HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY FOR SIMULTANEOUS DETERMINATION OF NAPROXEN AND PANTOPRAZOLE IN PHARMACEUTICAL PREPARATION.
MATERIALS AND METHODS:
GENERAL PROFILE
A) Name: Naproxen
B) Chemical name: (+)-(S)-2-(6-methoxynaphthalen-2-yl) Propanoic acid
C) Formula: C_{14}H_{14}O_{3}
   i) Structure
   ![Structure of Naproxen](image)
   ii) Molecular weight: 230.259 g/mol
   iii) Description: white to off-white crystalline
   iv) Solubility: practically insoluble in water at low pH and freely soluble in water at high pH and also soluble in methanol.
   v) Melting point: 152-154 °C
   VI) Category: Anti-inflammatory

INTRODUCTION:
Naproxen is kind of nonsteroidal anti inflammatory drug. Naproxen reduces the pain caused by arthritis, tendinitis or bursitis. It is also used to reduce pain during menstrual cramps. If you are allergic to aspirin then do not use Naproxen. Overdose of Naproxen may cause the risk of heart attack. Do not use this drug immediately after your bypass surgery. Stomach or intestinal bleeding may be caused by this drug. Ask your doctor if you have a history of following:
   1. A history of heart disease
   2. A history of blood clot
   3. A history of high blood pressure
   4. A history of kidney disease
   5. A history of asthma
   6. Do not use this medicine during pregnancy or ask your doctor.

Use Naproxen as suggested by your doctor. Do not take overdose of Naproxen, follow what your doctor said to you.

PHARMACOLOGY OF NAPROXEN:
Pharmacodynamics
It is a kind of nonsteroidol Antiinflammatory drug and also called as Nsaid. It has analgesic and antipyretic properties. Sodium salt of Naproxen is commonly used as analgesic drug. Mostly sodium salt is used. Other Nsaid drugs are similar in mechanism as that of naproxen anion but its mechanasm is not completely understood but it is quite realated to PSI (prostaglandin synthetase inhibition).

**Pharmacokinetics**

Naproxen and its salt is completely absorbed in the body when it enters in circulation of the body. About 95% of drug is adsorbed by the body with its active effects. Dosage concentration is different and it has different action on the body then the AUC and Cmax i.e. Extent of absorption and Peak conc. respectively are bioequivalent. That’s why they are differing in their pattern of absorption. Naproxen and its formulation are related to these differences between naproxen products. According to pattern absorption difference observed half life of naproxen is not changed. In 4 to 5 days Naproxen reached to its steady state. It proves that change in absorption pattern of Naproxen plays a very negligible role to get its steady state level.

**Absorption**

Immediate Release

After consumption of Naproxen tablets, within 2 to 4 hrs peak plasma level is attained.

After consumption of Anaprox, within 1 to 2 hrs peak plasma is attained. This difference in their rates is due to sodium salt of this drug used in Anaprox. Plasma level in naprosyn in attained in 1 to 4 hrs.

**Delayed Release**

EC Naprosyn has pH sensitive coating which acts as a barrier between to lose disintegration in acidic environment of stomach and neutral environment of intestine. Above pH 6 Polymeric coating of Ec Naprosyn dissolves. Fasted subject consume Ec Naprosyn then peak plasma level is attained within 4 to 6 hrs and first dosage range is 2 to 12 hrs. Ec Naprosyn first dissolves in intestine than stomach studied according to scientists. That’s why the absorption of drugs not takes place until the stomach is emptied. When Ec naprosyn and naprosyn were given to patients (fasted patients) then plasma peak differences are observed in one week but no differences were observed in Cmax and AUC as given below (Listed in table). The table is given below and values are also motioned in the table:
<table>
<thead>
<tr>
<th></th>
<th>Ec naprosyn* 500 mg bid</th>
<th>Naprosyn* 500 mg bid</th>
</tr>
</thead>
<tbody>
<tr>
<td>cmax (μg/ml)</td>
<td>94.9 (18%)</td>
<td>97.4 (13%)</td>
</tr>
<tr>
<td>tmax (hours)</td>
<td>4 (39%)</td>
<td>1.9 (61%)</td>
</tr>
<tr>
<td>auc0-12 hr (μg•hr/ml)</td>
<td>845 (20%)</td>
<td>767 (15%)</td>
</tr>
</tbody>
</table>

*mean value (coefficient of variation)

GENERAL PROFILE
A) Name: Pantoprazone
B) Chemical name: \((RS)-6-(Difluoromethoxy)-2-[(3, 4-dimethoxypyridin-2-yl)methylsulfinyl]-1H-benzo[d]imidazole\)
C) Formula: \(\text{C}_{16}\text{H}_{15}\text{F}_{2}\text{N}_{3}\text{O}_{4}\text{S}\)
   i) Structure
   ii) Molecular weight: 383.37
   iii) Description: White crystalline powder
   iv) Solubility: Free soluble in water and also soluble in organic liquids like Ethyl acetate, Glacial acetic acid and methanol
   v) Melting point: 139-140°C
   VI) Category: Proton pump inhibitors (PPI)

INTRODUCTION:
It is kind of PPI drug. This drug prevents gastric acid secretion. If a person is suffering from acidity problem then Pantoprazone is used for the treatment.

