

QUANTITATIVE ANALYSIS OF DEXTROMETHORPHAN-HBr, GUAIFENESIN, AND DIPHENHYDRAMINE-HCl IN TABLET DOSAGE FORM BY RATIO DIFFERENCE SPECTROPHOTOMETRY METHOD

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ABSTRACT

Objective: This study aims to develop a spectrophotometric method with the Ratio Difference method using ethanol pro analysis solvent to obtain the results of Dextromethorphan Hydrobromide (HBr) levels of Guaifenesin and Diphenhydramine Hydrochloride (HCl) in tablets.

Methods: The Ratio Difference Spectrophotography method involves dividing the mixture spectrum by the standard spectrum of each analyte and reducing the ratio to obtain a spectrum that does not depend on the concentration of the analyte used as a divider and can directly determine the levels of Dextromethorphan HBr, Guaifenesin, and Diphenhydramine HCl in the range 200-400 nm wavelength using experimentally calculated absorbance.

Results: The maximum wavelengths of Dextromethorphan HBr, Guaifenesin, and Diphenhydramine HCl were obtained at 278 nm, 273 nm, and 252 nm, respectively. The average % accuracy obtained was 99.60% for Dextromethorphan HBr, 98.98% for Guaifenesin, and 100.32% for Diphenhydramine HCl in dosage forms.

Conclusion: This method was successfully applied with simultaneous estimation to determine Dextromethorphan HBr, Guaifenesin, and Diphenhydramine HCl levels in tablet preparations and met the validation requirements.

Keywords: Dextromethorphan HBr, Guaifenesin, Diphenhydramine HCl, Spectrophotometry, Ratio difference

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INTRODUCTION

Cough medicine preparations are already widely available in the market in various variations by combining or combining two or more active substances in one preparation, one of which is by combining Dextromethorphan HBr (DEX), Guaifenesin (GUA), and Diphenhydramine HCl (DIF). Combining two or more active ingredients means the drug can be more effective in achieving therapeutic targets [1-3].

Coughing is a physiological reflex that occurs as lung protection from mechanical trauma or a natural defense process to protect the respiratory tract by preventing foreign bodies from entering the respiratory tract. One of the drugs that can treat cough is *Gratusif Tablet* from Graha Farma, which is used as a cough medicine and contains three components, Dextromethorphan HBr 15 mg, Guaifenesin 100 mg, and Diphenhydramine HCl 5 mg [4, 5].

Dextromethorphan hydrobromide (DEX) is an antitussive agent commonly used in cough and cold medicines. DEX is chemically known as morphinean 3-methoxy-17-methyl-(9 α ,13 α ,14 α) hydrobromide monohydrate, combined with another ingredient, Guaifenesin (GUA) 3-(2-methoxyphenoxy)propane-1, 2-diol is a class of drugs called as expectorants and is mainly used to split up congestion and reduce mucus thickness and Diphenhydramine HCl (DIF), namely as a decongestant or as an anti-allergic, the combination of these three drugs is very effective for the treatment of cough [6-9].

Examination of active substance levels is a requirement that must be fulfilled in the process of making drugs and guaranteeing the quality of drug preparations. Excellent and appropriate drug preparations and ingredients will support the achievement of the expected drug therapeutic effect so that the drug is safe to use [10, 11]. The requirements that must be met for the quality of the ingredients of the drug are by the Indonesian Pharmacopoeia Edition VI of 2020, the requirements for the content of cough medicine tablets

containing three-drug combinations, namely dextromethorphan HBr 98.0%, diphenhydramine HCl not more than 102.0%, for guaifenesin tablets not less than 98.0% and not more than 102.0% [12].

In previous studies, several techniques for determining drug levels could be carried out using LC-MS/MS [13, 14], UV spectrophotometry [15, 16], gas chromatography [17, 18], HPLC [19, 20], HPTLC [21, 22], Infrared Infrared Infrared [23, 24], potentiometric and voltammetry [25, 26], can also be carried out with a simultaneous UV spectrophotometer for analysis in single or combined preparations. However, no studies have used a combination of 3 drugs, DEX, GUA, and DIF, using the Ratio Difference (RD) method.

RD method is used to analyze the difference in amplitude between two points on the spectrum, the ratio of the mixture is directly proportional to the concentration of the desired component, and the independence of the interfering components is the basic principle of the difference ratio method. This method can be chosen if a part of the extended spectrum has two wavelengths, which will be reduced by the amplitude of one of the analytes [27, 28]. Based on this explanation, the levels of the three-drug combinations DEX, GUA, and DIF in tablets can be determined using the RD spectrophotometry method.

