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# A VALIDATED UV SPECTROPHOTOMETRIC METHOD DEVELOPMENT FOR CENTHAQUINE CITRATE IN BULK AND INJECTION DOSAGE FORM

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## ABSTRACT

**Objectives:** A simple, precise, accurate UV Spectrophotometric method developed for the determination of Centhaquine citrate in bulk and injection dosage form. **Materials and Methods:** The wavelength used for quantification was 232nm in distilled water. Beer's law was obeyed at the concentration range 5-30µg/ml. The method is validated as per ICH guidelines. Stastical analysis proved that the method was accurate for centhaquine citrate. The wide linearity range, sensitivity, accuracy and simple procedure imply that the developed method can be used in the routine analysis of Centhaquine citrate in quality control laboratory. The different aliquots of Centhaquine citrate were prepared in the concentration range from 5-30µg/ml. The absorbance of the solution was measured at 232nm. The linearity procedure was repeated for six times. **Results:** From the linearity analysis, the calibration curve was plotted by using concentration against absorbance. The optical characteristics like Correlation Coefficient, slope, intercept were calculated. **Conclusion:** A validated UV spectrophotometric method

development for Centhaquine citrate in bulk and formulated injection dosage form. The method was validated according to ICH guidelines with various parameters like Precision, Accuracy, Limit of detection, Limit of quantitation etc.

**KEYWORDS:** Centhaquine citrate, Raw material, UV method and Injection dosage form.

## I. INTRODUCTION

Centhaquine citrate is a resuscitative agent for managing hypovolemic shock was found to be effective without causing arterial constriction or an increase in blood pressure by enhancing the output from the heart.[1-2] It stimulates $\alpha 2_B$ adrenergic receptor, increases blood pressure, cardiac output and enhances tissue blood perfusion by arterial dilation.[3-5] According to the literature survey for the quantification of Centhaquine citrate in bulk and injection dosage form, it was found that no method available for the estimation of Centhaquine citrate. Hence the present work aim to develop accurate, precise, specific, linear, simple and rapid method for the estimation of Centhaquine citrate in bulk and Injection dosage form. The developed method validated as per ICH guidelines and Stastical parameters.[6-7]

## II. MATERIALS AND METHODS

### Instrumentation

In the present study, Shimadzu Double beam UV-Visible spectrophotometer (Model: UV-1700) with spectral band width of 1nm was used . Shimadzu digital balance (AUX-200) was used for weighing.

### Reagents and Chemicals

Lyfaquin injection equivalent to 0.1 mg of Centhaquine citrate per ml (1mg/10ml) was used in the method. Distilled water obtained from double distillation unit in our laboratory was used as solvent.

### Selection of solvent

The Centhaquine citrate was found to be soluble in distilled water, Methanol and Sodium chloride 0.9 % w/v. Considering the economic factor and the drug was stable upto 72hours, distilled water was selected as a solvent for further analysis.

### Preparation of standard stock solutions

10 mg of centhaquine citrate was accurately weighed and transferred into a 100 ml volumetric flask and dissolved in distilled water to get final concentration of 100 µg/ml.

**Selection of wavelengths for estimation**

The standard stock solution was diluted with distilled water to get a final concentration of 10 µg/ml of Centhaquine citrate. The solution was scanned between 200 and 400 nm range by using distilled water as blank. From the UV spectra obtained, Centhaquine citrate showed maximum absorbance at 232 nm. The selected wavelength for quantification was 232nm.

**Preparation of calibration graph**

From the working standard solution of Centhaquine citrate containing 100 µg/ml. (0.5-3 ml) was transferred into series of six 10 ml volumetric flasks and made up to the volume with water to get the concentration range 5-30 µg/ml. The absorbance of different concentration solutions were measured at their selected wavelength. The calibration curve was constructed by plotting concentration against absorbance.

**Physical mixture**

10mg of Centhaquine citrate was dissolved in 10ml of distilled water.

**Quantification of physical mixture**

Pipetted out 1ml of physical mixture, transferred to 10 ml volumetric flask added minimum amount of distilled water to dissolve and made-up to the volume with distilled water further diluted to get final concentration of 15µg/ml. The absorbance measurements were made six times for physical mixture at 232 nm and the amount of Centhaquine citrate present in physical mixture was calculated from the slope and intercept of respective calibration curve.