EXPERIMENTAL
INSTRUMENTATION:
HPTLC system used was equipped with:
a) CAMAG LINOMAT IV automatic sample applicator.
b) CAMAG TLC SCANNER III
c) Computerized integrator controlled by CATS 4 software.
d) CAMAG twin trough glass chamber with stainless steel lid.

CHROMATOGRAPHIC CONDITIONS
SELECTION OF STATIONARY PHASE

In method development selection of stationary phase is one of the easiest step and mostly silica gel is used as a stationary phase. In 80% analysis silica gel is used. Silica gel is very reasonable and mostly used for all kinds of drugs. In market Precoated silica gel i.e. HPTLC plates are also available (Silica Gel 60 F254). In most of the method these plates are used. C2 and C18 are also used as stationary phases if silica gel plates are failed. There mechanisms are different as:

1. Polar normal phase bonded layer
2. Cellulose layers
3. Thicker preparative layers
4. Chiral layers are also used for the enantiomers
5. Impregnated layers

Guidelines for the selection of stationary phase:

<table>
<thead>
<tr>
<th>Stationary Phases</th>
<th>Type of sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silica gel</td>
<td>All classes of compounds</td>
</tr>
<tr>
<td>Aluminum oxide</td>
<td>All basic compounds like amines alkoids and some aromatic compounds,</td>
</tr>
<tr>
<td></td>
<td>compounds of carbons</td>
</tr>
<tr>
<td>Amino phase</td>
<td>Sugar compounds, some carboxylic acids, phenols etc</td>
</tr>
<tr>
<td>Cyano phase</td>
<td>All classes of compounds and esters</td>
</tr>
<tr>
<td>Diol phase</td>
<td>Hormones and steroids, All classes of compounds</td>
</tr>
<tr>
<td>RP C-2, C-8, C-18 phases</td>
<td>Polar substances, separation according to lipophilic properties and chain</td>
</tr>
<tr>
<td></td>
<td>length, steroids, tetracyclins, phthalates, barbiturates, nucleo bases,</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>aminophenols Polyamide Phenols, flavonoids, nitro compounds</td>
</tr>
<tr>
<td>--------------------------</td>
<td>------------------------------------------------------------</td>
</tr>
<tr>
<td>Silica gel impregnated with silver nitrate</td>
<td>PAH compounds, number of diol groups (boric acid), number of isolated double bonds.</td>
</tr>
<tr>
<td>Chiral phase</td>
<td>Enantiomers</td>
</tr>
</tbody>
</table>

**Silica gel 60 GF254 precoated aluminum plates; (Merck 20 x 10 cm) is selected as stationary phase.**

Chamber saturation: 10 min is time for chamber development.
Sample application: 6 mm band
Separation technique: In this case ascending technique is used.
Migration distance : 80 mm.

**SCANNER DETAILS:**

1) Slit dimension : 5 x 0.45 mm,
2) Scanning mode : absorbance reflectance
3) Scan length : 80 mm
4) Lamp : Deuterium
5) Scanning wavelength $\lambda$ : 278 nm
6) Off set : 8%
8) Scanning speed : 6 mm/sec.
9) Sensitivity : Automatic

**SOPE AND IMPORTANCE OF THIS METHOD:**

1. This HPTLC method development is less expensive and works with high speed.
2. As compared to the HPLC and GC method we can use this method for quantitative analysis of this combination.
3. Time required for HPTLC method development is half day but in case of HPLC 2-7 days are required.
4. For this method very less solvent is used that’s why this method is in the favor of Green chemistry.
5. This method gives more information about drug. It is easy to achieve ruggedness in case of this method but not very easy in case of HPLC method.
6. Very easy to prepare sample solution for this method but not for others.
7. Chromatographic system in this method is open; you can see what is happening in this method with naked eyes.
8. Results can be seen by machine and eyes.
9. In this method analysis takes place offline.
10. Time per analysis required for this method is 1-3 min but in case of other method it is 2-60 min.
11. There is no need to maintain eqpt. Each and every time.
12. This method is ideal for screening.
13. Stationary phase cost in HPTLC method development is Rs.3/sample and Rs 120/plate. But in case of HPLC method cost required for stationary phase Rs 15/Injection i.e 15000 column/500 Injection. (Best case)
14. Stationary phase cost in HPTLC method development is Rs.6/sample and Rs 120/plate. But in case of HPLC method cost required for stationary phase Rs 50/Injection i.e 25000 column/500 Injection. (Worst case)
15. System set up cost is nil in case of this method development.
16. System washing cost is nil in case of this method development.
17. Sample cleanup cost is nil in case of this method development.
18. Mobile phase cost required for this method development is negligible (1 ml/sample).
19. Gas Phase cost required for this method is Rs.2/- N₂ gas is used as a sample applicator.
20. Cost required per sample analysis is Rs 8-10 but in case of HPLC Cost required for HPLC is Rs 30-75.
21. By this method we can analyze different samples at a same time but this not possible in case of HPLC.
22. In parallel analysis we can analyze 100 samples but in case of HPLC only 1 sample analysis is possible.
Experimental:

Solvents and Chemicals:
Reference standard of Naproxen and Pantoprazole were collected from CDT lab. Mumbai and They were purchased from the medical shop. Ethyl acetate and glacial acetic acid were purchased from the market.

