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Short Communication

# DEVELOPMENT AND VALIDATION OF DOUBLE DIVISOR RATIO SPECTRA DERIVATIVE SPECTROPHOTOMETRY METHOD FOR TERNARY MIXTURE OF DEXTROMETHORPHAN HYDROBROMIDE, DOXYLAMINE SUCCINATE AND PSEUDOEPHEDRINE HYDROCHLORIDE IN TABLET DOSAGE FORM

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**ABSTRACT**

This study aimed to develop spectrophotometry method by double divisor ratio spectra derivative to determine the levels of dextromethorphan hydrobromide (DEX), doxylamine succinate (DOX) and pseudoephedrine hydrochloride (PSE) in tablet dosage form using ethanol as solvent. The method is based on the use of the coincident spectra of the derivative of the ratio spectra obtained using a double divisor and measuring at the wavelengths selected. Then, the method was applied to determine the levels of DEX, DOX and PSE in tablet dosage form. The selected wavelengths for determination of DEX, DOX and PSE are 277.0 nm, 243.0 nm, and 243.2 nm, respectively. The mean % recoveries were found to be in 100.88%, 100.05%, and 100.26% for DEX, DOX and PSE, respectively. The method is successfully applied to analyze DEX, DOX and PSE in pharmaceutical formulation with no interference from excipients as indicated by the recovery study. All validation parameters were within the acceptable range.

**Keywords**: Dextromethorphan hydrobromide, Doxylamine succinate, Pseudoephedrine hydrochloride, Spectrophotometry, Double divisor ratio spectra derivative


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Dextromethorphan hydrobromide (DEX) is an antitussive agent commonly used in cough and cold preparations. DEX is chemically known as 3-methoxy-17-methyl-(9α,13α,14α) morphinan hydrobromide monohydrate [1, 2]. Doxylamine succinate (DOX) is an antihistamine used to relieve symptoms of allergy and the common cold. DOX is chemically known as N,N-dimethyl-2-[1-phenyl-1-(2-pyridinyl)ethoxy]-butanedioate [2, 3]. Pseudoephedrine hydrochloride (PSE) is a nasal decongestant and also as bronchodilation. PSE is chemically known as (1S,2S)-2methylamino-1-phenylpropan-1-ol hydrochloride [4-6].

Reported methods for estimation of DEX individually and in combination with other drugs from bulk and its formulation include HPLC [7-9], RP-HPLC [10-12], spectrophotometry [13-15], HPTLC [16]. DOX was determined individually and in combination with other drugs from bulk and its formulation include RP-HPLC [17-19], spectrophotometry [20-22]. PSE was determined individually and in combination with other drugs from bulk and its formulation include RP-HPLC [23], spectrophotometry [24-26], TLC–densitometric [27].

Double divisor ratio spectra derivative spectrophotometry method for simultaneous estimation is simpler, rapid, accurate, and economic [28-30]. However, no references have been found for simultaneous estimation of DEX, DOX and PSE in their combined tablet dosage form by double divisor ratio spectra derivative spectrophotometry method. So, the objective is to develop double divisor ratio spectra derivative spectrophotometry method for estimation of DEX, DOX and PSE in the combined tablet dosage form.

Standard of DEX, DOX, PSE were from the National Agency of Drug and Food Control of the Republic of Indonesia, Ethanol (E. Merck), raw material of DEX, DOX and PSE from PT. Konimex, Sukoharjo, Central Java, Indonesia.

Ultraviolet-visible spectrophotometer (Shimadzu 1800, Japan), a set of Personal Computers (PC) equipped with UV-Probe 2.42 software

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

The solutions of DEX, DOX and PSE were prepared in diluent by appropriate dilution and spectrum was recorded. The absorption spectra of the solutions prepared at different concentrations of DEX (40–430 µg/ml), DOX (20–430 µg/ml), and PSE (230–630 µg/ml) were scanned in the range of 200–400 nm. The double divisor value in various concentrations is calculated to selected wavelength range analysis.

Content of 20 tablets was weighed accurately. A powder quantity equivalent to 15 mg DEX, 4 mg DOX and 30 mg PSE was accurately weighed and transferred into a volumetric flask of 25 ml capacity; 15 ml of ethanol was transferred into this volumetric flask and sonicated for 10 min. The flask was shaken and volume was made up to the mark with diluent. Filtered, pipette 1.16 ml of the filtrate and transfer to a 10 ml volumetric flask. filled with ethanol to the mark line to obtain a solution containing 70 µg/ml of DEX, 40 µg/ml of DOX, and 430 µg/ml of PSE. The resulting solution was analyzed by proposed method. The quantitation was carried out by keeping these values to the straight-line equation of calibration curve.

The methods were validated as per International Conference of Harmonization (ICH) guidelines with respect to linearity, accuracy, precision, limit of detection (LOD) and limit of quantitation (LOQ) [31].

Selection of analytical wavelength is carried out by dividing absorption spectra of solutions at different concentrations using the sum of the absorption spectra of solutions of DOX+PSE (430 µg/ml each in diluents), DEX+PSE (430 µg/ml each in diluents), and DEX+DOX (430 µg/ml each in diluents), respectively, for the determination of DEX, DOX, and PSE as double divisor to get the ratio spectra and their first derivatives were plotted with delta lambda 2 nm and scaling factor 1.0. The divided and derivatized spectra's as shown in fig. 1-3.

