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**Der Pharmacia Lettre, 2018, 10 [8]: 17-32**
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# Spectrophotometric Microdetermination of Methyldopa and Etilefrine Hydrochloride using Copper (II)-Neocuproine Reagent in Pure Form and Pharmaceutical Formulations

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## ABSTRACT

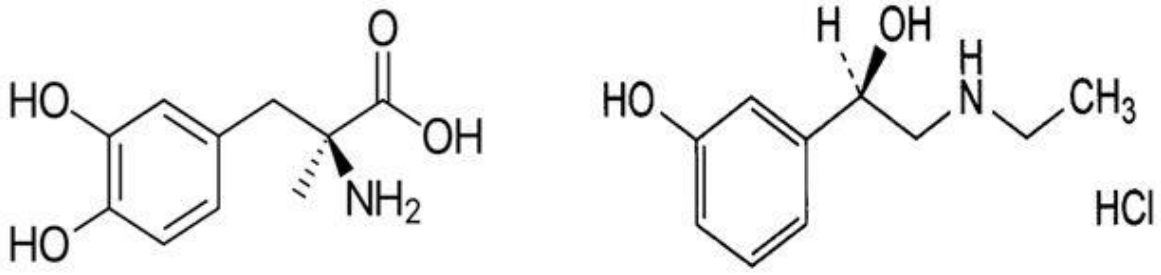
*This study characterizes a newly developed simple, rapid, sensitive and cost effective spectrophotometric method for the microdetermination of Methyldopa and Etilefrine hydrochloride in pure form and pharmaceutical formulations. The developed method is based on the reaction of copper (II) with studied drugs in the presence of neocuproine (NC) (2,9-dimethyl-1,10-phenanthroline) reagent. Copper (II) is reduced easily by both drugs to Cu (I)–neocuproine chelate, which shows an absorption maximum at 455 nm. Results showed that percentage recoveries for the suggested method were 99.822 ± 0.797 and 100.027 ± 0.469 within the concentration ranges of 0.4-3.6 and 1.2-6.8 µg/mL with a minimum detection limit (LOD) of 0.115 and 0.348 µg/mL and a quantification limit (LOQ) of 0.348 and 1.086 µg/mL for Methyldopa and Etilefrine hydrochloride respectively. The suggested method was applied successfully for determination of both drugs in pure form and pharmaceutical formulations without interference of common pharmaceutical excipients or additives with the assay results.*

**Keywords:** Methyldopa, Etilefrine HCl, Copper-neocuproine reagent, Spectrophotometry, Pharmaceutical formulations.

## INTRODUCTION

Methyldopa is a catechol derivative widely used for treatment of hypertension. It is a centrally acting a-2-adrenoceptor agonist, which decreases sympathetic tone and induces a fall in blood pressure [1]. Various methods have been published for estimation of methyldopa in pharmaceutical formulations, including high-performance liquid chromatography (HPLC) [2-5], titrimetry [6], visible spectrophotometry [7-11], kinetic measurements [12,13], flow injection analysis [14], voltammetry [15] and chemiluminescence [16,17] (Figure 1). Etilefrine hydrochloride is a direct-acting sympathomimetic with beta1-agonist properties, and some alpha-and beta2-agonist actions, used for the treatment of hypotensive states [18]. Etilefrine hydrochloride is official in British pharmacopeia [19] where it was determined by non-aqueous titration using perchloric acid and the end-point was determined potentiometrically (Figure 1).

Different methods were published for the determination of etilefrine hydrochloride including, spectrophotometry [20-26], Flow-injection spectrophotometry [27] spectroflourimetry [28] and flow-injection chemiluminometric assay [29]. Also an HPLC method has been reported [30].




