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Artículos originales

SmartAnalysis: A sustainable digital-image colorimetry method for ethanol determination in alcohol-based hand sanitizer

SmartAnalysis: Un método sostenible de colorimetría de imagen digital para la determinación de etanol en desinfectante de manos a base de alcohol

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Conflicto de intereses

Los autores declaran no tener conflictos de interés.

Resumen

Introducción: El consumo de desinfectantes de manos a base de alcohol ha aumentado significativamente después de la pandemia causada por el SARS-CoV-2. A pesar de la conclusión de la emergencia sanitaria declarada por la OMS en 2023, la costumbre de desinfectar las manos con geles sanitizantes a base de etanol ha sido adoptada a nivel mundial por la población. Dado que los métodos generales descritos en los compendios oficiales para la determinación del etanol, como la cromatografía de gases o la destilación, son laboriosos y no específicos para geles que contienen carbómero, este trabajo propone un método alternativo basado em la colorimetría de imágenes digitales.

Método: La imagen digital (proporcionada por la reacción etanol-fenolftaleína) fue capturada y transformada en una señal analítica basada en el sistema de colores Rojo-Verde-Azul. Las adquisiciones de imágenes se realizaron utilizando un smartphone Samsung Galaxy J6, y las señales se generaron mediante el programa gratuito Photometrix Pro®. El método fue validado de acuerdo con las directrices de la ICH y se aplicó en muestras comerciales. Además, el método propuesto fue evaluado por su impacto ambiental utilizando la herramienta Índice del Proceso Analítico Verde (GAPI). Se generaron pictogramas utilizando el programa gratuito ComplexGAPI®.

Resultados: El canal verde mostró una respuesta lineal en las curvas de calibración para concentraciones de etanol que van desde el 5 hasta el 40 % (p/p) en medio ácido. El método demostró linearidad, precisión, exactitud y robustez.

Conclusiones: El método propuesto presentó como principales ventajas el uso de dispositivos de bajo costo y fáciles de manejar, así como un consumo reducido de reactivos, de acuerdo con los principios de la química analítica verde.

Palabras clave: smartphone; imagen digital; gel desinfectante; análisis.

Abstract

Introduction: The consumption of alcohol-based hand sanitizers has increased significantly after the pandemic caused by SARS-CoV-2. Despite the conclusion of the health emergency declared by the WHO in 2023, the habit of sanitizing hands with ethanol-based gel sanitizers has been globally adopted by the population. Since general methods described in official compendia for ethanol determination such as gas chromatography or distillation are laborious and not-specific to carbomer-containing gels, this work proposes an alternative method based on digital image colorimetry.

Method: The digital image (provided by ethanol-phenolphthalein reaction) was captured and transformed into an analytical signal based on the Red-Green-Blue system. The image acquisitions were performed using a Samsung Galaxy J6 smartphone, and the signals were generated using the Photometrix Pro® free program. The method was validated in accordance with ICH and applied in commercial samples. Additionally, the proposed method was evaluated for its environmental impact using the Green Analytical Process Index (GAPI) tool. Pictograms were generated using the ComplexGAPI® free program.

Results: Green channel exhibited a linear response in the calibration curves for ethanol concentrations ranging from 5 to 40 % (w/w) in acidic medium. The method showed linearity, precision, accuracy, and robustness.

Conclusions: The proposed method presented as main advantages the use of low-cost and easy-to-handle devices and reduced reagent consumption, in accordance with green analytical chemistry principles.

Keywords: smartphone; digital image; gel sanitizer; analysis.

Highlights

Methods describe in literature for ethanol determination in pharmaceutical products involve a set of complex and high-cost instrumentation, making it difficult to implement in laboratories that produce on a small scale. The proposed method enables the quantification of ethanol in hand sanitizer samples based on a simple colorimetric reaction. Image acquisition by a smartphone and the generation of the analytical signal using the RGB system allows the implementation of this technique at a low cost and in a sustainable manner.

