Spectrophotometric determination of Methyldopa in pure form and in the pharmaceutical preparations

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Abstract

A simple, rapid and sensitive spectrophotometric method for determination of methyldopa (MD) using 4-chloro-7-nitrobenzo-2-oxa-1, 3-diazole (NBD-Cl) as reagent in an alkaline medium (pH 12.3). Absorbance of the resulting brown-colored product is measured at 470 nm. Beer's Law is obeyed in a concentration range of (1.6-17.6 µg/mL) with molar absorptivity (1.9337×10^{4} L/mol.cm), correlation coefficient 0.9988, and the limit of detection (5.536×10^{-3} µg/mL). The method has been successfully applied to the determination of Methyldopa in pharmaceutical preparations.

التقدير الطيفي للمركب الدوائي (ميثيل دوبا) بشكله النقي وفي مستحضراته الصيدلانية

قبس ناجي رشيد محسن حمزة بكر شيروان عمر بابان

الخلاصة

استخدمت طريقة بسيطة وسريعة وحساسة في تقدير عقار ميثيل دوبا باستخدام كاشف 4- كلورو - 7- نايتروبنزو- 2- اوكسا-3,1- دايازول في الوسط القاعدي وعند أس هيدروجيني (pH 12.3) لتكوين ناتج بني اللون له اعلى امتصاص عند طول موجي 470 نانوميتر. طبق قانون بير في مدى التراكيز (1.6-17.6 مايكروغرام/مل)، وبامتصاصية مولارية (1.9337×10^{4} لتر/مول.سم)، ومعامل ارتباط 0.9988، وحد كشف (5.536×10^{-3} مايكروغرام/مل). وقد طبقت الطريقة بنجاح في تقدير ميثيل دوبا في المستحضرات الصيدلانية.

Introduction

Methyldopa (MD), is a white powder, odorless, molar mass is 211.215 gm/mol, M.P.=290^{0}C, chemically known as α-methyl-3,4-dihydroxyphenylalanine, is a catechol derivative (catecholamine) widely used as an antihypertensive agent, (or high blood pressure) and gestational hypertension (or pregnancy-induced hypertension) and preeclampsia [(1)].

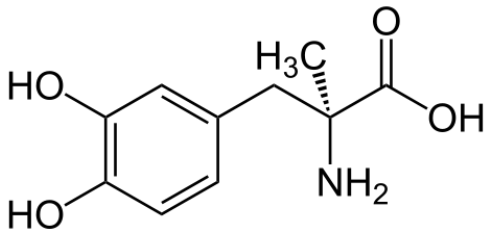

Fig. (1): Chemical structure of methyldopa

Several methods have been proposed for determination of methyldopa in pharmaceutical formulations, including high-performance liquid chromatography (HPLC) with UV detection[(2)], differential pulse polarography[(3)], titrimetry[(4)], UV[(5)] and visible spectrophotometry [(6-11)], flow injection analysis (FIA) [(12,13)], kinetic measurements [(14,15)], anodic voltammetry [(16)] and chemiluminescence [(17,18)]. 4-Chloro-7-nitrobenzo-2-oxa-1,3-diazol (NBD-Cl) has been proved to be a useful and sensitive analytical derivatizing agent for spectrophotometric analysis of pharmaceuticals bearing a primary or secondary amino group [19-21], for the fluorimetric assay of some amines and amino acids [(22)].

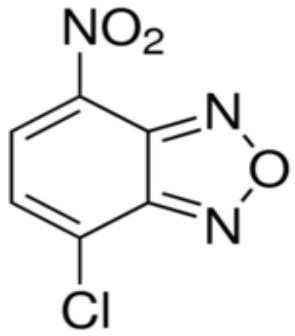

Fig. (2):- Chemical Structure of (NBD-Cl) reagent

Material and Methods

UV-VIS spectrophotometer single beam from A&E Lab (UK) -S60- Series with 1 cm quartz cells, pH meter from (Senz pH tester, China), Balance from Mettler AB 104-S (Switzerland).

