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# Sensitive spectrophotometric methods for microdetermination of dothiepin hydrochloride in pure form, pharmaceuticals and human urine and blood

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## ABSTRACT

Three new, rapid sensitive, economical and simple spectrophotometric methods (A-C) have been developed for the micro determination of dothiepin hydrochloride (DOT) in bulk samples, dosage forms and in post-mortem urine and blood samples. The methods are based on the on the reaction between (DOT) and three acidic (sulphonphthalein)) dyes; namely phenol red (PhR),cresol red(CR) and metanil yellow (MY) producing of a yellow colored ion-associates followed by their extraction with methylene chloride and measured at 396 nm,394 nm and 408 nm respectively. After optimization, Beer's law was obeyed in the concentration ranges 6.3-56.47 µg/mL, 3.16-41.42 µg/mL, and 3.12-28.18 µg/mL for methods A, B and C respectively. The molar absorptivityand Sandell sensitivity of the reaction produts were calculated. The results are well compared to those obtained by official method using students t-and F tests.

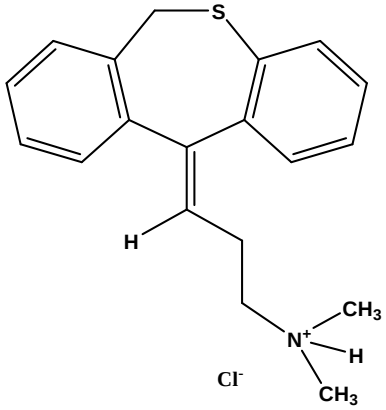


## KEYWORDS

Dothiepin hydrochloride;
Phenol red;
Cresol red;
Metanil yellow and post-mortem urine and blood sample.

## INTRODUCTION

Dothiepin (Dosulepin) hydrochloride is a tricyclic antidepressant with a noticeable action. It is chemically designated as 3(6H)dibenzo[b,e]thiepin-11-ylidene) propyldimethylamine hydrochloride[1-3]. It is indicated in the treatment of depression and anxiety. Several analytical methods described for the determination of Dothiepin (Dosulepin) hydrochloride in biological and pharmaceutical samples involve aspectrophotometric[4-10], a number of high-performance liquid chromatographic (HPLC)[11-16], GC–MS[17-19], LC–MS[20-21], potentiometric[22], Conductimetric[23] and




$C_{19}H_{21}NS.HCl$ 331.9

**Structures of Dothiepin Hydrochloride (DOT)**

capillary electrophoresis[24].

The present study describes simple, sensitive and economical spectrophotometric methods for the analysis of Dothiepin in pure, dosage forms and spiked urine and blood samples. The methods based on the methylene chloride soluble ion-associates complexes between the Dothiepin drug (DOT) and some acid dyes (PhR, CR and MY) were reported. Analytical criteria including linearity, sensitivity, precision, accuracy and recovery are discussed.

## EXPERIMENTAL

### Apparatus

The electronic absorption spectral were recorded on SHIMADZU 1601 UV-Vis spectrophotometer equipped with quartz cell of 1 cm optical path length with a resolution of 0.1 nm. The pH measurements of the prepared solutions were adjusted using pH-meter type Consort model P400.

### Materials

Dothiepin hydrochloride (DOT) and Prothiaden capsules (25 mg/cap) were provided from Cairo Pharmaceutical Company (Egypt)

### Reagents and chemicals

All chemicals used were of analytical reagent grade. Chemicals (suppliers) were as follows: Phenol red, Cresol red Metanil yellow, Sodium acetate trihydrate, Disodium hydrogen phosphate, Sodium hydroxide, Hydrochloric acid and Acetic acid are products of Merck chemical company while Sodium sulphate anhydrous is a product of BDH chemical company. The common solvents as Chloroform (Lab-Scan product), Methylene chloride (BDH product), Carbon tetrachloride, Benzene (Prolabo product), Petroleum ether, Toluene, n-Hexane and Cyclohexane (Merck products).

