Spectrophotometric Determination of Benzocaine by Azo-Dye Formation Reaction with N – (1-naphthyl)ethylenediamine as Coupling Agent

W. A. Bashir **N. S. Othman**
Department of Chemistry - College of Science
Mosul University

Received 17/5/2005 Accepted 17/7/2005

الخلاصة

يتضمن البحث طريقة طيفية بسيطة لتقدير البنزوكايين في الوسط المائي. تعتمد الطريقة على اقتران ملح الدايازونيوم للبنزوكايين مع الكاشف في الوسط الحامضي لتكوين صبغة آزوية ذات لون بنفسجي محمر. كانت الصبغة مستقرة وذائبة بالماء وتعطي أعلى امتصاص عند547.5 نانوميتر ولقد تراوحت حدود قانون بير في مدى التركيز 10 –100 مايكروغرام بنزوكايين في حجم نهائي 25 مللتر(0.4–4 جزء/مليون) حيث كانت الامتصاصية المولارية5.56×10^4 لترمول$^{-1}$.سم$^{-1}$، وخطأ نسبي تراوح بين+ 0.002 و– 0.660 وانحراف قياسي نسبي تراوح بين± 0.110 و±0.550 اعتماداً على مستوى التركيز. وقد تم تطبيق الطريقة بنجاح لتقدير البنزوكايين في مستحضرين دوائيين مصنعين.

ABSTRACT

A simple spectrophotometric method for the determination of benzocaine in aqueous solution is developed. The method based on the coupling of diazotized benzocaine with N–(1-naphthyl)ethylenediamine(N–NED) in acidic medium. The purplish – violet azo dye formed is water soluble, stable, and shows maximum absorption at 547.5 nm. Beer's law is obeyed over the range 10 – 100 µg / 25 ml, i.e, 0.4 – 4 ppm with a molar absorptivity of 5.56×10^4 $l.mol^{-1}.cm^{-1}$ and Sandell's sensitivity index of 0.0029 $\mu g.cm^{-2}$, a relative error of – 0.660 to + 0.002% and a relative standard deviations of ± 0.110 to ± 0.550% depending on the concentration. The method has been applied to determine benzocaine in two synthetic pharmaceutical preparations.

INTRODUCTION

Benzocaine is ethyl *p*- aminobenzoate which is a sparingly soluble local anaesthetic with a toxicity about one – tenth that of cocaine.Benzocaine dissolves slowly in the mouth, producing a local anaesthetic effect and is used to prevent nausea and vomiting.(1)

Benzocaine is used in different anerestic lozenges, and when introduced into body gives a maximum therapeutic effect with a minimum side effect for these properties, different methods have been used to determine benzocaine as free or in drugs.

Benzocaine in drug formulations has been determined photometrically. The method is based on treatment of benzocaine with sodium nitrite in acidic medium. After 5 minutes ethacridine lactate solution is added and the absorbance is measured at 508 nm (green filter). (2)

Zero – order and second – derivative (wavelength difference 3 nm) spectrophotometry between 320 and 220 nm have been used to analyse ethanolic solution of cocaine (5 to 20 mg / l) and benzocaine (2 to 15 mg / l) in mixture with ratio from 1:3 to 10:1. Benzocaine could be determined at 292.9 or 299.4 nm in the zero – order or derivative spectrum, respectively. Cocaine could not be determined by zero – order absorbance without interference from benzocaine.(3)

The UV determination of benzocaine in pharmaceutical preparations has been accomplished, based on measuring the absorbance at 290 nm in 1:1 ethanol – water mixture. The coefficient of variation is 1.15 % and 1.43 % for cream and ointment, respectively. Beer's law is obeyed from 10 to 50 μg / ml. Matrix interference does not occure.(4)

Benzocaine in extempore medicinal form has been determined spectrophotometrically; the sample in DMF is heated on a water bath with 1 % 5–nitrobarbituric acid in DMF and then the absorbance is measured at401nm.(5) The drugs benzocaine(I), procaine(II)and sulphanilamide(III) in complex dosage forms have been determined by colorimetry of the compound formed after treatment with nitrite and alkaline 2–naphthol. The method has been applied to these drugs after extraction of (I) into chloroform,(II) with hot water and(III) with 96% ethanol. The absorbance was measured at 445 nm and the error was better than ± 3 %.(6)

