Rafidain Journal of Science
Vol. 30, No. 3, pp. 1-11, 2021

DOI: 10.33899/rjs.2021.168906

https://rsci.mosuljournals.com

ISSN: 1608-9391
e-ISSN: 2664-2786

Received:26/12/2020
Accepted:22/2/2021

Using of 4-Chlororesorcinol as a Coupling Agent in Spectrophotometric Determination of Benzocaine

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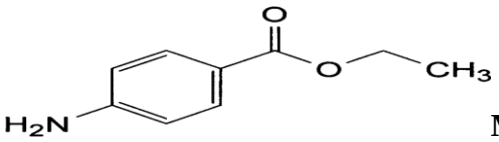
ABSTRACT

Simple and accurate spectrophotometric method for the estimation of benzocaine (BENZ) as pure form and in its formulation (ear drops) in aqueous solution has been developed. The method is based on the diazotization of BENZ, with equivalent amount of nitrite, in an acidic medium to yield the diazotized benzocaine. Then the diazotized benzocaine is coupled with 4-chlororesorcinol (4-CRL) reagent in basic medium to formed, an intense yellow azo dye, which is water-soluble and it has good stability. The yellow azo dye exhibits maximum absorption at 436 nm. The relationship between absorbance and concentration gave good range of determination from 10 to 50 µg BENZ in final volume of 10 ml i.e.,1 to 5 $\mu g.ml^{-1}$with a molar absorptivity and Sandell's sensitivity index values of 3.722×10^{4} $l.mol^{-1}.cm^{-1}$ and 0.0044 $\mu g.cm^{-2}$ respectively, a relative error of –1.06 to +2.47% and a relative standard deviation was not more than 0.921% depending on the concentration level of BENZ, low detection limit of 0.1924 $\mu g.ml^{-1}$ and low of quantitation value equal to 0.6416 $\mu g.ml^{-1}$ have been estimated. The method has been applied to the estimation of BENZ in ear drop (otocol drops).

Keywords: benzocaine**,** 4-chlororesorcinol**,** diazotization and coupling, spectrophotometric determination**.**

INTRODUCTION

Benzocaine, ethyl p-aminobenzoate or ethyl ester p-aminobenzoic acid has become widespread in the drugs industry, as it has a number of applications in this field, such as its use as an anesthetic before some endoscopic investigations. It is in a colorless or white, odorless crystalline form affected by light, benzocaine has the molecular formula $C_9H_{11}NO_2$ (Scheme 1). It is poorly soluble in water but it dissolves when adding six parts of ethanol 75% (The International Pharmacopoeia, 2016; British Pharmacopeia, 2009).



M.wt=165.2 g / mol

Scheme 1: The chemical structure of benzocaine.

From literature numerous analytical techniques or methods were described for the estimation of benzocaine including: RP-HPLC (Manikandan, *et al.*, 2019), LC-mass (Amelin and Bol'shakov, 2020), GC-mass and Gc-mass/mass (Fiorentin *et al.*, 2019), capillary electrophoresis (Junger *et al.*, 2019), also electrochemical sensing plate form (Mohamed *et al*., 2017) and single-sweep polarography (Plotycya *et al.*, 2018) methods were also reported and finally the spectrophotometric methods have been widely used in estimating the amount of benzocaine in its pharmaceutical preparations with accurate results (Al-Abdaly, 2009; Merey, 2016; Khayata and Zakri, 2018; Mhemeed, 2019; Bashir and Othman, 2005; Qadir, 2008; Mohammed and Othman, 2020 and Sarsam and Mohammed, 2011).

The purpose of new investigation is to suggest a simple and accurate spectrophotometric method for the estimation of benzocaine in its formulations by diazotization and coupling method.

EXPERIMENTAL

Apparatus

A UV / VIS spectrophotometer (JASCOV – 630, Japan), with two quartz cells (1cm) were used in this work. The sensitive balance used is BEL type.

Chemicals used

All chemicals and solvents used are of a high degree of purity.

Solutions

Benzocaine solution (200 µg. ml^{-1},1.2106x10^{-3}M)

The solution was prepared by dissolving 0.0200 g of benzocaine ($C_9H_{11}NO_2$) in 5 ml of ethanol and then the volume was supplemented with distilled water to 100 ml in a calibrated flask.

