Research Article

วิธีวัดสีโดยใช้ค่าอาร์จีบีสำหรับการวิเคราะห์ปริมาณเลโวซิทีริซีนในยาเม็ด

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การศึกษานี้ได้นำเสนอวิธีที่ง่ายและประหยัดเวลา ใช้หลักเทคนิคโดยการอ่านค่าสีอาร์จีบี โดยใช้งานร่วมกับความสามารถของโทรศัพท์มือถือที่สามารถถ่ายภาพดิจิทัล จากนั้นนำภาพไปใช้เพื่อการวิเคราะห์เชิงปริมาณ วิธีนี้เป็นการวัดความเข้มของสีและวัดประเมินผลโดยความสัมพันธ์ระหว่างสีที่วัดได้กับค่าความเข้มข้นของสาร **วัสดุและวิธีทดลอง:** วิธีที่นำเสนอนี้ได้ประสบผลสำเร็จในการตรวจวิเคราะห์ยาเลโวซิทีริซีน (LCTZ) ในรูปแบบยาเม็ด ซึ่งใช้โบโมครีซอลกรีน (BCG) เป็นสารทำให้เกิดสีโบโมครีซอลกรีนสารเดี่ยว ๆจะมีสีน้ำเงิน เมื่อเกิดสารประกอบเชิงซ้อนกับเลโวซิทีริซีน จะเกิดเป็นสารประกอบเชิงซ้อนที่มีสีเขียว-เหลือง ความเข้มของสารประกอบเชิงซ้อนสีเขียว-เหลืองที่เกิดนี้จะขึ้นกับความเข้มข้นของสารละลายตัวอย่างยา สารละลายของสารประกอบเชิงซ้อนที่ได้จะถ่ายเทใส่ในหลอด บรรจุตัวอย่างประเภทแก้ว ทำการบันทึกภาพด้วยโทรศัพท์มือถือ โปรแกรมการอ่านค่าพิกเซลอาร์เรย์ของสีจะนำมาใช้ในการอ่านค่า สีแดง-สีเขียว-สีน้ำเงิน(เป็นหน่วย อาร์จีบี) ซึ่งจะมีการเปลี่ยนให้เป็นความเข้มข้นด้วยการคำนวณด้วยความสัมพันธ์ของสมการเส้นตรงภายใต้สภาวะการทดลองที่เหมาะสมกราฟมาตรฐานระหว่างค่าอาร์จีบี และความเข้มข้นของสารมาตรฐานยาจะถูกสร้างขึ้น กระบวนการประเมินวิธีวิเคราะห์จะให้ความสำคัญการศึกษาในเรื่องความเป็นเส้นตรง ค่าความถูกต้อง และค่าความแม่นยำของวิธีที่พัฒนาขึ้น **ผลการศึกษา:** วิธีที่นำเสนอนี้ให้ความสัมพันธ์เป็นไปตามกฎของเบียร์มีค่าความเป็นเส้นตรงอยู่ในช่วงความเข้มข้น 2-40 ไมโครกรัมต่อมิลลิลิตร ของยาเลโวซิทีริซีน ด้วยค่าสหสัมพันธ์ความเป็นเส้นตรงที่ดี (r^2 = 0.9996) ค่าขีดจำกัดต่ำสุดของการวิเคราะห์ (LOD) และค่าขีดจำกัดต่ำสุดของการวิเคราะห์เชิงปริมาณ (LOQ) มีค่าเท่ากับ 1.64 และ 4.96 ไมโครกรัมต่อมิลลิลิตร ตามลำดับ จากการศึกษาสารปรุงแต่งที่อาจพบได้ในการผลิตยาพบว่าไม่แสดงผลการรบกวนในวิธีที่นำเสนอนี้ ผลของการวิเคราะห์ตัวอย่างยาด้วยวิธีที่นำเสนอนี้เมื่อเปรียบเทียบกับวิธีอ้างอิงแล้ว พบว่าไม่มีความแตกต่างกันในเชิงสถิติด้วยความเชื่อมั่นที่ 95 เปอร์เซ็นต์ (n=6) **สรุปผล:** วิธีที่พัฒนาขึ้นนี้พบว่ามีความแม่นยำ ถูกต้องและให้ผลการวิเคราะห์ซ้ำที่ดี ยังกล่าวได้ว่ามีความเป็นไปได้ที่จะนำเทคนิคการอ่านค่าสีแบบอาร์จีบี มาใช้ในการวิเคราะห์เชิงปริมาณในตัวอย่างยาในเภสัชภัณฑ์รูปแบบต่าง ๆ ด้วยขั้นตอนที่ง่าย ประหยัด และสามารถที่จะประยุกต์ใช้งานในด้านการวิเคราะห์หาปริมาณยาเป็นอีกวิธีทางเลือกหนึ่งได้

