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Original Article

QUANTITATIVE ANALYSIS OF DEXTROMETHORPHAN-HBr, GUAIFENESIN AND DIPHENHYDRAMINE-HCl IN TABLET DOSAGE FORM BY SUCCESSIVE RATIO DERIVATIVE SPECTRA METHOD

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ABSTRACT

Objective: This study aims to develop a spectrophotometric method with the successive ratio derivative spectra method using ethanol pro analysis solvent to obtain the results of the levels of Dextromethorphan (DEX) Hydrobromide (HBr), Guaifenesin (GUA) and, Diphenhydramine (DIF) Hydrochloric Acid (HCl) in tablet dosage form.

Methods: This method is straightforward to determine the levels of DEX HBr, GUA, and DIF HCl in the wavelength range of 200-400 nm using absorption, which is calculated experimentally using spectrophotometry UV-Vis.

Results: The maximum wavelengths of DEX HBr, GUA and DIF HCl were obtained at 278 nm, 273 nm, and 252 nm, respectively. The average % accuracy was obtained at 99.50% for DEX HBr, 99.91% for GUA, and 99.98% for DIF HCl in dosage forms.

Conclusion: This method was successfully applied to determine the levels of DEX HBr, GUA and DIF HCl in tablet preparations and met the validation requirements.

Keywords: Dextromethorphan HBr, Guaifenesin, Diphenhydramine HCl, Spectrophotometry, Succesive ratio derivative

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INTRODUCTION

Various cough medicine preparations are already widely available in the market that combines two or more active substances in one preparation, one of which is Dextromethorphan (DEX), Guaifenesin (GUA), and Diphenhydramine (DIF), a drug product containing a combination of two or more ingredients. Active means that the drug can more effectively reach the therapy target [1-3].

Coughing is a vital reflex to help clear the airways and prevent aspiration. Cough is also a troublesome clinical problem that often causes people to seek medical treatment [4, 5]. Gratusif tablets from Graha Farma are used as a cough medicine, which contains three components: DEX 15 mg, GUA 100 mg, and DIF 5 mg.

DEX is an antitussive drug commonly used as a cough suppressant that acts centrally to suppress the cough center in the medulla, in combination with GUA, usually used in expectorant cough medicines acting by reducing the surface tension and viscosity of mucus, and DIF, namely as a decongestant or as an anti-allergy, the combination of these three drugs is very effective in the treatment of cough [6-8].

In the manufacture of drugs, checking the levels of active substances is a requirement that must be met to ensure the quality of drug preparations. Good drug preparations will support the achievement of the expected therapeutic effect, so the drug is safe to use [9, 10]. According to the Indonesian Pharmacopoeia Edition VI of 2020, the content requirements for dextromethorphan HBr tablets, diphenhydramine HCI are not less than 98.0% and not more than 102.0%, for guaifenesin tablets, which are not less than 98.0% and not more than 102.0% [11].

In previous studies, several techniques for determining drug levels can be carried out using gas chromatography, UV spectrophotometry [12, 13], High-Performance Liquid Chromatography (HPLC) [14, 15], High-Performance Thin Layer Chromatography (HPTLC) [16], Infrared [17], Liquid Chromatography Tandem-Mass Spectrometry (LC-MS/MS) [18], and Ultra-Performance Liquid Chromatography-Electrospray Tandem Mass Spectrometry (UPLC-ESI-MS/MS) [19], Potentiometric and voltammetry [20] simultaneous UV spectrophotometer for analysis in single or combined preparations. However, no studies have used a combination of 3 drugs, DEX, GUA, and DIF, with the Succesive Ratio Derivative Spectra (SRDS) method.

The SRDS method is used to analyze the concentration of drug mixtures as successive reduction ratios. This method can be chosen when there is a broad spectrum of one of the analytes, but it may also represent a limitation. This method is also used to simultaneously determine three compounds in a ternary mixture without the need to know the concentration ratio of the species. It is based on the successive derivation of the spectral ratio in two consecutive steps [21, 22]. Based on this explanation, the levels of DEX, GUA, and DIF in tablets can be determined using the SRDS spectrophotometric method.