Sodium nitrite solution (6x10^{-3} M)

This solution was prepared by dissolving 0.0414 g of sodium nitrite in a small amount of distilled water, then the volume was supplemented with 100 ml of distilled water in a calibrated flask.

Diazotized benzocaine(D-BENZ) solution, 6x10^{-4} M**.**

This solution was prepared by mixing 10 ml of benzocaine (1.2106x10^{-3}M,200 µg. ml^{-1}) with 2 ml of sodium nitrite solution (6x10^{-3} M) in a 20 ml volumetric flask and then 1 ml of 1M HCl was added, then complete the volume with distilled water to the mark.

4-Chlororesorcinol(4-CRL) **solution (0.01%).**

This solution was prepared by dissolving 0.0050 g of 4-CRL in 50 ml of distilled water.

Potassium hydroxide solution (0.05M).

This solution was prepared by dissolving 0.2805 g of potassium hydroxide in distilled water and then completing the volume with distilled water in a volumetric flask to 100 ml.

Solution of pharmaceutical preparation.

Three containers of ear drop (otocol, 50 mg benzocaine / ml) solutions were mixed and then 1 ml was tacked and mixed with 2 ml of ethanol and then the volume completed with distilled water into a 100 ml in a volumetric flask. Using the above solution to prepare a working solution of benzocaine at a concentration 200 µg. ml^{-1} by drawing 10 ml into a 25 ml volumetric flask and then complete the volume with distilled water to the mark. Then the same procedure mentioned in preparation of D-BENZ solution to prepare 100 µg. ml^{-1} has been followed.

Procedure and Standard Curve

The standard curve for the determination of benzocaine was prepared as follows: Increasing volumes of the D-BENZ solution, at concentrations between 10-50 µg. BENZ were added to 10-ml volumetric flasks. Then 1.5 ml of 4-CRL (0.01%) and 1 ml of potassium hydroxide (0.05 M) were added to each of these flasks and the volume was completed with distilled water to the limit of the mark. The absorbance of each flask was measured at the wavelength of 436 nm against the blank solution and the linearity of the method is from 0.5 to 5µg.ml^{-1} Fig. (1). The molar absorptivity and Sandell's sensitivity values have been calculated and equal to $3.722x10^4$ l.mol^{-1} $cm^{-1}$and 0.0044 µg.cm^{-2} respectively.

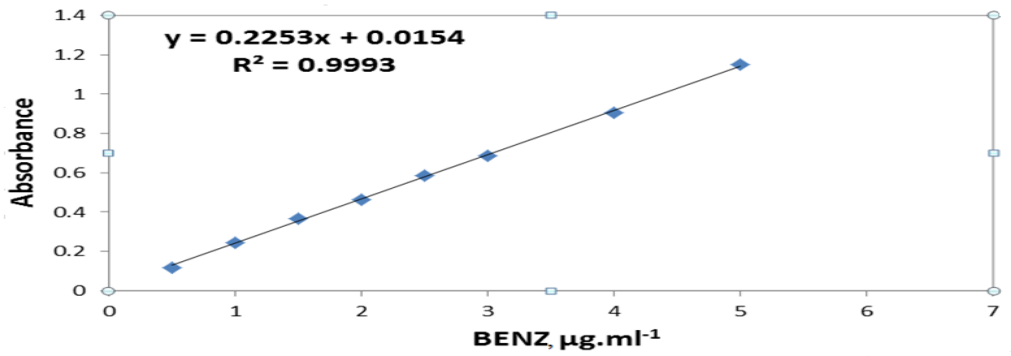


Fig. 1: The standard curve of benzocaine determination.

The detection limit (LOD) and the limit of quantitation (LOQ) of the proposed method were determined by measuring absorbance of ten replicates of low concentration solution (0.5µg.ml^{-1}) in calibration curve and then applying the mathematical relationships fixed in literature (De Levie,1997). (Table 1) shows the most important analytical parameters of the proposed method.

