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Development and Validation of a Rp-Hplc Method for Simultaneous Estimation of Domperidone and Naproxen Sodium in a Bulk and in Tablet Dosage Form

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ABSTRACT: Chromatography is a separation process that is achieved by distributing the substances to be separated between a moving phase and a stationary phase. HPLC is basically separating technique; it is always used in conjunction with another analytical tool for quantitative analysis. HPLC is high resolution, high pressure and high speed liquid chromatography. The separation mechanism in reversed phase chromatography depends on the hydrophobic binding interaction between the solute molecule in the mobile phase and the immobilized hydrophobic ligand, i.e. the stationary phase. . In this mode, the stationary phase is nonpolar and mobile phase is polar solvent. HPLC were developed for the simultaneous estimation of Domperidone and Naproxen in marketed formulation. The HPLC system used was WATERS Alliance series System with Rheodyne injector 20 µL and the column Inertsil ODS 250 x 4.6 mm, 5 µm. The mobile phase comprised of mixed phosphate buffer and Acetonitrile in the ratio of 40:60 v/v and flow rate of 1.0 ml/min with UV detection at 273 nm produced peaks of Domperidone and Naproxen in the chromatogram which were well resolved with retention time of 2.721 min and 3.974 min respectively. The developed HPLC method was validated for various parameters like accuracy, precision, specificity, LOD, LOQ, linearity, range and robustness as per ICH guidelines. The results obtained were well within the acceptance criteria for all the parameters. The proposed method was applied for simultaneous estimation of Domperidone and Naproxen in marketed formulations (Tablet). The assay results conformed to the label claim of the formulation. Hence the proposed method can be used for the routine analysis of Domperidone and Naproxen in their marketed tablet dosage formulations.

KEYWORDS: Domperidone, Naproxen, Chromatography, HPLC, Stationary Phase, Mobile Phase.

I. INTRODUCTION

Chromatography: Chromatography may be defined as a method of separating a mixture of components into individual components through equilibrium distribution between two phases a moving phase and a stationary phase. In a single procedure it can separate a mixture into its individual components and simultaneously determine quantitatively the amount of each component present.

[1] Principle of Chromatographic Separation: Chromatography Is A Separation Process That Is Achieved By Distributing The Substances To Be Separated Between A Moving Phase And A Stationary Phase. Those Substances Distributed Preferentially In The Moving Phase Pass Through The Chromatographic System Faster Than Those That Are Distributed Preferentially In The Stationary Phase. As A Consequence the Substances Are Eluted From the Column In Reverse Order of Their Distribution Coefficient With Respect To the Stationary Phase.

[2] Classification of Chromatographic Techniques: Chromatographic methods can be classified according to the nature of the stationary and mobile phase. The different types of chromatography are as:

1. Thin Layer Chromatography
2. Paper Chromatography
3. Ion Exchange Chromatography
4. Size Exclusion or Gel Permeation Chromatography
5. Adsorption Chromatography
6. Partition Chromatography

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[3,4] High Performance Liquid Chromatography:

Since HPLC Is Basically Separating Technique, It Is Always Used In Conjunction With Another Analytical Tool For Quantitative Analysis. HPLC Is High Resolution, High Pressure and High Speed Liquid Chromatography. It Has Several Times Resolving Power Than Open Column Liquid Chromatography Hence It Is Used For Speedy Resolution Of Complex Mixture, Separation And Determination Of Species In A Variety Of Organic, Inorganic And Biological Materials. With This Technology, Liquid Chromatography Can Give High Speed Separations When Compared With Many Cases Like Those Achieved By Gas Chromatography, With The Advantage That Non–Volatile Or Thermally Unstable Samples Can Be Chromatographer Without Decomposition Or The Necessity For Making Volatile Derivatives.[5]

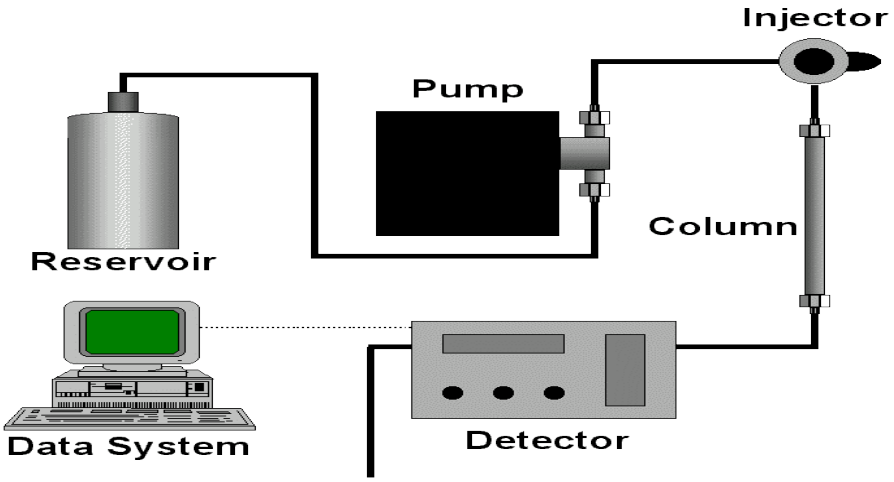

Fig.No.1: Typical Quaternary HPLC instrumentation.

Normal Phase HPLC: In the normal phase mode, the stationary phase is polar and the mobile phase is nonpolar in nature. In this technique, nonpolar compounds travel faster and are eluted first. This is because of the lower affinity between the nonpolar compounds and the stationary phase. Polar compounds are retained for longer times because of their higher affinity with the stationary phase. These compounds, therefore take more times to elute. Normal phase mode of separation is therefore, not generally used for pharmaceutical applications because most of the drug molecules are polar in nature and hence take longer time to elute.

Reverse Phase HPLC: The separation mechanism in reversed phase chromatography depends on the hydrophobic binding interaction between the solute molecule in the mobile phase and the immobilized hydrophobic ligand, i.e. the stationary phase. In this mode, the stationary phase is nonpolar hydrophobic packing with octal or octa decyl functional group bonded to silica gel and the mobile phase is polar solvent. An aqueous mobile phase allows the use of secondary solute chemical equilibrium (such as ionization control, ion suppression, ion pairing and complexation) to control retention and selectivity. The polar compound gets eluted first in this mode and nonpolar compounds are retained for longer time. As most of the drugs and pharmaceuticals are polar in nature, they are not retained for longer times and hence elute faster. The different columns used are octa decyl silane (ODS) or C_{18}, $C_{8,}$ C_4, etc., (in the order of increasing polarity of the stationary phase).

[5] Principle: Reversed phase chromatography based on gradient elution.

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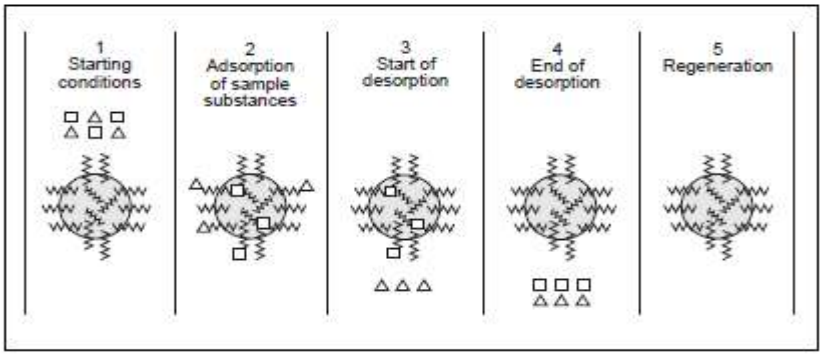

Fig. No.2: Principle of reversed phase chromatography with gradient elution

[6,7] Various components of HPLC are:

a) A solvent delivery system, including pump,
b) Sample injection system,
c) A chromatographic column,
d) A detector,
e) A strip chart recorder,
f) Data handling device and microprocessor control.

a) Solvent delivery system: A mobile phase is pumped under pressure from one or several reservoir and flows through the column at a constant rate. For normal phase separation eluting power increases with increasing polarity of the solvent but for reversed phase separation, eluting power decreases with increasing polarity. A degasser is needed to remove dissolved air and other gases from the solvent.

HPLC Pumps: The pump is one of the most important components of HPLC, since its performance directly affects retention time, reproducibility and detector sensitivity.

The main types of pumps are used in HPLC to propel the liquid mobile phase through the system.

i. Displacement pump: It produces a flow that tends to independent of viscosity and back pressure and also output is pulse free. But it possesses limited capacity (250 ml).

ii. Reciprocating pump: It has small internal volume (35 to 400 µl), their high output pressure (up to 10,000 psi) and their constant flow rates. But it produces a pulsed flow.

b) Sample injection system: Insertion of the sample onto the pressurized column must be as a narrow plug so that the peak broadening attributable to this step is negligible. The injection system itself should have no dead (void) volume.

There are three important ways of introducing the sample into injection port.

- Loop injection: In which, a fixed amount of volume is introduced by making use of fixed volume loop injector

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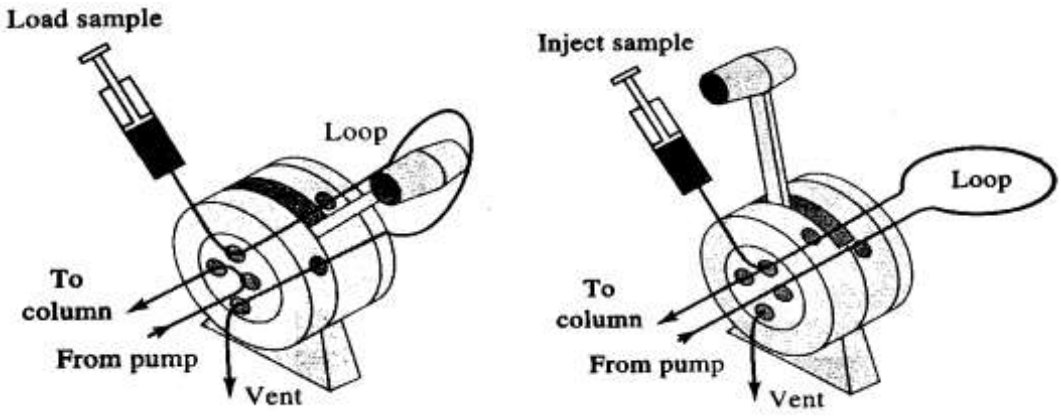

Fig. No.3: Loop injection

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- Valve injection: In which, a variable volume is introduced by making use of an injection valve.
- On column injection: In which, a variable volume is introduced by means of a syringe through a septum, same as above.

c) Chromatographic column: The column is usually made up of heavy glass or stainless steel tubing to withstand high pressure. The columns are usually 10-30 cm long and 4-10 mm inside diameter containing stationary phase at particle diameter of 25 µm or less.

d) Detectors: A detector is required to sense the presence, and measure the amount, of a sample component in the column effluent. Detectors are usually of two types:

i. Bulk property detectors: It compares overall changes in a physical property of the mobile phase with and without an eluting solute. e.g. refractive index, dielectric constant or density.

ii. Solute property detectors: It responds to a physical property of the solute which is not exhibited by the pure mobile phase. e.g. UV absorbance, fluorescence or diffusion current. Such detectors are about 1000 times more sensitive giving a detectable signal for a few nanograms of sample. [7]

[8] System Suitability Tests For Chromatographic Methods:

System suitability tests are an integral part of gas and liquid chromatographic methods. They are used to verify that the resolution and reproducibility of the chromatographic system are adequate for the analysis to be done. The 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.

Table No. 1: System Suitability Parameters and Recommendations

Parameter	Recommendation
Capacity Factor (k')	The peak should be well-resolved from other peaks and the void volume, generally k'>2.0
Repeatability	RSD </= 1% for N >/= 5 is desirable.
Relative retention	Not essential as long as the resolution is stated.
Resolution (R_s)	R_s of > 2 between the peak of interest and the closest eluting potential interferent (impurity, excipient, degradation product, internal standard, etc.
Tailing Factor (T)	T of </= 2
Theoretical Plates (N)	In general should be > 2000

The parameters that are affected by the changes in chromatographic conditions are:

- Resolution (R_s)
- Capacity factor (k')
- Selectivity (a)
- Column efficiency (N)
- Peak asymmetry factor (A_s)

- **Resolution (R_S):** Resolution is the parameter describing the separation power of the complete chromatographic system relative to the particular components of the mixture. The resolution, R_s, of two neighboring peaks is defined as the ratio of the distance between two peak maxima. It is the difference between the retention times of two solutes divided by their average peak width. For baseline separation, the ideal value of R_s is 1.5. It is calculated by using the formula,

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EXPERIMENTAL RESOLUTION

$$Rs = \frac{t_{R(2)} - t_{R(1)}}{1/2\ (w_1 + w_2)}$$

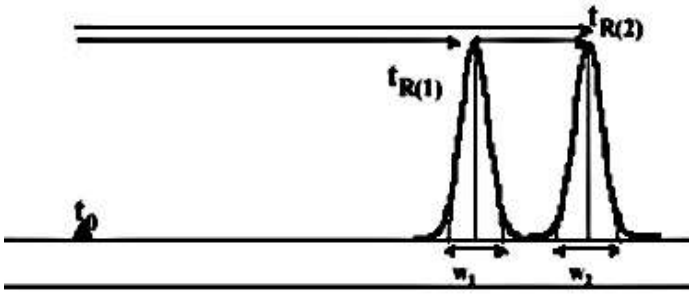

Fig. No.4: Resolution between two peaks

Where, Rt_1 and Rt_2 are the retention times of components 1 and 2 and

W_1 and W_2 are peak width of components 1 and 2.

