New Spectrophotometric Reduction–Oxidation System for Methyldopa Determination in Different Pharmaceutical Models

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Received: January 24, 2024; Accepted: February 07, 2024
DOI: 10.17721/moca.2024.45-53

Two spectrophotometric methods have been developed for the determination of methyldopa in the pure form and pharmaceutical formulations, both two methods based on the oxidation of the drug with an excess of N-Bromosuccinimide (NBS) and then reduction with 3,3-Diaminobenzidine (DAB), Absorbance of the resulting Magenta colored product is measured at 513 nm, the linearity ranged between (0.5 to 10) mg L^{-1} for the first spectroscopy method, and (0.5 to 15) mg L^{-1} for the second microfluid method. The detection limits (LOD) are 0.171, and 0.180 µg mL^{-1} for methyldopa in two methods spectroscopies, and microfluidic respectively. The limits of quantities (LOQ) are 0.571, and 0.600 µg mL^{-1} for methyldopa in two methods spectroscopies, and microfluidic respectively. The molar absorptivity (Ɛ) 2.58 ×10^4, 2.112×10^3 L $mol^{-1}cm^{-1}$ for methyldopa in two methods spectroscopies, and microfluidic respectively. No interference was observed from common excipients in formulations. The results show a simple, accurate, fast, and readily applied method to the determination of methyldopa in pharmaceutical products. The proposed method was applied successfully for the determination of the drug in their pharmaceutical formulations.

Keywords: N-bromosuccinimide (NBS), 3,3-diaminobenzidine (DAB); methyldopa (M.dopa), spectrometry; microfluidic; pharmaceutical samples

Introduction

Microfluidic chips, which originally debuted in the early 1990s, are reshaping the landscape of analytical research [1-10]. Current technologies provide “laboratories on a chip” that have already exhibited various advantages, such as reductions in reagent and sample use [4, 5], greater efficiency [7, 8], increased throughput and speed, adaptability, integration, and improved parallelism and automation [4,11,12]. Current research efforts are focused on enhancing the designs of various microfluidic devices for biochemical applications by combining chemical and physical techniques [5, 9]. Methyldopa (M.dopa) is a catecholamine derivative used for the treatment of hypertension. It is one of the most often used antihypertensive drugs during pregnancy, especially in difficult situations of pregnancy and renal failure. It preserves renal blood flow since it does not influence both uterine and placental circulations [13,14], and it is chemically known as 3-hydroxy-a-methyl-L-tyrosine sesquihydrate. Its antihypertensive property is principally attributed to its ability to trick alpha-adrenoreceptors in the lower brain stem into accepting its metabolite-methyl-norepinephrine instead of norepinephrine, resulting in neurotransmitter activation, which reduces nerve sympathetic activity and leads to reduced blood pressure. [15]. Methyldopa is a white or yellowish-white, crystalline powder that is moderately soluble in water but very barely soluble in alcohol and practically insoluble in ether. M.dopa dissolves well in dilute mineral acids [16]. Only the presence of a methyl group distinguishes alanine (dopa) from other amino acids on the a-carbon side of the chain in dihydroxyphenyl [17]. Sleepiness is a common adverse effect. Red blood cell disintegration and liver issues are more serious adverse effects. In 1960, methyldopa was found [18]. It is on the World Health Organization’s Essential Medicines List [19]. In industrial laboratories, spectrophotometry is by far the most often used instrumental method. Because of their simplicity, frequently requiring low-cost equipment and allowing for easy automation of trace analytical techniques, a variety of UV-Visible Spectrophotophotometric methods for methyldopa determination exist [20]. For determining the trace amounts of M.dopa in various applications, many standard techniques have been utilized, including spectrophotometry, and microfluidic technique, spectrophotometry has become a popular approach because of its advantages over other techniques, such as precision, simplicity, accuracy, and speed, especially in developing countries [21]. Several analytical techniques for determining M.dopa were mentioned in the literature review. High-performance liquid chromatography [22], liquid chromatography combined with mass spectrometry [23], electro-chemical methods [24-27], chemiluminescence [28], and other techniques [29,30] are among these technologies. Ternary complex formation is thought to play a significant function in spectrophotometric

analysis [31, 32]; they are classified into two types: ion association and mixed-ligand complex. Ion-pair complexes have been used to investigate a variety of medicinal substances [33, 34]. Meanwhile, the production of ternary compounds is gaining popularity [35, 36]. The proposed approach is used to identify M. dopa in bulk powder and pharmaceutical dose forms. The current work describes a simple, precise, sensitive, quick, specific, and cost-effective spectrophotometric approach for determining the concentration of (M. dopa) in bulk and medicinal dose formulations. The suggested approach is based on the synthesis of a compound, which is a combination of two regents, NBS and DAB, and M. dopa. At 513 nm, the magenta-colored product demonstrated the highest absorption. The suggested technique has none of the drawbacks associated with excipient interference that is commonly reported with M. dopa in tablet dosage formulations, and it requires no extraction or heating procedures. M. dopa in pharmaceutical formulations was determined using this approach.

