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Using of Hantzsch Condensation Reaction in Spectrophotometric Determination of Benzocaine

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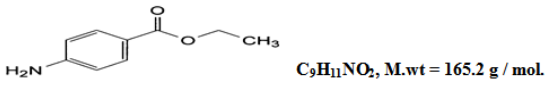
ABSTRACT

An accurate spectrophotometric method had been suggested for the determination of benzocaine (BEN) as pure form and in its pharmaceutical preparation (ear drop). The method depends on using Hantzsch reaction, the reaction included condensation of Benz with acetyl acetone-formaldehyde (Ac-Ac-FD) reagent. The suggested reagent was prepared by the reaction of Ac-Ac with FD, the reaction requires heating in a water bath at boiling point for 5 minutes. The condensation of BEN with Ac-Ac-FD needs heating in a water bath at a temperature of 40^0C for 25 minute, an intense yellow colored product was formed, which is stable and water-soluble. The yellow product has a maximum absorption at 417 nm. The linearity of Beer's law was observed within the range (2-48 µg BEN.ml^{-1}).The values of a molar absorptivity of $1.82x10^3$ l.mol^{-1}.cm^{-1} and Sandell's sensitivity index values of 0.0907µg .cm^{-2}.The relative error, relative standard deviation, low detection limit and low of quantitation values have been estimated. An application part of the suggested method in estimation of Benz in ear drop (otocol drops).

1. Introduction

BEN is ethyl p-aminobenzoate has become extensive in the drugs formulation industry, Benz was used as an anesthetic previously in some endoscopic investigations. It is white and odorless crystalline form, BEN has the chemical structure as shown in Scheme1[1,2].



Scheme 1: The formula and chemical structure of benzocaine.

Through literary survey, different analytical techniques were labelled for the estimation of BEN these techniques including: RP-HPLC[3] ,LC-mass[4], GC–MS, GC–TMS and LC–MS/MS [5],electrochemical sensing plate form[6], single-sweep polarography[7], capillary electrophoresis[8], the spectrophotometric methods were also reported [9-16].

Hantzsch reaction has been used for determination of various compounds containing primary amine as the main function group[17- 21].

The current project included a condensation reaction of Ac-Ac-FD. reagent with primary amine of benzocaine to produce a yellow colored product which was used as a basis for construction a confirmed spectrophotometric method for determination of Benz as pure form and in it is formulation.

2. Experimental

2.1. Apparatus

The absorbance and absorption spectrum were measured using a UV/VIS spectrophotometer (JASCOV – 630, Japan) and a water bath was also used to heat the samples.

2.2. Chemicals and solutions used

All chemicals used in this investigation are of a high purity.

Benzocaine standard solution (400 µg.ml^{-1}):

It was prepared by dissolving 0.0400 g. of benzocaine in 5 ml of ethanol and then the volume was

completed with distilled water to 100 ml with distilled water in a calibrated flask.

Sodium acetate solution, 0.2 M:

This solution was prepared by dissolving 2.7200 grams of sodium acetate (CH3COONa.3H2O) in distilled water then completing the volume with distilled water in a 100 ml volumetric flask.

Acetic acid solution (1 M):

This solution was prepared from diluting 5.75 ml of concentrated acetic acid (17.4M) with distilled water to 100 ml in a volumetric flask.

Acetic acid solution(0.2M):

This solution was prepared by mixing 20 ml of prepared (1M) acetic acid to a 100 ml volumetric flask and complete the volume to the mark with distilled water.

Reagent solution (Ac-Ac-FD):

This reagent was prepared by mixing 3.9 ml of acetyl acetone with 7.5 ml of formaldehyde (36%) with 8 ml of sodium acetate (0.2 M) with 17 ml of acetic acid (0.2 M) and then put the mixture in a water bath at boiling point for 5 minutes, then it was cooled and its acidity was set at pH equal to 4.3 using sodium hydroxide (1 M), then completed its volume with distilled water to 50 ml in a volumetric flask, knowing that the reagent solution was unstable and thus it must be prepared daily[18,21].