There are three fundamental parameters that influence the resolution of a chromatographic separation:

- capacity factor (k')
- selectivity (α)
- column efficiency (N)

These parameters provide you with different means to achieve better resolution, as well as defining different problem sources

- **Capacity Factor (K'):** Capacity factor is the ratio of the reduced retention volume to the dead volume. Capacity factor, k', is defined as the ratio of the number of molecules of solute in the stationary phase to the number of molecules of the same in the mobile phase. Capacity factor is a measure of how well the sample molecule is retained by a column during an isocratic separation. The ideal value of k' ranges from 2-10. Capacity factor can be determined by using the formula,

RETENTION FACTOR or CAPACITY RATIO

$$k' = \frac{t_R - t_0}{t_0} \qquad k' = \phi \frac{C_s}{C_m}$$

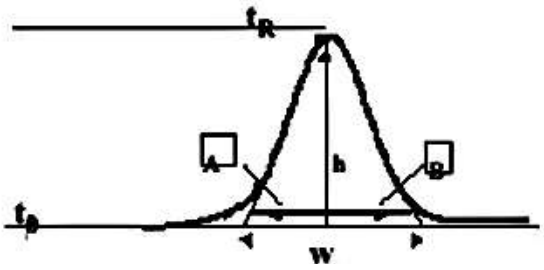

Fig. No.5: Retention Factor

Where, t_R = retention volume at the apex of the peak (solute) and

t_0 = void volume of the system.

- **Selectivity (A):** The selectivity (or separation factor), a, is a measure of relative retention of two components in a mixture. Selectivity is the ratio of the capacity factors of both peaks, and the ratio of its adjusted retention times. Selectivity represents the separation power of

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particular adsorbent to the mixture of these particular components.

This parameter is independent of the column efficiency; it only depends on the nature of the components, eluent type, and eluent composition, and adsorbent surface chemistry. In general, if the selectivity of two components is equal to 1, then there is no way to separate them by improving the column efficiency.

The ideal value is 2. It can be calculated by using formula,

$$a = V_2 - V_1 / V_1 - V_0 = k_1' / k_2'$$

Where, V_0 = the void volume of the column,
V_1 and V_2 =the retention volumes of the second and the first peak respectively.

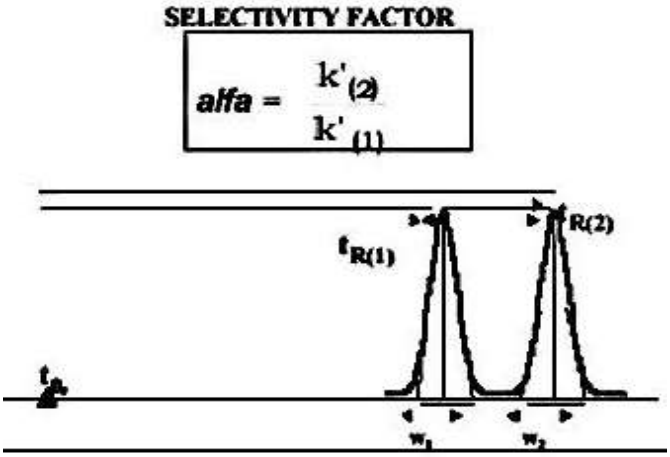

Fig. No.6: Selectivity

- **Column Efficiency/ Band Broadening:** Efficiency, N, of a column is measured by the number of theoretical plates per meter. It is a measure of band spreading of a peak. Similar the band spread, higher is the number of theoretical plates, indicating good column and system performance. Columns with N ranging from 5,000 to 100,000 plates/meter are ideal for a good system. Efficiency is calculated by using the formula,

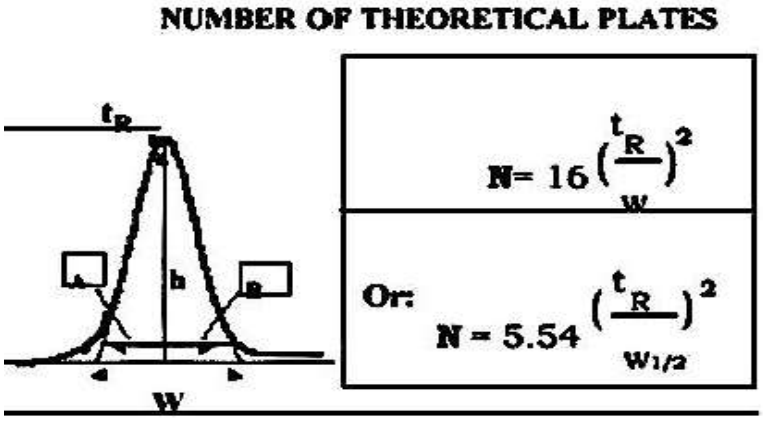

Fig. No.7: Number of Theoretical Plates

Where, t_R is the retention time and
W is the peak width.

- **Peak Asymmetry Factor (T_F):** Peak asymmetry factor, T_f, can be used as a criterion of column performance. The peak half width, b, of a peak at 10% of the peak height, divided by the corresponding front half width, a, gives the asymmetry factor.

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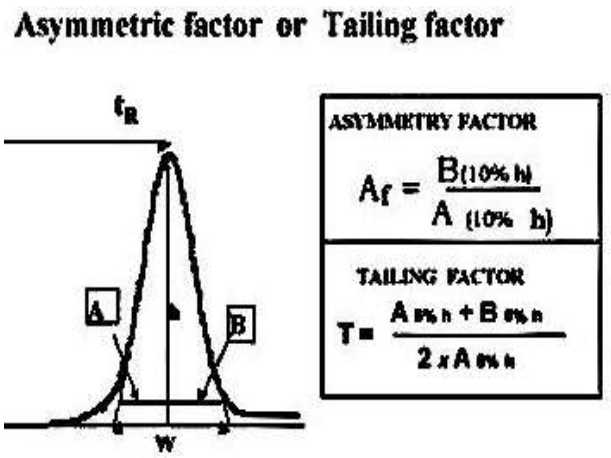

Fig. No.8: Asymmetric Factor

For a well packed column, an asymmetry factor of 0.9 to 1.1 should be achievable.[8,9,10]

[9,10] INTRODUCTION TO VALIDATION:

Validation is documented evidence that provides a high degree of assurance that a specific process will consistently produce a product that meets its predetermined specifications and quality attributes.

Method of Validation: The developed methods were validated by following steps: [11,12]

- Accuracy
- Precision
- Specificity
- Limit of detection
- Limit of quantification
- Linearity and Range
- Ruggedness
- Robustness

II. PREPARATION OF STANDARD STOCK SOLUTIONS:

Selection of Solvent: The ideal property of a solvent should be that the drug should be completely soluble in the solvent used. Drug should be stable in the solvent used and should be economical. Various solvents were studied for the solubility of Domperidone and Naproxen. Both the drugs were very soluble in methanol and freely soluble in acetonitrile and slightly soluble in other non-polar solvents. Therefore methanol was selected as a solvent of choice for both drugs. Both the drugs were stable in methanol.

For UV: The stock solution of Domperidone and Naproxen was prepared separately by dissolving accurately weighed 20 mg in 20 ml of methanol mix well & sonicate. Taken 0.1ml from above stock solution dilute with 10 ml methanol to obtain a final concentration of 10µg/ml.

For HPLC: The stock solution of Domperidone was prepared by dissolving accurately weighed 10 mg in 100 ml of methanol mix well & sonicate after that 10ml transferred in to the 100ml volumetric flask & make up the volume up to 100ml by methanol to obtain a final concentration of 10µg/ml. The stock solution of Naproxen was prepared by dissolving accurately weighed 250 mg in 100 ml of methanol mix well & sonicate after that 10ml transferred in to the 100ml volumetric flask & make up the volume up to 100ml by methanol to obtain a final concentration of 250µg/ml. This stock solution used for system suitability study.

Mixed Stock Solution: The stock solution of Domperidone and Naproxen was prepared by dissolving accurately weighed 10 mg of Domperidone & 250mg of Naproxen in 100 ml of methanol mix well & sonicate after that 10 ml of standard stock solution of Domperidone and Domperidone was taken and transferred it into a 100ml volumetric flask and diluted to 100 ml with the diluents to get a concentration of 10µg/ ml of Domperidone and 250µg/ ml of naproxen.

Selection of Wavelength: The wavelength at which Domperidone and Naproxen showed maximum absorption was selected by obtaining overlay spectra of Domperidone and Naproxen of 10 µg/mL concentration in methanol.

Procedure: Domperidone and Naproxen 10 µg/mL solutions were scanned individually on a Shimadzu UV–Visible spectrophotometer in the wavelength range of 200 to 400 nm.

Selection of Mobile Phase: Domperidone and Naproxen are marketed as combined dosage formulation. The proposed method for estimation

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of Domperidone and Naproxen required adequate resolution between the two drug peaks in the chromatogram. Several solvent systems were tried to obtain optimum resolution.

Table No.2: Mobile phase combinations used

Mobile Phase used	Ratio
Water : Methanol	30:70
Water :Acetonitrile	30:70
Mixed phosphate buffer : Acetonitrile	30:70
Mixed phosphate buffer : Acetonitrile	50:50
Mixed phosphate buffer : Acetonitrile	45:55
Mixed phosphate buffer : Acetonitrile	40:60

Peaks of Domperidone and Naproxen were well resolved with the solvent system of Acetonitrile : Mixed phosphate buffer in the ratio of 60:40.

Determination of Retention Time: The time at which Domperidone and Naproxen showed peaks with good resolution was determined by injecting 10µg/mL and 250µg/mL solution into a chromatogram at the flow rate of 1.0 mL/min, UV detection at 273 nm.

(A) Determination of Retention Time for Domperidone: 10 mg of Domperidone was weighed into a clean and dry 100 mL volumetric flask, dissolved with sufficient volume of mobile phase. The volume was then made up to 100 mL with mobile phase to get a concentration of 100µg/mL (Stock I).10 mL of the stock solution was further diluted to 100 mL with mobile phase to get a concentration 10 µg/mL (Stock II).

Procedure: 20 µl of working standard solution of Domperidone (10µg/mL) was injected into the chromatograph with the flow rate of 1 mL/min.

(B) Determination of Retention Time for Domperidone and Naproxen in combination.

Procedure: 20 µl of working standard mixture solution of Domperidone (10 µg/mL) and Naproxen (250µg/mL) was injected into the chromatograph with the flow rate of 1 mL/min.

Validation of analytical method for the assay of Domperidone and Naproxen: Validation of an analytical method is a process to establish that the performance characteristics of the developed method meet the requirements of the intended analytical application.

The developed HPLC method for simultaneous estimation of Domperidone and Naproxen using Inertsil ODS column Enable 250mm x 4.6mm, 5µm, mobile phase Mixed phosphate buffer: Acetonitrile (40:60), detection wavelength at 273 nm, at a flow rate of 1.0 mL/min was validated for typical analytical parameters like Accuracy, Precision, Specificity, Detection Limit, Quantification Limit, Linearity and Range, Robustness.

Preparation of Standard Stock solution: Accurately weighed 10 mg of Domperidone and 250 mg of Naproxen was transferred into a clean and dry 100 mL volumetric flask and dissolved with sufficient volume of mobile phase, and then volume was made up to 100 mL with mobile phase to obtain concentration of 100 µg/mL and 2500 µg/mL .10 mL of the stock solution was further diluted to 100 mL with mobile phase to get a concentration 10µg/mL and 250µg/ml.

Preparation of Sample Stock Solution:

Powder contents from 20 tablets were taken and weighed. The powder equivalent to 10 mg of Domperidone and 250 mg of Naproxen was accurately weighed into clean, dry 100 mL volumetric flask. The powder was first dissolved in few mL of mobile phase by sonication and filtered through a Whatmann filter. The volume of filtrate was made up to 100 mL with mobile phase to obtain the concentration 100 µg/mL and 2500µg/ml. 10 mL of the stock solution was further diluted to 100 mL with mobile phase to get a concentration 10µg/mL and 250µg/mL.

Preparation of working stock solutions:

- **Solution I:** 10mL sample stock solution and 5mL standard stock solution containing 10µg/mL of Domperidone and 250µg/mL of Naproxen was transferred into 100 mL

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volumetric flask and volume made up with mobile phase to get the 50% spike.

- **Solution II:** 10mL sample stock solution and10mL standard stock solution containing 10µg/mL of Domperidone and 250µg/mL of Naproxen was transferred into 100 mL volumetric flask and volume made up with mobile phase to get the 100% spike.
- **Solution III:** 10mL sample stock solution and 15mL standard stock solution containing 10µg/mL of Domperidone and 250µg/mL of Naproxen was transferred into 100 mL volumetric flask and volume made up with mobile phase to get the 150% spike

Procedure: The resulting solutions I, II and III were injected repeatedly in to the chromatograph the peak area and chromatograms obtained were recorded. Accuracy is measured as the percentage of the analyses recovered by the assay. Spiked samples were injected with triplicate at three intervals a range of 50-150% of the target concentration and injected in to the HPLC system.

Acceptance criteria: percentage recovery should be within 98-120%w/v.

- **PRECISION:** The precision of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The precision of method and system was determined by replicate injection of standard solution and the %RSD was calculated for the results.

i. **System Precision:** The system precision is checked by using standard drug substance to ensure that the analytical system is working properly. The peak area of six estimations is to be measured and %RSD should be calculated.