### Preparation of buffer solution

Acetate buffer solutions of pH values (3.0–6.0) were made from a mixture of 0.1 M acetic acid (1050 g/L) and 0.1 M sodium acetate trihydrate (13.6 g/L). Phosphate buffer solutions of pH values (7.0–11) were made from a mixture of 0.1 M disodium hydrogen phosphate (14.2 g/L), 0.1 M HCl and 0.1 M NaOH.

### Procedure for calibration curve

Into 125 ml separating funnel, 5.0 mL of reagents CR, MY ($1.0 \times 10^{-3}$ M) and PHR ($5.0 \times 10^{-3}$ M) were added to different volumes of solution containing ($1.0 \times 10^{-3}$ M) of DOT respectivelyand 3.0 mL of buffer solution were added and the volume was made up to 10 mL with bidistilled water. The yellow formed ion–associates were extracted by separating funnel with 10 mL methylene chloride by shaking for two minutes and allowed to separate into two layers. Filter the organic layer over anhydrous sodium sulphate, then completed to 10 mL with the same solvent. The absorbance of the extract was measured at the recommended wavelength ($\lambda$max) against a blank (treated in the same way in absence of the examined drug).

### Procedure for the assay of pharmaceutical capsules

For the analysis of DOT in [Prothiaden capsules 25 mg/cap] and, five content of capsules were weighed into a small dish, powdered and mixed well, then dissolved in 100 mL bidistilled water, a turbid solution was shaken well and filtered through a filter paper to obtain a clear solution. Then, the clear solution was diluted with bidistilled water in a 100 mL calibrated measuring flask. Successive dilutions were prepared for carrying out the subsequent studies. The drug content of these solutions was obtained by applying the general procedure to aliquot containing different volumes of drug solution as described above.

### Procedure for post-mortem urine

Five milliliters of post-mortem urine free from investigated drug taken in a 125 ml separating funnel was spiked with different volumes of solution containing ($1.0 \times 10^{-3}$ M) of DOT then 3.0 mL of buffer solution were added and the volume was made up to 10 mL with distilled water. The drug content of these solutions was obtained by applying the general procedure to aliquot containing different volumes of drug solutions as descried above.

### Procedure for post-mortem blood

Five mL of investigated drugs free post-mortem blood taken in a 125 mL separating funnel was spiked with different volumes of solution containing ($1.0 \times 10^{-}$

Full Paper

$^{3}$ M) DOT then 3.0 mL of buffer solution were added and the volume was made up to 10 mL with bidistilled water followed by 10 ml of methylene chloride. The content was shaken for 5 min. The organic layer was collected and dried with anhydrous sodium sulphate and transferred into a dried beaker and evaporated on a hot water bath, the residue was dissolved in 1.0 mL of acetone and 3 mL of buffer solution and the volume was complete to 10 mL with distilled water. An aliquot of resulting solution was analyzed following the procedures described above, using blood treated by the same way without adding the substance as a blank.

### Procedure for stoichiometric relationship

Job's method of continuous variation was employed; 2.0 x $10^{-3}$ M solutions of investigated drugs were mixed with 2.0 x $^{10-3}$ M solution of each selected reagent in which the total volume of drug and reagent was kept constant[25]. The reagents were mixed with each drug in various proportions along with the chosen buffer solution, which then diluted in calibrated flask with the appropriate solvent and the method was accomplished as previously illustrated in the general procedure.

## RESULTS AND DISCUSSION

In proposed methods, some variables in the reaction conditions were studied and the influence of these variables on the reaction was tested.

### Selection of wavelength

The absorption spectra for DOT ion-associates with PhR, CR and MY reagents show a maximum at 396nm, 394 nm and 408 nm respectively (Figure 1). The wavelengths maximum absorbencies (λmax) of the drug-coloring reagent ion-associates were recorded and tested against reagent blanks (prepared in the same manner without the addition of drug).