**Methyldopa Etilefrine HCl**

**Figure 1:** The chemical structures of Methyldopa and Etilefrine HCl.

Spectrophotometric methods for the determination of some reductants. This reagent have been used for estimation of cysteine [31], trace amounts of reducing agents [32], vitamin E [33], isoniazide [34], ascorbic acid [35], some proton pump inhibitors [36], ceftazidime [37] and captopril [38]. Spectrophotometry is the most convenient analytical technique for routine analysis because of its simplicity, low cost and wide availability in routine quality control laboratories. The spectrophotometric methods previously published for the determination of methyldopa and etilefrine hydrochloride suffer from some disadvantages such as

low sensitivity, complex procedures and time consumption. In this paper new simple, accurate, reproducible, and sensitive spectrophotometric method have been developed for the determination of the cited drugs in pure form and pharmaceutical formulations that overcome these drawbacks. The mechanism of reaction is that these drugs, as reducing agents, enhance reduction of Cu (II), followed by treating the Cu (I) with chromogenic reagent neocuproine.

## MATERIALS AND METHODS

***Chemicals and reagents***

- A stock solution ($1 \times 10^{-3}$ M) of copper sulphate (El- nasr Co., Egypt) was prepared by dissolving 0.159 gm in distilled water in a 100 mL volumetric flask and diluting to the mark with the same solvent.
- A stock solution ($5.0 \times 10^{-3}$ M) of neocuproine hemihydrate (Merck) was prepared by dissolving 0.054 g in ethanol in a 50 mL volumetric flask and diluting to the mark with the same solvent. Solution should be stored in cool place; it was stable for two weeks.
- Hydrochloric acid and ethanol were purchased from El-Nasr Co. (Egypt).
- Methyldopa raw material was obtained from Pharco Pharmaceuticals Inc. (Alexandria, Egypt).
- Etilefrine hydrochloride raw material was obtained Chemical Industrial Development (CID), Egypt.

***Pharmaceutical dosage forms***

- Aldomet ® tablets contain 250 mg methyldopa per tablet (Pharco Pharmaceuticals Inc., Alexandria, Egypt).
- Effortil® Drops contain 7.5 mg etilefrine hydrochloride per 1 mL solution (Chemical Industrial Development (Cid) under the licence of Boehringer Ingelheim, Germany)

***Instrumentation***

All of the spectrophotometric measurements were carried out using a Shimadzu UV-1800 with matched 1 cm quartz cells (Japan).

***General procedures***

**Preparation of stock and standard working solutions**

**Methyldopa:** Stock solution (1 mg/mL) was prepared by dissolving 25 mg of pure drug in 2 mL of 4 M Hydrochloric acid and dilution to 25 mL in 25 mL volumetric flask by distilled water. Standard working solution (0.01 mg/mL) was prepared by taking 1 mL of the stock solution then dilution to 100 mL with distilled water. Working solution of Methyldopa was stable for one week.

**Etilefrine HCl:** Standard working solution (1 mg/mL) was prepared by dissolving 25 mg of pure drug in 25mL of distilled water. Standard working solution (0.02 mg/mL) was prepared by taking 1 mL of the stock solution then dilution to 50 mL with distilled water. Working solution of Etilefrine HCl was stable for one week.

**Construction of calibration curves:** To the specified volumes of $5.0 \times 10^{-3}$ M neocuproine solution, the specified volumes of $1.0 \times 10^{-3}$ M Cu (II) solution and 0.5 mL acetate buffer solution (pH 5.0) in case of methyldopa where added to aliquots of working solutions, ranging from 0.2 to 1.8 and 0.3 to 1.7 ml of methyldopa only and etilefrine Hydrochloride, respectively in a 10 mL volumetric flask and mixed. The contents were heated in water bath at 90°C for the specified times. After cooling, the mixture was diluted to 5 ml with distilled water. Absorbances of the colored solutions were measured at 455 nm against reagent blank treated similarly (Figure 2).