MATERIALS AND METHODS

Materials

DEX, GUA and DIF raw materials were obtained from the Indonesian Food and Drug Supervisory Agency. *Grantusif®* tablets containing 15 mg Dextromethorphan HBr, 100 mg Guaifenesin, and 5 mg Diphenhydramine HCl (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 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 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). The ratio difference concentrations is calculated to selected wavelength range analysis.

Assay of tablet formulation by ratio difference spectrophotometry method

The content of 20 tablets was weighed accurately. A powder quantity equivalent to Dextromethorphan HBr 15 mg, Guaifenesin 100 mg and Diphenhydramine HCl 5 mg was accurately weighed and transferred into a volumetric flask of 50 ml capacity; solvent was transferred into this volumetric flask and sonicated for 10 min. The flask was shaken, and the volume was made up to the mark with

diluent. Filtered the solution with whatman® filter paper no. 42, discarded the first 10 ml of filtrate. Pipette 0.4 ml of the filtrate into a 25 ml volumetric flask and add solvent to the marked line. The resulting solution was analyzed by the proposed method. The quantitation was carried out by keeping these values to the straight-line calibration curve equation. The absorbance was then measured according to the optimization results procedure using the Ratio Difference method.

Method validation

The methods was validated was validated based on linearity, accuracy, precision, LOD and LOQ referring to International Conference of Harmonization (ICH) guidelines (29–33).

RESULTS AND DISCUSSION

Selection of analytical wavelength

The maximum wavelength is chosen by looking for the assay value with the RD method of each spectrum with different concentrations. Based on fig. 1 and 2, DEX showed a linear response at λ_{max} 278 nm, GUA 273 nm and DIF was measured at 252 nm, as shown in fig. 1 and 2.

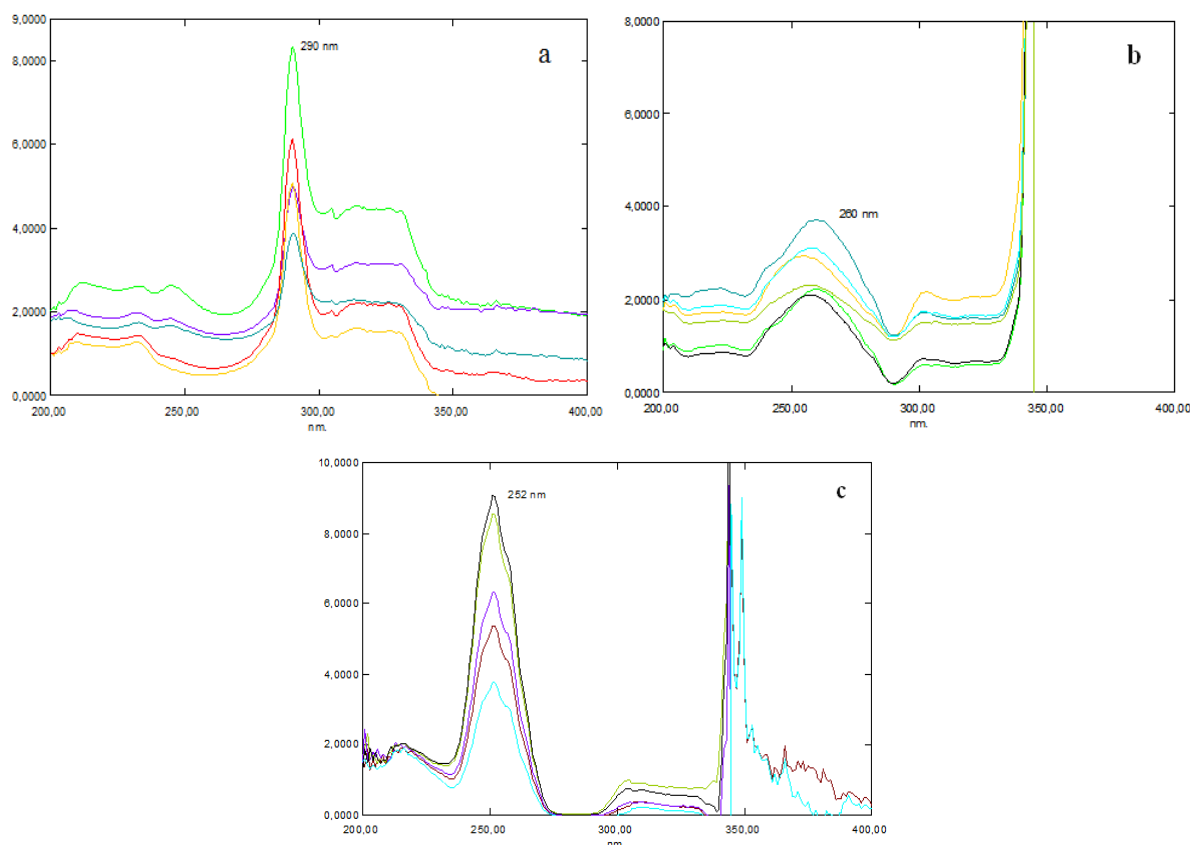


Fig. 1: Absorption spectra of DEX (a), GUA (b), and DIF (c) at various concentrations