Materials

Methyldopa from (SDI Samarra-Iraq), 4-Chloro-7-nitrobenzo-2-oxa-1,3-diazol (NBD-Cl) from (Solarbio), sodium hydroxide (NaOH) from (GCC), Ethanol from (Scharlau).

Solutions

Methyldopa Stock solution (1000 µg/mL): An accurately (0.1 gm) of (MD) standard were dissolved in (100 ml) distilled water.

NBD-Cl (8×10^{-3}M): were prepared by dissolving (0.1596 gm) of NBD-Cl in (100 ml) ethanol.

NaOH (1M): were prepared by dissolving (4 gm) of NaOH in (100 ml) distilled water

Procedure

A 1.0 ml of 200 µg/mL of (MD) were transferred into 25 ml volumetric flask, 1.5 ml of 8×10^{-3}M (NBD-Cl) were added and followed by 1.0 ml of NaOH 1M. After (5 min.), the volume were completed to volume with distilled water, and the resulting solution were measured at 470 nm against reagent blank treated similarly.

Procedure for stoichiometric ratio

The reaction stoichiometry between the studied drug and NBD-Cl has been determined spectrophotometrically by applying molar ratio and continuous variation methods. In the former method, equimolar solutions of (MD) and NBD-Cl (2×10^{-3} M) were used. Different aliquots of NBD-Cl were added to fixed aliquots of drug solution -total volume (25 ml) and the absorbance were measured at 470 nm against the reagent blank treated similarly. While in the latter method, a series of MD-NBD-Cl solutions were kept at (5 ml) (0:5, 0.5:4.5, 1:4, 1.5:3.5, 2:3, …… 5:0). The absorbance of the resulting solutions were measured at 470 nm against the reagent blank treated similarly.

Results and Discussion

Absorption spectra of MD-NBD-Cl system against reagent blank in an alkaline medium at room temperature (25^{o}C) were produced brown colored product which absorbs maximally at 470 nm, the result shown in Figure (3), and reagent blank against ethanol, the result shown in Figure (4).