**Procedure for dosage forms:**

**Aldomet® tablets:** Weigh and finely powder 20 tablets. Extract an accurately weighed portion of the fine powder equivalent to 25 mg of methyldopa with 2 mL 4 M hydrochloric acid by occasional shaking for 10 minutes then make extraction with 2 × 10 ml of double distilled water, completed to 25 ml with double distilled water then filtered. Procedures were completed as in general procedures by applying the standard addition technique.

**Effortil® drops:** A specific volume of drop solution equivalent to 10 mg pure drug were placed in 10 mL volumetric flask and diluted to 10 mL with distilled water to obtain stock solution with concentration of 1 mg/mL. Working solution was prepared by further dilution of the stock solution until reach concentration of 0.02 mg/mL. Procedures were completed as in general procedures by applying the standard addition technique.

## RESULTS AND DISCUSSION

When the copper (II)–neocuproine complex is used as a reagent, an orange-yellow Cu (I)–neocupoine chelate is formed once. The reduction of Cu (II) to Cu (I) by the studied drugs in the presence of neocuproine and subsequent complex formation between Cu (I) and the chromogenic reagent neocuproine need heating at 90ºC for the specified times to ensure complement of the reaction. The absorption spectra of the reaction products of the cited drugs showed maximum absorption at 455 nm but with different absorptivities. Figure 2 shows the absorbance spectra of the reaction products. At the selected wave length, 455 nm, the drug has no absorption and the reagent has very small absorbance.

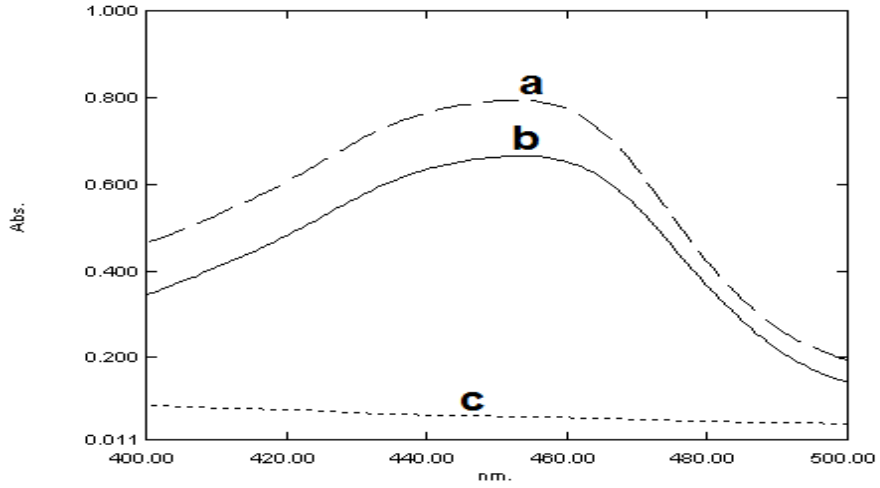



**Figure 2:** Absorption spectra of: (a) 1.8 µg/ml Methyldopa and (b) 5.5 µg/ml Etilefrine HCl against (c) reagent blank. Factors affecting the reaction product formation were optimized.

**Effect of pH of Buffer**

Experimental results show that no need for addition of buffer solution in case of etilefrine HCl as it has no effect on the reaction. In contrast, in case of methyldopa, buffer solution has a great effect on the reaction progression. The effect of pH on the reduction of Cu (II) by methyldopa and formation of Cu (I)–neocuproine complex was studied over the pH range of 3-7 of acetate buffer solutions. The absorbance increases with increasing pH up to pH 5 and after that, by increasing the pH of acetate buffer solution, there were an observed decrease in the obtained absorbances results. So, acetate buffer pH 5 was selected as the optimum pH for the complement of the reaction.

**Volume of Acetate Buffer (pH 5)**

Different volumes of acetate buffer (pH 5) were tried in the range of 0.2-3 mL. It was found that 0.5 mL is the optimum volume for complement of the reaction as shown in Figure 3. It was found that increasing the buffer solution volume did not affect the color intensity.

