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Indirect Spectrophotometric Determination of Metformin Via Decolorization Eriochrome Black-T

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A B S T R A C T

The research included the description of a simple indirect spectrophotometric method for the determination of Metformin, which depended on the oxidation of Metformin with an amount of N-bromosuccinimide in the presence of acetic acid and the excess amount of it oxidized the dye Eriochrome black-T, and the absorption was measured at 520 nm, and the molar absorption coefficient was 4 × 104 L / mol. cm, indicating the sensitivity of the method. And compliance with Beer's law for concentrations ranged from (2-16) micrograms. ML-1, the method was characterized by good accuracy and compatibility, with a recoverability of 100.155%. While the relative standard deviation was less than 1.0.The method was applied to pharmaceutical preparations of Metformin hydrochloride.

Introduction

This Word Metformin, a widely prescribed oral medication, has emerged as a cornerstone in the management of type 2 diabetes mellitus (T2DM). This drug, known under brand names such as Glucophage and Glumetza, has revolutionized the field of diabetes treatment, offering a multifaceted approach to blood glucose regulation. Beyond its primary indication, metformin has garnered considerable attention for its potential therapeutic applications in various medical domains.

This introduction offers a comprehensive look at metformin, covering its historical evolution, how it works, its uses in clinical settings, and its growing importance in the field of healthcare. It also includes references to support its importance.

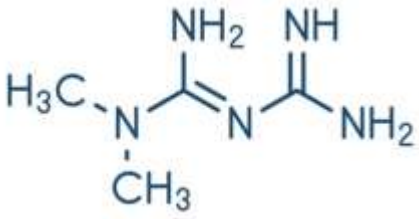
Metformin's journey into clinical practice can be traced back to the 1920s when its glucose-lowering properties were first discovered. However, it was not until the latter half of the 20th century that metformin gained prominence as a key tool in the management of diabetes. A pivotal moment in metformin's history is well-documented in Bailey CJ and Turner RC's influential 1996 paper in the New England Journal of Medicine [1]. This paper reflects on the historical evolution of metformin and its significance in diabetes care.

The effectiveness of metformin lies in its unique mechanism of action. Unlike many other antidiabetic medications, metformin does not stimulate insulin secretion from the pancreas. Instead, it primarily operates by inhibiting hepatic gluconeogenesis and improving insulin sensitivity in peripheral tissues. Rena G, Pearson ER, and Sakamoto K's 2013 paper in Diabetologia [2] provides a comprehensive overview of the molecular mechanisms underlying metformin's action, shedding light on its distinctive approach to regulating blood glucose levels.

Metformin's clinical applications extend beyond the realm of diabetes management. While it is a first-line therapy for T2DM, it finds utility in other clinical scenarios. Notably, metformin is employed in the treatment of polycystic ovary syndrome (PCOS) and gestational diabetes. The National Institute for Health and Care Excellence (NICE) has outlined its diverse clinical applications in its guidance on the management of type 2 diabetes in adults [3].

Metformin's influence transcends diabetes treatment. It has garnered attention for its potential benefits in weight management, cardiovascular risk reduction, and cancer prevention. Rena G, Hardie DG, and Pearson ER's 2017 paper in Diabetologia [4] dives into the mechanisms underlying metformin's potential benefits. Meanwhile, Campbell JM, Bellman SM, Stephenson MD, and Lisy K's 2017 systematic review and meta-analysis [5] discuss the broader impact of metformin on all-cause mortality and diseases of aging, independent of its effect on diabetes control.

In the subsequent sections, we will delve deeper into these facets of metformin, exploring its diverse clinical applications and the evolving body of evidence supporting its multifaceted role in healthcare.The Metformin chemical structure has the following form:

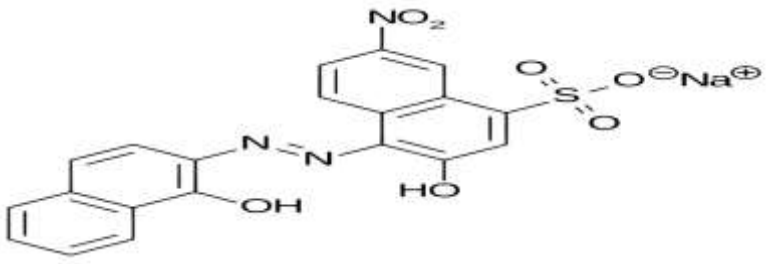


C4H12ClN5
M.Wt.165.62 g/mol.

