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GREENNESS ASSESSMENT OF CONTINUOUS FIA-SPECTROPHOTOMETRIC METHOD FOR QUANTITATION OF OXYMETAZOLINE IN BULK AND PHARMACEUTICAL FORMS

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ABSTRACT. An accurate and cost-effective method utilizing continuous flow injection spectroscopy has been developed for analyzing oxymetazoline hydrochloride in pharmaceutical and bulk powder forms. This new method involves reacting drug in an alkaline solution with diazotized 4-chloroaniline to produce an orange dye with a maximum absorption at λ_{max} of 483 nm. The flow injection analysis approach was extensively studied in terms of both chemical and physical characteristics to achieve high sensitivity. Within a concentration range of 50-150 µg/mL and a detection limit of 10.4 µg/mL, oxymetazoline hydrochloride follows Beer's law when the experimental variables are optimized. The method's effectiveness was confirmed by achieving high reproducibility of less than 2% (n = 5). A statistical analysis comparing the proposed method to the standard spectrophotometric technique using F and t tests shown no significant differences in accuracy and precision. Additionally, the analytical greenness assessment and the green analytical procedure index were applied to assess the method's greenness and the results demonstrated that the flow injection analysis method satisfies the criteria of green analytical methodologies.

KEY WORDS: Flow injection analysis, Diazotization reaction, 4-Chloroaniline, Oxymetazoline hydrochloride

INTRODUCTION

Oxymetazoline hydrochloride (OXY), chemically named, 3-[(4,5-dihydro-1H-imidazol-2-yl)methyl]-6-(1,1- dimethyl ethyl)-2,4-dimethyl-phenol hydrochloride [1], is an adrenergic drug. By targeting adrenergic receptors, it has been utilized as eye and nasal drops to induce significant vasoconstriction and elevate blood pressure [2]. It is used to reduce eye itching and irritation, as well as to relieve redness, burning, and dryness caused by wind, sun, and other minor irritants [3]. Various analytical methods have been proposed for the determination of OXY, including voltammetry [4, 5], capillary electrophoresis [6, 7], FIA [8-10], high-performance liquid chromatography [11, 12], and spectrophotometry [13-17]. Due to their sensitivity and ease of use, spectroscopic techniques like UV-Vis spectrophotometry are widely employed [18-20]. Additionally, continuous flow injection analysis (FIA) has been established, which reduces reagent use and automates the process, increasing efficiency and cost-effectiveness [21-27].

Flow injection methods have gained significant attention and widespread application due to their simplicity, high reproducibility, and low cost of instrumentation [28-33]. FIA is commonly employed for repetitive analysis of various compounds. Compared to batch techniques, FIA is more adaptable, offers better precision, uses fewer chemicals, and has a higher sampling rate [34-39]. Engaging these two techniques facilitates the reliable quantification of many compounds, particularly drugs, in both pharmaceutical formulations and bulk powder forms [40-42]. The aim of this work is to develop a rapid and simple and green flow injection-spectrophotometric approach for estimating OXY in pharmaceutical forms. The work is based on reacting the drug with diazotized 4-chloroaniline in a basic medium to produce an azo dye, which has a maximum wavelength absorption at 483 nm.

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EXPERIMENTAL

Chemicals and reagents

The standard oxymetazoline drug utilized in this study was provided from the state company for drug industries and medical appliances (Samarra-Iraq). Sodium hydroxide, sodium nitrite and hydrochloric acid 35% (w/w) were obtained from BDH. *p*-Chloroaniline (purity 98%) was purchased from Sigma-Aldrich.

