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Spectrophotometric Determination of Benzocaine by Azo-Dye Formation Reaction

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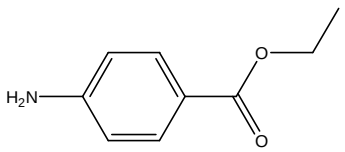
Abstract :A spectrophotometric method for the assay of trace amounts of benzocaine was based on the reaction of benzocaine with nitrite ion to form the corresponding diazonium salt followed by coupling reaction with ethyl cyano acetate to form a stable and a soluble yellow azo dye with maximum absorption at 405 nm. Beer's law was obeyed over the range 5-250μg/25ml, i.e, (0.2-10ppm) of benzocaine and correlation coefficient 0.998 with a molar absorptivity of 3.1 ×104 L.mol-1.cm-1, a relative error of 0.0 to 0.25 % and a relative standard deviation of ± 0.03 to ± 0.38 %, depending on the concentration level. The method has been successfully applied for the assay of benzocaine in one pharmaceutical preparation (Lozenges).

Key words : Spectrophotometric , Benzocaine , Azo-Dye Formation Reaction

Introduction:

Benzocaine (4-aminobenzoic acid ethyl ester)(synonym: ethyl aminobenzoic acid , is a local anaesthetic of the ester type with a poor solubility in water which is used for superficial anesthesia, for the local and temporal rely of pain ,among other disorders, to buccal effections (1). For such reasons , it is a drug extensively used in odontology(2-3). It is used in Cattle , sheep ,swine and horses for local and prolonged low epidural anaesthesia . Benzocaine acts on the central nervous system ,cardiovascular system , neuromuscular junctions and ganglion synapse .Its mechanism of action is to prevent the generation and condition of the nerve impulse. It has been proposed that the drug penetrates cell membranes in its uncharged form and binds to putative intracellular receptors. Various spectrophotometric (4-10) and chromatographic(11-16) methods for determination of benzocaine have been reported. In the present work an attempt was made to develop a rapid and sensitive method for the determination of benzocaine in pharmaceutical formulation.



4-amino benzoic ethyl ester

Experimental

Instruments

All spectrophotometric measurements were performed on Shimadzu UV-Visible Recording Spectrophotometer UV-160 using 1 cm silica cell, pH meter type Philips PW 9420 was used for pH measurements.

Reagents

All chemicals used in this investigation are of analytical – reagent grade, benzocaine standard material is provided from general establishment for medical appliance and drugs / NDI – Mosul / Iraq.

Solutions

Benzocaine (100 μg/ml): 0.01g was dissolved in ethanol solution transferred into a 100 ml volumetric flask, and diluted to the mark with distilled water.

Ethyl cyano acetate, 2% (v/v), was prepared freshly daily by dissolving 2 ml of ethyl cyano acetate in 100 ml distilled water.

Sodium nitrite solution, 1% (w/v), was prepared by dissolving 1 g of sodium nitrite (BDH) in 100 ml distilled water.

Sulphamic acid solution, 3% (w/v), was prepared by dissolving 3 g of sulphamic acid (Fluka) in 100 ml distilled water.

Hydrochloric acid solution, 1N. was prepared by diluting 8.47ml of concentrated acid (11.8 N) to 100 ml with distilled water.

General procedure

To a series of 25-ml calibrated flasks, transfer 0.05 – 2.5 ml of benzocaine solution, then 1 ml of 1M hydrochloric acid and 0.3 ml of 1% (w/v) sodium nitrite solution were added and the mixture was allowed to stand for 2 minute and then 1.0 ml of 3% (w/v) sulphamic acid solution was added with occasional shaking for 3 minutes. After that a 1.0 ml of 2% (v/v) ethyl cyano acetate was added. Then the solutions was let to stand for 1 minute at room temperature before adding 2ml of 1M ammonium hydroxide then the volumes were completed to the mark with distilled water, The absorbance was read at 405 nm against the reagent blank. A linear calibration graph was obtained over the concentration range of 5 – 250 µg benzocaine / 25 ml (0.2-10 ppm) and a concentration above 250 µg / 25 ml gave a negative deviation (Fig. 1). The molar absorptivity has been found to be 3.1 ×104 L. mol-1. cm-1.