A review of the existing literature shows that there are numerous High-Performance Liquid Chromatography (HPLC) techniques available for quantifying metformin. However, a significant majority of these methods rely on either ion-pair reagents or cation exchange columns [6-17]. In addition to these approaches, various alternative methods for metformin determination have been documented. These alternative methods include conductometric titration [18], flow-injection chemiluminescence [19-21], capillary electrophoresis [22], and ion-selective electrode techniques [23].

Eriochrome Black-T dye and its uses:

This substance is recognized for its distinct blue hue and angular shape, and it finds application in the investigation of complex formations. When it interacts with calcium or magnesium, it results in the formation of a complex with a red color. Upon reaching the endpoint, it reverts to its original blue color. The dye is characterized by the structural formula shown below:



Mwt=461.381g/mol
3-hydroxy-4-[(1-hydroxy-2-naphthalnyl)azo]-7-nitro-1-naphthylene sulfonic acid mono sodium salt

Experimental Section:

Equipment Utilized:

For conducting the spectroscopic analysis of the solutions, a Shimadzu UV-1800 spectrophotometer with a dual-beam photometer was employed. Glass cells with a 1 cm width were utilized. Precise measurements of materials were carried out using a sensitive ADAM balance, and an elekto-mag water bath was employed for solution heating.

Solutions of Chemical Substances Used

All the materials employed were of exceptional purity.

Metformin Solution (100 µg/ml)

This solution was created by dissolving 0.01 grams of pure metformin in a limited quantity of distilled water. Subsequently, the solution was transferred into a volumetric flask, and its volume was adjusted to 100 milliliters with distilled water.

Eriochrome Black-T (EBT) Dye Solution (500 µg/ml)

The dye solution was prepared by dissolving 0.05 grams of the pure Eriochrome Black-T substance in a small portion of distilled water. It was then transferred to a volumetric flask, and its volume was brought up to 100 milliliters with distilled water.

Solutions of Acids

Solutions of various acids, including hydrochloric acid, phosphoric acid, sulfuric acid, and nitric acid, were prepared at a concentration of 1.0 molar. This was achieved by dissolving the requisite quantities of each acid in 100-milliliter volumetric flasks with distilled water.

Surfactant Solutions

Solutions of surfactants, including Tween 20, SDS, and CPC, were all formulated at a concentration of 0.1%. This concentration was achieved by dissolving 0.1 grams of each surfactant in distilled water. Subsequently, the solutions were transferred to volumetric flasks, and their volumes were adjusted to 100 milliliters with additional distilled water.

In the initial phase of our study, we aimed to develop an indirect spectroscopic method for estimating metformin in both its pure form and pharmaceutical preparations. This method involved exploring the applicability of Eriochrome Black-T (EBT) dye in an oxidation process. To establish the optimal amount of dye that would yield maximum absorption, we conducted experiments.

First, we constructed a standard curve for the EBT dye by testing various volumes (ranging from 0.02 to 2 ml) of a 500 microgram per milliliter Eriochrome Black-T solution. Each volume was added to a volumetric flask and diluted with distilled water to a total volume of 25 ml. The results indicated that the highest absorption occurred at 517 nm, as depicted in Figure (1). Within the concentration range of (0.4-40) micrograms per milliliter, the dye adhered to the Beer limits, as shown in Figure (2). An optimal concentration of 40 micrograms per milliliter was chosen for subsequent experiments.

Next, we determined the ideal quantity of dye to facilitate the oxidation reaction in both acidic and basic environments separately. We introduced 1.0 ml of 1.0 molar solutions of acitic acid and sodium hydroxide into the mix, alongside molar quantities of the oxidizing agent. Our findings indicated that the acidic medium was more effective in accelerating the dye's oxidation, yielding the highest absorption value.

Upon conducting experiments where microgram amounts of metformin reacted with the optimal quantities of the dye and oxidizing agent in an acidic medium, we observed a linear increase in the absorption of Eriochrome Black-T dye at 520 nm with the rise in metformin concentration. Consequently, we determined that this method could be employed for the estimation of the drug and its pharmaceutical preparations.