Benzocaine is determined by colorimetric determination of its reaction product with bindone [2 – (2,3 – dihydro – 3- oxo – 1 Hinden – 1 – ylidene) – 1H indene – 1, 3(2H) – dione] at 482 nm.(7)

Benzocaine can be diazotized and coupled with ethyl acetoacetate to form yellow products with absorption maximum at 385 nm, the range of determination is 2 to 15 μg / ml and the method has been applied to eye-drop, tablets and ointment.(8)

Benzocaine can be determined by it's reaction with 4–dimethylaminocinnamaldehyde and trichloroacetic acid in methanolic medium at 544 nm Beer's law is obyed the concentration of 2.5-12.5μg , the coefficient of variation was ± 1.03 % and mean recovery of 0.5 to 2.2 μg / ml is 99.6 %. However aminobenzoic acid produces a red color similar to that given by benzocaine.(9)

Determination of benzocaine and procaine by usiny a time-resolved luminescence method using terbium (III) in the dry reagent format. Benzocaine and procaine release p-aminobenzoic acid after hydrolysis in alkaline medium which reacts with terbium (III) giving luminescent chelate . The luminescence intensity measurememts are made at 288 nm (excitation) and 545 nm (emission)(10).

Local anaesthetics procaine hydrochloride (I),benzocaine (II) and tetracaine hydrochloride (III) were determined by the technique of sequential injection analysis (SIA) with chemluminescence (CL) detection. The CL was emitted during the oxidation of the analyses by permanganate in aqueous sulphuric acid in the presence of various CL enhancers. The limits of detection were 0.3 mg for (I) and (II) and 0.1 mg/ml for (III) (11).

Determination of benzocaine in biological fluids has been made by flow injection technique and chemiluminescence detection. The method was used dinitrobenzofuroxan derivative as a reagent. Beer's law is obeyed over the range 0.08-5.0 mg/L and the absorbance measured at 510nm (12).

From the above literature survey some of the above methods need organic medium (3,4,5and 9) another need extraction(6), it may seems desirable to develop a method that with more analytical satisfaction than those of the present days. The present method involves the diazotization of benzocaine and subsequent coupling with N–(1-naphthyl)ethylenediamine to form a highly colored dye that has proved successful for the assay of benzocaine in throat lozenges and lozenges of benzocaine compound B.P.C–synthetic pharmaceuticals.

EXPERIMENTAL

Apparatus

All measurements are performed using Shimadzu UV-Visible Recording Spectrophotometer UV-160 with 1 – cm matched silica cells.

Reagents

All chemicals used are of the highest purity available.

Working benzocaine solution, 50 μg / ml. A 0.01 g of benzocaine (BDH) is dissolved in 2 ml ethanol and 30 ml distilled water (heating is necessary to increase solubility), and the volume is completed to 200 ml in a volumetric flask, and this solution is stoppered and kept in a brown bottle in a refrigerator. Under these conditions the solution should be stable for at least one week.

Hydrochloric acid solution, 1 N. This solution is prepared by diluting 8.5 ml of the concentrated acid to 100 ml with distilled water.

Sodium nitrite solution, 1 %. This solution is prepared by dissolving 1 g of sodium nitrite in 100 ml distilled water.

Sulphamic acid solution, 3 %. A 3 g of sulphamic acid is dissolved in 100 ml distilled water.

N – NED solution, 0.1 %. This solution is prepared by dissolving 0.1 g of the compound [$C_{10}H_7NHCH_2NH_2.2HCl.CH_3OH$] in distilled water in a 100 ml volumetric flask.

Lozenges of Benzocaine compound B.P.C. This lozenges is prepared by weighing (100 mg of benzocaine + 50 mg of borax + 3 mg of menthol)(1) and dissolving this components in 2 ml ethanol + 20 ml distilled water, heating is necessary to complete dissolution, and the volume is completed to 100 ml in volumetric flask after cooling.

Throat lozenges This lozenges is prepared by dissolving the mixture of (5 mg benzocaine and 2 mg cetlypyridine chloride) (1) in 2 ml ethanol with 20 ml distilled water and heating, then the solution is completed to 100 ml in a volumetric flask after cooling.