คำสำคัญ: เลโวซีทีริซีน, รูปถ่ายดิจิทัล, ความเข้มของอาร์จีบี, วิธีวัดสี

RGB Colorimetric Method for the Quantitative Analysis of Levocetirizine Tablets

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Abstract

RGB Colorimetric Method for the Quantitative Analysis of Levocetirizine Tablets

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This study presented a simple and not time-consuming method coupled with a RGB colorimetric technique using the built-in function of mobile phone digital imaging devices for quantitative analysis. The method was based on the detection of color intensities and the evaluation of relationship between measured color and concentration of sample. **Materials and methods:** The proposed method was applied successfully to analyze levocetirizine (LCTZ) quantitatively in pharmaceutical tablet dosage forms using bromocresol green (BCG) as a coloring agent. Bromocresol green solution only is a blue color. When it reacted with levocetirizine it formed a yellow-green complex. The intensity of yellow-green complex color was depending on the concentration of drug sample. The complex solutions were transferred into standard glass cuvettes and then the images were recorded by mobile phone. The pixel array reader program was applied to evaluate the red-green-blue intensities (RGB scale) of the recorded images that were converted to the concentration which was calculated from the relationship of linear equation. Under the optimized condition, a calibration curve was established by the RGB scale and concentration of drug standard. Validation procedure was achieved concerning linearity, accuracy, and precision of developing method. **Results:** The proposed method obeyed Beer's law and show linear relationship in concentration range of 2 - 40 µg mL^{-1} of levocetirizine with a good coefficient of determination (r^2 = 0.9996). The limit of detection (LOD) and limit of quantification (LOQ) were found to be 1.64 and 4.96 µg mL^{-1}, respectively. The common excipients used as additives in the pharmaceutical dosage form had shown no effect on the proposed method. The results acquired by the proposed method were compared favorably with those acquired by the reference method at a 95% confidence level with no significant difference (n=6). **Conclusion:** The developed method had shown accurate, precise, and reproducible. Moreover, this demonstrates the possibility of RGB colorimetric technique in the quantitative analysis of drugs in their pharmaceutical dosage forms with a simple procedure, low cost, and more applications for pharmaceutical analysis are expected to be use as an alternative method.

Keywords: Levocetirizine, Digital imaging, RGB intensities, Colorimetric method

Introduction

Nowadays, the analysis method was concerned on pollution awareness of the environment and the technologies of cleaner production, following the green chemistry is increasing significantly. And it also becomes an impressive goal for chemists to research the new techniques and processes based on the green process. Red-green-blue (RGB) colorimetric method is one of the new modernized technique based on digital image colorimetric analysis principle which has been used as an alternative for analytical sciences. This technique applies common digital imaging devices for the laboratory quantitative analysis of colored solutions. The RGB technique is relied on monitoring the pixel values of the red, green, and blue intensities that are related to the concentrations of the compound. RGB represents the colors by three numbers from 0 to 255 and gives more than 16 million different colors (16,777,216 colors). RGB technique can be applied to read RGB pixels value of each color from the colorimetric reaction directly and/or recorded digital images which were read the intensities by color-analysis software. It is also a notable trend in the development of out-of-the-lab analytical methodologies and technologies (Ohta and Robertson, 2005).