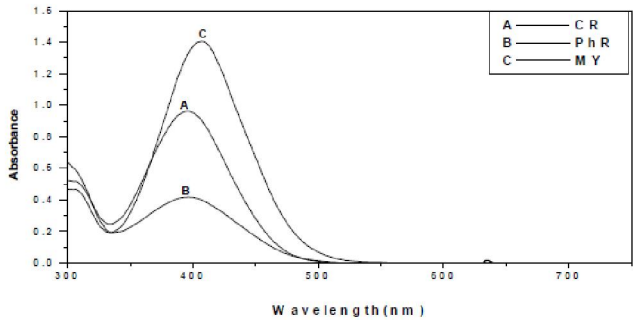



**Figure 1 : Absorption spectra of DOT ion-associates with CR, PhR and MY**

### Effect of pH

The effect of pH was studied using different buffer systems such as phosphate and acetate buffers. The maximum color intensity was observed in the pH ranges (3.0-4.0), (3.0-4.0), and (2.0-4.0) for DOT ion associates with CR, PhR and MY, respectively as shown in Figure (2).

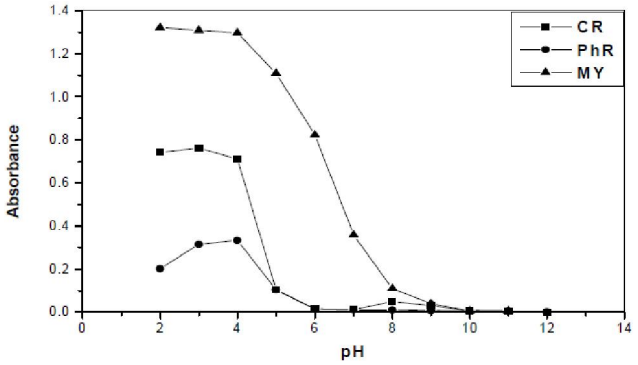



**Figure 2 : Effect of pH on DOT ion-associates with CR, PhR and MY**

### Selecting of extracting solvents

Several water-immiscible organic solvents including benzene, toluene, carbon tetrachloride, methylene chloride, petroleum ether, chloroform, cyclohexane and n-hexane had been checked to select the most convenient solvent of the highest absorbance. It was found that methylen chloride is the most suitable solvent for the extraction of drug- coloring reagent ion - associates in all cases as shown in (Figure 3).

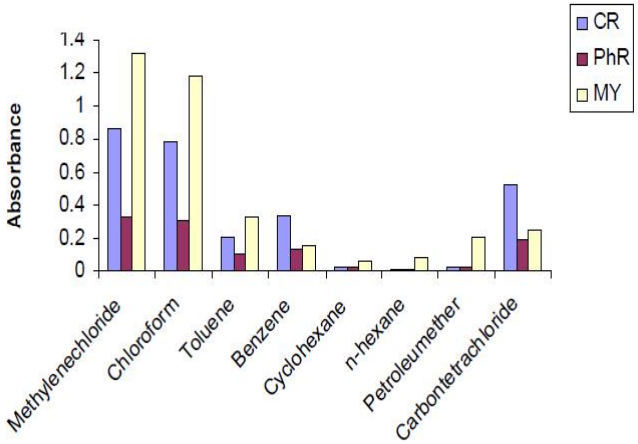



**Figure 3 : Effect of extracting solvents on DOT ion-associates with CR, PhR and MY**

## Effect of dye concentration

The effect of dye concentration on the intensity of the color developed atselected wavelengths was tested using different milliliters of the reagent. The results showed that 4.0 mL of 1 x$10^{-3}$ M of CR, MY, and 5.0 mL of 5 x$10^{-3}$ M PHR reagent, were found to be optimum for these proposed methods and excess of these dyes do not affect the color of the complex or the absorbance as shown in (Figure 4).