Pharmaceutical preparation solution: (400 µg.ml^{-1}):

The solution of the pharmaceutical preparation was prepared from the ear drop (otocol drops, 50 mg BEN / ml) by withdrawing 0.8 ml of the drug and adding to it 2 ml of ethanol in a 100 mL volumetric flask. After mixing, complete the volume with distilled water to the mark.

3. Procedure and standard curve

The standard curve was drawn by preparing several 10-ml flasks, each of which was added a different amount of the standard benzocaine solution within the range (20-480 µg /10 ml) and then added to each of them 2 ml of reagent solution (AcAc-FD) and all these flasks were placed In a water bath at 40 ^{o}C for a period of 25 minutes waiting, then the volumes of these volumes were completed with distilled water to the point of the mark and the absorbance was measured at the wavelength of 417 nm. The linear following of Beer's law was observed within the range (2-48 µg.ml^{-1}) so that outside this range a deviation occurred from Beer's law linearity (Figure1).

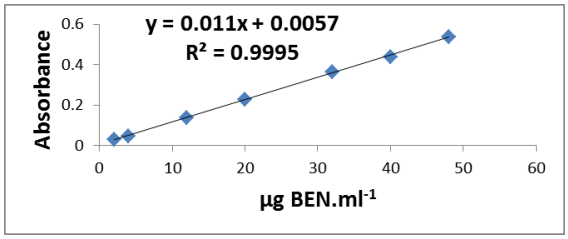


Fig. 1: Calibration curve

The molar absorptivity and Sandell's index values have been calculated and their values 1.82x103 l.mol.-1.cm.-1 and 0.0907 µg. cm-2 respectively. Also the limit of detection and limit of quantitation were calculated (LOD= 0.2279 µg.ml-1; LOQ = 0.7595 µg.ml-1) for estimating benzocaine according to the proposed method by applying the procedure and mathematical relationship fixed in literature [22].

4-Results and discussion

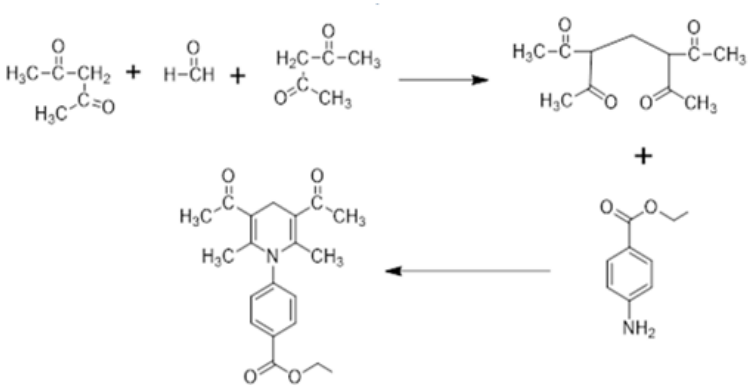
4.1. Preliminary study

The absorption spectrum of the reagent complex (AcAc-FD) with benzocaine was studied by mixing 0.5 ml of the sample (benzocaine 400 µg. ml-1) with 2 ml of reagent in a 10 ml volumetric flask and then put the mixture in a water bath at 400C for 15 minutes, then complete the volume to the point of the mark with distilled water, as it was observed that a bright yellow solution was formed. The measuring the absorption against the blank solution, gave the highest absorbance at the wavelength of 414 nm.The wavelength 414 nm fixed in the next determinations

4.2. Principle of the method

The method included two steps:

The first step included preparation of Ac-Ac-FD reagent then condensation of reagent with benzocaine to produce the yellow complex



Yellow complex **BEN**

4.3. Setting the optimum conditions for the reaction

In order to obtain the optimum conditions for the reaction and for the purpose of obtaining the highest sensitivity of the method and the highest stability of the resulting product, the all parameter that affected the intensity of yellow product have been studied as follows:

4.3.1. Investigate the optimum amount of reagent (Ac-Ac-FD)

Several 10 ml volumetric flasks were prepared and 0.5 ml of BEN was added to each of them with different volumes of reagent(1-2.5 ml) and these flasks were placed in a water bath at 40 oC for 15 minutes, then the volume was completed with distilled water and the absorbance was measured for each solution against its blank solution at 417 nm, the results are illustrated in Table 1.

