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Reverse flow injection spectrophotometric method for determination Tenoxicam using 3-Methyl-2-benzothiazolinone hydrazine hydrochloride monohydrate

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ABSTRACT
The concentration of tenoxicam (TX) in pure and pharmaceutical tablets was measured by the use of an oxidative coupling reaction in a one-of-a-kind research study that utilised the reverse flow injection method (rFIA). Tenoxicam is used in the process, which entails reacting it with 3-Methyl-2-benzothiazolinone hydrazine hydrochloride monohydrate in the presence of sodium periodate. The end result is the creation of a product that has a green colourpattern.Absorbance was found to be at its maximum at a wavelength of 607 nm, according to the findings.Two to one hundred micrograms per millilitre is the range of concentration linearity that is taken into consideration. A thorough examination of the parameters, which encompasses the concentration of the reagent, the various types of oxidants, the concentrations of the oxidative agents, the flow velocity, the injection volume, and the reaction coil, among other things.

Keywords: 3-methyl-2-benzothiazoline hydrazone, Tenoxicam compound, spectrophotometric methods, Reverse flow injection methods.

INTRODUCTION

Reagent (3-methyl-2-benzothiazolinone hydrazone hydrochloride) is a pharmaceutical reagent and a chromogenic reagent used to measure a variety of pharmaceutical amines using spectrophotometry. Other names for reagent are (MBTH, Besthorn's reagent and Sawicki's reagent) [1]. Molecular mass: 233.72g $.mol^{-1}$, Solubility: 1% solution in water is clear and pale yellow color. Molecular formula: $C_8H_9N_3SHCl.H_2O$. Produced exceptionally colored yields by combining a specific through an oxidative process with aromatic amines, heterocyclic bases, phenols, and molecules with active methylene group [2,3]. use of high-purity reagent for estimating carbonyl compounds in analytical chemistry like substituted aromatic amines [5], chemical structure shown in Figure (1) .

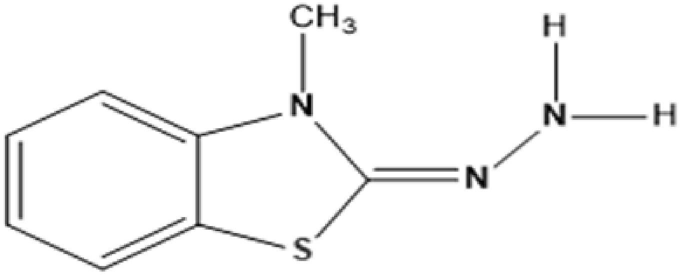

Figure 1: Chemical structure of MBTH reagent.

Tenoxicam (TX), is a nonsteroidal anti-inflammatory and analgesic substance that belongs to the oxicams . chemical Iupac name is [[4-hydroxy-2,methyl-N-2-pyridyl-2H-thieno(2,3-e) - 1,2-thiazine-3-carboxamide-1,1-dioxide]]. It's practically insoluble in water (14.1 µg.mL^{-1}), soluble in ethanol, chemical structure [6,7] shows in Figure (2).

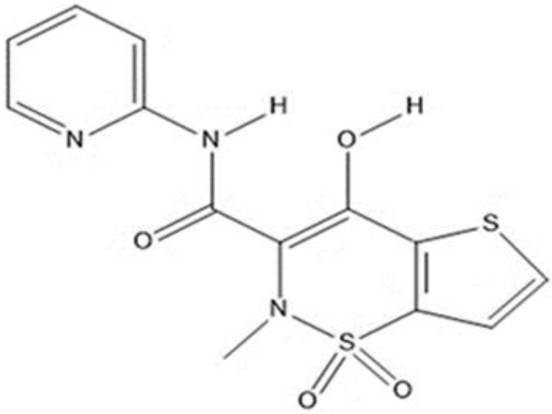

Figure 2. Chemical structure of Tenoxicam

TX used for treatment of chronic rheumatic diseases [8]. It been determined with the different methods such as flow injection spectrophotometric [9], high-performance liquid chromatography [10]. TLC analysis [11], ultraviolet spectrophotometry [12], potentiometric ion-selective membrane electrodes [13], infrared spectrophotometry [14], electroanalytical [15].Examples of oxidative coupling pharmacological reactions. Following the oxidation of these components, intermediary chemicals are produced, which combine to produce a colored product that can be spectrally detected[16,17].The suggested method include oxidative coupling reaction between TX drug with MBTH reagent in presence of $NaIO_4$ to generate a green liquid complex[18-21] .Using spectroscopic reverse flow injection method and study all physical and chemical parameters. This is advantage from this reaction for the aim of quantification of this compound.

