

NEW COLORIMETRIC METHOD DEVELOPMENT AND VALIDATION OF  
BENZOCAINE BY VISIBLE SPECTROSCOPYP. Salomi<sup>\*1</sup>, S. Arshiya Banu<sup>2</sup>, B. Gowthami<sup>2</sup>, R. Spandana<sup>2</sup>, S. Sivaiah<sup>2</sup>, M. Rajesh Kumar<sup>2</sup><sup>1</sup>Associate Professor, Department of Pharmaceutical Analysis, P. Rami Reddy Memorial College of Pharmacy, Kadapa, A.P – 516003.<sup>2</sup>B.Pharmacy, P. Rami Reddy Memorial College of Pharmacy, Kadapa, A.P – 516003.**\*Corresponding Author: P. Salomi**

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**ABSTRACT**

**Background & objectives:** Benzocaine is a local anesthetic used to relieve pain caused by conditions like mouth ulcers or sores. The present study has been undertaken in order to develop new simple, rapid efficient and reproducible method for the analysis of Benzocaine by using NED as a coloring reagent. **Methods:** With the help of NED *N*-(1-Naphthyl) ethylenediamine reagent a new colorimetric method was developed and the color intensity of the resulting solution is measured by using Visible spectroscopy. **Results:** The proposed U.V. spectrophotometric method was validated by evaluation of validation parameters. The proposed colorimetric methods give good reproductive results for the estimation of Benzocaine. This is very simple, rapid and nowhere involves complicate sample preparation. **Conclusion:** It is simple and rapid, selective, precise. Hence, suitable for application in routine analysis of pharmaceutical preparation.

**KEYWORDS:** Benzocaine, NED, Colorimetry, Validation.**INTRODUCTION**

Benzocaine (Mucopain gel) is typically used to relieve pain caused by conditions like mouth ulcers or sores. In some cases, it may also be prescribed and used as a mild local anesthetic to numb a localized area in preparation for medical examinations and is used when impacted ear wax is removed.<sup>[1]</sup> It is the ethyl ester of *p*-aminobenzoic acid (PABA). It can be prepared from PABA and ethanol<sup>[2]</sup> by Fischer esterification or via the reduction of ethyl *p*-nitro benzoate. Benzocaine is sparingly soluble in water; it is more soluble in dilute acids and very soluble in ethanol, chloroform, and ethyl ether. The melting point of benzocaine is 88-90°C<sup>[3]</sup>, and the boiling point is about 310°C. The density of benzocaine is 1.17 g/cm<sup>3</sup>.

Different methods have been used for the determination of benzocaine and one of those methods Benzocaine has been determined by using the photometric method in an aqueous acidic medium with *p*-benzoquinone to form a charge-transfer complex. The range of determination is 5.0-70µg.ml<sup>-1</sup> and the molar absorptive is 1.7×10<sup>3</sup> l.mol<sup>-1</sup>.cm<sup>-1</sup> (Amin and El-Didamony, 2003).<sup>[4]</sup>

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 µg.ml<sup>-1</sup> (Song, 1990).<sup>[5]</sup> A colorimetric method is used to determine Benzocaine in

some dosage forms. It is 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 µg. ml<sup>-1</sup> (Tan. *et al.*, 1977)<sup>[6]</sup>

