Spectrophotometric determination of drug compounds in pure forms and in the pharmaceutical preparations

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Abstract. A “Simple, sensitive and rapid” spectrophotometric method for determination of drug compounds (I) “Dextromethorphan HBr (Dex)” and (II) Trimethoprim (Tri)” in both pure forms and pharmaceutical formulations. These methods were based on the oxidation of the studied drugs in presence of acidic medium by a known excess of “N – Bromosuccinimide” as an oxidizing agent and subsequent determination of unreacted oxidant by reacting it with: (I) Phenosafranine (PS) dye [3,7-Diamino-5-phenylphenazin-5-ium chloride] to produce red product at \( \lambda_{\text{max}} \) 532 nm. The linearity range was found to be (6 -56) \( \mu \)g/mL and molar absorptivity \( 4.686 \times 10^3 \) L/mol.cm, correlation coefficient 0.9983 and the limit of detection 0.763 \( \mu \)g/ml, with slope, intercept and \%RSD were 0.0133, 0.3069 and 0.367 respectively. (II) Crystal Violet (CV) dye [4 [bis [4 (dimethylamino) phenyl] methylidene] cyclohexa-2,5-dien-1-ylidene]-dimethylazanium;chloride to produce blue product at \( \lambda_{\text{max}} \) 590 nm. The linearity range was found to be (5 -65) \( \mu \)g/mL and molar absorptivity \( 8.042 \times 10^3 \) L/mol.cm, correlation coefficient 0.9980 and the limit of detection 1.07 \( \mu \)g/ml, with slope, intercept and \%RSD were 0.0277, 0.2558 and 0.273 respectively. These methods were successfully applied for the determination of (Dex) and (Tri) in tablets formulations.

Key Words: Dextromethorphan HBr, Trimethoprim, Phenosafranine dye, Crystal Violet dye, spectrophotometric.

1. Introduction

Dextromethorphan HBr ‘figure 1’, “is a cough suppressant that acts on the cough centre in the brain to suppress a dry cough. There may be more than one active ingredient in your medicine, such as paracetamol, ibuprofen, a nasal decongestant or an antihistamine. Is used to temporarily relieve cough caused by the common cold, the flu, or other conditions. Dextromethorphan will relieve a cough but will not treat the cause of the cough or speed recovery. Dextromethorphan is in a class of medications called antitussives. It works by decreasing activity in the part of the brain that causes coughing.” [1,2], Molar mass is 352.316 gm/mole, M.P. = about 125 °C with decomposition, is a white, crystalline powder. Dextromethorphan HBr is freely soluble in ethanol, and sparingly soluble in water [3]. Chemically known is (9α,13α,14α)-3-Methoxy-17-methylmorphinan hydrobromide [4]. Several methods have been proposed for determination of this drug, such as HPLC [5-7], TLC [8,9], HPTLC[10],Voltammetry[11],UV-spectrophotometry[12-15],UV-Vis.pectrophotometry [16,17].

Trimethoprim ‘figure 2’, “is an antibiotic used mainly in the treatment of bladder infections. Other uses include for middle ear infections and travelers’ diarrhea [18]. It is primarily used in the treatment of urinary tract infections, although it may be used against any susceptible aerobic bacterial species. It may also be used to treat and prevent Pneumocystis jiroveci pneumonia. It is generally not recommended for the treatment of anaerobic infections such as Clostridium difficile colitis (the leading cause of antibiotic-induced diarrhea)[19], Trimethoprim has been used in trials to treat retinitis [20].
Molar mass is 290.32 gm/mole, M.P. = 199-203 °C, is a white or yellowish white powder. Trimethoprim is very slightly soluble in water, slightly soluble in ethanol [3]. Chemically known is 2,4-Diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine [21]. Several methods have been proposed for determination of this drug, such as HPLC [22,23], TLC [24], Voltammetry [25,26], UV-Vis.pectrophotometry [27-29].

The research aims at finding a “simple, fast and economical spectral methods” for determination of “Dextromethorphan HBr and Trimethoprim” by using two dyes with an oxidizing agent in the acid medium, As well as, the stoichiometry was studied by mole ratio and job method for each, and the success of the proposed methods for determination of the pharmaceutical preparations (as tablets).