Solutions preparations

Oxymetazoline hydrochloride solution (500 µg/mL) was prepared as follow: A 0.05 g of the pure medication was dissolved in 100 mL of bidistilled water to prepare a standard OXY solution. Diazotized p-chloroaniline (0.01 M) was prepared in a 100 mL volumetric flask. A 0.0638 g of standard p-chloroaniline was dissolved in 5 mL of ethanol, followed by 3 mL of hydrochloric acid (1 M) in an ice bath while keeping the solution temperature at 0-5 °C. Then, 0.0345 g of $NaNO_2$ (0.01 M) was added and shaken for three minutes, and the flask was then completely filled with bidistilled water [21]. Hydrochloric acid solution (1 M), prepared by diluting an appropriate volume of concentrated HCl (36.4% w/w) with bidistilled water to a total volume of 100 mL, followed by standardization. Sodium hydroxide solution (1 M), prepared by dissolving an appropriate amount of the base in 100 mL of bidistilled water, followed by standardization. Pharmaceutical applications of OXY (Alerjon®-0.025%, Edol/Portugal, and OXYMET®-0.05%, PHARAONIA/Egypt) were obtained from the local pharmacies. A stock solution with a concentration of 100 µg/mL was made by combining the contents of five commercial OXY nasal drops vials. Then, aliquots of 10 mL and 20 mL from this stock solution were each diluted to a final volume of 50 mL with bidistilled water in volumetric flasks.

Instruments

The UV-Visible-single beam spectrophotometer model Shimadzu/1240 was employed for both scanning and measurement of absorbance. Continuous FIA system primarily consisted of quartz cell (50 µL), a peristaltic pump (Ismatec/Switzerland) and a six ports-injection valve (Rheodyne/USA). A few microliters of drug sample were injected into the manifold through injection valve. Meanwhile, chemicals solutions were steadily pushed through flexible vinyl tubes and mixed within a reaction coil (RC) composed of Teflon tubes with an internal diameter of 0.5 mm. Using a dual-channel manifold (Figure 1), diazotized 4-chloroaniline (DCA) was introduced through one channel, while NaOH solution was delivered through the other. The OXY solution was introduced into the DCA reagent stream through the injection valve, then mixed with NaOH solution in the RC. The pump facilitated the movement of the solutions, and the absorbance of the resulting azo-dye was monitored using a spectroscopic detector.

Procedure of analysis by FIA manifold

Using a syringe, 100 µL of the OXY solution, at a concentration rage of 50-150 µg/mL, was injected via the injection valve. The OXY solution were introduced into a 0.005 M of DCA stream. The resulting solution was subsequently combined with a 1 M NaOH solution in the RC (100 cm). Solutions of reactant were pumped by a peristaltic pump at a flow rate of 5.3 mL/min, and the orange product's absorbance was determine at 483 nm. A 100 µg/mL OXY solution was used to investigate the optimal conditions of flow injection approach.

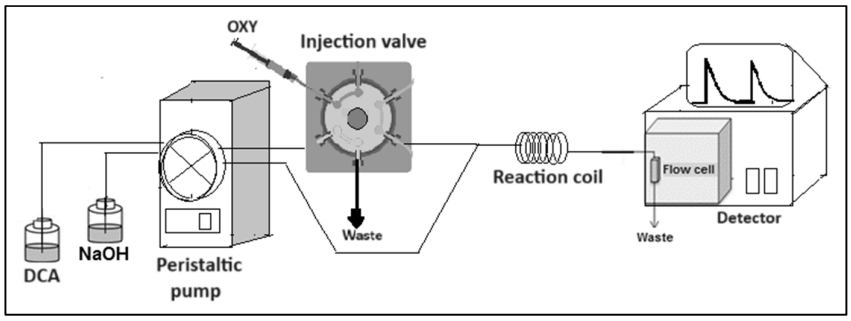


Figure 1. Continuous FIA manifold.

RESULTS AND DISCUSSION

A flow injection method has been proposed as an automated and sensitive spectrophotometric technique for determining OXY. This approach involves combining OXY and DCA in a basic medium to produce an orange-colored product.

Absorption spectra and the reaction mechanism

The product's and the blank's absorption spectra were manually recorded prior to the reaction being automated. This involved caring out the reaction in 25 mL measuring flask with 50 µg/mL of OXY, 2 mL of 0.01 M of DCA, and 1 mL of 0.5 M NaOH. The azo-dye formed instantly once the solutions were mixed and stirred. After five minutes of mixing the reagents, the absorption spectra ranging from 350 to 1100 nm were recorded. The highest absorption was observed at 483 nm compared to the reagent blank, which show minimal absorbance at the same wavelength (Figure 2). The influence of various physical and chemical FIA parameters was extensively studied.

