Spectrophotometric Determination of Benzocaine via Oxidation and Bleaching Colour of Rifampicin Dye

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Abstract

Benzocaine (BENZ) has been estimated by indirect spectrophotometric method. The suggested method based on oxidation of BENZ via N-bromosuccinamidamide (NBSA) in presence of hydrochloric acid, then the unreacted or the excess NBSA was immerse in bleaching the colour of rifampicin dye (REFPD), the absorbance of unreacted REFPD has been estimated at wavelength 476 nm (the highest absorption of REFPD). The measured absorbance is directly proportional to the amount of BENZ in the solution. All parameters affected the oxidation of BENZ and bleaching the colour of REFPD and the optimum values have been fixed. Beer's law (linearity) of the method is in the range of 2 to 15 μg.ml⁻¹, the molar absorptivity value equal to 4.295 x10³ l.mol⁻¹.cm⁻¹ and the Sandall's index for sensitivity is calculated and equal to 0.0384 μg.cm⁻². The suggested method has an application part included estimation of BENZ in its pharmaceutical formulation (sterile ear drops).

Keywords: Benzocaine, rifampicin, N-bromosuccinimide, spectroscopy, bleaching.

Introduction

BENZ is one of a local anesthetic drug for local and temporal relief of pain. BENZ is used as a local to loss the sensitivity and to eliminate sensation of the hard pain, also it so-called pain sensitivity [1,2]. BENZ is ethyl p-aminobenzoate; ethyl 4-aminobenzoate or it called ethylaminobenzoate, it is white crystal, colourless and odorless has the following structure in Scheme 1 [3].

\[ \text{M.wt} = 165.2 \text{ g/mol.} \]

Chemical formula : \( \text{C}_9\text{H}_{11}\text{NO}_2 \)

Scheme 1. The chemical formula and structure of BENZ
Various techniques or method have been used in estimation BENZ as free or in its pharmaceutical formulations. These techniques included HPLC[4,5], HPLC-FIA-amperometric detector[6], batch injection analysis-amperometric detector [7], single sweep polarography[8], capillary electrophoresis[9]. According to availability of devices used in spectroscopy and are not expensive, and also BENZ contains the amine group in its structure that is considered one of the encouraging groups for researchers, as it enters into many known reaction that are sensitive analytically [10-18]. The dye used in this investigation is rifampicin dye, a reddish-brown or brownish-red, crystalline powder, slightly soluble in acetone, water and soluble in methanol[3]. REFPD has been used in estimating paracetamol via the, oxidation and bleaching the colour[19].

The main objective of the current research is to estimate BENZ in an easy and sensitive spectrophotometric method and apply it in estimation BEBNZ in its pharmaceutical preparation (sterile ear drops).

**Experimental**

**Apparatus**

A JASCOV – 630 UV / VIS spectrophotometer (Japan), with matched quartz cells (1cm) were used for all measurement. The balance BEL ENGINEERING was used in this investigation.

**Material and solution**

All material used in this research are of analytical grad.

**Benzocaine solution, 100 μg .ml⁻¹**

BENZ in 100 μg .ml⁻¹ was prepared by dissolving 0.0100 g of BENZ [C₉H₁₁NO₂] in 5 ml ethanol and then the volume was completed to mark of the 100 ml volumetric flask with distilled water.

**N-bromosuccinimide solution (0.01M)**

This solution was prepared by dissolving 0.1779 g of NBSA in a 30 ml of distilled water, then complete the volume to the mark of 100 ml volumetric flask with distilled water.

**Rifampicin solution (0.1%)**

This solution was prepared by dissolving 0.05 g of REFPD with 2 ml ethanol (95%), then the volume was completed with distilled water in a 50 ml volumetric flask.

**Hydrochloric acid solution (1M)**

8.4 ml of concentrated hydrochloric acid was diluted to 100 ml with distilled water in a volume flask.

**Pharmaceutical preparation, 100 μg .ml⁻¹**

Three container of otocol sterile ear drops were mixed (each ml contain 50 mg of BENZ, 1 ml of the mixture was mixed with 2 ml of ethanol and then the volume was completed with
distilled water to 100 ml in a volumetric flask, 5 ml from above solution diluted into 25 ml in a volume flask with distilled water to prepare 100 μg BENZ.ml⁻¹ solution.