Table 1: The importance analytical variable of the method

Parameter	Value
Range of linearity µg. ml^{-1}	0.5-5
Maximum wavelength, nm	436
Molar absorptivity, l.$mol^{-1}cm^{-1}$	$3.722x10^4$
Linear relationship	y = 0.2253x + 0.0154 y = Absorbance x = Concentration (µg. 10 $ml^{-1)}$ Slop = 0.2253 Intercept = 0.0154
LOD, µg.ml^{-1}	0.1924
LOQ, µg.ml^{-1}	0.6416

RESULTS AND DISCUSSION

Preliminary Study

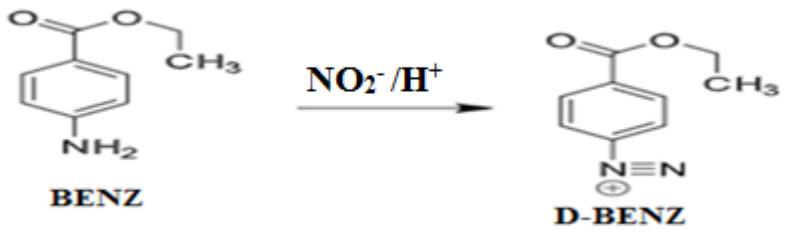
A 0.5 ml of the D-BENZ solution was mixed with 1 ml of 4-CRL, then 0.5 ml of NaOH (0.1M) in a 10 ml volumetric flask was added, then the volume was completed with distilled water

to the mark, as it was observed that a bright yellow azo dye was formed. The scanning of the resulting product against the blank solution gave the highest absorption at the wavelength of 438 nm and this wavelength is used in the next experiments.

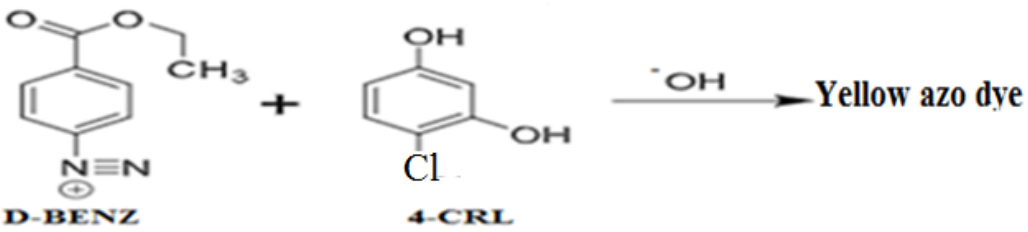
The Principle of the Present Method

The suggested method included two steps:

1- Diazotization of BENZ to corresponding BENZ diazotized(D-BENZ).



2- Coupling D-BENZ with 4-CRL in alkaline medium.



The Optimum Conditions for the Reaction

In order to obtain the optimum conditions for the preparation of the diazotized of benzocaine and the formation of the azo dye and for the purpose of obtaining the highest sensitivity and stability of the azo dye, all the factors affecting on the reaction were studied and the optimum conditions were followed.

This study was divided into two parts:

1- The optimal conditions for preparing the diazotized benzocaine, this part included the important conditions below:

The Effect of Temperature

As previously known and proven in the literature (Othman and Othman, 2013; Shlear *et al*., 2020), that the preparation of the diazotized agent at a high concentration requires cooling the solution to a temperature between 0 to 5 °C. In this study the D-BENZ was prepared by taking two flasks and to each one all the reagents which mentioned before to preparation D-BENZ, except $NaNO_2$ which used different temperature when added it, then 0.5 ml of each prepared diazotized solutions were transferred into two flasks, then 1 ml of the 4-CRL, and 0.5 ml of 1M NaOH were added and the volume was completed with distilled water. The absorbance of each solution was measured at 438 nm. (Table 2).

Table 2: Effect of temperature in preparation of D-BENZ.

Temperature, ^{o}C	5	Room temp. (25±2)
Absorbance	0.677	0.688

Through the results which obvious in (Table 2) that the temperature effect on the absorbance of the formed azo dye according to use the two diazotized of benzocaine which prepared at different temperature was not significant. The room temperature was fixed in the subsequent preparations.

The Effect of Acid Type in the Preparation of Diazotized Benzocaine

D-BENZ was prepared via the addition of sodium nitrite to BENZ in an acidic medium, so different types of acids (hydrochloric acid, sulphuric acid, and nitric acid) were used, and then each D-BENZ (prepared with different acid solution) coupled with 4-CRL in alkaline medium. The results obvious in (Table 3).