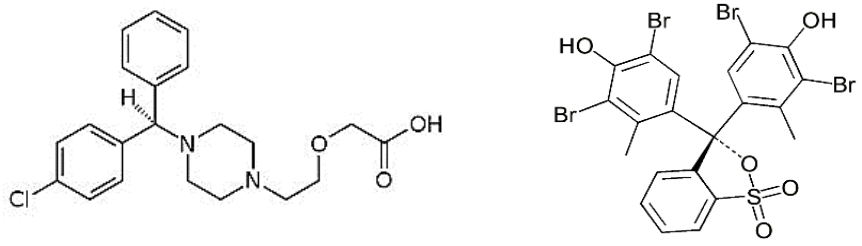
Popularization of smartphone in the world has provided new opportunities for pharmaceutical analysis and environmental monitoring. This is because the mobile phone can achieve rapid, real-time detection with acceptable accuracy. By the digital camera, an inexpensive smartphone could take a photograph for the sample under rapid analysis at anytime and anywhere. The digital image could be changed to the properties of light such as color intensity. Some of software were provide simply for the colorimetric readout scale such as RGB scale. The previously publications were reported the determination of mycotoxin (Liu *et al*., 2020), digoxigenin (Ruppert *et al*., 2019), glucose (Ping *et al*., 2018), ibuprofen (Ji *et al*., 2017) and ascorbic acid (Kong *et al*., 2020) using smartphone-based analysis.

Allergic diseases are increasing in many parts of the world, including the Asia-Pacific (APAC) region. Allergic rhinitis and urticaria are common allergic diseases that may have a negative influence on human's everyday life, work performance, or school activities as well as on the health issue. In the treatment of allergic rhinitis and urticaria, second-generation antihistamines are the first-line treatment option (Brozek *et al*., 2010).

Levocetirizine Dihydrochloride (LCTZ) is the dihydrochloride salt form of the active levorotatory enantiomer of cetirizine, levocetirizine; a third generation, non-sedating, selective histamine H1 receptor antagonist, with antihistamine, anti-inflammatory and potential anti-angiogenic activities. It has been demonstrated that levocetirizine has better efficacy than other second-generation antihistamines like desloratadine and fexofenadine. Moreover, it has less sedative, and duration of action is longer than other antihistamines. Half the strength of levocetirizine (2.5 mg) has a competitive activity to normal strength of cetirizine (5.0 mg). Hence, cetirizine has been replaced by levocetirizine in clinical treatment (De Jong, 2002). Triphenylmethane acid dyes can be used as coloring agents in the analysis of organic drug molecules containing quaternary ammonium groups such as levocetirizine (Issa, Sherif, and Abo, 2013).

Various methods have been described to determine the assay of levocetirizine including titrimetry (Indian pharmacopeia, 2010), spectrophotometric method (Shende *et al*., 2010; Raghu and Basavaiah, 2012; Almamoun and Abdel, 2017), chromatographic method (Birendra and Sumit, 2010; Rathore, Sathiyanarayanan and Mahadik, 2010; Homoss, Elzein and Haidar, 2011) and other spectral methods of analysis (Patel and Pancholi, 2011). All previously method involved with sophisticated and expensive techniques. But, the proposed RGB colorimetric method was provided a new, simple, rapid, portable, environmentally friendly, and inexpensive colorimetric

method to analyze levocetirizine quantitatively in tablet dosage forms. The aim of this study was developed the simply method which was based on colorimetric reaction between drug (levocetirizine) and color reagent (bromocresol green). Chemical structures of levocetirizine and bromocresol green were shown in Figure 1. It was gave a yellow-green complex which was recorded the digital image with smartphone. The RGB scales of digital image was read using in-house software. Results of the analysis were validated using univariate method. The proposed method was applied for determination levocetirizine in pharmaceutical formulation.



(a) **(b)**

Figure 1 Chemical structures of levocetirizine (a) and bromocresol green (b).

Materials and methods

Apparatus and software

1. Android smartphone, Mi 6X; People's Republic of China.
2. Standard glass cuvettes
3. Analytical balance, SI-234, Denver instruments; USA.
4. UV-visible spectrophotometer, Shimadzu, Japan.
5. Deionized water unit,18.2 $m\Omega \cdot cm$, Millipore® Milli-Q; USA.
6. Pixel array reader program (GetPixel Version 1.0); *in-house* software.