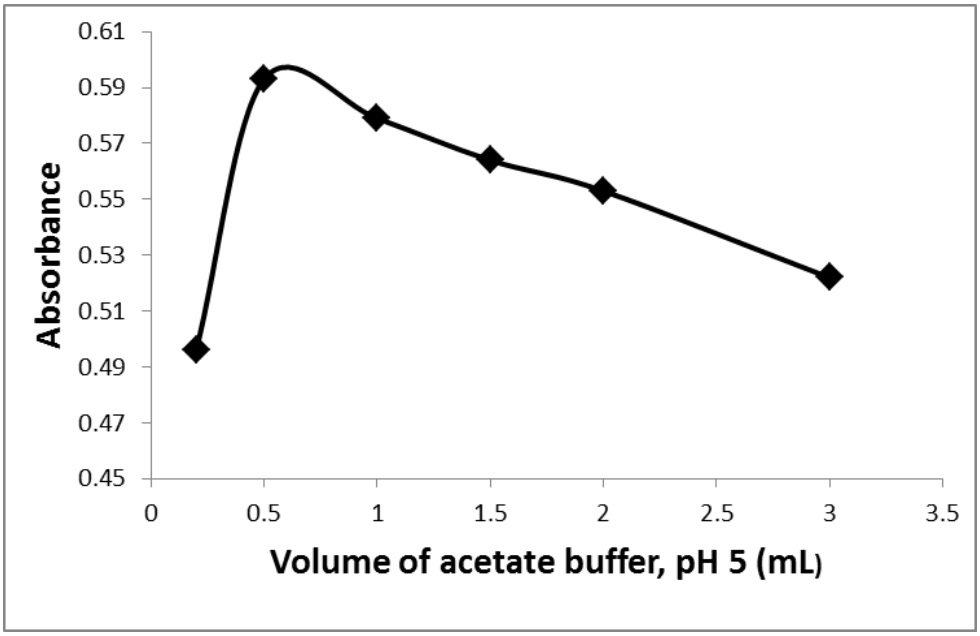



**Figure 3:** Effect of volume of acetate buffer (pH=5) on the absorbance of the formed product in the presence of 2 µg/mL Methyldopa.

**Effect of Cu (II) volume**

The effect of Cu (II) $1 \times 10^{-2}$ M volume on the absorbance was studied in the range of 0.05-1.5 mL. In a solution containing neocuproine, the power of oxidation of Cu (II) is affected by the ease of formation of Cu (I)-neocuproine complex. So, any excess of Cu (II) can exert an affinity for neocuproine, as a result preventing the quantitative formation of the resulted complex. Thus, excess of Cu (II) competes with Cu (I) for complex formation with neocuproine. Results showed that 0.25 and 0.5 mL of $1 \times 10^{-2}$ M Cu (II) solution was the optimum volume for complement of the reaction and reaching maximum color intensity. Results are shown in Figure 4.

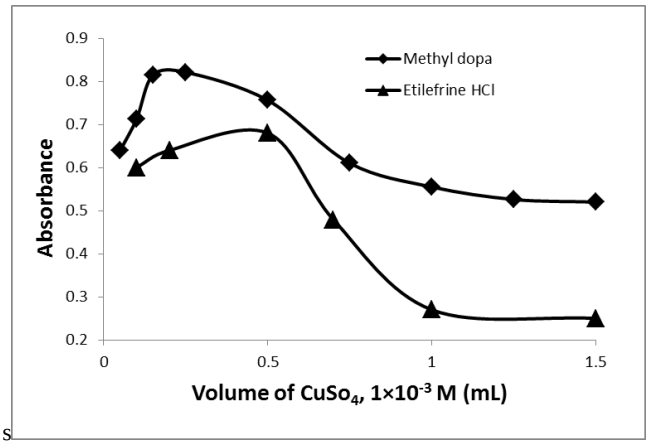



**Figure 4:** Effect of volume of copper sulphate (1 × $10^{-3}$ M) on absorbance of the formed product in the presence of 3 µg/mL for Methyldopa and 5 µg/mL Etilefrine HCl**.**

