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# VALIDATION PARAMETERS OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF NAPROXEN SODIUM AND ESOMEPRAZOLE MAGNESIUM TRIHYDRATE

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## ABSTRACT

An isocratic RP-HPLC method was developed and validated for the Simultaneous estimation of Naproxen sodium and Esomeprazole magnesium trihydrate in Pharmaceutical tablet dosage form. The separation was achieved by using a reversed-phase C 18 column(Thermo eletrole, ODS, 250mm × 4.6 mm i.d, 5µm) at ambient temperature with mobile phase consisting of Phosphate buffer (pH adjust to 3.8using OPA): Acetonitrile : Methanol (30:50:20v/v). The flow rate was 1.0 ml/min. Detection was carried out at a wavelength of 220 nm. Retention time of Naproxen sodium and Esomeprazole magnesium trihydrate were found tobe2.417 and 3.903min respectively. The proposed method was validated for selectivity, precision, linearity and accuracy. The assay method was found to be linear from 75-175µg/ml and 3-7µg/ml for Naproxen sodium and Esomeprazole magnesium trihydrate respectively. All validation parameters were within the acceptable range.

**Keywords:** Naproxen sodium and Esomeprazole magnesium trihydrate, Thermo eletrole, selectivity, precision, linearity and accuracy etc.

## INTRODUCTION

RP-HPLC operates on the principle of hydrophobic interactions, which originate from the high symmetry in the dipolar water structure and play the most important role in all processes in life science. RP-HPLC allows the measurement of these interactive forces. The binding of the analyte to the stationary phase is proportional to the contact surface area around the non-polar segment of the analyte molecule upon association with the ligand on the stationary phase. This solvophobic effect is dominated by the force of water for "cavity-reduction" around the analyte and the $C_{18}$-chain versus the complex of both.(1) The energy released in this process is proportional to the surface tension of the eluent (water: $7.3\times10^{-6}$ J/cm², methanol: $2.2\times10^{-6}$ J/cm²) and to the hydrophobic surface of the analyte and the ligand respectively. The retention can be decreased by adding a less polar solvent (methanol, acetonitrile) into the mobile phase to reduce the surface tension of water. (2)Gradient elution uses this effect by automatically reducing the polarity and the surface tension of the aqueous mobile phase during the course of the analysis.

## MATERIALS AND METHODS

### *Validation parameters*

### *Precision*

The precision of an analytical method is the degree of agreement among individual test results obtained when the method is applied to multiple sampling of a homogenous sample in a same day. The precision is expressed by,(3,4) %RSD = SD x 100 Mean.

### *Preparation of standard solution*

Aliquots of standard stock solutions of Naproxen sodium and Esomeprazole magnesium trihydrate (1 mL of 1000 µg/mL for NAP and 1 mL of 40 µg/mL for ESO) were transferred into a 10 mL standard flask and made up to the mark with mobile phase (100 µg/mL for NAP and 4.0 µg/mL for ESO). 20 µL of the solution was injected and the chromatograms were recorded. The procedures were repeated for five times. The peak areas were measured and calculated the %RSD. (5)

### *Method Precision*

The method precision of an analytical method is the degree of agreement among individual test results obtained when the method is applied to multiple sampling of a homogenous sample in different days. The method precision is expressed by,(6) %RSD = SD x 100 Mean

### *Preparation of standard solution*

Aliquots of standard stock solutions of Naproxen sodium and Esomeprazole magnesium trihydrate (1 mL of 1000 µg/mL for NAP and 1 mL of 40 µg/mL for ESO) were transferred into a 10 mL standard flask and made up to the mark with mobile phase (100 µg/mL for NAP and 4.0 µg/mL for ESO). (7,8) 20 µL of the solution was injected and the chromatograms were recorded. The procedures were repeated for five times in five different days. The peak areas were measured and calculated the % RSD.

### *Accuracy*

From the standard stock solution prepared the three concentrations of samples containing 90, 110, 130 µg/mL for Naproxen sodium and 3.6, 4.4, 5.2 µg/mL for Esomeprazole magnesium trihydrate. 20 µL of the (90, 110, 130 µg/mL) those concentrations for Naproxen sodium and (3.6, 4.4, 5.2 µg/mL) those concentrations for Esomeprazole magnesium trihydrate solutions were injected. The chromatograms were recorded.(9)

### *Linearity*

This is the method's ability to obtain results which are either directly or after mathematical transformation proportional to the concentration of the analyte within a given range.

