



Research into spectroscopic techniques through their application in pharmaceutical preparations

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Abstract

This study investigated the optimal conditions for developing and improving the formulation of a high-quality medicinal product, as well as its efficacy. Excellent results were obtained, particularly as the product exhibited a high relative standardisation index; the study revealed that the colouring agent was present in a 1:1 ratio, which is considered an excellent ratio in terms of detectability and drug performance. The study was conducted using the Jop method, applying highly suitable conditions, including a temperature below 25 °C for a duration of 6 minutes; the resulting chromophore demonstrated good stability for approximately one and a half hours.

Keywords: Drug; Reagent; Oxidizing Agent; Base; Spectrophotometry; GUE.

1. Introduction

The simplest and the most widespread method of analytical technique in pharmaceutical sciences, spectrophotometry is used in various ways in the qualitative and quantitative drug identification. Actually, spectrophotometry can be described by the statement according to which the light absorbed by any compound at specific wavelength is proportional to the concentration of the particular substance [1] [2]. Such quantitative correlation plays a vital role in the process of drug verification and control of its content and purity. Being cheap, affordable and relatively easy to perform spectrophotometric techniques are often required. Such techniques allow for testing of medicines; they need minimum sample preparation and small quantities of the sample for investigation [9]. In comparison with complex procedures of HPLC, which require huge theoretical and practical background, these techniques can be considered as a nice and convenient alternative in the process of regular quality control [3] [4] really 3Such methods can be easily incorporated in the laboratory routine because of their simplicity and easy performance. Spectral analysis is essential for quality control, drug development, and compliance because it may detect trace contaminants and degradation products that might compromise the safety and effectiveness of medications (3). One of the most popular analytical methods for identifying the combination of chemical compounds in pharmaceutical [5], biological [6], and environmental [7] materials is spectral analysis, particularly in the UV and visible ranges. Nonetheless, indications play a significant role in the present pharmaceutical quality control procedure, which begins with raw material identification and concludes with final product testing [8].because they may determine the drug's purity, stability, and concentration—thereby ensuring the safety and effectiveness of pharmaceutical products—indications are very adaptable and essential owing to the necessity to maintain quality standards. Because it can identify biomarkers in biological materials, it also aids in medical diagnosis. Because spectrophotometry is multidimensional and incorporates quantitative analysis, it is one of the main tools used in the pharmaceutical sector to guarantee the quality of pharmaceutical goods 9.Spectra have been obtained for hundreds of years through spectrophotometry, the study of the interaction between light and matter. Although the sections of current research offer a short timeline of spectrophotometry's growth, these paragraphs emphasize some of the basic concepts and methods that underlie it. One of the foundations of modern spectroscopy, Beer-Lambert's law, is the result of concepts that have evolved over time. This is the outcome of the 18th-century research of Bouguer and Lambert, which describes how the thickness of the

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absorbing media affects the intensity of absorbed light. Near the end of the eighteenth century, the component of concentration was added to the well-known formula that linked absorbance to concentration and route length. This steady advancement in knowledge of light assimilation [11] [13] prompted the creation of quantitative spectroscopy. Over the course of the 20th century, spectroscopy saw several equipment advancements. When the traditional infrared spectrometer was first launched in the 1940s, it was a dispersive device. Since these devices could only measure one frequency (although only once), it would have taken some time to capture the whole spectrum. The 1960s saw a complete transformation with the advent of the interferometric method in Fourier Transform (FT) devices (such as FT-IR). This allowed for the simultaneous collection of data from every frequency. This represents a lengthy history of innovation in spectroscopic analysis, mostly due to advancements in the pharmaceutical sciences' need for sophisticated and quick analytical tools. [14] [15] [16].

2. Ingredients and instructions for preparation

2.1. 250 µg/mL of standard guaifenesin solution

The solution was prepared by dissolving 0.035 grams of GUE in distilled water and filling the volumetric flask to the 175 mL mark with distilled water. For around ten days, the GUE solution was steady.

2.2. Reagent solution of Hydroxy-4-Nitrochalcone (4-HNC) 25×10^{-3} M

This solution was prepared by dissolving 0.0600g of 4-HNC in EtOH and distilling water to make

2.3. 1×10^{-3} M ferric chloride (FeCl₃)

It was made by dissolving 0.01622g of FeCl₃ in distilled water and adding distilled water to a 100 mL volumetric flask.