**Effect of Volume of Neocuproine (5 × $10^{-3}$)**

The effect of neocuproine volume was studied over the range 0.2-3 mL. The results are shown in Figure 5. It was noticed that, the absorbance due to Cu (I)-neocuproine complex decreases at high concentrations of neocuproine. This may be attributed to the fact that high concentrations of neocuproine would result in interference from Cu (II) which could have arisen from incomplete conversion of Cu (I) into the Cu (I)–neocuproine complex [31]. Results showed that 1 and 1.5 mL of 5 × $10^{-3}$ M neocuproine solution was the optimum volume for complement of the reaction and reaching maximum color intensity.

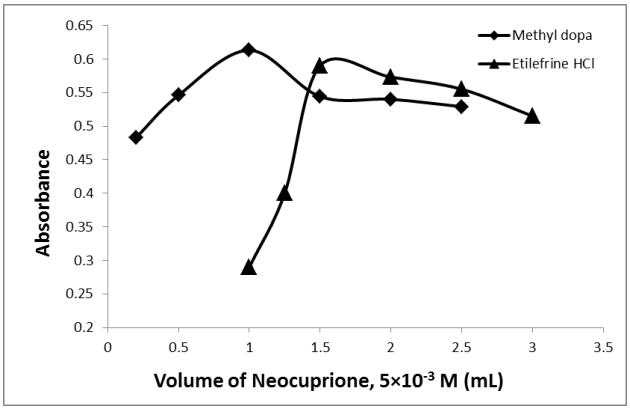



**Figure 5:** Effect of volume of Neocuproine (5 × $10^{-3}$M) on absorbance of the formed product in the presence of 2 µg/mL for Methyldopa and 4 µg/mL Etilefrine HCl

**Effect of time and temperature**

The reaction rate was found to increase with increasing temperature with a subsequent increase in the slope of calibration graph. It was observed that, above 90°C unwanted chemical changes might occur, so 90°C was chosen as the optimum temperature. Complete color development was attained in water bath of 90°C after 30 and 10 minutes for Methyldopa and Etilefrine HCl, respectively (Figures 6 and 7).

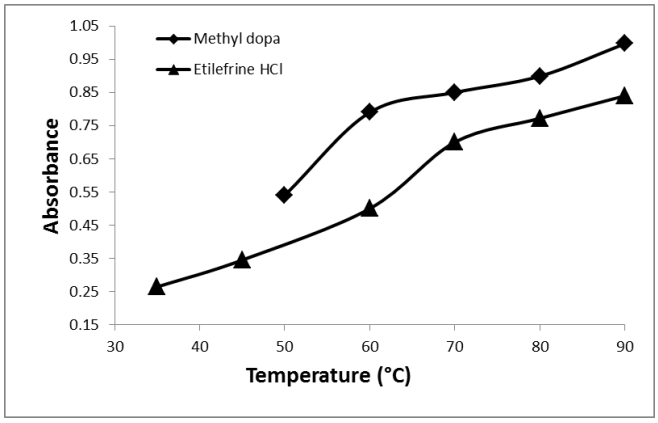



**Figure 6:** Effect of different temperatures on absorbance of the formed product in the presence of 3 µg/mL for Methyldopa and 6 µg/mL Etilefrine HCl respectively.

