3.1 RP-HPLC-PDA procedure by simultaneous identification of Paracetamol, Famotidine, Diclofenac potassium and Chlorzoxazone in non-marketed and marketed formulation.

3.1.1 Drug Profile – Paracetamol

3.1.1.1 Description:

Paracetamol is the derivative of acetanilide, also known as acetaminophen. This is utilized as pain relieving furthermore antipyretic. Its helpful impacts are about like that of salicylates, however it needs calming impact, hostile to platelet and gastric ulcerative effect (Pubchem CID 1983 – Acetaminophen; Drugbank -DB00316, Acetaminophen).

3.1.1.2 IUPAC Name:

N-(4-hydroxyphenyl) acetamide

3.1.1.3 Structure:
3.1.1.4 Molecular Formula:
C₈H₉NO₂

3.1.1.5 Composition:
C 63.57%, H 6.00%, N 9.27% and O 21.17%

3.1.1.6 Molecular Weight:
151.16

3.1.1.7 Melting Point:
169-170.5°C  
(Acetaminophen - drugfuture.com)

3.1.1.8 Route of Administration:
Oral, Rectal, Intravenous  
(Paracetamol – Wikipedia)

3.1.1.9 Mechanism of Action:
Acetaminophen acts fundamentally in the CNS, this expanding torrent edge by hindering the isoforms of cyclooxygenase, (COX-1,2,3) compounds required in the prostaglandin production. Dissimilar to NSAIDs, acetaminophen not ready to hinder cyclooxygenase especially in fringe tissues and hence, has no fringe mitigating impacts. Studies reveles if peroxide presents the indirect blockade of COX by acetaminophen is ineffective. These might clarify about acetaminophen impact in the CNS and in endothelial cells yet not in platelets and insusceptible cells which have large amounts of peroxides. Previous studies shows that the acetaminophen blocks only a variant of the COX enzyme that is different from other variants like COX-1 and COX-2. So it is COX-3. This actual mechanism for this action is unknown till nowin any case, future examination may give further understanding into how it functions. The antipyretic properties of medication because of direct impacts on the warmth controlling focuses situated on hypothalamus bringing about fringe vasodilation and sweating.

3.1.1.9 Contraindication:
Allergic to Paracetamol, Liver problems, alcoholics and interactions with certain other medicaments (Paracetamol - wisegeekhealth.com).

3.1.1.10 Formulation:
3.1.1.11 Storage:
Store below 30°C; freezing and refrigeration will affect medicament; diluted parenteral solutions should use within one hour (Paracetamol 589 – drugsupdate).

3.1.1.12 Examples of brands:
- Dolo – 250mg, 500mg, 650mg – Tablet; Dolo – 156.25mg – Suspension – Micro Labs Ltd.
- Paracip - 300mg – injection – Cipla Limited
- Paracetanol – 170mg – Suppository – Zydus Vaccicare (Zydus Cadila Healthcare Ltd)
- Paradol (60ml) – 250mg/5ml – Syrup – Biomedica International

(Drug "Paracetamol" Price list - medindia.net)
3.1.2 Drug Profile - Famotidine

3.1.2.1 Description:
Famotidine is an aggressive histamine H2-receptor enemy. Also, its primary pharmacodynamic impact is the hindrance of gastric discharge (Pubchem CID 3325, Famotidine).

3.1.2.2 IUPAC Name:
3-[(2-[(diaminomethylidene)amino]-1,3-thiazol-4-yl)methyl)sulfanyl]-N' sulfamoylpropanimidamide.

3.1.2.3 Structure:

![Structure of Famotidine]

(Drugbank DB00927, Famotidine)

3.1.2.4 Molecular Formula:
C₈H₁₅N₇O₂S₃

3.1.2.5 Percent Composition:
C 28.47%,  H 4.48%,  N 29.06%,  O 9.48%,  S 28.51% (Famotidine – drugfuture.com).

3.1.2.6 Molecular Weight:
337.44(Pubchem CID 3325, Famotidine).

3.1.2.7 Melting Point:
163 - 164°C (Famotidine – drugfuture.com).

3.1.2.8 Route of Administration:
Oral and Parenteral (Famotidine – Wikipedia; Famotidine information from Drugs Update).

3.1.2.9 Mechanism of Action:
Famotidine intensely inhibits H2-receptors of histamine in this manner dropping basal, nocturnal and stimulated gastric secretion. Pepsin discharge is diminished resultant in diminished peptic action. It efficiently recuperates duodenal and gastric ulcers and anticipates repeat (Famotidine information from DrugsUpdate).