Table1: The optimum amount of Ac-Ac-FD reagent.

Reagent amount (ml)	1.0	1.5	2.0	2.5
Absorbance	0.060	0.178	0.194	0.192

From the results obtained above, indicated that a volume of 2 ml of the reagent was chosen according to the highest absorbance and it to be adopted in the remaining measurements.

4.3.2. Study the optimum pH:

The effect of the acidity of the reagent was studied to give the highest absorbance value by preparing several reagent solutions with different values of the acidity (by raising the pH with a solution of NaOH (1M) or by reducing the acidity with acetic acid (0.2M), then several 10-ml volumetric flasks were prepared each contains 0.5 ml of 400 µg BEN in 10 ml (20 µg /ml) with 2 ml of reagent (with different acidity) for each flask was added, after placing the flasks in the water bath at 400C for 15 minutes then completed volumes with distilled water and the absorbance was measured at 417 nm and the results were as in the Table 2.

Table 2: The optimum value of pH in preparing the reagent.

pH value of reagent	2.5	4.3	6	8.5
Absorbance	0.097	0.198	0.137	Turbid

From the results of the Table 2, the reagent solution with the pH = 4.3 was selected for the rest of the measurements.

4.3.3. Study the effect of temperature:

After the appropriate value of the acidity of the reagent (AcAc-FD) was fixed, the optimum temperature was studied for several samples, each containing 0.5 ml of 400 µg BEN in 10 ml (20 µg /ml) with 2 ml of the reagent, the results were in Table 3.

Table 3: Effect of temperature.

Temperature, ^{0}C	27	40	60
Absorbance	0.032	0.196	0.193

From the results in Table 3, the best temperature that can be adopted in the subsequent experiments is 40 °C it gave the highest intensity.

4.3.4. Study of the incubation time of samples inside the water bath:

The time of being of the samples inside the water bath was studied by leaving the solutions for different times at 40°C to choose the most suitable ones, so that each of these samples contains 0.5 ml of 400 µg BEN in 10 ml (20 µg /ml) and 2 ml of the reagent and the results are listed in Table 4.

Table 4: Effect of heating time.

Time (min.)	**Absorbance**
5	0.136
10	0.158
15	0.198
20	0.204
25	0.219
30	0.212
35	0.210
40	0.211

Thus, based on the above results, the time of 25 minutes was chosen as the most appropriate period for the flasks inside the water bath to give a complete reaction and the highest absorbance.

4.3.5. The medium of reaction (type of solvent):

The effect of different types of solvents used in dilution of solutions on the absorption spectrum of 200 µg. of BEN in 10 ml (20 µg./ml) was studied according to the procedure of proposed method, and the results are shown in Figure (2) as well as in Table (5) .

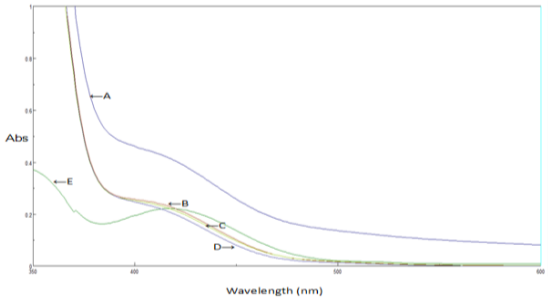


Figure 2: Effect of solvents on spectrum: A: Methanol, B: Propanol, C: Ethanol, D: Acetone, E: Water

Table 5: Effect of solvents.