METHODOLOGY

Chemicals

- **Tenoxicam (TX) stock standard :(**200 g. mL^{-1}) was prepared in a controlled manner weighted 0.02 g of TX and solve in 10 mL of ethanol, followed by the addition of 90 mL of low-mineral water and filling up a 100 mL volumetric flask to the mark.
- **• Medications tablets:** The TX tablets were purchased at a pharmacy in Baghdad, Iraq, and two samples, (Tilcotil:Tenoxicam 20 mg, 10 film-coated tablets, Switzerland) and (Tenoxam 20mg Flim-coated tablet, Turkey), were analyzed using the suggested methodology. Weighed each sample 0.02 g, then solve in 10 mL of ethanol solvent with 90 mL of low-mineral water, filled a 100 mL volumetric flask with it, filtered out the additives, and then diluted it in a 25 mL volumetric flask.Finally, liquid from two samples was taken and placed in two volumetric flasks (size 25 ml).
- **MBTH reagent:** Prepare 0.3% (w/v) solution, 0.3g of MBTH reagent was weighed, transferred to a 100 ml calibrated volumetric flask, dissolved in low mineral water, and the volume was built up to the mark.
- **Sodium period ate $NaIO_4$:**Molar mass: 213.89 g/mol, purity 99%, company: (BDH , England). Weighted 0.4278 g from substance (0.02 M) , dissolve in 100 ml distilled water than full in (size 100 ml volumetric flask).

Instrumentation

- A Shimadzu UV mini -1240 digital single beam spectrophotometer with a movement cell with a 50 l internal volume and a 1 cm path length is used to measure the absorbance of colored products.
- Sensitive balance (ISO LAB laboratory).
- Distilled set (Model –WD 1004)
- Standard rFIA practice: A stream of oxidant (0.02 M NaIO4) and a portion of the reagent of MBTH (0.3%) were coupled with a stream of TX (50 g mL-1). 150 l of sample was injected, and the reaction coil was 50 cm in length. The total flow rate was 2.5 mL min-1. The colored dye's subsequent absorbance was calculated using its maximum wave length. A calibration graph using experimental parameters was created for the specified range.

RESULTS AND DISCUSSION

Absorption spectra

To measure absorbance must prepare 50 µg.ml^{-1} conc. of TX .Experimentally prepared 0.003M of MBTH reagent and 0.02M of $NaIO_4$ oxidant agent for oxidation process . To produce the high intensity color, the addition was tested in various quantities from prepared solutions figure (3) show two volumetric flask: green liquid for TX complex and colorless is blank .Absorbance measured by UV-Vis spectrophotometer and recorded maximum wave length at 607 nm. While figure (4) explain mechanism of coupling .After that Complete study the experimental conditions to reach to optimization.