2.4. 1×10^{-2} M sodium hydroxide (NaOH) solution

To make it, dissolve 0.045g of NaOH in 150 ml of distilled water..

2.5. GUE tablet formulation solution sample (250 µg/mL)

Five Indonesian-made Triman® pills, each containing 120 mg of GUE, were meticulously weighed. After careful mixing and crushing, the weighed value was 1.66g. After being crushed and dissolved in distilled water, 0.460g of the crushed material—equivalent to 0.035g of the drug—was moved into a 150 mL volumetric flask and filled to the necessary volume with distilled water.

2.6. General procedure

In a basic medium, 2.5 mL of 4'-Hydroxy-4-Nitrochalcone (4-NHC), 2.5 mL of GUE standard solution (250 µg/mL), and 1.75 mL of FeCl₃ solution were combined with 1.5 mL of sodium hydroxide. An orange-reddish product was produced by diluting this combination with distilled water up to a 25 mL volumetric flask. The resulting solution exhibits an absorption spectrum with a peak absorbance at a wavelength of 510 nm when compared to the corresponding blank solution. The resulting product's absorption spectra are displayed in Figure (1).

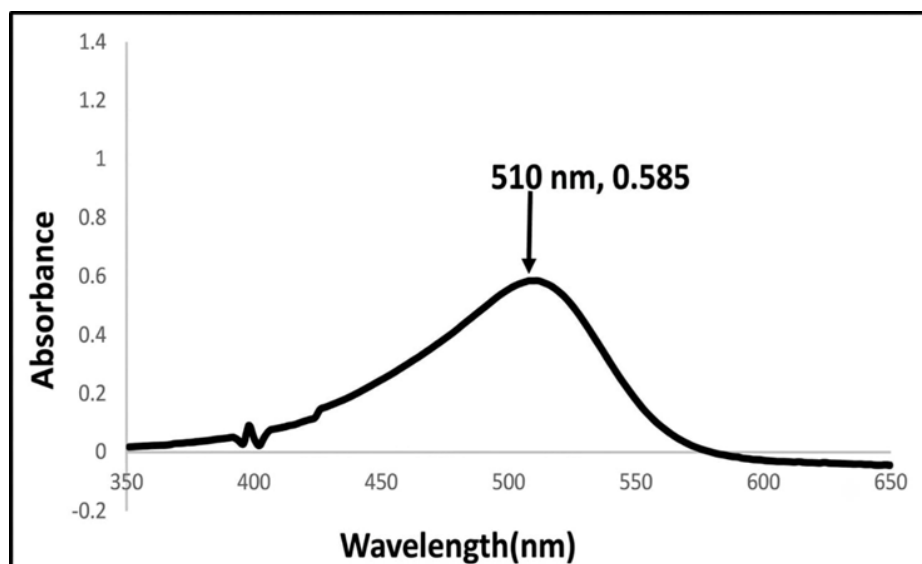


Figure 1 Absorption range of the colorful product

3. Findings and Conversation

3.1. Common circumstances

The following optimum conditions were identified after analyzing the effects of several elements on the color dye's absorbance intensity following GUE's interaction with 4-HNC:

3.2. Choosing the ideal coupling agent

Several chemicals were explored to find the best coupling reagent that produces the highest color intensity. The best reagent, according to the findings, is the 4-HNC reagent at a concentration of 25×10^{-3} M. As Table (1) makes clear, it was discovered that this reagent offers a significant variation in color intensity compared to other reagents.