Introduction

The use of commercial alcohol-based hand sanitizers (ABHS) had a significant growth among the population during the COVID-19 pandemic, becoming indispensable for care against the coronavirus, along with the use of face masks and distancing[1,2]. Despite the conclusion of the health emergency declared by the World Health Organization in 2023, the habit of sanitizing hands with alcohol-based hand sanitizers has been globally adopted by the population.

According to Brazilian Pharmacopoeia 6th ed. - National Formulary 2nd ed., hydroalcoholic antiseptic gels included water, ethanol, carbomer 980 and triethanolamine[3,4]. In addition, the ethanol content is required to be at least 70 % (w/w). Mass fractions below 70 % (w/w) do not guarantee effectiveness against microorganisms and can only be used for cleaning surfaces.

In general procedures described in the Brazilian Pharmacopoeia 6th ed. (2019), the alcoholmeter method is indicated for ethanol quantification in hydroalcoholic solutions. However, the same procedure cannot be applied to products containing carbomer as thickening agents. The other two general methods described in official compendia for ethanol determination (gas chromatography and distillation) are laborious and costly. Some authors have described alternative methods for the determination of ethanol in ABHS using near infrared spectroscopy[5,6] or gas chromatography tandem mass spectrometry (GC-MS/MS)[7]. These techniques involve a set of complex and high-cost instrumentation, making it difficult to implement in laboratories that produce on a small scale or compounding pharmacies. In addition, high consumption of energy and toxic solvents are not in compliance with the principles of Green Analytical Chemistry (GAC)[8].

In order to overcome these disadvantages, techniques such as digital image colorimetry (DIC) bring great advances to analytical procedures, as they involve reduced reagent consumption, minimal sample preparation and low cost. Furthermore, these techniques also show portability, accessibility in relation to data processing and reduction in time of analysis[9]. The image acquisition using a smartphone, referred to for the first time in this article as "SmartAnalysis," combines concepts of colorimetry, digital imaging, miniaturized methods, and the principles of green analytical chemistry (Figure 1).

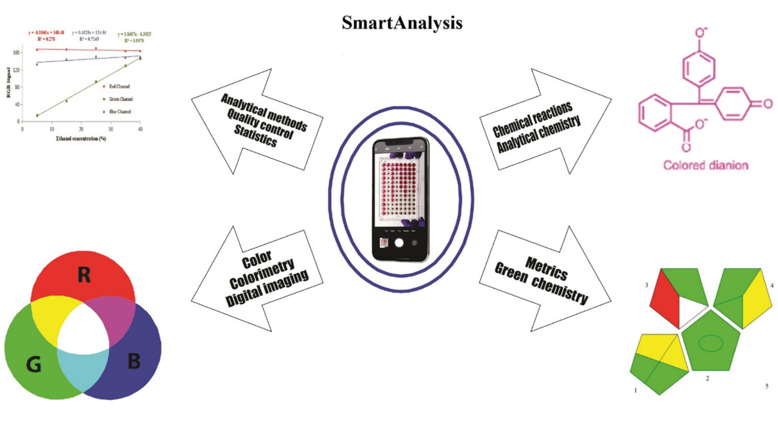

Figure 1. SmartAnalysis

Furthermore, freely available programs can be used in digital image acquisition for: (a) image processing (Photometrix Pro®), and (b) evaluating the conformity of the proposed method with the GAC principles(10,11). DIC is based on digital image capture, decomposition in the Red-Green-Blue (RGB) system, and the determination of a linear correlation between the sample concentration and the RGB signal(12). In the proposed study, the determination of ethanol using colorimetric methods such as DIC could be achieved through its reaction with phenolphthalein (PHP) in a basic medium, resulting in the production of a pink-colored product. PHP, when solubilized in polar protic solvents such as water, exists predominantly in the colored form (quinoid), while a loss of color occurs at dielectric constants lower than that of water, such as ethanol. Thus, in hydroalcoholic solutions, a quantitative relationship can be established based on the intensity of the dye's color, which decreases proportionally with the concentration of ethanol in the solution(13,14).