Figure 3. Green liquid for drug complex and colorless for blank

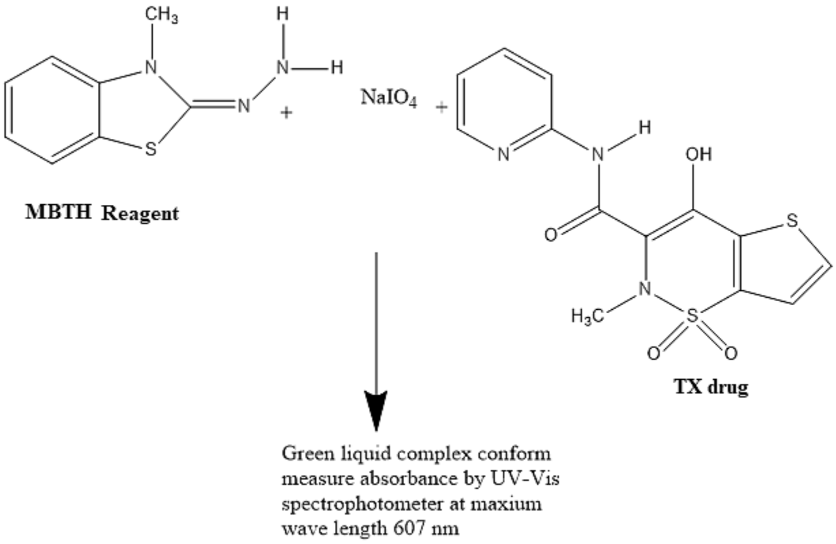

Figure 4. Mechanism of Oxidatve coupling between TX

Optimization of coupling conditions

For determination of TX drug using rFIA method must study experimental parameters to perform the coupling between TX drug and MBTH chromogenic reagent in presences $NaIO_4$oxidative agent through. The experimental chemical and physical parameters are described in Table 1 together with the TX concentration that was set on in all subsequent tests (50 g.mL^{-1}).

Table 1: Exploratory parameters

Parameter	**Value**
Conc. of MBTH (M)	0.003
Conc. of $NaIO_4$ (M)	0.02
Rate of flow (mL.min^{-1})	2.5
Coil of reaction (cm)	50
Sample volume (µL)	150

Order of mixing

Two paths rFIAmethod are (A and B) manifolds used for coupling of TX drug. The rFIA manifold contained two channels for $NaTO_4$ and TX solutions. After defining every criterion, it was discovered that the (A) approach provided the greatest absorbance when compared to the (B). After merging with the TX stream solution in flowing carrier to the injected volume of MBTH. In order to find the ideal circumstances, the rFIA manifold (A) was created to examine the chemical and physical variables. Figure (5) diagram proposed method and table (2) written the absorbance result for A and B manifolds, the higher absorbance chosen for complete study.

Table 2. Types Manifold used for proposed method

Manifold	Absorbance
A	0.589
B	0.224

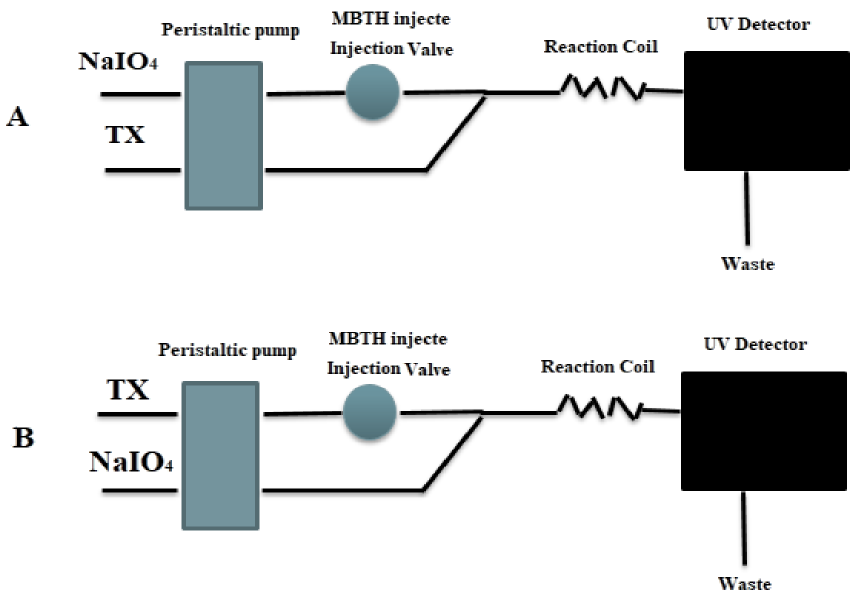

Figure 5: rFIA manifolds (A and B) proposed for determination of TX , maximum absorbance was shown in a manifold A.

Effect of parameters

1-Effect of MBTH concentration and oxidant (chemical parameters)

Series of MBTH reagent concentrations range (1×10^{-3} to 7×10^{-3} M) was studied. Results drown in Figure (6) recorded maximum absorbance at 0.003 M but the absorbance decreased after this concentration because MBTH used as accepter equivalent concentration to coupling.