Table 1 Choosing the optimal coupling agent

Reagent(5×10^{-3} M)	Variable	λ_{max} ,nm	$\Delta\lambda^*$, nm	Absorbance
4'-Hydroxy-4-Nitrochalcone	SB	510	87	0.590
	BW	423		0.090
4-'Amino-4-Nitrochalcone	SB	472	127	0.450
	BW	345		0.090
4'-Amino-2-Bromochalcone	SB	433	78	0.395
	BW	355		0.075

* $\Delta\lambda$ = color contrast, = $\lambda_{\text{max}} S - \lambda_{\text{max}} B$, where the S = Sample and B = blank", SB: GUE absorption spectrum sample vs, blank, and BW: blank absorption vs. D.W

3.3. The effect of reagent volume

A study was conducted to estimate the amount of reagent solution (4-HNC) needed to produce the color product's peak absorption in order to establish the standard amount. The volumetric flasks containing 2.25 mL GUE (250 $\mu\text{g/mL}$), 1.25 mL FeCl_3 (1×10^{-2} M), and 1.25 mL NaOH were filled with different volumes (0.25–1.5 mL) of 4-HNC reagent (2×10^{-3} M), with the final volume set to 30 mL. Lower quantities resulted in incomplete complex development, as Table (2) shows. The absorbance rose with increasing doses, reaching its maximum when a 2.5 mL 4-HNC solution was used.

Table 2 Impact of reagent quantity

mL of Reagent (2×10^{-3} M)	Absorbance	
	SB	BW
0.25	0.468	0.077
0.5	0.499	0.080
0.75	0.540	0.080
1	0.580	0.085
1.25	0.515	0.079
1.5	0.460	0.090

3.4. Choosing the ideal oxidizing agent

The studies used 1.0 mL of different oxidants (1×10^{-3} M), 2.5 mL of 4-HNC (2×10^{-3} M), and 1.5 mL of NaOH solutions. The FeCl₃ solution was selected for the subsequent experiment because it displayed the product's greatest intensity at a wavelength of 510 nm. Table (3) presents the findings.

Table 3 best oxidizing reagent

Oxidizing agent (1×10^{-3} M)	Absorbance	λ max
	SB	
Ferric chloride	0.585	510
Potassium persulfate	0.397	489
Potassium iodate	0.349	497
Potassium periodate	0.327	445

3.5. The impact of FeCl₃ concentration

An experiment was carried out to identify the proper amount of FeCl₃ (1×10^{-3} M) when added to GUE solution (2.0 mL), 4-HNC reagent solution (2.0 mL), and sodium hydroxide (1.0 mL) in flasks. The results showed that the most appropriate quantity (0.75-3 mL) of FeCl₃ was found. The quantity was increased to 25 mL using distilled water after five minutes to allow the reaction to finish, and the wavelength was discovered to be 510 nm. It was determined that the greatest absorbance is observed when the quantity of FeCl₃ applied is 1.0 mL.

Table 4 Impact of oxidizing agent volume

ml of FeCl ₃ (1×10^{-3} M)	Absorbance	
	SB	BW
0.75	0.493	0.061
1.5	0.579	0.077
2.25	0.489	0.079
3	0.456	0.086

3.6. The basic type solution's impact

The influence of various bases (1.0 mL, 1×10^{-2}) was studied to determine the base that produces the highest absorbance since the coupling reaction of reagent 4-HNC with GUE is performed under basic conditions. The sodium hydroxide produces the maximum absorbance, as per the values found in Table (5). Thus, the subsequent trials were conducted using it.

Table 5 The base type solution's impact

Oxidizing agent ($1 \times 10^{-2} \text{M}$)	Absorbance		λ_{Max}
	SB	BW	
Na_2CO_3	0.575	0.075	487
NaOH	0.589	0.85	510
KOH	0.547	0.96	468

3.7. The impact of sodium hydroxide solution concentration

The 1.0 mL of NaOH solution causes maximum Abs at pH 10.6, as found out from the experiment performed to determine the normal volume of the base solution by using varying volumes (0.5-2.5 mL) of sodium carbonate. This volume is taken into consideration for further experimentation. Observations are provided in Table (6).

Table 6 The impact of sodium hydroxide solution concentration

ml of NaOH ($1 \times 10^{-2} \text{M}$)	Absorbance		
	SB	BW	pH
0.5	0.495	0.08	9.2
1	0.538	0.077	9.75
1.5	0.579	0.082	10.75
2	0.510	0.089	11.25
2.5	0.449	0.075	12.45

3.8. The addition's effect order

The order of addition was examined to determine the ideal sequence that would result in the dye's maximal absorption. Table (7) lists a few addition orders; order No. 3.0 was found to be the most effective. As a result, it was chosen for more research.