MATERIALS AND METHODS

Materials

UV-Vis 1800 Spectrophotometer (Shimadzu) and a set of Personal Computers (PC) equipped with UV-Probe 2.42 software. DEX, Guaifenesin GUA, and DIF raw materials were obtained from the Indonesian Food and Drug Supervisory Agency. Grantusif® tablets containing 15 mg DEX, 100 mg GUA, and 5 mg DIF (produced by Graha Farma, Surakarta, Indonesia), and pro-ethanol analysis (e-Merck).

Instrumentation

UV-Vis 1800 Spectrophotometer (Shimadzu) and a set of Personal Computers (PC) equipped with UV-Probe 2.42 software.

Preparation of standard solution

Accurately weighed 50 mg of DEX, GUA, and DIF standard was separately transferred into a 50 ml volumetric flask and dissolved in ethanol to give solutions containing 1000 µg/ml DEX, GUA, and DIF.

Selection of analytical wavelength

The solutions of DEX, GUA, and DIF were prepared in diluent by appropriate dilution, and the spectrum was recorded. The maximum wavelength was selected by measuring at 200 to 400 nm with concentrations of DEX (28, 42, 56, 71, 85 µg/ml), GUA (16, 24, 32, 40, 48 µg/ml), and DIF (7, 10, 13, 16, 19 µg/ml).

Assay of tablet formulation by succesive ratio derivative spectra (SRDS)

Twenty tablets were weighed and then crushed in a mortar until smooth and homogeneous. Accurately weighed 25 mg DEX powder (weighing was carried out six times repetition) and calculated the equivalence of GUA, and DIF contained therein. The weighed powder was put into a 50 ml volumetric flask, dissolved in ethanol p. a., and homogenized with a sonicator for 15 min until it was up to the mark line. The solution is filtered, approximately 10 ml of the first filtrate is discarded, and the next is accommodated. Then pipetted as much as 0.4 ml, put into a 25 ml volumetric flask, and diluted with ethanol p. a up to the mark line. Absorbance was measured at a wavelength of 200-400 nm. The absorbance was then measured according to the optimization results procedure using SRDS.

Method validation

The method validation was carried out by determining the wavelength (λ) using SRDS method analysis with samples of DEX, GUA, and DIF by looking at the best correlation coefficient value. The regression line equation and validated method are seen based on the values of linearity, accuracy, precision, Limit of Detection (LOD), and Limit of Quantification (LOQ) referring to ICH guidelines [23,24], then preparing a DEX sample solution (56 µg/ml), GUA (32 µg/ml), and DIF (13µg/ml) in ethanol solvent. The DEX, GUA, and DIF solutions were measured with a spectrophotometry UV-Vis, and the absorption factor values were obtained [25-28].

RESULTS AND DISCUSSION

Selection of analytical wavelength

The maximum wavelength is chosen by looking for the assay value with the SRDS method of each spectrum with different concentrations. Based on fig. 1 and 2, DEX showed a linear response at λmax 278 nm, GUA 273 nm and DIF was measured at 257 nm, as shown in fig. 1 and 2.