Solvent	**Absorbance**	**λ_{max}**	**Ɛ , L.ml^{-1}.cm^{-1}**
Acetone	0.235	406	1958
Methanol	0.445	406	3708
Ethanol	0.241	411	2008
Propanol	0.246	404	2050
n-butanol	Turbid	———	———
Water	0.221	417	1841

From the results of Fig. 2 and Table 5 above, water was adopted as a solvent in subsequent experiments,

despite giving it a lower absorption compared to other solvents, due to its cheap price and abundance.

5. Ultimate absorption spectrum:

After the optimum conditions were fixed for this method, the final spectrum curve was drawn by preparing 200 µg of the standard solution of BEN in a 10-ml volumetric flask and adding to it 2 ml of reagent solution (AcAc-FD) and placed it in a water bath at 40 °C for 25 minute's wait, then completed the volumes of these volumetric flasks with distilled water to the point of the mark, and it was measured against the blank solution once and against the distilled water again. The absorption spectrum of the blank solution versus the distilled water was also measured, and the results were as in Figure 3.

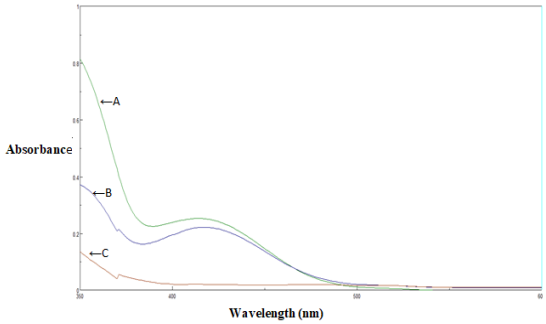


Fig. 3: The final absorption spectrum of determination of 200 µg BEN: Sample versus Distilled water (A), Sample versus Blank (B) and Blank versus Distilled water

6. The stability of yellow product.

The stability of the reaction product solution over time was studied by tacking two concentrations of BEN and the recommended procedure applied, and then studying the effect of time on them(Table 6).

Table 6: Effect of time on stability of product.

Time (min.)	Absorbance / µg Benzocaine in ml	
	10	20
Immediately	0.109	0.219
5	0.112	0.221
10	0.114	0.222
15	0.113	0.221
20	0.113	0.222
25	0.114	0.223
30	0.115	0.223
35	0.115	0.223
40	0.115	0.223
45	0.116	0.224
50	0.116	0.225
55	0.116	0.224
60	0.116	0.226

From the results in Table (6) it is clear to us that the reaction product is characterized by high stability.

7. Accuracy and precision

The accuracy and precision of the proposed method for estimating three different concentrations of BEN (8.0, 20.0 and 32.0 µg. ml-1) with five readings for each of them have been calculated, and then calculate the relative error and relative standard deviation to know the accuracy and precision of the method (Table 7).

Table7: Calculations of accuracy and precision values of standard benzocaine

Sample	Amount taken, µg/ml	RE %	RSD %
Standard benzocaine solution (400 µg /ml)	8.0	+1.95	3.75
	20.0	+2.98	1.86
	32.0	+1.69	3.24

Through the satisfactory results of RE% and RSD %, it becomes clear to us the feasibility of the proposed method for estimating benzocaine.

8. Application part

The application of suggested method was carried out on the estimation of BEN in it is pharmaceutical formulation (otocol drops,50 mg/ml) by using standard addition method. The method included preparing two series of volumetric flasks of 10 ml, adding to each of them different concentrations of standard BEN (0-280 µg). then 100 µg of BEN drug(otocol drops solution) to each flask of the first series, then 200 µg of BEN drug solution to each flask of the second series, then 2 ml of reagent solution(AcAc-FD) to each of the two series flasks and put all these flasks in a water bath at 40 0C for a period of 25 minutes waiting, then completed the volumes of these volumetric flasks with distilled water to the extent of the mark and were measured at the wavelength of 417 nm, the results were as in Figure (4).

