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Spectrophotometric method for the determination of chlordiazepoxide, diazepam and alprazolam drugs by using oxidation coupling reaction

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***Abstract*---**Sensitive, accurate, precise and efficient spectrophotometric"method for the determination of chlordiazepoxide, Diazepam and Alprazolam drug (CH, DIA, ALP) in pure as well as in dosage form was described. "The suggested method was based on oxidation coupling reaction of suggested drug (CH, DIA, ALP) drug using acidic buffer "then"coupling with"3-methyl-2-benzothiazolinone hydrazone hydrochloride"(MBTH) in the presence of ferric chloride. Spectrophotometric measurement was established by recording the absorbance of the blue colored product at (663,609,619) nm. Using the optimized reaction conditions, beer' s law was obeyed in the range of"(2.5-25) µg/mL "(5-30) µg/mL, and "(5-30) µg/mL with good correlation coefficient of (0.9997, 0.9979, 0.9965) and the LOD and LOQ (CH,DIA,ALP) values were(0.1750, 5.834 , 0.676 and 2.253 ,0.881and 2.938)µg/mL for chlordiazepoxide, Diazepam and Alprazolam drug respectively. The accuracy and precision of the proposed method represented by recovery and relative standard deviation were satisfactory; about 99.33% and)") 0.502and 0.195, 0.46) µg/mL for chlordiazepoxide, Diazepam and Alprazolam drug respectively. The "proposed" method "was" applied "for" determination"

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of (CH, DIA, ALP) in "its" pharmaceutical" "forms" and"the "results" compared successfully "with" the "obtained" by "standard" method (British"pharmacopeia"method).

***Keywords*---**chlordiazepoxide, Diazepam and Alprazolam, MBTH, Oxidative coupling reaction.

Introduction

Chlordiazepoxide, 7-chloro-2-methylamino-5-phenyl-3H-1, 4-benzodiazepine-4-oxide [1-2].It has an empirical formula of $C_{16}H_{14}ClN_3O$ and has a molecular weight of 299.80g/mol. It was the first benzodiazepine to be used clinically in the treatment of generalized anxiety disorder. It has characteristics comparable to diazepam and is used in the treatment of anxiety disorders in the short term and insomnia [3].

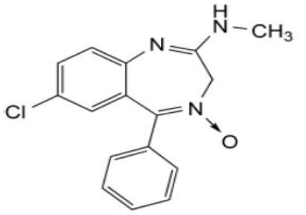

Fig. 1 The structure of chlordiazepoxide

For the purpose of detecting chlordiazepoxide in a variety of samples using an ultraviolet detector, high-performance liquid chromatography has been developed [8–12]. DOX was extracted from plasma samples using a solid-phase extraction method, with a Zorbax CN column serving as the stationary phase [5]. Ion-pair and charge-transfer complexation processes were performed to determine the amount of DOX in tablets using spectrophotometric techniques [6].

Alprazolam is an anxiolytic medication that belongs to the benzodiazepine chemical family. The UPAC name of the drug is, 8-Chloro-1-methyl-6-phenyl-4H-(1,2,4) triazolo(4,3-)(1,4)-benzodiazepine. It used as anxiety disorders, agoraphobia, panic disorders, and depression are all treated with it. [4-5]

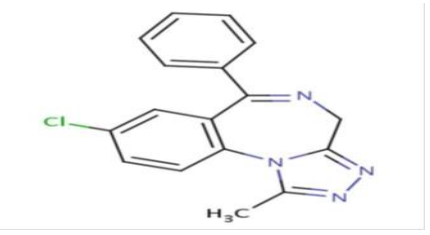

Fig.2 The structure of Alprazolam

Diazepam 7-chloro-1, 3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one is the chemical name for diazepam (figure- 3). Diazepam (DIA) is a benzodiazepine that is commonly used as a muscle relaxant, hypnotic, and anxiolytic. For abrupt convulsions and protracted status epilepticus, DIA is commonly recommended as the first-line therapy. [6-7]

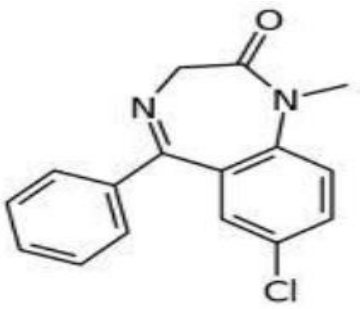

Fig. 3 The structure of Diazepam

HPLC[8-12] and Micellar electrokinetic chromatographic technique have been reported to be used to estimate chlordiazepoxide (CH) alone or in combination with other medicines. Amperometric determination [13], HPLC[14],HPLC[15-16], and Micellar electrokinetic chromatographic technique are used to determine the concentration of chlordiazepoxide alone or in combination with other medications. A review of the literature indicated that Methods for estimating HP-TLC [17] and RP-HPLC [18-22),have been reported .In pharmaceutical formulations, alprazolam is used.

MBTH (also known as Sawicki's reagent or Besthorn's reagent) is a 3-Methyl-2-Benzothiazolinone Hydrazone

Besthron created 3-Methyl-2-Benzothiazolinone Hydrazone ($C_8H_{10}ClN_3S$, Molecular Weight 215.70g/mol for the first time in 1910. In order to generate very excellent yields, Huning and Fritsch defined this reagent's oxidative coupler with aromatic amines, heterocyclic bases, phenols, and compounds having active methylene group in 1957. Sawicki et al. proposed MBTH as a practical reagent for the quantification of carbonyl compounds in analytical chemistry in 1961. Additionally, phenols, polyhydroxy compounds, aldehydes, aromatic amines, and amino hetero aromatic compounds like indoles, carbazoles, and phenothiazines can be revealed and estimated using this method. Fig.4 depicts the chemical composition of MBTH reagents 215.70[23].