Standard stock solutions:
Weigh accurately 10 mg of standard Naproxen and Pantoprazole into two separate 10 ml volumetric flask, sonicated for 15 min. and dilute solution to the mark by using methanol as a solvent.

Sample solution:
5 tablets of Naproxen and Pantoprazole are taken and crushed it. 10 mg from it is taken into 10 ml vol. flask (label claim: 20mg of pantoprazole and 250mg of Naproxen), sonicated and diluted solution up to the mark by using methanol.

SELECTION AND OPTIMIZATION OF MOBILE PHASE:
Selection and optimization of mobile phase depends on analyst’s own experience. Literature review about particular drug is important for selection and optimization of mobile phase, also trial and error method is important. Mostly diethyl ether, methylene chloride, chloroform are used with hexane in combined, together in case of normal phase. Selection of mobile phase is also depends on nature of drug means physical properties of drug. If water is used with methanol then method is considered as Reverse phase method. Lab. Of CAMAG suggested four steps for selection and optimization of mobile phase. In first step use of seven or twelve solvents take place and then proper solvent is selected for drug which separates that drug powerfully. After this solvents are decided on the basis of Rf values they produce. The difference between two drugs or Rf values must be less than 0.5. Solvents are classified according to their Rf values as:

1. Group A solvents------- 0.2<Rf<0.8
2. Group B solvents------- Rf>0.8
3. Group C solvents------- Rf < 0.2

After this, hexane or water are used to decrease or increase Rf value. Strength of the B group solvent is decreased by solvent which is weak in strength solvents i.e. hexane or cyclohexane. If that solvent runs below the solvent front then the solvent is diluted first, then 1:4 ratio is used for trial. If samples have higher Rf values but they get separated then 1:1 ratio is used for trial. Acid is used to increase the strength of the
solvents. These are called as Polar modifier. Generally acetic acid or formic acid are used as a modifier. Sometimes bases are also used as modifiers. Generally diethyl amine or ammonia is used as a base modifier. There are used to avoid tailing in the method development. First used 1:1 ratio, if result is improved and if result is not better then use another ratio of the solvent. First avoid combination because it disturbs the resolution. Try one combination at a time. If problem get solved then quit the procedure. If not then try another method. Solvent from group B or group C are combined at first level. At third level, 1:1 ratio is good but addition of 10% of the B group C solvent. If Rf value is satisfactory then go with fourth level. In fourth level, minor adjustment takes place. In fourth level, acid or base is used to avoid tailing in rf values. If analytical results are not satisfactory then try with different stationary phases. For multi component drugs, mobile phase is prepared in a small bottle with lid to small amount of solvent is used.

**TRIAL AND ERROR (MOBILE PHASE DETECTION):**

1st trial: Naproxen and Pantoprazole is separated by trial and error method. There are so many solvents are used in different proportions. Both drugs are polar that’s why non polar solvent are used to separate the compounds. First 5 ml of n-hexane is tried as a mobile phase. N-hexane is non polar organic solvent. Spots on TLC plate are applied manually then plate is dried by using hair dryer. In twin through glass chamber n-hexane is added by using pipette. Time set for chamber saturation is 10 min. After 10 min plate is dried by using hair dryer. In presence of U.V light no spots are observed. n-hexane is discarded as a mobile phase. Polarity index for n-hexane is 0.1.

2nd trial: Naproxen and Pantoprazole is separated by trial and error method. There are so many solvents are used in different proportions. Both drugs are polar that’s why non polar solvent are used to separate the compounds. In 2nd trial 5 ml of toluene is tried as a mobile phase. Toluene is non polar organic solvent. Spots on TLC plate are applied manually then plate is dried by using hair dryer. In twin through glass chamber toluene is added by using pipette. Time set for chamber saturation is 10 min. After 10 min plate is dried by using hair dryer. In presence of U.V light no spots are observed. Toluene is discarded as a mobile phase. Polarity index for Toluene is 2.4.

3rd trial: Naproxen and Pantoprazole is separated by trial and error method. There are so many solvents are used in different proportions. Both drugs are polar that’s why non polar solvent are used to separate the compounds. First 5 ml of Diethyl ether is
tried as a mobile phase. Diethyl ether is non polar organic solvent. Spots on TLC plate are applied manually then plate is dried by using hair dryer. In twin through glass chamber Diethyl ether is added by using pipette. Time set for chamber saturation is 10 min. After 10 min plate is dried by using hair dryer. In presence of U.V light poor spot is observed. But with Diethyl ether cannot be used as mobile phase. Polarity index for Diethyl ether is 2.4.