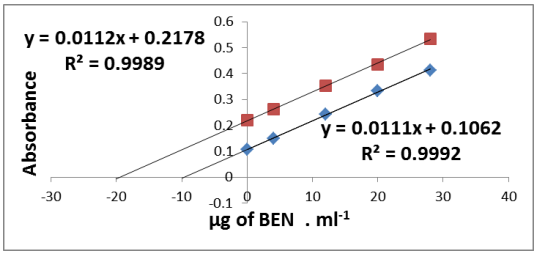


Fig. 4: Standard addition method plot of application part.

The values of the recovery, relative error and the relative standard deviation were also calculated for drug application by repeating three readings for each concentration of the two concentrations used in the application (10 and 20 µg/ml), and the results were as shown in Table (8).

Table 8: The results of application part.

Drug	Amount taken, µg/ml	Recovery %	RE %	RSD %
Otocol/drops (400 µg/ml)	10	95.68	-4.32	1.925
	20	97.23	-2.77	0.730

Through the results shown in Table (8), we note the possibility of adopting the proposed method for estimating BEN in it is formulation with acceptable analytical results.

Comparison of methods

The comparison between the important analytical variables for the present method with the same of two literatures spectrophotometric methods illustrated in Table 9.

Table 9: Comparison of the methods

Analytical parameters	Present method	Literature method [11]	Literature method [12]
Type of reaction	Condensation	Oxidation Bleaching	Oxidative Coupling
Reagent	Ac-Ac-FD	Rifampicin	Promethazine
λ max (nm)	417	476	615
Beer's law range(µg . ml^{-1})	2-48	2-15	5-300
Molar absorptivity l.mol^{-1}.cm^{-1}	1.82x103	4.295 x10 3	1.77 x10 3
Application of the method	Pharmaceutical preparation (sterile ear drops)	Pharmaceutical preparation (sterile ear drops)	Two synthetic pharmaceutical preparations

The results above in Table 9 indicated that the method in reference[11] is more sensitive than in [12] and present method (sensitivity for method in reference[11] >Present method > in [12])and the present method applied in estimation of BENZ in its pharmaceutical formulation(sterile ear drops) with good results..

9. Conclusion:

The suggested method is respect to their good sensitivity, accuracy, precision, the colored product has good stability and it is suitable for the estimation of BEN in ear drop with satisfactory results.

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استخدام تفاعل (Hantzsch) التكثيفي في التقدير الطيفي للبنزوكائين

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الملخص

تم اقتراح طريقة طيفية دقيقة لتقدير البنزوكائين (BEN) بشكله النقي وفي مستحضره الصيدلاني (قطرة الأذن). تعتمد الطريقة على استخدام تفاعل Hantzsch . تعتمد طريقة العمل على تفاعل تكثيفي للبنزوكائين مع كاشف أستيل أسيتون– فورمالديهايد (Ac-Ac-FD). تم تحضير الكاشف المقترح عن طريق تفاعل Ac-Ac مع الفورمالديهايد يتطلب التفاعل التسخين في حمام مائي عند درجة الغليان لمدة 5 دقائق. يحتاج تكثيف BEN مع Ac-Ac-FD إلى تسخين في حمام مائي عند درجة حرارة 40 درجة مئوية لمدة 25 دقيقة ، وتم تكوين ناتج أصفر اللون ، وهو مستقر وقابل للذوبان في الماء. المنتج الأصفر لديه أقصى امتصاص عند 417 نانومتر. الخطية لقانون بير ضمن المدى من 2الى 48 مايكروغرام بنزوكائين.مل$^{-1}$ وكانت قيمة الامتصاصية المولارية $1.82x10^{3}$لتر مول$^{-1}$ سم$^{-1}$ وقيم معامل الحساسية ساندل 0.0907 ميكروغرام سم$^{-2}$. تم تقدير الخطأ النسبي والانحراف المعياري النسبي وحد الكشف وحد التقدير الكمي. استخدمت الطريقة المقترحة في تقديرالبنزوكائين في قطرة الأذن (قطرات الاوتوكول).