Standard stock solution of Domperidone and Naproxen: Accurately weighed 10mg of Domperidone and 250mg of Naproxen was transferred into a clean and dry 100 mL volumetric flask and dissolved with sufficient volume of mobile phase, and then volume was made up to 100 mL with mobile phase to obtain concentration of 100 µg/mL and 2500 µg/mL .

Working solution: 10 mL of the stock solution was further diluted to 100 mL with mobile phase to get a concentration 10µg/mL and 250µg/ml.

Procedure: Successive six injections of 20 µl working standard stock solution of Domperidone (10µg/mL) and Naproxen (250µg/mL) were injected into the chromatograph and the chromatograms were recorded. The % relative standard deviation was calculated for the peak area of drug in replicates.

Acceptance criteria: The relative standard deviation should be within 2% w/v

ii. **Method Precision (repeatability):** Method precision indicates whether a method is giving consistent results for a single batch, usually applied to standardization of methodology.

Standard stock solution of Domperidone and Naproxen: Accurately weighed 10mg of Domperidone and 250mg of Naproxen was transferred into a clean and dry 100 mL volumetric flask and dissolved with sufficient volume of mobile phase, and then volume was made up to 100 mL with mobile phase to obtain concentration of 100 µg/mL and 2500 µg/mL .

Working solution: 10 mL of the stock solution was further diluted to 100 mL with mobile phase to get a concentration 10µg/mL and 250µg/ml.

Procedure: Successive six injections from six different vial of 20µl working standard solutions of Domperidone (10µg/mL) and Naproxen (250µg/mL) were injected into the chromatograph and chromatograms were recorded. The % relative standard deviation was calculated for the peak area of the drug.

Acceptance criteria: The relative standard deviation should be within 2% w/v

- **RUGGEDNESS:** The ruggedness of an analytical method is determined by analysis of aliquots from homogeneous samples in different laboratories, by different analysts, using operational and environmental conditions that may differ but are still within the specified parameters of the assay. The degree of reproducibility of test results is then determined as a function of the assay variables. This reproducibility may be compared to the precision of the assay under normal conditions to obtain a measure of the ruggedness of the analytical method.

Standard stock solution of Domperidone and Naproxen: Accurately weighed 10mg of Domperidone and 250mg of Naproxen was transferred into a clean and dry 100 mL volumetric flask and dissolved with sufficient volume of mobile phase, and then volume was made up to 100 mL with mobile phase to obtain concentration of 100 µg/mL and 2500 µg/mL .

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Working solution: 10 mL of the stock solution was further diluted to 100 mL with mobile phase to get a concentration 10µg/mL and 250µg/ml.

Procedure: 20µl of mixed working standard solution were injected in different laboratories, by different analysts and chromatograms are recorded .

Acceptance criteria: the relative standard deviation should be within 2% w/v.

- **SPECIFICITY:** Specificity is the ability to assess unequivocally that the analyze in the presence of components which may be expected to be present; typically these might include impurities, degradation products and matrix components.

Procedure: To perform the specificity parameter blank solution was injected, chromatogram recorded. Standard sample of Domperidone and Naproxen was injected, chromatogram recorded. Stressed samples in acidic condition were injected, chromatogram recorded.

- **LIMIT OF DETECTION (LOD):**Limit of detection is the lowest concentration of the analyze that can be detected by injecting decreasing amount, not necessarily quantity by the method, under the stated experimental conditions.

The minimum concentration at which the analyze can be detected is determined from the standard deviation of the response and slope by applying the formula

Limit of detection $= \frac{\sigma}{S} \times 3.3$

- **LIMIT OF QUANTIFICATION (LOQ):** Limit of quantification is the lowest concentration of the analyze in a sample that can be estimated quantitatively. By injecting decreasing amount of drug, with acceptable precision and accuracy under the stated experimental conditions of the method.

Limit of quantification can be obtained from the standard deviation of the response and slope by applying the following formula.

Limit of quantification $= \frac{\sigma}{S} \times 10$

Linearity and Range: The linearity of an analytical procedure is its ability to obtain test results which are directly proportional to the concentration of analyze in the sample.

Each working standard solution of Domperidone and Naproxen were injected into the chromatograph. The peak areas and chromatograms were recorded. A linearity graph was plotted with concentration variable on x – axis and peak areas on y – axis for Domperidone and Naproxen. The Correlation coefficient and Percentage curve fittings were calculated.

Standard stock solution of Domperidone and Naproxen:

Accurately weighed 10mg of Domperidone and 250mg of Naproxen was transferred into a clean and dry 100 mL volumetric flask and dissolved with sufficient volume of mobile phase, and then volume was made up to 100 mL with mobile phase to obtain concentration of 100 µg/mL and 2500 µg/mL .

Working Standard Solution: Aliquots from standard stock solution were withdrawn in the volumes of 2.5, 5, 7.5, 10, 12.5 and 15mL in separate 100 mL volumetric flasks. The volume was made up with the mobile phase to get concentrations ranging from 2.5-15 µg/mL for Domperidone and 62.5-375 µg/mL for Naproxen**.**

Standard stock solution of Domperidone and Naproxen: Accurately weighed 10mg of Domperidone and 250mg of Naproxen was transferred into a clean and dry 100 mL volumetric flask and dissolved with sufficient volume of mobile phase, and then volume was made up to 100 mL with mobile phase to obtain concentration of 100 µg/mL and 2500 µg/mL .

Working solution: 10 mL of the stock solution was further diluted to 100 mL with mobile phase to get a concentration 10µg/mL and 250µg/ml.

a) **Change in flow rate:** For the method developed, flow rate of 1mL/min was used. For Robustness study, flow rate of 0.9 and 1.1mL/min were selected. Three injections of 20 µl of each working standard solution of Domperidone and Naproxen at flow rate of 0.9 and 1.1 mL/min were injected into the chromatograph, the peak area and chromatogram obtained were recorded and the % assay was calculated

b) **Change in Buffer ratio: Procedure:** 20µl of mixed working standard solution were injected in different chromatographic condition of buffer ratio and chromatograms are recorded. The results obtained are presented

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Table No.3: Acceptance criteria for different validation parameters

Validation Parameter		**Acceptance Criteria**
Specificity		**No interference by the degraded components at the drug retention time**
Precision	System	**NMT 2% (% RSD)**
	Method	
	Intra day	
	Interday	
Robustness		**within 90-110% (%Assay)**
Accuracy		**within 90-110% (%Recovery)**

Application of the developed HPLC method for Simultaneous Estimation of Domperidone and Naproxen in Marketed Formulation

The developed and validated method has been applied for Simultaneous Estimation of Domperidone and Naproxen in marketed formulation.

Sample: Domperidone and Naproxen Tablet.

Brand name: 'Naxdom-250' (Domperidone 10 mg and Naproxen 250 mg)

Manufacturer: Atoz life sciences.

Table No.4: Chromatographic Conditions for Sample Analysis

Instrument	WATERS Alliance series.
Injector	Rheodyne
Column	Inertsil ODS Column 250mm(length) x 4.6mm(I.D) , 5µm (Particle Size)
Wavelength	273nm
Detector	UV Detector
Flow rate	1.0 mL/min
Injection volume	20µl
Mobile Phase	Mixed phosphate buffer : Acetonitrile (40: 60)

Preparation of Sample Stock Solution: Weighed 20 Tablet; take Avg. wt. of it powdered equivalent to 10 mg of Domperidone and 250mg of Naproxen was accurately weighed and transferred into clean, dry 100mL volumetric flask. The powder was first dissolved in few mL of mobile phase by sonication, the volume was made up to 100mL and then filtered through a Whatmann filter to obtain the concentration 100µg/mL and 2500µg/mL for Domperidone and Naproxen respectively (Stock I).

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Working Sample solutions: From the above stock, volumes of 10mL were transferred into 100mL volumetric flasks and volume made up to 100mL with the mobile phase to get the concentration of 10µg/mL for Domperidone and 250µg/mL for Naproxen respectively.

Procedure: 20 µl solution of the working sample solutions were injected repeatedly into the chromatograph, at a flow rate of 1 ml/min and detection at 273 nm, the chromatograms obtained were recorded.

The amount of drug per Tablet & % assay is calculated by following formula

$$\text{Amount /Tablet} = \frac{\text{sample peak avg.}}{\text{std peak avg.}} * \frac{\text{std wt}}{\text{sample wt}} * \frac{\text{std factor}}{\text{sample factor}} * \text{avg. wt} * \frac{\text{std purity}}{100}$$

$$\% \text{ Assay} = \frac{\text{amount per Tablet}}{\text{label claim}} * 100$$

III. RESULTS

Stability indicating RP-HPLC method for simultaneous estimation of Domperidone and Naproxen: A stability indicating RP- HPLC method for simultaneous determination of Domperidone and Naproxen was developed and validated at Bio-leo analytical labs India pvt. Limited, prashanti nagar, Hyderabad.

Table No.5: Instrument Specification:

Instrument	WATERS Alliance series.
Injector	Rheodyne
Column	Inertsil ODS column 250mm(length) x 4.6mm(I.D), 5µm (Particle Size)
Detector	UV Detector
Wavelength	273nm
Injection volume	20µL
Flow rate	1.0 mL/min
Mobile phase	Mixed phosphate buffer : Acetonitrile (40:60)

Chemicals and Reagents used:

1. Millipore water
2. Methanol, HPLC grade (Merck, Mumbai)
3. Acetonitrile, HPLC grade (RFCL Ltd.)
4. Potassium dihydrogen orthophosphate (RFCL Ltd.)
5. Dipotassium hydrogen phosphate anhydrous (Merck, Mumbai)
6. Domperidone (Bio-leo analytical labs India pvt. Limited, Hyderabad)
7. Naproxen (Bio-leo analytical labs India pvt. Limited, Hyderabad)

Naxdom-250 Tablet was manufactured by Atoz life sciences, India purchased from the local market.

Development of RP-HPLC Method for Simultaneous estimation of Domperidone and Naproxen: A method was developed for simultaneous estimation of Domperidone and Naproxen on HPLC by selecting the appropriate λ max, optimum mobile phase and flow rate which gives good and sharp resolution.

Selection of Wavelength: Standard solutions of Domperidone and Naproxen (10 µg/ml) were scanned in the wavelength range of 200-400

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nm using Methanol as diluents and UV spectrum obtained is presented below.

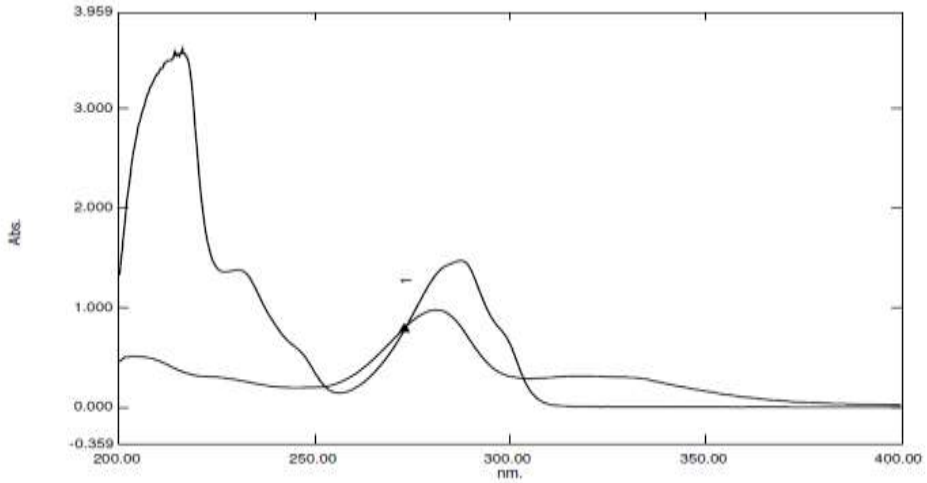

Fig. No.9: Overlay UV Spectrum of Domperidone and Naproxen

No.	Wavelength	Absorbance	Description
1.	273.20	0.794	Domperidone & Naproxen

Report: The 10µg/mL of Domperidone and Naproxen solutions showed λ max at 273.20 nm which was selected as wavelength maxima for determination of Domperidone and Naproxen.