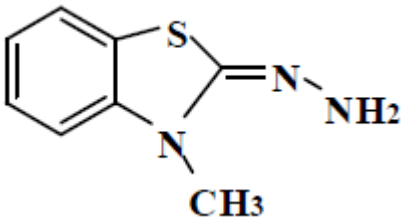

Fig. 4 Depicts the chemical structure of MBTH

The suggested procedure relies on the oxidation coupling of the drugs chlordiazepoxide, diazepam, and alprazolam with MBTH in the presence of ferric chloride as an oxidizing agent.

Experimental

Apparatus

--UV-Visible Spectrophotometer SHIMADZU-1800, (Kyoto, Japan) with 1.0 cm quartz cells was used for all absorption measurements.
- HANA 300 pH meter
- Water Bath ISO 9001
-Sensitive balance (Sartorius BL 210S)

Standards and reagents

Throughout this experiment, only analytical-grade chemicals and reagents and freshly made solutions were employed.

-Chlordiazepoxide, Alprazolam and Diazepam (CH , ALP, DIZE) solution 1000 µg/mL

The preparation of all Standard medications involved dissolving 0.0100 g of each medication in a 100 ml volumetric flask using ethanol as the solvent.

- Hydrochloride methylbenzothiazoline-2-one hydrazone, 0.2% w/v (14) (Sigma-Aldrich)

In a 100 ml volumetric flask, the MBTH reagent was prepared by dissolve 0.200 g of the reagent with distilled water to get $1.12x10^{-3}$ M . The solution should be kept in 5-8 C^0 at least one weak.

-**Ferric Chloride . $FeCl_3$ $3.3x10^{-3}$M**

0.162g of anhydrous Fe^{+3} of the oxidant were dissolved in 100 mL of a 1 % HCl solution to create $3.3x10^{-3}$ M of this acidic solution. Daily preparation of the working solution involved proper dilution with the same solvent.

-Hydrochloric acid 0.2M aqueous solution.

The solution was prepare by dilution of appropriate volume of concentrated solution of the acid.

Medication Formulations

The various pharmaceutical concoctions were bought from a commercial vendor in the neighborhood market:

Twenty tablets (LIBROXIDE 10 Chlordiazepoxide 10gm) SDI Iraq (XANAX, Pfizer, compressed Oral) each containing 0.5 mg of alprazolam, AL-Kindi Co. For pharma .Ind .Baghdad-Iraq VAL IUPAM 5 TABLETS Diazepam USP 5 mg.

Solutions of pharmaceutical preparations

Ten tablets from each of the selected companies were carefully weighed and then crushed and mixed well using a ceramic mortar. A weight equivalent to 0.1000 g of the active substance was taken from them for the concentration of 10mg and the same equivalent weight of the active substance for the preparation that had the concentration of the active substance equal to mg10. Dissolve it in the previously prepared solvent and in a beaker volume of 50 mL and after checking the dissolution process using an ultrasound device for a quarter of an hour The mixture was filtered by 45 micron filter paper, then the filtrate was transferred to a 50 mL volumetric vial, and the volume was completed to the mark with the same solvent previously prepared to obtain a concentration of 1000 µg/mL then we perform the dilution process to obtain the working concentrations. . For the

proposed method, the content of a tablet was calculated using the corresponding regression equation of the appropriate calibration graph.

Recommended procedure

In a series of 10 ml volumetric flasks, 2mL for CH,DIZ,ALO (100 µg/mL) ,then 1.5 mL of ($1.12x10^{-3}$ M) was added and shake well. A 1 ml ($3.3x10^{-3}$M) of $FeCl_3$ was added and left the reaction for 10 min , then 0.1 ml of concentrated HCl was added, leave the solution for 10 min . The solution make up to the mark with distilled water. The spectrum of the colored compounds was recorder in the range of (400- 800nm) which get a maximum absorption verses reagent blank prepare in the same way expect of absence drug(30).

Results and Discussion

Benzodiazepines group containing drugs are very important kinds of drugs, but the spectrophotometric determination of these drugs (CH, ALP and DIZE) is not easy because of the poor affinity of these drugs to react directly with other coupling reagents. The present work depends on a simple oxidative coupling reaction between (CH, ALP and DIZE) and MBTH in the presence of an efficient oxidant ($FeCl_3$). A blue colored product was formed and have a maximum absorbance at (663nm, 619nm, 609nm) for CH, ALP and DIZE respectively .and it was used in all subsequent experiments, The absorption spectra of the reaction product and the reagent blank are presented in Figure 5,6,7.