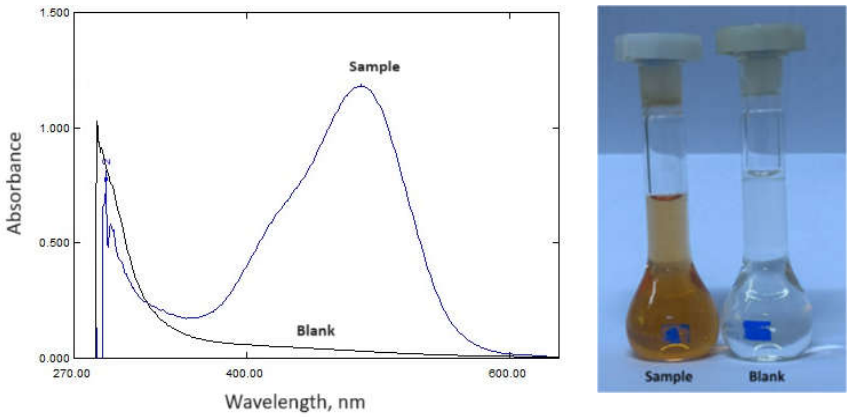
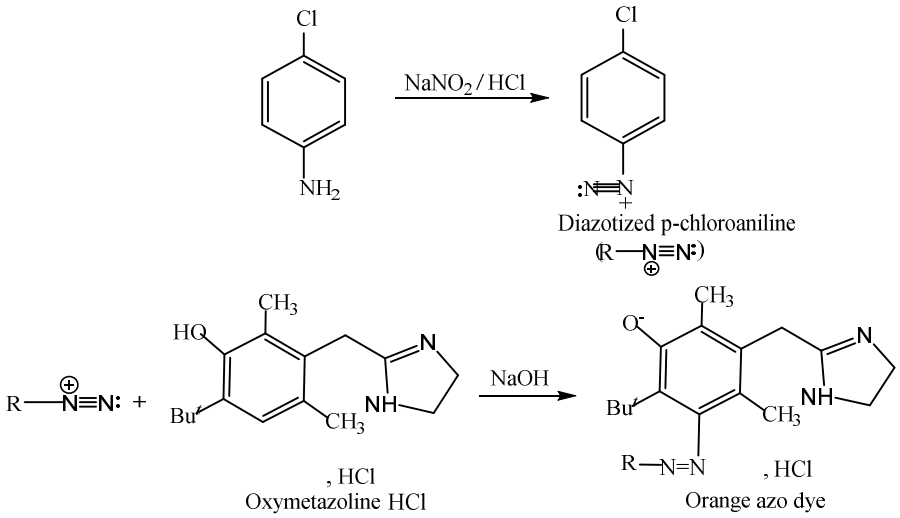


Figure 2. (Left) UV-Vis scan and the spectra of (a) azo-dye formed after reaction OXY with DCA/NaOH and (b) the blank measured against bidistilled water. (Right) color of reaction.

Scheme 1 illustrated the proposed reaction mechanism [43-45]. In the diazotization reaction with HNO_2, the aromatic-NH_2 group in *p*-chloroaniline is quickly converted to a diazonium salt. Subsequently, in an alkaline medium, the diazonium salt reacts with phenolic drug (OXY). This mechanism was validated by using equimolar concentrations (1×10^{-3} M) of the OXY and DAC utilized the ideal conditions and determining the molar ratio of reagent to drug using Job's method [46]. A stoichiometry of 1:1 (OXY: DCA) was observed.



Scheme 1. Proposed reaction mechanism.

Study of chemical and physical parameters

Manifold design

Research was conducted to assess the impact of different chemical and manifold FIA factors on the sensitivity assessment of OXY. The crucial parameter to optimize was the FIA manifold. To investigate various reaction pathways for OXY in an alkaline medium with DCA, several manifold configurations (double-channel manifolds) were examined. The findings showed that manifold 2 in Figure 3 achieved the highest absorbance and was chosen for subsequent studies. As depicted in Figure 3, OXY was injected in the DCA stream before mixing with NaOH solution using a mixing coil.

