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Indirect Spectrophotometric Determination of Benzocaine in Pharmaceutical Preparations*

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ABSTRACT

An indirect spectrophotometric method is developed for the determination of benzocaine as pure and in pharmaceutical preparations. The method is based on the oxidation of benzocaine with iron (III) in acidic medium, and the liberated iron (II) reacts with 1,10 -phenanthroline to produce ferroin complex which has a maximum absorption at 510 nm against reagent blank. Beer's law is valid over the concentration range of 5-80 µg benzocaine/25 ml and the molar absorptivity is 5.7×10^4 $l.mol^{-1}.cm^{-1}$, indicating high sensitivity. The common excipients and additives do not interfere in benzocaine determination. The proposed method is successfully applied to the assay of benzocaine in two synthetic preparations.

التقدير الطيفي الغير مباشر للبنزوكائين في مستحضراته الدوائية

الملخص

تم تطوير طريقة طيفية غير مباشرة لتقدير البنزوكائين وتعتمد الطريقة على أكسدة البنزوكائين بواسطة الحديد الثلاثي في وسط حامضي ومفاعلة الحديد الثنائي الناتج مع الكاشف 10,1- فينانثرولين لينتج معقد الفيرون الذي أعطى أعلى شدة امتصاص عند الطول ألموجي 510 نانوميتر مقابل المحلول الصوري. اتبعت هذه الطريقة قانون بير في مدى التركيز من 5 إلى 80 مايكرو غرام/25 مللتر. إن الطريقة ذات حساسية عالية حيث كانت الامتصاصية المولارية 5.7×10^4 لتر. مول$^{-1}$. سم$^{-1}$. للبنزوكائين. تم دراسة تأثير المتداخلات (مواد السواغ) على طريقة التقدير المقترحة ولوحظ بان الطريقة لاتعاني من وجود هذه المواد وأمكن تطبيق الطريقة بنجاح في تقدير البنزوكائين في مستحضرين دوائيين محضرين.

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INTRODUCTION

Benzocaine (ethyl p- aminobenzoate) is a local anesthetic of the ester type used as a dry powder to decrease painful skin ulcers (Rang and Dale, 1989) and is used when impacted ear wax is removed (Lannon and Arcangelo, 1986).

Different methods have been used for the determination of benzocaine, in one of these, benzocaine has been determined by photometric method in an aqueous acidic medium with p-benzoquinone to form a charge-transfer complex. The range of determination
is 5.0-70 $\mu g.ml^{-1}$ and the molar absorptivitiy is 1.7×10^3 $l.mol^{-1}.cm^{-1}$ (Amin and El-Didamony, 2003).

Determination of benzocaine in pharmaceutical preparation has been accomplished, based on measuring the absorbance of the compound in ethanolic-aqueous solution at 290 nm. Beer's law is obeyed over the concentration range 10- 50 $\mu g.ml^{-1}$ (Song, 1990).

A colorimetric method is used to determine benzocaine in some dosage forms. The method based on the formation of a red Schiff's base results from the reaction of benzocaine with p-dimethylaminocinnamaldehyde in an aqueous acidic medium. The intensity of the product is measured at 544 nm and Beer's law is obeyed over the concentration range 0.025 - 2.3 $\mu g.ml^{-1}$ (Tan. *et al.*, 1977).

Benzocaine was extracted as a 1:1 ion pair complex with dicyclohexyl-18-crown-6 and calmagite in acidic medium into chloroform followed by spectrophotometric determination at 486 nm. Beer's law is obeyed over the concentration range 1.65-123 $\mu g.ml^{-1}$ (Madrakian *et al*., 2002).

A spectrophotometric method has been used for the determination of benzocaine involving diazotisation of benzocaine followed by coupling with ethyl acetoacetate to form a yellow product is developed. The range of determination is 2-15 $\mu g.ml^{-1}$ (Belal *et al*., 1978), m-Aminophenol reagent in basic medium forming an orange product with a maximum absorption at 471 nm is worked out. Beer's law is obeyed over the concentration range 5-400 µg/25 ml (Al-Hddeady, 2005). Also, phloroglucinol reagent is used (Othman and Zakaria, 2004).