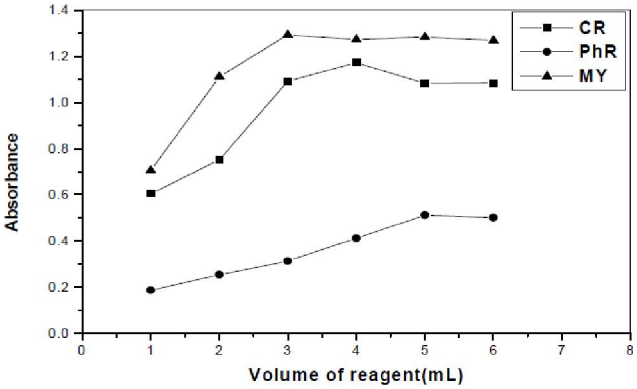



**Figure 4 : Effect of Reagent concentration on DOT ion-associates with CR, PhR and MY**

## Effect of sequence of addition

Several different possible sequences of addition were studied to select the most suitable one for developing the most stable concerned ion-associates showed the highest absorptivity. The best sequence of mixing was "drug-reagent-buffer-solvent" for the highest absorbance and stability. Other sequences needed longer time in addition to lower stability.

## Effect of time

The effect of time on the formation of the ion-associates was studied by measuring absorbance of the extracted ion-associates at increasing time intervals. The results showed that the ion-associates were formed almost instantaneously and the developed color remained stable for several hours as shown in and the developed color remained stable for several hours which are 10, 12and 14 for DOT ion associates with CR,PhR and MY respectivety. After these intervals, a decrease in color intensity occurred. The effect of time on the stability of the ion–associates is represented graphically in (Figure 5).

## Effect of temperature

The effect of temperature on ion-associates formation was studied at temperature range 25-100°C. The results showed that the ion-associates were formed almost instantaneously in all cases at room temperature 25+ 5 °C and remained constant up up to 55°C for all reagents. The effect of temperature on the stability of ion–associates is shown in (Figure 6).

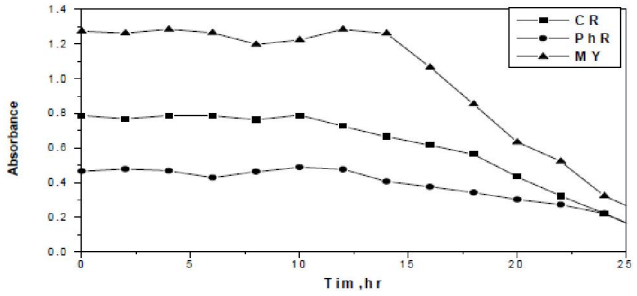



**Figure 5 : Effect of time on DOT ion-associates with CR, PhR and MY**

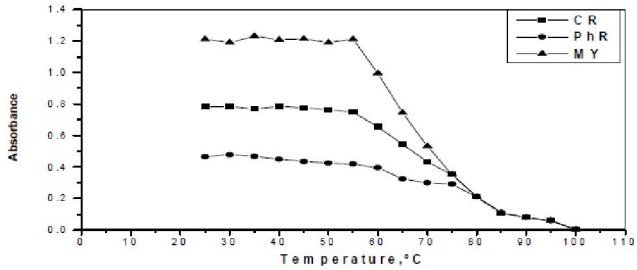



**Figure 6 : Effect of temperature on DOT ion-associates with CR, PhR and MY**

## The composition of ion-associates complex

The stoichiometry of the ion-associates formed between investigated drugs and reagents has been determined by implementing the molar ratio method and continuous variation method[25,26]. It was found that the results obtained from molar ratio results matched with those obtained from continuous variation results which confirm the obtained stoichiometry of the reactions by both methods. Results showed that the existence of 1:1 incase of PHR and MY and found 2:1(drug: reagent) in case of CR with DOT as shown in (Figure 7, 8).