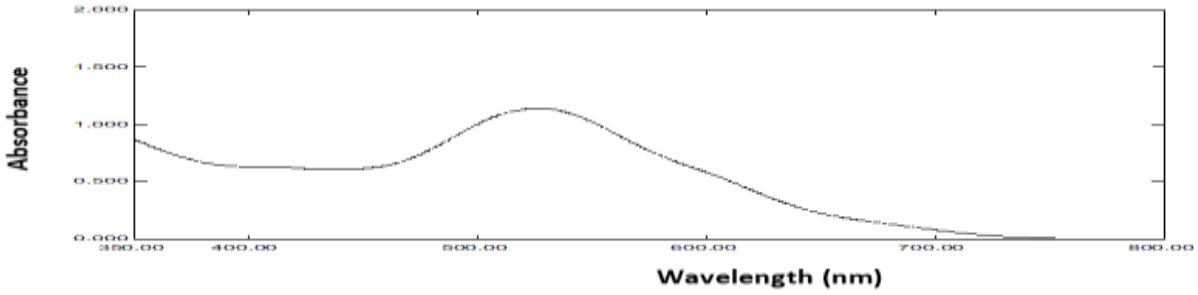


Figure 1. Absorption spectrum of 40 micrograms. 1 ml Erochrome Black dye - T

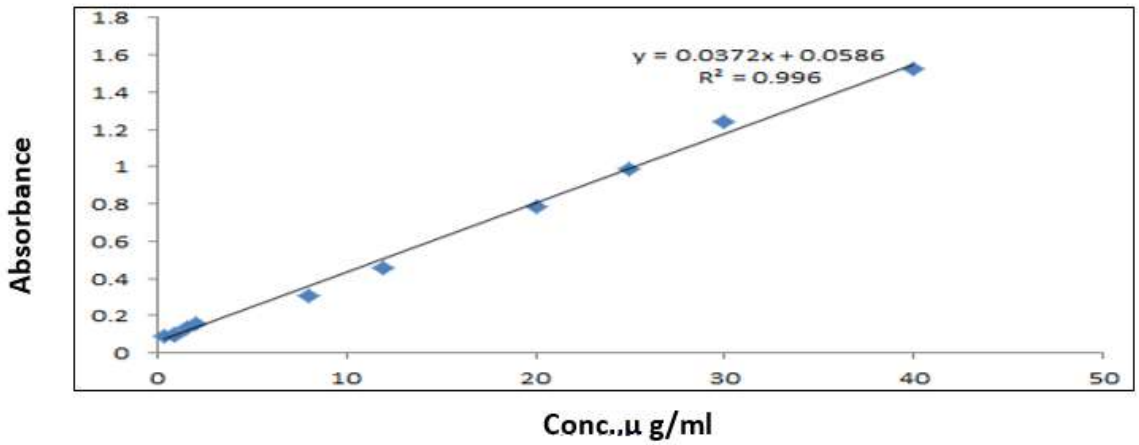


Figure 2. Standard curve of Eerochrome Black-T dye

Establishing Ideal Parameters

It is aimed to investigate the influence of the type and quantity of the oxidizing agent on the reduction of Eriochrome Black T dye. A set of various oxidizing agents was prepared at a constant concentration of 2 x 10-2 M. Microgram quantities of these agents were added to the optimal amount of dye and allowed to react for five minutes. Subsequently, 1.0 ml of 1.0 M acetic acid was introduced, followed by dilution with distilled water in

volumetric flasks to a total volume of 25 ml. The absorbance was measured ten minutes after dilution at 520 nm. The results presented in Table (1) indicate that N-bromosuccinimide exhibited the highest efficacy in inhibiting the dye.

Table 1. Effect of oxidizing agents on shortening the dye Eriochrome Black T-

Oxidant 2 x 10-2 M	Without	NBS	KIO4	K2CrO4
Absorbents	1.052	0.323	1.062	1.084

To determine the ideal quantity of the oxidizing agent, N-bromosuccinimide, we varied the volumes from (0-2) milliliters while following the previously outlined procedure. As illustrated in Table (2), the optimal volume for achieving maximum reduction of the dye was found to be 2 milliliters. This quantity was subsequently employed in all subsequent investigations.