Chemicals

1. Levocetirizine dihydrochloride standard, AR, Sigma; Germany.
2. Bromocresol green, AR, BDH; England.
3. Absolute ethyl alcohol, AR; Merck; Germany.
4. Starch, AR, Sigma; Germany.
5. Lactose, AR, Sigma; Germany.
6. Polyvinyl pyrrolidone, AR, Sigma; Germany.
7. Magnesium stearate, USP, Sigma; Germany.
8. Hypromellose, USP, Sigma; Germany.
9. Talcum powder, USP, Fisher Scientific; USA.

Procedures

Solutions preparation

Stock standard solution of levocetirizine (100 µg mL^{-1}) was prepared in 20 mL of absolute ethanol and diluting with deionized water. A working standard solution (40 µg mL^{-1}) was prepared from stock solutions diluting with deionized water. The designed working standard was obtained by appropriate dilution of the working standard solution in the range concentration of 2 - 40 µg mL^{-1}.

The bromocresol green solution (0.025%) was prepared daily by dissolving an accurate weight of 0.025 g of bromocresol green powder in deionized water and made up to 100 mL in a calibrated flask with the same solvent to the graduate mark.

Sample preparation

For the determination of levocetirizine (5 mg/tablet) in tablet samples, twenty tablets were weighed and calculate the average weight. Then, they were powdered and mixed well. The equivalent weight as a tablet was accurately weighed and dissolved in 10 mL of absolute ethanol shaking for 15 min. The mixture was filtered and made up to 100 mL with deionized water to get a solution claimed to contain 50 µg mL^{-1} of levocetirizine and diluted to 15 µg mL^{-1} of levocetirizine with deionized water before analysis sample.

Preparation of an experimental standard calibration curve

Each standard solution (5 mL) was taken into the separate tubes and 1 mL of 0.025% of the bromocresol green solution was added into the tubes. The blank solution was prepared in the same procedure with deionized water instead of the standard solution. The RGB scale from blank was compensated for each standard solution. Each standard solution was transferred into a glass cuvette and take a color digital image with an android mobile phone afterward using RGB software to readout the RGB scale.

RGB software

The software used in this proposed method was the GetPixel program (Version 1.0). *The Getpixel program was developed in-house using Microsoft Visual Basic 6.0.* It was written and supported by Assist. Prof. Senee Kruanetr, Department of Chemistry, Faculty of Sciences, Mahasarakham University, Thailand. The GetPixel program integrates unique combinations of R, G, and B values exhibiting from different color intensities. This software fetches the RGB color values of the pixel at the *x-y* coordinate of the mouse cursor. It is critical to select the sampling point in the same position for each sample, and that point should be a typical representative to get accurate and precise measurements. This program can be applied to analyze the color intensities of the image which concerns the theoretical basis for quantitative analysis (Labounmi, Kruanetr and Ruengsitagoon, 2018).

Quantitative analysis using the RGB technique

The reaction between standard or drug samples and bromocresol green were reacted and leave it stand for 30 min. These solutions were transferred into the standard glass cuvettes and they were arranged side-by-side. The color images were taken using an android smartphone. Standard glass cuvettes were placed 50 cm distance far from the lens of the smartphone (Kehoe and Penn, 2013) and images were recorded. The recording was proceeded under the natural light and do not need photography or flash light. RGB scales were read from the images using the GetPixel program (Figure 2), then, the data was placed in the Microsoft Excel spreadsheet where data organization and calculation was carried out.