4th trial: Naproxen and Pantoprazole is separated by trial and error method. There are so many solvents are used in different proportions. Both drugs are polar that’s why non polar solvent are used to separate the compounds. First 5 ml of Carbon tetrachloride is tried as a mobile phase. Carbon tetrachloride is non polar organic solvent. Spots on TLC plate are applied manually then plate is dried by using hair dryer. In twin through glass chamber n-hexane is added by using pipette. Time set for chamber saturation is 10 min. After 10 min plate is dried by using hair dryer. In presence of U.V light no spots are observed. Carbon tetrachloride is discarded as a mobile phase.

5th trial: Naproxen and Pantoprazole is separated by trial and error method. There are so many solvents are used in different proportions. Both drugs are polar that’s why non polar solvent are used to separate the compounds. First 5 ml of Chlorobenzene is tried as a mobile phase. N-hexane is non polar organic solvent. Spots on TLC plate are applied manually then plate is dried by using hair dryer. In twin through glass chamber n-hexane is added by using pipette. Time set for chamber saturation is 10 min. After 10 min plate is dried by using hair dryer. In presence of U.V light no spots are observed. Chlorobenzene is discarded as a mobile phase.

6th trial: Naproxen and Pantoprazole is separated by trial and error method. There are so many solvents are used in different proportions. Both drugs are polar that’s why non polar solvent are used to separate the compounds. First 5 ml of THF is tried as a mobile phase. THF is non polar organic solvent. Spots on TLC plate are applied manually then plate is dried by using hair dryer. In twin through glass chamber n-hexane is added by using pipette. Time set for chamber saturation is 10 min. After 10 min plate is dried by using hair dryer. In presence of U.V light no spots are observed. THF is discarded as a mobile phase.

7th trial: Naproxen and Pantoprazole is separated by trial and error method. There are so many solvents are used in different proportions. Both drugs are polar that’s why non polar solvent are used to separate the compounds. First 5 ml of n-hexane is tried
as a mobile phase. THF is non polar organic solvent. Spots on TLC plate are applied manually then plate is dried by using hair dryer. In twin through glass chamber n-hexane is added by using pipette. Time set for chamber saturation is 10 min. After 10 min plate is dried by using hair dryer. In presence of U.V light no spots are observed. N-hexane is discarded as a mobile phase. Polarity index for n-hexane is 0.1.

8th trial: Naproxen and Pantoprazole is separated by trial and error method. There are so many solvents are used in different proportions. Both drugs are polar that’s why non polar solvent are used to separate the compounds. First 5 ml of Ethyl acetate is tried as a mobile phase. Ethyl acetate is non polar organic solvent. Spots on Ethyl acetate plate are applied manually then plate is dried by using hair dryer. In twin through glass chamber n-hexane is added by using pipette. Time set for chamber saturation is 10 min. After 10 min plate is dried by using hair dryer. In presence of U.V light no spots are observed. Ethyl acetate is discarded as a mobile phase.

9th trial: Naproxen and Pantoprazole is separated by trial and error method. There are so many solvents are used in different proportions. Both drugs are polar that’s why non polar solvent are used to separate the compounds. First 5 ml of chloroform is tried as a mobile phase. Chloroform is non polar organic solvent. Spots on TLC plate are applied manually then plate is dried by using hair dryer. In twin through glass chamber n-hexane is added by using pipette. Time set for chamber saturation is 10 min. After 10 min plate is dried by using hair dryer. In presence of U.V light no spots are observed. Chloroform is discarded as a mobile phase.

10th trial: Naproxen and Pantoprazole is separated by trial and error method. There are so many solvents are used in different proportions. Both drugs are polar that’s why non polar solvent are used to separate the compounds. First 5 ml of n-hexane is tried as a mobile phase. N-hexane is non polar organic solvent. Spots on TLC plate are applied manually then plate is dried by using hair dryer. In twin through glass chamber n-hexane is added by using pipette. Time set for chamber saturation is 10 min. After 10 min plate is dried by using hair dryer. In presence of U.V light no spots are observed. n-hexane is discarded as a mobile phase.

11th trial: Naproxen and Pantoprazole is separated by trial and error method. There are so many solvents are used in different proportions. Both drugs are polar that’s why non polar solvent are used to separate the compounds. First 5 ml of Acetone is tried as a mobile phase. Acetone is non polar organic solvent. Spots on TLC plate are applied manually then plate is dried by using hair dryer. In twin through glass
chamber n- Acetone is added by using pipette. Time set for chamber saturation is 10 min. After 10 min plate is dried by using hair dryer. In presence of U.V light no spots are observed. Acetone is discarded as a mobile phase.