Chromatographic methods have been also utilized for the determination of benzocaine such as high performance liquid chromatography (HPLC) for the determination of benzocaine in ear and eye drops and ointments (Sadana and Ghogare, 1991). Another HPLC method has been developed for the simultaneous determination of benzocaine, the mobile phase consists of mixture of menthol and glacial acetic acid (10% ,v/v) at different preparations (Perez-Lozano *et al.*, 2005). Another HPLC using methanol-10 mM triethylamine as amobile phase (Joseph-Charles *et al*., 2001).

Reversed-phase-HPLC is used for the determination of benzocaine and N-acetylbenzocaine in fillet of rainbow trout with an isocratic mobile phase and UV detection (Meinertz *et al*., 1999).

The present work describes a simple spectrophotometric method for the determination of benzocaine, based on the oxidation of benzocaine with iron (III) and the liberated iron (II) reacts with 1,10 -phenanthroline in aqueous solution to form a highly coloured complex that has proved successful for the assay of benzocaine in two synthetic drugs.

EXPERIMENTAL

Spectral absorbance measurements are carried out on double beam spectrophotometer Shimadzu (UV-160A) and UV-Visible spectrophotometer CECIL-CE 1021 digital single beam using 1-cm silica cells.

Reagents

Standard solution of benzocaine, 100µg.ml^{-1}.

This solution is prepared by dissolving 0.01g of benzocaine in distilled water and then the solution is made up to 100 ml in a volumetric flask with distilled water, working solutions are prepared by further dilutions of stock solution.

Iron (III) solution, 0.03M.

This solution is prepared by dissolving 1.21g of ferric nitrate nanohydrate in distilled water in the presence of 7 ml of 1M nitric acid and then the volume is completed to 100 ml in a volumetric flask with distilled water (Al-Sabha, 2007).

1,10-phenanthroline solution, 0.05M.

This solution is prepared by dissolving 0.99g of 1,10-phenanthroline monohydrate in 10 ml of ethanol and the solution is made up to100 ml in a volumetric flask with distilled water.

Lozenges of benzocaine compound.

This lozenges is prepared by dissolving 10 mg of benzocaine +50 mg of borax +0.3 mg menthol in 2 ml ethanol and 20 ml distilled water, heating is necessary to complete dissolution and volume after cooling is completed to 100 ml with distilled water in a volumetric flask (Clowes and Sons, 1973).

Throat lozenges

This solution is prepared by dissolving 5 mg benzocaine + 2 mg cetylpyridinum chloride in 2 ml ethanol and 20 ml distilled water with heating. The solution after cooling is completed to 100 ml with distilled water in a volumetric flask (Clowes and Sons, 1973).

Procedure and calibration graph

To a series of 25-ml calibrated flasks, 0.1-1.6 ml of 50 µg.ml.$^{-1}$ benzocaine solution are transferred then1.0 ml of 0.03 M Fe (III) solution is added, followed by addition of 3.5 ml of 0.05 M 1,10 -phenanthroline solution after which the volumes are completed to the mark with distilled water. The solutions are allowed to stand for 40 minutes in water bath adjusted at 90ºC, then the solutions left to stand for 15 minutes at room temperature before the absorbances of red coloured product are measured at 510 nm against the reagent blank. A linear calibration graph is obtained over the concentration range of 5-80 µg benzocaine/25 ml and concentration above 80 µg /25 ml gives a negative deviation (Fig.1). The molar absorptivity has been found to be 5.7×10^4 l. mol^{-1} cm $^{-1}$.