### *Preparation of standard solutions*

Aliquots of standard stock solution of Naproxen sodium and Esomeprazole magnesium trihydrate (0.2 mL – 1.2 mL) were transferred into six 10 mL standard flasks and made up to the mark with mobile phase.(10) A solution containing 20, 40, 60, 80, 100 and 120 µg/mL for Naproxen sodium and 0.8, 1.6, 2.4, 3.2, 4.0 and 4.8 µg/mL for Esomeprazole magnesium trihydrate respectively. 20µl of the standard solutions were injected and the chromatograms were recorded. The calibration was done by external standard calibration method. Linearity was observed between the selected concentrations. Calibration graph was obtained by plotting peak area versus concentration.(11,12)

### *Limit of Detection*

This is the lowest concentration in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions. The limit of detection is important for impurity tests and the assays of dosages containing low drug levels and placebos. LOD was calculated by using formula,

**LOD = 3.3x std.dev / slope (13)**

In the above formula the standard deviation was taken from the precision data and the slope was taken from the linearity curve. The LOD was found to be 0.445 µg/mL for Naproxen sodium and 0.2071 µg/mL for Esomeprazole magnesium trihydrate respectively.(14,15)

### *Limit of Quantification*

This is the lowest concentration in a sample that can be detected and quantified. LOQ was calculated by using formulae,

**LOQ = 10 x std.dev / slope**

In the above formula the standard deviation was taken from the precision data and the slope was taken from the linearity curve. The LOQ was found to be 1.3624µg/mL for Naproxen sodium and 0.6339µg/mL for Esomeprazole magnesium trihydrate respectively.(16)

### *Robustness*

Robustness is the degree of reproducible results obtained by the analysis of the sample under a different test conditions i.e. wavelength and flow rate.

### *Preparation of standard solution*

Aliquots of standard stock solutions of Naproxen sodium and Esomeprazole magnesium trihydrate (1 mL of 1000 µg/mL for NAP and 1 mL of 40 µg/mL for ESO) were transferred into a 10 mL standard flask and made up to the mark with mobile phase (100 µg/mL for NAP and 4.0 µg/mL for ESO). 20 µL of the solution was injected and the chromatograms were recorded by changing the wavelength 234 nm and 238nm and changing the flow rate 0.9 mL/min and 1.1 mL/min.

### *Ruggedness*

Defined by the USP as the degree of reproducibility of results obtained under a variety of conditions, such as different laboratories, analysts, instruments, environmental conditions, operators and materials. Ruggedness is a measure of reproducibility of test results under normal, expected operational conditions from laboratory to laboratory and from analyst to analyst.(17)

### *Preparation of standard solution*

Aliquots of standard stock solutions of Naproxen sodium and Esomeprazole magnesium trihydrate (1 mL of 1000 µg/mL for NAP and 1 mL of 40 µg/mL for ESO) were transferred into a 10 mL standard flask and made up to the

mark with mobile phase (100 µg/mL for NAP and 4.0 µg/mL for ESO). 20 µL of the solution was injected by two different analysts. The peak areas were measured and calculated the % RSD.