2. Experimental

2.1. Apparatus
T90 UV-VIS spectrophotometer “double beam from PG Instruments Ltd, with 1 cm quartz cells”, UV-VIS spectrophotometer “single beam from Genesys UV 10, pH meter InoLab pH/INO735 from Jenway 3310, Balance Kern 770GS/GJ from Sartorius BL210S, Oven from Memmert, Schutzart DIN 40050-IP20.

2.2. Materials
Dextromethorphan HBr %99, Trimethoprim %99 from (SDI Samarra-Iraq), N-Bromosuccinimide %99 from merck, Phenosafranine (dye) %80 from Merck, Crystal Violet (dye) %90 from Merck, Ethanol %99.9 from Scharlau, H2SO4 98% from GCC, HCl %36 from Thomas baker, HNO3 %70 from GCC, CH3COOH %98 from Scharlau.

2.3. Solutions
Dextromethorphan HBr Stock solution (1000 µg/ml): An exactly (0.1000 gm) of (Dex) “standard” are dissolved in (100 ml) ethanol. Trimethoprim Stock solution (1000 µg/ml): An exactly (0.1000 gm) of (Tri) “standard” were dissolved in (100 ml) ethanol. N-Bromosuccinimide (NBS): Prepare at a concentration of 1x10-2 M by dissolving 0.1779 g of them in 100 ml ethanol. Phenosafranine (PS) dye: Prepare at a concentration of 5 x10-4 M by dissolving 0.0161 g of them in 100 ml distilled water. Crystal Violet (CV) dye: Prepare at a concentration of 5x10-5 M by dissolving 0.0020 g of them in 100 ml distilled water. Sulfuric acid solution: prepare with an approximate concentration of 1.0 molar by diluting 5.4 ml of concentrated acid (18.4 M) to 100 ml distilled water. Hydrochloric acid solution: prepare at an approximate concentration of 1.0 molar by diluting 8.6 ml of concentrated acid (11.64 M) to 100 ml distilled water. Nitric acid solution: prepare at an approximate concentration of 1.0 molar by diluting 6.3 ml of concentrated acid (15.78 M) to 100 ml distilled water. Acetic acid solution: prepare with an approximate concentration of 1.0 molar by diluting 5.8 ml of concentrated acid (17.33 M) to 100 ml distilled water.

2.4. Procedures
2.4.1 (I) Dextromethorphan HBr
After initial testing, optimal conditions were obtained, transferring 2.0 ml of 500 µg/ml (Dex) to a 25 ml volumetric flask, then add 2.0 ml of 10-2 M from oxidizing agent (NBS). After 15 minutes, add 0.5 ml of 1.0 M H2SO4 acid. After 10 minutes, add 2.5 ml of 5 x10-4 M Phenosafranine dye, and after 15 minutes, the volume is supplemented with distilled water to 25 ml, the highest absorption of the resulting compound (red color) is 532 nm.

2.4.2 (II) Trimethoprim

After initial testing, optimal conditions were obtained, transferring 0.5 ml of 500 µg/ml (Tri) to a 10 ml volumetric flask, then add 0.5 ml of 10-2 M from oxidizing agent (NBS). After 15 minutes, add 0.5 ml of 1.0 M HCl acid. After 10 minutes, add 3.0 ml of 5x10-5 M Crystal Violet dye, and after 15 minutes, the volume is supplemented with ethanol to 10 ml, because after 15 minutes the color of the blank disappears who prepared the same way except for adding the drug, the highest absorption of the resulting compound (blue color) is 590 nm.

2.4.3 Procedures for "stoichiometric ratio"

The reactions of equivalence between these drugs and the reagent (dyes), have been estimated by carrying out "molar ratio" and "continuous variation method". In these methods, "equimolar" solutions of (Dex) and “PS dye” (1 x 10-3 M), (Tri) and “CV dye” (5x10-5 M) are used. Varying aliquots of “PS dye” and “CV dye” were added to constant aliquots of drug solutions, final volumes (25ml) and (10ml) and the absorbance were measured at 532 nm and 592 nm, respectively, opposite the “blanks treated similarly”. While in the latter methods, a series of Dex-PS and Tri-CV dye solutions were kepts at (5ml) (0:5, 0.5:4.5, 1:4, 1.5:3.5, 2:3, …… 5:0).