**Quantification of formulation**

Pipetted out 1ml from 10ml of Lyfaquin injection equivalent to 1.0 mg and transferred to 10 ml volumetric flask added minimum amount of distilled water to dissolve and made-up to the volume with distilled water further diluted to get final concentration of 15µg/ml. The absorbance measurements were made six times for formulation at 232 nm and the amount of Centhaquine citrate present in formulation was calculated from the slope and intercept of respective calibration curve.

**Validation**

The proposed method was validated as per ICH guidelines.

**Linearity**

The linearity was checked for Centhaquine citrate in the concentration range of 5 to 30 µg/ml and was found to be linear in the specified concentration range. A calibration curve was plotted with concentration versus the absorbance value.

**Limit of Detection and Limit of Quantification**

The linearity study was carried out for six times. The LOD and LOQ were calculated based up on the calibration curve method. The LOD and LOQ were calculated using the average slope and intercept.

**Accuracy**

Recovery studies were conducted using the standard addition method at three different levels (50%, 75%, and 100%) to evaluate the recovery studies of the suggested approaches. The recovery study was carried out for six times. The recovery study findings of physical mixture and formulation are shown in Table 4 & 5 as a percent recovery was good.

**Precision**

The reproducibility of this method was determined by analyzing physical mixture and formulation (Intra-day assay precision) at different time intervals on same day in triplicates and (Inter-day assay precision) on three different days. The procedure was repeated for six times. The standard analytical error, relative standard deviations (RSD) were found to be acceptable.

**Robustness**

Robustness of the method was confirmed by the analysis of formulation unaffected by small, but deliberate variations in the method parameters and provides an indication of its reliability during normal usage.

**Ruggedness**

Ruggedness of the method was confirmed by the analysis of formulation by different analyst and different instrument. The amount of drug and the percentage RSD values were calculated.

## III. RESULTS AND DISCUSSION

For our studies, the Centhaquine citrate analysis of a new drug was used. The UV Spectroscopic Method has advantages that it takes less time and consume less solvent when

estimating a single dosage form. To ensure the % purity in single dosage form of thedrug, the UV-spectroscopy was developed.

A accurate, fast, simple and precise UV Spectrophotometric method was developed and validated. The standard Centhaquine citrate was identified by melting point and IR spectroscopy. From the solubility studies Distilled water was selected as a solvent for UV spectrophotometric method, because of its solubility, stability and easy availability.

Centhaquine citrate was dissolved in distilled water and made further dilutions with the distilled water to get the concentration 10µg/ml. The solution was scanned between the wavelength ranges of 200-400nm using distilled water as a blank.

From the spectrum obtained, 232nm was chosen as a maximum wavelength for the further analysis of Centhaquine citrate. The UV spectrum was shown in Figure 1. The stability of the drug was studied by measuring the absorbance at the different time intervals. The drug was stable up to 72 hours in distilled water.

The different aliquots of Centhaquine citrate were prepared in the concentration range from 5-30µg/ml. The absorbance of the solution was measured at 232nm. The linearity procedure was repeated for six times.

From the linearity analysis, the calibration curve was plotted by using concentration against absorbance were shown in Figure 2. The optical characteristics like Correlation Coefficient, slope, intercept were calculated, and shown in Table 1.

Lyfaquin formulation containing 1.0 mg of CEN was selected for estimation. From the linearity, the nominal concentration of CEN i.e. 15 µg/ml was prepared. Six test solution were determined based on the absorbance of the solutions was measured at 232 nm. The % purity of physical mixture and formulation were found to be was 100.07 ± 0.5531 & 99.67 ± 0.4991. The results of analysis are shown in Table 2 & 3.

Accuracy studies of the developed UV method was confirmed for physical mixture and formulation. The % recovery ranged from 99.84 to 100.02% and 99.90 to 100.07 respectively. Lower % RSD values indicated that the developed UV spectroscopic method was more accurate. The results was shown in Table 4 & 5.

Precision of the developed UV method was studied by making repeated analysis (Intraday and Interday). The % RSD values for Intraday and Interday analysis of physical mixture were found to be 0.0926 & 0.0760 and for formulation 0.0912 & 0.0957 were found. The results are shown in table 6 & 7.