Recommended Procedure and Calibration Graph

To a series of 25 ml volumetric flasks aliquots covering the range of 10 – 100 μg benzocaine are transferred, 2.0 ml of 1 N HCl is then added and the mixtures are shaken. Then 0.5 ml of 1 % sodium nitrite solution is added and the mixtures are allowed to stand for 5 minutes. Then 0.5 ml of 3 % sulphamic acid solution is added and the mixtures are occasionally stirred for 5 minutes. Then 1 ml of 0.1 % N – NED solution is added and the volumes are completed to the mark with distilled water. After 10 minutes, the absorbances are measured at 547.5 nm against blank solution or distilled water using 1 – cm matched cells. (Fig. 2) shows the calibration curve which indicates that Beer's law is obeyed over the concentration range 10 – 100 μg / 25 ml final volume, i.e., 0.4 – 4 ppm and above 100 μg / 25 ml gives negative deviation . The molar absorptivity is 5.56×10^4 $l.mol^{-1}.cm^{-1}$ and the Sandell's sensitivity index 0.0029 $\mu g.cm^{-2}$

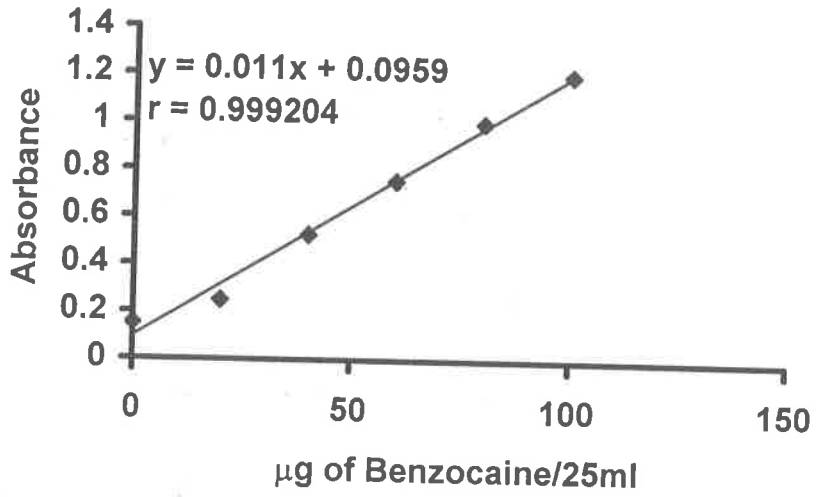


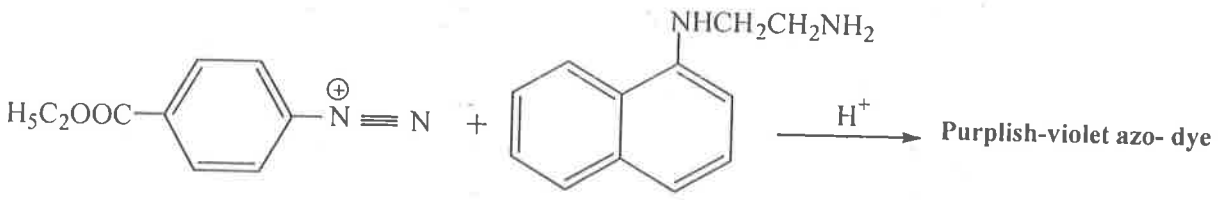
Fig 1.: Calibration graph for benzocaine determination using N – NED as a coupling reagent

RESULTS AND DISCUSSION

For the subsequent experiments, 50 µg of benzocaine is taken in 25 ml final volumes and absorbance measurements are performed at 547.5 nm.

Principle of the method

Benzocaine is reacted with excess nitrite in acidic medium to form the corresponding diazonium salt, and after removal of residual nitrite with sulphamic acid, the diazotized benzocaine is then coupled with N – NED in acidic medium to form, an intensely –purplish- violet azo-dye.



Benzocaine diazonium salt N - NED

Study of the Optimum Reaction Conditions

The various parameters affecting and related to the color intensity of the dye have been studied and optimum conditions are selected.

Choice of coupling agent

Several aromatic coupling agents have been tested for optimum conditions. The results in Table 1 show that N – NED give the most sensitive reaction (ε = 51790.20 $l.mol^{-1}.cm^{-1}$) in acidic medium. Therefore, it has been selected for subsequent experiments.