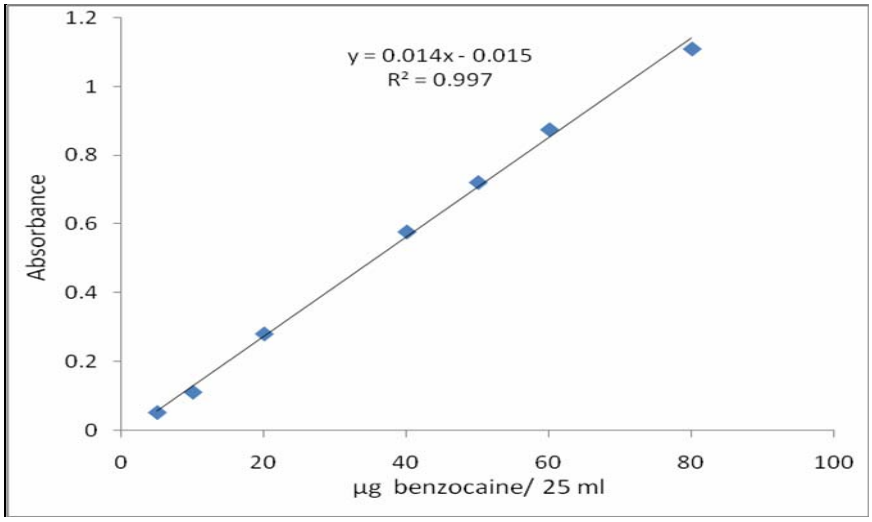

Fig.1: Calibration graph of benzocaine determination

Optimum reaction conditions

The optimum reaction conditions for quantitative determination of benzocaine is established doing the following experiments.

Effect of pH

Different types and amounts of acid added are investigated and the results indicate that the components of reaction without acid added (pH 3.385) give high intensity of the complex formed.

Effect of temperature

The effect of temperature on the colour intensity of the resulting complex is investigated. The results indicated that the absorbance of the complex increased with an increase in temperature (Table 1).

Table 1: Effect of temperature

Temperature (Cº)	50	60	70	80	90	100
Absorbance	0.166	0.235	0.259	0.269	0.281	0.210

The results in Table 1 indicate that the temperature of 90ºC gives highest intensity of the complex.

Effect of ferric ion amount

The effect of changing the ferric ion amount on the absorbance of solution containing 50 µg benzocaine/25 ml has been studied and it is evident from (Table 2) that the absorbance increased with increasing ferric ion amount and reached maximum when using 1.0 ml of 0.03M ferric ion solution. More than this amount leading to a decrease in the absorbance. Therefore, 1.0 ml of 0.03M ferric ion solution is used in all subsequent experiments.

Table 2:The effect of ferric ion amount on absorbance

Ml of 0.03 M Ferric solution	0.3	0.5	0.7	1.0	1.5	2.0
Absorbance*	0.140	0.194	0.240	0.275	0.109	Turbid

*Using 1 ml of 0.05 M 1,10-phenanthroline

Effect of 1,10-phenanthroline reagent amount

The effect of changing 1,10-phenanthroline amount on the absorbance of solution containing the same amount of benzocaine is studied. It is evident that the absorbance increases with increasing reagent concentration and reached maximum on using of 3.5 ml of 0.05M 1,10-phenanthroline, above this amount a slight decrease in the absorbance is observed. Therefore, 3.5 ml of 0.05M 1,10-phenanthroline is used in all subsequent experiments (Table 3).

Table 3:The effect of 1,10-phenanthroline amount on absorbance

Ml of 1,10-Phenanthroline (0.05M)	1.0	2.0	2.5	3.0	3.5	4.0	5.0
Absorbance	0.271	0.330	0.597	0.672	0.698	0.645	0.650

Order of addition

The order of addition of reagents should be followed as given under the general procedure, otherwise a lose in colour intensity takes place.

Stability

The stability of the coloured complex is investigated under the optimum conditions for the determination of benzocaine. The results (Table 4) show that the coloured complex formed from three different amounts of benzocaine is complete after 15 minutes after removing the flasks from water bath and the absorbance remained constant at least for one hour.

Table 4: Effect of time on absorbance

Benzocaine (µg /25ml)	**Absorbance/ min. standing time**							
	5	**10**	**15**	**20**	**30**	**40**	**50**	**60**
30	0.293	0.297	0.310	0.312	0.315	0.316	0.314	0.313
50	0.704	0.708	0.715	0.716	0.716	0.718	0.720	0.718
60	0.831	0.836	0.840	0.840	0.842	0.843	0.839	0.830

Final absorption spectra

Under the above optimized conditions, absorption spectra of the red coloured tris-1,10-phenanthroline - iron (ll) (ferroin) against reagent blank show maximum absorption at 510 nm as shown in Fig.(2).