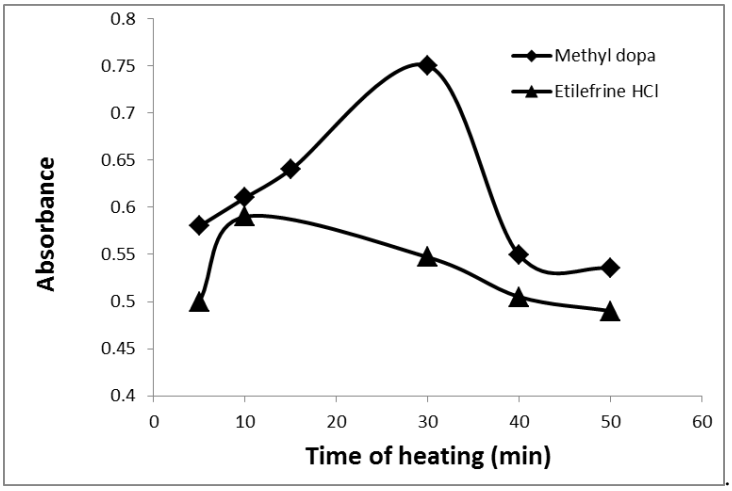



**Figure 7:** Effect of reaction time at 90°C on absorbance of the formed product in the presence of 3 µg/mL for Methyldopa and 3.6 µg/mL Etilefrine HCl.

**Validation of the proposed method**

***Linearity***

Calibration curves for determination of the cited drugs were constructed by plotting absorbance against drug concentrations. (Figure 8) showed that linear calibration curves were obtained in the range of 0.4-3.6 and 1.2-6.8 µg/mL for Methyldopa and Etilefrine HCl, respectively with good correlation coefficients indicating excellent linearity over this ranges. Results were summarized in Table 1.

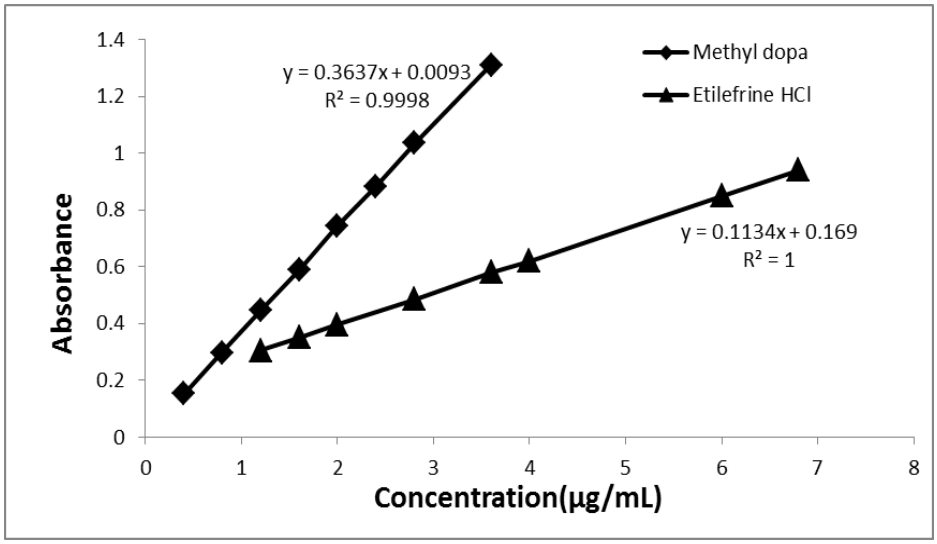



**Figure 8:** Calibration curves for determinationof Methyl dopa and Etilefrine HCl using Copper (II)-neocuproine reagent.

**Table 1:** Analytical parameters for the reaction of Methyldopa and Etilefrine HCl with copper (II)-neocuproine reagent.