Table 2. Amount of oxcidizing agent

Ml of NBS(M)	without	0.2	0.5	0.8	1	1.5	2
Absorbance	1.212	1.085	0.916	0.540	0.415	0.143	0.083

Choosing the appropriate acid

Because Eriochrome Black-T dye is oxidized in an acidic environment, different types of acids (hydrochloric acetic acid, sulfuric acid, phosphoric acid, and nitric acid) were studied in the estimation, where 1.0 milliliters of each acid were added at fixed concentrations of 1.0 molar, each separately to volumetric bottles of capacity. 25 ml containing a microgram amount (8 micrograms. ml-1) of metformin and in the presence of both the dye and the oxidizing agent in their optimum quantities. The absorption was measured after dilution with distilled water at 520 nm, and Table (3) shows that hydrochloric acid is optimal in the oxidation process because it gives the maximum absorption. Accordingly, it was used in subsequent studies.

Table 3. Effect of acid type

1.0 ml of acid	HCL	CH3COOH	H2SO4	H3PO4	HNO3
Absorbance	0.234	0.480	0.390	0.320	0.291

Effect of concentration and amount of acetic acid

To reach the optimal concentration of acetic acid, different concentrations were prepared, ranging from (0.5-3) molar, and added to 25 ml volumetric bottles containing 8 micrograms. 1 ml of metformin and 2 ml of N-bromosuccinamide, the concentration of 2 x 10-2, and wait for five minutes, followed by adding 40 micrograms. 1 ml of Eriochrome Black-T dye, and the absorbance was measured at 520 nm ten minutes after filling the bottles with distilled water. Table (4) shows that the concentration of 1.0 molar was optimal. Accordingly, it was adopted in subsequent studies.

Table 4. Effect of acetic acid concentration

Molarity of acid	0.5	1.0	1.5	2.0	2.5	3.0
Absorbance	0.320	0.490	0.390	0.302	0.294	0.282

To determine the ideal quantity of acid, we experimented with varying volumes of acid, ranging from 0.5 to 3.0 milliliters, each at a concentration of 1.0 molar. By replicating the same procedural steps as previously described, we observed that 1.0 milliliter yielded the highest absorption, which has been adopted as the standard in subsequent investigations. Table (5) provides a summary of the optimum volume of hydrochloric acid.

Table 5. Effect of volume of acetic acid

Volume of acid ml	0.5	1.0	1.5	2.0	2.5	3.0
Absorbance	0.390	0.491	0.399	0.386	0.380	0.202

The effect of temperature and time on the oxidation reaction and stability of the Eriochrome Black-T dye

The effect of temperature and stability time of the product was studied at temperatures (37 °C, 50 °C) in the presence of a fixed amount of metformin, 8 micrograms. 1 ml and 1.0 ml of 1.0 molar concentration of acetic

acid, followed by adding 2 ml of the oxidizing agent N-bromosuccinamide and waiting for five minutes, then adding the optimal amount of eriochrome black-T dye and measuring the absorbance at 520 nm after completing the volume with distilled water to 25 ml. Figure (4) shows the optimal temperature for stability and estimation of metformin, which is the laboratory temperature (37 °C) because it gave the highest absorption ten minutes after the start of the reaction.

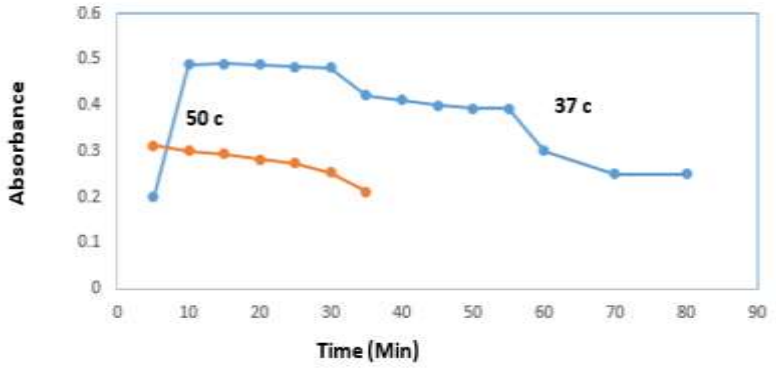


Figure 4. Effect of temperature and settling time

Effect of surfactant

To determine the effect of surfactants on the absorption of the Ariochrome Black-T dye when determining metformin, different types of surfactants were studied by adding 1.0 ml of each substance at a concentration of 0.1%. Table (6) shows the negative results of these substances. Therefore, I was excluded from the study.