Effect of reagent and base concentrations

Chemical variables such as reagent concentration, type, and base concentration were investigated. The impact of DCA concentration on dye absorption was examined. A 100 µL sample of OXY was introduced through the diazotized reagent stream, with concentrations ranging from 0.001 M to 0.01 M, and was pumped and transferred through FIA manifold. The best analytical signal was produced by 0.005 M, which was selected as the ideal value based on the results (Figure 4A). Previous research shows that the reaction between OXY and DCA requires an alkaline environment. This requirement is likely due to the phenolic drug OXY being converted into reactive phenoxide form. Diverse bases, included NH4OH, NaOH, and Na_2CO_3, were evaluated, with sodium hydroxide yielding the highest absorbance. Consequently, sodium hydroxide was

chosen for subsequent experiments (Figure 4B). A range of 0.3-1.5 M was used to examine the effects of varying the NaOH concentration. 1M sodium hydroxide produced the best results (Figure 4C), hence it was chosen for the subsequent trials.

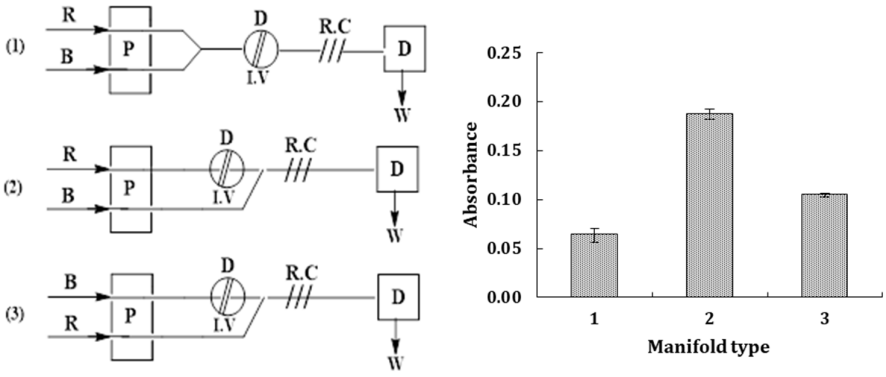


Figure 3. (Left) FIA manifold (D: drug, R: reagent, B: base, I.V: injection valve; P: pump; R.C: reaction coil; D: detector; W: waste). (Right) Effect of manifold.