## Conditional stability constant ($k_f$) of the ion-associates

Theconditional stability constant ($k_f$) of the ion-association complex was calculated from the continuous variation data using the following equation:

$$k_f == \frac{A / Am}{[1- (A / Am)]^{n+2} C_M (n)^n}$$

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Where A and $A_m$ were the observed maximum absorbance and the absorbance value when all the drug is associated, respectively[27]. $C_M$ is the mole concentration of the drug at the maximum absorbance and n is the stoichiometry which CR, PhR and MY ion associates with DOT. The log $k_f$ values were found 3.862, 3.953 and 4.378 for DOT ion associates with CR, PhR and MY, respectively. From the results the obtained ion associates complex is high stability

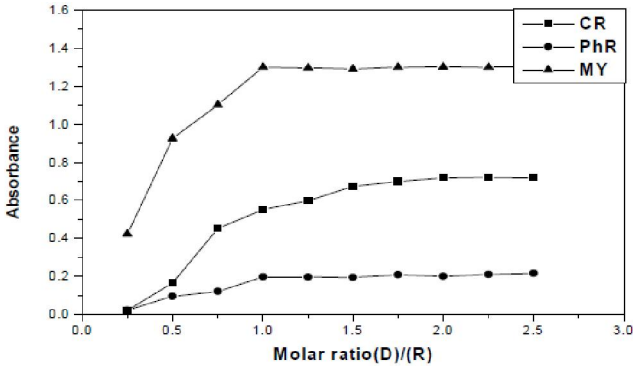


**Figure 7 : Molar ratio of DOT ion-associates with CR, PhR and MY**

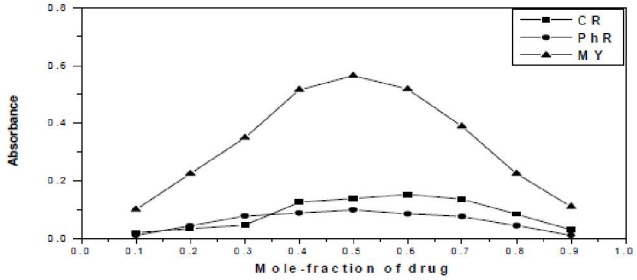


**Figure 8 : Continuous variation of DOT ion-associates with CR, PhR and MY**

## Influence of foreign ions

No significant interference was observed in the determination of DOT with reagents: CR, PhR and MY from the presence of excipients commonly used such as glucose, lactose, starch, sucrose, magnesium stearate, methyl paraben and propyl paraben.

## Method validation

- **Linearity of the calibration curves**

Under the experimental conditions described, calibration graphs were obtained for each proposed methods (Figure 9). The calibration graph in each case is described by the equation: A = a + bc where A= absorbance, a = intercept, b = slope and c = concentration in µg/mL, Correlation coefficient, intercept

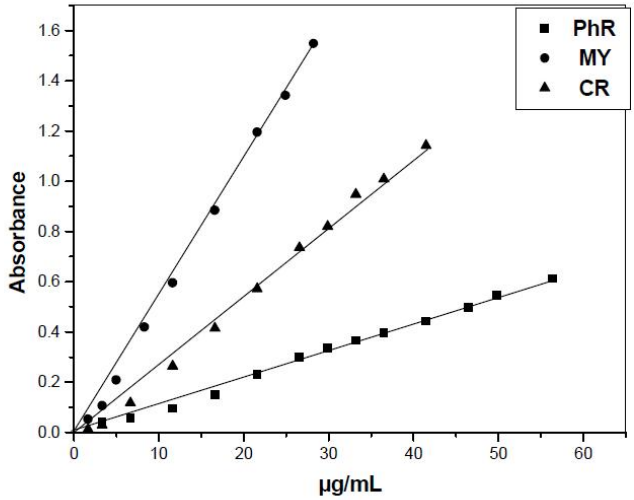


**Figure 9 : Standard curves of DOT ion-associates with PhR, MY and CR**

and slope for the calibration data are summarized in TABLE 1. Also molar absorpitiveties (Є) Sandell's sensitivity were evaluated and recorded in TABLE 1.

For more accurate analysis Ringbom optimum concentration ranges were determined by plotting log [drug] in µg/mL against percent transmittance from which the linear portion of the curve gives accurate range of determination (TABLE 1).