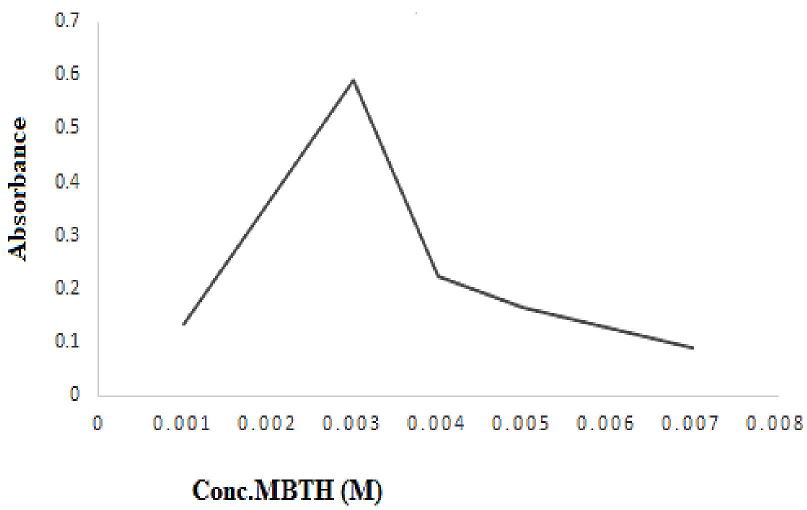

Figure 6. Effect of MBTH concentration on absorbance of coupling product

A good oxidizing agent for the oxidative coupling reaction has been discovered to be $NaIO_4$,figure (7) among five oxidants studied like $K_3[Fe(CN)_6]$, $K_2S_2O_8$, $FeCl_3.6H_2O$ and $(NH_4)_2[Ce(NO_3)_6$ which not affective with reagent for coupling with TX drug . Different concentrations rang (0.005 to 0.05 M) of $NaIO_4$ used for study effect chemical parameter Figure (8).Indicating that absorbance would increase when oxidant concentrations were raised, but after the chosen concentration of 0.02 M, the absorbance fell since there was less reagent available.

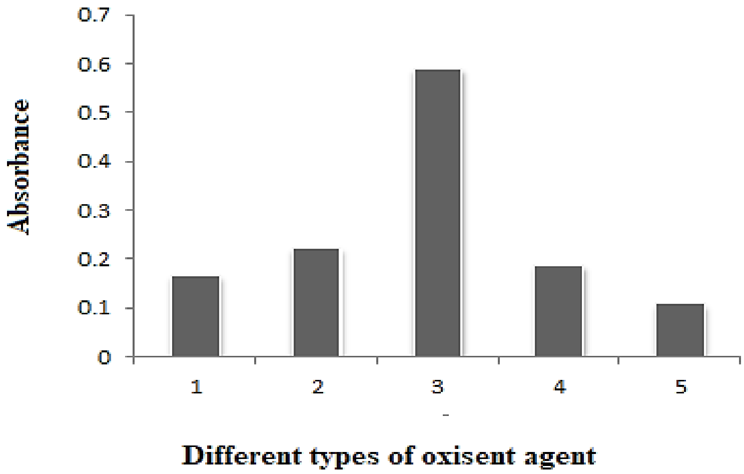

Figure 7. Oxidative agents include: [1. $K_3[Fe(CN)_6]$, 2. $K_2S_2O_8$, 3. $NaIO_4$ .4. $FeCl_3.6H_2O$, 5. $(NH_4)_2[Ce(NO_3)_6$, the higher column for no 3 was sodiumperiodate recorded maximum absorbance .