Table 6. Shows the negative effect of surfactants

Surfactant	without	CPC	SDS	Tween-20
Absorbance	0.490	Turbid	Turbid	0.270

Effect of addition sequence

Five variable-order addition sequences were studied to obtain the best sequence that gives the highest absorption. The results recorded in Table (7) show that the sequence (I) is the best and was followed in studies of stabilization of conditions.

Table 7. Effect of addition sequence

Order Number	Reaction of component	Absorbance
I	D + A + O + E	0.491
II	D + O + A + E	0.398
III	E + D + O + A	0.400
IV	E + A + O + D	0.343
V	E + O + D + A	0.324

Metformin(D), Acitic Acid(A), N-Bromosuccinamide(O), Eriochrome Black-T(E)

Final absorption spectrum

After reaching the optimal conditions for metformin determination and stabilization, the final absorption spectrum of different concentrations was plotted against their mock solutions. The highest absorption peaks were obtained at 520 nm, as shown in Figure (5). Table (8) shows the optimal conditions that were reached for metformin.

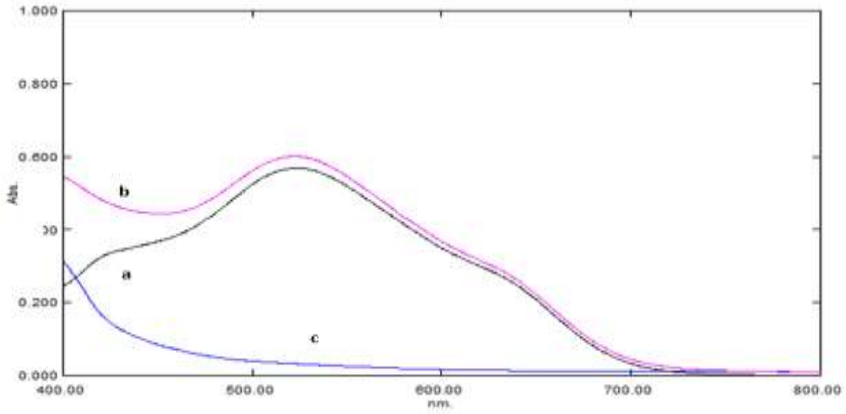


Figure 5. Final absorption spectrum

a. The reaction product of 10 µg.mL-1 of metformin versus the mock solution

b. Reaction product: 10 micrograms. 1 ml of metformin versus distilled water

c. Mock solution versus distilled water.

Table 8. Summary of optimal conditions for metformin

Experimental condition	Metformin
λ max (nm)	520
Color	Dark purple
Conc .of acetic acid (M)	1
Vol. of acetic acid (ml)	1
EBT (500µg/ml) amount (ml)	2
Conc.of N- bromosuccinimide(M)	2×10-2
Vol.of N- bromosuccinimide(ml)	2
Oxidizing time (min)	5
Development time after dilution(min)	10

How to make a standard curve for metformin estimation.

Increasing amounts of metformin (0.5-4 ml) at a concentration of 100 micrograms were added. ml-1 to a group of 25 ml volumetric bottles, then add 1.0 ml of acetic acid solution, followed by adding 2 ml of N-bromosuccinamide at their optimum molar concentrations, shaking and waiting for five minutes, then adding 2 ml of the Eriochrome Black-T dye solution at its optimal molar concentration. 500 micrograms. ml-1, and the absorbance was measured after ten minutes of dilution with distilled water against the mock solution at 520 nm, at laboratory temperature (37°C). Figure (6) shows the standard curve for metformin, which complies with Beer's law at concentrations ranging from (2-16) micrograms. ml-1, the molar absorption coefficient was 4 x 104 l/mol.cm, and the detection limit was 0.167 micrograms. ml-1 and the limit of quantity is 0.557 micrograms. ml-1.