Experimental part

Apparatus. The following devices were utilized in both spectroscopic and microfluidic approaches; all absorbance measurements were performed using the Biochrome Libra S60 double-beam spectrophotometer. The Oakton 2100 Series pH/mV/Ion/0C/0F Meter was used to determine the pH value. An Ohaus PA214 Pioneer Analytical Balance was used to weigh all samples. Shimadzu UV-1700 spectrophotometer was used to determine the maximum wavelength. Materials were loaded via Teflon pipes. The flow cell employed is 450 L, and the solutions were pushed using an Ismatic peristaltic pump. A UV-Visible detector (OPTIMASP300) was used to get the resulting peak. In this investigation, the recorder was also used to record the signal Pen Siemens C1032 Hitter Ardeas 51. ethanol, water as solvents, and the procedures were utilized to identify the reagent and M. dopa chemical.

Chemicals. In the Spectroscopy method, and In Microfluidic method all chemicals were analytical grade and were used without additional purification, N-Bromosuccinimide (NBS) (purity 99.9%) was obtained from London-UK. 3,3-Diaminobenzidine (DAB) (purity ≥ 98) was obtained from Sigma-chemicals company (Germany). The M. dopa standard was obtained from Iraq (Samara). Pharmaceutical drug (Methyldopa purity 98 % were purchased from the market Iraq-Samara. The solutions in this study were prepared using distilled water and ethanol.

Methyldopa pharmaceutical preparations. Pharmaceutical preparation containing methyldopa, discovered as five samples (methyl dopa, aldosam, aldomet, methyl dopa safa, and methyl dopa Moroccan) each tablet comprises 250 mg given by Samarra Pharmaceuticals Corporation (SDI-IRAQ). The tablets solution was made by crushing 10 tablets (4.38) g and dissolving 0.0526 g of this amount in a little amount of boiling water in a 100 mL volumetric flask. The resultant solution was filtered, and the residue was rinsed with hot distilled water several times. The filtrate volume was completed to the mark using distilled water to get a solution with a concentration of 1000 g/mL. 30 mL of the produced solution was put into a 100 mL volumetric flask, and the volume was supplemented to the limit with distilled water, yielding a 300 g/mL solution.

Preparation of the reagent N-Bromosuccinimide (NBS), 3,3-Diaminobenzidine (DAB): Both reagents were prepared in very simple and uncomplicated ways, where the N-Bromosuccinimide (NBS) reagent was prepared by dissolving it in water at a low temperature while stirring the solution, and the 3,3-Diamino-benzidine (DAB) reagent was prepared by dissolving it in absolute ethanol.

Preparation of standard stock solutions. In the spectroscopy method, and in microfluidic method.

M. dopa drug solution 100 mg L^{-1}: 0.01 g of M. dopa was dissolved in 100 mL of distilled water to make a stock solution. Further dilution resulted in working solutions.

NBS solution 1×10^{-2} mol L^{-1}: 0.0889 g of the reagent was dissolved in 50 mL of water to make the solution.

DAB solution 1×10^{-3} mol L^{-1}: 0.0107 g of reagent was dissolved in 50 mL of ethanol to make a stock solution. As needed, more diluted reagent solutions were produced.

Interference solutions. All interference samples solutions were made in 100 mg L^{-1} concentration in the spectroscopy and microfluidic methods by dissolving quantities (0.01, 0.01, 0.01, 0.0208, 0.0391, 0.367, and 0.0894 g) of ascorbic acid, glucose, starch, $ZnCl_2$, $MgCl_2$, $CaCl_2.2H_2O$, and $AlCl_3.6H_2O$, respectively in distilled water that certain pH and completing the volume to 100 mL to get on ascorbic acid, glucose, starch, Zn^{+2}, Mg^{+2}, Ca^{+2}, and Al^{+3} as interference ions. There were two concentrations of each foreign interference examined. In the first test, the interference ion concentration was low at 5 mg L^{-1}, but in the second test, the interference ion concentration was high at 50 mg L^{-1}.