**N(1-NAPHTHYL) ETHYLENE DIAMINE**

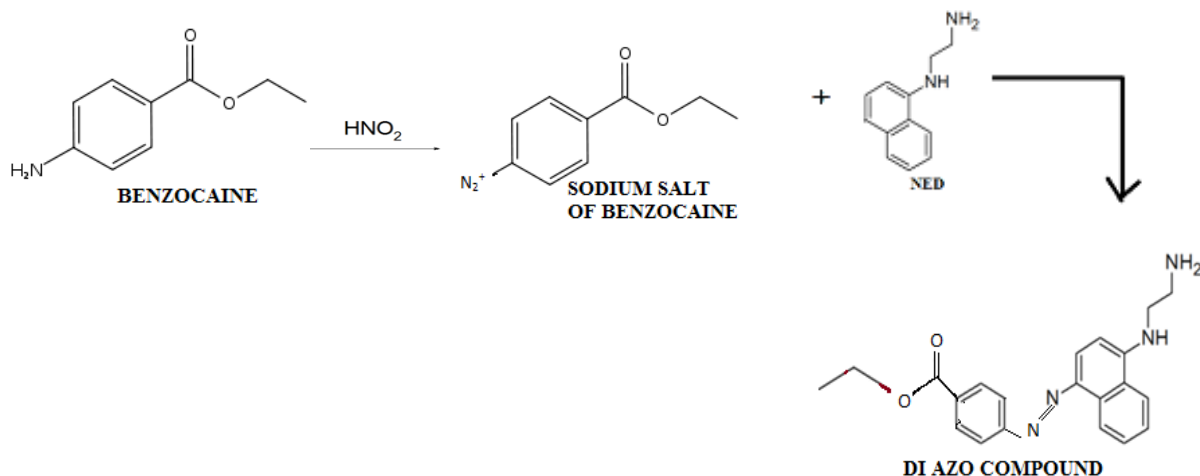
*N*-(1-Naphthyl)ethylenediamine is an organic compound. It is commercially available and finds application in quantitative inorganic analysis of nitrates, nitrite, and sulfonamide in blood. It undergoes most reactions typical to naphthylamine and primary amines such as diazotation. Similar to its analogue ethylenediamine, it can also act as a bidentate ligand to give several coordination compounds. This compound can be prepared by the reaction of 1-naphthylamine with 2-chloroethanamine. It is commercially available as the dihydrochloride salt. It is widely used in the quantitative analysis of nitrate and nitrite in water samples by colorimetry. It readily undergoes a diazonium coupling reaction in the presence of nitrite to give a strongly colored azo compound. The present study describes a simple spectroscopic method for the method development of Benzocaine using NED as a coloring reagent.

**MATERIALS AND METHOD****The instrument employed (UV)**

Make : SYSTRONICS  
 Model no : 2203 smart  
 Detector : photomultiplier tube  
 Source of light : Sodium vapor lamp

**REAGENTS USED**

1. NED (1-naphthyl ethylenediamine) : 0.1 gm  
 2. Sodium nitrite : 0.1 gm  
 3. Sulphuric acid : 4N H<sub>2</sub>SO<sub>4</sub>  
 4. 95% Ethanol : 5 ml

**METHOD OF PREPARATION****Procedure****Preparation of stock solution**

- 100 mg of benzocaine powder(standard) was accurately weighed & transferred to a volumetric flask & the volume is made up to 100 ml with water. This solution gives the concentration of 1000µg/ml.
- 10 ml of this solution was taken & the volume was made up to 100ml with water in another volumetric flask. The concentration of the resulting solution is 100 µg/ml.
- From the above solution, 5 ml was taken and the volume was made up to 10 ml with water to get the solution of the concentration of 50µg/ml.
- Thus the stock solution was prepared.

**Preparation of 4N H<sub>2</sub>SO<sub>4</sub>**

22.8ml of conc. H<sub>2</sub>SO<sub>4</sub> taken and the vol. was made up to 100ml with water to get 4N H<sub>2</sub>SO<sub>4</sub>

**Preparation of 0.1% NaNO<sub>2</sub> solution**

Weighed accurately 0.1gm of NaNO<sub>2</sub> and transferred to a volumetric flask. The volume was made up to 100ml with water to get 0.1% NaNO<sub>2</sub> solutions.

**Preparation of 0.1% NED (1-naphthyl ethylene diamine)**

Weighed accurately 0.1gm NED & transferred it into a volumetric flask and the volume was made up to 100ml with water to get 0.1%NED solutions.