Table 3: The results of using the two diazotized of BENZ in formation the azo dye

ml acid used (1M)	HCl	H_2SO_4	HNO_3
Absorbance	0.697	0.664	0.679

It was noticed from the results in (Table 3) that hydrochloric acid gave the highest absorbance and thus it was fixed as an acid in preparation the D-BENZ solution. Also, the amount of hydrochloric acid was studied, and the results in (Table 4) indicated that 1.5 ml was the optimum amount.

Table 4: The optimum amount of hydrochloric acid.

ml of HCl(1M)	0.5	1.0	1.5	2.0	3.0
Absorbance	0.699	0.706	0.721	0.694	0.699

The Second Part of Studding the Optimum Conditions for Preparation of Azo Dye Included: Study the Effect of 4-CRL Amount

The effect of different volumes of 4-CRL reagent on the absorbance was added to form azo dye was studied by preparing several 10-ml volumetric flasks and adding to each one different volumes of D-BENZ (0.25-1.5 ml), also added different volumes (0.75-2ml) of 4-CRL then 0.5 ml of NaOH (0.1M) was added and the volume was completed with distilled water to the mark. Then the absorbance measured at 438 nm and the results shown in (Table 5) indicated that the optimum volume of 4-CRL was 1.5 ml.

Table 5: Effect of the amount of 4-CRL

ml of 4-CRL (0.01 %)	**Absorbance/ (µg.ml^{-1})**					**R^2**
	2.5	**5**	**7**	**10**	**15**	
0.75	0.388	0.733	1.047	1.103	1.002	0.5536
1.0	0.362	0.724	1.032	1.432	1.454	0.8525
1.5	0.360	0.721	1.021	1.469	2.153	0.9996
2.0	0.372	0.710	1.018	1.433	2.346	0.9954

Selection of the base and it's amount

Different types of base were used to identify the best ones to give the highest absorbance of formed azo dye by preparing several 10-ml volumetric flasks and adding to each one 0.5 ml of the D-BENZ with 1.5 ml of the reagent and finally 0.5 ml of 0.1M base (sodium hydroxide, potassium hydroxide, and sodium carbonate) for each flask was added individually(Table6).

Table 6: Selection of the Base

Base (0.1M)	NaOH	KOH	Na_2CO_3
Absorbance	0.718	1.136	0.724
λ_{max}	438	436	437

Based on the results illustrated in (Table 5), potassium hydroxide KOH was chosen in the subsequent experiments because KOH gave the highest absorbance of the azo dye formed. Also, the amount of KOH has been studied and the results in (Table 7) indicated that 1ml was the optimum amount.

Table 7: The optimum amount of KOH.

KOH (ml,0.05M)	0.5	0.7	1.0	1.5	2.0
Absorbance	0.221	0.687	1.125	1.102	1.107

Study the effect of solvent

Different types of solvents have been used in dilution to choose the most suitable ones Fig. (2) and (Table 8).

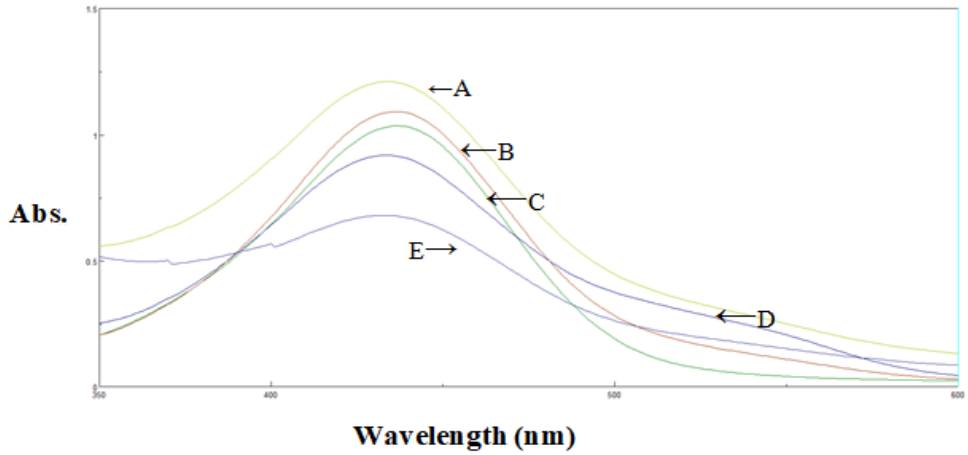


Fig. 2: Effect of solvents on spectrum A: Methanol, B: Water, C: Ethanol, D: Acetone, E: Propanol