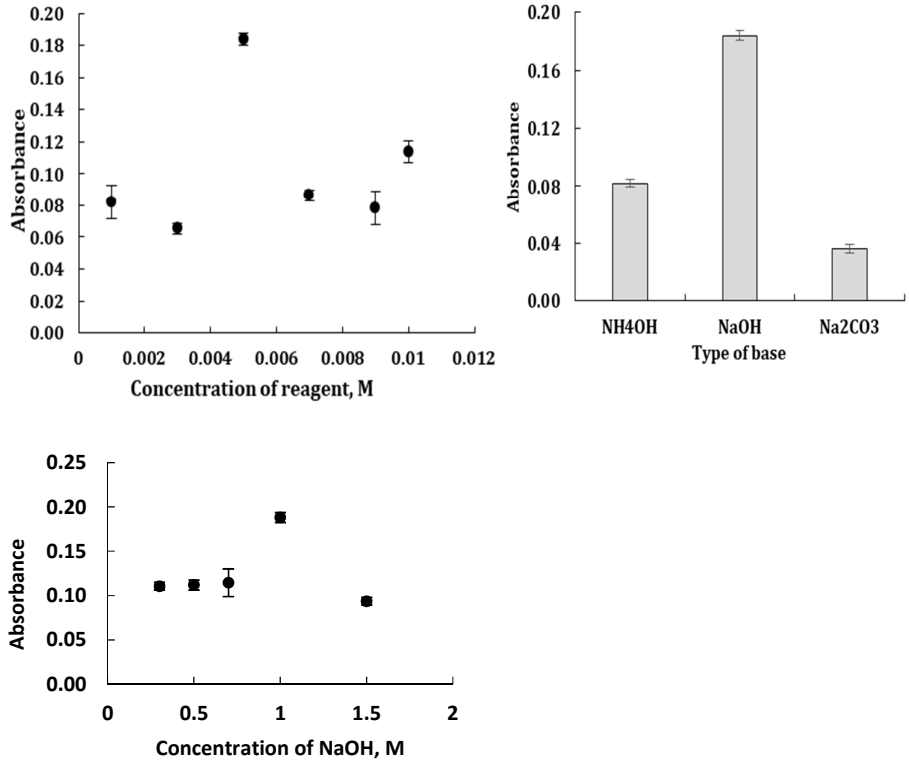


Figure 4. Effect of (A) concentration of reagent, (B) type of bases, (C) concentration of NaOH.

Effect of physical variables

The study also examined physical variables, including flow rate, sample volume, and reaction coil. Flow rate is considered to have the most significant impact on sample frequency and reaction sensitivity. Therefore, to optimize this parameter, various rates ranging from 2.9 to 7.5 mL/min were tested. Figure 5A demonstrated that the analytical response improved up to 5.3 mL/min and then gradually decreased with higher rates due to increased dispersion. Dispersion refers to the consistent yet dynamic mixing of a sample zone with a reagent zone and/or carrier fluid, driven by the flow patterns created by fluid dynamics as they move through narrow-bore tubing. Consequently, a flow rate of 5.3 mL/min was chosen at the optimal rate. The impact of the mixing coil length was examined over lengths between 25 and 200 cm. An obvious decrease in analytical signal was detected once the coil length exceeded 100 cm (Figure 5B). The rapid coupling reaction between the reactants likely reduces the requirement to extend the reaction's steady time by elongating the mixing coil. Consequently, 100 cm was selected for the subsequent work. To establish the optimal volume of injected OXY solution, samples ranging from 75-200 µL were tested in the DCA stream while keeping other variables without changing. The findings (Figure 5C) indicated that 100 µL formed the maximum analytical response and was therefore selected as the ideal volume.