## RESULTS

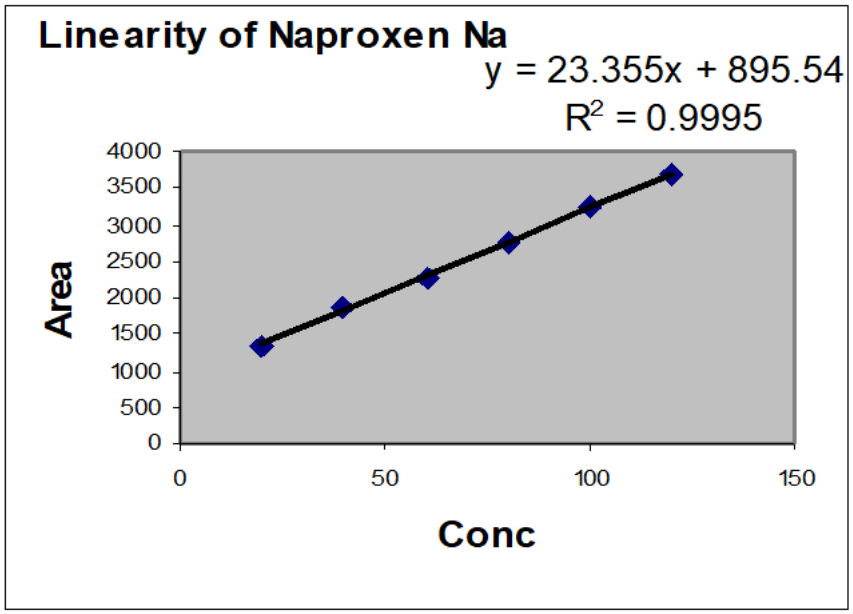


**Fig 1: Linearity Graph for Naproxen Sodium**

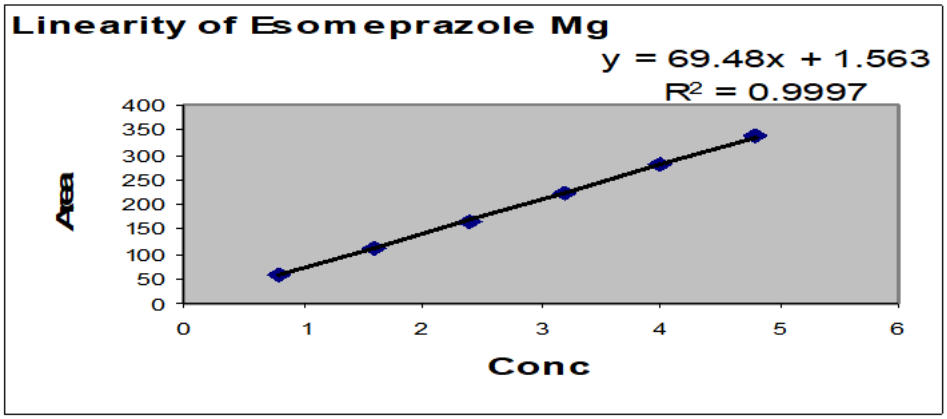


**Fig 2: Linearity Graph for Esomeprazole Magnesium Trihydrate**

**Method Precision**

| S.No | Drugs | Concentration | Avg Peak Area | %Recovery |
|---|---|---|---|---|
| 1 | NAP | 90 µg | 3282.24 | 99.94% |
| 2 | | 110 µg | 3609.86 | 99.98% |
| 3 | | 130 µg | 4265.60 | 99.97% |
| 1 | ESO | 3.6 µg | 256.84 | 99.51% |
| 2 | | 4.4 µg | 313.96 | 99.53% |
| 3 | | 5.2 µg | 370.85 | 99.48% |

**Table 1:**

| S.No | Naproxen sodium | | Esomeprazole Magnesium | |
|---|---|---|---|---|
| | Rt | Peak area | Rt | Peak area |
| 1 | 3.13 | 3280.59 | 4.367 | 280.60 |
| 2 | 3.13 | 3281.61 | 4.347 | 286.96 |
| 3 | 3.143 | 3283.82 | 4.357 | 286.37 |
| 4 | 3.177 | 3281.40 | 4.367 | 283.40 |
| 5 | 3.177 | 3283.70 | 4.367 | 284.10 |
| Avg | 3.1514 | 3282.22 | 4.361 | 284.29 |
| SD | 0.0239 | 1.45424 | 0.0089 | 2.5455 |
| %RSD | 0.76 | 0.04 | 0.21 | 0.90 |

**Table 2: Linearity Study**

| Drugs | Concentration | $r^2$ | Slope | LOD | LOQ |
|---|---|---|---|---|---|
| NAP | 20-120 µg/mL | 0.9995 | 23.35 | 0.445 | 1.362 |
| ESO | 0.8-4.8 µg/Ml | 0.9997 | 69.48 | 0.207 | 0.633 |

**Table 3:Robustness Study**

| Parameter | | Retention time for NAP | Retention time for ESO |
|---|---|---|---|
| Flow rate | 0.9 mL/min | 2.867 | 3.967 |
| | 1.1 mL/min | 3.472 | 4.820 |
| wavelength | 234 nm | 3.140 | 4.347 |
| | 238 nm | 3.123 | 4.347 |

**Table 4: Accurancy Study**

| Analysts | Retention time of NAP | Retention of ESO |
|---|---|---|
| Analyst 1 | 3.130 | 4.367 |
| Analyst 2 | 3.177 | 4.367 |
| **Avg** | **3.153** | **4.637** |
| **SD** | **0.0332** | **0** |
| **%RSD** | **1.053** | **0** |

## DISCUSSION

### *Method validation*

When a method has been it must be validation before practical use. By following the ICH guidelines for analytical method validation $Q_2$ ($R_1$), the system suitability test was performed and the validation characteristics were addressed.

The system suitability test ensures the validity of the analytical procedure as well as confirms the resolution between different peaks of interest. System suitability parameters like retention time, tailing factor, efficiency, capacity factor and resolution were performed.

The precision study was conducted for the NAP and ESO standard stock solutions. The concentrations of 100 µg/mL for NAP and 4 µg/mL for ESO sample solutions were prepared. The samples were injected 5 times into the HPLC system and the retention time was recorded from that %RSD was calculated it was found to be 0.25 for NAP and 0.11 for ESO. It was found to be within the specified limit and it shows that the drugs are having good precision and the chromatograms.