3.1.2.10 Adverse impacts: Migraine, unsteadiness, clogging, looseness of the bowels, sickness, rash, GI inconvenience, weakness, gynaecomastia, barrenness (Famotidine data from DrugsUpdate).

3.1.2.11 Warning and Precautions: Alert ought to be practiced in patients with history of kidney or liver issues, any sensitivity, who are taking different meds, elderly, amid pregnancy and breastfeeding (Famotidine - Drug Information - medindia.net)

3.1.2.12 Drug Interactions:
Assimilation of famotidine is reduced with antacids therefore administration should be disconnected by two hour. Famotidine reduces the assimilation of antifungal drugs. Ethanol may cause stomachic mucosal irritation. (Famotidine information from DrugsUpdate).

3.1.2.13 Contraindication:
Hypersensitivity, lactation (Famotidine information from DrugsUpdate; Famotidine - Drug Information - medindia.net)

3.1.2.14 Formulation:
3.1.2.15 Storage:

Store it at controlled room temperature and in a hermetically sealed compartment. Avoid youngsters. Injection: store at 2-8°C. Do not freeze (Famotidine information from DrugsUpdate; Famotidine - Drug Information - medindia.net).

3.1.2.16 Examples of brands: (Drug "Famotidine" Price list - medindia.net).

- Blocacid – 20mg, 40mg Tablet – IPCA Laboratories Ltd.
- Acidosh – 20mg, 40mg Capsule – Osho Pharma Pvt. Ltd.
- Femidin (40mg) – 20mg/1ml - injection – Taj Pharmaceuticals Ltd
3.1.3 Drug Profile - Diclofenac

3.1.3.1 Description:
A non-steroidal anti-inflammatory agent with analgesic and antipyretic actions. It is basically accessible as the sodium salt (Pubchem CID3033, Diclofenac).

3.1.3.2 IUPAC Name:
2-[(2,6-dichloro phenyl)amino]phenyl)acetic acid.

3.1.3.3 Structure:

(Drugbank DB00586, Diclofenac)
3.1.3.4 Molecular Formula:
\[ C_{14}H_{11}Cl_{2}NO_{2} \]

3.1.3.5 Composition:
- C - 56.78%
- H - 3.74%
- Cl - 23.94%
- N - 4.73%
- O - 10.80%

3.1.3.6 Molecular Weight:
296.15

3.1.3.7 Melting Point:
156 - 158°C (Diclofenac - drugfuture.com)

3.1.3.8 Route of Administration:
Oral, rectal, intramuscular, intravenous (renal and gall stones) and topical (Diclofenac –Wikipedia).

3.1.3.9 Mechanism of Action:
The mitigating impacts of diclofenac should be because of hindrance of leukocyte relocation and the catalyst cylooxygenase (COX-1 and COX-2), foremost to the peripheral blockade of prostaglandin synthesis. As prostaglandins ready the pain receptors, restraint of their combination is responsible for the pain relieving impacts of diclofenac. Antipyretic impacts might be because of activity on the hypothalamus, resultant in peripheral dilation, expanded cutaneous blood stream, and resulting heat dissemination (Drugbank DB00586, Diclofenac).