Therefore, this work proposes the determination of ethanol in ABHS containing carbomer using DIC. The digital image from the sample added to the colorimetric reagent was captured by a smartphone camera and decoded in the RGB system using the Photometrix PRO® program. From the correlation between ethanol concentration and the green channel, the analytical method was optimized and validated according to International Council for Harmonization (ICH) guidelines(15). To assess the agreement of the proposed method with the GAC principles, a pictogram was constructed using the ComplexGAPI tool(10,11).

Methods

Samples, reagents and solutions

PHP (Synth, Brazil), glycine (Sigma-Aldrich, Brazil), sodium chloride (NaCl) (Vetec, Brazil), and sodium hydroxide (NaOH) (Vetec, Brazil) were employed for the preparation of the colorimetric reagent. Absolute grade ethanol p.a (Dinâmica, Brazil), HCl p.a. (Vetec, Brazil) and carbomer 980 (Dinâmica, Brazil) were used in the preparation of calibration curves. All reagents used were of analytical grade, and the solutions were prepared daily in the laboratory. PHP solution 0.1 % (w/v) in a glycine buffer medium was utilized as the colorimetric reagent. The glycine buffer (0.2 M, pH 11.5) was prepared by dissolving glycine and NaCl in distilled water(13). The pH adjustment of the buffer was carried out using 1.0 M NaOH. Compounding samples containing carbomer 980 (0.5 %, w/w) and ethanol concentrations ranging from 30 % to 80 % (w/w) were prepared according to the instructions in the Brazilian Pharmacopoeia 6th edition (2019) - National Formulary (2012) and used after dilution (1:1) with deionized water(3,4). These samples were referred to as ABHScompound and were employed in the development and validation of the proposed method. These samples were generously donated by Dermaroma® Compounding Pharmacy (Uruguaiana - RS, Brazil), and the reported content was considered the real value. Commercial ABHS containing a declared ethanol concentration of 70 % (w/w) were purchased from a local drugstore. These samples were denoted as ABHScommercial and were employed after dilution (1:1) with deionized water. For these samples, the content was considered as indicated on the product label, since there are no official methodologies available for ethanol quantification. Standard solutions containing 5 % to 40 % (w/w) of ethanol in: (a) 0.1 M HCl medium, (b) aqueous medium and (c) polymeric medium (carbomer 980, 0.5 %) were prepared daily and used in the development and validation of the proposed method.

Disposable for image capture

The colorimetric reactions for ethanol determinations were carried out in disposable polystyrene 24-well microplates (Nest Biotech, China). Digital images were captured using a homemade colorimetric box. The system comprised a dark chamber (25 × 20 × 20 cm) containing: (a) LED light (5V - 6500K) enclosed in a white paper box attached at the bottom; (b) an opening at the top for smartphone coupling; and (c) an ON/OFF switch. The intensity of the LED light was adjusted and maintained at a stable brightness of 12275 Lux inside the box during image capture. Micropipettes were used to dispense reagent/sample aliquots into each well. Digital images were captured using a digital camera on a Samsung

Galaxy J6 smartphone (13Mpx camera resolution) and processed using the Photometrix PRO®[16]. All measurements were performed in triplicate. The inverse intensity I (I = 255 - signal) was applied to the RGB signals, which were inversely proportional to the increase in ethanol concentration.