| Parameters | Methyldopa | Etilefrine HCl |
|---|---|---|
| Molarity of Copper sulphate | $1 \times 10^{-2}$ M | |
| Vol. of Copper sulphate (mL) | 0.25 mL | 0.5 mL |
| Vol. of neocuprione reagent, $5 \times 10^{-3}$ M (mL) | 1 mL | 1.5 mL |
| Temperature (C˚) | 90°C | 90°C |
| Time of reaction (min.) | 30 min. | 10 min. |
| $\lambda_{max}$, nm | 455 | 455 |
| Beer's law limits ( µg/mL) | 0.4-3.6 | 1.2-6.8 |
| Regression equation* | | |

| | | |
|---|---|---|
| Intercept | 0.0093 | 0.169 |
| Slope | 0.3637 | 0.1134 |
| **Determination coefficient** | 0.9998 | 1 |
| **Note:** *A = a + b C, where C = Concentration of drug in µg/mL, A = Absorbance, a = Intercept, b = Slope | | |

**Detection, and quantitation limits**

The LOD was determined by evaluating the minimum level of the analyte which could be detected while the LOQ was the minimum level of the analyte which could be quantitatively determined with acceptable accuracy and precision. The LOD and LOQ were evaluated using the following equations according to ICH guidelines [39]:

$LOD=3.3\frac{\sigma}{s}$ ; $LOQ=10\frac{\sigma}{s}$ Where, σ = The standard deviation of replicate blank responses and S = The calibration graph slope. LODs and LOQs were calculated and listed Table 2.

**Table 2:** Results of the analysis for determination of Methyl dopa and Etilefrine HCl using Copper (II)–neocuproine reagent.

| **Parameters** | **Methyldopa** | | | **Etilefrine HCl** | | |
|---|---|---|---|---|---|---|
| | **Taken µg/mL** | **Found µg/mL** | **Recovery %** | **Taken µg/mL** | **Found µg/mL** | **Recovery %** |
| | 0.4 | 0.395 | 98.776 | 1.2 | 1.208 | 100.676 |
| | 0.8 | 0.794 | 99.223 | 1.6 | 1.596 | 99.757 |
| | 1.2 | 1.198 | 99.83 | 2 | 2.002 | 100.088 |
| | 1.6 | 1.591 | 99.447 | 2.8 | 2.787 | 99.521 |
| | 2 | 2.023 | 101.141 | 3.6 | 3.624 | 100.676 |
| | 2.4 | 2.402 | 100.094 | 4 | 3.977 | 99.427 |
| | 2.8 | 2.82 | 100.721 | 6 | 6.005 | 100.088 |
| | 3.6 | 3.576 | 99.342 | 6.8 | 6.799 | 99.984 |
| Mean | | | 99.822 | | | 100.027 |
| S D | | | 0.797 | | | 0.469 |
| RSD | | | 0.798 | | | 0.469 |
| SE | | | 0.282 | | | 0.166 |
| LOD µg/mL | | | 0.115 | | | 0.357 |
| LOQ µg/mL | | | 0.348 | | | 1.086 |
| Molar Absorptivity L.Mol-1.cm$^{-1}$ | | $7.799 \times 10^4$ | | $3.688 \times 10^4$ | | |
| **Note:** * Average of three different determinations | | | | | | |

**Accuracy**

Accuracy of the measurements of the suggested method was determined using the calibration curves of the cited drugs, where mean percentages of 99.822 and 100.027 for Methyldopa and Etilefrine HCl, respectively, were obtained indicating high accuracy of the method. Results are listed in Table 2.

**Precision**

Intraday precision and inter-day reproducibility were evaluated by calculating relative standard deviations and recoveries of three replicate determinations using two different concentrations of the cited drugs. However, the obtained results by the suggested method were found to be acceptable. Results are listed in Table 3.