Table 8: Effect of solvents

Solvent	**Absorbance**	**λ_{max}**	**ϵ , L.mol^{-1}.cm^{-1}**
Acetone	0.917	434	30566
Methanol	1.209	434	40300
Ethanol	1.034	436	34466
Propanol	0.679	433	22633
n-Butanol	Turbid	———	----------
Water	1.118	436	37266

Through the results shown in Fig. (2) and (Table 8), including the fact that the absorbance resulting from dilution with methanol in this experiment is greater than the absorbance resulting when using water, but water was adopted as a solvent for next experiments because of its abundance, cheap and safe compared to other solvents.

Effect of Surfactants

The effect of different types of surface-active substances has been studied with different addition sequences to identify the possibility of obtaining the highest absorbance of the resulting azo dye and the results show that there is no improvement in the absorbance value or the value of color contrast.

Study the Effect of Time on Stability

The stability of the yellow azo dye is one of the important variables that was studied, which gave results in (Table 9).

Table 9: Effect of time on stability of azo dye

Time	Absorbance/µg D-BEN in 10 ml	
	25	50
Immediately	0.575	1.118
5	0.588	1.123
10	0.590	1.138
15	0.591	1.145
20	0.592	1.140
25	0.592	1.137
30	0.592	1.140
35	0.592	1.140
40	0.592	1.140
45	0.592	1.141
50	0.592	1.142
55	0.592	1.144
60	0.592	1.144

Through the results above, the azo dye stabled at least 60 minutes.

The Optimum Conditions of the Method

The optimum values of all components of the reaction were illustrated in (Table 10).

Table 10: The optimum condition of the method

Variable	The optimum
Temperature in preparation D-BENZ.	Room temperature, 25 ±2 ^{o}C
Type of acid(M) and amount in diazotization benzocaine.	HCl (1M),1.5 ml
Amount of 4-CRL reagent.	1.5 ml
Type of base(M) and amount.	KOH (0.05),1ml
Medium of reaction.	Aqueous

Absorption Spectrum

After completing the study of optimum conditions, the final absorption spectrum was achieved, by following the optimum conditions indicated in the (Table 9). The results in Fig. (3) indicated that the maximum wavelength of the yellow azo dye was 436 nm, and this wavelength was fixed in the next experiments.

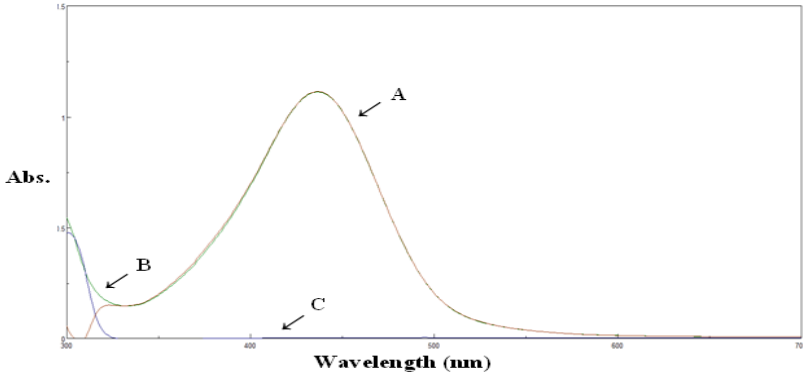


Fig. 3: The final absorption spectrum of benzocaine. A: Sample versus Distilled water, B: Sample versus Blank and C: Blank versus Distilled water.