2.4.4 Application of the proposed methods

"Ten tablets" are weighed and average weights were computed. These tablets were grinded into exact powders. An precisely weighed amounts of powders were transferred into a beakers and they were shaken with 50 ml of solvents and filtered. The filtrates and the washings were collected in a 100ml “volumetrics flask”. These filtrates and the washings were diluted up to the mark with solvents to obtain final concentrations as 1000µg/ml. These suggested methods were successfully implemented for the determination of (Dex) and (Tri) in various commercial tablets

3. Results and Discussion

3.1. (I) For Dextromethorphan HBr

Absorption spectra of “color product of Dex” against the blank at room temperature (25oC) producing a red colored product where absorbs maximally at 532nm ‘figure 3’, and the blank against distilled water ‘figure 4’.

Figure 3. Absorption spectrum of “color product of Dex” system against blank.

Figure 4. Absorption spectrum of the blank against distilled water.

3.1.2. Optimum conditions:
3.1.2.1. Effect of oxidation agent volume
After testing for the best mixture ratio of the oxidizing agent on the product composition. It is clear from ‘figure 5’ that the best added volume of the oxidizing agent is 2.0 ml which is used in subsequent studies.

![Figure 5](image)

Figure 5. Effect of oxidative agent volume (NBS) on absorption of the (Dex) product.

3.1.2.2. Effect of the use of different acids
The acids are used (H$_2$SO$_4$, HCl, HNO$_3$, CH$_3$COOH), with a concentration of 1.0 M for each, as well as the same volume added 0.5 ml, to know which acid gives the best absorption when product formation, (table 1) shows that the best acid used to form the product is Sulfuric acid.

| The acid used | Product absorption values |
|---------------|---------------------------|
| HCl           | 0.135                     |
| H$_2$SO$_4$   | 0.834                     |
| HNO$_3$       | 0.382                     |
| CH$_3$COOH    | 0.195                     |

3.1.2.3. Effect of the use of different acids
Different and increasing volumes of H$_2$SO$_4$ are used at a concentration of 1.0M, To know which volume gives the best absorption, as shown in ‘figure 6’, the optimal added acid volume is 0.5 ml, and when the added volume increases, the color of the formed product disappears gradually.

3.1.2.4. Effect of dye volume
‘Figure 7’ shows the effect of adding different volumes of the (PS) dye on the absorption of the product. The best added volume was 2.5 ml.

![Figure 6](image)

Figure 6. Effect of H$_2$SO$_4$ on the absorption values of the product.

![Figure 7](image)

Figure 7. Effect of (PS) dye volumes on product absorption values.

3.1.2.5. Effect of time on product stability
The effect of stability of the product was studied for its importance in knowing the period of time in which it remains constant, the interaction with time is followed using optimum conditions every ten minutes for 60 minutes. (Table 2) shows the stability of the absorption values at $\lambda_{\text{max}}$. with time, the absorption value of the product is fixed for 1.0 hour.

**Table 2.** Effect of time on stability of product.

| Time (min.) | Absorbance |
|-------------|------------|
| 0.0         | 0.811      |
| 10          | 0.816      |
| 20          | 0.834      |
| 30          | 0.844      |
| 40          | 0.832      |
| 50          | 0.831      |
| 60          | 0.831      |
| 70          | 0.780      |
| 80          | 0.716      |

3.1.2.6. Effect of Interferences

The effect of interferences on the composition of the product was studied, and not observed any effect, as shown in (Table 3).

**Table 3.** Effect of Interference.

| Interference          | Added con. µg/mL | % RE | Added con. µg/mL | % RE |
|-----------------------|------------------|------|------------------|------|
| Lactose mono hydrate  | 20               | 1.92 | 40               | -0.48|
| Manitol               | 20               | -4.08| 40               | -3.84|
| Sodium benzoate       | 20               | -3.72| 40               | -4.68|

3.1.2.7. Effect of the reactions

Under the "optimum conditions", "the stoichiometry" of the reaction between (Dex) and the dye was studied by mole–ratio and continuous variation methods. The equivalence between dye and this drug is 1:1 ‘figure 8, 9’.