Standard and curved working method

The calibration curve for the suggested method was prepared by adding various volumes of BENZ (100 μg.ml⁻¹) to cover the range of BENZ concentration from 2 to 15 μg.ml⁻¹ to a serious of 10 ml volumetric flasks; then 1 ml of 1M HCl, and 0.3 mL of NBSA (0.01M) were added and after a period waiting for 20 minutes, 0.5 ml of REFPD solution then 2 ml of ethanol (95%) for each flask (to increase stability) were added. Let the flasks for 5 minutes before the volume completed with distilled water to the mark. The absorbance of solutions was measured at the wavelength of 476 nm and the results were as in Figure 1 indicated that the linearity is from 2 to 15 μg.ml⁻¹

Figure 1. The calibration curve of determination BENZ via suggested method.

The molar absorptivity and the Sandall's index values are calculated and equal to 4.295 x 10³ l.mol⁻¹ cm⁻¹ and 0.0384 μg.cm⁻² respectively.

Results and discussion

The preliminary experiment involved knowing the maximum wavelength for REFPD by taking 1 ml of (0.1%) solution and diluting it into 10 ml with DW, and the spectrum from 250-700 nm against DW (Fig. 2) indicated that 476 nm was the maximum absorption of REFPD, and it is fixed in the next experiments.
Figure 2. The spectrum of REFPD.

The optimum amount of REFPD

Absorbance values for solutions prepared from various amount of REFPD were used in the constructing of standard curve of REFPD (Fig 3).

Figure 3. The calibration curve of REFPD

REFPD volume 0.5 ml was adopted in subsequent experiments.

Selection of oxidizing agent

Also, one of the preliminary experiments that must be performed is to know the type of oxidizing agent that gives a highest colour bleaching of REFPD. Figure 4 shows that the NBSA solution gives the best results (maximum bleaching) when compared with KIO₄, and was chosen in subsequent experiments.
The optimum conditions for the reaction:
In order to obtain the ideal conditions that give the highest sensitivity and the highest stability of the unbleached – REFPD using NBSA as an oxidant, the optimum conditions that achieve this have been studied as follows:

The optimum amount of NBSA

Different volumes of 100 μg . ml⁻¹ BENZ solution (to cover the concentration from 25-200 μg BENZ .10 ml⁻¹) have been added to several 10 ml volumetric flasks then 0.5 ml of 1 M HCl has been added. NBSA solution was added with various volumes then after good shaking, left these volumetric for a waiting period of 10 minutes and then 2 ml of the REFP (0.1%) and 2 ml of ethanol (95%) were added. After waiting for 5 minutes, complete the volume with distilled water and the absorbance was measured against the blank solution at 476 nm and the results were as in Table 1.

Table 1. The optimum amount of NBSA.

| 0.01M of NBSA (ml) | μg of Benzocaine /10 ml | R²    |
|---------------------|------------------------|-------|
|                     | 25         | 50   | 100  | 150  | 200  |       |
| 0.1                 | 0.3392     | 0.3955| 0.4057| 0.4085| 0.4106| 0.5667|
| 0.2                 | 0.2104     | 0.2962| 0.3753| 0.3951| 0.4038| 0.8077|
| 0.3                 | 0.0731     | 0.1366| 0.2513| 0.3652| 0.3995| 0.9685|
| 0.4                 | 0.0194     | 0.0639| 0.2107| 0.2785| 0.3318| 0.9621|
| 0.5                 | 0.0180     | 0.0283| 0.0831| 0.2130| 0.2667| 0.9623|

From the results in Table 1, although 0.1 and 0.2 ml of NBSA gave highest intensity, but the stability is bad and they gave lowers values of determination coefficient (R²), therefore 0.3 ml of the oxidant (NBSA) was chosen to give it the highest absorbance of the excess REFPD and
the highest value of determination coefficient ($R^2$), which indicates that most of the oxidizing agent is used to oxidize BENZ, and the volume 0.3 ml was fixed in subsequent experiments.

**Selection the optimum acid**
The effect of different types of acids has been studied to give the highest absorbance value by preparing several samples, each containing 0.5 ml of acid with 1 ml of BENZ (100 μg) with 0.3 ml of NBSA (0.01M). Then, after shaking, left the flasks for 10 minutes, then add 0.5 ml of REFPD and 2 ml of ethanol (95%), with a wait of 5 minutes before completing the volumes with distilled water to the marks. The absorbance was estimated at 476 nm (Table 2).