Table 1. Selection of coupling agent

Coupling agent, 0.1 %	In the absence of NaOH			In the presence of NaOH		
	Molar absorptivity $l.mol^{-1}.cm^{-1}$	λ_{max} (nm)	pH	Molar absorptivity $l.mol^{-1}.cm^{-1}$	λ_{max} (nm)	pH
m- Aminophenol	4543.00	459.0	1.37	23954.00	469.5	12.62
Resorcinol	.....	.....	1.42	29818.60	491.5	12.69
Phloroglucinol	11316.20	420.5	1.42	44273.60	419.5	12.48
α - Naphthol	3221.40	462.0	1.46	30892.40	515.0	12.70
β - Naphthol	.....	.....	1.46	6029.80	506.0	12.70
N - NED	51790.20	547.5	1.42	2552.34	501.0	12.70

..... = colorless standard and blank solutions

Effect of diazotization acid

The effect of the amount of different acids (weak and strong) for the diazotization of benzocaine, have been investigated (Table2).

Table 2. Effect of diazotization acid on absorbance

Acid used (1 N)	Absorbance / ml of acid used for the diazotization reaction						
	0.5	1.0	1.5	2.0	3.0	4.0	5.0
HCl	0.620	0.631	0.643	0.650	0.648	0.634	0.623
$HClO_4$	0.605	0.618	0.608	0.602	0.599	0.601	0.600
H_2SO_4	0.565	0.581	0.604	0.607	0.598	0.589	0.585
H_3PO_4	0.583	0.589	0.599	0.614	0.620	0.623	0.627
HNO_3	0.621	0.621	0.630	0.628	0.631	0.630	0.632
CH_3COOH	0.542	0.558	0.555	0.554	0.554	0.568	0.575
HCOOH	0.375	0.318	0.251	0.206	0.157	0.115	0.091
Without	0.446						

The results in Table 2 show that 2 ml of 1 N HCl produces the highest intensity for the dye, so it has been selected in the subsequent experiments.

Effect of nitrite amount and time

The effect of nitrite amount and its reaction time with benzocaine have been investigated to verify its optimum amount which give the higest intensity of the resulting azo-dye. A 0.5 ml of 1 % nitrite solution with 5 minutes reaction time have been incorporated for the subsequent steps (Table3).

Table 3. Effect of nitrite amount and time on absorbance

Ml of 1% $NaNO_2$	Absorbance / minute of diazotization time						
	0	1	2	3	5	7	10
0.1	0.619	0.645	0.622	0.629	0.625	0.624	0.628
0.3	0.638	0.621	0.632	0.638	0.630	0.623	0.607
0.5	0.621	0.630	0.629	0.641	0.646	0.645	0.645
0.7	0.577	0.565	0.598	0.608	0.587	0.549	0.457
1.0	0.578	0.529	0.526	0.513	0.504	0.467	0.431
2.0	- 0.007	- 0.012	- 0.006	- 0.008	- 0.013	- 0.001	- 0.005

Effect of sulphamic acid amount and time

The presence of unreacted nitrite is undesirable in diazotization reaction.(13) Therefore it should be removed by sulphamic acid which fastly reacts with nitrite(Table4).

Table 4. Effect of sulphamic acid amount and time on absorbance

Ml of 3% sulphamic acid solution	Absorbance / minute standing time							
		0	1	2	3	5	7	10
0.0	Sample = S Blank = B	0.009 0.070	-0.013 0.081	-0.011 0.064	-0.000 0.062	-0.002 0.088	0.011 0.073	0.000 0.053
0.25	S B	0.006 0.076	0.622 0.014	0.662 -0.003	0.660 0.007	0.652 0.002	0.586 0.010	0.638 0.010
0.50	S B	0.569 0.040	0.653 -0.001	0.615 0.010	0.614 0.014	0.654 0.002	0.649 -0.004	0.621 0.010
0.75	S B	0.596 0.018	0.644 -0.001	0.611 0.013	0.637 -0.010	0.637 0.008	0.658 0.003	0.623 0.010
1.00	S B	0.588 0.017	0.645 0.004	0.614 0.020	0.629 0.011	0.625 0.004	0.635 0.015	0.636 0.006
2.0	S B	0.699 0.002	0.681 0.008	0.672 0.012	0.661 -0.000	0.615 0.013	0.618 0.017	0.620 0.011

The results in Table 4 show that 0.5 ml of 3 % sulphamic acid solution with 5 minutes standing time are considered to be the most suitable, and therefore are selected subsequently.