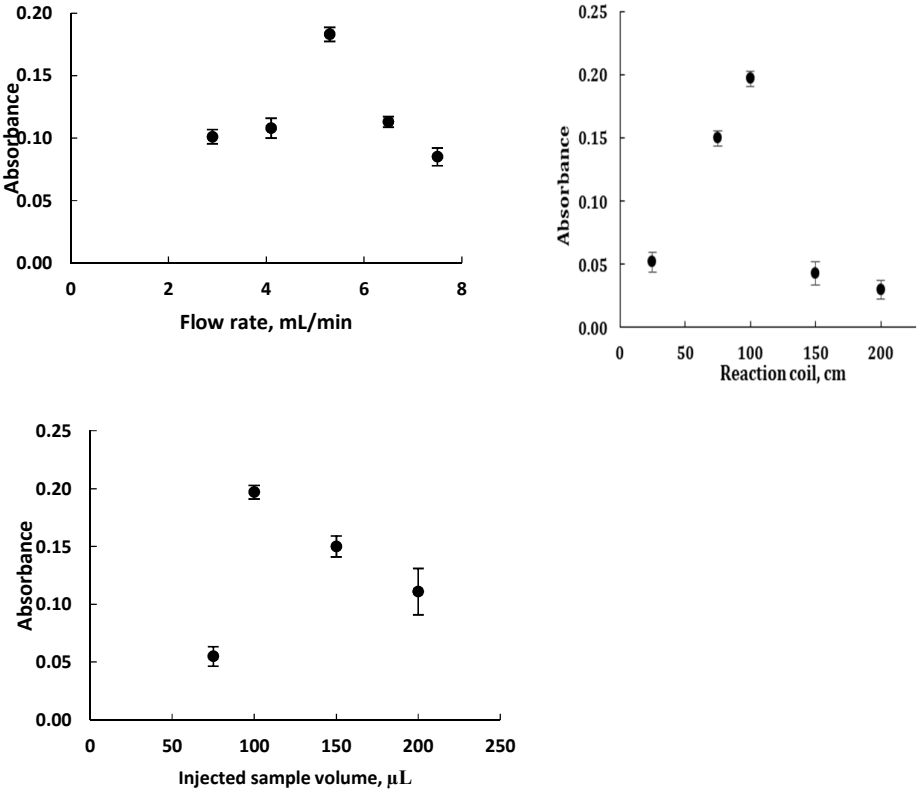


Figure 5. Study the optimum value of (A) manifold flow rate, (B) mixing coil length, and (C) injected OXY volume on produced the azo-dye.

Method validation

Linearity and sensitivity

Using FIA approach, a linear correlation between the concentration of OXY (ranging from 50 to 150 µg/mL) and absorbance at λmax was established under optimal conditions. The calibration curve is expressed by the equation y = bx + a, (y represents absorbance, a is the intercept, b is the slope, and x denotes the concentration (µg/mL)), determined using the least squares method. Linearity range, coefficient of determination, slope and intercept values are documented in Table 2. The molar absorptivity and Sandell's sensitivity values, along with the detection and quantification limits (LOD and LOQ), were determined according to current ICH guidelines [45, 47] and are presented in Table 1. These values were estimated using the subsequent formulas in accordance with the same guidelines: LOQ = 10σ/s and LOD = 3.3σ/s, (where s is the calibration curve's slope and σ is the standard deviation of five reagent blank determinations). The obtained values indicate the excellent sensitivity of the proposed method.

Table 1. Analytical characteristics of FIA method

Variables	Value
Regression equation	y = 0.0032x - 0.1163
Linearity range, µg/mL	50-150
Correlation coefficient, r	0.9983
LOD, µg/mL	10.43
LOQ, µg/mL	34.76
Molar absorptivity, L/mol cm	0.95×10^{3}
Sandell's sensitivity, µg/cm^2	0.31
Through-put, h^{-1}	55
Slope, b	0.0032
Intercept, a	- 0.1163
$S_{y/x}$	6.85×10^{-3}
S_b	7.13×10^{-5}
S_a	7.79×10^{-3}
Dispersion value	2.35

Evaluated the accuracy and precision of the method

The precision of the FIA method was evaluated using prepared solutions with three different concentrations of OXY, each tested in five replicates. The obtained results is summarized in Table 2. To assess repeatability (intra-day precision), the percentage RSD % data was obtained within the same day, and to assess intermediate precision (inter-day precision), they were acquired over three separate days. Small values of the relative standard deviation (RSD % less than 2%) and percentage relative error (RE %) referred to precise and accurate of the proposed approach. The recovery values (97.7-102.9%), which are close to 100% as shown in Table 2, demonstrate the excellent accuracy of the present approach.

Table 2. Evaluated the accuracy and precision of the suggested FIA approach

Added conc. (µg/mL)	Intra-day (n = 5)				Inter-day (n = 3)			
	Found conc. (µg/mL)	RE (%)	Recovery (%)	RSD (%)	Found conc. (µg/mL)	RE (%)	Recovery (%)	RSD (%)
60	60.40	0.67	100.67	1.98	61.76	2.93	102.93	1.88
110	107.44	-2.33	97.67	1.55	108.64	-1.24	98.76	0.98
140	137.39	-1.86	98.14	0.99	140.82	0.59	100.59	0.96

Analysis of pharmaceutical formulations

The FIA approach was effectively applied to analyze OXY in pharmaceutical formulations (nasal drops), with the results presented in Table 3. The declared substance and the obtained findings are in good agreement. The suggested method was evaluated through recovery experiments for various application, and the results showed recovery values ranging from 97.7 to 102.2%. The results achieved from a reference UV method [1] for the analysis of OXY drug were compared with the recovery values obtained from FIA procedure. According to the t and F-test results at 95 % confidence level [48], there was no significant difference between the assay of OXY in dosage forms using FIA method and the conventional method.