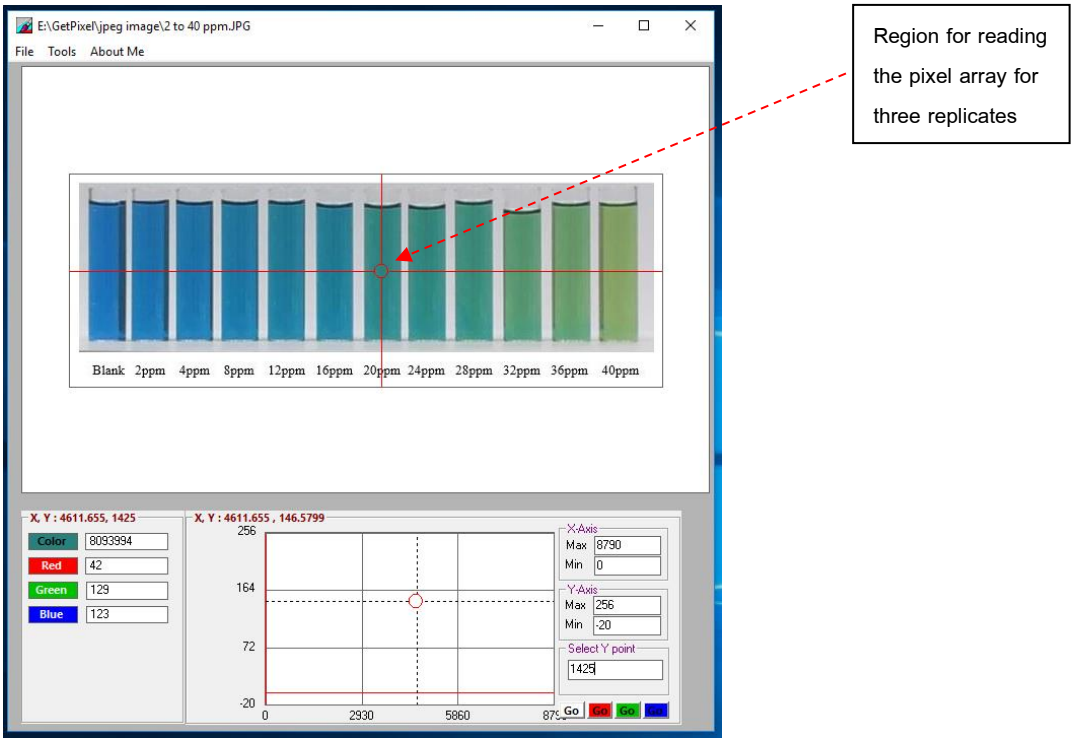


Figure 2 The example of a complex color image in glass cuvettes used to readout the RGB scale which was generated by the GetPixel program.

Study of Interferences

The proposed method was aimed to analyze levocetirizine quantitatively in pharmaceutical tablet dosage forms. Therefore, the effects of the most commonly used as excipients were carefully studied. The excipients examined were starch, lactose, polyvinyl pyrrolidone, magnesium stearate, hypromellose, and talcum powder. For this study, ten times greater amount of all excipients than active levocetirizine drugs were mixed with levocetirizine into a homogenous solution for 15 minutes, diluted to required concentration of levocetirizine, and analyzed under the same conditions by the proposed method.

Method validation

Linearity was studied between the RGB scales and the concentration ranges of levocetirizine. The calibration curve was constructed in the concentration range of 2 - 40 µg mL^{-1}. To examine the accuracy and precision of the proposed methods, the parameters of the linear regression equation, repeatability, and percentage recoveries were examined. Limit of detection (LOD) and limit of quantification (LOQ) were investigated corresponding to the guidelines (ICH guidelines, 2005). The RGB value was then normalized using mathematical deconvolution to achieve delegate data to represent the concentration scale of each standard. The delegate data was then plotted against concentration to obtain the calibration curve where the suspected samples were correlated to predict its unknown concentration. The proposed method was compared with the reference method for evaluation of the accuracy.

The reference method using a spectrophotometric method (Shende P *et al*., 2010). The grinded drug powder equivalent to 5 mg of levocetirizine hydrochloride was transferred into 50 ml volumetric flask dissolved in phosphate buffer pH 7.0. The solution was then filtered through Whatman filter paper No 40. Aliquots of the sample were removed and diluted to 10 ml of phosphate buffer 7.0 to obtain strengths of 5 mg/ml determined at the respective absorbance of 231nm against the phosphate buffer 7.0 as blank. Afterward, the resulting concentration of levocetirizine was compared with the proposed RGB method.