The detection limit **(**LOD) and the limit of quantitation, (LOQ) for the proposed method were calculated using the following equations

$$\text{LOD} = \frac{3\,S}{k}$$

**TABLE 1 : Characteristics and analytical data of (DOT) ion-associates with PhR, CR and MY**

| Parameter | DOT-PHR | DOT-CR | DOT-MY |
|---|---|---|---|
| $\lambda_{max}$ (nm) | 396 | 394 | 408 |
| Beer's law range (µg $mL^{-1}$) | 6.3-56.47 | 3.16-41.42 | 3.12-28.18 |
| Molar absorpitivity (ε) (L $mol^{-1}$ $cm^{-1}$) x $10^4$ | 0.355 | 0.878 | 1.77 |
| Ringbom range(µg $mL^{-1}$) | 6.63-56.2 | 3.3-41 | 3.31-24.8 |
| Sandell sensitivity(µg $cm^{-2}$) | 0.09 | 0.026 | 0.018 |
| Limit of detection(µg$mL^{-1}$) | 2.3 | 1.92 | 0.89 |
| Limit of quantification (µg$mL^{-1}$) | 6.2 | 5.81 | 2.937 |
| Intercept | -0.0171 | -0.0798 | -0.0643 |
| Slope | 0.01126 | 0.02986 | 0.05721 |
| Correlation Coefficient | 0.99806 | 0.99818 | 0.9996 |

**TABLE 2 : The intra-day and inter-day precision and accuracy to determine (DOT) by the proposed methods**

| Method | Intra-day | | | | | Inter-day | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Added µg mL$^{-1}$ | Recovery %[a] | Precision RSD% | Accuracy Er% | Confidence limit[b] | Recovery % | Precision RSD% | Accuracy Er% | Confidence limit[b] |
| DO-PhR | 15 | 99.55 | 0.37 | -0.45 | 14.93 + 0.06 | 99.58 | 0.51 | -0.51 | 14.93+ 0.08 |
| | 30 | 100.73 | 0.56 | 0.74 | 30.22 + 0.19 | 99.36 | 0.33 | -0.64 | 29.81 + 0.12 |
| | 35 | 101.68 | 0.17 | 1.68 | 35.59 + 0.19 | 99.71 | 0.60 | -0.82 | 34.71+ 0.23 |
| | 40 | 101.87 | 0.29 | 1.8 | 40.75+ 0.13 | 99.10 | 0.45 | -0.9 | 39.64+ 0.20 |
| DO-CR | 5 | 100.20 | 1 | 0.2 | 5.01 + 0.05 | 98.40 | 0.81 | - 1.6 | 4.92 + 0.045 |
| | 10 | 98.40 | 0.6 | -1.4 | 9.84 + 0.067 | 98.90 | 0.47 | - 1.1 | 9.89 + 0.08 |
| | 15 | 100.93 | 0.66 | 0.94 | 15.14 + 0.12 | 98.86 | 0.47 | - 1.13 | 14.83 + 0.08 |
| | 20 | 99.20 | 0.25 | -0.80 | 19.84+ 0.056 | 100.75 | 0.39 | 0.75 | 20.15+ 0.09 |
| DO-MY | 8 | 98.87 | 0.3 | -1.12 | 7.91 + 0.025 | 98.75 | 0.45 | -1.2 | 7.90 + 0.04 |
| | 12 | 100.33 | 0.49 | 0.33 | 12.04 + 0.067 | 99.00 | 0.42 | - 1 | 11.88 + 0.056 |
| | 18 | 99.33 | 0.27 | 0.66 | 17.88+ 0.05 | 99.34 | 0.20 | - 0.67 | 17.88+ 0.04 |
| | 24 | 101.50 | 0.77 | 1.5 | 24.36 + 0.21 | 102.37 | 0.61 | 2.3 | 24.57 + 0.16 |