Selection of Mobile Phase: Several solvent systems were tried to get optimum resolutions of Domperidone and Naproxen in the present method. The observations obtained with various mobile phases in different ratios are given below

Table No.6: Different Mobile Phases used and their observations

Mobile Phase used	Ratio
Water : Methanol	30:70
Water : Acetonitrile	30:70
Mixed phosphate buffer : Acetonitrile	30:70
Mixed phosphate buffer : Acetonitrile	50:50
Mixed phosphate buffer : Acetonitrile	45:55
Mixed phosphate buffer : Acetonitrile	40:60

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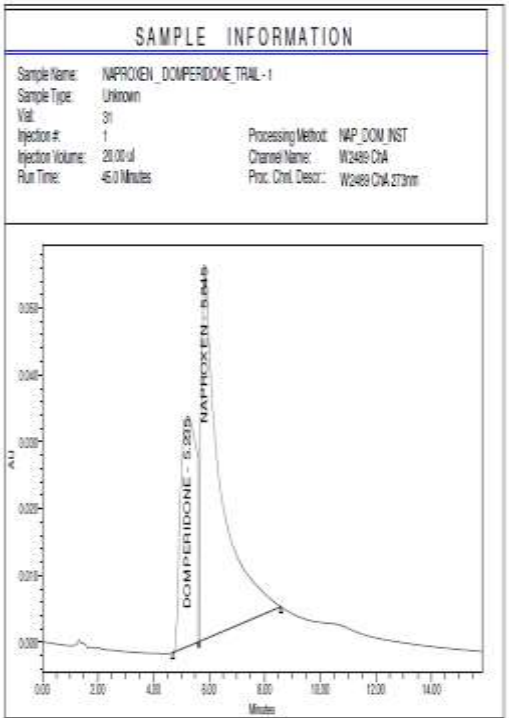

Fig. No.10(a): Chromatogram for comb. of and Methanol (30:70)

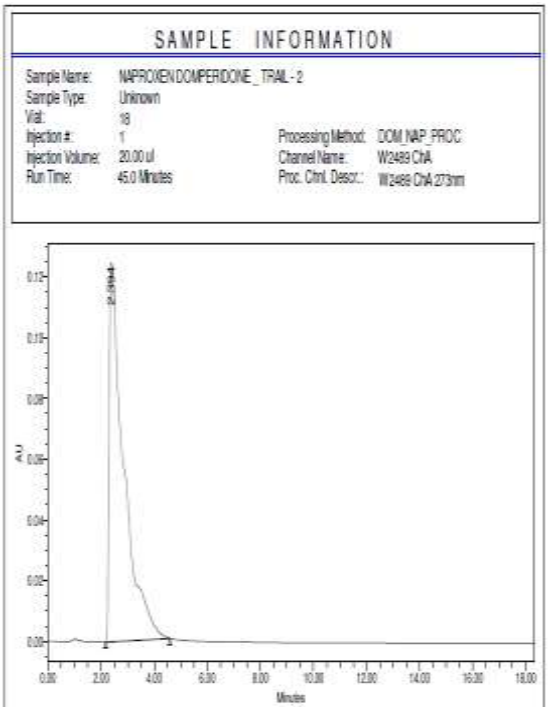

Fig. No.11 (b):Chromatogram for comb. of water water and Acetonitrile (30:70)

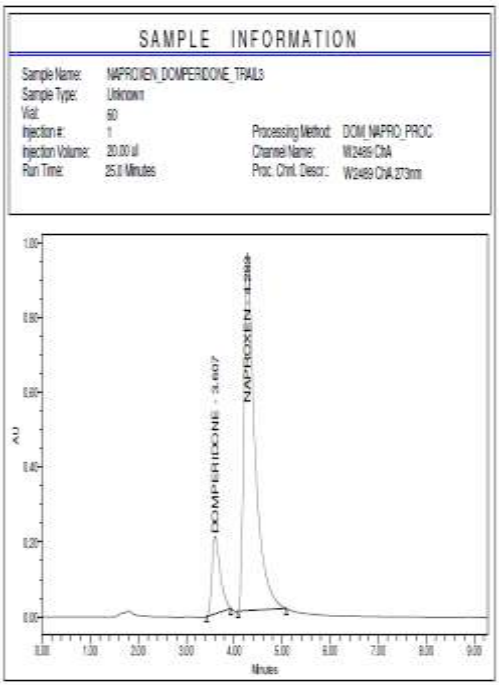

Fig. No. 12(c): Chromatogram for comb. of Phosphate Buffer and Acetonitrile (30:70)

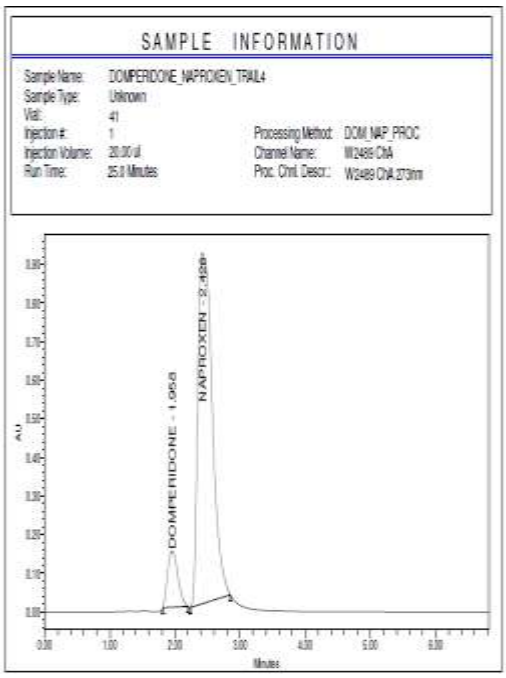

Fig. No.13(d): Chromatogram for comb. Of Mix Mix Phosphate Buffer and Acetonitrile (50:50)

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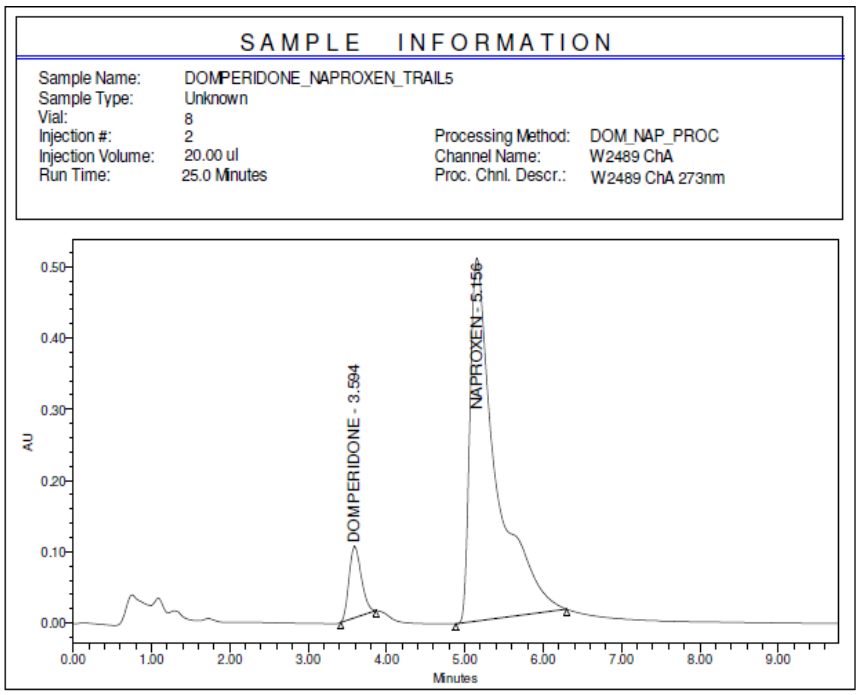
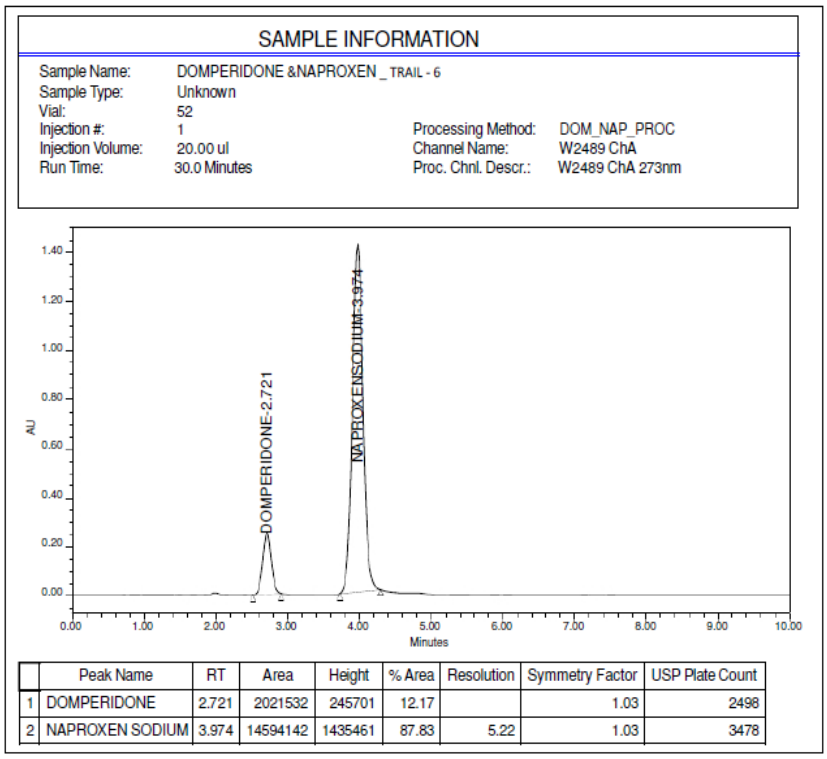


Fig. No.14(e): Chromatogram for comb. of Mix Phosphate Buffer and Acetonitrile(45:55)



	Peak Name	RT	Area	Height	% Area	Resolution	Symmetry Factor	USP Plate Count
1	DOMPERIDONE	2.721	2021532	245701	12.17		1.03	2498
2	NAPROXEN SODIUM	3.974	14594142	1435461	87.83	5.22	1.03	3478

Fig. No. 15(f): Chromatogram of Domperidone and Naproxen peaks showing good resolution with mobile phase Mix Phosphate Buffer and Acetonitrile (40:60)

Report: In this trail I got proper resolution i.e. 5.22 also proper retention time is 2.721 for Domperidone and 3.974 for Naproxen and plate count are within limit so I finalized this trial as

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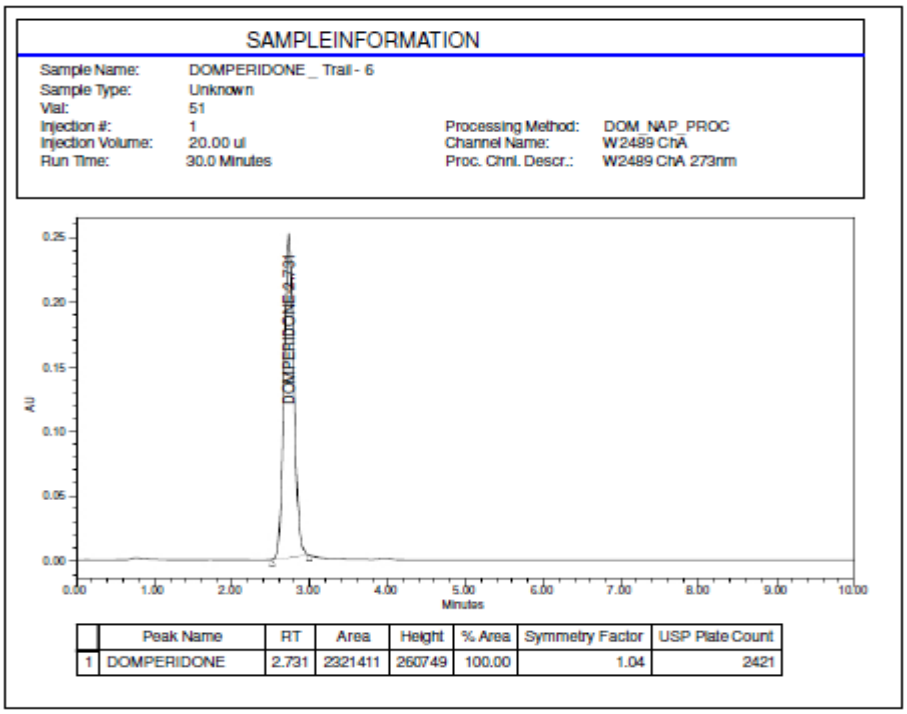
optimized method for Domperidone and Naproxen drug.

Determination of Retention Time: The optimum retention time at which Domperidone and Naproxen showed good resolution was selected for the proposed method. The chromatograms obtained individually for Domperidone and Naproxen and in combination are presented below.

a) Determination of Retention Time for Domperidone: 20µl of standard solution Domperidone (10 µg/mL) was injected into the chromatograph at a flow rate of 1.0 mL/min and UV detection at 273 nm. The retention time and chromatogram obtained is presented below.



	Peak Name	RT	Area	Height	% Area	Symmetry Factor	USP Plate Count
1	DOMPERIDONE	2.731	2321411	260749	100.00	1.04	2421

Fig. No. 16: Chromatogram for retention time of Domperidone

Report: The Retention time for Domperidone was found to be 2.731 min.

a) Determination of Retention Time for Domperidone and Naproxen. 20µl of Domperidone (10 µg/mL) and Naproxen (250µg/mL) mixture solution was injected at a flow rate of 1mL/min using mobile phase of Mix Phosphate Buffer and Acetonitrile in the ratio of 40:60 v/v and UV detection at 273 nm. The retention time and chromatogram obtained are presented below.