In ruggedness studies, Different Instrument 1,2 and 3 with three different concentrations (50%, 75% and 100%) were employed. For Instrument 1, the % RSD values were found to be 0.0983, 0.1687, 0.1126. For Instrument 2, 0.0987, 0.2134, 0.3510 and For Instrument 3, the values 0.2060, 0.1769, 0.1549 were found. Different Analyst 1 and 2 with three different concentrations were found to have % RSD values of 0.0983, 0.1687,0.1126 and 0.0987, 0.2134, 0.3510 respectively. Lower % RSD values indicated, method was more rugged shown in Table 8 & 9.

**Table 1: Melting point.**

| Standard value | Observed average value* |
|---|---|
| 140°C | 140ºC |

Average of three observations.

**Table 2: Solubility profile for centhaquine citrate.**

| S. NO | Solvents | Centhaquine citrate |
|---|---|---|
| 1 | Distilled Water | Soluble |
| 2 | Methanol | Soluble |
| 3 | 0.9% NaCl solution | Soluble |

**Table 3: Stability studyof centhaquine citrate by uv spectroscopic method.**

Solvent: Distilled water

Concentration of Centhaquine citrate: 10 µg/ml

| S. No. | Time | Absorbance of Centhaquine citrate (232 nm) |
|---|---|---|
| 1 | 0 min | 0.547 |
| 2 | 15min | 0.546 |
| 3 | 30 min | 0.544 |
| 4 | 45 min | 0.548 |
| 5 | 60 min | 0.549 |
| 6 | 1hour30min | 0.548 |
| 7 | 2 hours | 0.546 |
| 8 | 2 hour 30 min | 0.547 |
| 9 | 3 hours | 0.545 |
| 10 | 4 hours | 0.544 |

| | | |
|---|---|---|
| 11 | 24 hours | 0.549 |
| 12 | 48 hours | 0.546 |
| 13 | 72 hours | 0.545 |

**Table 4: Average of linearity values.**

| S.NO | Concentration (µg/ml) | Absorbance (nm) |
|---|---|---|
| 1. | 5 | 0.225 |
| 2. | 10 | 0.458 |
| 3. | 15 | 0.676 |
| 4. | 20 | 0.906 |
| 5. | 25 | 1.120 |
| 6. | 30 | 1.346 |

**Table 5: Optical characteristics of centhaquine citrate at 232nm.**

| Parameters | AT 232 nm* |
|---|---|
| Beer's law range (µg/ml) | 5-25 |
| Molae absorptivity (L $mol^{-1}cm^{-1}$) | 23798.25 |
| Regression equation(y= mx + c) | y= (0.0448)x + 0.0034 |
| Slope (m) | 0.0448 |
| Intercept (c) | 0.0034 |
| Loss of detection (µg/ml) | 0.0038 |
| Loss of quantification (µg/ml) | 0.0118 |
| Sandells sensitivity (µg/$cm^2$/0.001 A.U) | 0.0222 |
| Standard error | 0.0052 |
| Correlation coefficient ($r^2$) | 0.9999 |

*Mean of six observations

**Table 6: Quantification of centhaquine citrate for physical mixture.**

| Sample | Sample number | Amount taken (µg/ml) | Amount found | % Purity* (% w/w) | Mean purity (% w/w) | SD | % RSD |
|---|---|---|---|---|---|---|---|
| CEN | 1 | 15 | 14.99 | 99.97 | 100.07 | 0.5535 | 0.5531 |
| | 2 | 15 | 15.13 | 100.87 | | | |
| | 3 | 15 | 14.90 | 99.38 | | | |
| | 4 | 15 | 14.99 | 99.97 | | | |
| | 5 | 15 | 15.08 | 100.57 | | | |
| | 6 | 15 | 14.95 | 99.68 | | | |

*Mean of six observations

**Table 7: Quantification of centhaquine citratefor formulation.**

| Sample | Sample number | Amount taken (µg/ml) | Amount found | % Purity* (% w/w) | Mean purity (% w/w) | SD | % RSD |
|---|---|---|---|---|---|---|---|
| CEN | 1 | 15 | 14.92 | 99.53 | 99.67 | 0.4975 | 0.4991 |
| | 2 | 15 | 14.88 | 99.23 | | | |
| | 3 | 15 | 14.99 | 99.97 | | | |
| | 4 | 15 | 14.97 | 99.83 | | | |
| | 5 | 15 | 14.86 | 99.08 | | | |
| | 6 | 15 | 15.06 | 100.42 | | | |