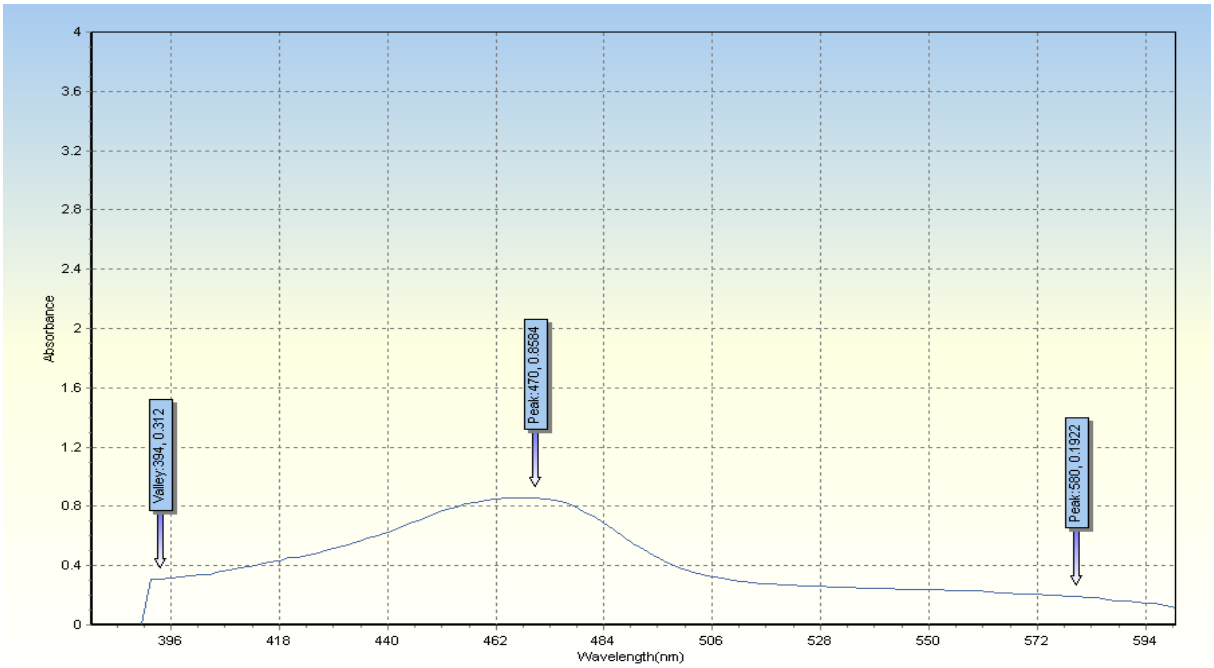

Fig. (3):- Absorption spectrum of MD-NBD-Cl system against reagent

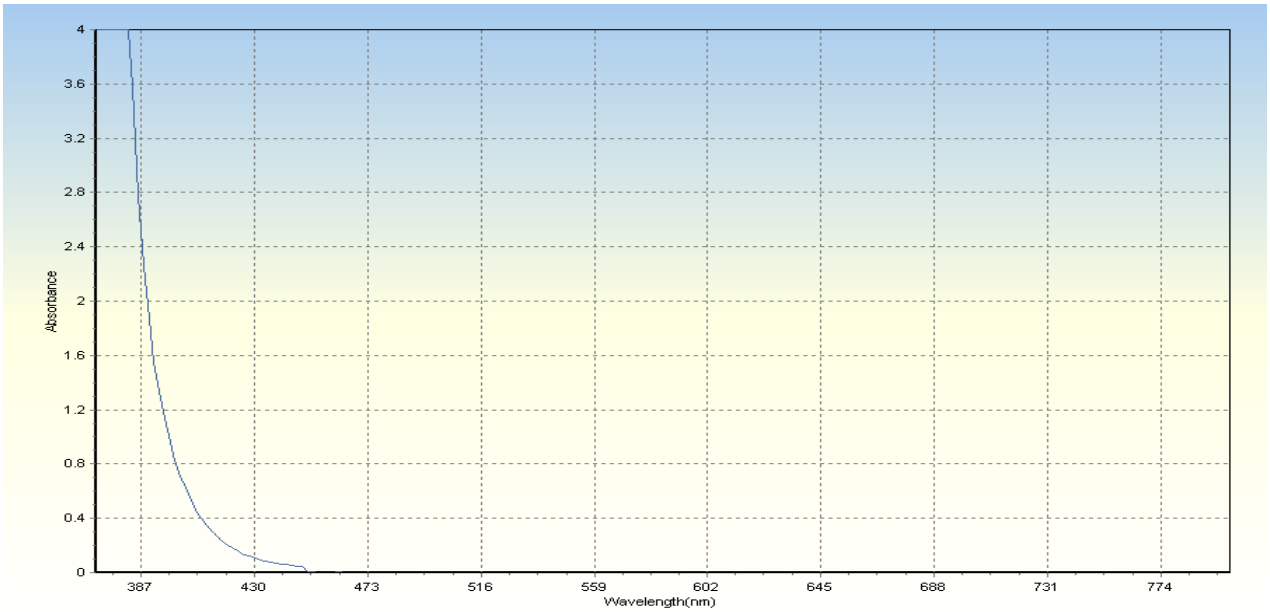

Fig.(4):- Absorption spectrum of reagent blank against ethanol

Optimization of reaction variables

In order to establish optimum experimental conditions, necessary for rapid and quantitative formation of colored product with maximum stability and sensitivity, the effect of various parameters such as volumes of NBD-Cl, in addition of alkaline medium, the reaction time and the stability of colored product were studied at room temperature (25^0C).