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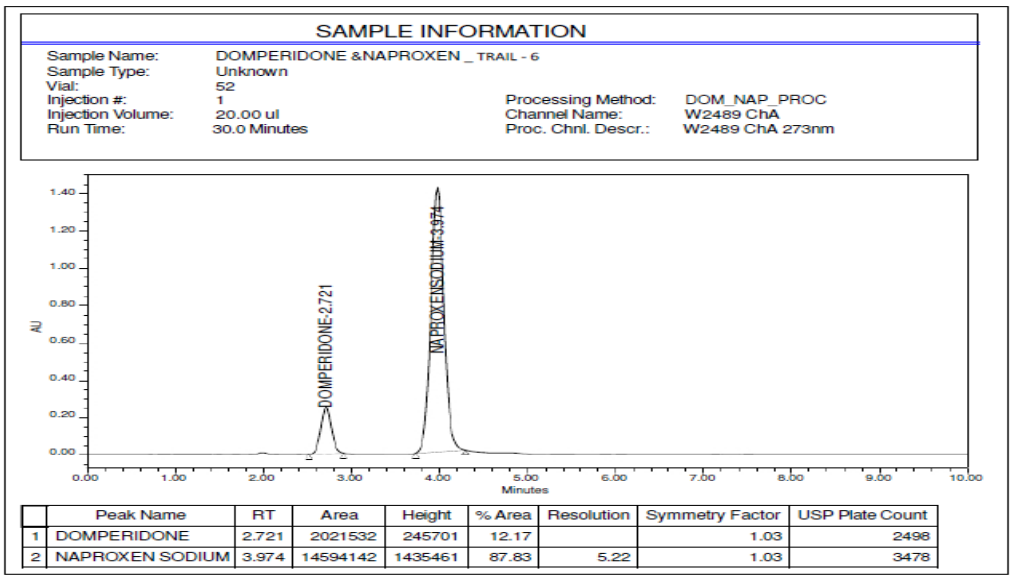
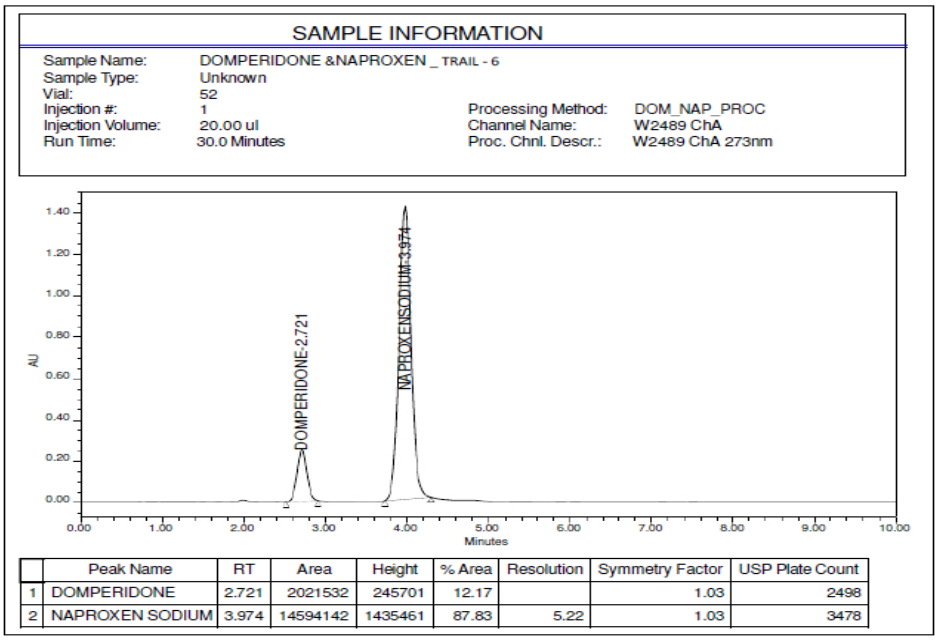


	Peak Name	RT	Area	Height	% Area	Resolution	Symmetry Factor	USP Plate Count
1	DOMPERIDONE	2.721	2021532	245701	12.17		1.03	2498
2	NAPROXEN SODIUM	3.974	14594142	1435461	87.83	5.22	1.03	3478

Fig. No.17: Chromatogram for retention time of Domperidone and Naproxen in combination

Report: The retention time for Domperidone was found to 2.721min and the retention time for Naproxen were found to be 3.974 min indicating no change in retention time for Domperidone and Naproxen in combination.

System suitability: This parameter ensures that the analytical system is working properly and can give accurate and precise results. A 20µl solution of Domperidone and Naproxen (10µg/mL and 250µg/mL) each was injected and the system suitability parameters like theoretical plates per column, tailing factor, HETP and resolution were calculated from the following chromatogram.



	Peak Name	RT	Area	Height	% Area	Resolution	Symmetry Factor	USP Plate Count
1	DOMPERIDONE	2.721	2021532	245701	12.17		1.03	2498
2	NAPROXEN SODIUM	3.974	14594142	1435461	87.83	5.22	1.03	3478

Fig. No. 18: Chromatogram of System Suitability Parameters for Domperidone and Naproxen

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Table No.7: System Suitability Data of Parameters for Domperidone and Naproxen

System Suitability Factor	Domperidone	Naproxen	Acceptance Criteria
Tailing factor	1.03	1.03	2
HETP(mm)	0.10008	0.0718	-
Resolution	5.22		-
Theoretical plates	2498	3478	>1500

Report: The obtained system suitability parameters for Domperidone and Naproxen were found to be within the acceptance criteria.

Validation of analytical method for the assay of Domperidone and Naproxen: The HPLC method developed was validated by performing the various method validation parameters like specificity, LOD, LOQ, linearity, range, precision, robustness, accuracy, and system suitability parameters as per ICH guidelines. This is to ensure that the performance characteristics of the HPLC method developed meets the requirements for the intended analytical applications.

Following parameters were performed for method validation:

1. Accuracy
2. Precision
   a) System precision
   b) Method precision
   c) Intermediate precision (Ruggedness)
3. Specificity
4. Limit of Detection (LOD)
5. Limit of Quantification (LOQ)
6. Linearity and Range
7. Robustness

1. Accuracy: This parameter is performed to determine the closeness of test results with that of the true value which is expressed as % recovery. These studies were performed at three different levels (50%, 100% and 150%) and the % recovery of Domperidone and Naproxen was calculated. 20µL of three different solutions of Domperidone and Naproxen were injected repeatedly into chromatograph, the peak area and chromatogram were recorded and are presented below.

Table No.8: Recovery study data for Domperidone and Naproxen

Standard and Sample	Domperidone	Naproxen
Standard 1 peak area	2159055	15304237
Standard 2 peak area	2138009	15274129
Standard 3 peak area	2184062	15255184
avg. std peak area	2160375.333	15277850
sample peak area	2115945	15263693

Table No.9: Recovery study data for Domperidone and Naproxen in 50% spike

50% spike	3193679	22863993
	3194236	22998063
	3197498	22811677
Avg.	3195137.667	22891244.33
amt.recovered	**49.95**	**49.93**
%Recovery	**99.91**	**99.85**

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Table No.10: Recovery study data for Domperidone and Naproxen in 100% spike

100%spike	4270221	30622016
	4308264	30637471
	4233801	30581811
Avg	4270762	30613766
amt.recovered	**99.74**	**100.47**
%Recovery	**99.74**	**100.47**

Table No.11: Recovery study data for Domperidone and Naproxen in 150% spike

150%spike	5339012	38000448
	5274748	38015235
	5344143	38126979
Avg	5319301	38047554
amt.recovered	**148.28**	**149.13**
%recovered	**98.85**	**99.42**

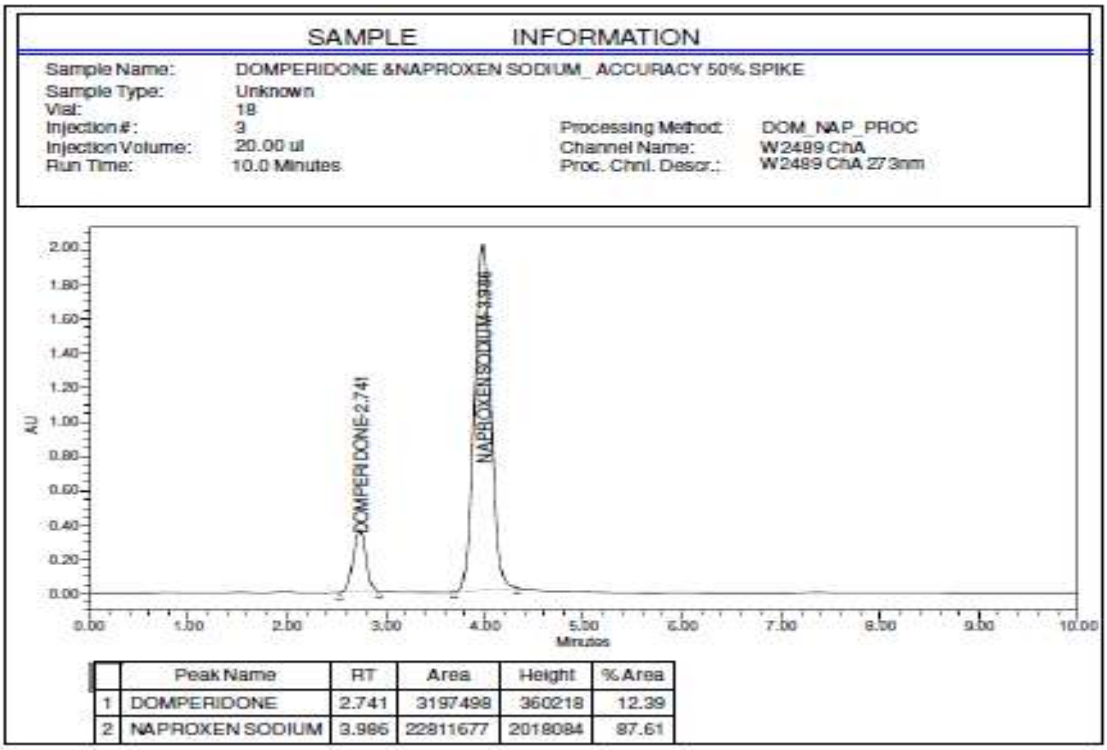

Fig. No.19: Chromatogram for recovery Studies at 50% spike

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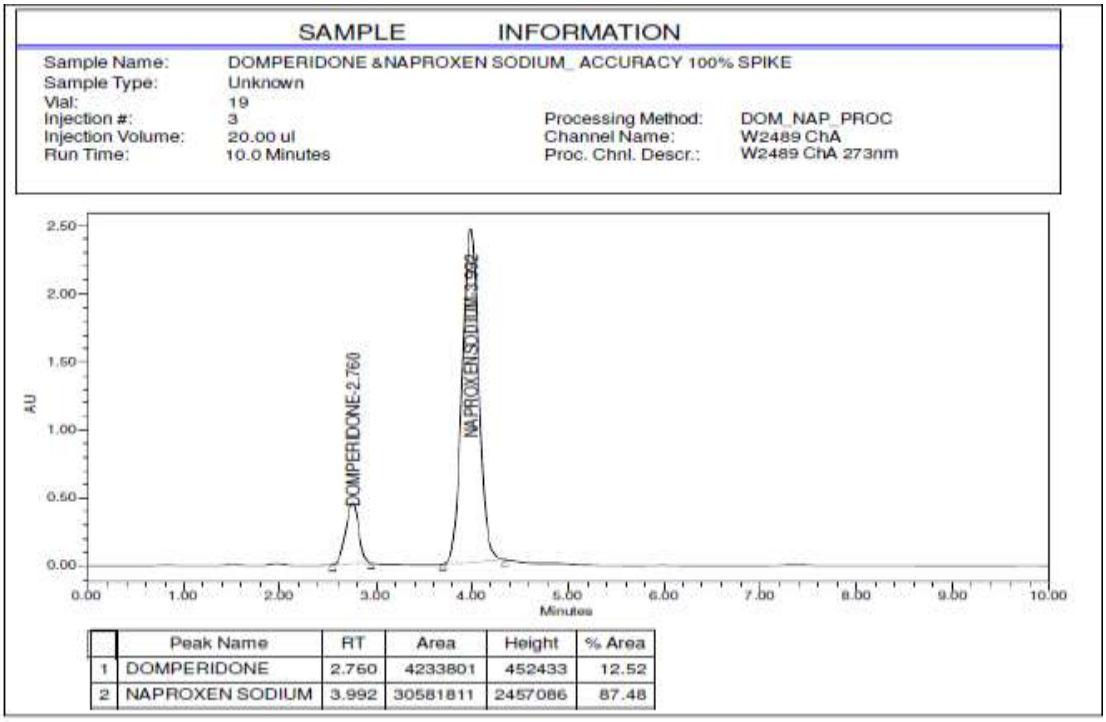
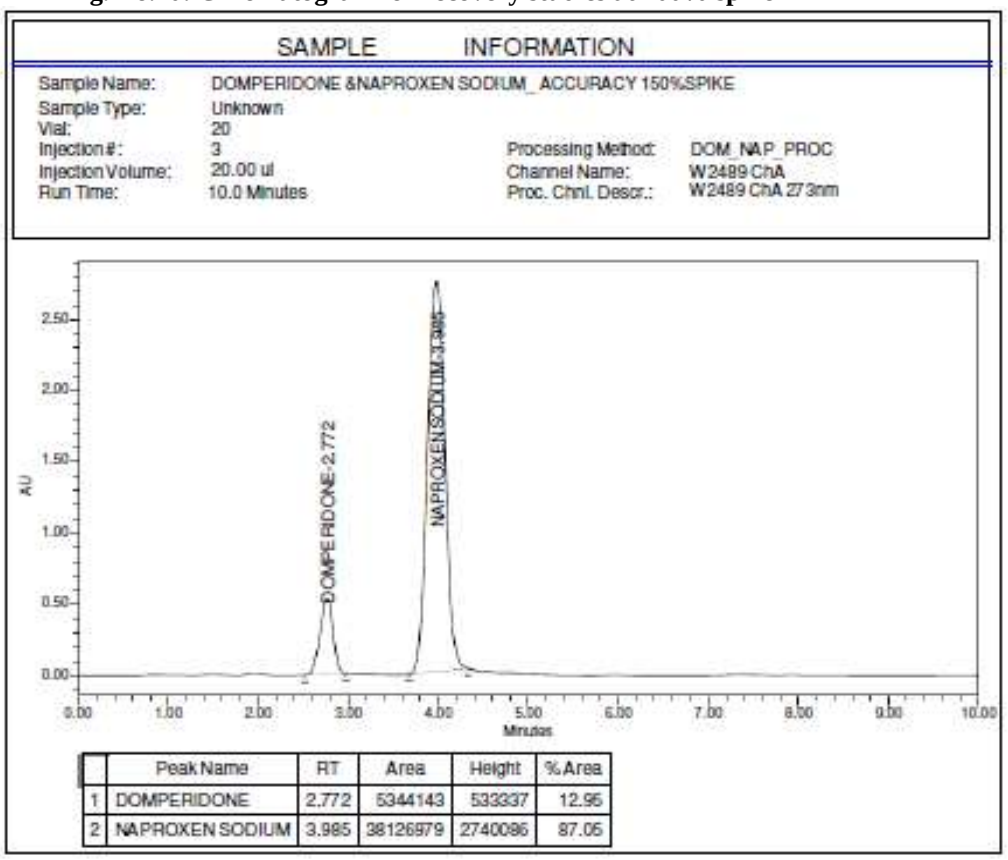