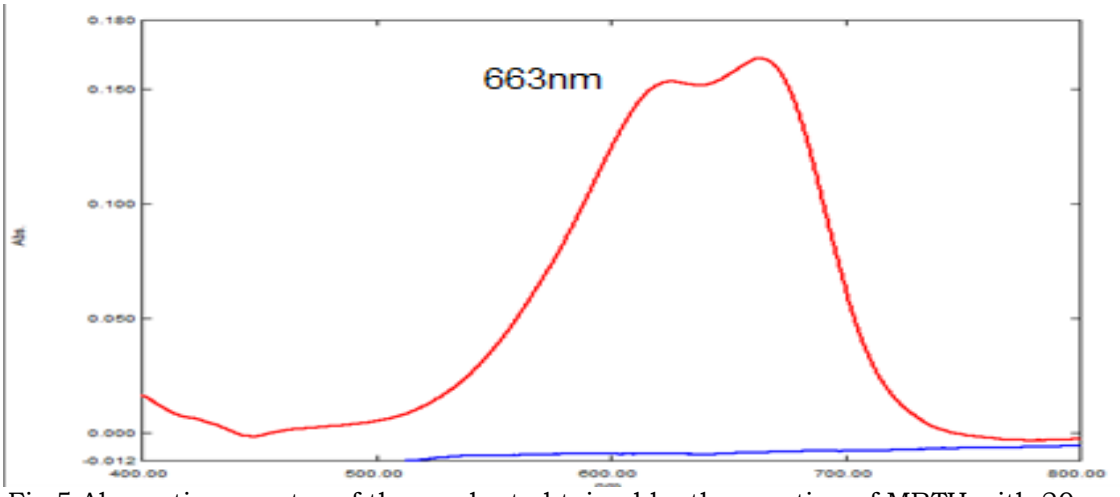

Fig.5 Absorption spectra of the product obtained by the reaction of MBTH with 20 µg/mL of CH in presence of $FeCl_3$ versus reagent blank

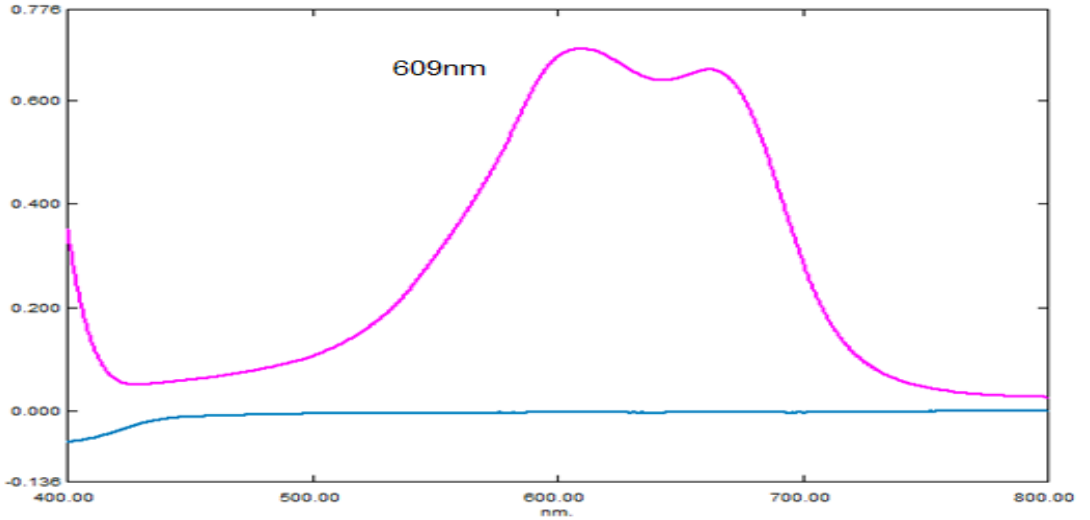

Fig.6Absorption spectra of the product obtained by the reaction of MBTH with 20 µg/mL of DIZE in presence of $FeCl_3$ versus reagent blank

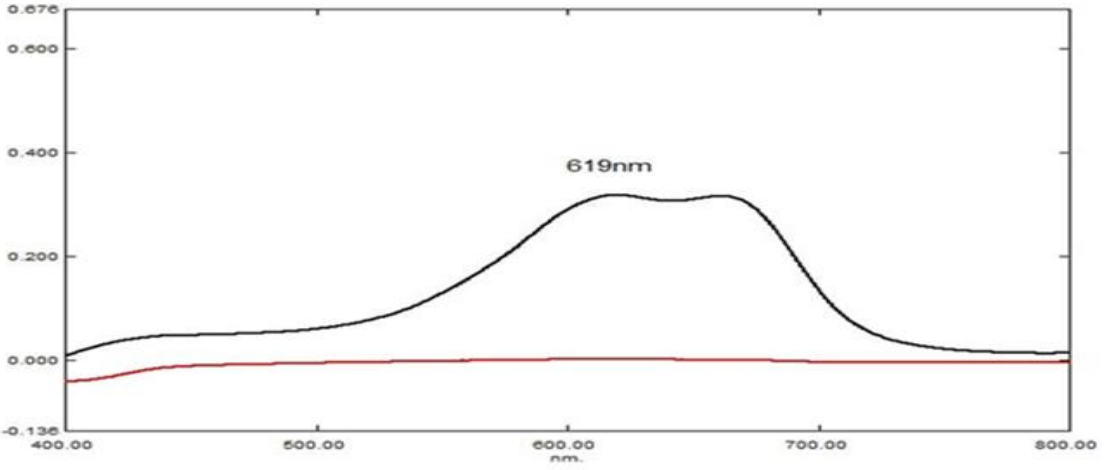

Fig.7 Absorption spectra of the product obtained by the reaction of MBTH with 20 µg/mL of reduced ALP in presence of $FeCl_3$ versus reagent blank.