DIC method optimization

According to prior research conducted by Filgueiras et al. (2022) and Marinho et al. (2019), PHP in an alkaline medium has the potential to be utilized for the quantification of ethanol in alcoholic beverage samples[13,14]. This is due to the direct correlation between the color change observed and the concentration of the analyte. Since ABHS has a similar matrix to the previously described sample, PHP was chosen as the colorimetric reagent in this study. Previous research was conducted to establish the analytical parameters for signal acquisition. These investigations assessed the test solutions in terms of visible color change (observed by the naked eye) and RGB signal. By analyzing the visual coloration and RGB signal, the optimal correlation between the volume of the standard solution and the volume of the colorimetric reagent was established. The parallelism between the analytical curves constructed in the polymeric and acidic media was investigated to assess the feasibility of utilizing an external curve (prepared in the acidic medium) for routine analyses. Both the analytical curves in polymeric medium and the blank gel samples were produced with carbomer 980 at a concentration of 0.5 % (w/w). Once the analytical conditions were determined, the method was validated based on the parameters defined by the International Council for Harmonization (ICH). The following criteria were evaluated: linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ) and robustness. Linearity was assessed by performing linear regression analysis utilizing the least-square regression method. Calibration curves were generated using hydroalcoholic solutions in a 0.1 M HCl medium (ethanol concentrations ranging from 5 to 40 %; w/w). The data was subjected to analysis of variance (ANOVA) using independent analytical curves. Data processing was carried out using Microsoft Excel® (Microsoft Corporation, USA). Sensitivity was evaluated by determining the limits of detection (LOD) and quantification (LOQ), which were derived from the calibration curves. The precision was evaluated by repeatability (intraday precision) and intermediate precision (inter-day precision) tests. The accuracy was evaluated by calculating the percent recovery (R %) of the analyte. The robustness was performed by making minor modifications to the optimized conditions. The altered conditions included the distance, final volume in the well, and colorimetric reagent: sample proportion.

Green Analytical Chemistry metric

The Green Analytical Procedure Index (GAPI) is a metric used to assess the environmental impact of analytical methodologies. It employs visual representation in the form of color-coded pictograms to indicate the environmental impact associated with each step of the procedure. This approach enables a comprehensive evaluation of environmental sustainability and facilitates the comparison of different analytical methods in terms of their environmentally friendly practices. To assess the adherence of the proposed method to the principles of GAC, pictograms were generated using the ComplexGAPI tool (free software available in: mostwiedzy.pl/complexgapi)[10,11].

Results and Discussion

Ethanol-PHP reaction

PHP is extensively employed as a colorimetric reagent for acid-base reactions due to its pH-responsive color alteration. In an aqueous solution, PHP exists in three distinct structural forms. Within the pH range of 8.3 to 12, PHP assumes a quinoid form, resulting in a red-pink coloration. In polar protic solvents like water, the quinoid form prevails, whereas solvents with lower dielectric constants, such as ethanol, exhibit a diminished color intensity. Hence, by modifying of the water/ethanol ratio, a color variation directly correlated to the ethanol concentration can be achieved, which is attributed to the opening/closing of the lactone ring. To maintain the reaction equilibrium unaffected by acidic species, it is essential for the colorimetric reaction to occur in a buffered alkaline medium.

Development of the proposed analytical method

This work proposes a colorimetric method for quantifying ethanol in ABHS samples containing the carbomer 980 polymer as a thickening agent. Prior to this study, potential interference from the acidic nature of the polymer in the colorimetric reaction equilibrium was considered. Therefore, preliminary experiments were conducted to assess the concentration of the colorimetric reagent, buffering capacity, and sample volume within the well. Visual observation and RGB signal analysis were performed for different solutions.

Results revealed that hydroalcoholic solutions containing PHP 0.1 % exhibited a color scale ranging from purple to light pink, directly correlated with ethanol concentration. Polymeric and acidic hydroalcoholic solutions (500 µL) containing PHP 0.1 % (200 µL) displayed a similar color scale, ranging from purple to light pink, with a linear correlation between ethanol concentration and the green channel. The green channel was selected for further studies due to its higher sensitivity and linearity compared to the other RGB channels (as shown in figure 2).