	Peak Name	RT	Area	Height	% Area
1	DOMPERIDONE	2.760	4233801	452433	12.52
2	NAPROXEN SODIUM	3.992	30581811	2457086	87.48

Fig. No.20: Chromatogram for recovery studies at 100% spike



	Peak Name	RT	Area	Height	% Area
1	DOMPERIDONE	2.772	5344143	533337	12.95
2	NAPROXEN SODIUM	3.985	38126979	2740086	87.05

Fig. No.21: Chromatogram for recovery studies at 150% spike

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Report: The mean percentage recovery for Domperidone and Naproxen at three different levels was found to be between 99.91-98.85% and 100.47-99.42% respectively, which are well within the limit and hence the method was found to be accurate.

2. Precision:

Precision of an analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) it is performed to see the closeness of agreement between the series of measurements. It is determined by assaying sufficient number of samples and their relative standard deviation is determined.

(a) System precision:

This method validation parameter was performed to ensure the closeness of results between true value and experimental value. Successive Six injections of 20µl of concentration of 10µg/mL and 250µg/mL for Domperidone and Naproxen respectively were injected into the system. The peak area was recorded and is presented below.

Table No.12: System Precision Data for Domperidone and Naproxen

Replicates	Domperidone	Naproxen
	Peak Area	**Peak Area**
1	2097527	15410390
2	2092212	15202292
3	2102277	15240941
4	2123572	15278315
5	2107466	15328188
6	2103968	15261539
Average	2104504	15286944
Standard Deviation	10745.98	73405.85
%RSD	**0.51**	**0.48**

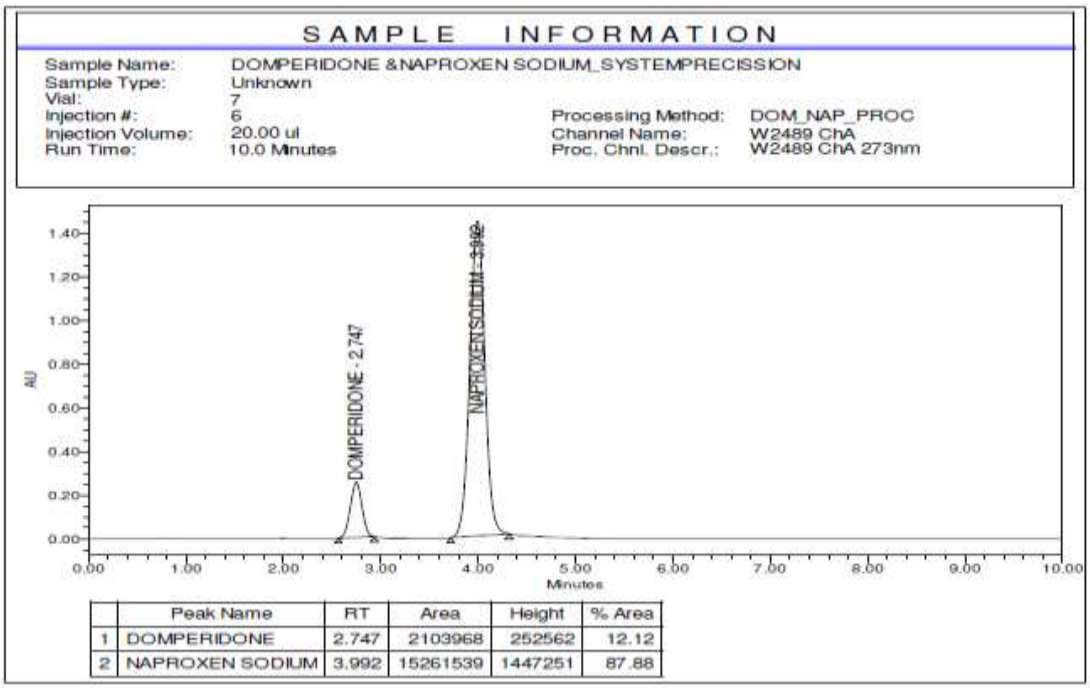

Fig. No. 22: Chromatogram of Domperidone and Naproxen for System Precision

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Report: The % RSD values of peak area for six replicate injections of Domperidone and Naproxen were found to be **0.51** and **0.48** respectively which are well within the acceptance criteria limit of NMT 2%.

(b) Method Precision (Repeatability): The method precision was performed to standardize methodology i.e. to check whether the developed method is precise i.e. whether the method is giving consistent results. Successive six injections from six different vial of 20µl working standard solution of Domperidone and Naproxen were injected. The peak area and Chromatograms were recorded and presented below. The % RSD for peak area and was calculated.

Table No.13: Method Precision Data Domperidone and Naproxen

Replicates	Domperidone	Naproxen
	Peak Area	**Peak Area**
1	2115561	15203684
2	2079163	15101282
3	2100554	15130777
4	2081103	15029695
5	2069052	14872741
6	2081383	14862330
Average	2087803	15033418
Standard Deviation	17002.65	140126.3
%RSD	**0.81**	**0.93**

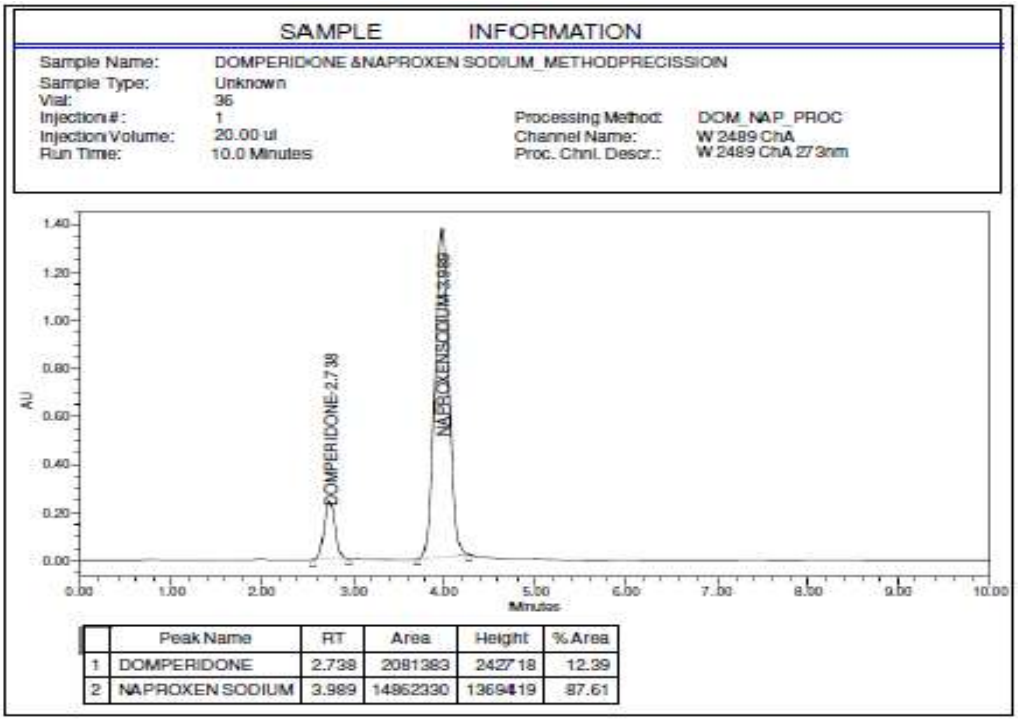

Fig No.23: Chromatogram of Domperidone and Naproxen for Method Precision

Report: The % RSD values of concentration for six replicate injections of Domperidone and Naproxen were found to be **0.81** and **0.93** respectively which are well within the acceptance criteria limit of NMT 2%.

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iii. Specificity: Specificity was performed to assess and ensure that the impurities, degraded products do not interfere with peaks of analyses.

Procedure: Volume of 20µl of blank working standard solution of Domperidone and Naproxen and stressed samples in alkaline condition were injected into the chromatograph and the chromatograms were recorded and presented below.

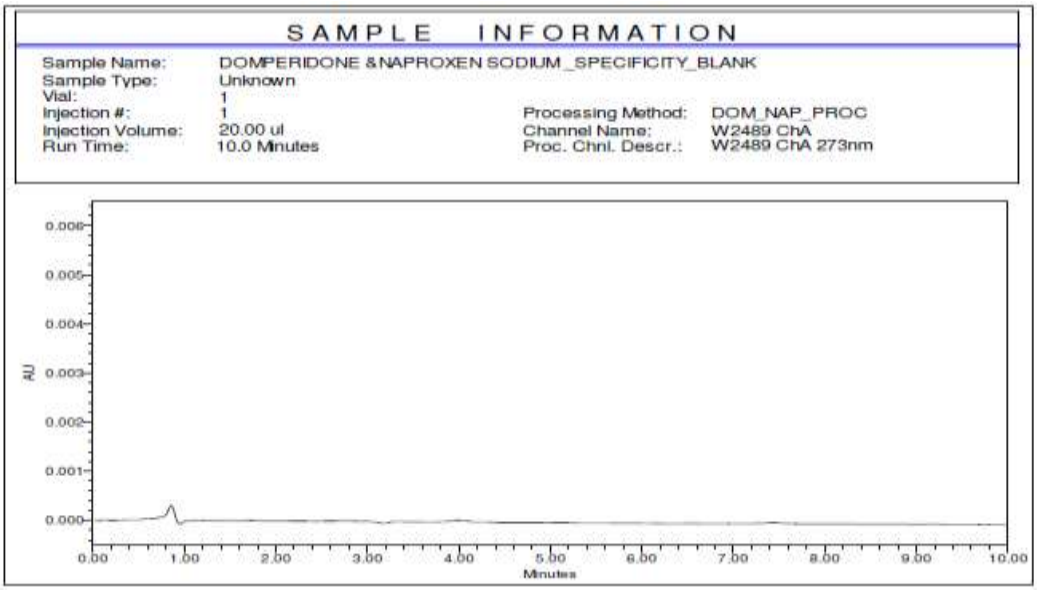
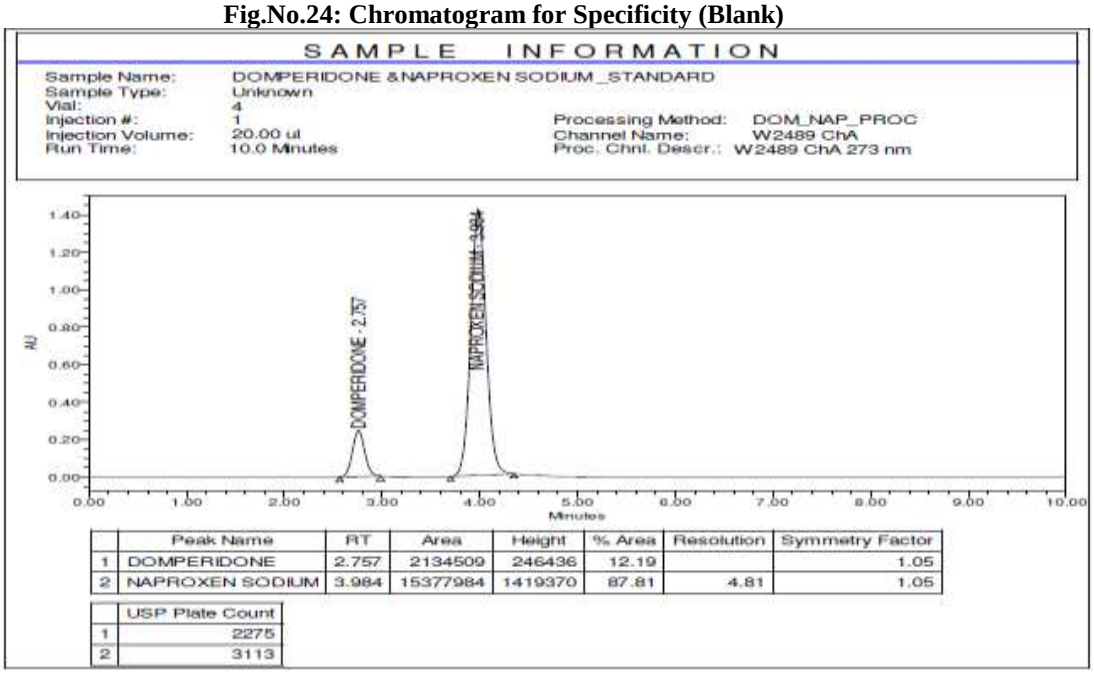