Table 3. Assay of OXY in nasal drops using the FIA and UV techniques.

Pharmaceutical application	FIA method					UV method				
	Added conc. (µg/mL)	Found conc. (µg/mL)	Error %	Rec. (%)[a]	RSD (%)[a]	Added conc. (µg/mL)	Found conc. (µg/mL)	Error %	Rec. (%)[a]	RSD (%)[a]
Alerjon®	70	68.37	-2.33	97.67	0.68	25	24.66	-1.36	98.64	0.98
	100	102.16	2.16	102.16	1.49	35	34.78	-0.63	99.37	1.22
	130	132.16	1.66	101.66	0.92					
OXYMET®	70	68.69	-1.87	98.13	1.35	25	25.05	0.20	100.20	0.52
	100	101.35	1.35	101.35	1.13	35	34.88	-0.34	99.66	0.44
	130	131.08	0.83	100.83	0.69					
t (2.776)[b]	1.657									
F (19.000)[b]	5.530									

a: for five determinations, b: theoretical value.

Evaluation the greenness of FIA method

The FIA-spectrophotometric method aligns with the principles of Green Analytical Chemistry (GAC) due to its low solvents and reagents usage, as well as minimal waste production. Two different metric tools, analytical greenness assessment (AGREE) [49] and and the green analytical procedure index (GAPI) [50], were used to evaluate the environmental friendliness of FIA system. The two parameters in the GAPI pictogram (Figure 6) that represent the solvents and reagents used (parameter 7) and the health risk of the reagent (parameter 10) are colored red. This outcome is due to the use of non-green solvents and reagents in the diazotized reagent preparation process, specifically ethanol and p-chloroaniline, as well as the toxicity of the reagent, which can cause temporary incapacitation.

The AGREE pictogram (Figure 6) indicated that parameter 10 (source of reagents) was highlighted in red and parameters 3 (type of measurement) in orange due to the use of toxic reagents and the fact that the measurement was taken at line. Yellow or various shades of green

were used to color-code the other. The suggested approach demonstrated an overall green profile, as indicated by the overall evaluation number of 0.74 that was automatically created at the center of the AGREE pictogram. Conclusively, the suggested FIA approach meets the requirements set forth by GAC and has the potential to be employed in standard analyses within pharmaceutical quality assurance laboratories.

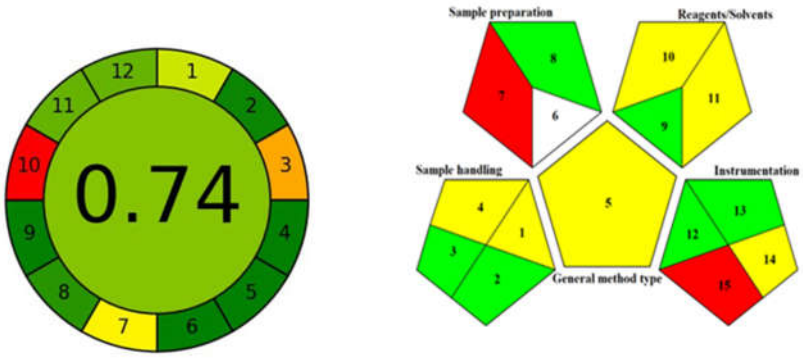


Figure 6. Assessment the greenness degree of FIA approach with (left) AGREE, and (right) GAPI tools.

CONCLUSION

A rapid and eco-friendly FIA-spectrophotometric approached has been established using DCA as a coupling agent to determine OXY in nasal drops and bulk drugs. This method has been validated according to the latest ICH guidelines. Unlike many previously reported OXY methods, the current FIA–spectrophotometric approach is straightforward, inexpensive, and avoids time-consuming, labor-intensive extraction steps and the use of organic solvents. Additionally, it offers high selectivity and sensitivity. The test method also boasts advantages such as temperature independence, accuracy, reproducibility, minimal drug sample requirement (100 µL), and a high sampling rate. These benefits support the application of the proposed method in regular quality control analyses of OXY in its dosage forms.

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