**Preparation of standard solution**

- 5ml of the above stock solution was taken in a 30ml volumetric flask and 5 ml of 4N H<sub>2</sub>SO<sub>4</sub> was added to it.

- Then 1 ml of 0.1%NaNO<sub>2</sub> solution was added and the mixture was allowed to stand for 3 minutes.
- After 3 minutes, 5 ml of 95% alcohol was added to destroy excess nitrous acid and the mixture was allowed for 2 minutes longer.
- After 2 minutes 1ml of 0.1% NED was added and the mixture was allowed to stand for 5 minutes. Then the solution gets the pink colour.
- From the above solution 1ml,2ml,3ml,4ml & 5ml was taken individually & volume was made up to 10 ml to get the solutions of the concentrations 10,20,30,40 & 50( µg/ml) respectively.
- The absorbance of each solution was measured at 545nm and noted.

**Preparation of sample solution**

- 250mg of sample i.e mucopain gel (containing 50µg of benzocaine) was taken in a 50 ml volumetric flask.
- 5ml of 4N H<sub>2</sub>SO<sub>4</sub> & 1 ml of 0.1% NaNO<sub>2</sub> was added to it and the mixture was allowed to stand for 3 minutes.
- Then 5 ml of 95% alcohol was added to destroy the excess nitrous acid and the mixture was allowed to stand for 2 minutes longer.
- 1 ml of 0.1% 1-naphthyl ethylene diamine was added and the mixture was allowed to stand for 5 minutes.
- The volume was made up to 50 ml with distilled water the solution gets the concentration of 1000µg/ml.

- From the above solution, 10 ml was taken and made up to 100 ml with water to get the concentration of 100µg/ml.
- From the above solution 1.2ml was taken and the volume was made up to 10ml to get the concentration of 12µg/ml.
- In the same way 1.5ml was taken and the volume was made up to 10ml with water to get the solution of a concentration of 15µg/ml.
- The absorbance of each solution was measured at 545nm and recorded the values.

## RESULTS

### Experimental Section

#### 1.1 System Suitability Testing

System suitability testing is an integral part of many analytical procedures. These tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. The following are the parameters obtained from the system suitability testing.

**Table 1.**

S.NO.	Parameter	Benzocaine
1.	$\lambda$ max (nm)	545nm
2.	Slope (m)	0.004
3.	Regression coefficient ( $r^2$ )	0.998
4.	LOD ( $\mu\text{g/ml}$ )	0.231 $\mu\text{g/ml}$
5.	LOQ ( $\mu\text{g/ml}$ )	0.700 $\mu\text{g/ml}$
6.	Linearity range	10-50 $\mu\text{g/ml}$
7.	Regression equation:	Y=mx+c Y=12 $\mu\text{g}$ m=0.004 c=0.024

### Calibration

**Table-3: Accuracy Data.**

S.No.	Conc. ( $\mu\text{g/ml}$ )	Abs. (nm)	Conc. Obtained ( $\mu\text{g/ml}$ )	% Purity	Mean Percentage purity	Relative error (R.E)	% Relative error
1.	10 $\mu\text{g}$	0.065	10.25 $\mu\text{g/ml}$	102.5%	101.8%	0.018	1.8%
2.	12 $\mu\text{g}$	0.073	12.25 $\mu\text{g/ml}$	102%			
3.	15 $\mu\text{g}$	0.085	15.25 $\mu\text{g/ml}$	101.6%			

### 1.4 PERCENTAGE RECOVERY STUDIES

**Table-4.**

S.No	Percentage of drug added	Expected concentration	Absorbance (nm)	Concentration obtained( $\mu\text{g}$ )	Percentage purity	Average
1	100%	10	0.065	10.25 $\mu\text{g/ml}$	102.5	102
2	120%	12	0.073	12.25 $\mu\text{g/ml}$	102	
3	150%	15	0.085	15.25 $\mu\text{g/ml}$	101.6	

### 1.5 PRECISION

Validation of tests for assay and for quantitative determination of impurities includes an investigation of precision.