General Procedure for Microfluidic method

Designing of micro valve

The new microfluidic chip is designed with high efficiency and is made of cheap materials available in local markets. A new design of μFIA and new microfluidic chip are showing in details in Figure 1. The microfluidic chip with two symmetrical and two-dimensional micro-channels with lengths of 7.0, and 9.0 cm with volumes 54.95, and 70.00 μL, with (id 0.5 mm). The conduits of the reagent and drug solution are merged inside the microfluidic chip to mix the solutions in the mixing coil. Three-way sub-valves, installed outside the chip can carry out the loading and injection control process.

The working stages

The determination of methyldopa was reached through three stages, including the injection and the loading of sample and reagent into the microchip and pumped of carrier stream of distilled water, as showed in the Figure 2.

Sample collection

The suggested method was applied on pure sample from the pharmaceutical factory in Iraq in Samarra Province Where the value of the pure drug was compared with drugs that contain M. dopa from different companies, and this value was considered as a measured value of M. dopa in samples for comparison with the suggested method.

Results and Discussion

The spectrophotometric approach that was suggested for this investigation relied on the molecule that was formed when M. dopa was combined with N-Bromosuccinimide (NBS) and 3,3-Diamino-benzidine (DAB) [37], as Figure 3 illustrates. This combination produced a magenta color and had a maximum absorbance at 513 nm. The optimum conditions for this reaction for two spectroscopy methods were studied carefully in order to increase the accuracy of the results.

The wavelength of the maximum absorbance of (N-Bromosuccinimide (NBS), and 3,3-Diamino-benzidine (DAB)) and its compound

Using a Shimadzu UV-1700 spectrophotometer, the maximum absorbance of NBS, and DAB, with M. dopa drug was tested in different mediums in order to achieve the greatest sensitivity. The results showed that the best spectra were in an acid medium. The maximum absorbance of the M. dopa compound appeared at 513 nm and the maximum absorbance of the reagent was found at 281 nm. According to these results, 513 nm was chosen as the maximum wavelength (λ) in this study.

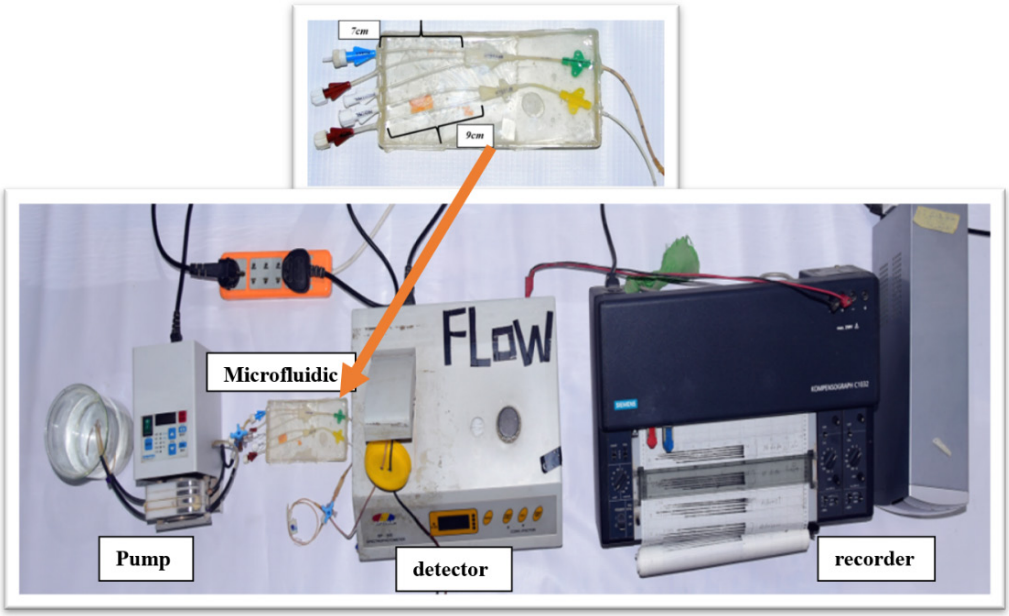

Figure 1. New design of μFIA System with microfluidic chip.

