

ESTIMATION OF CAFFEINE AND SODIUM BENZOATE IN CAFFEINE AND SODIUM BENZOATE INJECTION BY ISOABSORPTION METHOD (ISOBESTIC METHOD)

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ABSTRACT

Caffeine and Sodium benzoate present in Caffeine and Sodium Benzoate injection was estimated by isoabsorption assay method using UV spectrophotometer. Maximum absorbance of Caffeine and Sodium Benzoate was recorded at 273nm and 224nm and the isobestic point is at 242nm. Each ml of injection solution consists of 125mg of caffeine and 125mg of sodiumbenzoate, a concentration of 10 μ g/ml of Caffeine and Sodium Benzoate was prepared and absorbance was recorded at 273nm and 224nm. Pure Sodium Benzoate, Caffeine solutions were prepared at concentration of 20 μ g/ml and the absorbance is measured in the region of 300-200nm.

Key words: Caffeine, Sodium benzoate, Isobestic, UV Spectrophotometer.

INTRODUCTION

Caffeine and Sodium Benzoate injection is a clear, non-pyrogenic solution of caffeine alkaloid. Sodium benzoate is added to increase the solubility of caffeine. This injection is used as intra muscular or slow intravenous administration and it is used in conjunction with supportive measures to create respiratory depression associated with overdose with CNS depressant drugs. The USP specifies gravimetric assay for caffeine and titrimetric method for sodium benzoate. Our main objective is to develop the most acceptable isobestic assay method for the estimation of Caffeine and Sodium Benzoate present in the formulation fabricated in the laboratory and comparison of the result with USP assay method.

MATERIALS AND METHODS

Chemicals and reagents

The various chemicals that are used belong to analytical grade Caffeine, Sodium benzoate, hydrochloric acid, sodium hydroxide, water.

Assay of Caffeine and Sodium benzoate injection by isobestic method

Caffeine and Sodium benzoate present in caffeine and sodium benzoate injection was estimated by iso absorption assay method using UV spectrophotometer. Each ml of injection contains 125mg of anhydrous caffeine and 125mg of Sodium benzoate, by means of dilution, of injection with water a concentration of 10 μ g/ml each of Sodium Benzoate and Caffeine were prepared. This absorbance of the resulting solution were recorded at 273nm and 242nm. Pure Sodium

Benzoate 100mg accurately weighed, mixed and diluted to 10ml with water and 2ml of this was diluted further with water to 100ml to get a working standard of 20µg/ml. Similarly standard solution of anhydrous Caffeine (20µg/ml) was prepared separately. The UV spectra of both the solutions were recorded from 320 to 220nm. The spectra of both the solutions were recorded on the same chart to find the isobestic point. From the individual spectrum of caffeine the λ_{max} was found to be 273nm and isobestic point at 242nm. The absorbance values of the diluted sample of the injection at 273nm and 242nm were employed for calculating the amount of Caffeine and Sodium benzoate.

RESULTS AND DISCUSSION

Isobestic assay method

Determination of Caffeine and Sodium Benzoate present in the injection by isobestic assay method is established. As the injection was not available in the market, the injection solution was fabricated in the laboratory within the composition of each ml of injection contained 125mg of anhydrous caffeine and 125mg of sodium benzoate and the pH was adjusted between 7.2 to 6.2 with HCl and NaOH.

Sample preparation

Caffeine working standard solution

Accurately weighed 100mg of anhydrous Caffeine in 100ml volumetric flask, mixed and diluted to 100ml with water. 2ml of this solution was transferred to another 100ml volumetric flask, mixed and diluted to 100ml with water. This is the working standard for

anhydrous caffeine. The UV spectrum of this solution was recorded from 320nm to 210nm on UV spectrophotometer.

Sodium Benzoate working standard solution

Accurately weighed 100mg of Sodium benzoate in 100ml volumetric flask, mixed and diluted to 100ml with water. 2ml of this solution was transferred to another 100ml volumetric flask, mixed and diluted to 100ml with water. This is the working standard for Sodium benzoate. The UV spectrum of this solution was recorded from 320 to 210nm on UV spectrophotometer.

Determination of isobestic point

The UV spectrums of Caffeine and Sodium Benzoate were recorded on the same chart to find the isobestic point and it was found at 242nm. Caffeine and Sodium benzoate having same absorbance at this wavelength resulted from the same concentration and both spectra are intersected at 242nm.

Assay of injection

The given sample of injection contains anhydrous caffeine 125mg and Sodium Benzoate 125mg per ml.