### 1.2 Linearity

Linear relationships should be evaluated across the range of the analytical procedure. Linearity should be evaluated by visual inspection of a plot signals as a function of analyte concentration or content. If there is a linear relationship, test results should be evaluated by appropriate statistical methods.

The correlation coefficient, Y-intercept, the slope of the regression line and should be described by an appropriate function of the concentration.

**Table – 2: Linearity.**

S.NO.	CONCENTRATION ( $\mu\text{g/ml}$ )	ABSORBANCE (nm)
1.	10 $\mu\text{g}$	0.049nm
2.	20 $\mu\text{g}$	0.099nm
3.	30 $\mu\text{g}$	0.145nm
4.	40 $\mu\text{g}$	0.188nm
5.	50 $\mu\text{g}$	0.230nm

### 1.3 Accuracy

Accuracy should be established across the specified range of the analytical procedure. It should be assessed using a minimum concentration of three concentration levels covering the specified range.

### REPEATABILITY

a) A minimum of determinations covering the specified range for the specified range for the procedure.

b) A minimum of 6 determinations at 100% of the test concentration.

**PRECISION****Table-5.**

S.No.	Absorbance (nm)	Concentration took. ( $\mu\text{g/ml}$ )	Concentration obtained ( $\mu\text{g/ml}$ )	Percentage purity	Average	Standard deviation
1.	0.065	10 ( $\mu\text{g/ml}$ )	10.25( $\mu\text{g/ml}$ )	102.5%	101.6%	0.115
2.	0.064	10 ( $\mu\text{g/ml}$ )	10.0( $\mu\text{g/ml}$ )	100%		
3.	0.065	10 ( $\mu\text{g/ml}$ )	10.25( $\mu\text{g/ml}$ )	102.5%		
1.	0.073	12	12.25( $\mu\text{g/ml}$ )	102%	102.6%	
2.	0.074	12	12.5( $\mu\text{g/ml}$ )	104%		
3.	0.073	12	12.25( $\mu\text{g/ml}$ )	102%		
1.	0.085	15	15.25( $\mu\text{g/ml}$ )	101.6%	101%	
2.	0.084	15	15.0( $\mu\text{g/ml}$ )	100%		
3.	0.085	15	15.25( $\mu\text{g/ml}$ )	101.6%		

**1.6 Assay**

**Active substance:** Several methods of determining accuracy are available.

- Application of an analytical procedure to synthetic mixtures of the product components to be analysed have been added.
- Comparison of the results of the proposed analytical procedure with those of a second well-characterized procedure, the accuracy of which is stated and/or defined.
- Accuracy may be inferred once precision, linearity, and specificity have been established.

**Medical Product:** Several methods for determining accuracy are available.

- Application of the analytical procedure to synthetic mixtures of the product components to which known quantities of the substance to be analysed have been added.
- In cases where it is impossible to obtain samples of all product components, it may be acceptable either to add known quantities of the analyte to the product or to compare the result obtained from a second, well-characterized procedure, the accuracy of which is stated and/or defined (independent procedure).
- accuracy may be inferred once precision. linearity and specificity have been established.

**Table-6.**

S.No.	Concentration ( $\mu\text{g/ml}$ )	Label claim (mg)	Amount present (mg)	Percentage purity
1.	10 $\mu\text{g}$	200mg	205 mg	102.5%
2.	12 $\mu\text{g}$	200mg	204 mg	102%
3.	15 $\mu\text{g}$	200mg	203.2 mg	101.6%

**1.7 Statistical Validation**

The proposed method is statistically validated for its Relative error, standard deviation, standard error, the