**n, number of determination, R.S.D. %, percentage relative standard deviation, Er%, percentage error.; [a] mean of five determination; [b] confidence limit at 95% confidence level and five degrees of freedom**

**TABLE 3 : Spectrophotometric determination of (DOT). Prothiaden capsules (25 mg/cap)**

| reagent | Pure solution | | | Prothiaden capsule 25 mg / cap | | | Urine samples | | | Blood samples | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Taken µg mL$^{-1}$ | found µg mL$^{-1}$ | Recovery %[a] | Taken µg mL$^{-1}$ | found µg mL$^{-1}$ | Recovery %[a] | Taken µg mL$^{-1}$ | found µg mL$^{-1}$ | Recovery %[a] | Taken µg mL$^{-1}$ | found µg mL$^{-1}$ | Recovery %[a] |
| PhR | 20 | 20.13 | 100.65 | 20 | 19.88 | 99.40 | 20 | 19.83 | 99.45 | 20 | 19.64 | 98.20 |
| | 25 | 25.26 | 101.04 | 25 | 25.17 | 100.68 | 25 | 24.85 | 99.40 | 25 | 24.43 | 97.72 |
| | 32 | 32.64 | 102.00 | 32 | 32.20 | 100.62 | 32 | 31.68 | 99.00 | 32 | 31.43 | 98.21 |
| | 45 | 45.71 | 101.58 | 45 | 44.50 | 98.88 | 45 | 44.75 | 99.44 | 45 | 44.42 | 98.71 |
| | Mean recovery ± RSD* | | | Mean recovery ± RSD* | | | Mean recovery ± RSD* | | | Mean recovery ± RSD* | | |
| | 101.58 ± 0.22 | | | 99.89 ± 0.33 | | | 99.32 ± 0.23 | | | 98.21 ± 0.65 | | |
| CR | 6 | 6 | 100.00 | 6 | 6.04 | 100.66 | 6 | 5.91 | 98.50 | 6 | 5.73 | 95.50 |
| | 12 | 11.89 | 99.08 | 12 | 12.12 | 101.00 | 12 | 11.88 | 99.00 | 12 | 11.73 | 97.75 |
| | 18 | 18.12 | 100.66 | 18 | 9.16 | 100.84 | 18 | 17.88 | 99.34 | 18 | 17.64 | 98.00 |
| | 25 | 25.14 | 100.56 | 25 | 12.15 | 103.00 | 25 | 24.87 | 99.49 | 25 | 24.52 | 98.08 |
| | Mean recovery ± RSD* | | | Mean recovery ± RSD* | | | Mean recovery ± RSD* | | | Mean recovery ± RSD* | | |
| | 100.07 ± 0.48 | | | 101.37± 0.37 | | | 99.08± 0.33 | | | 97.33± 1.83 | | |
| MY | 10 | 10.10 | 101.00 | 10 | 9.90 | 99.00 | 10 | 9.84 | 98.40 | 10 | 9.54 | 95.40 |
| | 15 | 15.12 | 100.80 | 15 | 14.90 | 99.34 | 15 | 14.84 | 98.93 | 15 | 14.63 | 97.53 |
| | 20 | 20.12 | 100.60 | 20 | 19.88 | 99.40 | 20 | 19.90 | 99.50 | 20 | 19.54 | 97.70 |
| | 25 | 25.12 | 100.48 | 25 | 25.12 | 100.48 | 25 | 24.74 | 98.96 | 25 | 24.61 | 98.44 |
| | Mean recovery ± RSD* | | | Mean recovery ± RSD* | | | Mean recovery ± RSD* | | | Mean recovery ± RSD* | | |
| | 100.73± 0.40 | | | 99.55± 0.27 | | | 98.94± 0.20 | | | 97.26± 1.31 | | |

**[a]Mean of six determination, RSD%, percentage relative standard deviation.**

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$$LOQ = \frac{10S}{k}$$

Where S is the standard deviation of replicate determination values under the same conditions as the sample analysis in the absence of the analyte and k is sensitivity, the slope of the calibration graph. The detection limits were calculated and recorded in TABLE 1 and the results indicate the reasonably high sensitivity of the method. Also the linear equations for absorbance versus concentration, together with the correlation coefficients, indicate excellent linearities.