3.1.3.10 Pharmacokinetics:
It is rapidly absorbed from oral, parenteral and topical routes. It is completely absorbed from the GIT. The volume of distribution is 1.3 L/kg. It penetrates synovial fluid and a small amount enters in to the breast milk. It shows more than 99% protein binding. It is subjected to hepatic metabolism and converted in to metabolites. Its half life is 2 hours. It is excreted as glucuronide and sulfate conjugates of the metabolites. About 60% is excreted in urine and 35% in bile. Small amount is excreted through urine (Drugbank DB00586, Diclofenac; Diclofenac information from DrugsUpdate)

3.1.3.11 Adverse impacts:
GI unsettling influences, cerebral pain, dazedness, rash; GI dying, peptic ulceration; variations from the norm of kidney capacity. Torment and tissue harm at the infusion site (IM); neighborhood disturbance.
3.1.3.12 Warning and Precautions:

Alert ought to be practiced in patients with history of liver or kidney ailment, swelling of the hands, feet, lower legs or lower legs, hypertension, lack of hydration, blood issue, smoking propensity, liquor mishandle, any hypersensitivity, elderly, youngsters, who are taking different pharmaceuticals, amid pregnancy and breastfeeding. Alert is required in patients with history of heart issues; this solution can altogether build the danger of a heart assault or a stroke. It might bring about unsteadiness or sluggishness, don't drive an auto or work apparatus while taking this prescription. Screen kidney, liver capacities, blood electrolyte levels, finish platelet numbers and circulatory strain consistently while taking this pharmaceutical (Diclofenac - Drug Information - medindia.net)

3.1.3.13 Contraindication:

Contraindicated in patients with asthma or other hypersensitivity reactions after having NSAIDs. In pregnancy it is contraindicated also when used during lactation. Active peptic ulcer, treatment of perioperative pain in CABG surgery. Not allowed to apply onto damaged & non intact skin (Diclofenac information from DrugsUpdate; Diclofenac - Drug Information - medindia.net).

3.1.3.14 Formulation:

Tablet, Dispersable tablet, Capsule, Injection, Suppository, Gel (Drug "Diclofenac" Price list - medindia.net).

3.1.3.15 Storage:

Store at room temperature and in hermetically sealed container. Avoid from more heat and water content. (Diclofenac - Drug Information -medindia.net).

3.1.3.16 Examples of brands:

Jonac - 25mg/ml injection, 100mg & 12.5mg Suppository – Zydus Cadila (German Remedies Division).
Naclo – 100mg Capsule/Tablet – Sun Pharmaceuticals (Solares Division).
Oxalgin. Sr – 100mg – SR. Capsule/Tablet – Zydus Cadila
Voveran – 75mg – CR Capsule/Tablet – Novartis
Zobid. D – 25mg, 50mg – Dispersable Tablet – Nicholas Piramal (Sarabhai Piramal)
3.1.4 Drug Profile - Chlorzoxazone

3.1.4.1 Description:
Muscle relaxants that are acting centrally and with sedative assets. It is asked for to hinder muscle spasm by applying an impact essentially at the level of the spinalcord and subcortical zones of the brain (Pubchem CID 2733, Chlorzoxazone).

3.1.4.2 IUPAC Name:
5-chloro-2, 3-dihydro-1, 3-benzoxazol-2-one

3.1.4.3 Structure:
3.1.4.4 Molecular Formula:

C\textsubscript{7}H\textsubscript{4}Cl NO\textsubscript{2} (Drug bank DB00356, Chlorzoxazone)

3.1.4.5 Composition:

C 49.58\%, H 2.38\%, Cl 20.91\%, N 8.26\%, O 18.87\%

3.1.4.6 Molecular Weight:

169.57

3.1.4.7 Melting Point:

191-191.5°C (Chlorzoxazone – drugfuture.com)

3.1.4.8 Route of Administration:

Oral (Chlorzoxazone – Wikipedia).

3.1.4.9 Mechanism of Action:

Chlorzoxazone impedes mast cell degranulation, then blocks the arrival of histamine and moderate responding material of hypersensitivity (SRS-A), go betweens of sort I unfavorably susceptible responses. Chlorzoxazone may decrease the deleveranve of inflammatory leukotrienes. Chlorzoxazone act by hindering calcium and potassium inward pull which would prompt neuronal hold and muscle unwinding. Information accessible from animal tests and also human study determine that chlorzoxazone demonstrations essentially at the level of the spinal cord and subcortical territories of the
brain where it hinders multisynaptic reflex circular segments included in creating and sustaining skeletal muscle spasms ((Drug bank DB00356, Chlorzoxazone).