*Mean of six observations

**Table 8: Intraday and Interday analysis of centhaquine citrate (Physical mixture).**

| Sample | Sample number | % Purity* (% w/w) | | SD | | % RSD | |
|---|---|---|---|---|---|---|---|
| | | Intraday | Interday | Intraday | Interday | Intraday | Interday |
| CEN | 1 | 99.92 | 99.88 | 0.0926 | 0.0760 | 0.0926 | 0.0760 |
| | 2 | 99.95 | 99.95 | | | | |
| | 3 | 99.98 | 99.97 | | | | |
| | 4 | 99.94 | 99.94 | | | | |
| | 5 | 99.90 | 99.98 | | | | |
| | 6 | 99.86 | 99.94 | | | | |
| Mean purity (%w/w) | | 99.92 | 99.94 | | | | |

*Mean of six observations

**Table 9: Intraday and Interday analysis of centhaquine citrate (Formulation).**

| Sample | Sample number | % Purity* (% w/w) | | SD | | % RSD | |
|---|---|---|---|---|---|---|---|
| | | Intraday | Interday | Intraday | Interday | Intraday | Interday |
| CEN | 1 | 99.95 | 99.92 | 0.0911 | 0.0956 | 0.0912 | 0.0957 |
| | 2 | 99.92 | 99.88 | | | | |
| | 3 | 99.63 | 99.91 | | | | |
| | 4 | 99.69 | 99.94 | | | | |
| | 5 | 99.98 | 99.94 | | | | |
| | 6 | 99.91 | 99.89 | | | | |
| Mean purity (%w/w) | | 99.84 | 99.91 | | | | |

*Mean of six observations

**Table 10: Recovery study for physical mixture.**

| Sample | % Concentrationn | Sample amount* (µgml) | Amount spiked* (µg/ml) | Estimated amount* (µg/ml) | Recovered amount* (µg/ml) | Average* % recovery | SD | % RSD |
|---|---|---|---|---|---|---|---|---|
| CEN | 50 | 15 | 7.5 | 22.51 | 7.50 | 100.00 | 0.3118 | 0.3118 |
| | 75 | 15 | 11.25 | 23.13 | 11.23 | 99.84 | 0.2317 | 0.2321 |
| | 100 | 15 | 15 | 26.24 | 15.00 | 100.02 | 0.2102 | 0.2101 |

*Mean of six observations

**Table 11: Recovery study for formulation.**

| Sample | % Concentrationn | Sample amount* (µg/ml) | Amount spiked* (µg/ml) | Estimated amount* (µg/ml) | Recovered amount* (µg/ml) | Average* % recovery | SD | % RSD |
|---|---|---|---|---|---|---|---|---|
| CEN | 50 | 15 | 7.5 | 22.51 | 7.49 | 99.90 | 0.2923 | 0.2925 |
| | 75 | 15 | 11.25 | 26.26 | 11.25 | 100.03 | 0.2317 | 0.2316 |
| | 100 | 15 | 15 | 30.03 | 15.01 | 100.07 | 0.2031 | 0.2029 |

*Mean of six observations

**Table 12: Ruggedness study of centhaquine citrate formulation.**

| Sample | Type of ruggedness | Average* % recovery | SD | % RSD |
|---|---|---|---|---|
| CEN | Instrument-1 | | | |
| | 50 | 100.74 | 0.0991 | 0.0983 |
| | 75 | 100.71 | 0.1699 | 0.1687 |
| | 100 | 100.80 | 0.1135 | 0.1126 |
| | Instrument-2 | | | |
| | 50 | 100.34 | 0.0991 | 0.0987 |
| | 75 | 100.14 | 0.2137 | 0.2134 |
| | 100 | 100.06 | 0.3512 | 0.3510 |
| | Instrument-3 | | | |
| | 50 | 100.11 | 0.2063 | 0.2060 |
| | 75 | 99.91 | 0.1768 | 0.1769 |
| | 100 | 99.89 | 0.1547 | 0.1549 |