3.1.2.8. Calibration curve

‘Figure 10’ shows the linearity of the calibration curve obtained at optimal conditions, where the linearity was within concentrations (5-50) µg/ml, which is equal to the volumes within (0.1-1.0) ml.
3.1.2.9. Construction of calibration curve
Calibration curve was constructed according to the optimum conditions in (table 4).

**Table 4.** Optical characteristics of the calibration curve for spectrophotometric determination of (Dex) product.

| Parameter                        | (Dex)  |
|----------------------------------|--------|
| $\lambda_{max}$(nm)              | 532    |
| Beer’s law ($\mu$g/ml)           | 6-56   |
| Molar absorptivity(L/mol.cm)     | $4.686 \times 10^3$ |
| Correlation coefficient (r)      | 0.9983 |
| Limit of Detection ($\mu$g/ml)   | 0.763  |
| Slope                            | 0.0133 |
| Intercept                        | 0.3069 |
| %RSD                             | 0.367  |

3.1.2.10. Application of the proposed methods
In (table 5), the result of determination of (Dex) in the pharmaceutical preparations (as tablets).

**Table 5.** Determination of (Dex) in commercial tablets by spectrophotometric method.

| pharmaceutical preparations | Content(mg) declared | Found(mg) by proposed method | %RE  | %Recovery |
|------------------------------|----------------------|------------------------------|------|----------|
| SEDILAR                      | 15                   | 14.95                        | 0.33 | 99.67    |
|                              | 15                   | 15.09                        | 0.60 | 100.6    |
|                              | 15                   | 14.98                        | -0.13| 99.87    |
| TUSSILAR                     | 15                   | 15.12                        | 0.8  | 100.8    |
|                              | 15                   | 14.99                        | -0.07| 99.93    |
|                              | 15                   | 15.06                        | 0.4  | 100.4    |

3.1.2.11. Suggested interactions
The proposed reaction can be based on how the (Dex) drug is oxidized by N-Bromosuccinimide [30] and reduced dye [31]:

![Figure 10. Calibration curve of (Dex) product.](image)

**Figure 10.** Calibration curve of (Dex) product.
3.2. (II) For Trimethoprim

If Absorption spectra of “color product of Tri” against the blank at room temperature (25°C) producing a blue colored product where absorbs maximally at 590 nm ‘figure 11’, and the blank against distilled water ‘figure 12’.

3.2.1. Optimum conditions

3.2.1.1. Effect of oxidation agent volume

After testing for the best mixture ratio of the oxidizing agent on the product composition. It is clear from ‘figure 13’ that the best added volume of the oxidizing agent is 0.5 ml which is used in subsequent studies.

Figure 11. Absorption spectrum of “color product of Tri” system against blank.

Figure 12. Absorption spectrum of the blank against ethanol.

Figure 13. Effect of oxidative agent volume (NBS) on absorption of the (Tri) product.
3.2.1.2. **Effect of the use of different acids**

The acids are used (\( \text{H}_2\text{SO}_4, \text{HCl}, \text{HNO}_3, \text{CH}_3\text{COOH} \)), with a concentration of 1.0 M for each, as well as the same volume added 0.5 ml, to know which acid gives the best absorption when product formation, (table 6) shows that the best acid used to form the product is Hydrochloric acid.

### Table 6. Effect of different types of acids on absorption values of the (Tri) product.

| The acid used | Product absorption values |
|---------------|---------------------------|
| HCl           | 0.949                     |
| \( \text{H}_2\text{SO}_4 \) | 0.137                     |
| HNO\(_3\)     | 0.482                     |
| CH\(_3\)COOH  | 0.003                     |

3.2.1.3. **Effect of acid volume**

Different and increasing volumes of HCl are used at a concentration of 1.0M, To know which volume gives the best absorption, as shown in ‘figure 14’, the optimal added acid volume is 0.5 ml, and when the added volume increases, the color of the formed product disappears gradually.

3.2.1.4. **Effect of dye volume**

‘Figure 15’ shows the effect of adding different volumes of the (CV) dye on the absorption of the product. The best added volume is 3.0 ml.