Fig.No.24: Chromatogram for Specificity (Blank)



	Peak Name	RT	Area	Height	% Area	Resolution	Symmetry Factor
1	DOMPERIDONE	2.757	2134509	246436	12.19		1.05
2	NAPROXEN SODIUM	3.984	15377984	1419370	87.81	4.81	1.05

	USP Plate Count
1	2275
2	3113

Fig No.25: Chromatogram for Specificity (standard)

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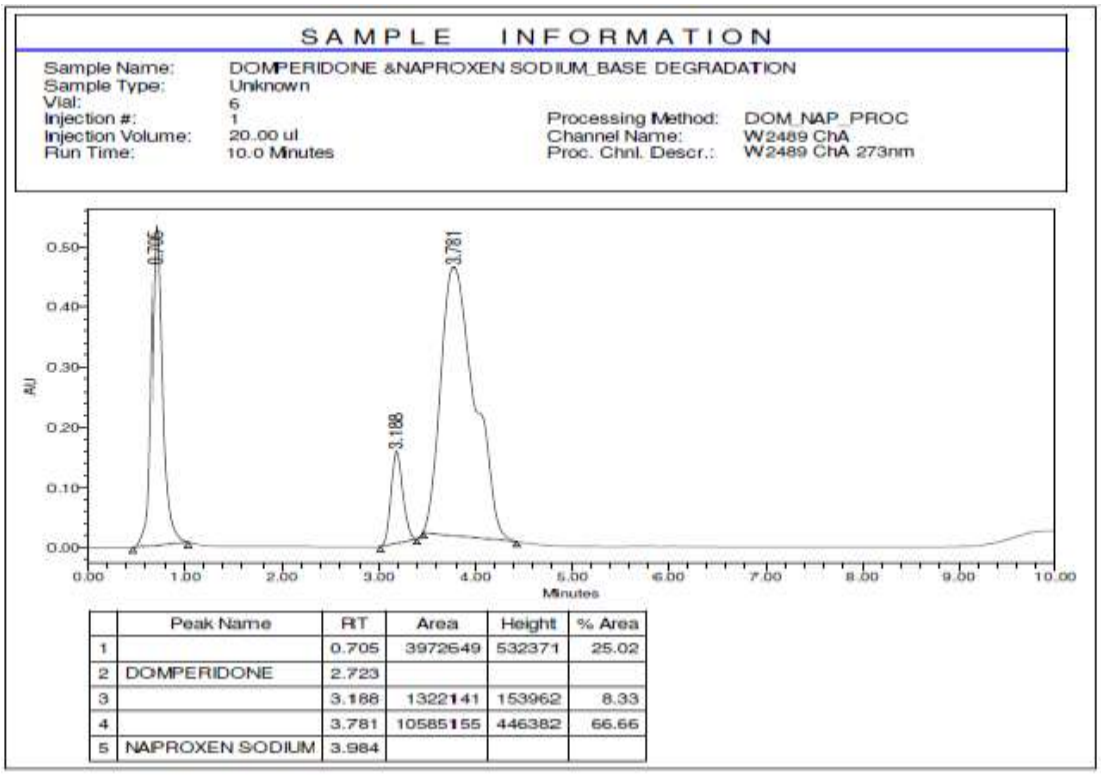

	Peak Name	RT	Area	Height	% Area
1		0.705	3972649	532371	25.02
2	DOMPERIDONE	2.723			
3		3.188	1322141	153962	8.33
4		3.781	10585155	446382	66.66
5	NAPROXEN SODIUM	3.984			

Fig No.26: Chromatogram for specificity (Alkali)

Observation: The analyses do not show any interference with the degraded components.

Report: As there is no interference with the degraded components at retention time of 2.723 (Domperidone) and 3.984 min (Naproxen), hence the proposed method was specific for the detection of Domperidone and Naproxen in combination product.

iv. Limit of Detection (LOD): Limit of detection is the lowest concentration of the analyze that can be detected by injecting decreasing amount, not necessarily quantity by the method, under the stated experimental conditions. The minimum concentration at which the analyze can be detected is determined from the standard deviation of the response and the slope by applying the formula.

Limit of detection = $\frac{\sigma}{S}$ **x 3.3**

Report: The lowest concentration of Domperidone that can be detected, was determined from standard curve was **1.680µg/ml**.

The lowest concentration of Naproxen that can be detected was determined from standard curve was **4.002µg/mL**

v. Limit of Quantification (LOQ): Limit of quantification is the lowest concentration of the analyze in a sample that can be estimated quantitatively. By injecting decreasing amount of drug, with acceptable precision and accuracy under the stated experimental conditions of the method. Limit of quantification can be obtained from the standard deviation of the response and the slope by applying the following formula.

Limit of quantification = $\frac{\sigma}{S}$ **x 10**

Report: The lowest concentration at which peak can be quantified is called LOQ, was found to be **5.245µg/mL** for Domperidone.

The lowest concentration at which peak can be quantified is called LOQ, was found to be **12.491µg/mL** for Naproxen.

vi. Linearity and Range: The linearity was performed to ensure that the test results are directly proportional to the concentration of analyze sample.

20µl of each of working standard solution of Domperidone and Naproxen were injected in to the chromatograph. The results obtained are tabulated below.

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TableNo.14: Linearity Range Data for Domperidone.

Volume of stock solution (mL)	Volume made up to (mL)	Concentration (µg/mL)		Domperidone Peak Area	Naproxen Peak Area
		Dom.	Napro.		
2.5	100	2.5	62.5	517297	3760541
5	100	5	125	1049354	7575341
7.5	100	7.5	187.5	1586590	11327012
10	100	10	250	2108946	15193024
12.5	100	12.5	312.5	2646962	18951972
15	100	15	375	3147217	22641464

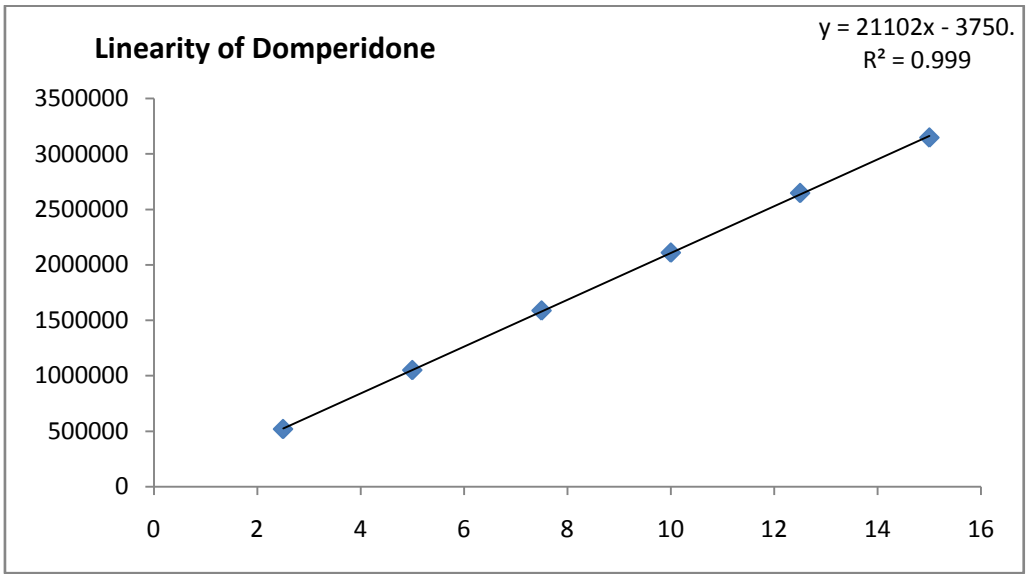

Fig No.27: Linearity Range Graph of Domperidone

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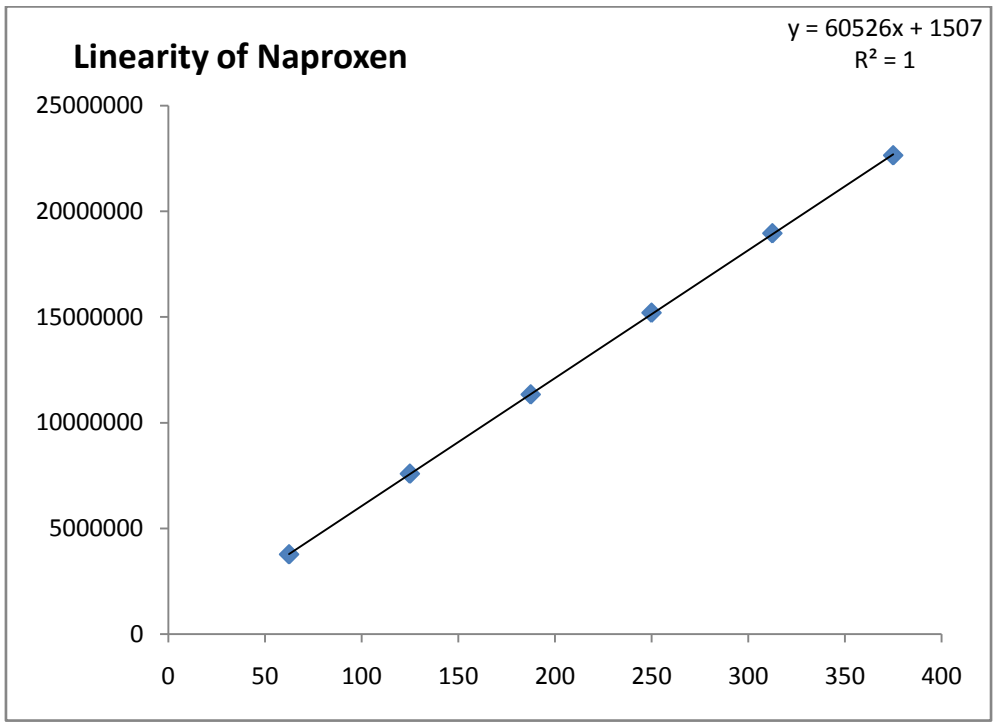

Fig No.28: Linearity Range Graph of Naproxen

Table No.15: Linearity Range data for Domperidone and Naproxen

Parameters	Domperidone	Naproxen	Acceptance Criteria
Linearity Range	2.5-15 µg/mL	62.5-375 µg/mL	-
Regression Equation	y = 21102x+3750	y = 60526x+1507	-
Correlation Coefficient	0.9999	1	0.999
Percentage curve Fitting	100%	99.9%	99.0%
Intercept	3750	1507	-
Slope	21102	60526	-

Report: The linearity in response for Domperidone and Naproxen was observed in the concentration range of 2.5 to 15µg/mL and 62.5 to 375µg/mL respectively, with percentage curve fittings found to be well within the limits of acceptance criteria (99%).

Robustness: Robustness was performed to check the ability of the system to give unaffected results for small deliberate changes in system parameters and method parameters.

(a) Change in Flow Rate: 20µl working standard solutions prepared in mobile phase and were injected in chromatograph at flow rate 0.9 and 1.1mL/min, the % assay was calculated and presented below.

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Table No.21: Robustness Data for Change in Flow Rate

Drug	Change in flow Rate	Peak Area	% Assay
Domperidone and Naproxen	0.9 mL	5468403	101.37
	1.1 mL	4879784	104.63

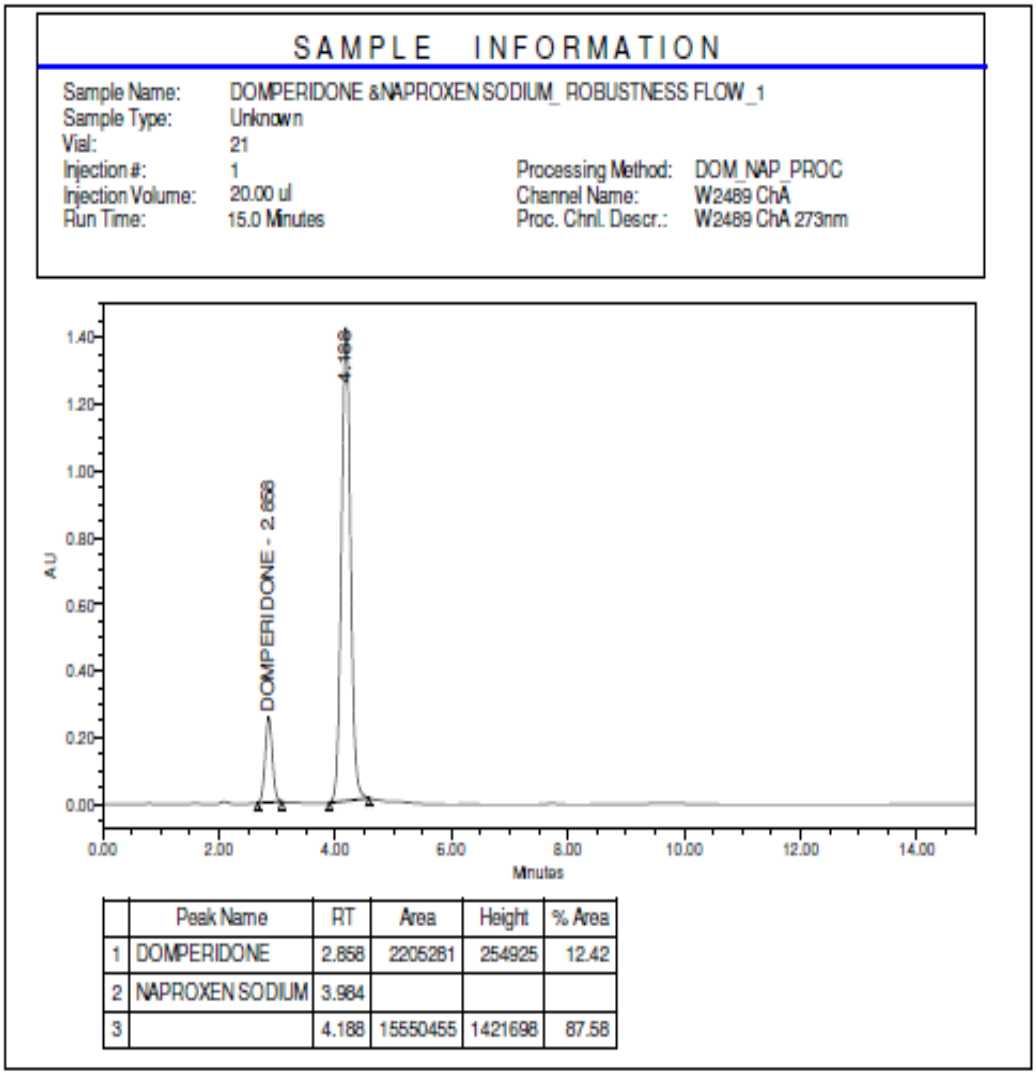

Fig No.29: Chromatogram for Change inflow rate (0.9mL/min)