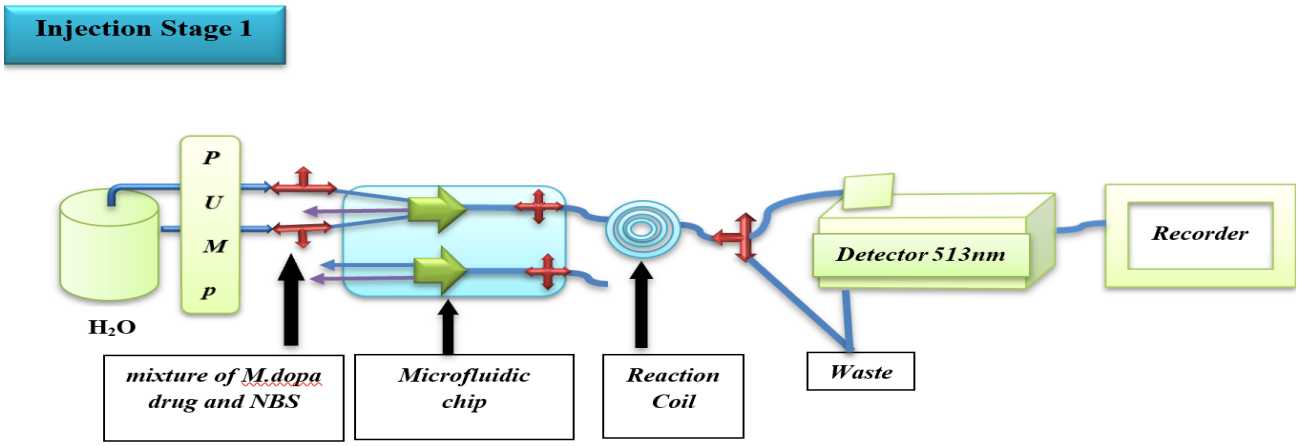

Figure 2. Loading of mix (M. dopa drug and NBS) solutions in loop1 and DAB in loop2 on microfluidic chip.

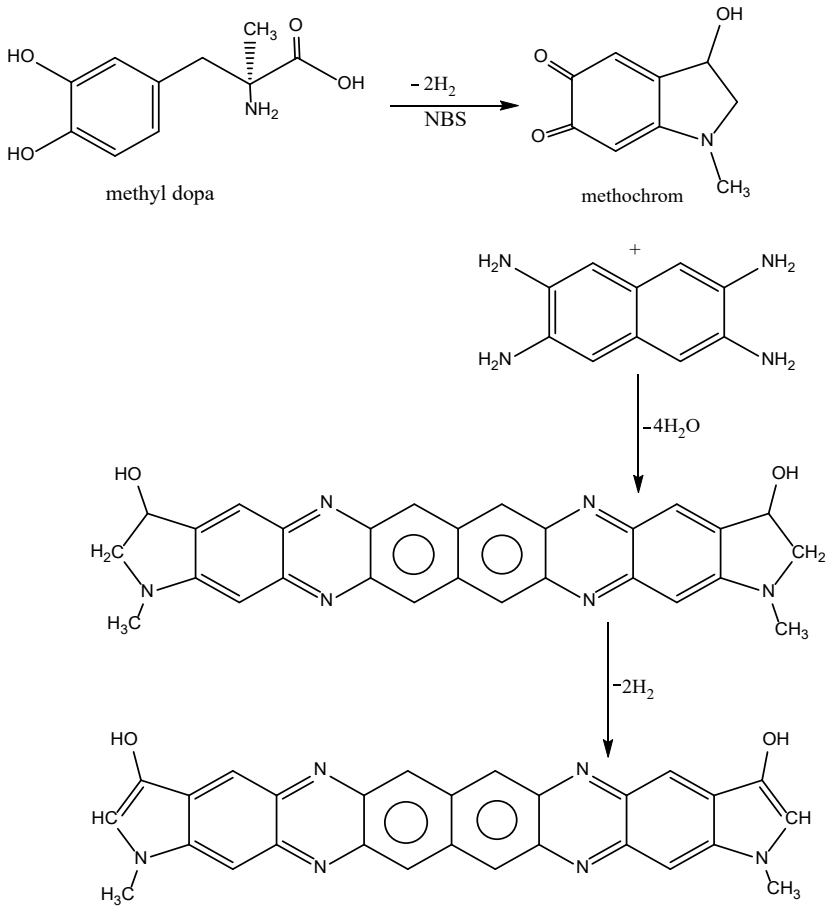

Figure 3. Proposal mechanism between M. dopa drug and the mixture of (NBS), and (DAB) reagent**.**

Determining the conditions for the spectroscopy technique

Effect of pH on the M.dopa reaction. This test was performed with a constant concentration and volume of each reactant, M.dopa (10 mg L^{-1}, 1 mL), NBS ($1 \cdot 10^{-2}$ mol L^{-1}, 0.15 mL), and DAB ($1 \cdot 10^{-3}$ mol L^{-1}, 0.15 mL) respectively. To determine the optimal pH of formation compound, the pH of the medium is changed from (3 to 7) using NaOH, HCl and pH meter. The production of the compound is clearly favored by pH=5, this value is selected as the optimum pH for the tests as shown in Figure 4.