Results and Discussions

Experimental optimization

The Effect of reaction time between levocetirizine (15 µg mL^{-1}) and bromocresol green was examined in the range of 0-90 min. The reaction time of drug-reagent complex of 0, 15, 30, 45, 60 and 90 min provided the RGB of 1.45, 1.51, 1.54, 1.61, 1.66 and 1.81 ($x10^6$ RGB Scale), respectively. It was found that the reaction time between drug and bromocresol green reagent solution is slightly increase. For the acceptable RGB signal and time consuming of reaction time prior to take an image for 30 min was chosen as the proposed method (Figure 3).

The example of preliminary test the reaction between levocetirizine and bromocresol was produced the variety of colors of drug between 5-40 µg mL^{-1} (Figure 4). The specific color was depending on the concentration of levocetirizine and bromocresol green.

The effect of various concentrations of bromocresol green solutions (0.005%-0.100%) was investigated. The RGB scale exhibited the greatest RGB signal was found to be 0.025% of bromocresol green concentration. Then, the concentration of the bromocresol green solution at 0.025% was chosen for further study (Figure 5).

The effect of various bromocresol green reagent volumes for using as a complexing agent was investigated between 0.5 to 5.0 mL. The RGB scale was increased with bromocresol green reagent volume up to 1.0 mL and decreased when the bromocresol green reagent volume more than 1.0 mL. The most selective bromocresol green reagent volume for further use was 1.0 mL (Figure 6).

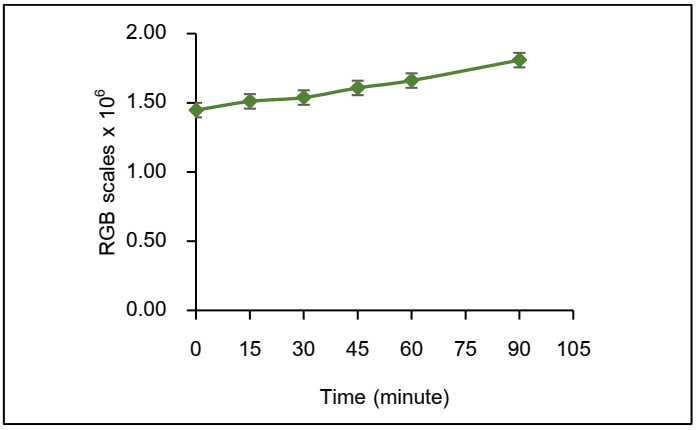


Figure 3 Effect of reaction time between levocetirizine and bromocresol green (*n*=3).

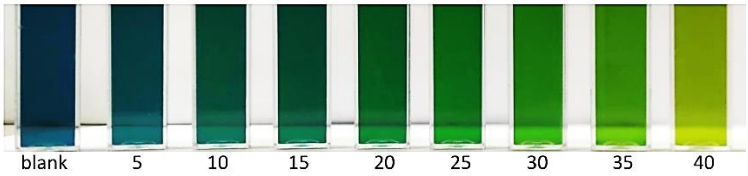


Figure 4 Example of preliminary test the reaction between levocetirizine and bromocresol green using levocetirizine in concentration range of 5-40 µg mL^{-1}.

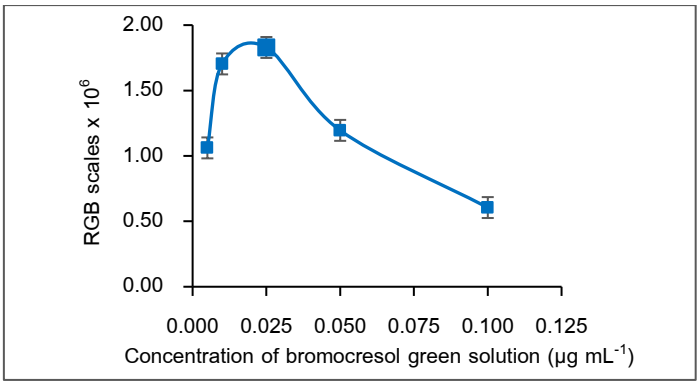


Figure 5 Effect of concentration of bromocresol green solution (1 mL) on the reaction scheme with levocetirizine (15 µg mL^{-1}, *n*=3).