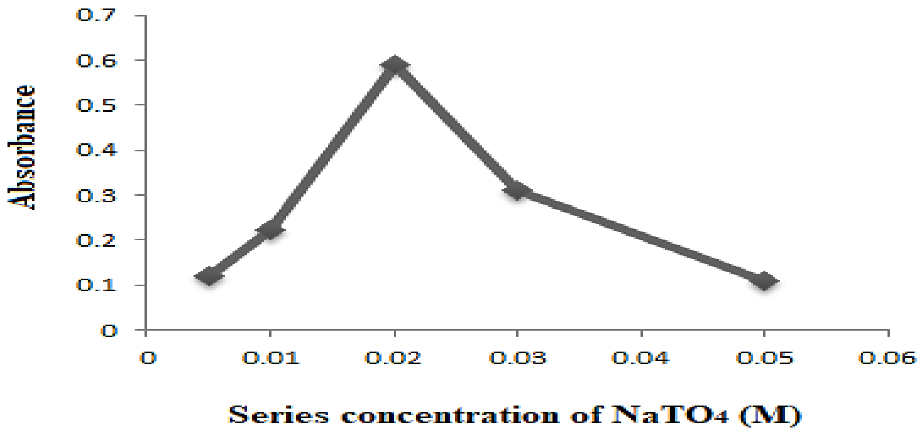

Figure 8. Effect of various concentration of $NaIO_4$ (M) recorded 0.03M great effect in coupling

2-Physical characteristics [reaction coil length, flow rate, and injected volume]

Reaction coil was evaluated with the best physical parameters between (25-250 cm). Having the best absorption value observed over a length of 50 cm and a low dispersion figure (9), the result is satisfactory. Sample dilution and dispersion may have increased, causing the absorbance to drop. In this experiment, several injector loop volumes were tested. The range (60-200 L) of the injected reagent volume was investigated. When the injection volumes were increased to 150 L in order to finish the studies, the absorbance increased. Figure (10), but later decreased due to a drop in absorbance and TX concentration. The maximum flow rate, which was studied in the range of 0.25 to 3.5 $mL.min^{-1}$, was shown to give the greatest absorbance value by the sensitivity and sampling rate experiments. Because the recommended reaction is highly unstable, a high flow rate is preferred. However, a flow rate that is higher than the chosen value results in a drop in absorbance due to the dispersion of the sample zone. Results appeared sample throughput rate and sensitivity, give total flow rate at (2.5 $mL.min^{-1}$) in figure (11) was used.