*Mean of three observations

**Table 13: Ruggedness study of centhaquine citrate formulation.**

| Sample | Type of ruggedness | Average* % recovery | SD | % RSD |
|---|---|---|---|---|
| CEN | Analyst-1 | | | |
| | 50 | 100.74 | 0.0991 | 0.0983 |
| | 75 | 100.71 | 0.1699 | 0.1687 |
| | 100 | 100.80 | 0.1135 | 0.1126 |
| | Analyst-2 | | | |
| | 50 | 100.34 | 0.0991 | 0.0987 |
| | 75 | 100.14 | 0.2137 | 0.2134 |
| | 100 | 100.06 | 0.3512 | 0.3510 |

*Mean of three observations

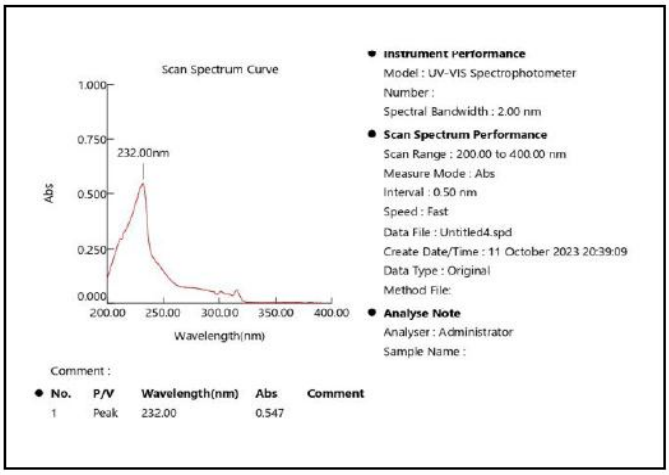


**Fig. 1: UV Spectrum of Centhaquine citrate(10µ/ml).**

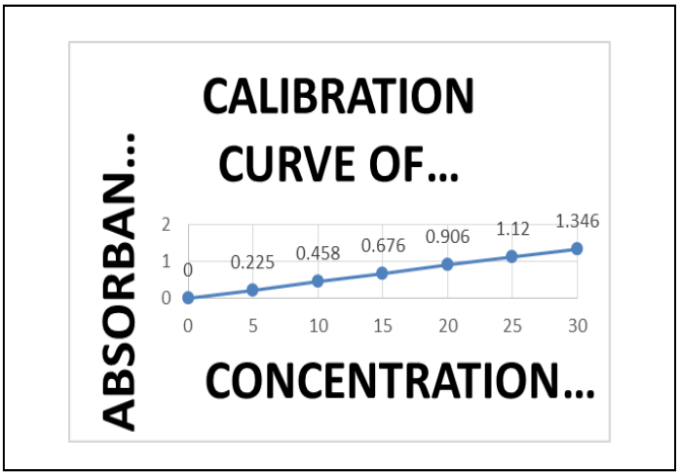


**Fig. 2: Calibration curve of Centhaquine citrate at 232nm.**

Centhaquine citrate was obtained from Steril Gene Life Sciences, Puducherry as a gift sample. The standard Centhaquine citrate was identified by melting point and IR spectroscopy.

The solubility of Centhaquine citrate was determined as per Indian Pharmacopoeia From the solubility studies, Centhaquine citrate was freely soluble in Distilled water, Methanol and soluble in Sodium chloride 0.9% w/v.

From the above solubility studies Distilled water was selected as a solvent for UV spectrophotometric method, because of its solubility, stability and easy availability.

Centhaquine citrate was dissolved in distilled water and made further dilutions with the distilled water to get the concentration 10µg/ml. The solution was scanned between the wavelength ranges of 200-400nm using distilled water as a blank.

From the spectrum obtained, 232nm was chosen as a maximum wavelength for the further analysis of Centhaquine citrate.

The stability of the drug was studied by measuring the absorbance at the different time intervals. The drug was stable up to 72 hours in distilled water.

The different aliquots of Centhaquine citrate were prepared in the concentration range from 5-30µg/ml. The absorbance of the solution was measured at 232nm. The linearity procedure was repeated for six times.

From the linearity analysis, the calibration curve was plotted by using concentration against absorbance. The optical characteristics like Correlation Coefficient, slope, intercept were calculated.

## IV. CONCLUSION

A validated UV spectrophotometric method development for Centhaquine citrate in bulk and formulated injection dosage form. The method was validated according to ICH guidelines with various parameters like Precision, Accuracy, LOD, LOQ etc. The advantage of the method was highly selective, sensitive, Economic, Precise and Accurate method.

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