Effect of N – NED amount

The effect of N – NED amount on the intensity of the dye has been studied (Table 5).

Table 5. Effect of N- NED amount on absorbance

Ml of 0.1 % N - NED	Absorbance / µg benzocaine present					Correlation coefficient (r)
	10	30	50	70	100	
0.1	0.042	0.238	0.360	0.461	0.579	0.981258
0.5	0.144	0.375	0.537	0.875	1.203	0.995747
1.0	0.138	0.382	0.667	0.888	1.217	0.998626
2.0	0.130	0.381	0.668	0.884	1.230	0.998832

From the results illustrated in Table 5, it can be observed that 1 ml of 0.1% N–NED is the more suitable amount since it gives the highest value of correlation coefficient (0.998626) and good intensity.

Effect of time on color development

The effect of time on the development and stability period of the colored dye is investigated under optimum conditions for determination of benzocaine (Table 6).

Table 6. Effect of benzocaine amount and time on absorbance

µg benzocaine/ 25 ml	Absorbance / minute standing time							
	0	5	10	20	30	40	50	60
10	0.084	0.155	0.154	0.155	0.153	0.155	0.155	0.155
50	0.458	0.640	0.668	0.667	0.667	0.668	0.668	0.667
100	0.851	1.224	1.237	1.237	1.232	1.232	1.230	1.228

From the experimental data illustrated in the above table, it can be shown that the complete formation of the colored dye is obtained from three different amounts of benzocaine after 10 minutes and the absorbance remained constant for at least 1 hour.

Final Absorption Spectra

Under the above optimized conditions, absorption spectra of the dye formed from the reaction of diazotized benzocaine with N – NED in acidic medium against its corresponding reagent blank, since the reagent blank shows no absorption in the visible region (Fig. 2). The wavelength of maximum absorption of the colored dye at 547.5 nm has been used in all subsequent experiments.

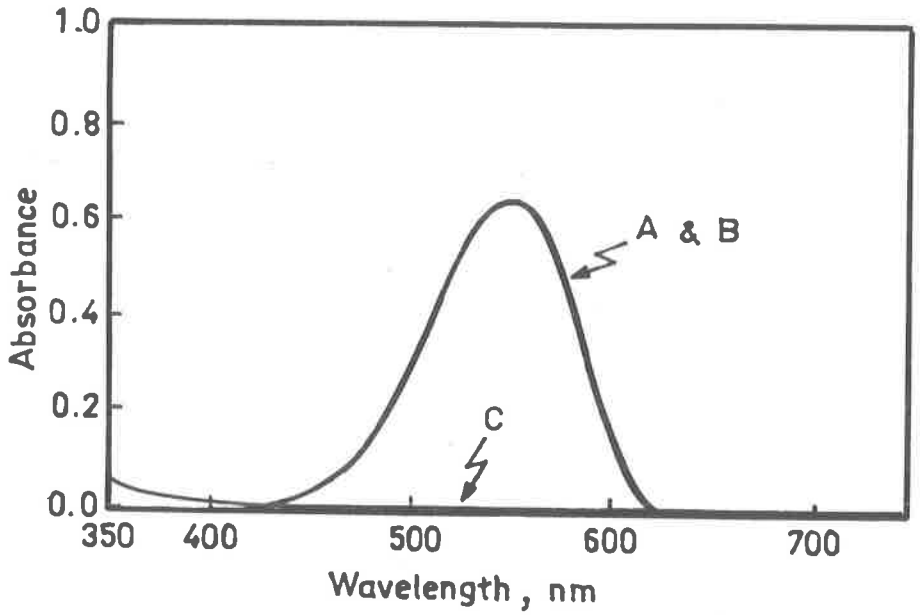


Fig. 2: Absorption spectra of 50 µg benzocaine / 25 ml treated according to the recommended procedure and measured against (A) blank, (B) distilled water and (C) blank measured against distilled water.

Accuracy And Precision

Three different concentrations of benzocaine are used in the investigation of the accuracy and precision of the method; the results shown in Table 7 indicate that the method has good accuracy and precision.