**Table 2. The effect of acid.**

| Acid (1M)       | HCl  | H$_2$SO$_4$ | HNO$_3$ | CH$_3$COOH |
|-----------------|------|-------------|---------|------------|
| Absorbance      | 0.2506 | 0.2013      | 0.2296  | 0.1988     |

The hydrochloric acid was chosen for subsequent experiments to give it the highest absorbance, as well as the effect of the optimum amount of hydrochloric acid was studied and the results in the Table 3 show that the 1 ml was the optimal for the reaction.

**Table 3. The optimum amount of hydrochloric acid.**

| 1M HCl, ml | 0.2  | 0.5  | 0.7  | 1.0  | 2.0  | 3.0  |
|------------|------|------|------|------|------|------|
| Absorbance | 0.2497 | 0.2511 | 0.2524 | 0.2531 | 0.2526 | 0.2528 |

The effect of the time needed to oxidize the BENZ and to bleach the colour of REFPD
To serious of volumetric flasks, 1 ml of BENZ solution with 1 ml of HCl and 0.3 ml of NBSA were added, then each solution was left for different waiting times, then 0.5 ml of REFPD was added with 2 ml of ethanol, the solution was left again for different waiting periods, and the absorbance was measured against the blank solution (Table 4).

**Table 4. The time for oxidation of BENZ and bleaching the colour of REFPD.**

| Time (minutes) after adding NBSA | 5  | 10 | 15 | 20 | 30 |
|----------------------------------|----|----|----|----|----|
| 5                                | 0.2306 | 0.2285 | 0.2261 | 0.2254 | 0.2249 |
| 10                               | 0.2541 | 0.2533 | 0.2520 | 0.2511 | 0.2496 |
| 15                               | 0.2683 | 0.2675 | 0.2664 | 0.2668 | 0.2645 |
| 20                               | 0.2849 | 0.2841 | 0.2833 | 0.2825 | 0.2816 |
| 30                               | 0.2842 | 0.2845 | 0.2836 | 0.2827 | 0.2821 |
Through the results in Table 4, a waiting time of 20 minutes was adopted after adding the oxidizing agent, and also 5 minutes after adding the REFPD before diluting with DW, then the absorbance measured at 476 nm.

**Study addition sequences**
The effect of different sequences of additives of solution components was studied as shown in the Table 5.

**Table 5. The order of addition.**

| Order of addition | Absorbance |
|-------------------|------------|
| І : BENZ + HCl(H) + NBSA + REFPD | 0.2852 |
| ІІ : BENZ + NBSA + H + REFPD | 0.2850 |
| ІІІ : BENZ + NBSA + REFPD + H | 0.2736 |
| ІV : BENZ + H + REFPD + NBSA | 0.1973 |

The results in Table 5 show that the order from І to ІІІ have approximately the same absorbance and order ІV, a decrease in the absorbance value occurred due to the competition between BENZ and the REFPD on oxidation with the oxidizing agent(NBSA), therefore, the order number І used in previous experiments was recommended for the next experiments.

**Study the effect of time on stability**
The stability of the unreacted REFPD was studied and the results illustrated in Table 6.

**Table 6. The stability of unreacted REFPD.**

| Time  | Absorbance / μg BENZ in 10 ml |
|-------|-----------------------------|
|       | 50                          | 100                          |
| Immediately | 0.1524                  | 0.2836                     |
| 5      | 0.1516                      | 0.2824                     |
| 10     | 0.1513                      | 0.2822                     |
| 15     | 0.1513                      | 0.2819                     |
| 20     | 0.1512                      | 0.2816                     |
| 25     | 0.1509                      | 0.2816                     |
| 30     | 0.1507                      | 0.2814                     |
| 35     | 0.1506                      | 0.2813                     |
| 40     | 0.1506                      | 0.2811                     |
| 45     | 0.1505                      | 0.2809                     |
| 50     | 0.1503                      | 0.2807                     |
| 55     | 0.1504                      | 0.2807                     |
| 60     | 0.1503                      | 0.2807                     |

The results in Table 6 indicated that excellent stability for at least 1 hour.