Optimum reaction conditions

Optimization of the experimental conditions
Effect of the reagent MBTH

MBTH is an efficient coupling reagent for many drugs. In order to study the effect of the amount of reagent, different volumes in the range (0.5-3)mL MBTH of $1.2x10^{-2}$ M was examined in the presence of 1.5mL of ferric chloride(3.3 x^{-3} M). The results Figure. 8 show that 1.5 mL of the MBTH solution was enough to obtain a maximum absorbance, and it was used in the subsequent experiments.

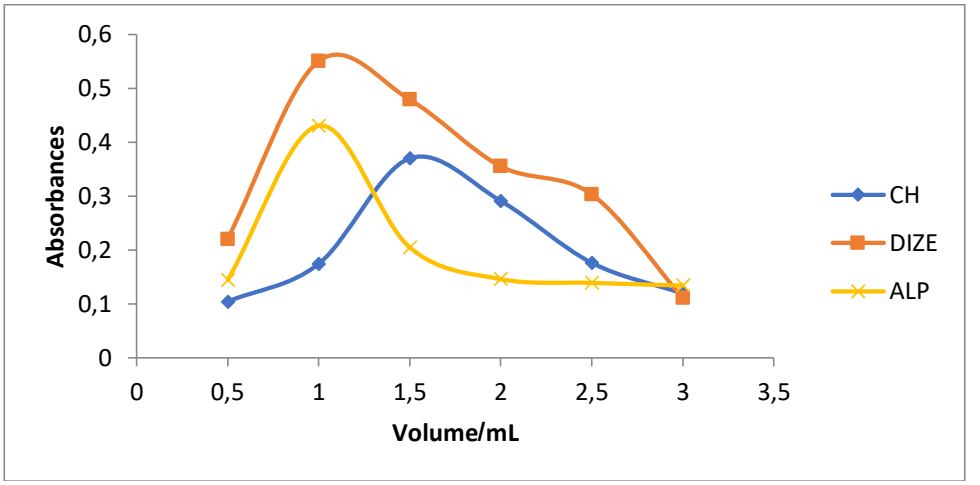

Fig. 8: Effect of the reagent MBTH

Effect of the reagent$FeCl_3$:

Different volumes in the range (0.5-3)mL of 3.3 x^{-3} M **$FeCl_3$** is an efficient oxidation reagent where used to study the effect of the amount of reagent, 1.5 mL MBTH of $1.2x10^{-2}$ M was used .The results Figure. 9 show that 1.5 mL of the 3.3 x^{-3} M **$FeCl_3$** solution was enough to obtain a maximum absorbance, and it was used in the subsequent experiments.

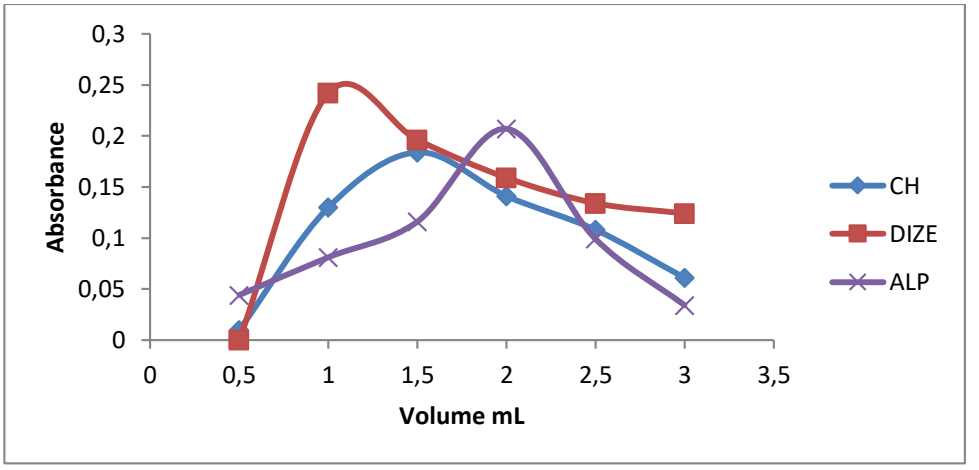

Fig.9:- Effect of the reagent$FeCl_3$

The Effect of Order addition

The order of addition is also studied under the obtained optimum results. According to the results, it was found that the order of addition of reagents (Drug CH, ALP, DIZE + MBTH + $FeCl_3$) as shown in respectively gave the maximum absorbance and stability in measurement as shown in Table .1

Table.1 The effect of order of addition of three drugs

DURG	Order of addition	Absorbance
chlordiazepoxide	Drug+MBTH+ $FeCl_3$	0.469
	Drug+ FeCl3+ MBTH	0.416
	MBTH+ FeCl3+ Drug	0.260
Alprazolam	Drug+MBTH+ $FeCl_3$	0.429
	Drug+ FeCl3+ MBTH	0.289
	MBTH+ FeCl3+ Drug	0.110
Diazepam	Drug+ MBTH+ FeCl3	0.577
	Drug+ FeCl3+ MBTH	0.517
	MBTH+ FeCl3+ Drug	0.274

Effect of temperature

The effect of temperature on the oxidative coupling reaction was also studied using three different temperatures (0, 25,35, 50, 65)c. Figure .10 shows that a maximum absorbance and a good stability were obtained hen the formed product developed at ambient temperature (25c).

