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Research Article

# Validated Mean Centering Ratio UV Spectrophotometric Method for the Determination of Bromhexine Hydrochloride and Guaifenesin in Syrup Dosage Form

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## ABSTRACT

Bromhexine hydrochloride and guaifenesin are frequently combined in syrup formulations; however, their zero-order UV absorption spectra exhibit considerable overlap, which limits direct spectrophotometric analysis. This study aimed to develop and validate a simple and selective mean centering of ratio spectra (MCR) method for the simultaneous determination of bromhexine hydrochloride and guaifenesin in syrup dosage form. Zero-order absorption spectra were recorded over the wavelength range of 200–400 nm, and the overlapping spectra were resolved using ratio spectra followed by mean centering. The method exhibited linear responses over concentration ranges of 54–74 µg/mL for bromhexine hydrochloride and 74–94 µg/mL for guaifenesin. Method validation was performed in accordance with International Council for Harmonisation (ICH) guidelines. Accuracy, evaluated using the standard addition method at three concentration levels (80%, 100%, and 120%), showed satisfactory recoveries, while precision studies demonstrated low relative standard deviation values. The obtained limits of detection and quantification confirmed adequate method sensitivity. The proposed MCR method is simple, accurate, precise, and cost-effective, making it suitable for routine quality control analysis of bromhexine hydrochloride and guaifenesin in combined syrup formulations.

**Keywords:** Bromhexine Hydrochloride, Guaifenesin, Mean Centering Of Ratio Spectra, Syrup Dosage Form

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## INTRODUCTION

Bromhexine hydrochloride (BH) and guaifenesin (GF) are widely used pharmaceutical agents in the treatment of respiratory disorders. BH acts as a mucolytic by reducing the viscosity of bronchial secretions and enhancing mucociliary clearance, thereby facilitating expectoration, while GF functions as an expectorant by increasing the hydration and volume of respiratory tract secretions to relieve chest congestion[1,2]. Their combination in syrup dosage forms is commonly employed for the management of productive cough in both pediatric and adult patients due to their complementary pharmacological effects and favorable clinical efficacy[3].

Various analytical methods have been reported for the determination of BH and GF, either individually, in their binary combination, or with other drugs. These methods include HPLC[4], UV–visible spectrophotometry[5], and LC–MS/MS[2], which have been applied for quality control and bioanalytical purposes due to their high selectivity, accuracy, and sensitivity[2,4-5]. However, overlapping UV absorption of BH and GF in syrup formulations can limit the selectivity of conventional spectrophotometric methods, highlighting the need for chemometric approaches[6, 7].

The mean centering of ratio spectra (MCR) method is a chemometric spectrophotometric approach that enables simultaneous determination of components in overlapping spectra without prior separation. By transforming ratio spectra and applying mean centering, spectral interference is minimized, improving selectivity and analytical performance. This approach is particularly suitable for syrup formulations,

where overlapping UV absorption of active ingredients is frequently encountered[8-10].

To the best of our knowledge, no studies have reported the application of an MCR method for the simultaneous determination of bromhexine hydrochloride and guaifenesin in syrup dosage forms. Therefore, the present study aims to develop and validate a simple, rapid, and accurate MCR-UV spectrophotometric method for the simultaneous quantification of BH and GF in syrup, suitable for routine quality control analysis in the pharmaceutical industry.

## MATERIAL AND METHODS

### Instrumentation

UV–Visible spectrophotometric analysis was performed using a Shimadzu UV-1800 spectrophotometer (Shimadzu, Japan) equipped with a personal computer and MATLAB® software (R2015a). An analytical balance (Ohaus, USA), ultrasonic bath (Branson 1510, USA), and calibrated glassware (Iwaki Pyrex, Japan).

### Chemicals and Reagents

BH and GF raw materials were obtained from PT Pharos, Indonesia. Certified reference standards of BH and GF were provided by the Indonesian National Agency of Drug and Food Control. Ethanol of analytical grade (E. Merck, Germany) was used as the solvent for all preparations.

### Preparation of Standard Solutions

Accurately weighed quantities (50 mg) of BH and GF reference standards were separately transferred into 50 mL volumetric flasks, dissolved in ethanol, and diluted to volume to obtain standard stock solutions with a final concentration of 1000 µg/mL.