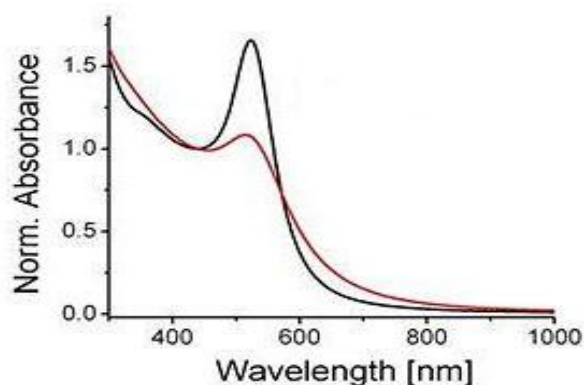
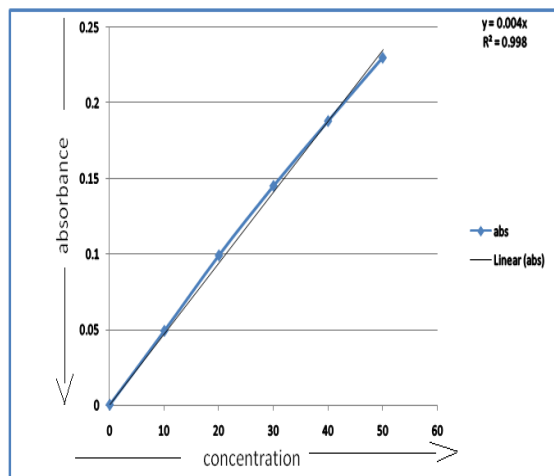
coefficient of variance, the percentage of standard deviation, relative standard deviation.

**Table -7.**

S. No.	Assay	average mean	Std. deviation (S.D)	Relative standard deviation (R.S.D)	% (R.S.D)	Standard Error (S.E)	Relative error (R.E)	coefficient of variance
1.	102.5%	102%		0.0027	0.27%	0.161	0.02	0.27
2.	102%							
3.	101.6%							

**LINEARITY GRAPH**

Concentration	Absorbance
0 $\mu$ g	0 nm
10 $\mu$ g	0.49 nm
20 $\mu$ g	0.099 nm
30 $\mu$ g	0.145 nm
40 $\mu$ g	0.188 nm
50 $\mu$ g	0.23 nm



**Fig.2: Wavelength Determination.**

**DISCUSSION**

The proposed colorimetric methods give good reproductive results for the estimation of Benzocaine. This is very simple, rapid and nowhere involves complicate sample preparation.

A new colorimetric method was developed using NED which has been proved to give good and effective results and the solution also was stable for maintaining. A detecting wavelength at 545nm showed better selectivity.

System suitability testing, linearity, accuracy, assay, precision, repeatability, statistical validation has been done. The absorbance value of Benzocaine has shown their relationship with their concentrations. The corresponding concentrations were represented in **Table-2** and the corresponding linearity graph is shown in **Fig.2** respectively.

The precision of the method was demonstrated by repeatability studies. That means the precision of the assay was determined. The accuracy of the proposed U.V method was expressed in terms of recovery. The recovery studies were carried out and the results expressed as percentage recovery (%) was shown in the **Table-4**. The proposed method was performed on the pharmaceutical topical dosage form. The statistical validation was shown in **Table-7**.

Hence the proposed method was found to be simple, precise, accurate, specific and less time-consuming.

**CONCLUSION**

In the present study, we made an attempt to develop new simple, rapid efficient and reproducible method development and validation of the local anesthetic having a primary amino group i.e. Benzocaine topical dosage form like gel. Benzocaine is marketed as Benzocaine gel (mucopain).

The proposed U.V. spectrophotometric method was validated by evaluation of validation parameters. The LOD, LOQ values, the standard deviation of the slope, correlation coefficient within and between day reproducibility other factors for this technique was obtained. The assay was performed within a short analysis time, a reliable and cost-efficient method for the development of Benzocaine. The results obtained were reproducible and reliable. The validity and precision of the methods were evident from the statistical and analytical parameters obtained.

The result obtained by this method is summarized in table- 7.

It is simple and rapid, selective, precise. Hence, suitable for application in routine analysis of pharmaceutical preparation.

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