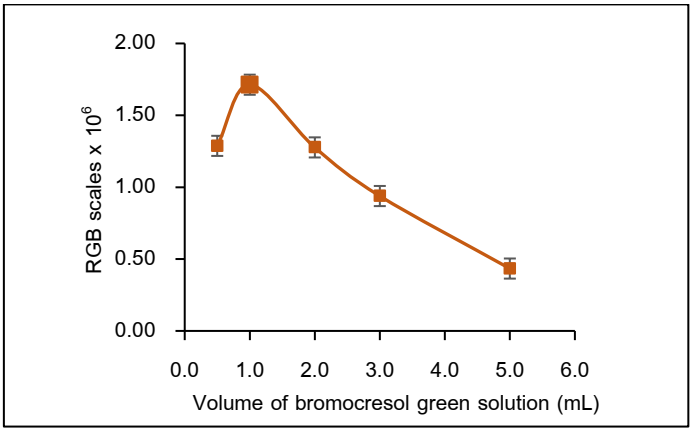


Figure 6 Effect of volume of bromocresol green solution (0.025%) on the reaction scheme. with levocetirizine (15 µg mL^{-1}, *n*=3).

Possible mechanism

Chemical structure of levocetirizine and bromocresol green had shown in Figure 6. The possible mechanism was proposed to be levocetirizine interacts with the bromocresol green via the formation of an ion–pair through the negatively charged sulfonic acid group of bromocresol green and the positively charged quaternary ammonium group of the drug. Interestingly, the formation of a number of hydrogen bonds provide more stability for the formed product (Dena and Hassan, 2016).

Analytical Characteristics

The proposed RGB method for the analysis of levocetirizine using bromocresol green was optimized and validated according to the current ICH guidelines (ICH guidelines, 2005). Based on the validation data, 1 mL of 0.025% bromocresol green was found to be the optimum condition for the analysis of levocetirizine that produced the colored complex product which was took the digital image after leave it stand for 30 min. The concentration of levocetirizine in bromocresol green complexes was linear in the range of 2 – 40 µg mL^{-1} (Table1, Figure 7). The analytical characteristics of the reference method and the proposed RGB method were presented in Table 2. Over the above concentrations range, linear regression of the levocetirizine's RGB value (y) and concentration of levocetirizine (x) expressed the equation as $y = 1.5486x + 0.9188$ ($r^2 = 0.9996$, $n = 5$) for levocetirizine. The detection limit of an individual analytical procedure defines the lowest quantity of analyte in a sample that gave the different signals from the blank by an amount equal to three times the standard deviation of the blank signal. These were found to be 1.64 µg mL^{-1}. The quantification limit is defined as the analyte producing a signal that is at least ten times the standard deviation of the blank signal and was shown to be 4.96 µg mL^{-1}.

Table 1 RGB scale data of standard levocetirizine solution from 2 – 40 µg mL^{-1}, (n=5).

Concentration of levocetirizine (µg mL^{-1})	RGB scales					Average value
	#1	#2	#3	#4	#5	
2	457739	457716	457739	417777	343955	426985
4	720902	720902	720902	749592	717626	725985
8	1309705	1309705	1309705	1327736	1291690	1309708
16	2555909	2555909	2555909	2595161	2533994	2559376
32	4976118	4976118	4976118	5012864	4937388	4975721
40	6348748	6348748	6348748	6386294	6313255	6349159