Study the Nature of the Azo Dye

Job's method (Valcarcel, 2000) was used to find the ratio of reaction of the azo dye formed between D-BENZ and 4-CRL reagent, as follows:

A series of solutions were prepared containing increased volumes of benzocaine (6×10^{-4} M, D-BENZ) within the range 1.25-0.25 ml and decreasing volumes with complementary quantities of 4-CRL reagent with the same concentration of benzocaine in the range 0.25-1.25 ml with optimal conditions of KOH volume, then completing the volume with distilled water in 10-ml volumetric flasks. The absorbance was measured at the wavelength of 436 nm against the blank's solutions. Fig. (4) indicated that the ratio of D-BENZ to the 4-CRL was1:1. The azo dye composition was confirmed by applying the mole ratio method (Valcarcel, 2000) by taking 1 ml of the D-BENZ and increasing volumes of 4-CRLwith a range from 0.25 to 2 ml and the Fig. (5) shows that the ratio is 1: 1 benzocaine: 4-CRL.

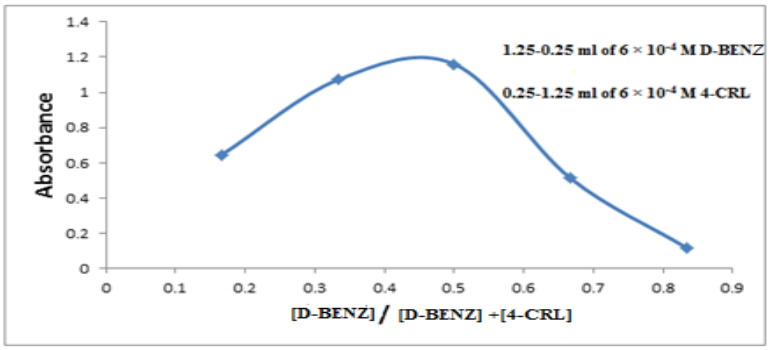


Fig. 4: Job's method plot.

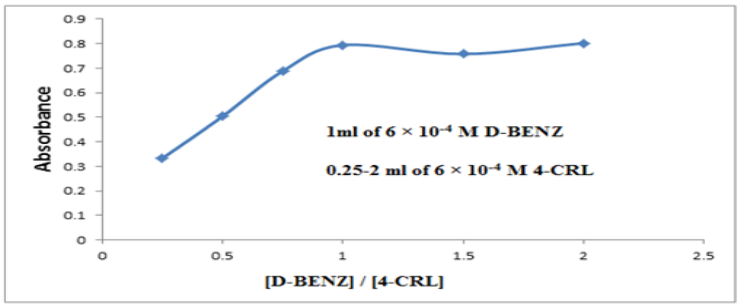
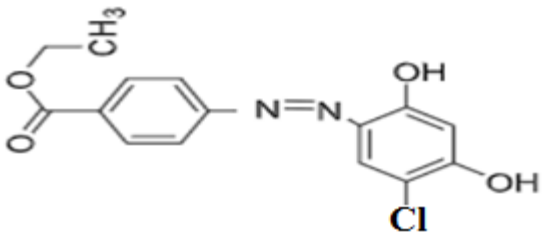


Fig. 5: The mole-ratio method plot.

According to the results above the azo dye has the following suggested structure in scheme 2.



Scheme 2: The chemical structure of yellow azo dye.

Application Part

The suggested method was applied to determine benzocaine on ear drop (otocol, 50 mg benzocaine/ ml). Into two series of 10-ml volumetric flasks 0 to 4.0 ml of the D-BENZ standard solution (100 µg.ml^{-1}) was added, then 0.1 ml of the D-BENZ ear drop solution was added to each flask in series No. 1 and 0.2 ml to each flask in series No.2 (contain 0 to 3.0 ml of the D-BENZ standard solution) then 1.5 ml of 4-CRL reagent (0.01%) and 1 ml of potassium hydroxide (0.05 M) were added, and the volumes were completed with distilled water to the limit of the marks. The

absorbance was measured at 436 nm. The results in Fig. (6) and (Table 11) indicated that the proposed method gave satisfactory results.