Based on Figure, DEX, GUA, and DIF were measured at 278 nm, 273 nm, and 252 nm, respectively; absorption spectra of DEX, GUA, and DIF with various concentrations show that these concentrations do not change the solvent; both DEX, GUA, and DIF obtained stable spectra. 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 RD method is used.

The Ratio Difference Method is used to determine three compounds in a mixture simultaneously. The amplitude difference between two points on the mixture ratio spectrum is directly proportional to the concentration of the desired component, as in research conducted

by Emam *et al.* 2018 [34]. The distribution concentration used in this study was guaifenesin 32µg/ml. The basis for choosing a division concentration is that there is no difference in the location of the maximum wavelength of a substance in the spectrum; the only difference is the resulting absorption value, and this concentration is the concentration for the maximum wavelength according to research by Mansour, 2018 [35].

Each spectrum is divided by its respective divider. After all the spectra were divided based on their respective spectra, a comparison of the DEX, GUA, and DIF spectra was obtained, which can be seen in fig. 2.

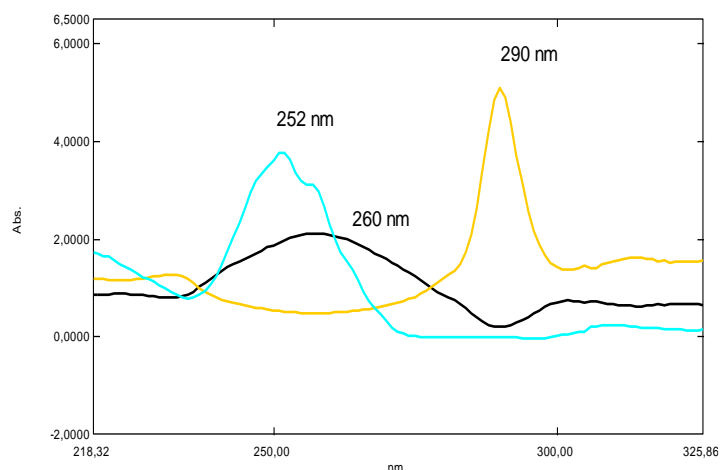


Fig. 2: Overlapping spectrum of RD method of DEX (—), GUA (—), and DIF (—)

Table 1: Validation parameters for DEX, GUA and DIF

No	Parameter	DEX	GUA	DIF
1	Linearity	0.9984	0.9982	0.9994
2	Accuracy(%)	99.60%	98.98%	100.32%
3	Precision %	0.02757	0.59600	0.05230
4	LOD ($\mu\text{g/ml}$)	2.9502	2.5456	2.5178
5	LOQ ($\mu\text{g/ml}$)	5.3864	4.6475	4.5969

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 the percentage of the recovery. Since method validation is between 98%

and 102%, the percentage recovery obtained is certified as meeting the accuracy standar (36). The precision results obtained have a value of less than 2 percent, which meets the precision requirements for the method Validation [37].

Application of the method in tablet dosage form

The proposed method was applied for the determination of DEX, GUA and DIF in their combined tablet and the results are shown in table 2.

Table 2: DEX, GUA and DIF contents in tablet

Component of drugs	Contents %	Level requirements (%)
DEX	100.4035 \pm 0.5104	98-102
GUA	99.2467 \pm 0.7499	98-102
DIF	99.3712 \pm 0.5225	98-102

Data are given as mean \pm SEM, n = 3.

The levels of DEX, GUA and DIF obtained can be seen in table 2 above and are still within the range of requirements in the Indonesian Pharmacopoeia VI edition. This proves that the levels of DEX, GUA and DIF obtained meet the standard levels of the Indonesian Pharmacopoeia Edition VI (38).

CONCLUSION

The RD spectrophotometric method is a simple, accurate, precise, sensitive and easy-to-apply spectrophotometry method. This method can be applied to the analysis of DEX, GUA and DIF 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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