Effect of NBD-Cl concentration

The effect of NBD-Cl concentration on the reaction were studied at room temperature (25 ± 5°C). The reaction of (MD) with NBD-Cl were dependent on the concentration of NBD-Cl reagent. So, the reagent concentration in solution were studied by varying the NBD-Cl volume of (8×10^{-3}M) NBD-Cl, while the (MD) concentration were maintained constant at 8 µg/mL. The study revealed that the reaction were dependent on concentration of NBD-Cl reagent. The highest absorption intensity were attained when the volume of NBD-Cl were (1.5 ml, 4.8×10^{-4}M) of (8×10^{-3}M) NBD-Cl, and decrease in the absorbance at volume large than 1.5 ml of NBD-Cl, the result shown in Figure (5).

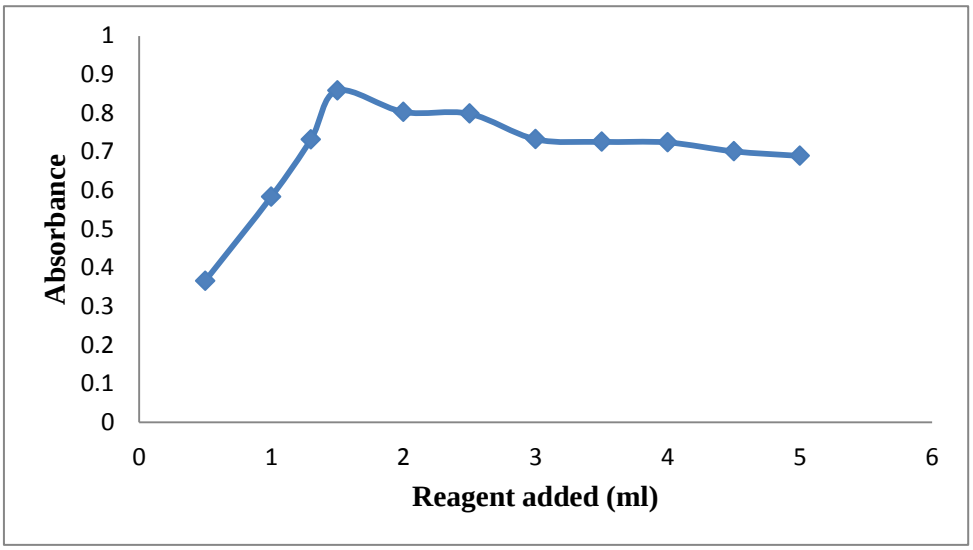

Fig. (5):- Effect of volume of NBD-Cl 8×10^{-3}M

Effect of temperature

The effect of temperature on the reaction of (MD) with NBD-Cl in alkaline medium was studied at different values (20-80°C) by continuous monitoring of the absorbance at 470 nm. It was found that the reaction with NBD-Cl was not affected by increasing the temperature, the result shown in Figure (6).