### Zero-Order Absorption Spectra of BH and GF

Zero-order absorption spectra of BH (84 µg/mL) and GF (64 µg/mL) were recorded over the wavelength range of 200–400 nm using ethanol as a blank.

### MCR Method

The absorption spectra of BH and GF at various concentrations (54–74 µg/mL for BH and 74–94 µg/mL for GF) were recorded within the wavelength range of 200–400 nm. For the determination of BH, each recorded spectrum was divided by the spectrum of GF at 84 µg/mL, followed by mean centering of the resulting ratio spectra. Similarly, the spectra of GF were divided by the standard spectrum of BH at 64 µg/mL and subsequently mean-centered.

### Sample Preparation

An accurately measured volume of syrup, equivalent to 4 mg of BH and 100 mg of GF per 5 mL, was transferred into a 50 mL volumetric flask, and ethanol was added to dissolve the active components. The mixture was thoroughly homogenized and filtered through Whatman® no. 42 filter paper, discarding the first 5 mL of filtrate to remove any particulate matter. A 0.5 mL aliquot of the filtrate was then transferred into a 25 mL volumetric flask, and the volume was made up to the mark with ethanol. The absorbance of the resulting solution was measured according to the optimized MCR procedure.

### Method Validation

The proposed MCR method was validated according to the International Council for Harmonisation (ICH) guideline[8-11].

### Linearity

Linearity was evaluated over concentration ranges of 54–74 µg/mL for BH and 74–94 µg/mL for GF. Calibration curves were constructed by plotting the mean-centered ratio amplitudes against the corresponding concentrations, followed by regression analysis[11, 12].

### Accuracy

Accuracy was assessed by recovery studies using the standard addition method at three concentration levels (80%, 100%, and 120%). Percentage recoveries were calculated to evaluate the accuracy of the method[8, 11].

### Precision

Precision was expressed as relative standard deviation (%RSD) and calculated using the following equation:

$$RSD = \frac{SD}{X} \times 100\%$$

where SD is the standard deviation and X is the mean value[8, 9].

### Limit of Detection (LOD) and Limit of Quantification (LOQ)

The sensitivity of the method was evaluated by calculating the LOD and LOQ according to ICH guidelines using the following equations:

$$LOD = 3.3x\frac{\sigma}{S}$$

$$LOQ = 10x\frac{\sigma}{S}$$

where σ is the standard deviation of the response and S is the slope of the calibration curve[10, 11].

## RESULTS AND DISCUSSION

### Zero-Order Absorption Spectra of BH and GF

The zero-order UV-Vis absorption spectra of BH and GF were recorded in the 200–400 nm range, as shown in Figure 1. BH exhibits a characteristic absorption peak at ~248 nm, while GF shows peaks around 224 nm and 274 nm, reflecting their UV-active chromophores. These features indicate that both compounds absorb in overlapping regions, making direct determination at a single wavelength impractical[7, 9].

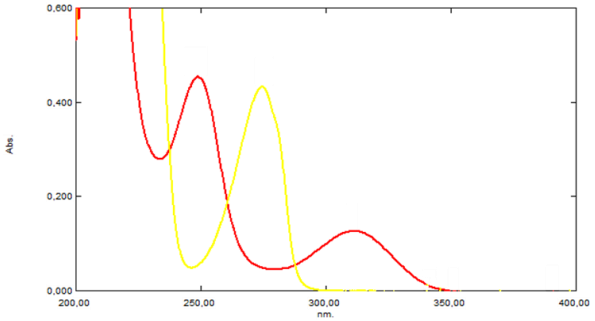


**Figure 1:** Zero-order absorption spectra of BH and GF

As seen in Figure 1, the spectra of the two compounds significantly overlap, so the measured absorbance at each wavelength is a combination of both contributions according to the Beer-Lambert law. Such overlap is common in multicomponent analysis and requires mathematical or chemometric approaches, such as MCR, to selectively resolve and accurately quantify each component without prior separation[8-12].