- **Precision and accuracy**

The precision of the proposed methods was calculated in terms of intermediate precision (intra-day and inter-day). Four different concentrations of DOT were analyzed in five replicates during the same day (intra-day precision) and five consecutive days (inter-day precision).

Percentage relative standard deviation (R.S.D. %) values of intra-day and inter-day found low values indicating good precision and reproducibility (repeatability) of the proposed methods (TABLE 2).

Percentage relative error (Er %) as accuracy of the suggested methods was calculated using the following equation:

$$Er\ \% = \left[\frac{Found - added}{Added}\right] X100$$

The results obtained are compiled in (TABLE 2) and show that the accuracy was good. The average percent recoveries were in range (98.40%- 102.37%) indicating good accuracy of the proposed methods.

- **Analytical applications**

Six replicate determinations, using reported coloring reagents at different concentration ranges, were carried out for pure DOT Prothiaden capsules (25 mg/cap) and their spiked urine samples. The overall recoveries are in the range (98.40-102.00%) reflecting a high accuracy of the results (TABLE 3).

The mean values obtained and the calculated standard deviations are compared with those obtained by the official methods[28] and by applying the t- and F-tests[29,30]. It was found that there are no significance difference between proposed and the official methods

**TABLE 4 : Statistical treatment of data obtained for determination of (DOT) applying the proposed methods in comparison with the reference methods**

| Parameters | Official method | DOT-PhR | DOT-CR | DOT-MY |
|---|---|---|---|---|
| Pure solution | | | | |
| 15 µg m L-1 | | 100.25±0.11 | 100.42±0.17 | 100.33±0.13 |
| X + SD | 100.01 ± 0.228 | 6 | 6 | 6 |
| n | 6 | 0.81 | 0.84 | 0.91 |
| t-value* | | 4.3 | 1.8 | 3.07 |
| F-value | | | | |
| Prothiaden capsules 25 mg / cap | | | | |
| 15 µg mL-1 | | 99.80 ± 0.107 | 100.32 ± 0.12 | 99.60±0.17 |
| X + SD | 99.93 ± 0.23 | 6 | 6 | 6 |
| n | 6 | 0.91 | 1.14 | 0.15 |
| t-value* | | 4.62 | 3.38 | 1.83 |
| F-value | | | | |
| Urine | | | | |
| 15 µg m L-1 | | 99.13± 0.13 | 99.46 ± 0.15 | 99.13 ± 0.14 |
| X + SD | 98.86 ± 0 .10 | 6 | 6 | 6 |
| n | 6 | 0.72 | 1.46 | 0.7 |
| t-value* | | 1.82 | 2.25 | 1.96 |
| F-value | | | | |
| Blood samples | | | | |
| 15µg m $L^{-1}$ | | 97.60 + 0 .19 | 96.80 + 0 .11 | 96.94 + 0 .25 |
| X ± SD | 97.00 ± 0 .17 | 6 | 6 | 6 |
| n | 6 | 1.3 | 0.43 | 0.14 |
| t-value* | | 1.24 | 2.38 | 2.16 |
| F-value | | | | |

***: theoretical value at 95% confidence level; n: number of replicates**

(TABLE 4). So the present methods are accurate, precise, highly sensitive, rapid, and simple and their results are in good agreement with those of the official methods.

## CONCLUSION

The proposed methods for the determination of DOT with different reagents (CR, PhR and MY) were successfully applied to determination of investigated drug in pure, dosage forms and spiked urine samples. The results are compared statistically with the official methods. High recoveries, accuracy, in addition to the high precision indicated by very low values of relative standard deviations have been achieved. Also these methods, less time consuming and need simple reagents which are available, thus offering an economic method for routine determination of the cited drugs.

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