![Figure 14. Effect of HCl on the absorption values of the product.](image1)

![Figure 15. Effect of (CV) dye volumes on product absorption values.](image2)

3.2.1.5. **Effect of time on product stability**

The effect of stability of the product was studied for its importance in knowing the period of time in which it remains constant, the interaction with time is followed using optimum conditions every ten minutes for 60 minutes. (Table 7) shows the stability of the absorption values at \( \lambda_{\text{max}} \). with time, the absorption value of the product is fixed for 1.0 hour.

### Table 7. Effect of time on Stability of product.

| Time (min.) | Absorbance |
|-------------|------------|
| 0.0         | 0.896      |
| 10          | 0.931      |
| 20          | 0.945      |
| 30          | 0.949      |
| 40          | 0.946      |
| 50          | 0.948      |
| 60          | 0.947      |
| 70          | 0.864      |
| 80          | 0.842      |
3.2.1.6. Effect of Interferences
The effect of interferences on the composition of the product was studied, and not observed any effect, as shown in the (table 8).

| Interference            | Added con. µg/mL | % RE  | Added con. µg/mL | % RE  |
|-------------------------|------------------|-------|------------------|-------|
| Lactose mono hydrate    | 50               | -2.00 | 100              | -4.12 |
| Manitol                 | 50               | -4.85 | 100              | -3.90 |
| Sodium benzoate         | 50               | 0.12  | 100              | 2.21  |

3.2.1.7. Equivalent of the reactions
Under the "optimum conditions", "the stoichiometry" of the reaction between (Tri) and the dye was studied by mole–ratio and continuous variation methods. The equivalence between dye and this drug is 2:1 ‘figure 16, 17’.

3.2.1.8. Calibration curve
‘Figure 18’ shows the linearity of the calibration curve obtained at optimal conditions, where the linearity was within concentrations (5-65) µg/ml, which is equal to the volumes within (0.1-1.3) ml.

3.2.1.9. Construction of calibration curve
Calibration curve was constructed according to the optimum conditions in (table 9).
Table 9. Optical characteristics of the calibration curve for spectrophotometric determination of (Tri) product.

| Parameter                        | (Tri)  |
|----------------------------------|--------|
| \(\lambda_{\text{max}}\) (nm)   | 590    |
| Beer’s law (\(\mu\)g/ml)        | 5.65   |
| Molar absorptivity (L/mol.cm)    | \(8.042 \times 10^3\)  |
| Correlation coefficient (r)      | 0.9980 |
| Limit of Detection (\(\mu\)g/ml) | 1.07   |
| Slope                            | 0.0277 |
| Intercept                        | 0.2558 |
| %RSD                             | 0.273  |

3.2.1.10. Application of the proposed methods

In (table 10), the result of determination of (Tri) in the pharmaceutical preparations (as tablets).

Table 10. Determination of (Tri) in commercial tablets by spectrophotometric method.

| Pharmaceutical preparations | Content(mg) declared | Found(mg) by proposed method | %RE | %Recovery |
|-----------------------------|----------------------|------------------------------|-----|-----------|
| Septrin                     | 80                   | 80.95                        | 1.19| 101.19    |
|                            | 80                   | 80.07                        | 0.09| 100.09    |
|                            | 80                   | 79.89                        | -0.14| 99.86     |
| Methoprim                   | 80                   | 80.88                        | 1.10| 101.11    |
|                            | 80                   | 79.93                        | -0.09| 99.91     |
|                            | 80                   | 80.40                        | 0.5 | 100.5     |

3.2.1.11. Suggested interactions

The proposed reaction can be based on how the (Tri) drug is oxidized by N-Bromosuccinimide \(^{[32]}\) and reduced dye \(^{[33]}\):

\[ (\text{Tri}) + 2 \overset{\text{HCl}}{\rightarrow} (\text{NBS}) \]

4. Conclusion

These methods described here are simple, rapid, convenient and do not requires special working conditions unlike many other reported methods. The procedures showed shorter reactions time, stable colored species with inexpensive reagents. The determination can be performed at room temperature
and do not require heating step. The proposed methods can be applied to determination of (Dex) and (Tri) in pharmaceutical preparations (Tablets).

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