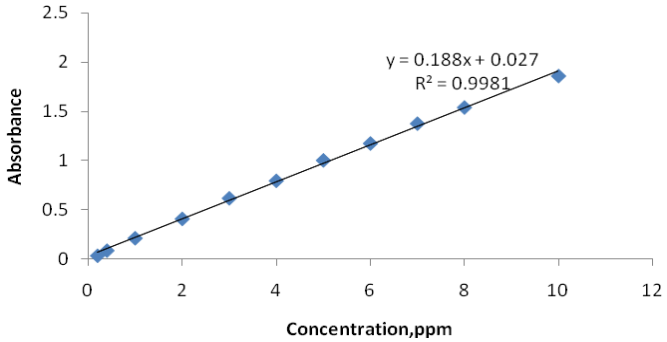


Fig. 1. Calibration graph of benzocaine determination at 405nm.

Results and Discussion

Study of the optimum reaction conditions

Effect of acid

Different amounts and types of acids have been used in diazotization of benzocaine; the results showed that 1 ml of 1 M HCl has been selected for subsequent experiments (Table 1).

Table 1. Effect of acids on absorbance and colour contrast

1M Acid solution used	Abnsorbance/ml of acid added				
	0	0.5	1.0	1.5	2
HCl	0.339	0.387	0.406	0.387	0.380
HNO_3	0.339	0.128	0.109	0.084	0.070
H_2SO_4	0.339	0.402	0.261	0.084	0.071
CH_3COOH	0.339	0.380	0. 377	0.095	0.098

Effect of sodium nitrite amount and time

The maximum absorbance reading was obtained by adding 0.3 ml of 1% sodium nitrite for 2 minutes of reaction time (Table2).

Table 2. Effect of sodium nitrite amounts and time on the absorbance of benzocaine

ml of 1% $NaNO_2$ solution	Absorbance / minute standing time					
	0	1	2	3	4	5
0.1	0.387	0.371	0.376	0.377	0.391	0.388
0.3	0.375	0.389	0.402	0.358	0.380	0.385
0.5	0.379	0.387	0.384	0.369	0.382	0.389
0.7	0.378	0.377	0.387	0.384	0.379	0.398
1.0	0.393	0.378	0.390	0.381	0.354	0.383

Effect of sulphamic acid amounts and time

The excess of nitrite can be removed by the addition of sulphamic acid solution. The effect of sulphamic acid amount and time has been studied. (Table3)

Table 3. Effect of sulphamic acid amounts and time on the absorbance of benzocaine

ml of 3% Sulphamic acid solution	Variable	Absorbance/minute standing time					
		0	1	2	3	4	5
0.1	Sample = S	0.383	0.390	0.387	0.380	0.395	0.377
	Blank = B	0.00	-0.010	-0.008	-0.006	-0.004	-0.018
0.3	S	0.389	0.387	0.408	0.379	0.383	0.395
	B	-0.003	-0.012	-0.001	0.009	0.000	-0.014
0.5	S	0.385	0.394	0.387	0.384	0.387	0.372
	B	-0.007	-0.010	-0.008	0.000	0.008	-0.006
0.7	S	0.377	0.391	0.408	0.374	0.376	0.387
	B	0.001	-0.007	-0.005	-0.003	0.000	-0.008
1.0	**S**	0.365	0.368	0.409	0.379	0.388	0.370
	B	0.007	-0.014	-0.006	0.001	-0.006	-0.013

The results in the table 3 indicated that 1.0 ml of sulphamic acid solution (3%, w/v) with 2 minutes as standing time for the reaction gave the most suitable effect on the intensity of the azo-dye.

Effect of ethyl cyano acetate amount on absorbance

The effect of ethyl cyano acetate amount on the absorbance of the dye has been studied. From the results, it can be observed that 1.0 ml of 2% ethyl cyano acetate with 1 minute of reaction time was the more suitable which gave the highest value of intensity for the azo-dye (Table 4).

Table 4. Effect of coupling agent amount on absorbance

Ml of ethyl cyano acetate solution (2%)	Absorbance/min. standing time		
	0	1	3
0.5	0.368	0.393	0.376
1.0	0.392	0.406	0.373
3.0	0.375	0.385	0.362
5.0	0.385	0.357	0.361
6.0	0.366	0.361	0.377

Effect of time

The coloured azo dye developed rapidly after addition of ethyl cyano acetate and the stability period (within the first hour of stability) was sufficient to perform several measurements and the results are given in table 5. It is shown from table (5) that the maximum absorbance was obtained during the first five minutss and declined gradually.

Effect of amount and type of base

The preliminary experiments have shown that the azo- dye developed only completely in alkaline medium. Different amounts of bases (strong and weak) have been used (table 6).