12th trial: Naproxen and Pantoprazole is separated by trial and error method. There are so many solvents are used in different proportions. Both drugs are polar that’s why non polar solvent are used to separate the compounds. First 5 ml of 1-propanol is tried as a mobile phase. 1-propanol is non polar organic solvent. Spots on TLC plate are applied manually then plate is dried by using hair dryer. In twin through glass chamber 1-propanol is added by using pipette. Time set for chamber saturation is 10 min. After 10 min plate is dried by using hair dryer. In presence of U.V light no spots are observed. 1-propanol is discarded as a mobile phase.

13th trial: Naproxen and Pantoprazole is separated by trial and error method. There are so many solvents are used in different proportions. Both drugs are polar that’s why non polar solvent are used to separate the compounds. First 5 ml of Ethyl acetate and toluene are tried as a mobile phase. Ethyl acetate and toluene are non polar organic solvent. Spots on TLC plate are applied manually then plate is dried by using hair dryer. In twin through glass chamber Ethyl acetate and toluene are added by using pipette. Time set for chamber saturation is 10 min. After 10 min plate is dried by using hair dryer. In presence of U.V light no spots are observed. Ethyl acetate and toluene are discarded as a mobile phase.

14th trial: Naproxen and Pantoprazole is separated by trial and error method. There are so many solvents are used in different proportions. Both drugs are polar that’s why non polar solvent are used to separate the compounds. First 5 ml of THF and ethyl acetate is tried as a mobile phase. THF and ethyl acetate are non polar organic solvent. Spots on TLC plate are applied manually then plate is dried by using hair dryer. In twin through glass chamber n-hexane is added by using pipette. Time set for chamber saturation is 10 min. After 10 min plate is dried by using hair dryer. In presence of U.V light no spots are observed. THF and ethyl acetate are discarded as a mobile phase.

15th trial: Naproxen and Pantoprazole is separated by trial and error method. There are so many solvents are used in different proportions. Both drugs are polar that’s why non polar solvent are used to separate the compounds. First 5 ml of Ethyl acetate and Chloroform is tried as a mobile phase. Ethyl acetate and Chloroform are non polar organic solvent. Spots on TLC plate are applied manually then plate is dried by
using hair dryer. In twin through glass chamber Ethyl acetate and Chloroform are added by using pipette. Time set for chamber saturation is 10 min. After 10 min plate is dried by using hair dryer. In presence of U.V light no spots are observed. Ethyl acetate and Chloroform are discarded as a mobile phase.

**16th trial:** Naproxen and Pantoprazole is separated by trial and error method. There are so many solvents are used in different proportions. Both drugs are polar that’s why non polar solvent are used to separate the compounds. First 5 ml of Ethyl acetate and chlorobenzene are tried as a mobile phase. Ethyl acetate and chlorobenzene are non polar organic solvent. Spots on TLC plate are applied manually then plate is dried by using hair dryer. In twin through glass chamber Ethyl acetate and chlorobenzene are added by using pipette. Time set for chamber saturation is 10 min. After 10 min plate is dried by using hair dryer. In presence of U.V light no spots are observed. Ethyl acetate and chlorobenzene are discarded as a mobile phase.

**17th trial:** Naproxen and Pantoprazole is separated by trial and error method. There are so many solvents are used in different proportions. Both drugs are polar that’s why non polar solvent are used to separate the compounds. First 5 ml of Acetone and chloroform are tried as a mobile phase. Acetone and chloroform are non polar organic solvent. Spots on TLC plate are applied manually then plate is dried by using hair dryer. In twin through glass chamber Acetone and chloroform are added by using pipette. Time set for chamber saturation is 10 min. After 10 min plate is dried by using hair dryer. In presence of U.V light no spots are observed. Acetone and chloroform are discarded as a mobile phase.

**18th trial:** Naproxen and Pantoprazole is separated by trial and error method. There are so many solvents are used in different proportions. Both drugs are polar that’s why non polar solvent are used to separate the compounds. First 5 ml of ethyl acetate and acetic acid are tried as a mobile phase. Ethyl acetate and acetic acid are non polar organic solvent. Spots on TLC plate are applied manually then plate is dried by using hair dryer. In twin through glass chamber ethyl acetate and acetic acid are added by using pipette. Time set for chamber saturation is 10 min. After 10 min plate is dried by using hair dryer. In presence of U.V light no spots are observed. Ethyl acetate and acetic acid are accepted as a mobile phase.

**LAYER PREWASHING**

HPTLC silica plates should be handled very carefully to avoid contamination; all plates are checked for any damage or impurities on the HPTLC plate under white light.
or UV light. In general analysis plates are used without any pre-treatment but this is very important to check these plates before use to avoid error in method development. Robustness and reproducibility is improved if prewashing takes place. Methanol is the best solvent for prewashing or mixture of methanol and ethyl acetate or mobile phase of method is used for prewashing. Prewashed plates must be dried at 120°C for 20 min in an oven.

**SAMPLE APPLICATION**

Sample application technique is very important in method development. There are two types of technique in sample application.