Table 7 Impact of addition sequences

Order	The addition	Absorbance	
		SB	BW
1	Ox + Re + D + Ba	0.549	0.075
2	Ox + D + Ba + Re	0.555	0.069
3	D + Re + Ox + Ba	0.580	0.078
4	D + Ox + Re + Ba	0.565	0.090
5	Re + Ba + D + Ox	0.545	0.075
6	Ox + Ba + D + Re	0.530	0.088

Drug solution" (D), reagent solution" (Re), oxidizing agent solution" (Ox), and base solution" (Ba)

3.9. The spectrum of ultimate absorption

The absorbance spectrum of GUE (250 $\mu\text{g/ml}$), 4-HNC ($2 \times 10^{-3} \text{M}$), and FeCl_3 ($1 \times 10^{-3} \text{M}$) was determined in the presence of $1 \times 10^{-2} \text{M}$ sodium hydroxide solution at 25°C in 2 ml volume each. The mixture was allowed to react for five minutes. Then absorbance was read against the blank in a 25 ml volumetric flask.

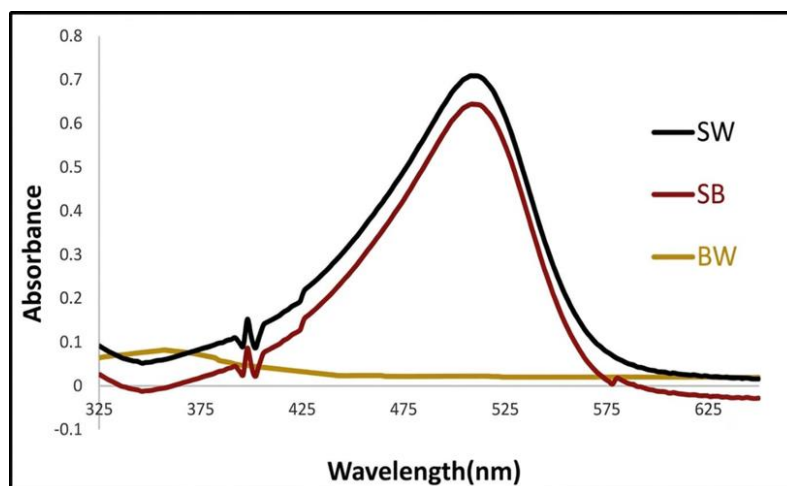


Figure 2 The ultimate spectrum of absorption

3.10. The developer containing PHE in the composition of the composite ionic dye

The chemical composition of the product was analysed using the continuous difference technique, also known as the Jop technique. This technique is used to determine the characteristics of the final product and the binding affinity ratios of the drug to the reagent (33). From the results shown in Figure 3, it can be concluded that the ratio between the ionic dye

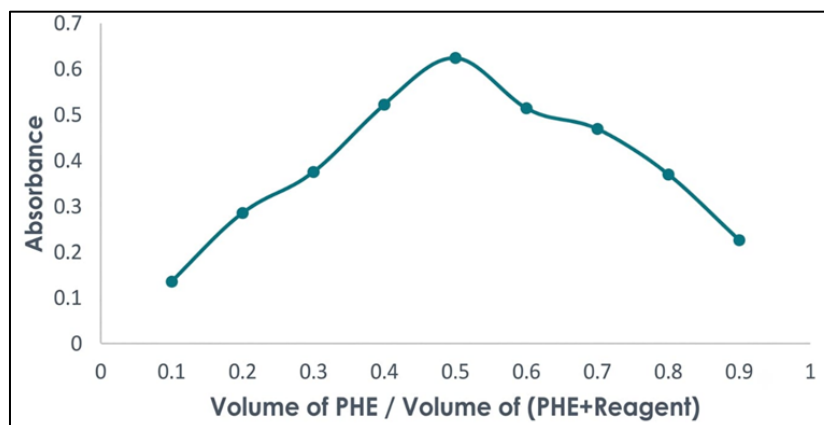


Figure 3 The continuous variation method for the formation of ionic compounds.