Sample preparation

1ml of injection was diluted to 100ml with water and 0.80ml of the above dilution was transferred to another 100ml volumetric flask and diluted to 10ml with water and mixed. The UV spectra of this solution was recorded from 320 to 210nm.

Calculation

The absorbance ratio method was applied to estimate the concentration of caffeine and sodium benzoate present in the injection. This was done by solving two equations (1) and (2) as given below

$$C_I = \frac{Q_0 - Q_{II}}{Q_I - Q_{II}} \times \frac{A}{a}$$

$$C_{II} = \frac{Q_0 - Q_I}{Q_{II} - Q_I} \times \frac{A}{a}$$

C_I is the concentration (g/l) of component (1) – Caffeine

C_{II} is the concentration (g/l) of component (2) – Sodium Benzoate

Q_0 is the ratio of absorbance value of binary mixture

Absorbance of the sample at 2 different wavelengths were recorded, one of which is the isobeistic point (242nm) and the other is the λ_{max} of caffeine (273nm)

TERMS	VALUES
Q_0 - sample	1.772
Q_1 - Pure caffeine	1.96
Q_{II} - Pure sodium benzoate	0.357

A is the absorbance value of the sample at isoabsorption wavelength at 242nm

a is the absorptivity of component at isoabsorption wavelength at 242nm

Substituting the above values in the equation caffeine and sodium benzoate contents were determined.

Caffeine content

$$C1 = \frac{Q_0 - Q_{II}}{Q_1 - Q_{II}} \times \frac{A}{a} = 0.00997 \text{ g/L}$$

The given injection contains = $\frac{0.00997}{0.010} \times 100\text{gm}$ of the standard amount of anhydrous caffeine

The sample dilution made for the assay contains $10\mu\text{g/ml}$ i.e. = $\frac{10 \times 1000}{1000 \times 1000} = 0.01 \text{ g/L}$

Result: The given injection sample contains 99.7% of the standard amount of anhydrous caffeine

Sodium Benzoate content

$$CII = \frac{Q_0 - Q_1}{Q_{II} - Q_1} \times \frac{A}{a} = 0.00102 \text{ g/L}$$

On substitution of values of Q_0 , Q_1 , Q_2 and A/a and on solving, for C_2 the amount of sodium benzoate in gm/l of solution taken for the analysis was given by 0.0012gm/L

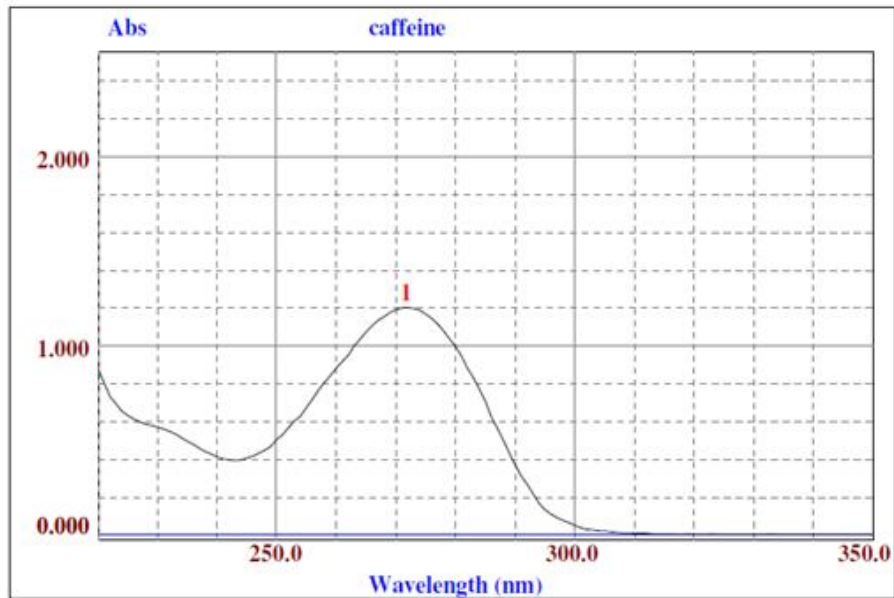
Therefore the percentage of sodium benzoate of the stated amount is = $\frac{0.00102 \times 100}{0.01}$