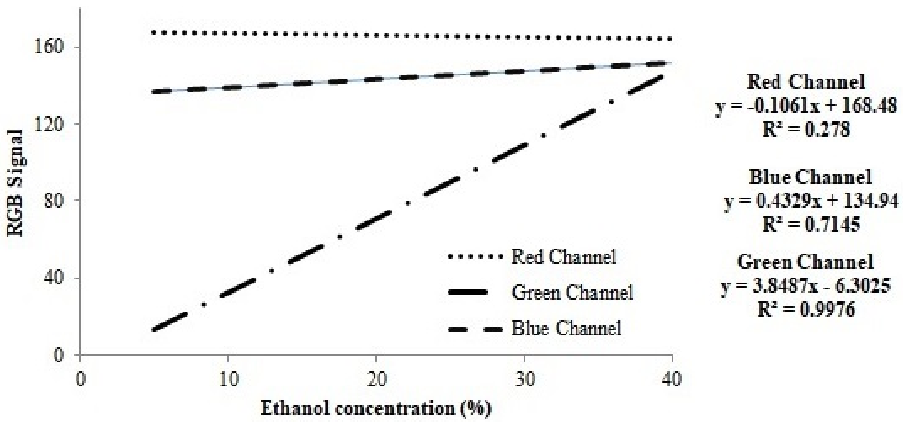

Figure 2. RGB calibration curves

The pH of each colorimetric reaction was monitored to confirm that changes in color were due to the reaction with ethanol. A reaction time of 5 minutes was established, as the analytical signal remained constant after this duration.

The method was considered selective since there was no variation in the green (G) signal for blank gel samples. Analytical curves in acidic (y=3.8487x-6.3025; r^2=0.998) and polymeric (y=3.8735x-5.4917; r^2=0.997) media were constructed to evaluate matrix interference. The slope ratios for calibration curves were found to be between 0.91 and 1.2, indicating parallelism of the external (acidic medium) and matrix (polymeric medium) curves. This characteristic allows for the construction of calibration curves in an acidic medium during routine analyses, simplifying the process and reducing costs. The analytical conditions for DIC method are shown in Table 1.

Table 1. Analytical conditions

Analytical Conditions	Value
Reaction time (min)	5
Distance (cm)	10
Sample volume in the -well microplate (µL)	650
PHP 1% solution in the -well microplate (µL)	200
Working solution (% ethanol, w/w)	35

Figures of merit

The proposed method exhibited a linear response for ethanol concentrations ranging from 5 to 40 % (w/w) in acidic medium (HCl 0.1M). Solutions containing ethanol above 40 % did not exhibit a linear relationship with the observed analytical signal for the G channel under the tested conditions. This linear range (with an upper limit at 40 %) required prior sample dilution (1:1), resulting in a theoretical sample concentration of 35 % (ethanol, w/w). Analytical curves obtained from the G channel showed correlation coefficients close to 1. According to the ANOVA test, the data showed significant linear regression and satisfies linearity condition.

Precision was evaluated by repeatability (intraday precision) and intermediate precision (inter-day precision). For the repeatability assay, six replicates of ABHScompound70 sample were evaluated on the same day and by the same analyst, and the relative standard deviation (RSD) of the measurements was evaluated. The intermediate precision test was performed on different days and by different analysts. The intra- and inter-day precision was considered adequate for RSD less than 2 %. As shown in table 2, the RSD values were considered satisfactory, both for the repeatability and intermediate precision tests. Therefore, a good agreement between the two assays performed independently was obtained despite the use of different analysts and day of analysis.

Table 2. Repeatability and intermediate precision assays

	% Ethanol (w/w)	RSD (%)
Day 1 (n=6)	35.32±0.41	1.15
Day 2 (n=6)	34.98±0.44	1.25
Day 3 (n=6)	34.93±0.35	0.99
Total (n=18)	35.08±0.41	1.18

The accuracy was evaluated by calculating the percent recovery (R %) of the analyte. The proposed method establishes that a volume of 650 µL of the diluted sample should be added to the well. Thus, the recovery test was conducted by adding increasing volumes of ethanol standard solution (35 %, w/w) into the wells of the microplate containing decreasing volumes of ABHS sample solution 35 % (ethanol, w/w), totaling 650 µL. The volumes added to each well are described in Table 3. After this procedure, 200 µL of PHP was added, and the analyte concentration was determined.