Based on fig. 1-3, we can conclude that DEX, DOX and PSE can be quantitatively determined at wavelength 277.0 nm, 243.0 nm, 243.2 nm, respectively in their ternary mixture without any interference from each other. According to this result, the double divisor ratio spectra derivative spectrophotometry method can be used to simultaneously determine the drug content consisting of more than one component [29, 32].

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

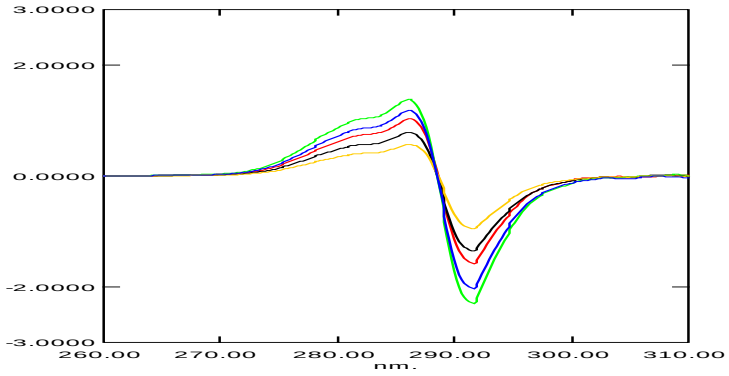


**Fig. 1: Overlay first derivative ratio spectra of DEX at 277.0 nm (DOX 430 µg/ml+DOX 430 µg/ml used as double divisor)**

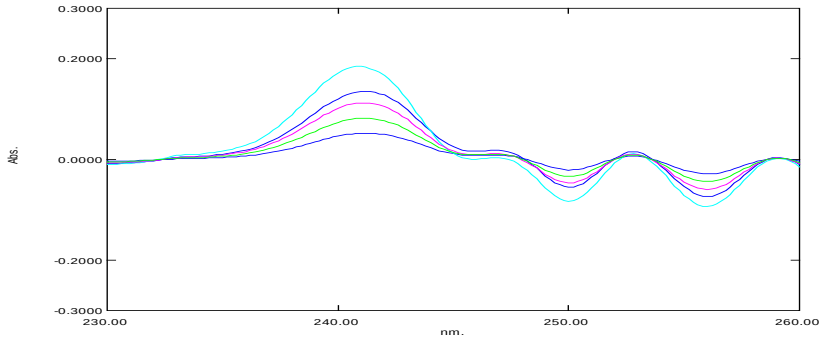


**Fig. 2: Overlay first derivative ratio spectra of DOX at 243.0 nm (DEX 430 µg/ml+PSE 430 µg/ml used as double divisor)**

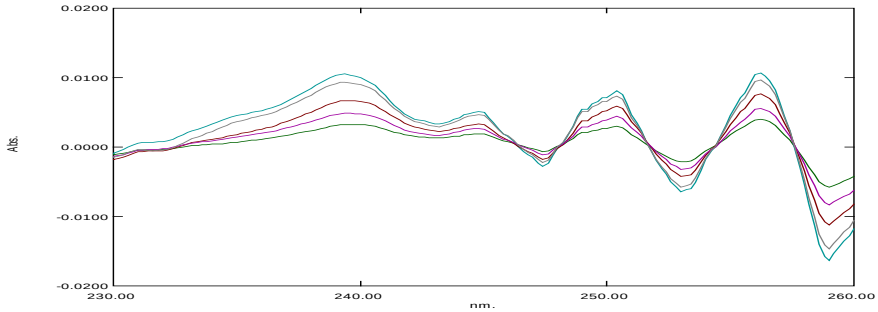


**Fig. 3: Overlay first derivative ratio spectra of PSE at 243.2 nm (DEX 430 µg/ml+DOX 430 µg/ml used as double divisor)**

**Table 1: Validation parameters for DEX, DOX and PSE**

| Parameters | DEX | DOX | PSE |
|---|---|---|---|
| Linearity | 0.9997 | 0.9999 | 0.9983 |
| Accuracy (%) | 100.88 | 100.05 | 100.26 |
| Precision (%) | 0.21 | 0.48 | 0.28 |
| LOD (µg/ml) | 2.9827 | 0.5715 | 44.7632 |
| LOQ (µg/ml) | 9.94 | 1.91 | 149 |

Based on table 1, this research has good result validation method for simultaneous DEX, DOX and PSE in their combine tablet dosage form because all parameters of validation test have according to the validation requirements of ICH. It means the methods have appropriated the validation requirements. Some studies have been reported about spectrophotometry by double divisor ratio spectra derivative method and gave good validation method result [29, 30].

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

**Table 2: Statistical calculation of DEX, DOX and PSE in tablet dosage form**

| Component of drug | Statistical calculation | Content in etiquette | Level requirements |
|---|---|---|---|
| DEX | 100.85±0.71% | 15 mg | (98–102)% |
| DOX | 100.08±0.69% | 4 mg | (98–101)% |
| PSE | 99.53±0.14% | 30 mg | (98–102)% |

Based on table 2, can be seen that the method of double divisor ratio spectra derivative have levels that meet requirements according to Indonesian Pharmacopeia Edition V. It means that the method can be used to simultaneous determine the mixture of DEX, DOX, and PSE in tablet dosage form. However, some studies have been reported [30], and according to this result, the proposed method is potential to use in routine drug analysis, especially drugs with contained the combination of several drugs.

The proposed method gives simpler, rapid, accurate, and precise results for determination of DEX, DOX and PSE as a ternary mixture without prior separation and is easily applied for routine analysis.

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**AUTHORS CONTRIBUTIONS**

All the authors have contributed equally.

**CONFLICT OF INTERESTS**

Declared none

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