Result: The given injection contains 102% of stated amount of Sodium Benzoate

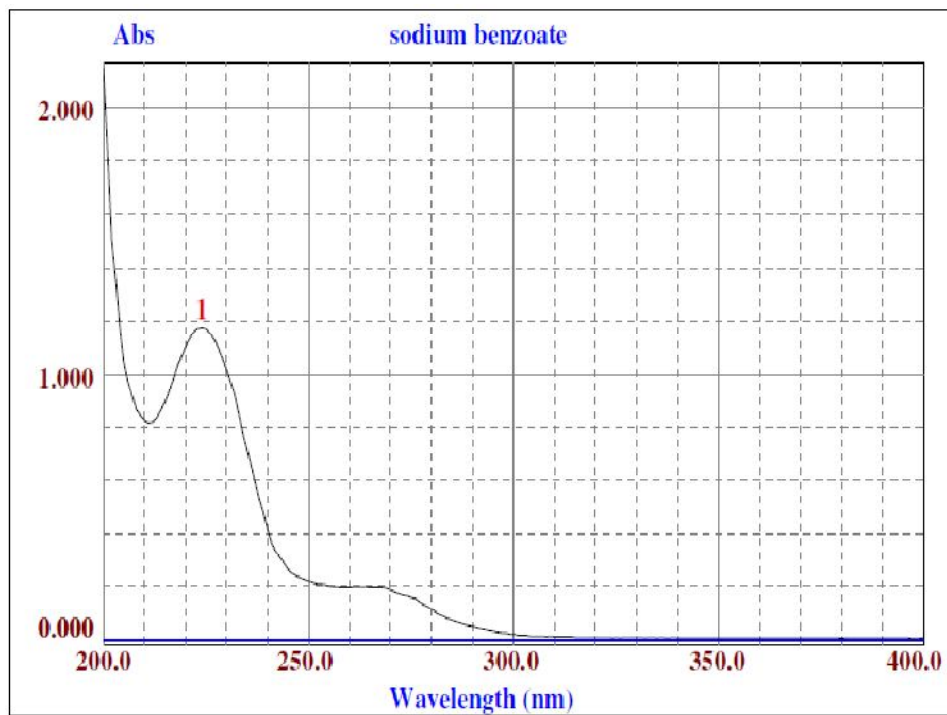
RESULTS OF THE ASSAY OF CAFFEINE AND SODIUM BENZOATE INJECTION

Iso absorption assay	USP Method
Caffeine - 99.7%	Caffeine - 97.6%
Sodium Benzoate - 102.0%	Sodium Benzoate - 99.71%

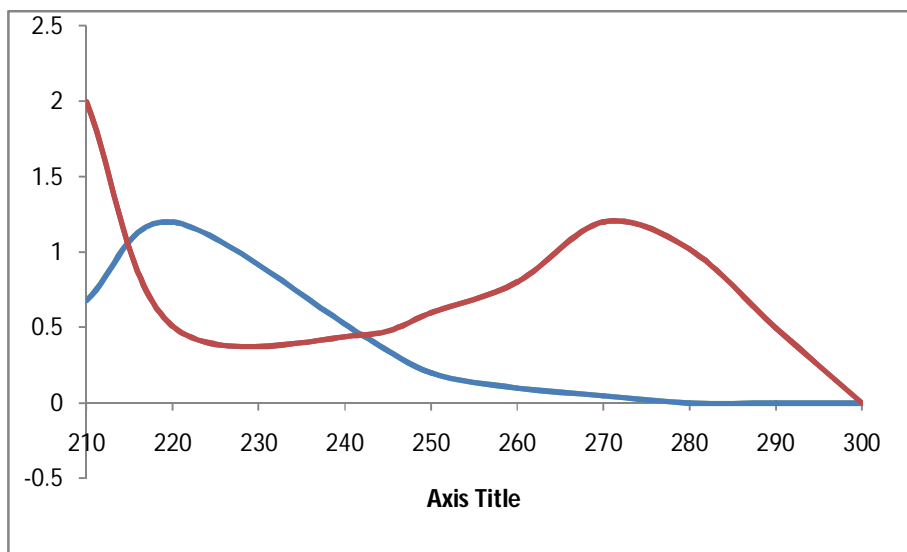
Caffeine absorbance



Sodium benzoate absorbance



Isobestic point of Caffeine & Sodium benzoate



Red line indicates Caffeine

Blue line indicates Sodium benzoate

CONCLUSION

Caffeine and Sodium Benzoate present in Caffeine and Sodium Benzoate injection was estimated by isoabsorption assay method using UV spectrophotometer. The result of this method was validated against USP assay method. Gravimetric estimation for caffeine and titrimetric method for Sodium Benzoate after separating these two by extraction was given in the USP assay method as the gravimetric method for Caffeine takes longer period of time for about 4 to 6 hours. The result of the present isoabsorption assay were comparable with the values of USP assay method. The estimation of Caffeine and Sodium Benzoate in the injection formulation can be conveniently carried out by the isoabsorption assay method. It is faster, reliable, precise and no interference was found.

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