Table 3. Recovery test for the proposed method

	ABHS sample sol. 70 % (µL)	Ethanol standard sol. 70 % (µL)	Theoretical concentration (% Ethanol)	Found concentration (% Ethanol)	Recovery (%)
-well microplate 1	650	-	70.0	71.1	-
-well microplate 2	600	50	71.0	71.3	100.42
-well microplate 3	500	150	70.8	72.3	102.11
-well microplate 4	325	325	70.5	71.6	101.53
-well microplate 5	150	500	70.3	72.0	102.41

The proposed method was considered accurate as the recovery percentage was ranged between 100.4-102.4 %. This range is in accordance with that provided for colorimetric assays using digital images[12-14].

The limits of detection and quantification were determined as LOD = 2.58 % (w/w) and LOQ = 8.60 % (w/w), respectively. These values showed the ability of the method to quantify samples containing ethanol concentrations lower than declared on the label.

The robustness test was carried out by means of small modifications in the optimized conditions. As shown in Table 4, the method was considered robust since the adopted variations were shown to have any effect on the reliability of the method.

Table 4. Robustness test

Condition	Modification	Ethanol (%)
Distance (cm)	9.2	71.41
	10.8	71.76
Final Volume (µL)	800	70.89
	900	71.24
Phenolphthalein:sample (µL: µL)	190:660	71.41
	210:640	71.59
Analytical condition*		71.24
	Mean ± SD	71.36±0.28
	RSD (%)	0.393

* As shown in Table 1

The proposed method was applied to ethanol determination in samples from drugstores (ABHScommercial) and compounding pharmacies (ABHScompound), and relative error was calculated for each sample as shown in the Table 5. Since there are no official methods for ethanol quantification in ABHScommercial, it was considered the label-declared content.

Table 5. Ethanol quantification in commercial samples

Samples	Label claim (% Ethanol)	DIC (% Ethanol)	DIC (%)	Relative error
ABHScompound1	32.8	30.3	92.4	-7.60
ABHScompound2	46.9	49.9	106.4	6.40
ABHScompound3	56.2	58.6	104.3	4.30
ABHScompound4	72.2	72.7	100.7	0.74
ABHScompound5	75.0	74.7	99.6	-0.36
ABHScommercial1	70.0	70.65	100.9	0.92
ABHScommercial2	70.0	71.33	101.9	1.90
ABHScommercial3	70.0	73.46	104.9	4.94
ABHScommercial4	70.0	70.64	100.9	0.91
ABHScommercial5	70.0	73.15	104.5	4.50
ABHScommercial6	70.0	71.68	102.4	2.40
ABHScommercial7	70.0	71.14	101.6	1.63
ABHScommercial8	70.0	71.07	101.5	1.52

Several analytical methods for ethanol quantification in ABHS using GC have been recently published in the literature, especially after the massive use of this type of sample during the pandemic [18-22]. The results indicated a high number of samples not conforming to the standards established by official codes, reinforcing the need for regulation of samples produced on a large scale. Additionally, the presence of impurities such as methanol should be monitored, and sensitive analytical methods based on gas chromatography are most suitable for this purpose. However, gas chromatography is costly, which makes its use as a quality control tool in compounding pharmacies unfeasible. Thus, the proposed analytical method, although not yet recognized in official codes, could be used as an alternative, provided that impurities of toxicological interest are monitored in the ethanol raw material. Furthermore, the proposed method is in accordance with the 12 principles of GAC, with a lower environmental impact compared to methods involving gas chromatography, as it is miniaturized, does not involve sample preparation, and does not require specific instrumentation.

Greenness evaluation of proposed method

One of the tools currently used to assess the environmental impact of an analytical method and its alignment with the 12 principles of green analytical chemistry is the GAPI. This tool allows for the evaluation, through a pictogram, of the environmental impact caused by an analytical methodology. The pictograms generated by GAPI consist of five pentagrams covering 15 processes in an analytical method, as shown in Table 6. For each pentagram, a green, yellow, or red color can be assigned, indicating the degree of environmental impact that each step of the analytical method presents, enabling a better visualization of areas that need to be revised and improved in terms of their environmental effect. The GAPI figure generated from the conditions established for the proposed method is shown in Figure 3.