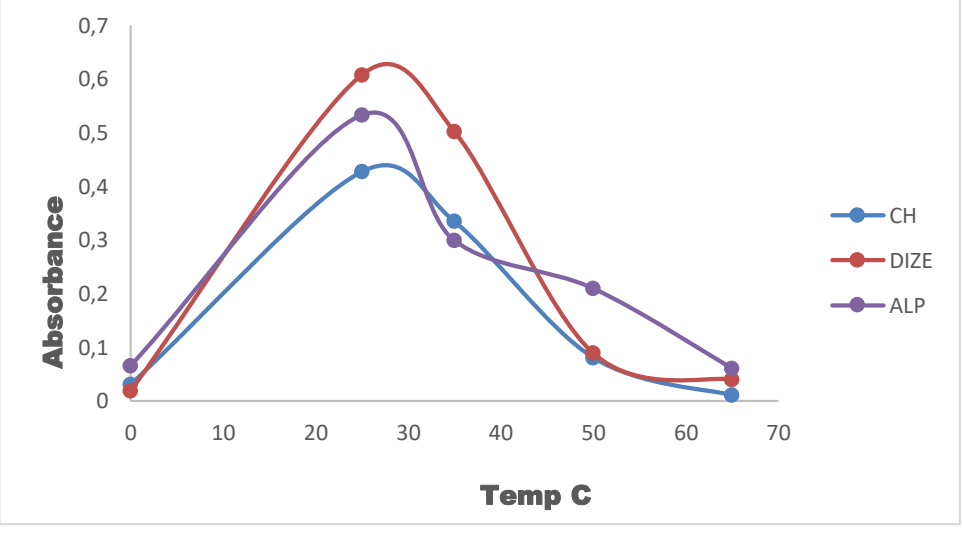

Fig.10: The effect of temperature on the reaction

Effect of reaction time

The drugs (20 µg/ mL, 2 mL 0f 100 µg/ mL) solution was reacted with MBTH and $FeCl_3$ in aqueous medium after 10 minutes. After 15 minutes, the color intensity stabilized and remained steady for at least 30 minutes (Figure.11). The experiment was repeated with the same outcomes after all reaction parameters were improved.

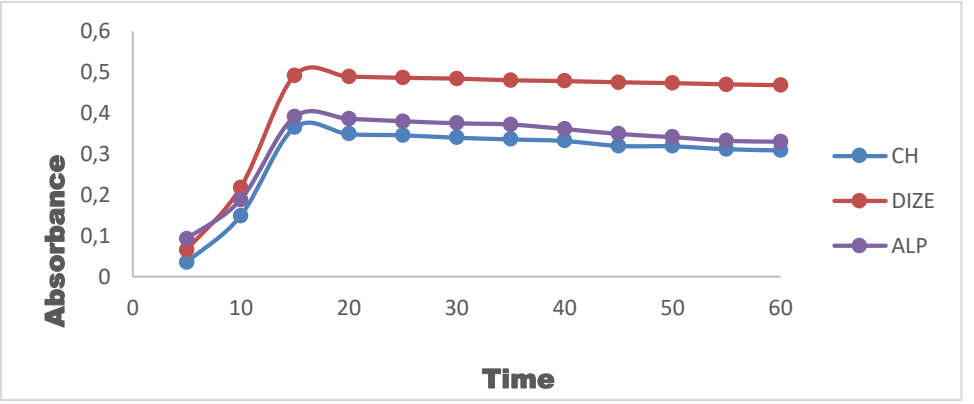

Fig.11 Absorbance versus time graphs for CH, DIZE, ALP with MBTH, $FeCl_3$

Plotting of Calibration curves

An increasing volumes of standard drug solution in the range (0.5- 3) mL of 100µg/mL CH were transferred into a series of 10 mL volumetric flasks to obtain the concentration range of (5-30) µg/mL. To each flask 1.5 mL of MBTH reagent (1.12×10^{-2}M) and 1.5 mL of $FeCl_3$ (3.3×10^{-3} M) HCl were added. After 15 min the contents were diluted to the mark with distilled water and mixed well. The absorbance of the colored product was measured after 15 min at 663 nm,609nm,619nm (CH,DIZE,ALP) respectively against the corresponding reagent blank prepared similarly omitting the drug content.