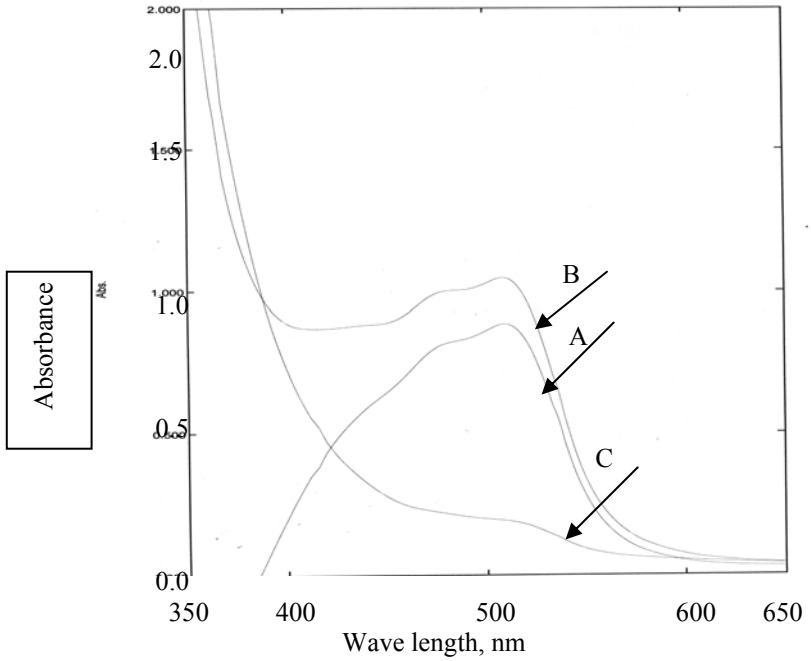

Fig. 2: Absorption spectra of 50 µg benzocaine/25 ml treated according to the recommended procedure and measured against (A) reagent blank, (B) distilled water and (C) blank measured against distilled water

Accuracy and precision

Five replicate determinations are performed for each of three different concentrations of benzocaine. The results of relative error and relative standard deviation indicate that the proposed method having high precision and accuracy (Table 5).

Table 5: The accuracy and precision

Amount of benzocaine taken (µg /25ml)	Relative error,%*	Relative standard deviation,%*
20	-0.21	±2.64
40	-0.69	±0.82
60	-0.34	±1.44

*Average of five determinations

Stoihiometry of the reaction

The stoichiometry of the product formed from the reaction of benzocaine with ferric ion is investigated by applying the continuous variations method (Job's

method). The results indicate that the product is formed in the ratio of 1 benzocaine: 3 Fe^{+3} (Fig. 3).

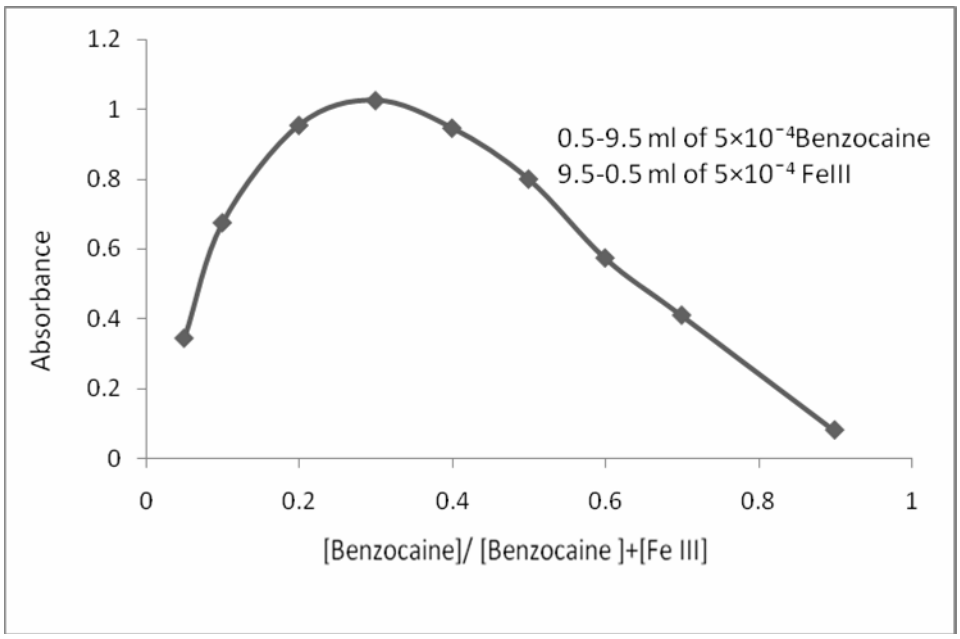

Fig. 3: Job's plot for benzocaine-FeIII

Interferences

In order to assess the possible analytical application of the proposed method, the effect of some foreign substances which often accompanied the pharmaceutical preparations are studied by adding different amounts of foreign substances to 50 µg benzocaine/25 ml. It is found that the studied foreign species didn't interfere in the present method (Table 6).