Effect of reagent N-bromosuccinimide (NBS) concentration on the formation of M. dopa reaction. To show the effect of the concentration of the reagent, different solutions are prepared ($1 \cdot 10^{-2}$, $5 \cdot 10^{-2}$, and $1 \cdot 10^{-1}$) mol L^{-1}. The results showed that the absorbance decreased rapidly without any precipitate in solution. These result of the concentrations of the reagent showed ideal reagent concentration is $1 \cdot 10^{-2}$ mol L^{-1}, as shown in Figure 5, this test at a constant concentration and volume of each reactants; M.dopa (10 mg L^{-1}, 1 mL), NBS (0.15 mL), and DAB ($1 \cdot 10^{-3}$ mol. L^{-1}, 0.15 mL) with pH=5.

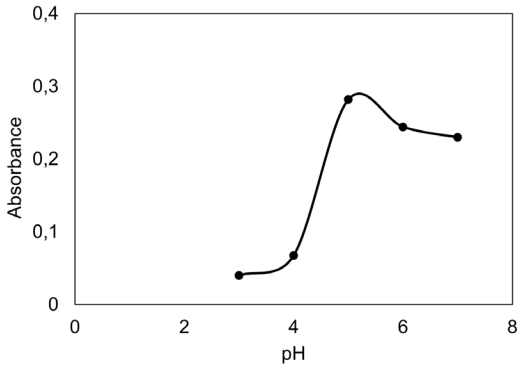

Figure 4. The effect of acidity function on M.dopa oxidation – reduction reaction.

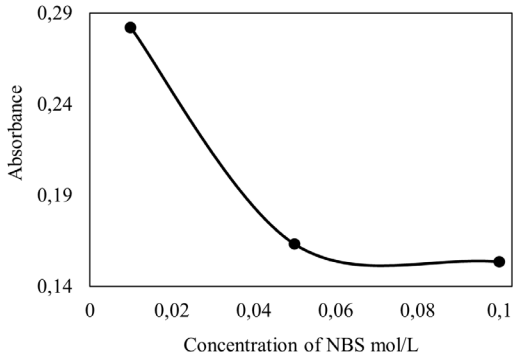

Figure 5. The effect of NBS concentration on M.dopa reaction.

Effect of DAB reagent concentration on M. dopa oxidation – reduction reaction. The effect of reagent concentration was tested at a constant concentration and volume of M.dopa (10 mg L^{-1}, 1 mL) and NBS ($1 \cdot 10^{-2}$ mol L^{-1}, 0.15 mL) respectively and volume of DAB (0.15 mL) with pH=5, different solutions of the reagent concentration were prepared ($1 \cdot 10^{-4}$, $5 \cdot 10^{-4}$, $1 \cdot 10^{-3}$) mol L^{-1}. The results showed that the absorbance increased without any precipitate in solution, The ideal reagent concentration was $1 \cdot 10^{-3}$ mol L^{-1} for DAB as shown in Figure 6.

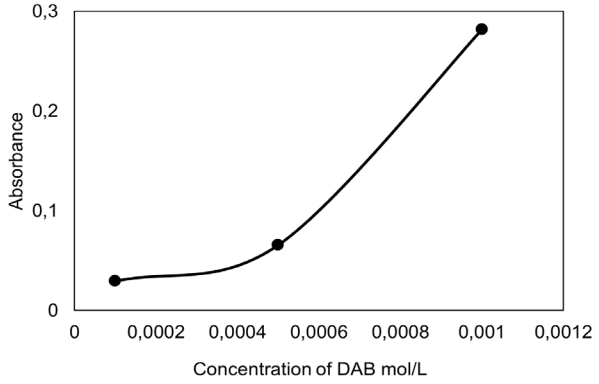

Figure 6. The effect of DAB Concentration on M. dopa oxidation – reduction reaction.

Time effect on M. dopa oxidation – reduction reaction. The effect of time on the production and stability of M. dopa compound was studied under the optimum conditions in order to define the suitable time of absorbance measurement. The absorbance of the compound was measured at different times: directly after the formation process, after 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 100, 140 and 170 min, and the result indicated that compound was formed and remain nearly stable for 170 min. According to the results, the suitable time for taking absorbance measurement of the compound was directly after preparing it as shown in Figure 7.

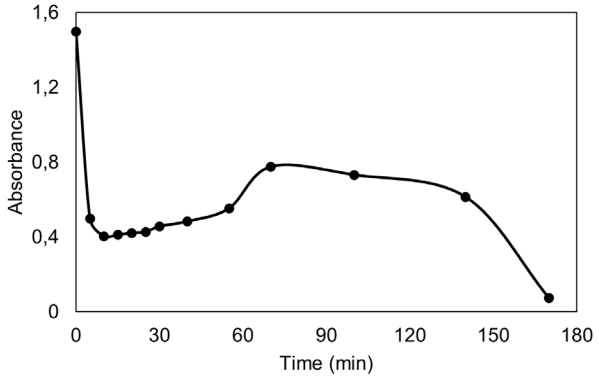

Figure 7. The effect of time on the reaction of M. dopa reaction.