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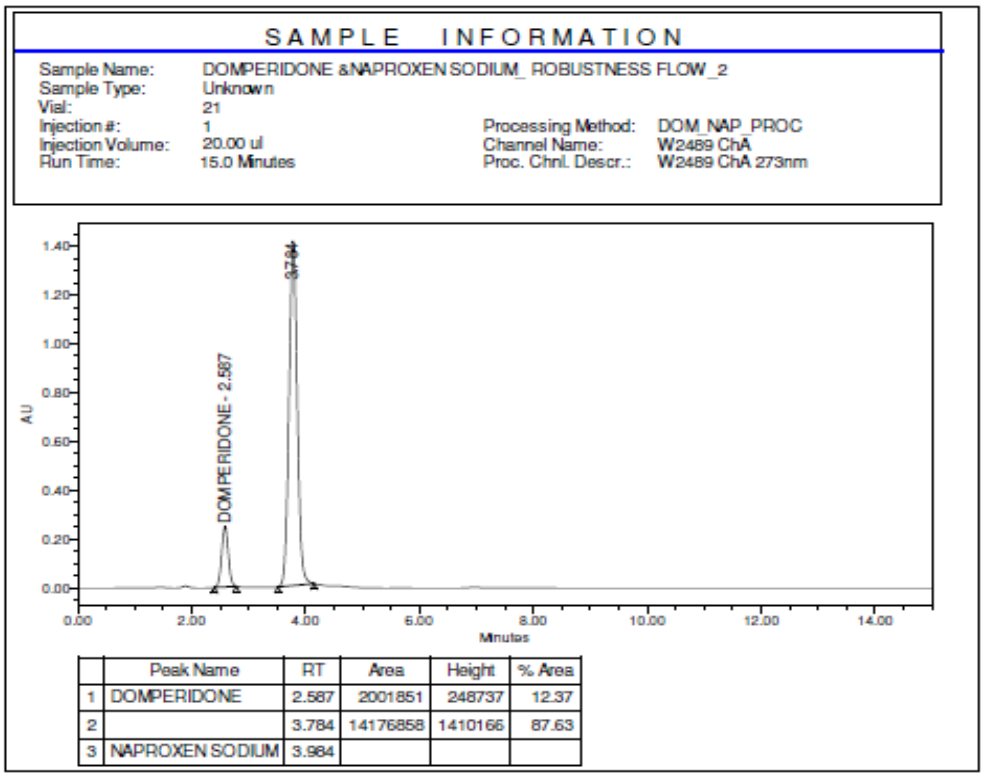

Fig No.30: Chromatogram for Change in Flow rate (1.1mL/min)

Report: The % assay were found to be 101.37%, 104.63% for Domperidone and Naproxen when the flow rate was deliberately changed to 0.9 mL and 1.1mL respectively, indicating that the method was found to be robust with deliberate change in flow rate.

Application of developed RP-HPLC method for the simultaneous estimation of Domperidone and Naproxen in marketed dosage form

The RP-HPLC method developed and validated for determination of Domperidone and Naproxen was applied to determine Domperidone and Naproxen was in marketed formulations (Tablet)

Brand name: Naxdom-250 (Domperidone 10mg and Naproxen 250mg)

Manufacturer: Atoz life sciences.

Table No. 16: Instrument Specifications

Instrument	WATERS Alliance series.
Injector	Rheodyne
Column	Inertsil ODS Column 250mm(length) x 4.6mm(I.D) , 5µm (Particle Size)

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Wavelength	273nm
Detector	UV Detector
Flow rate	1.0 mL/min
Injection volume	20µl
Mobile Phase	Mixed phosphate buffer : Acetonitrile (40: 60)

Procedure: 20 µl solution of the working sample solutions were injected repeatedly into the chromatograph, at a flow rate of 1 ml/min and detection at 273 nm, the chromatograms obtained were recorded and the amount of drug per Tablet & % assay is calculated by following formula

Table No.17: Assay report of Domperidone and Naproxen

Domperidone		**Naproxen**
Std	2165152	15306900
	2179730	15459578
	2200659	15517358
Avg	2181847	15427945
Spl	2171412	15676168
	2199606	15518996
Avg	2185509	15597582
LC	10 mg	250 mg
Std .wt	10	250
spl. Wt	392	392
std.fac	0.001	0.001
spl.fac	0.001	0.001
std. purity	99.82	99.65
Avg. wt	392	392
amt./tab	9.999 mg	251.86 mg
%assay	99.99 %	100.75 %

Report: The % assay of Domperidone and Naproxen in marketed formulation was found to be in range of **99.99%** and **100.75%** respectively. The results show that there is no interference from excipients and no impurities were observed in samples for the proposed method. Hence the developed HPLC method was found to be accurate, precise, and specific and can be routinely used for simultaneous determination of Domperidone and Naproxen in marketed formulations.

IV. CONCLUSION

Stability indicating HPLC were developed for the simultaneous estimation of Domperidone and Naproxen in marketed formulation. The HPLC system used was WATERS Alliance series System with Rheodyne injector 20 µL and the column Inertsil ODS 250 x 4.6 mm, 5 µm. The mobile phase comprised of Mixed phosphate buffer and Acetonitrile in the ratio of 40:60 v/v and flow rate of 1.0 mL/min with UV detection at 273 nm produced peaks of Domperidone and Naproxen in the chromatogram which were well resolved with

retention time of 2.721 min and 3.974 min respectively.

The developed HPLC method was validated for various parameters like accuracy, precision, specificity, LOD, LOQ, linearity, range and robustness as per ICH guidelines. The results obtained were well within the acceptance criteria for all the parameters. The proposed method was applied for simultaneous estimation of Domperidone and Naproxen in marketed formulations (Tablet). The assay results conformed to the label claim of the formulation. Hence the proposed method can be used for the routine analysis of Domperidone and Naproxen in their marketed tablet dosage formulations.

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All the authors have equal contribution.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1]. A S Patil, M S Kondawar, " RP HPLC Method For simultaneous estimation of Esomeprazole magnesium and Naproxen in pharmaceutical dosage form", Inventi Rapid: Pharm Analysis & Quality Assurance , Vol. 2012 , page no 328.

[2]. Md. Shozan Mondal, Md.Ahsanul Haque , Mohammad Safiqul Islam, S.M. Ashraful Islam., "Development and validation of RP-HPLC method for the simultaneous estimation of Domperidone and Naproxen in tablet Dosage form", Journal of Applied Pharmaceutical Science, 01 (07), 2011, page no 145-148.

[3]. Vani.P, Kalyana Seela kottapalli, "RP-HPLC Method For simultaneous estimation of Naproxen and Esomeprazole in pharmaceutical dosage form", Internationl Journal of Pharmacy and Technology, Dec-2011, Vol. 3, Issue No.4, page no 3446-3455.

[4]. Gauri Rani Das, Md Ahsanul Haque, Mohammad Shahriar and S.M. Ashraful Islam, "RP-HPLC method for the simultaneous estimation Ranitidine Hydrochloric and Domperidone in combine dosage form", Pharma Science Monitor online Publishedition 2011, page no 1894-1906.

[5]. Rakesh kumar singh , S Ramakrishna, Pragya Gupta, " RP-HPLC method development and validation for simultaneous estimation of Ranitidine Hydrochloride and Domperidone in combined tablet dosage form", International Journal of Pharmaceutical Sciences and Research (2010), Vol. 1, Issue 8, page no 77-87.

[6]. Shubhangi M. Pawar, Jitendra D. Fegade, Rajesh Y. Chaudhari,"Validated RP-HPLC method for simultaneous quantitation of Domperidone Maleate and Naproxen Sodium in bulk drug and formulation", Scholars Research Library Der Pharmacia Lettre, 2010, 2(5), page no 229-236.

[7]. Shubhangi M. Pawar, Bharat S. Patil, Ravindra Y. Patila, "Validated HPTLC method for simultaneous quantitation of Domperidone Maleate and Naproxen Sodium in bulk drug and formulation", Eurasian Journal of Analytical Chemistry, 5(3), 2010, page no 284-292.

[8]. Shweta S.Sabins, Nilesh D. Dhavale, Vijay Y. Jadhav, Santosh R. Tambe, Santosh V. Gandhi, " Spectrophotometric simultaneous determination of Rabeprazole Sodium and Domperidone in combined tablet dosage form" Eurasian Journal of Analytical Chemistry, Vol:3, 2008, page no 236-241.

[9]. Tripathi K.D.; Essentials of Meditionical Pharmacology, 5th edition. 2003; Jaypee Brothers Meditionical Publisher (P) Ltd. page no 601-604; 183-188.

[10]. T. Sivakumar, R. Manavalan, K. Valliapage no.an, "RP- HPLC method for simultaneous determination of Domperidone and Pantoprazole in pharmaceutical dosage forms" Acta Chromatographica, No. 18, 2007 page no 130-142.

[11]. Sharma B.K.; Instrumental methods of chemical analysis, 23rd edition.; Goel publishing House, Meerut; 2004; page no 7-8.

[12]. Skoog D. A., West D. M. and Holler F. J.; Fundamentals of Analytical Chemistry, Saunders College Publishing, New York; 6th edition; page no 713.

[13]. Jeffery G.H.; Basset J.; Mendham J.; Denney R.C.; Vogel's textbook of quantitative analysis, 5th edition.; Longman Scientific and Technical; 1989; page no 668.

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[14]. Vinay Wamokar, Pendota Santosh, Manjunth S.Y., " Validated spectroscopic method for estimation of Naproxen from tablet formulation", Journal of Pharmacy Research 2011, 4(8), page no 2633-2635.
[15]. Skoog D. A., West D. M. and Holler F. J.; Fundamentals of Analytical Chemistry, Saunders College Publishing, New York; 6th edition; page no 713.
[16]. Jeffery G.H.; Basset J.; Mendham J.; Denney R.C.; Vogel's textbook of quantitative analysis, 5th edition.; Longman Scientific and Technical; 1989; page no 668.
[17]. M.Veralakshmi, J.Vijya Ratna, K.Krishna Chaitanya, D.Samson, "RP- HPLC method development and validation of domperidone maleate in formulations and bulk drugs", International Journal of Pharmaceutical Research and Devlopment, Vol 3(3), 2011, page no 61-64.
[18]. Rajnish Kumar, Pinderjit Singh, Harinder Singh, " RP- HPLC method for simultaneous estimation of Naproxen and Pantoprazole in pharmaceutical dosage form", International Journal of Pharma Research and Development, Vol-2, Issue-12 (2011), page no 227-232.
[19]. A.G.Tated, F.A.Khan, C.K.Gadewar, R.L.Bakal, A.P.Dewani, A.V. Chandewar**, "**Method development and validation for the simultaneous determination of Naproxen and Pantoprazole Sodium in capsule dosage form by RP-HPLC", International Journal of Pharmaceutical Research and Devlopment, Vol 3(5), 2011 page no 8-14.
[20]. Hardman Joel G.; Limbird Lee E.; Gilman Alfredition Goodman; The Pharmacological Basis of Therapeutics, 10th edition 2001; Mc Graw Hill Publishing Division; page no 710-712; 1031.
[21]. Udaykumar Padmaja; Meditionical Pharmacology, CBS Publishers and Distributors, New Delhi; 3rd edition 2011; page no 370.
[22]. Barar F.S.K; Chand S.; Essentials of Pharmacotherapeutics, 4th edition 2007; page no 127; 533- 534.
[23]. Syeda kulsum, M.Padmalatha1, K.Sandeep, B.Saptasila, G.Vidyasagar, "Spectrophotometric Methods for the Determination of Naproxen sodium in Pure and Pharmaceutical Dosage Forms" International Journal of Research in Pharmaceutical and Biomedical Sciences, Vol. 2 (3), 2011, page no 1303-1307.