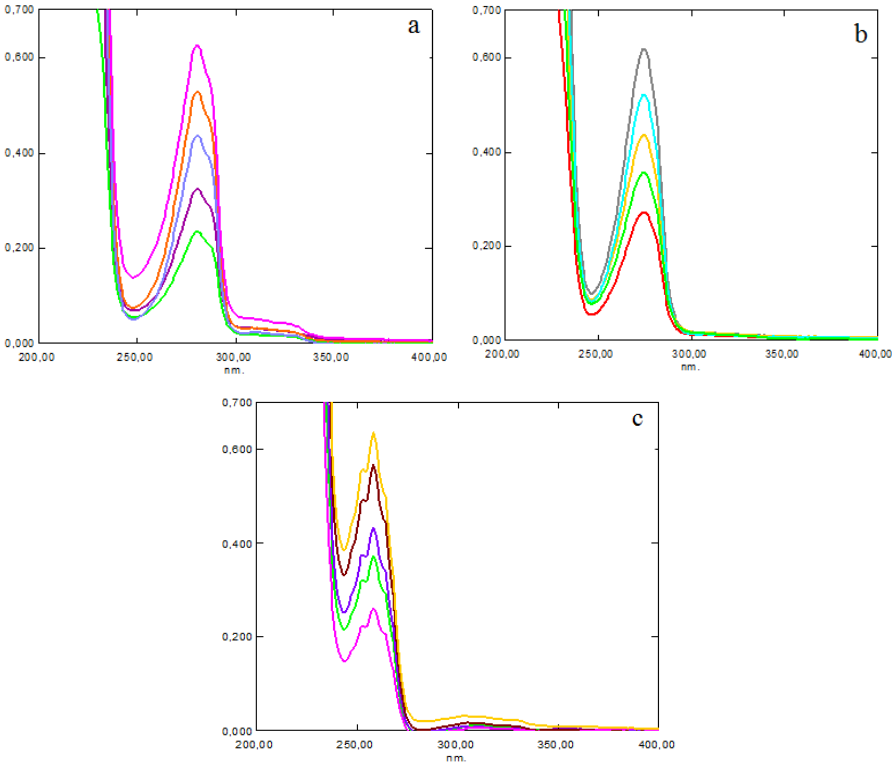

Fig. 1: Absorption spectra (a) DEX (b),GUA (c) DIF

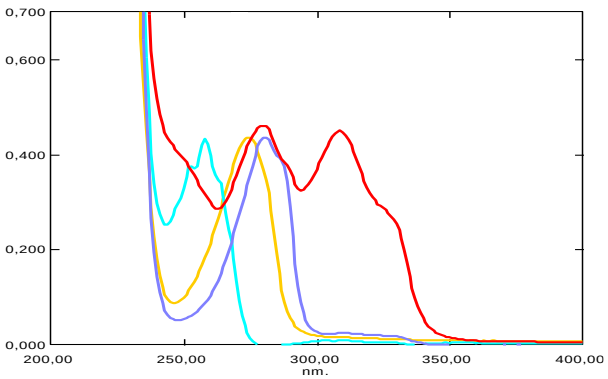

Fig. 2: The absorption spectrum of DEX overlaps 56µg/ml ( ), GUA32µg/ml ( ), DIF 13µg/ml ( ), mixture DEX+GUA+DIF ( )

Based on fig. 1, DEX, GUA, and DIF are at 278 nm, 273 nm, and 257 nm, respectively; seen in fig. 2, the absorption spectra of DEX, GUA, and DIF with various concentrations show that the concentrations do not change the solvent. Solvents, both DEX, GUA, and DIF, obtained stable spectra. The spectrum of the mixture of DEX, GUA, and DIF produces a spectrum that is different from this because the spectrum of the mixture is a combination of the spectra of the constituent substances. Ordinary spectrophotometric methods, such as ultraviolet spectrophotometry, cannot be used to determine DEX, GUA, and DIF levels in tablet mixtures because their spectra overlap. Ordinary spectrophotometric methods such as ultraviolet spectrophotometry cannot be used to determine the levels of DEX, GUA, and DIF in tablet mixtures because their spectra overlap, so the SRDS method is used.

The SRDS method is used to simultaneously determine three compounds in a mixture without knowing the ratio of the concentrations of the species. It is based on successfully reducing the spectral ratio in two consecutive steps [29].

The distribution concentration used in this study was guaifenesin 32µg/ml. Record the first ratio spectrum, which is then converted into derivative one at Δλ 4. Then, the stored spectrum is divided again with 32µg/ml guaifenesin to record the second ratio spectrum, and the results are transformed into derivative two at Δλ 4 [30]. Then the maximum or minimum wavelength is selected which provides the best correlation value, where in this case, the wavelength chosen for DEX is 284.00 nm, GUA 271.90 nm, and DIF 243.10 with a correlation coefficient value of DEX 0. 9996, GUA 0.9992, and DIF oride 0.9997 are given in the spectrum of derivative one at Δλ 4 which can be seen in fig. 3.