Calibration curve of M. dopa compound

Under optimal conditions the calibration curve of drugs determination by preparing a series of M. dopa solutions ranging from (0.05 to 50 mg L^{-1}), which are prepared using distilled water at pH= 5. At a constant volume of its 1 mL, and a constant concentration and volume of NBS ($1 \cdot 10^{-2}$ mol L^{-1}, 0.05 mL), and DAB ($1 \cdot 10^{-3}$ mol L^{-1},0.30 mL) respectively .The absorbance of the compound is measured at 513 nm, Beer's law is obeyed over the range of 0.5-10 mg L^{-1}. Calibration curve: y = 0.1225x + 0.2768; R^2 = 0.9988. Some properties from curve shown in Table 1.

Table 1. Properties of the calibration curve of M. dopa compound using mixture of (NBS, and DAB) as reagent**.**

Linearity, µg mL^{-1}	(0.5-10)
Correlation coefficient (R^2)	0.9988
Limit of Detection (LOD), µg mL^{-1}	0.171
Limit of Quantitation (LOQ), µg mL^{-1}	0.571
Sensitivity Slope	0.1225
Sandell sensitivity (S), µg cm^{-2}	0.008
Molar absorptivity (Ɛ), L $mol^{-1}cm^{-1}$	$2.58 \cdot 10^4$

The limit of detection (LOD) and the limit of quantification are calculated by the equations below [38]:

LOD =3*SD of blank / Slope ……..…. (1)

LOQ =10*SD of blank / Slope ……..…… (2)

SD: Standard deviation of blank

Slope: Slope of the calibration curve

The effect of interference ions

The effect of different cations and anions on the determination of M. dopa is studied in low and high concentrations. The taken amounts of foreign ions were 5 mg L^{-1} and 50 mg L^{-1}, while the concentration of M. dopa drug remained constant throughout the experiment at 5 mg L^{-1}, the interference such as (Starch, Ascorbic acid, glucose, $ZnCl_2$, $MgCl_2$, $CaCl_2.2H_2O$, and $AlCl_3.6H_2O$). The results show that all the cations and anions used did not interfered at both concentrations on the determination of the M. dopa drug.

Applications M. dopa drug

The proposed methodology is applied to the determination of M. dopa in five samples including (methyl dopa, aldosam, aldomet, methyl dopa safa, and methyl dopa Moroccan), The spectrophotometric method is used to determine the concentration of the drug at 5 mg L^{-1} in various pharmaceutical samples. pure value is used as the standard method. The comparison between the suggested method and

the pure value gives good accuracy with a recovery percentage as shown in Table 2 below, the following equations show the results.

$$R.E\% = (Mv - Pv)/(Pv) \times 100, \quad (3)$$

$$Recovery\ \% = 100 \pm R.E\%, \quad (4)$$

where Mv - Measured value, Pv - pure value.

Parameters for microfluidic system

Effect of flow rate. The flow rate is a very important factor. It effects on the dilution of the solution and conversely, affects the response. The effect of the flow rate on the absorbance value was studied over a range of (1.8 to 12.8 mL min^{-1}) higher flow rates produced absorbance, the best rate (10.2 mL min^{-1}) as shown in Figure 8. At a constant conc of M. dopa 5 mg L^{-1}, a constant concentration and volume of NBS ($1 \cdot 10^{-3}$ mol L^{-1}, 54.95 µl), and DAB ($1 \cdot 10^{-3}$ mol L^{-1}, 54.95 µl) respectively, the reaction occur at pH=5.

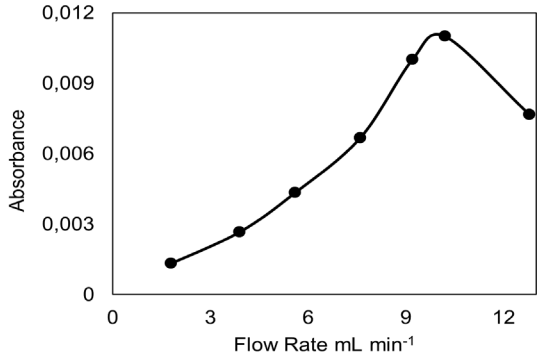

Figure 8. The effect of flow rate on the absorption value of M. dopa compound.