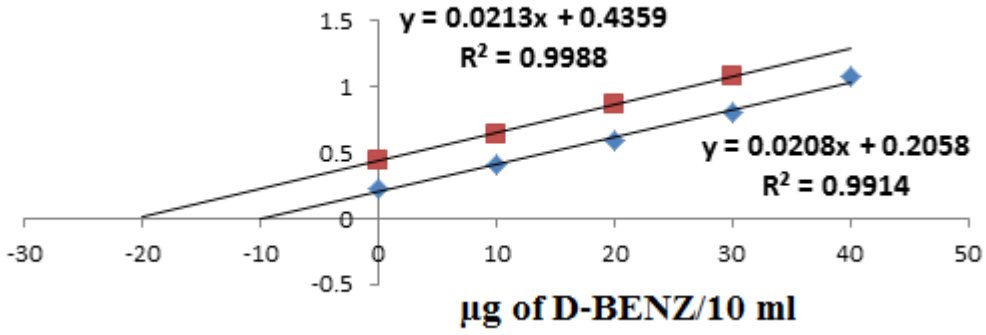


Fig. 6: The standard addition plots for determination of benzocaine in otocol drops.

Table 11: The application of the method

Drug	Amount taken, µg	Recovery	RE%	RSD%
Otocol/drop (50 mg/ml)	10	98.94	-1.06	0.921
	20	102.47	+2.47	0.631

Comparison with other Spectrophotometric Methods

Table 12 gives the results of comparison between some of analytical parameters of present method with the same parameters of literatures spectrophotometric methods.

Table 12: The results of comparison

Analytical parameters	Present method	Literature method, Zakaria,2003	Literature method, Bashir and Othman,2005
Type of reaction	Diazotisation and coupling	Diazotisation and coupling	Diazotisation and coupling
Reagent used	4-Chlororesorcinol	Phloroglucinol	N-(1-naphthyl) ethy-lenediamine dihydrochloride
Maximum wavelength, nm	436	419.5	547.5
Molar absorpitivity, l.mol.^{1}. cm.$^{-1}$	3.722 x 10^{4}	4.9807×10^{4}	5.56x10^{4}
Linearity, Mg.ml-1	1-5	0.2-10	0.4-4
RSD%	≤0.921%	0.38% ≤	0.55% ≤
RE%	-1.06 to +2.47	0.62 to -+0.5	0.7<
LOD	0.1924	----------	----------
LOQ	0.6416	----------	----------
Application	Ear drop (otocol drops)	Synthetic drugs	Synthetic drugs

The results in the (Table 12) shown that the proposed method is sensitive and not less important in terms of application as it was applied to tear drop**.**

CONCLUSION

An accurate and simple spectrophotometric method had been suggested for the estimation of benzocaine by diazotization and coupling method. The method was applied in assay of benzocaine in its formulation ear drops with analytical accepted results

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استخدام كاشف الاقتران 4- كلوروريسورسينول في التقدير الطيفي للبنزوكائين

الملخص

طريقة طيفية بسيطة ودقيقة لتقدير البنزوكائين (BENZ) بشكله النقي وفي مستحضره الصيدلاني (قطرة الأذن، اوتوكول). تعتمد الطريقة على ازوتة البنزوكائين مع كمية مكافئة من النتريت، في وسط حامضي لتحضير البنزوكائين المؤزوت. ثم يقترن البنزوكائين المؤزوت وفي الوسط المائي بوجود هيدروكسيد البوتاسيوم بالكاشف 4-كلوروريسورسينول لينتج صبغه ازوية صفراء اللون، ذائبة في الماء ولها استقراريه عاليه. تعطي صبغة الآزو الصفراء أقصى امتصاص عند 436 نانومتر. وكانت العلاقة بين الامتصاص والتركيز في مدى تقدير من10 إلى50 ميكروغرام من بنزوكائين في الحجم النهائي 10 مل أي من 1الى5 ميكروغرام. مل $^{-1}$مع امتصاصية مولارية 3.722 x 10^4 لتر. مول $^{-1}$. سم$^{-1}$ وقيمة معامل ساندل للحساسية 0.0044 مايكروغرام. سم $^{-2}$ وكانت قيمة الخطأ النسبي من −1.06 إلى +2.47٪ والانحراف القياسي النسبي ليس اكثر من 0.921% اعتمادًا على مستوى التركيز، وكانت قيمة حد الكشف 0.1924 وقيمة الحد الكمي 0.6416 مايكروغرام. مل $^{-1}$. تم تطبيق الطريقة لتقدير البنزوكائين في قطرة الأذن (اوتوكول).

الكلمات الدالة: البنزوكائين و4- كلوروريسورسينول والازوتة والاقتران والتقدير الطيفي.