1. Contact application
2. Spray-on application

Selection technique is depends on analytical task, type of sample. Also depends on work load. In case of contact application type Circular chromatography is applied but the spray-on technique is preferred because of its accuracy. There are so many drawbacks of contact application technique.

**CHROMATOGRAM DEVELOPMENT**

HPTLC method development takes place in twin through chambers. Horizontal development chambers are also used in method development. Twin through chambers are made in a way that it requires very low volume of mobile phase or another conditioning liquid. During method development vapors or gas is produced which influence the result of the mobile phase development. The gas phase depends on the saturation condition of the mobile phase and also depends on the size and type of twin through chamber used in method development. Unsaturated chambers are avoided during mobile phase development because it may affect the reproducibility of the result. Two dimensional methods is used for the complex separation in which sample mixture is injected on the lower side of the HPTLC plate. First the plate is developed with first mobile phase and then with second mobile phase.

**Chromatography:**

In HPTLC method development, 20 x10 cm twin through glass chamber is used (Camag). Mobile phase is ethyl acetate: glacial acetic acid (4.8:0.2) (v/v). Saturation time is 10 min and hair dryer is used to dry the plates. Deuterium lamp (200nm to 400nm) is used as source of radiation. Slit dimension and scanning speed was 5 x 0.45nm and 20mms⁻¹. Naproxen and Pantoprazole were developed up to 8 cm and then chromatogram of standard and sample was compared with each other. The curve
plotted with concentration Vs area shows good linearity in the concentration range of 250-1500 µg for Naproxen and 50-300 µg for Pantoprazole respectively.

DETECTION
After mobile phase development the plate is removed from the twin through chamber and dried by using hair dryer or heating plate then it is seen under UV light of range 254nm to 366nm. It is non destructive technique this method is generally used in HPTLC detection technique simultaneously so many plates are analyzed in this technique.

![Fig.1 Densitogram of naproxen and pantoprazole](image)

Conditions maintained during Rf value determination:
1. Polarity of solvent affects the Rf value of this experiment. Hence non polar solvent is used in this experiment because both the drugs are Polar.
2. Poor spotting is avoided in this experiment because there is a possibility of weak or poor separation. Sometime absorptivity of some drugs are too weak, at that time over spotting is necessary to avoid poor separation.
3. Room temperature is maintained during experiment because temperature also affects on the experiment. Temperature increases Rf value is also increases. Rf value is independent on the temperature if miscible solvents are used.

4. Multiple solvents are used for this experiment to determine mobile phase.

**Rf values for Naproxen and Pantoprazole are as:**

The R$_F$ values are as: Naproxen = 0.65, Pantoprazole = 0.3 and

**VALIDATION OF DEVELOPED METHOD**

Method validation is a part of validation process and it goes through laboratory studies and it gives properties of the method. Validation of the method simply denotes the fitness of the method. Standard validation figures are different according to different organizations and these organizations are as:

1. Current GMP
2. GLP
3. Good clinical practices
4. ICH
5. Food and Drug administration
6. EPA
7. United states pharmacopeia
8. ISO (International standards organization)

Method must be validated according to ICH guidelines. In validation, sample preparation, reference standard preparation, application of samples, HPTLC separation, detection procedures, and quantification and calculation of the final results

**LINEARITY:**

It is nothing but mathematical transformation. Linearity is directly proportional to the conc of sample. conc. is plotted on X-axis and area is plotted on Y-axis then we get the graph and it is called as linearity graph. Five or six injection is takes place during linearity and their conc. Range is 80-120%. But this conc. depends upon the test method purpose. First series of samples are prepared by weighing, transferring and diluting then they are injected on HPTLC plate one by one serially. Basic calibration curve must be obtained from individual sample. In this case, most calibration curves are non linear so Lambert beer law can’t be applied to this method.

**PROCEDURE:**

1. 5 series of conc are decided first
2. Weigh accurately the sample in series.
3. Transfer the sample in clean conical flask and the add methanol to the conical flask and dilute up to the mark and shake well.
4. Repeat this procedure for all series of sample.
5. Inject the sample through Hamiltonian syringe.

In this case, appropriate volume. The correlation coefficient and other factors should be submitted. Standardization may provide by means of the calibration curve, if the relationship between responses and concentration is not linear.

For different concentrations of analyte we get different areas and this plot is nothing but linearity. Plot of concentration of an analyte Vs peak must be linear. Linearity for Naproxen is in the range of 50 to 300 µg/ml. Linearity for Pantoprazole is in the range of 250 to 1500 µg/ml.