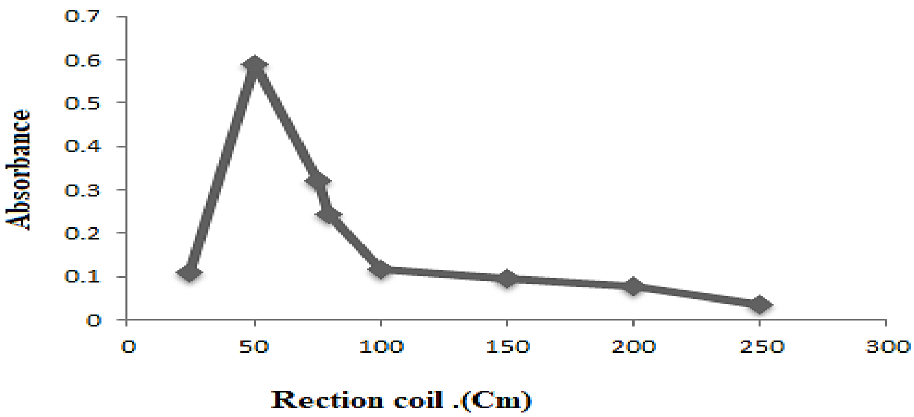

Figure 9. various coil tested

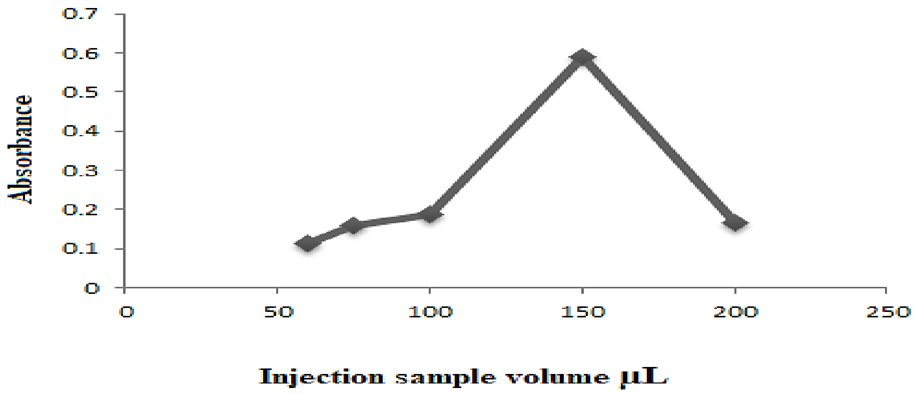

Figure 10. Effect of injected sample volume

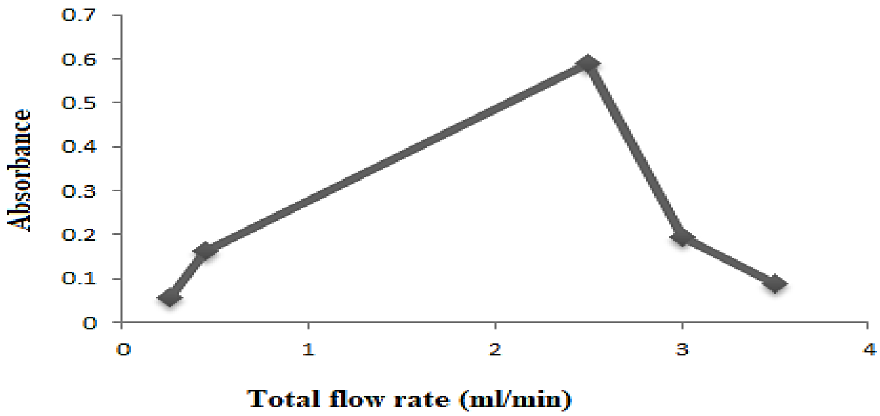

Figure 11. Effect of total flow rate

Analytical characteristics

After optimized all parameters, To check the linearity of TX concentrations (triplicate injections), a calibration graph was created. Table (3) lists the analytical results from the calibration graph, which showed high linearity and low statistical detection limits of 18. Figure (12) displayed different TX concentrations utilized at different ranges (2-100 l.ml-1). For the indicated approach used (20,50,70 l.ml-1), excellent precision and good reproducibility were attained. The maximum absorbance could not be seen until 20 seconds had passed.

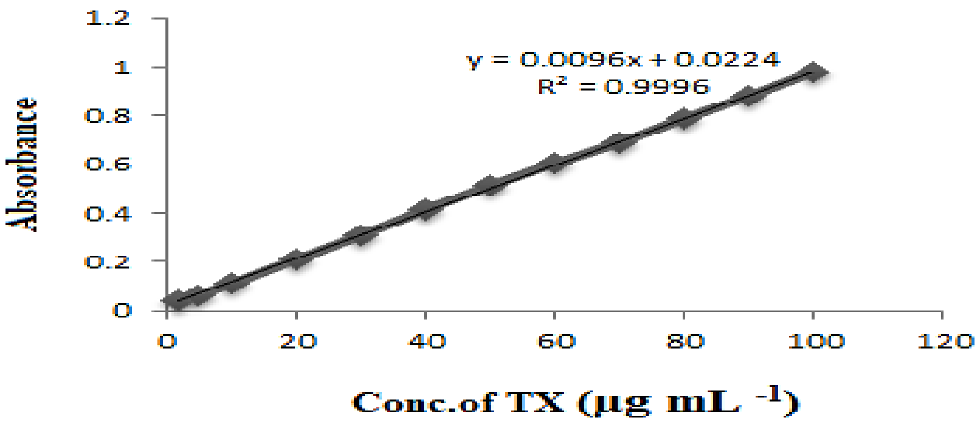

Figure 12. Calibration curve for TX drug obtained by reverse flow injection analysis

Table 3: shows the analytical findings from the suggested approach for determining TX.