Effect of NBS reagent concentration

The effect of NBS concentration on oxidation – reduction of M. dopa compound was investigated. The NBS concentration in the range of ($1 \cdot 10^{-4}$ to $1 \cdot 10^{-2}$) mol L^{-1}. The greatest signal of absorption was observed when the reagent concentration was ($1 \cdot 10^{-2}$) mol L^{-1} for NBS at room temperature, as shown in Figure 9.

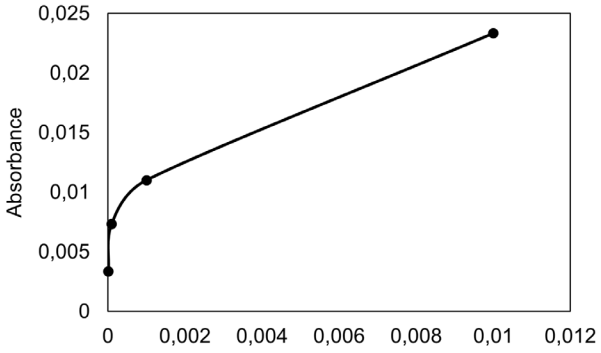

Figure 9. The effect of NBS reagent concentration on the reaction of M. dopa compound**.**

The conditions under which the experiment was conducted were; M. dopa concentration 5 mg L^{-1}, NBS volume 54.95 µ, concentration and volume of DAB (1×10^{-3} mol L^{-1}, 54.95 µl), flow rate was 10.2 mL min^{-1}, pH = 5 and mixing coil length 10 cm.

Effect of DAB reagent concentration

To determine NBS concentration on oxidation – reduction of M. dopa, used DAB concentration from the range of (1×10^{-6} to 1×10^{-3}) mol. L^{-1}. The best concentration which gives the best and highest sensitivity was 1×10^{-5} mol L^{-1}. The optimal conditions for this experiment were as follows: room temperature, mixing coil length 10 cm, M. dopa concentration 5 mg. L^{-1}, concentration and volume of NBS (1×10^{-2} mol L^{-1}, 54.95 µl) and, DAB (54.95 µl) respectively, flow rate 10.2 mL min^{-1}, and pH=5. The results are shown in the Figure 10.

Table 2. Accuracy of proposed method compared with pure value for determination of M. dopa in deferments pharmaceutical samples.

No	Sample	Company	True value mg L^{-1}	Measured value mg L^{-1}	E%	Recovery%
1	Methyl dopa	Accord - UK	5	5.156	3.1	103.1
2	Aldosam	General Company for Pharmaceutical Industries and Medical Supplies, Samarra - Iraq	5	5.039	0.8	100.8
3	Aldomet	Algorithm- Lebanon	5	5.731	5.1	105.1
4	Methyl dopa safa	Al-Safaa Pharmaceutical Production Company Diyala Iraq	5	5.712	4.7	104.7
5	Methyl dopa Moroccan	Pharma 5	5	5.325	5.7	105.7

* Average of three times

The original dose for the five medications is 250 mg per tablet

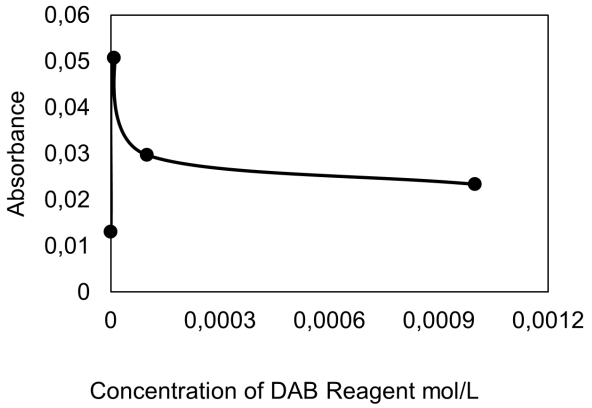

Figure 10. The effect of DAB reagent concentration on the reaction of M. dopa compound.

Effect of pH

The effect of the acidic medium on the absorbance values was studied in the range from (3 to 6) for the drug solution. The results show that the compound was stable at pH = 5 at room temperature, and mixing coil length 10 cm. when M. dopa conc. 5 mg L^{-1}, a constant concentration and volume of NBS (1×10^{-2} mol L^{-1}, 54.95 µl), and DAB (1×10^{-5} mol L^{-1}, 54.95 µl) respectively, and flow rate was 10.2 mL min^{-1}.

Calibration curve

After determining the optimal conditions and λmax = 513 nm. The calibration graph for M. dopa compound was generated by preparing a series of solutions containing concentrations ranging from (0.05 to 50) mg L^{-1} It became clear through the study that the linearity was 0.5-15 mg L^{-1}. Calibration curve: y = 0.01x + 0.0021; R^2=0.9946. Through the curve, a number of characteristics of the estimation method proposed in the study were deduced as shown in the Table 3.