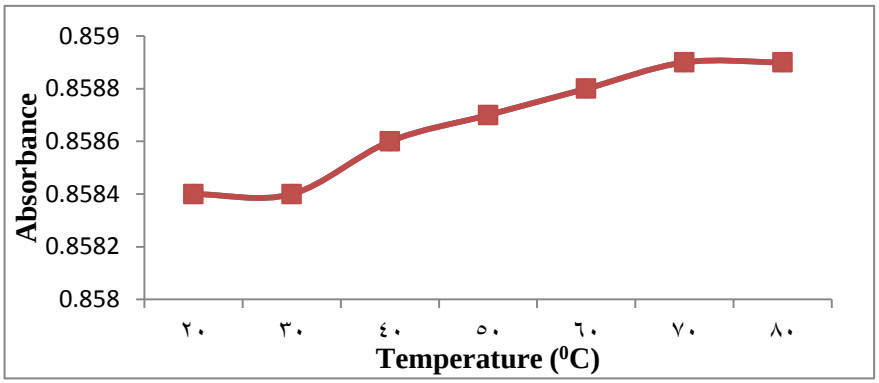

Fig. (6):- Effect of temperature

Effect of pH

An alkaline medium was necessary, since the results revealed that (MD) does not react with NBD-Cl in acidic media, the results revealed that the absorbances at pH < 8 were close to 0, indicating that under acidity, (MD) has difficulty to react with NBD-Cl. Different concentrations from NaOH were tested, Best results were obtained in the case of higher concentrations of NaOH (1M), the result shown in Figure (7).

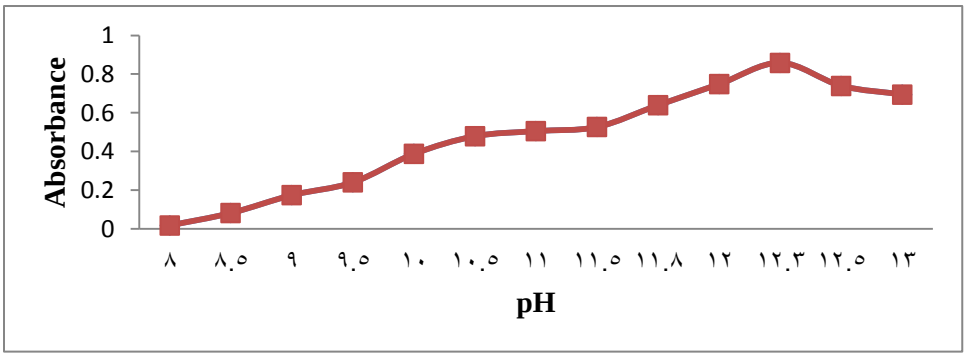

Fig. (7):- Effect of pH

Effect of Time

Under the above described optimum conditions, the absorbance-time curve for the reaction of (MD) with NBD-Cl in alkaline medium were constructed, and the product remained stable for (3h.), the result shown in Figure (8).

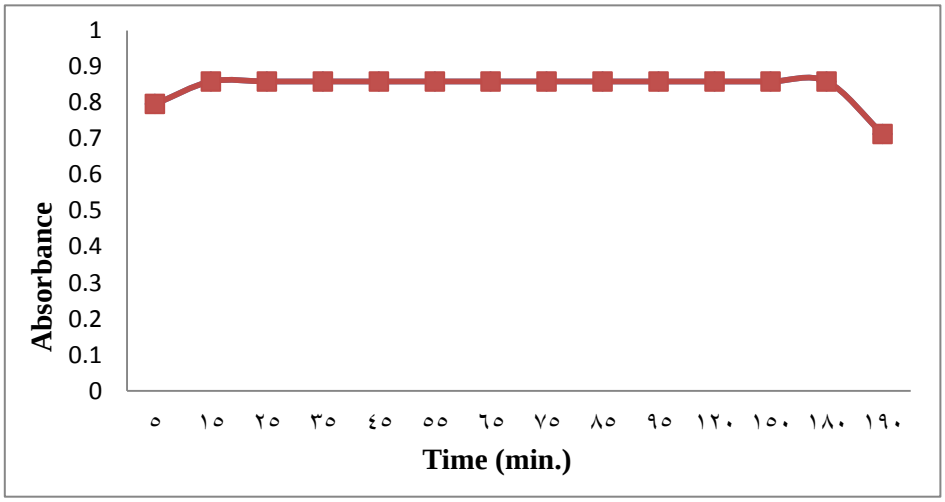

Fig. (8):- Absorbance-time curve for the reaction of (MD) with NBD-Cl in alkaline medium

Stoichiometry of the reaction

Under the optimum conditions, (cons. of NBD-Cl, pH, temperature, time) the stoichiometry of the reaction between (MD) and NBD-Cl were investigated by mole–ratio and continuous variation methods [23]. The stoichiometric ratio between NBD-Cl and (MD) was found to be 1:1, the results shown in Figures (9, 10).