Parameter	**Value for rFIA method**
Equation for regression.	y = 0.0096x + 0.0224
Coefficient of correlation, r.	0.9996
Percentage of linearity, r %.	99.96
Linearity range. (µg mL $^{-1}$)	2-100
Molar absorptivity. (Lmol^{-1} cm^{-1})	5433.72
LOD. (µg mL^{-1})	1.45
LOQ .(µg mL^{-1})	2.30

Table 4. The recommended approaches' accuracy and precision

Pure standard	**Conc.of TX (µg mL $^{-1}$)**		**Rec.%**	**RSD%**
	Present	**Found**		
Tenoxicam	20	20.35	101.75	0.43
	50	50.26	100.52	0.25
	70	70.13	100.19	0.36

Analytical application

The suggested method successfully were applied to analysis two pharmaceutical samples containing TX , two pharmaceutical tablets from various origins tested (Tilcotil (Tenoxicam 20 mg, Switzerland) and (Tenoxam 20mg Flim-coated tablet Turkey) .It's a good relative standard deviation and Recovery as shown in Table(5), The results obtained were calculate statistically[22-25].

Table 5. Direct applications of suggested method in pharmaceutical tablet.

Tablet	**Conc.of. TX. (µg mL $^{-1}$)**		**Rec.%**	**RSD%**
	Present .	**Found.**		
Tilcotil (Tenoxicam 20 mg, Switzerland)	20	20.07	100.35	0.23
	50	50.16	100.32	0.21
	70	70.03	100.04	0.08
Tenoxam (20mg Flim-coated tablet Turkey)	20	20.22	101.1	0.42
	50	50.31	100.62	0.20
	70	70.29	100.41	0.15

The method including comparsion between proposed method and method used another reagent and agent with new reaction .Table (6) shows the results for the method .

Table 6. Comparison suggested method rFIA analysis and method for determination TX worked by me.

Method	Conc .rang (of TX (µg .mL $^{-1}$)	Regent	Reaction
Suggested method	2-100	0.003 M MBTH reagent and NaIO4 agent	Oxidative coupling reaction ,color product recorded maxiumam wave lengh at 607 nm ,Correlation coefficient, r (0.9996)
rFIA method[27]	2-140	0.005 M for MFS and $K_2S_2O_8$ agent	Charge transfer interaction ,color product recorded maxiumam wave lengh at 530 nm , Correlation coefficient, r (0.9997)

Applied studying the effect of Tenoxicam on biological samples tracking different concentrations (0, 0.2, 0.4, 0.6, 0.8) mg /dl (shows in calibration below figure (13)) by adding to blood samples Adults to obtain the effect of the drug on uric acid. Using Ultraviolet-visible (UV-Vis) spectrophotometry for analysis samples.

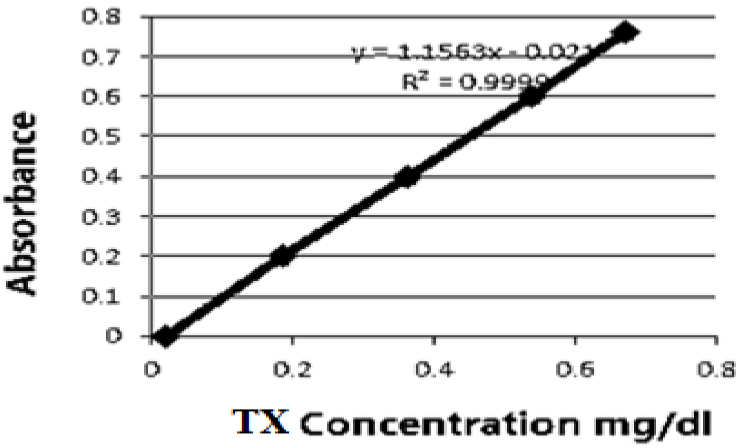

Figure 13. Calibration curve for TX using UV-Visible spectrophotometer

The last analysis was done and published at 2023[28] .while This research appeared r-FIA analysis proposed method for determination of TX in pure and pharmaceutical tablets was complete study of TX .

CONCLUSION

Tenoxicam is coupled with the pharmaceutical reagent MBTH utilizing the reverse-flow injection approach to determine the drug concentration in pharmaceutical samples, the oxidative coupling reaction is successful at the maximum wave length of 607 nm. Decreased reagent use, less sample volumes, linearity, and precision were examined. With positive outcomes and a correlation coefficient of (r: 0.9996), this method was applied to the analysis of TX in real samples at a variety of concentration levels. The techniques are straightforward and affordable, but data recording requires attentiveness.

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