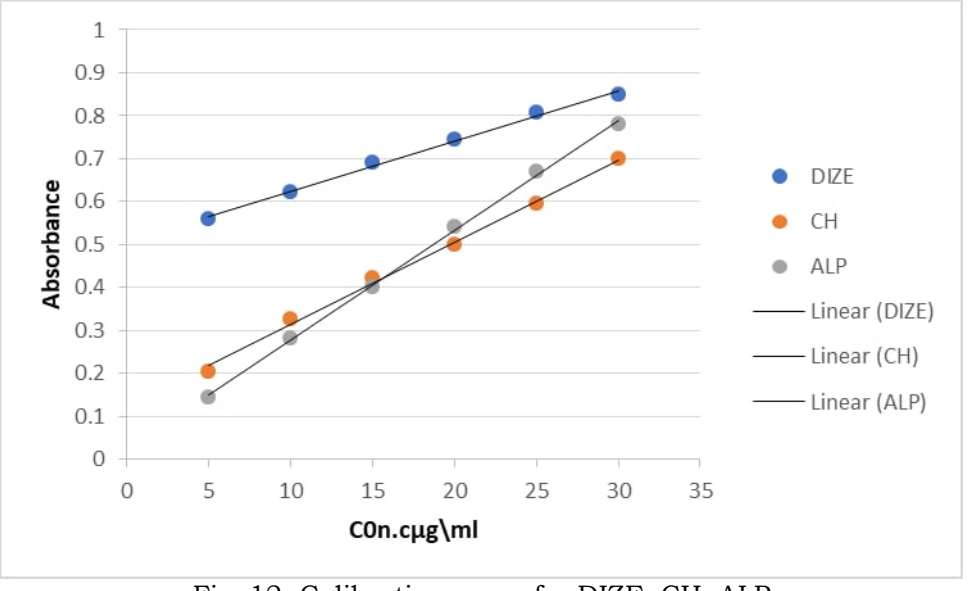

Fig. 12: Calibration curve for DIZE, CH, ALP

Stoichiometry of the coupling reaction using Job's method and mole ratio Method

The stoichiometry of the oxidative coupling reaction was calculated using 1ml of CH and 1.5ml of reduced MBTH at a constant 1.5ml oxidant concentration depending on Job's continuous change method and mole ratio methods. Job's method was used for continuous contrast of stoichiometric solutions: 1mL of standard solution of CH and reduced MBTH was used. A series of solutions were prepared in which the total volume of CH and MBTH was kept at 10 mL. The medicine and reagent were mixed with a different supplement.

Proportions (0:5, 1:4, 2:3....5:0, inclusive) and completed as directed under the recommended procedure. The absorbance of the resultant product was measured at optimum wavelength. In mole ratio method an increasing volume of MBTH (0.5, 1, 1.5, 2.....3mL) was added to 2mL,1mLand 1ml of reduced Chlordiazepoxide, alprazolam and Diazepam respectively, at constant oxidant concentration 1.5ml of $FeCl_3$. In varying proportions of both drug and reagent, the solutions were mixed and diluted with distilled water in 10mL volumetric flask, then the absorbance was measured at optimum wavelength and under optimal time 15min and room temperature against a reagent blank.

The proposed method was optimized to achieve complete reaction formation, highest sensitivity and maximum absorbance. All the experimental parameters were optimized using 100 µg/mL of All CH, DIZE and ALP

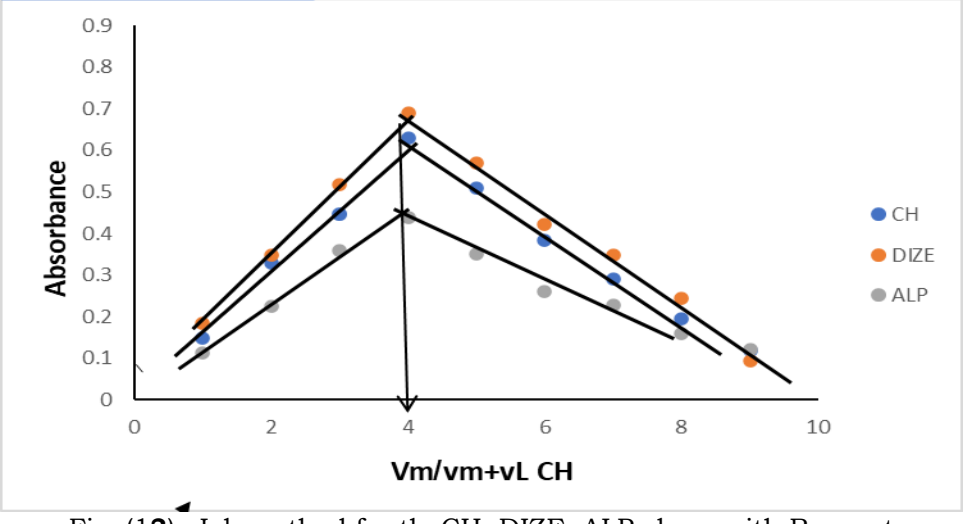

Fig. (1**3**): Job method for theCH, DIZE, ALP drug with Reagent
Vm: volume of Drug CH DIZE ALP / VL: volume of reagent MBTH