The optimum conditions obtained through previous experiences are listed in Table 7.
Table 7. The optimum condition of suggested method.

| Parameter                                | The optimum |
|------------------------------------------|-------------|
| Maximum wavelength of REFPD ,nm          | 474         |
| Acid used, concentration and amount      | Hydrochloric acid, 1M and 1 ml |
| Amount of REFPD, ml                      | 0.5         |
| Time of oxidation, minute                | 20          |
| Time of colour bleaching, minute         | 5           |
| Stability, minute (with 2 ml ethanol)    | 60          |

Accuracy and precision

Table 8 contains values calculated from reading absorbance of 5 replicates for 3 concentrations of BENZ, as the recovery percentage values are expressed in terms of accuracy and the values of the relative standard deviation (RSD %) express the precision, and the results indicate that the proposed method has good accuracy and precision.

Table 8. Accuracy and precision of proposed method.

| µg BENZ taken in 10 ml | µg BENZ found in 10 ml | Recovery*% | RSD % |
|------------------------|------------------------|------------|-------|
| 50                     | 49.2                   | 98.40      | 0.389 |
| 75                     | 75.45                  | 100.60     | 0.184 |
| 100                    | 103.85                 | 103.85     | 0.351 |

*Average of five determinations.

Application of the method

Two series of 10 ml volumetric flasks were prepared and different volumes of standard BENZ (0 to 1 ml of 100 µg) was added, then 0.2 ml of drug solution (otocol drops) to each flask of series No 1, as well as 0.4 ml of the drug solution (otocol drops) was added to each flask of the series No 2, then the flasks proceed as mentioned in the standard and curved working method. The absorbance of these solutions was measured at the wavelength of 476 nm, drawing absorbance vs. concentration of standard BENZ gave Figure 5.

Figure 5. The standard addition curves for determination of BENZ in otocol drops.
The results in Table 9 from using the standard addition method indicated that the suggested method yielded results for the percentage of recovery of the BENZ in its pharmaceutical preparations (sterile ear drops). The obtained result is very close to the result confirmed by the manufacturer of the drug and within the permissible analytical error.

Table 9. The results of determination of BENZ in sterile ear drop.

| Drug                  | Certified contained | $\mu g$ BENZ.ml$^{-1}$ taken | $\mu g$ BENZ.ml$^{-1}$ founded | Recovery % |
|-----------------------|---------------------|------------------------------|-------------------------------|------------|
| Otocol Sterile ear drops. | 50 mg BENZ.ml$^{-1}$ | 20                           | 20.868                         | 104.34     |
|                       |                     | 40                           | 39.448                         | 98.62      |

Comparison of method

Table 10 shows the comparison between main analytical variables for the suggested method with those of two literatures spectrophotometric methods.

Table 10. Comparison of the method

| Analytical parameters                  | Present method | Literature method [11] | Literature method [18] |
|----------------------------------------|----------------|------------------------|------------------------|
| $\lambda_{max}$ (nm)                    | 476            | 615                    | 510                    |
| Beer's law range ($\mu g$ . ml$^{-1}$)  | 2-15           | 5-300                  | 0.2-3.2                |
| Molar absorptivity Lmol$^{-1}$.cm$^{-1}$| $4.295 \times 10^{3}$ | $1.77 \times 10^{3}$ | $5.7 \times 10^{4}$ |
| Stability of the colour, minutes       | 60             | ..........              | 45                     |
| Medium of method                       | Acidic         | Acidic                 | Acidic                 |
| Reagent                                | Rifampicin     | Promethazine           | 1,10 -Phenanthroline   |
| Type of reaction                       | Oxidation –Bleaching | Oxidative Coupling   | Oxidation -Reduction  |
| Application of the method              | Pharmaceutical preparation (sterile ear drops) | Two synthetic pharmaceutical preparations | Two synthetic pharmaceutical preparations |

The results indicate that the present method is sensitive and gave successful estimation of BENZ in its pharmaceutical formulation (sterile ear drops).
Conclusion

The present method included a sensitive, simple and accurate spectrophotometric method for the estimation of BENZ via oxidation with NBSA in acidic medium and the excess amount of NBSA used in bleaching the colour of REFPD. The method applied in estimation of BENZ in ear drops with satisfactory results.

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