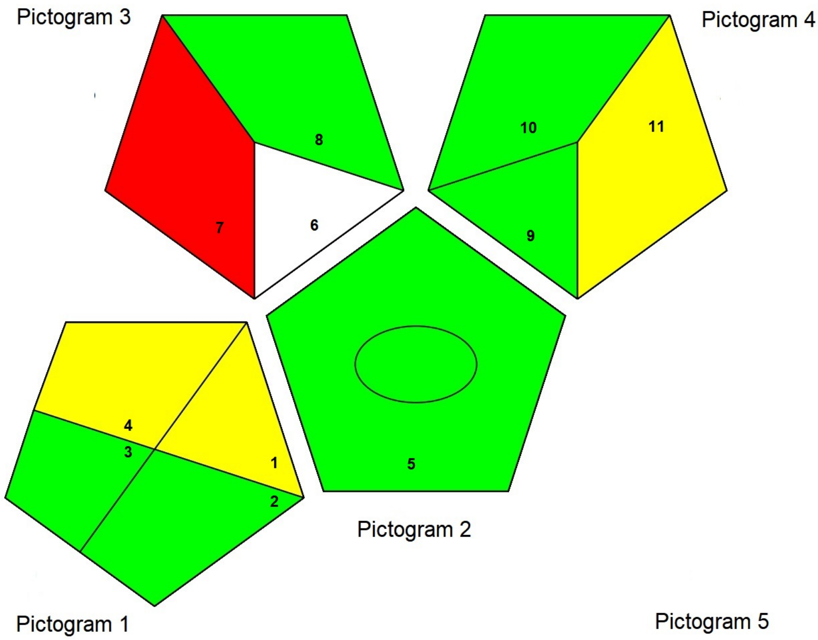

Figure 3. GAPI – DIC method

According to Vazquez et al (2022), colorimetric methods using digital images do not require specific analytical instruments[7]. For this reason, pictogram 5 is not drawn in the GAPI figure (Figure 3). The parameters used in pictograms construction are shown in Table 6. The method showed agreement with GAC principles, except for the use of non-green diluents (Process 7 – pictogram 3). However, this impact was limited since the diluents were used in volumes smaller than 10 mL (Process 9 – pictogram 4).

Table 6. Agreement with GAP principles

	Pictogram	Process	Result	Color assigned
Sample preparation	1	Collection	Online or at line	Yellow
		Preservation	None	Green
		Transport	None	Green
		Storage	Under normal conditions	Yellow
	2	Type of method	No sample preparation	Green
	3	Scale of extraction	n.a.	-
		Solvents/reagents used	Non-green solvents/reagents	Red
		Additional treatments	None	Green

	Pictogram	Process	Result	Color assigned
Reagents and solvents	4	Amount	<10 mL (<10g)	Green
		Health hazard	Slightly toxic; slight irritant; NFPA health hazard score 0 or 1. No special hazard.	Green
		Safety hazard	Highest NFPA flammability or instability score = 2 or 3, or a special hazard is used.	Yellow
Instrumentation	5	Energy	n.a.	Colorimetric methods using digital images do not require specific analytical instruments, thus pictogram 5 is not drawn in the GAPI figure.
		Occupational hazard	n.a.	
		Waste	n.a.	
		Waste treatment	n.a.	
Method Type	Presence (quantitative method) or absence (qualitative method) of the circle into pictogram 2	-	Quantitative	Circle into Pictogram 2

Conclusion

In this work, a DIC method was developed for the quantification of ethanol in ABHS using PHP as the colorimetric reagent. The method was validated in accordance with ICH guidelines and can be explored in routine analysis. The proposed method offers several key advantages, including the utilization of cost-effective and easily manageable devices, as well as reduced reagent consumption, aligning with the principles of green analytical chemistry.

Although the use of digital images is not considered a mode of acquiring analytical signal in official codes, the use of the methodology proposed in this work may be useful for quantifying the content of samples in compounding pharmacies. Furthermore, it drives innovation and eco-friendly solutions in the pharmaceutical analysis, fostering a more sustainable future for the healthcare and the environment.

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