Table 3. Properties of the calibration curve of M. dopa compound using mixture of (NBS, and DAB) reagents.

Linearity, µg mL^{-1}	(0.5-15)
Correlation coefficient (R^2)	0.9946
Limit of Detection (LOD), µg mL^{-1}	0.180
Limit of Quantitation (LOQ), µg mL^{-1}	0.600
Sensitivity Slope	0.010
Sandell sensitivity (S), µg cm^{-2}	0.100
Molar absorptivity (Ɛ), L $mol^{-1}cm^{-1}$	$2.112\cdot10^3$

Interferences

The effects of various cations and anions on the determination of M. dopa were studied such as (Starch, ascorbic acid, glucose, $ZnCl_2$, $MgCl_2$, $CaCl_2.2H_2O$, and $AlCl_3.3H_2O$) The use of any cations or anions has no influence on the determination of M. dopa.

Applications

The proposed system was applied within the study to M. dopa estimated in different drug models to cover the accuracy of the method and the laboratory-designed system, and the results were as shown in Table 4.

Table 4. Accuracy of suggested method for evaluation of M. dopa.

No	Sample	Company	True value mg L^{-1}	Measured value mg L^{-1}	E %	Recovery %
1	Methyl dopa	Accord - UK	5	5.137	2.8	102.8
2	Aldosam	General Company for Pharmaceutical Industries and Medical Supplies, Samarra - Iraq	5	4.809	-3.8	96.19
3	Aldomet	Algorithm- Lebanon	5	4.618	-7.6	92.4
4	Methyl dopa Moroccan	Pharma 5	5	5.074	1.5	101.5
5	Methyl dopa safa	Al-Safaa Pharmaceutical Production Company Diyala Iraq	5	4.936	-1.3	98.8

*Average of three Times

The original dose for the five medications is 250 mg per tablet

Comparing the outcomes of the microfluid method using a combination of two reagents and the spectrophotometric method

After determining the best conditions for both methods within the study (spectrophotometric and microfluidic), a comparison was made between them, as it became clear through the results that the microfluidic method was the best in terms of low consumption of materials in volume and concentration. However, the spectral method had better sensitivity than the microfluid method, while the linearity in the microfluidic method was the best as shown in Table 5.

Conclusion

This work spectrophotometric method has been suggested a new, simple, cheap, accurate, high sensitivity and selectivity system to determine M. dopa with mixture of NBS, and DAB as a new reagent. The design of the microflow injection system to determination M. dopa does not require expensive equipment compared to other technologies, because the lab-designed and local unit features rapid analysis and a wide range of concentrations and does not consume chemicals in large quantities. The limit of detection (LOD), the limit of quantification (LOQ) and Shandell's sensitivity were calculated to be 0.171 mg L^{-1}, 0.571 mg L^{-1} and 0.008μg cm^{-2} respectively for spectroscopy method, and 0.180 mgL^{-1}, 0.600 mg L^{-1} and 0.100μg cm^{-2} respectively for microfluidic method. The proposed method obtained a recovery percentage of the various pharmaceutical samples ranging from 100.780% to 105.736 % in spectroscopy method, and 92% to 102% in microfluidic method. My results were good with all the parameters studied compared to published research [39,40].

Table 5: The comparison between the results of the two Spectrophotometric methods by mixture of two reagents, and the Microfluid method for the M. dopa determination.

Parameter	**Spectral Method**		**Microfluidic Method**	
Reagent	NBS	DAB	NBS	DAB
Conc of reagent mol L^{-1}	1×10^{-2}	1×10^{-3}	1×10^{-2}	1×10^{-5}
Vol of reagent μl	0.05 mL, 0.00005μl	0.30 mL, 0.0003μl	54.95 μl	54.95 μl
pH	5		5	
Reaction temperature °C	at room temperature		at room temperature	
Flow Rate mL min^{-1}	…….		10.2	
Reaction Coil cm	…….		10	
Linearity range mg L^{-1}	(0.5 – 10)		(0.5 – 15)	
Limit of detection LOD mg L^{-1}	0.171		0.180	
Limit of quantitation LOQ mg L^{-1}	0.571		0.600	
Correlation coefficient R^2	0.9988		0.9946	
Sensitivity Slope (b)	0.1225		0.010	
Molar absorptivity (Ɛ) L mol^{-1} cm^{-1}	2.587×10^4		2.112×10^3	
Sandell's sensitivity (S) μg cm^{-2}	0.008		0.100	
Regression equation	y=0.1225x+0.2768		y=0.01x+0.0021	
Recovery average %	(100.780 to 114.620)		(92.372 to 102.754)	

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