**Table 3:** Inter-day and intra-day results for determination of Methyldopa and Etilefrine HCl using Copper (II)–neocuproine. reagent.

| ER% | RSD% | Recovery% ± SD | Found (µg/mL) | Added (µg/mL) | Drug | |
|---|---|---|---|---|---|---|
| -0.226 | 1.318 | 99.774 ± 1.315 | 3.592 | 3.6 | Intraday | Methyldopa |
| -0.725 | 1.479 | 99.275 ± 1.468 | 1.588 | 1.6 | | |
| 1.251 | 1.886 | 101.251 ± 1.909 | 3.645 | 3.6 | Interday | |
| -0.038 | 1.983 | 99.962 ± 1.982 | 1.599 | 1.6 | | |
| 1.195 | 1.29 | 101.195 ± 1.305 | 6.881 | 6.8 | Intraday | Etilefrine HCl |
| -1.773 | 1.247 | 98.227 ± 1.225 | 3.536 | 3.6 | | |
| 1.195 | 1.29 | 101.195 ± 1.305 | 6.881 | 6.8 | Interday | |
| 0.349 | 1.988 | 100.349 ± 1.995 | 3.613 | 3.6 | | |

**Specificity**

The specificity studies revealed that the presence of the additives and common excipients in the tablet dosage forms of the two cited drugs didn't show any kind of impurity interference, since mean recoveries lied in the range of 98.192-101.747 as illustrated in Table 4.

**Table 4:** Application of standard addition technique for determination of Methyldopa and Etilefrine HCl and in their tablets dosage form using Copper (II)-neocuproine reagent.

| **Methyl dopa (Aldomet® Tablets)** | | | **Etilefrine HCl (Effortil® drops)** | | |
|---|---|---|---|---|---|
| **Taken (µg/mL)** | **Added (µg/mL)** | **Recovery %*** | **Taken (µg/mL)** | **Added (µg/mL)** | **Recovery %*** |
| | ..... | 98.192 | 1.2 | …….. | 99.941 |
| | 0.4 | 100.747 | | 0.8 | 99.647 |
| | 0.8 | 101.509 | | 2.4 | 101.411 |
| | 1.2 | 101.141 | | 3.6 | 99.574 |
| | 2 | 101.114 | | 4.8 | 101.558 |
| | 2.4 | 101.363 | | 5.6 | 101.281 |
| 0.8 | 2.8 | 100.029 | | | |
| Mean ± SD | 100.984 ± 0.535 | | 100.569 ± 0.941 | | |
| RSD | 0.529 | | 0.936 | | |
| SE | 0.218 | | 0.421 | | |
| V | 0.286 | | 0.886 | | |
| **Note:** *mean of three different experiments | | | | | |

**Statistical analysis of the pharmaceutical formulation**

Aldomet® tablets and Effortil® drops have been successfully analyzed by the suggested method. Results obtained were compared to those obtained by applying comparison methods [11,19] where Student's t-test and F-test were performed for comparison. Results obtained showed that the calculated t and F values were less than tabulated values at p= 0.05, which indicate that there is no significant difference between suggested method and comparison methods relative to precision and accuracy. Results are illustrated in Table 5.

**Table 5:** Statistical analysis of results obtained by the proposed method applied on Aldomet® tablets and Effortil® drops tablets compared with reported methods.

| Parameters | Aldomet ® tablets | Reference method [11] | Effortil® drops | Reference method [19] |
|---|---|---|---|---|
| Mean Recovery | 100.984 | 100.33 | 100.569 | 100.06 |
| Variance | 0.286 | 1.102 | 0.886 | 0.429 |
| ± S.D. | 0.535 | 1.05 | 0.941 | 0.655 |
| ± R.S.D. | 0.529 | 1.046 | 0.936 | 0.655 |
| ± S.E. | 0.218 | 0.469 | 0.421 | 0.267 |
| Student-t | 1.341 (2.262)[a] | | 1.057 (2.262)[a] | |
| F-test | 3.853 (5.19)[b] | | 2.065 (5.19)[b] | |
| **Note:** A and B are the Theoretical Student t-values and F-ratios at p-0.05. | | | | |

## CONCLUSION

The current method for the determination of Methyldopa and Etilefrine HCl in pharmaceutical formulations has the advantage of simplicity, low cost, high sensitivity, repeatability, and reproducibility. In addition, It is useful for practical quality control analysis of both drugs in pure and in pharmaceutical formulations without interference from common additives.

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