The experimental data showed that ammonium hydroxide gave better sensitivity than sodium hydroxide and sodium carbonate. So that 2.0 ml of1M NH4OH is recommended for the subsequent experiments

Table 5. The effect of time and benzocaine amount on absorbance

µg of benzocaine present	Absorbance / minute standing time									
	0	5	10	15	20	25	35	45	55	60
5	0.051	0.060	0.061	0.057	0.060	0.059	0.058	0.058	0.065	0.060
25	0.211	0.213	0.221	0.206	0.219	0.212	0.211	0.210	0.216	0.208
50	0.397	0.406	0.405	0.406	0.404	0.406	0.406	0.406	0.406	0.396
100	0.792	0.790	0.790	0.792	0.793	0.792	0.792	0.793	0.792	0.784

Table 6. The effect of amount and type of base

Base used (1M)	Absorbance / ml of based used		
	1	2	3
NaOH	0.349	0.354	0.356
pH	12.10	12.56	12.71
Na_2CO_3	0.380	0.371	0.364
pH	9.57	10.02	10.17
NH_4OH	0.388	0.406	0.402
pH	8.92	9.47	9.74

Effect of order of additions

To obtain optimum results the order of additions of reagents should be followed as given under the general procedure, otherwise a loss in colour intensity was observed.

absorption spectra

The absorption spectra of the yellow azo dye formed by coupling of diazotised benzocaine with ethyl cyano acetate shows a maximum absorption at 405 nm. The reagent blank gives very weak absorption at this wavelength (Fig. 2).

Nature of the dye

The stoichiometry of the azo dye thus formed by reaction of diazotised benzocaine with ethyl cyano acetate was investigated by applying the continuous variations method (Job's method). The results indicated that the azo-dye was formed in the ratio of 1:1diazotised benzocaine to ethyl cyano acetate (Fig.3).

Interference

The effect of some foreign compounds which often accompanied pharmaceutical preparations were studied by adding three different amounts (50, 100 and 200µg) to 100µg benzocaine in a final volume 25ml (Table 7).

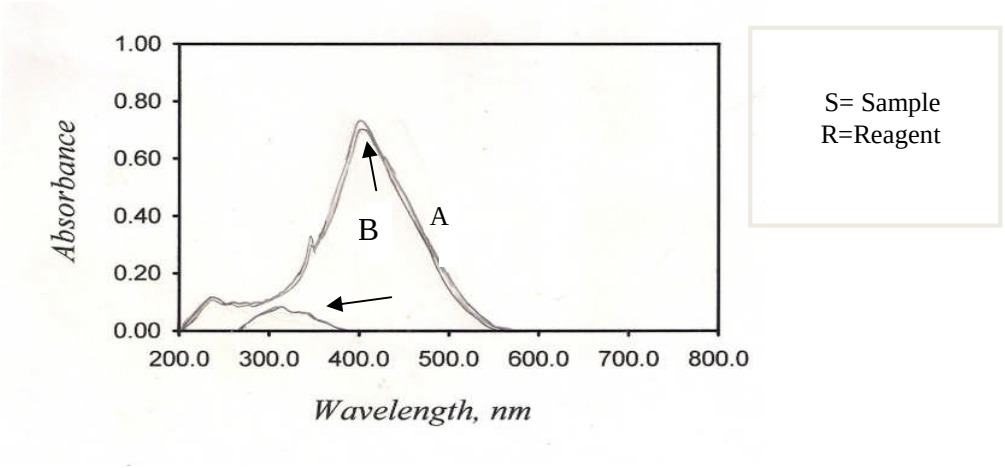


Fig.2: Absorption spectra of 100µg benzocaine / 25ml were treated according to the recommended procedure and measured against (A) reagent blank, (B) distilled water and (C) reagent blank measured against distilled water.