**Graph 1. Linearity of Naproxen**

![Graph 1: Linearity of Naproxen](image1)

\[ y = 3.267x + 783.7 \]
\[ R^2 = 0.997 \]

**Graph 2. Linearity of Pantoprazole**

![Graph 2: Linearity of Pantoprazole](image2)

\[ y = 14.12x + 290.4 \]
\[ R^2 = 0.995 \]

**PRECISION:**

It is the degree of agreements among test results. In this case, experimental values are close to each other. The student of analytical chemistry knows that good precision
doesn’t mean good accuracy. If experimental values are close to each other then experiment is precise we can say otherwise not. Precision is performed at three levels as:

1. Repeatability
2. Intermediate precision
3. Reproducibility

Analytical chemist must repeat the same procedure i.e. weighing, pretreatment requires sample preparation. Concept of accuracy and precision is as similar to the bulls eye concept. Precision is known to check analytical limits of the labeled claim and also to check level of impurities and also to inform other scientists whether the method is correct or labeled claim is true or false. There are two types of precision as:

1. Intraday precision
2. Interday Precision

Repeatability means if analysis is done in one or same laboratory by same analyst. In this case same reagents, same sample, same HPTLC plate and same time are considered. In case of repeatability of measurement RSD should not be more than 2% in the determination of assay. This parameter simply shows variation caused due to preparation of sample, application of sample and evaluation in one run within short time interval. Intraday precision is analysis is performed in same lab by different analyst (thrice) in working days (15 days). In this different reagent, HPTLC plates are used in the experiment. In this case, RSD is measured from peak area and which should not be more than 3%. It shows the effect of environment and different experimental condition on the experiment. Reproducibility means experiment performs in different labs. To verify that the method will give same results in different labs. In case of repeatability, at least 6 readings are taken of 3 different conc. (80%, 100% and 120% conc.). There are some factors affect on reproducibility of measurements as:

1. Humidity and room temp. difference
2. Different experiences of an analyst
3. Different characteristics of instruments
4. Instrumental conditions variation and variation in materials.
5. Flow of PH
6. Mobile phase flow rate
7. Compassion of mobile phase
8. Stationary phase supplied by suppliers
9. Reagents, solvents of quality difference.

It gives information about the random error.

**Table 1: Precision of Naproxen.**

<table>
<thead>
<tr>
<th>Amt µg/band</th>
<th>Intraday precision</th>
<th>Interday Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean area</td>
<td>R SD</td>
</tr>
<tr>
<td>500</td>
<td>2412</td>
<td>1.476269</td>
</tr>
<tr>
<td>750</td>
<td>3195</td>
<td>1.157593</td>
</tr>
<tr>
<td>1000</td>
<td>4112</td>
<td>1.611476</td>
</tr>
</tbody>
</table>

**Table 2: Precision of Pantoprazole.**

<table>
<thead>
<tr>
<th>Amount µg/band</th>
<th>Intra-day precision</th>
<th>Inter-day Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean area (AU)</td>
<td>RSD (%)</td>
</tr>
<tr>
<td>100</td>
<td>1712.9</td>
<td>1.476269</td>
</tr>
<tr>
<td>150</td>
<td>2365</td>
<td>1.157593</td>
</tr>
<tr>
<td>200</td>
<td>3058</td>
<td>1.611476</td>
</tr>
</tbody>
</table>

**LIMIT OF DETECTION AND LIMIT OF QUANTITATION**

**LOD:**

In case of LOD i.e (limit of detection), lowest concentration of an analyte is detected but it is not necessary to quantified it. It gives u a test whether an analyte is below or above the value. In case of LOQ i.e (Limit of quantitation), lowest concentration of
analyte is analyzed and it is quantified hence the name LOQ, acceptable accuracy and precision is determined in this case. In case of LOD and LOQ sample solution n\geq 3 is applied in decreasing order of concentration and also the same volume of a sample solution is applied as a blank. After this peak area is plotted against concentration applied then we get the graph. LOD and LOQ is depends on signal to noise ratio. Results of this should be noted as applied mass on the plate not the concentration. Limit of detection can be determined as a signal to noise ratio usually 2:1 to 3:1. It is determined by following formula as:

\[
LOD = 3.3(SD/S).
\]

\[
SD = \text{Standard deviation}
\]

\[
S = \text{Slope of the calibration curve.}
\]

**Limit of quantification**

LOQ can be determined as a signal to noise ratio, usually 10:1. Limit of detection is calculated as follows:

\[
LOQ = 10 (SD/S).
\]

In this case, final result is depends on an instrumental reading. Sometimes the detection is approximately twice the limit of detection\(^74\).

The limits of detection (LOD) and limit of quantization (LOQ) were calculated from slopes of the calibration plots and the standard deviation (SD) of the response by the use of the equations LOD 3.3 × SD/S and LOQ 10 × SD/S. The limit of detection and limit of quantization obtained by this method for Naproxen 13.52 & 40 and Pantoprazole 4.2 & 12.82 respectively.

**Recovery study (Accuracy)**

In case of accuracy closeness of experimental values are compared with the true values. The concept of accuracy is as similar to the bull’s eye experiment. In case of analytical chemistry no one knows the perfect true value but some values or standard values are given by the scientist are considered as true and experimental values are compared with these values.

