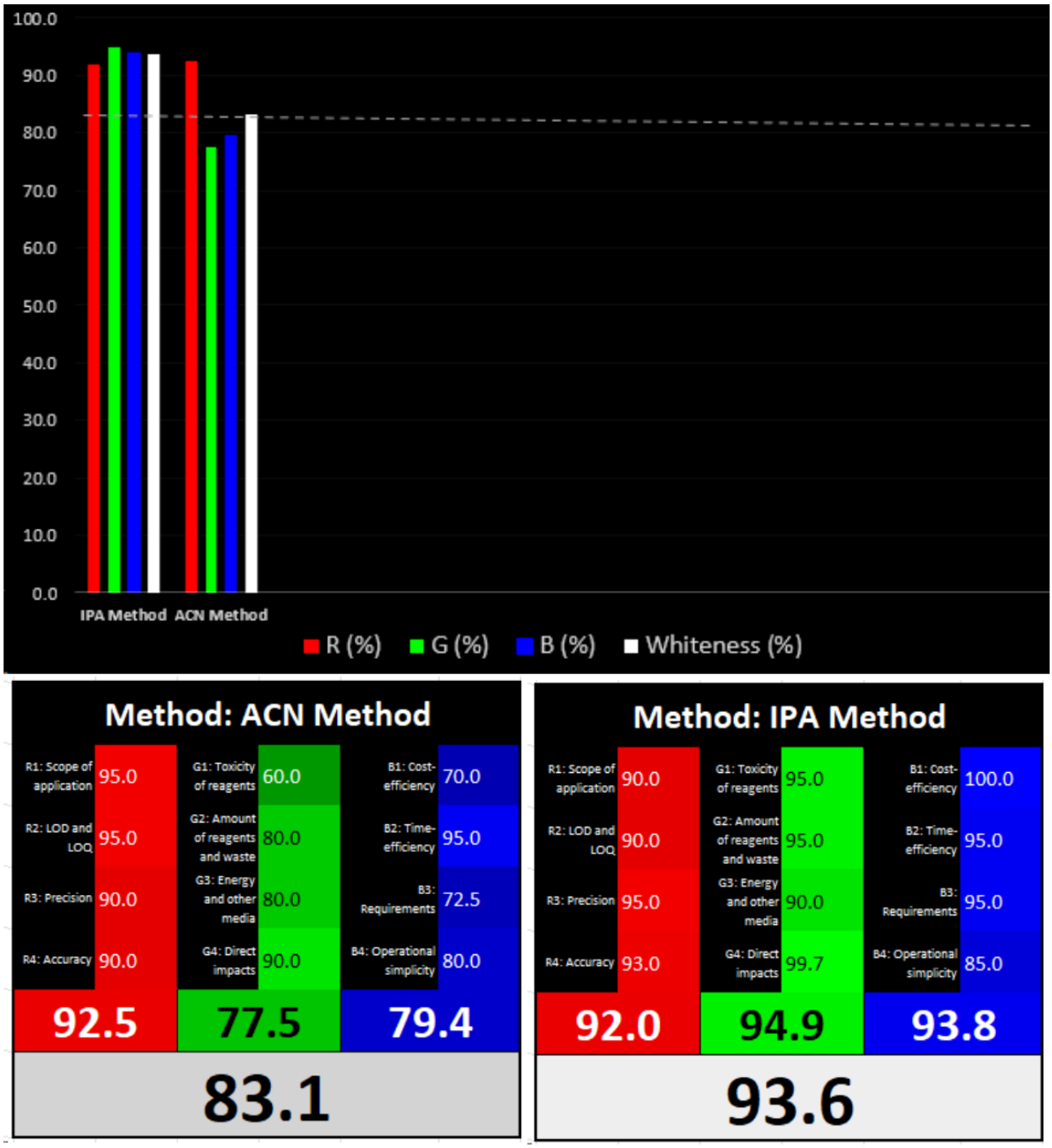


Figure 5. Assessment of the whiteness of the reference acetonitrile (ACN) and the newly developed isopropanol (IPA) methods based on the RGB12 tool, where the numerical values indicate improved whiteness.

4. Conclusions

This work shows a simple isocratic method for simultaneous separation and determination of dorzolamide, brinzolamide, and timolol within a time of analysis shorter than 8 min. Isopropanol is regarded as a green solvent and classified as green as ethanol, constituting an excellent replacement for acetonitrile or methanol in chromatography. The AGREE tool clearly showed a higher value of greenness for the developed isopropanol-based method in comparison to the reference method.

The results demonstrated the potential of isopropanol to replace environmentally hazardous nongreen organic solvents such as acetonitrile while maintaining method accuracy, precision, and efficiency. Despite similar levels of redness observed in both acetonitrile and isopropanol-based methods, the isopropanol-based approach significantly enhanced aspects of greenness and blueness according to the RGB12 tool. Although the limits of detection (LOD) and limits of quantification (LOQ) were slightly elevated, their adequacy for the intended application justified a commendable score. Accuracy percentages within the ranges of 99.1–101.0% for dorzolamide, 99.3–100.1% for brinzolamide, and 95.3–101.8% for timolol were obtained. The method demonstrated notable precision with a relative standard deviation (RSD)% < 0.1 for all three analytes. The paramount consideration remains the method's sufficiency for its intended purpose, attaining a favorable whiteness percentage. In summary, the developed isopropanol-based method is a simple, greener, and whiter method supporting the shift from classical RP-LC solvents to more sustainable ones.

Author Contributions: Conceptualization, S.E.D.; methodology, S.E.D.; software, M.K.P.; validation, S.E.D. and K.A.; formal analysis, S.E.D. and K.A.; investigation, S.E.D. and K.A.; resources, S.E.D. and M.K.P.; data curation, S.E.D. and K.A.; writing—original draft preparation, S.E.D. and K.A.; writing—review and editing, S.E.D., K.A., and M.K.P.; visualization, S.E.D. and K.A.; supervision, S.E.D.; project administration, S.E.D. and M.K.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Data are contained within the article.

Acknowledgments: We thank Merck KGaA, Darmstadt, Germany, for kindly providing the Purospher RP-HPLC column.

Conflicts of Interest: The authors declare no conflict of interest.

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