The method precision study was conducted for the NAP and ESO standard stock solutions. The concentrations of 100 µg/mL for NAP and 4 µg/mL for ESO sample solutions were prepared. The samples were injected into the HPLC system for 5 times in 5 different days and the retention time was recorded from that %RSD was calculated it was found to be 0.76 for NAP and 0.21 for ESO. It was found to be within the specified limit and it shows in the table-12 and the chromatograms.

The accuracy was confirmed by recovery studies by adding known amount of pure drug to the previously analysed formulation and the mixture was analysed by the proposed method and chromatograms were shown in the figures 29-40. The percentage recovery was found to be 99.94, 99.98, 99.97% for NAP and 99.51, 99.53, 99.48 % for ESO respectively.

The linearity study was conducted for the Naproxen sodium and Esomeprazole magnesium trihydrate standard stock solutions. For the construction of calibration curves, six calibration standard solutions were prepared over the concentration range of 20 to 120 µg/mL for NAP and 0.8 to 4.8 µg/mL for ESO. The results summarized in table-14, shows a good correlation between analytes peak area and concentration with r > 0.9997 (n=6).

The limit of detection and limit of quantification studies were determined based on taking standard deviation from the precision data and slope from the linearity data. The limit of detection was found to be 0.445 µg/mL for NAP and 0.207 µg/mL for ESO and the limit of quantification was found to be 1.362 µg/mL for NAP and 0.633 µg/mL for ESO. These are found to be within the limits.

The robustness was performed by changing the flow rate and wavelength. Prepared the sample solutions having the concentration of 100 µg/mL for NAP and 4 µg/mL for ESO. 20 µL solutions were injected in to the HPLC system by changing the flow rates of 1.1 mL/min and 0.9 mL/min and also changing the wavelengths of 234 nm and 238 nm and observed the retention time. It shows that there is no change in the retention time even after making deliberate change in the analytical procedure.

Ruggedness was performed by different analysts with the same sample. From the prepared standard stock solution 20 µL was injected in to the HPLC system and the chromatograms were observed. There is no change in retention time.

Specificity was performed by treating the sample with acid, base and heat. 20 µL of treated samples were injected in to the HPLC system and observed the degradation of drugs.

Assay (content estimation) was performed to determine the purity of the Naproxen sodium and esomeprazole magnesium trihydrate solutions. The solutions were prepared by using pure drug and sample. These are injected into the HPLC system and the area was recorded for the both standard and sample preparations. The percentage purity was found to be 100.78% for NAP and 99.89% for ESO respectively.

From the above all parameters combined with the simplicity and ease of operation ensures that the application of proposed method in the assay of drug in pharmaceutical dosage form. Therefore the developed method was accurate, precise, linear, robust, simple and rapid. Hence the RP-HPLC method may be applied for combination of Naproxen

sodium and Esomeprazole magnesium trihydrate bulk and in tablet dosage forms.

## SUMMARY AND CONCLUSION

A simple, precise and accurate RP-HPLC method was developed for the analysis of Naproxen sodium and Esomeprazole magnesium trihydrate in pure and tablet dosage form using the mobile phase consisting of mixed phosphate buffer (pH 6.8): Acetonitrile in the ratio of 55: 45 v/v. A wavelength of 236 nm was selected as a detection wavelength for the estimation of NAP and ESO in RP-HPLC system. The flow rate was found to be optimized at 1.0 mL/min. It reduces usage of mobile phase. The system suitability parameters like retention time, resolution, efficiency, capacity factor, tailing factor and % RSD were found to be within the limits for the optimized chromatogram. It is evident that the responses for NAP and ESO were found to be linear in the studied concentration ranges from 20-120 µg/mL and 0.8-4.8 µg/mL respectively and the correlation coefficient were found to be $r^2$=0.9997 and $r^2$=0.9995 for NAP and ESO respectively. LOD was found to be 0.445 µg/mL and 0.207 µg/mL and also LOQ was found to be 1.362 µg/mL and 0.633 µg/mL for Naproxen sodium and Esomeprazole magnesium trihydrate respectively. The recovery studies were also carried out to ensure the accuracy of the method by adding known concentration of drug to pre-analysed formulation. The average percentage recovery was found to be in the range of 99.94-99.98% and 99.48-99.53% for Naproxen sodium and Esomeprazole magnesium trihydrate respectively. In this nearly 100% recovery showed that the method was free from the interference of the excipients used in the formulation.

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