**Standard addition method**

In this case negative and positive changes are observed.

\[
\text{Recovery} = \frac{A - B}{C} \times 100
\]
Where,
A = Total drug estimated in mg.
B= Amount of drug contributed by tablet powder (As per proposed method).
C = Amount of pure drug added.

**RECOVERY:** Three different concentrations for Naproxen were taken. Recovery was found in between 99.50-101.15%. Recovery for Naproxen and Pantoprazole are shown in table 5 and 6 as:

**Table 3: Recovery of Naproxen**

<table>
<thead>
<tr>
<th>Amount of drug added (%)</th>
<th>Theoretical content (mcg)</th>
<th>Recovery (%)</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>400</td>
<td>500</td>
<td>100.2296</td>
<td>1.175355</td>
</tr>
<tr>
<td>500</td>
<td>500</td>
<td>99.8143</td>
<td>1.543492</td>
</tr>
<tr>
<td>600</td>
<td>500</td>
<td>99.28239</td>
<td>0.493749</td>
</tr>
</tbody>
</table>

**Table 4: Recovery of Pantoprazole.**

<table>
<thead>
<tr>
<th>Amount of drug added</th>
<th>Theoretical content (mcg)</th>
<th>Recovery (%)</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>100</td>
<td>99.94098</td>
<td>1.794321</td>
</tr>
<tr>
<td>100</td>
<td>100</td>
<td>101.152</td>
<td>1.457477</td>
</tr>
<tr>
<td>120</td>
<td>100</td>
<td>99.50031</td>
<td>0.774122</td>
</tr>
</tbody>
</table>

**ROBUSTNESS**

There are some important parameters in Robustness as:
1. The stability of analyte
2. Effect of temperature
3. Effect of humidity
Robustness must be considered before method development but it should not consider in mobile phase optimization. Optimization of mobile phase is one of the part of the method and robustness is the test of effect of some factors on the method. Most important parameter in HPTLC is Robustness. In this case, mobile phase is changed by small quantity.

In this case; it is the ability of the procedure to provide analytical result of acceptable accuracy and precision. Robustness was measured by analysis of the sample solution by making small changes to mobile phase composition. Ethyl acetate: Glacial acetic acid in the ratio 4.7:0.3 (v/v) and Ethyl acetate: Glacial acetic acid in the ratio of 4.9:0.1 (v/v) were selected.

Table 5: It shows robustness of Naproxen.

<table>
<thead>
<tr>
<th>Saturation Time</th>
<th>Mean</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 min</td>
<td>99.943</td>
<td>1.020</td>
</tr>
<tr>
<td>25 min</td>
<td>100.356</td>
<td>1.661</td>
</tr>
</tbody>
</table>

Condition

| Ethyl acetate: Glacial acetic acid (4.7:0.3) | 99.943 | 1.020 |
| Ethyl acetate: Glacial acetic acid (4.9:0.1) | 99.586 | 0.741 |

Table 6: It shows robustness of Pantoprazole.

<table>
<thead>
<tr>
<th>Saturation Time</th>
<th>Mean</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 min</td>
<td>100.511</td>
<td>0.917</td>
</tr>
<tr>
<td>25 min</td>
<td>100.989</td>
<td>1.141</td>
</tr>
</tbody>
</table>

123
<table>
<thead>
<tr>
<th>Condition</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl acetate: Glacial acetic acid (4.7:0.3)</td>
<td>100.800</td>
<td>1.183</td>
</tr>
</tbody>
</table>

**RUGGEDNESS:**

Ruggedness is measure of reproducibility of test results under normal, expected operating condition from instrument & from analyst, ruggedness was tested by analysis of 750, 150 mcg Naproxen and Pantoprazole for per band were listed in tables given below:

**Table 7: It shows Ruggedness for Naproxen.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analyst I</td>
<td>99.329</td>
<td>1.310</td>
</tr>
<tr>
<td>Analyst II</td>
<td>100.417</td>
<td>1.266</td>
</tr>
</tbody>
</table>

**Table 8: It shows Ruggedness for Pantoprazole.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ana I</td>
<td>99.596</td>
<td>1.688</td>
</tr>
<tr>
<td>Ana II</td>
<td>100.068</td>
<td>1.236</td>
</tr>
</tbody>
</table>

**SPECIFICITY:**

Reference standards and samples are analyzed and specificity are determined. Rf values of reference standards and samples are compared and combination drugs bands are confirmed. Uv spectra of these bands are determined and compared. Peak start, Apex peak and end peak of spectra are compared and peak purity are accessed

Specificity of the method is ascertained by analyzing reference standard and samples. The bands for Naproxen and Pantoprazole formulations were confirmed by comparing Rf values and U.V spectra of these separated bands with those from standard the peak
purity of Naproxen and Pantoprazole accessed by comparing the spectra acquired at the peak start(S) peak apex(N) and peak end(E) of a band.

CONCLUSION:
For the determination of paracetamol and Aceclofenac in combined dosage form, this HPTLC method is economic, accurate, simple and reproducible and used in daily analysis.