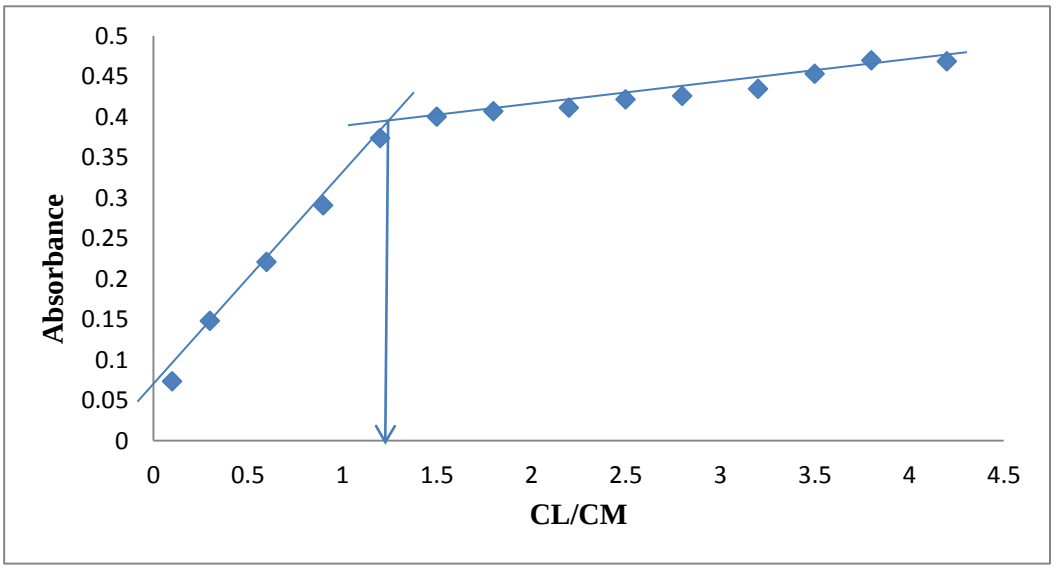

Fig. (9):- Mole-ratio method of MD-NBD-Cl complex

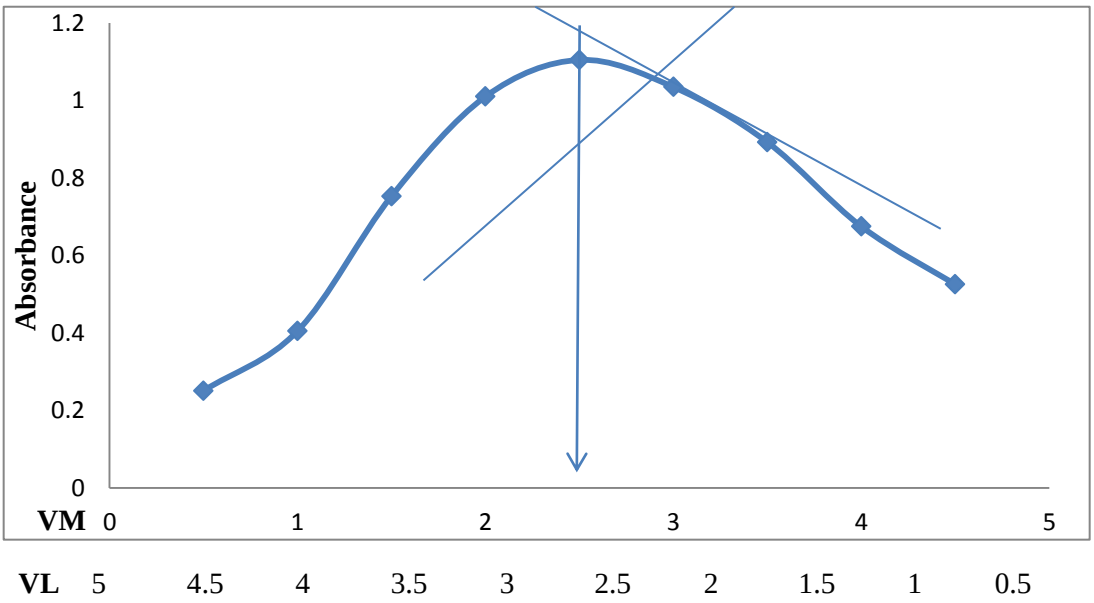

Fig. (10):- Continuous variation method of MD-NBD-Cl complex

Calibration curve

The calibration curves for (MD) pure form through complexation with NBD-Cl showed excellent linearity at concentration ranges of (1.6-17.6 µg/mL). The result shown in Figure (11).

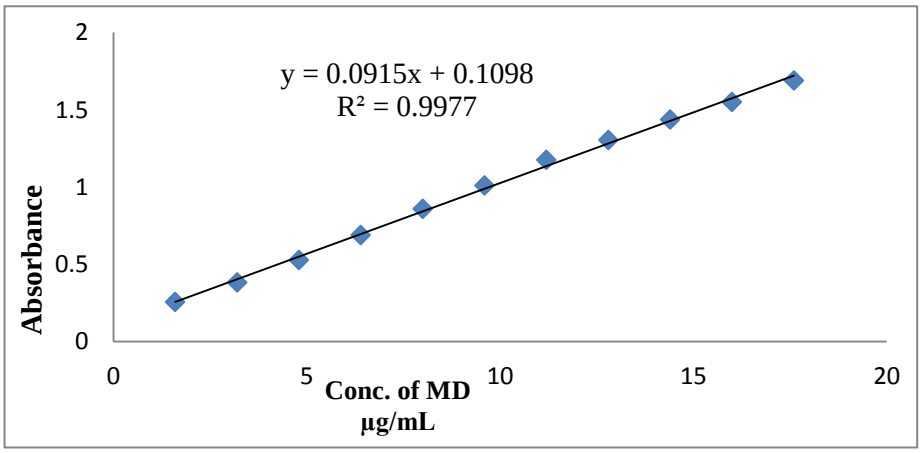

Fig. (11):- Calibration curve of MD-NBD-Cl (resulting product)

Construction of calibration curves

Calibration curves were constructed according to the optimum conditions in Table (1).

Parameter	Value
λmax.(nm)	470
Beer's law (µg/ml)	1.6-17.6
Molar absorptivity(l/mol.cm)	1.9337×10^{4}
Correlation coefficient (r)	0.9988
Limit of Detection (µg/ml)	5.536×10^{-3}
RSD%	0.033

Table (1):- Optical characteristics of the calibration curve for spectrophotometric determination of (MD) by NBD-Cl

Application of the method

Twenty tablets from each one of the pharmaceutical preparations were weighed and average weight were calculated. Tablets were crushed into fine powder. An accurately weighed quantity of powder equivalent to 250 mg of (MD) were transferred into a beaker and it were shaken with 50 ml of distilled water and filtered. The filtrate and the washing were collected in a 100 ml volumetric flask. The proposed method was successfully applied for the determination of (MD) in various commercial tablets , the results obtained are shown in Table (2).

Table (2): Determination of (MD) in commercial tablets by spectrophotometric method

Formulation	Content(mg) declared	Found(mg) by proposed method	Recovery%
Aldomet	250	249.69	99.88
Methyldopa	250	251.39	100.56
Apo-Methyldopa	250	249.72	99.89

Conclusion

The method described is simple, rapid, convenient and do not require special working conditions unlike many other reported methods. The procedure were showed shorter reaction time, stable colored species with inexpensive reagents. The determination can be performed at room temperature and do not require heating step. The proposed method can be applied to assay of (MD) in pharmaceutical preparations.

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