Method validation

The method was validated based on linearity, accuracy, precision, LOD and LOQ. The validation results are shown in table 1.

Table 1 shows that the linearity obtained meets the linearity requirements for method validation because there is a correlation coefficient value of ≤1. The test accuracy is measured in percentage recovery. Recoveries were carried out over three specific ranges with three repetitions. In this case, the specific ranges used are 80%, 100%, and 120%, where the composition consists of 70% sample and 30% standard. The accuracy value obtained shows that this method meets the method validation requirements. The percentage of recovery obtained is certified as meeting accuracy standards [31]. The precision results are less than 2 percent, which meets the precision requirements for method validation by looking at the closeness of the analysis results carried out in several repetitions. Precision indicates that it can be detected, while the quantitation limit is defined as the lowest concentration of analyte in a sample that can still meet the criteria for precision and accuracy [32].

Application of the method in tablet dosage form

The findings of using the suggested method to simultaneously determine.

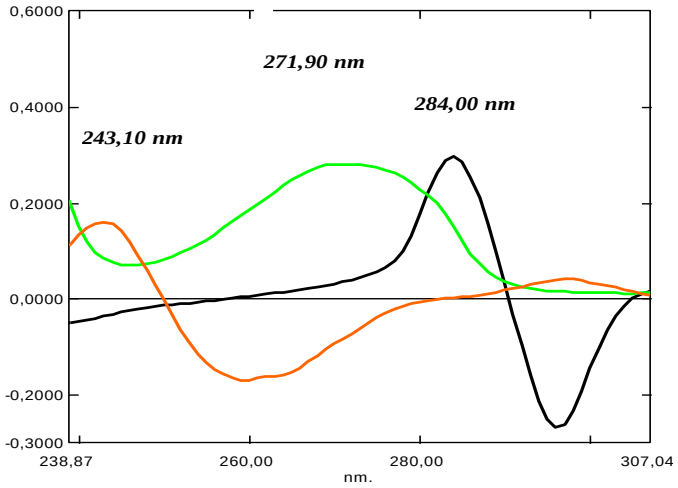

Fig. 3: Overlapping spectrum of the ratio derivative of DEX ( ), GUA ( ), and DIF ( )

Table 1: Validation results of DEX, GUA, and DIF

Component of drugs	Contents %	Level requirements (%)
DEX	100.33±0.7104	98-102
GUA	99.57±0.6499	98-102
DIF	99.12±0.6225	98-102

Data are given as mean±SEM, n = 3. Different letter superscripts indicate significant differences at p<0.05 in rows using the method ANOVA.

Table 2: DEX, GUA and DIF contents in tablet

No.	Parameters	DEX	GUA	DIF
1	Linearity	0.9996	0.9992	0.9997
2	Accuracy (%)	99.5055	99.9151	99.9864
3	Precision %	0.4302	0.3965	0.3815
4	LOD (µg/ml)	2.7391	1.2882	1.7910
5	LOQ (µg/ml)	9.1304	4.2741	5.9701

DEX, GUA, and DIF in tablets are displayed in table 2.

The levels of DEX, GUA and DIF obtained can be seen in table 2, and the tablet preparation meets the requirements where the substance levels are in the range of 98.0%-102.0%. This proves that the DEX, GUA, and DIF levels obtained meet the Indonesian Pharmacopoeia Edition VI standard levels [33].

CONCLUSION

The Succesive Ratio Derivative Spectra spectrophotometric method is a simple, accurate, precise, sensitive and easy-to-apply spectrophotometry method. This method can be applied to the analysis of Dextromethorphan, Guaifenesin, and Diphenhydramine simultaneously in combination with tablet preparations and meets the validation requirements and can be applied routinely DEX, GUA and DIF analysis.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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