## MCR Method

BH and GF often exhibit overlapping UV spectra, which complicates direct quantification in mixtures. To overcome this issue, the MCR method utilizes mean centering of ratio spectra, eliminating the need for derivative processing and improving the signal-to-noise ratio in complex multicomponent samples [11, 12]. In this approach, the absorption spectrum of BH was divided by the spectrum of GF, and vice versa, to generate the ratio spectra. These spectra were then mean-centered to remove common contributions from the divisor, enhancing selectivity and allowing the individual spectral features of each analyte to be clearly resolved. This method enables the separation of BH and GF in mixtures without requiring physical separation[8, 9].

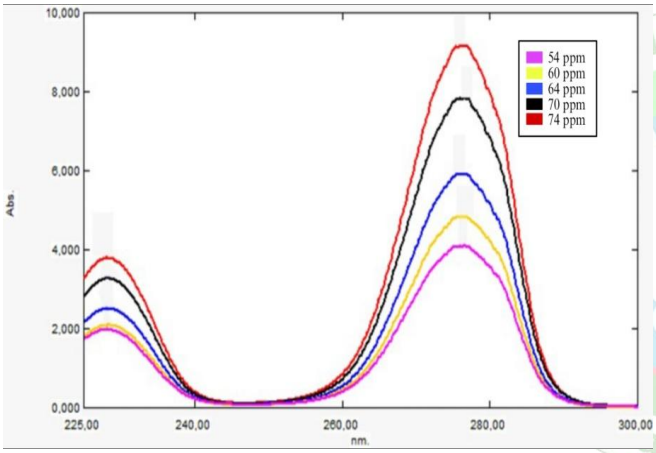


**Figure 2:** Ratio spectra of BH (54–74µg/mL) using GF (84µg/mL) as a divisor

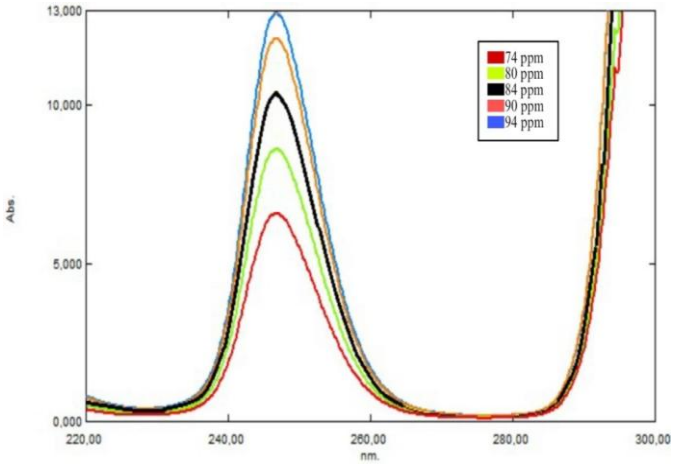


**Figure 3:** Ratio spectra of GF (74–94µg/mL) using BH (64µg/mL) as a divisor

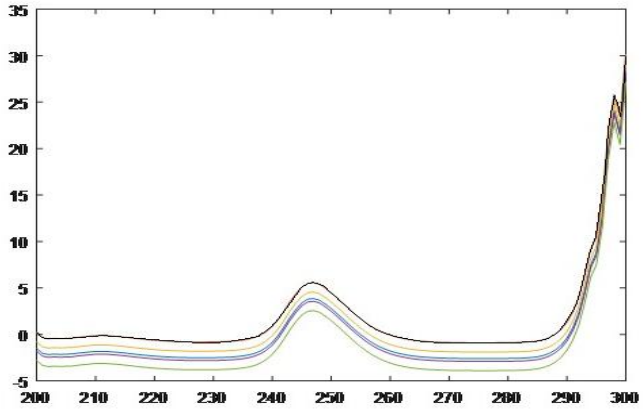

**Figure 4:** MCR of BH

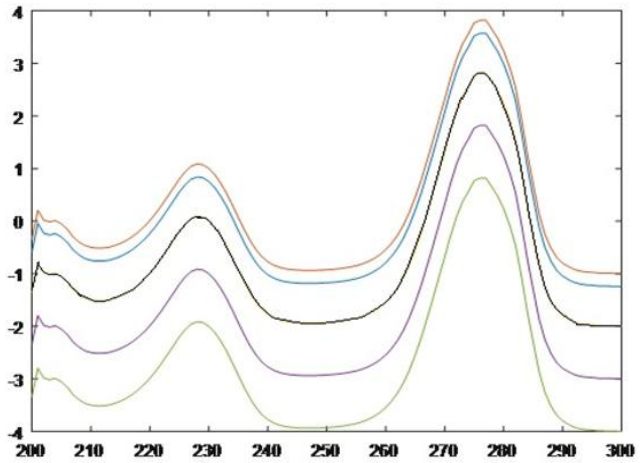

**Figure 5:** MCR of GF

As shown in Figures 2–3, the ratio spectra amplify the signals of the target analytes while suppressing interference from the other component or the sample matrix. After mean centering (Figures 4–5), the spectra of BH and GF were successfully resolved, with characteristic absorption maxima at 248 nm (BH) and 274 nm (GF). This clear separation allows for accurate simultaneous quantification of both compounds, demonstrating that the MCR method provides a selective, precise, and reliable approach for analyzing compounds with overlapping spectra without the need for additional prior separation[8-13].

## Application of the MCR Method to the Analysis of a Binary Mixture of BH and GF in Syrup Formulations

The applicability of the proposed MCR method for the simultaneous determination of BH and GF in syrup formulations was evaluated, and the results are summarized in Table 1. The assay values obtained for both active pharmaceutical ingredients were found to be within the acceptable limits specified by the Indonesian Pharmacopoeia, Sixth Edition, demonstrating good agreement with the labeled claims[8,14].

**Table 1:** Application of the MCR Method for the Simultaneous Determination of BH and GF in Syrup Dosage Form

| No | Component | Drugs Levels | | abel claim (per 5 mL) | Requirements According to the Indonesian Pharmacopeia | |