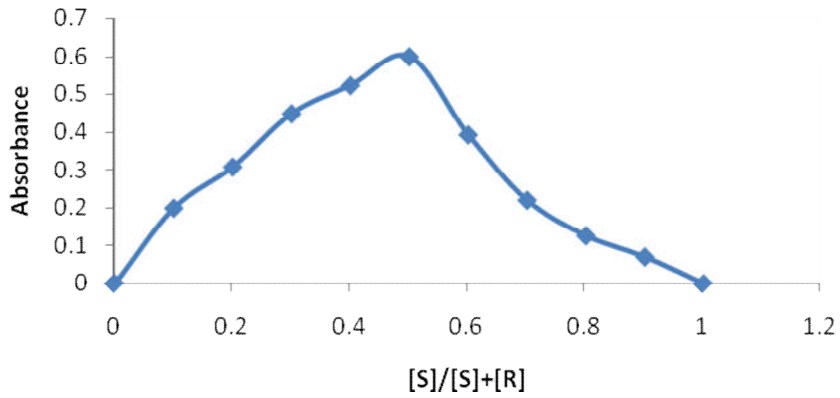
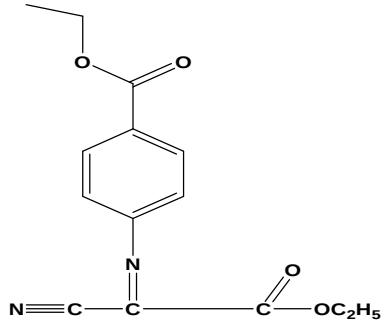


Fig.3: Job's plot for benzocaine - ethyl cyano acetate

Therefore the azo-dye may have the following suggested structure:



Yellow azo-dye

Table 7: Effect of foreign compounds for assay of benzocaine

Interferent	**Recovery (%) of 100 μg benzocaine / μg of interferent added**		
	50	**100**	**200**
Acaccia	98.28	99.70	99.6
Cetyl pyridenium chloride	99.60	99.26	95.7
Glucose	96.40	98.30	99.76
Lactose	98.52	97.05	100.1
Lindocaine	97.30	97.54	100.0
menthol	98.54	99.24	100.1
Starch	97.50	98.51	98.76

The results in table7 indicated that the studied foreign compounds did not interfere in determination of benzocaine using the proposed method.

Accuracy and precision.

To check the accuracy and precision of the method, benzocaine was determined at four different concentrations. The relative error% and relative standard deviation% results indicated the high accuracy and precision of the proposed method (Table 8).

Table 8. Accuracy and precision of determination of benzocaine using spectrophotometric method.

Amount of benzocaine taken, μg	**Relative error, %***	**Relative standard deviation, %***
25	0.00	± 0.23
50	+0.25	± 0.38
100	0.00	± 0.06
200	0.00	± 0.03

* Average of five determinations

Analytical application

The proposed method was applied to assay benzocaine in two Synthetic Pharmaceutical Preparations lozenges of benzocaine and throat lozenges solutions (E.Y. Hassen 2005). On applying proposed procedure, good recovery was obtained for lozenges of benzocaine solution only as shown in table 9.

Table9. Analytical applications of the spectrophotometric method in determination of benzocaine in Lozenges

Pharmaceutical preparation	**μg benzocaine present/25ml**	**Recovery* (%)**
Lozenges of benzocaine	50	97.3
	100	98.0
	150	99.0

*Average for five determinations

The proposed method for the determination of benzocaine in pharmaceutical preparation was simple and sensitive. The azo-dye formed was fairly soluble in aqueous solution. The statistical analysis of the results indicated that the method has good accuracy and good precision.

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التقدير الطيفي للبنزوكائين بواسطة تفاعلات تكوين صبغة الآزو

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الخلاصة

تضمن البحث طريقة طيفية لتقدير كميات متناهية في الصغر من البنزوكائيين. وأعتمدت الطريقة على أزوتة البنزوكائيين وذلك بمفاعلته مع ايون النتريت بوجود حامض الهيدروكلوريك ثم اقتران ملح الدايازونيوم الناتج مع كاشف الاقتران اثيل سيانو اسيتيت لتكوين صبغة آزوية صفراء مستقره وذائبة في الماء، تم قياس شدة الامتصاص للصبغة الناتجة عند الطول الموجي ٤٠٥ نانوميتر وكانت حدود قانون بير في مدى التركيز من ٥ إلى ٢٥٠ مايكروغرام من البنزوكائيين في حجم نهائي٢٥ مل (٠.٢ – ١٠جزء /مليون)وبمعامل ارتباط ٠.٩٩٨ وبلغت قيمة الامتصاصية المولارية 3.1 × ١٠ ٤ لتر. مول-١. سم-١، والخطأ النسبي تراوح بين 0.0 و 0.25 % والانحراف القياسي النسبي بين ± 0.03 و ± 0.38 % اعتمادا على مستوى تركيز البنزوكائيين. تم تطبيق الطريقة بنجاح لتقدير البنزوكائيين في احدى مستحضراته الصيدلانية المحضرة(Lozenges).