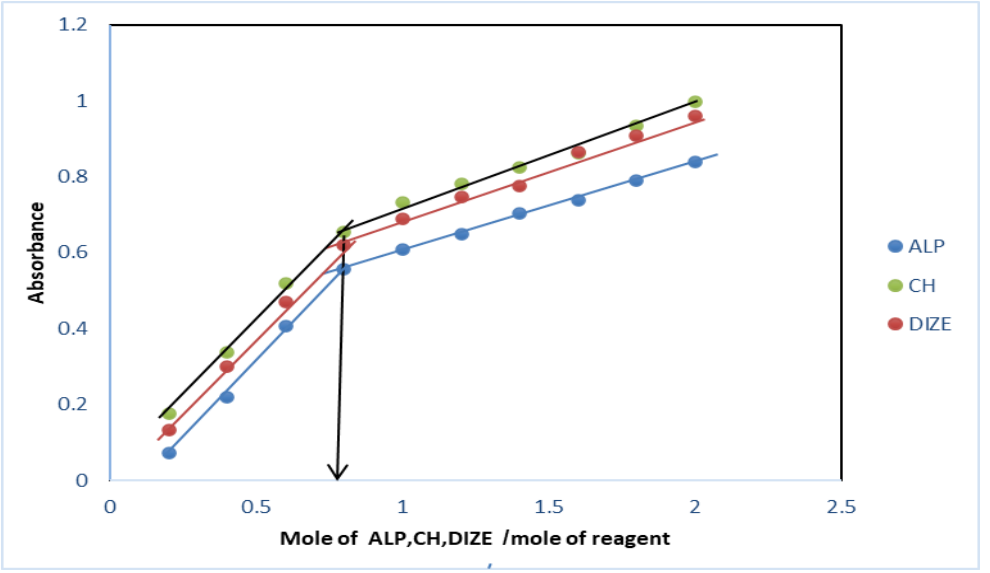

Fig. 14 : Mole ratio method for ALP CH DIZE with reagent

CH, ALP, and DIZE are reacted under normal circumstances to form a blue result when MBTH loses two electrons and one proton during the oxidation process. (16). Accordingly, the production process can be developed. Conditional stability constant (Kf) from The product and free energy of the Gibbs reaction conditional stability constant The product in blue color was calculated from Continuous variance data using the following equation (17)**:**

$$Kf = A/Am \frac{A/Am}{\left(1-\frac{A}{Am}\right)^{n+1} \; C^n N^n}$$

Where: A and Am are the maximum Continuous absorption of the contrast curve and the absorbance corresponding to the intersection Continuous change straight curve respectively. .n is a number Reagent molecules in the reaction Product (stoichiometric constant). ***C*** It is Molar concentration of CH, ALP, DIZE max Absorption. *Kf* is found equal to Table(4) liters mol^{2} This indicates stability.

Table.2 stability constant

Complex	Am	As	α	K_{st}
CH 100µg/ml	0.148	0.073	0.506	5.790×10^4
DIZE100µg/ml	0.177	0.076	0.570	3.782×10^4
ALP 50 µg/ml	0.211	0.014	0.788	1.038×10^4

Reaction product. Gibbs free energy The ΔG interaction was also calculated with the dependence of the following equation:

ΔG=-2.303RTlogKf

where R is the general gas constant (8.314 J Mole -1 degree -1). T is the absolute temperature (273 + 25 °C), Kf is the constant of formation interaction. The value

of ΔG turned out to be -33 kilojoules/mol. The negative value of ΔG indicates spontaneous reaction. Reaction rate also It is based on CH, ALP and DIZE concentrations. Comments Monitor at room temperature (25°C) with different concentrations of (CH, ALP and DIZE) in the range From with MBTH. FeCL3 concentrations are constant. Reaction The rate is found to obey the following equation: Rate = k' (CH, ALP and DIZE)^{n} 'where k' is the pseudo-order The rate is constant and n is the order of the reaction. The reaction rate can be estimated time-varying method [18] (differential prime rate method) as ΔA/Δt, where A is and t is the time in minutes. Taking stopper Logarithms of rates and concentration,

Validation of the proposed method

After optimized all the reaction conditions mentioned above, the calibration graph was plotted between the absorption intensity with the corresponding concentration of (CH, ALP and DIZE). Regression analysis and statistical parameters are calculated from the calibration plot using the least squares method. The slope, correlation coefficient, intercept for the calibration data and sensitivity parameters (molar absorption and Sandell sensitivity) are summarized in Table 2. A high correlation coefficient (r^2 =0.9979),(r^2=0.9997),(r^2 =0.9965) for (CH, ALP and DIZE) respectively, with a small intercept of the regression equation confirmed the linearity of the calibration curve. The small values of the statistical parameters computed from the regression equation indicate the high precision of the proposed method, the low dispersion of the calibration curve points and the high accuracy (Table 3).

Table 3: Summary of optical characteristics and statistics for the proposed metho

Parameter	Value
λmax (nm)	662nm,609nm,619nm respectively (CH,DIZE,ALP)
Color	Blue
Regression equation y = b x + a; Y = absorbance, x = concentration(µg/mL)	Y=0.017X+0.1641 y = 0.0245 X + 0.0003 Y=0.0118X+0. 5065 (CH, ALP, DIZE) respectively
Correlation coefficient, r	(r^2 =0.9979),(r^2=0.9997), (r^2 =0.9965) for (Ch, ALP, DIZE) respectively
Linearity range (µg/mL)	(5-25)for CH, (5-30)for (DIZE,ALP)
Molar absorptivity, ε (L/ mol cm)	5.575x10^3,3.359x103 ^{3} ,7.564x10 ^{3}, (Ch,DIZE, Alp) respectively
Slope, b (mL/µg)	0.017 ,0.0246, 0.0118 (CH, ALP, DIZE) respectively
Intercept, a (a = y– b x)	0.1641, 0.0003, 0. 5065 (CH, ALP, DIZE) respectively
Standard deviation of the slope, Sb	(0.0251, 0.013,0.009) respectively (CH,ALP ,DIZE)
Standard deviation of the intercept, Sa	0.824,0.904,0.0158

	(CH,DIZE,ALP)
Sandell's sensitivity, S (µg/cm)	(0.054,0.085, 0.041)(CH, ALP, DIZE) respectively

Sensitivity

According to the 3.3S/k and 10S/k criteria, respectively(19), the limit of detection (LOQ) and limit of quantitation (LOD) were calculated. S(0.001506) is the standard deviation of the response of the blank or the standard deviation of intercepts of regression lines, and k is the sensitivity, or the slope of the calibration curve. The values for the LOD and LOQ for al(CH,DIZE,ALP) were (0.1750 and 5.834, 0.676 and 2.253, 0.881 and 2.938) g/mL and 0.67 and 2.253, respectively.