3.11. The graph of calibration

The GUE solution, which had a concentration of 4–38 $\mu\text{g/mL}$, was quickly placed into a series of flasks (28 mL), with the proper experimental conditions listed in Table (7). The flasks were then filled with 2.25 mL of 4-HNC reagent, 1.5 mL of FeCl_3 , and 1.5 mL of NaOH . Five minutes passed throughout the reactions.

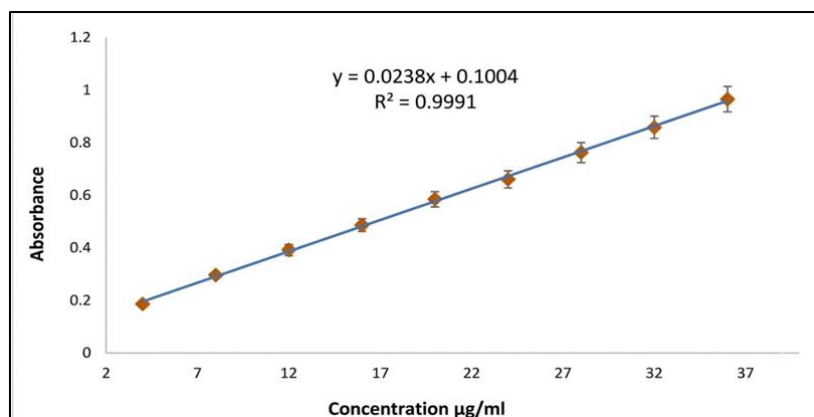


Figure 4 The GUE medication's calibration graph

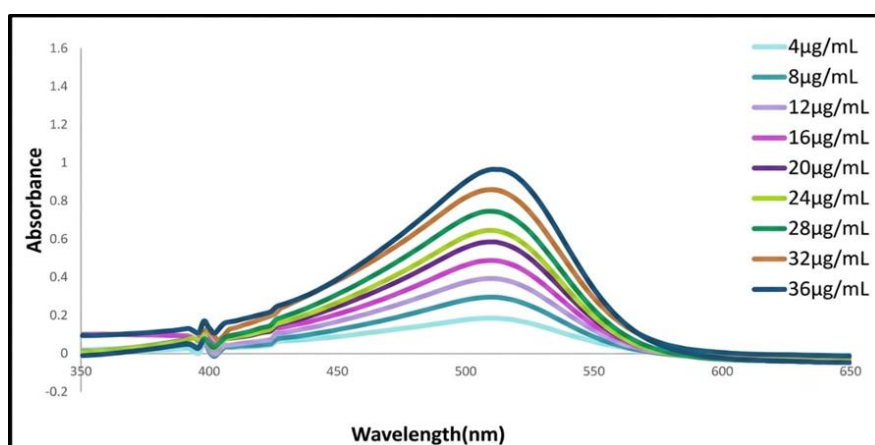


Figure 5 Calibration curve spectra for GUE calculation

4. Conclusion

There emerges a constant red-orange colored compound that can be analyzed quantitatively through this procedure. The absorbance of the produced species was found to be maximum at a wavelength of 510 nm under optimum conditions of experimentation. Optimum conditions for carrying out the experiment include 2.0 ml of GUE solution (250 µg/mL), 2.0 ml of 4-HNC (2×10^{-3} M), 1.0 ml of ferric chloride (1×10^{-3} M), and 1.0 ml of sodium hydroxide (1×10^{-2} M); maximum peak areas were observed with the above-mentioned order of mixing (drug-reagent-oxidizing agent-base). Colorimetry reaction completed in 5.0 minutes at 25°C. For up to 55 minutes, the developed chromophore remained stable. This procedure is appropriate for the quantitative determination of the range of 4 to 36 µg/mL from the validation. The quantitativity of the system is $4.712 \times 10^3 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ while its Sandell sensitivity is 0.0420 µg/cm^2 . The detection and quantification limits are 0.427 and 1.294 µg/mL, respectively. The high reproducibility and accuracy are assured by the precision which is determined in relative standard deviation values less than 1.01%, where the recovery value approaches to 100% (100.125% to 100.35%). The studies on equivalency prove that the complex forms 1:1 molar ratio of GUE and 4-HNC reagent through both Jop method and molar ratio equation. The analysis of the commercial product confirms the method applicability.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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