Table 7. Accuracy and precision of the method.

Amount of benzocaine taken, μg/25ml	Relative error %*	Relative standard deviation %*
25	+ 0.002	± 0.550
50	- 0.590	± 0.210
100	- 0.660	± 0.110

* Average of five determinations

Nature of the Dye

The composition of the intense purplish – violet dye that results from the reaction of diazotized benzocaine (B) with N- NED (N) has been established using the continuous variations and the mole – ratio methods (Fig. 3 and 4).

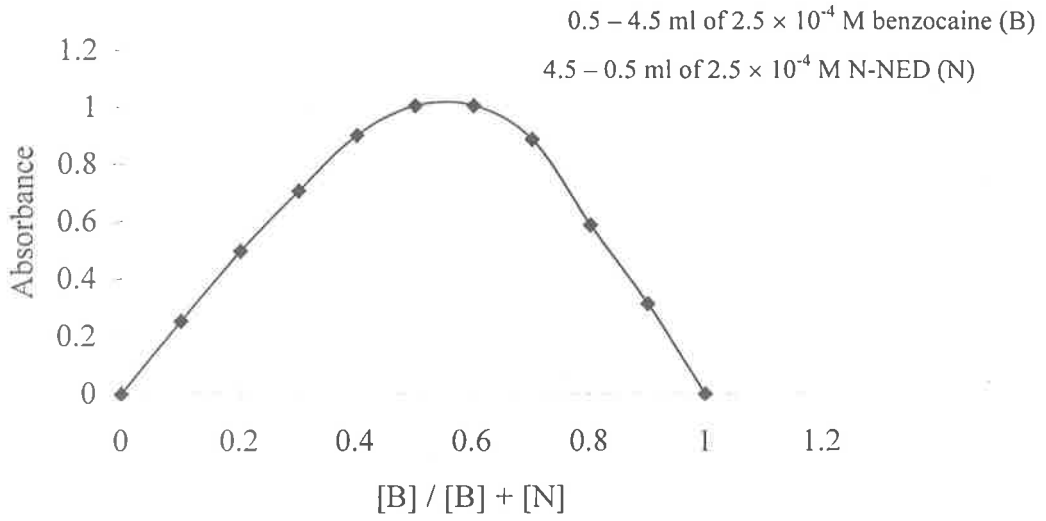


Fig. 3 : Job's plot for diazotized benzocaine – N – NED dye

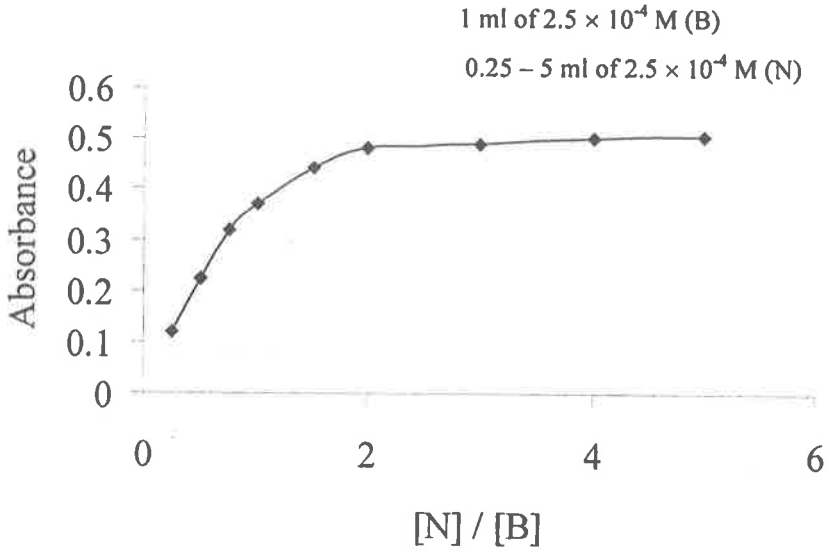
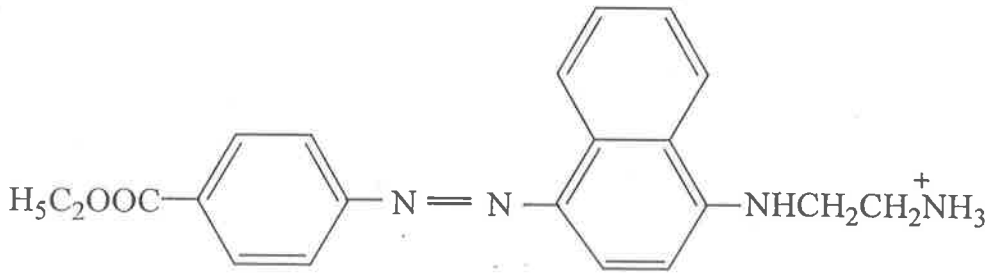