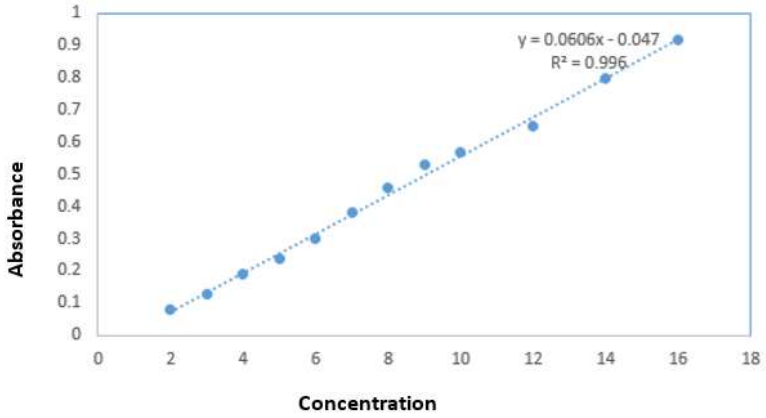


Figure (6) Standard curve for metformin hydrochloride

One can notice from the figure above that the value of the correlation coefficient equals 0.996, indicating that the standard curve has excellent linear specifications. Table (9) shows those specifications.

Table (9): Linear specifications for metformin

Parameters	Linearity range (µg/ml)	Intercept	Slope	Correlation coefficient (R2)	LOD (µg/ml)*	LOQ (µg/ml)*	Molar absorptivity (L.mol-1.cm-1)
Metformin	2-16	0.04	0.2423	0.996	0.167	0.557	4X104

*Average of ten determinations

Accuracy and compatibility of the method

The accuracy and compatibility of the proposed method were studied by calculating the recall and relative standard deviation using three replicates for three different concentrations of metformin. The results recorded in Table (10) show the accuracy and compatibility of the method.

Table 10. Method accuracy and compatibility

Compound	Amount added(µg/ml)	Recovery* (%)	Average recovery (%)	RSD*
Metformin	4	100.52	100.25	0.905
	8	98.69		0.009
	14	101.25		1.002

Average of three determinations*

Application of the method to pharmaceutical preparations

I took ten tablets of the medicinal preparation, weighed, ground, and mixed well. Then I took the equivalent of the weight of one tablet, dissolved it with distilled water, filtered, and completed the volume to 100 milliliters to obtain a concentration of 5000 micrograms. ml-1, withdraw 2 ml and dilute to 100 ml to prepare a solution with a concentration of 100 micrograms. 1 ml and take different concentrations of the solution (4, 8, 14) micrograms. mL-1 Then the concentration of the medicinal compound in the tablets was found through the standard curve of the pure substance and the results were recorded in Table (11).

Table 11. Application of the method to pharmaceutical preparations

Pharmaceutical preparation	Certified Value (mg)	Amount present (µg/ml)	Amount found (µg/ml)	Recovery (%)	Average recovery (%)	Average recovery (mg)
Metformin	500	4	3.95	98.75	99.3	496.5
		8	7.88	98.5		
		14	14.1	100.7		

Evaluation of the results of the developed method

Due to the lack of materials and devices of the British Constitution in assessing the two forms and to prove the accuracy of the proposed method. The proposed method was evaluated statistically through a t-test at a confidence level of 95% for three replicates by applying the law below.

$$t_{exp} = \frac{|\mu - \overline{X}| \times \sqrt{n}}{s}$$

Note that the real value (μ) equals 4 micrograms. ml-1, and the arithmetic mean is 0.191 for three replicates, with a standard deviation of 0.00173. The results are listed in Table (12)

Table (12) Evaluation of the results of the developed method for metformin

Drug	Pharmaceutical preparation	Recovery (%) Present method	t_{exp}
Metformin	Glucophage	99.3	3.7

From the above table, it is clear that the practical t value is less than its tabular value of 4.303 and has three degrees of freedom at a 95% confidence level. This indicates that the proposed analytical method is reliable and valid for application to pharmaceutical preparations.

Conclusion

A simple, indirect spectroscopic method was proposed for the determination of metformin. It relied on oxidizing metformin with an amount of N-bromosuccinimide in the presence of acetic acid, and the excess amount of it oxidized the dye Eriochrome Black-T. The absorbance was measured at 520 nm, and the molar absorption coefficient was 4 x 104 l/mol.cm, an indication of the sensitivity of the method. Complying with Beer's law for concentrations ranging from (2-16) µg.ml-1, the method was characterized by good accuracy and compatibility, as the recoverability reached 100.155%. While the relative standard deviation was less than 1.0, the method was also distinguished by its simplicity and ease, due to not using the extraction and heating processes. The method was applied to metformin pharmaceutical preparations and the results were found to be consistent with the standard addition method. When examining the validity of the application of the pharmaceutical preparation (tablets) and evaluating the result with the t-test, it was less than its tabular values.

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