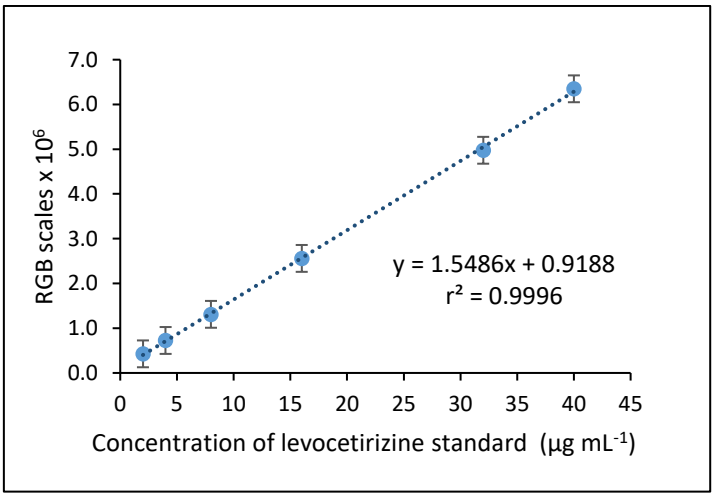


Figure 7 Calibration curve of levocetirizine standard in concentration range of 2-40 µg mL^{-1}.

The precision of the proposed method was studied through repeatability by investigating for three concentrations (5, 15 and 30 µg mL^{-1}) of the levocetirizine standard solution. The relative standard deviation (RSD) of this study was found to be 0.61, 1.05 and 1.43%, respectively. Accuracy was tested through recovery determination. The percentage recoveries for three concentrations (5, 15 and 30 µg mL^{-1}) of levocetirizine standard solutions were found to be in the range of 99.58 – 100.01% (Table 2).

The proposed method was applied to quantify levocetirizine from six commercial trade name and the results were compared to the reference spectrophotometric method (Table 3). The accuracy of proposed method was compared with the reference method. This was proved by using the Student's *t*-test. The calculated student's *t*-test value (0.60) was less than the theoretical value (2.57) at a 95% confidence level (P-value of 0.05). Acceptable agreement between the proposed method and the reference spectrophotometric method was found.

Table 2 Analytical characteristics for the determination of levocetirizine using propose method and comparison with reference method.

Parameters	**Optimum value**	
	Proposed RGB method	**Reference UV method**
Linearity of calibration curve	2 – 40 µg mL^{-1}	0.2 – 40 µg mL^{-1}
Linear regression equation (*n*=6)	y=1.5486x + 0.9188	y=0.0373x + 0.0023
Correlation coefficient, r^2	0.9996	0.9990
Limit of detection, LOD	1.64 µg mL^{-1}	0.10 µg mL^{-1}
Limit of quantification, LOQ	4.96 µg mL^{-1}	0.30 µg mL^{-1}
Repeatability *(n=6)*; RSD		
5 µg mL^{-1}	0.61%	0.31%
15 µg mL^{-1}	1.05%	0.18%
30 µg mL^{-1}	1.43%	0.14%
Percentage recoveries *(n=6)*		
5 µg mL^{-1}	99.58%	99.80%
15 µg mL^{-1}	100.01%	100.05%
30 µg mL^{-1}	99.61%	100.09%

Table 3 Accuracy of proposed RGB method compared with reference UV-visible spectrophotometric method for determination of levocetirizine.

Commercial trade name	**Levocetirizine dihydrochloride** (5 mg/Tablet)	
	Proposed RGB method	**Reference UV method**
A	4.97	5.00
B	5.01	4.97
C	4.95	5.00
D	4.97	4.97
E	4.99	5.00
F	4.99	5.00
t-test at 95% confidence level:		
t-calculation		-0.73
t-distribution at (*n*-1)=5		2.57

Conclusions

The study developed the simple and rapid RGB colorimetric technique to analyze the concentration of levocetirizine in pharmaceutical products. This proposed method used simple reagent in a simple and not time-consuming procedure. Interference studies showed that there was no effect with common excipients and other additives that are usually present in the tablet dosage forms at their regularly added levels. The statistical parameters and percentage of recoveries data had shown good accuracy and precision. The results acquired by the proposed method were compared generously with those acquired by the reference method at a confidence level of 95% with no significant difference (n=6). The proposed RGB colorimetric method was successfully used to determine levocetirizine in commercial tablet dosage forms. The proposed method is consisting of many advantages such as portable technique, cost-effective and environmental friendly that can be used as an alternative for analytical sciences in quantitative analysis of drugs in pharmaceutical products.

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