Precision and Accuracy

Solutions comprising three different concentrations of (CH, ALP, and DIZE) were produced and evaluated in three duplicates in order to evaluate the precision and accuracy of the proposed approach. Table.4 provides a summary of the investigation's analytical findings. The proposed method's precision and accuracy were computed using low values of percentage relative standard deviation (% RSD) and percentage relative error, respectively.

Table 4: Precision and Accuracy of the proposed method

Drug	Amount of CHtaken,(µg/mL)	Found* (µg/mL)	%Relative error*	%(Recovery	%RSD*
CH	5.00	4.9193	-2	98.39	0.502
	15.00	15.20	1.33	101.02	0.812
	25..00	24.901	-0.4	99.61	0.252
DIZE	5	5.077	1.523	101.4	0.195
	15	14.759	-1.60	98.4	0.106
	30	29.932	-0.227	99.8	0.039
AL0P	5	5.077	0.015	101.54	0.46
	15	14.755	-0.016	98.37	0.73
	25	24.932	- 0.272	99.73	0.35

*Average of three determinations, relative standard deviation RSD, RSD.

Effect of interferences

The examined drug (CH, ALP, DIZE) was determined in the presence of "diluents, excipients and additives that frequently accompany (CH, ALP and DIZE) in that [dosage forms such as fructose, starch, lactose]" in order to "evaluate the usefulness of the method," The experiment accomplished by measuring the absorbance of solution containing100 µg/mL of (CH,DIZE) and 50µg/mL of ALP in the presence of tenfold of excipient (100µg/mL). A good recovery ratio was obtained, indicating no interference were observed of any from these excipients

and additives, and a high selectivity for determining the (CH, ALP and DIZE)in its High selectivity for the determination of (CH, ALP and DIZE) in its dosage forms.

Table 5 : - (CH, ALP,DIZE) analysis in the presence "of common interferences by batch method

Excipients (20 µg/mL)	Conc. of Excipients (CH, ALP and DIZE), 100µg/ mL				%(Recovery ± SD)*		
	taken	CH found	ALP found	DIZE found	CH	ALP	DIZE
Starch	20	20.40	20.49	20.34	101.989±0.1	102.430.03 ±	101.7±0.800
Lactose	20	19.83	19.63	20.06	99.148±0.0195	98.172±0.019	100.28±0.285
Fructose	20	20.28	20.16	20.14	101.420±0.0196	100.813±0.008	100.68±0.700
Glucose	20	20.34	19.92	19.75	101.705±0.01	99.593±-0.004	98.7 ±0.012

* The Average of three decisions

Analytical applications

By directly analyzing three distinct concentrations of pharmaceutical preparations utilizing the analytical approach and employing conventional collection methods, the suggested method has been successfully used to determine (CH, ALP, and DIZE) in pharmaceutical preparations. Tables 4 and 5 present the findings. Results were compared with those from the usual BP method in order to evaluate the effectiveness and success of the suggested method**.**

The same drug formulations (CH, ALP, and DIZE) were examined using the conventional BP method. When degree of freedam (n=3) was present, the results from the two distinct techniques, as shown in Table 6, were statistically compared using the student t-test and variance ratio F-test at the 95% confidence level (15). There is no discernible difference between either method's precision and accuracy in determining (CH, ALP, and DIZE) in pharmaceutical preparations in all cases where the calculated values were less than the theoretical one.

Table .6 : f – test and t – test for Comparison of Accuracy and precision between proposed method and {standard method}for "all"CH(53), DIZE(54) and ALP(55)

"(((Pharmaceutical))) preparations (Containing) (CH10gm),(DIZE10gm) (ALP 0.5gm)	((Proposed method))		Standard method	
	("Recovery)% "	SD^2	Recovery %	SD^2
CH 5	98.4	0.025	98.96	1.16
CH 15	101.02	0.343	100.59	1.16
CH25	99.61	0.063	99.70	1.16
T=0.067,F=2.691	x^-= 99.669	Σ=0.431	x^- =99.75	Σ=1.16
DIZE 5	102.4	0.021	98.8	0.06
DIZE 15	98.95	0.031	99.73	0.06
DIZE 30	101.63	0.019	100.3	0.08

T= 1.015 ,F=0.317	x = 100.993	Σ = 0.071	x −= 99.75	Σ=0.200
ALP 4 ALP 6 ALP8	102.4 98.64 99.46	0.001 0.025 0.013	104.34 98.55 101.09	0.060 0.068 0.066
T= 0.938 F= 0.017	x = 100.18	Σ = 0.039	x - =101.33	Σ = 0.065

Conclusions

The suggested approach demonstrated strong sensitivity and a low detection threshold. Additionally, the information provided above demonstrates that the suggested procedure was accurate and sensitive with good precision. ; it can be successfully applied to the routine estimation of (CH, ALP and DIZE) in bulk and in pharmaceutical preparations. The values of relative standard deviation were satisfactorily low (less than 2%) with good recoveries which indicate the high reproducibility and accuracy for the proposed method. The reaction was adopted to suggest a new flow injection method for the determination of (CH, ALP and DIZE) in a separate work send for publication by the same authors.

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