Table 6: Effect of interferences

Foreign compound	Recovery % of 50µg benzocaine /25 ml per µg of foreign compound added		
	200	**500**	**1000**
Glucose	99.2	101.7	106.9
Fructose	100.3	100.8	105.5
Acacia	98.3	101.3	100.4
Starch	99.3	96.8	97.5

APPLICATION

To test the accuracy of the present method, it has been applied to determine benzocaine in two synthetic pharmaceutical preparations, throat lozenges and lozenges of benzocaine compound. The results indicate that the present method can be used for the determination of benzocaine in the two drugs with satisfactory recovery (Table7).

Table 7: The recovery of benzocaine in pharmaceutical preparation

Drug	µg Benzocaine present / 25 ml	µg Benzocaine measured/25ml	Recovery %
Throat lozenges	30.0	29.3	97.6
	50.0	51.6	103.1
	70.0	72.7	103.9
Lozenges-benzocaine	30.0	29.9	99.6
	50.0	50.8	101.6
	70.0	68.6	98.0

Comparison of the methods

According to the difficulties of availability of some reagents used in standard method for the determination of benzocaine in pharmaceutical preparations, we used standard addition method to prove that the proposed method can be applied to determine benzocaine in throat lozenges and lozenges (Table 8 and Fig. 3).

Table 8: Determination of benzocaine in pharmaceutical preparation by standard addition method

Pharmaceutical preparation	Benzocaine taken (µg /25 ml)	Benzocaine measrred (µg /25 ml)	Recovery,%*
Throat lozenges	10.0	10.6	105.5
	30.0	31.0	103.0
Lozenges of benzocaine	10.0	10.1	100.9
	30.0	30.1	100.4

*Average of three determinations

The results in Table 8 and Fig. 3 indicated that the proposed method can be used for the determination of benzocaine in different pharmaceutical preparations with satisfactory results.

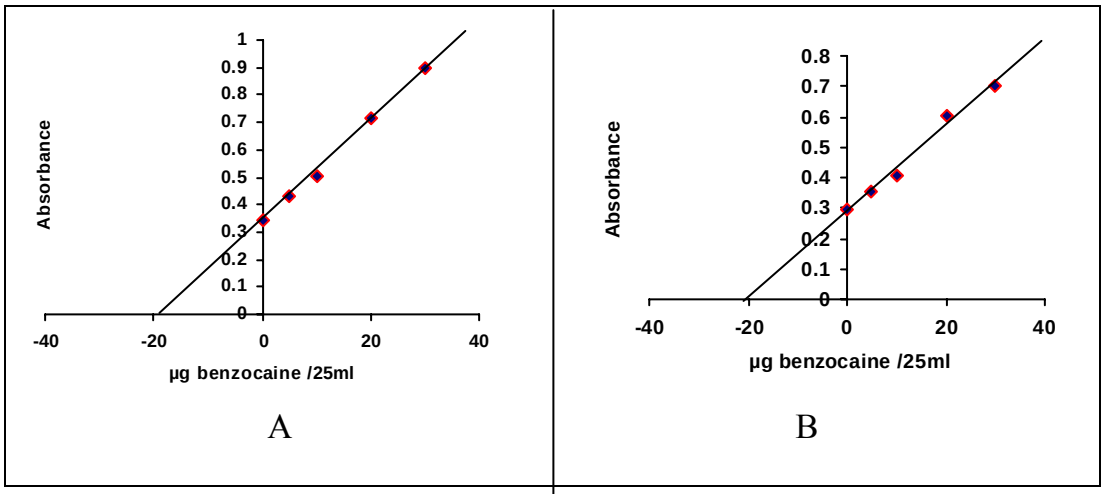

Fig. 3: Calibration standard addition graph for the determination of 20 µg/25 ml benzocaine in throat loznges (A) and in lozenges of benzocain

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