Fig. 4: Mole ratio plot for diazotized benzocaine – N- NED dye

From the results, the dye has a combination 1:1 ratio of diazotized benzocaine to N – NED. The formula of the azo dye may be suggested as:



Purplish – violet azo dye

Application of the Method

To test the applicability of the present method, it has been applied to determination of benzocaine in two synthetic pharmaceuticals, throat lozenges and lozenges of benzocaine compound B.P.C (Table 8).

Table 8. Determination of benzocaine in drugs

Drug	% Recovery
Throat lozenges	100.16
Lozenges of benzocaine compound B.P.C	99.19

The results in Table 8 indicated that the present method can be used to determine benzocaine in the above two drugs.

Comparison of Methods

Table 9 shows the comparison between the analytical variables for the present method with another spectrophotometric method[9].

Table 9. The comparison of methods

Analytical parameters	Present method	Literature method*
pH	1.42	acidic medium
Temperature (C°)	room temperature	...
Development time (minutes)	10	10
λ_{max} (nm)	547.5	544
Medium of reaction	aqueous	Nonaqueous
Reagent	N - NED	*p* – dimethylamino-cinnamaldehyde
Beer's law range (ppm)	0.4 - 4	0.025 – 2.5
Molar absorptivity($l.mol^{-1}.cm^{-1}$)	5.56×10^4	9.87×10^4
Relative error %	< 0.7	...
RSD (%)	< 0.6	0.0103
Color of the dye	Purplish - violet	Red
K. $Molar^{-1}$	0.154×10^6	...
Nature of dye	1 : 1	1 : 1
Application of the method	determination of benzocaine in two drugs	determination of benzocaine in two drugs

*S.I. Henry, A. Bruemmer and D. Shelton, J. Pharm. Sci., (1977), 66, 1037- 1039.

The results indicate that the present method is more sensitive and has application part.

REFERENCES

1. Clowes W. and Sons L., "British Pharmaceutical Codex," The Pharmaceutical Press, London, 82, 732 (1973).
2. Ivakhienko P. N., Lyashera N. N., Kilyakova G. M., Kruglyak I. V, Serbina L. V., Chigarenko L. S. and Dorofeenko G. N., Farmatsiya (Moscow), 30, 56 – 61(1981), (in Russian).
3. Martinez MI., Palanco JL., Lucas J. and Rojas MA., J. Anal. Toxicol, 13, 349 – 353 (1989).
4. Song N., Zhongguo-Yaoxue-Zazhi,25, 479 – 481 (1990).
5. Sadivs'kii VM. and Petrenko VV., Farm. Zh (Kiev), 1, 70 – 75 (1990).
6. Propov D. M. and Litvi A. A., Khim. Farm. Zh., 14, 108 – 111 (1980); Anal. Abst., 42, 1E48 (1982).
7. Petrenko V. V. and Tarkevich N. M, Farm. Zh (Kiev), 6, 63 – 65(1979).
8. Belal S., El-Sayed M. A., El-Nenecy A. and Soliman S. A., Talanta, 25, 290 – 291(1978).
9. Henry S. T., Bruemmer A. and Shelton D., J. Pharm. Sci., 66, 1037 – 1039(1977); Anal. Abst., 34, 2E35(1978).
10. Casas H., Agular AM,C., and Gomez H. , Anal. Chim. Acta, 452,169-175 (2002).
11. Pasekova H., and Polasek M., Talanta, 52,67-75 (2000).
12. Evgerev M., Garmonov S., and Shakirova L. , J. Anal. Chem ., 55, 6 96-699 (2000) .
13. Norwitz G. and Keliher P. N., Anal. Chem., 53, 56 – 60 (1981).