|---|---|---|---|---|---|---|
| | | (%) | (mg) | (mg) | (%) | (mg) |
| 1 | BH | 99.56±0.29 | 3.98 | 4 | 98.0-102.0 | 3.92–4.08 |
| 2 | GF | 100.34±0.09 | 100.34 | 100 | 98.0-102.0 | 98.0–102.0 |

As shown in Table 1, these findings confirm that the binary mixture of BH and GF in syrup dosage forms can be reliably quantified simultaneously without prior physical separation. The simplicity, selectivity, and cost-effectiveness of the MCR method make it a suitable alternative to chromatographic techniques for the quality control analysis of multicomponent syrup formulations with overlapping UV spectra[13, 14].

## Method Validation

The proposed spectrophotometric method was validated in accordance with the ICH guideline with respect to linearity, accuracy, precision, LOD, and LOQ. The validation results obtained for the simultaneous determination of BH and GF are summarized in Table 2.

**Table 2:** Validation parameters of MCR for BH and GF

| No | Parameter | BH | GF |
|---|---|---|---|
| 1 | Linearity (r) | 0.998 | 0.995 |
| 2 | Accuracy (%) | 99.56 | 100.34 |
| 3 | Precision (RSD) % | 0.29 | 0.18 |
| 4 | LOD (µg/mL) | 1.32 | 0.51 |
| 5 | LOQ (µg/mL) | 4.38 | 1.72 |

As shown in Table 2, the calibration curves for BH and GF exhibited good linearity over the investigated concentration ranges, indicating a direct proportionality between absorbance and concentration. The accuracy of the method was confirmed by satisfactory recovery values, demonstrating the absence of significant systematic errors. Precision studies, expressed as %RSD, revealed low variability for both analytes, reflecting good repeatability and intermediate precision. Furthermore, the calculated LOD and LOQ values indicated that the method possesses adequate sensitivity for the quantification of BH and GF in syrup dosage forms. Overall, all validation parameters complied with the acceptance criteria recommended by the ICH guidelines, confirming that the proposed method is reliable and suitable for routine quality control analysis of binary pharmaceutical formulations[11, 13, 15].

## CONCLUSION

The proposed MCR method was successfully applied for the simultaneous determination of BH and GF in syrup dosage forms. The method met ICH validation requirements and demonstrated satisfactory accuracy, precision, and sensitivity. Thus, it is suitable for routine quality control analysis of binary pharmaceutical formulations.

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