3.1.4.10 Pharmacokinetics:
Assimilation, T max is one to two hours; Protein binding: 13-18%; onset of action is one hr and the duration of the action is three to four hrs. Metabolised in liver through urine it excreted.

3.1.4.11 Indication:
For acute painful musculoskeletal conditions (Drug bank DB00356, Chlorzoxazone)

3.1.4.12 Dose and Administration:
Acute pain: Initial – five hundred milli gram three or four times daily; can be increase to 750mg whenever needed. Take the dose with meals to avoid gastro intestinal discomfort (Chlorzoxazone information from DrugsUpdate).

3.1.4.13 Adverse impacts:

3.1.4.14 Warning and Precautions:
Dodge use in patients with liver weakness; cease if indications of brokenness happen. May disable capacity to perform undertakings requiring mental or physical coordination, including driving. Languor is extremely normal. Avoid sharing this medication with other people. If there is no improvement in your health condition or if they become worse, consult with your doctor (Chlorzoxazone information from DrugsUpdate; Chlorzoxazone - Drug Information - medindia.net).

3.1.4.15 Drug Interactions:
Alcohol and other CNS depressants may cause additive effects
3.1.4.16 Contraindication:

Contraindicated in patients with hypersensitivity, liver disease, porphyria, lactation. Caution when used during pregnancy (Chlorzoxazone information from DrugsUpdate; Chlorzoxazone - Drug Information - medindia.net).

3.1.4.17 Formulation:

Tablet

3.1.4.18 Storage:

Store at 15-30°C. Store it in air tight container and keep away from children (Chlorzoxazone - Drug Information - medindia.net).

3.1.5 Aim and Objective

In this current study, endeavors were made to create and validate a new procedure for the same time estimation procedure of paracetamol, Famotidine, Diclofenac potassium and Chlorzoxazone in totality and combined tablet formulation.

3.1.6 Materials and Methods

3.1.6.1 Instrumentation
Chromatography was accomplished with Water's 2695 HPLC framework furnished with Hamilton Syringe, auto sampler and 2996 Photodiode array detector. Online degasser was equipped within the HPLC system which degasses the mobile phase and prevents the pressure fluctuations; along with this a column compartment was present in order to control the temperature. The HPLC system operates with Empower2 software and it is used for analysis, data acquisition followed by reporting the data.

3.1.6.2 Chemicals and Reagents:
Pharmaceutically genuinesamples of paracetamol, famotidine, diclofenac potassium and chlorzoxazone were acquired from range pharma research arrangements, hyderabad as blessing tests alongside their investigative reports. The chemicals required for the preparation of mobile phase i.e HPLC category acetonitrile, methanol was obtained from Merck, Mumbai. Millipore water (HPLC grade) used for the preparation of buffer and solutions was gotten from Milli-Q water cleansing system was obtained from Milli-Q water purification system. Commercial tablets of Andic-MR tablets (Label Claim: 325 mg of paracetamol, 20 mg of famotidine, 50 mg of diclofenac potassium and 250 mg of chlorzoxazone) were procured from Decisive Pharma Pvt. Ltd.

3.1.6.3 Standard stock solution preparation:
Thesesolutions are preparing with dissolving 325 milligram of paracetamol, 20 milligram of famotidine, 50 milligram of diclofenac potassium and 250 milligram of chlorzoxazone into a spotless and moisture free 50 ml volumetric jar, 35 ml of diluent was included, sonicated for about 5 minutes and volume increased upto 50 ml with diluent for the preparation of stock solution.

3.1.6.4 Preparation of working standard solutions
Aliquots of 0.125 ml, 0.25 ml, 0.375 ml, 0.5 ml, 0.625 ml and 0.75 ml are pipetted from stock solution into 10 ml volumetric flask independently and volume increased to 10 ml with diluent. This will give the solution of 81.25 microgram/ml, 162.5 microgram/ml, 243.75 microgram/ml, 325 microgram/ml, 406.25 microgram/ml and 487.5 microgram/ml accordingly for paracetamol, 5 microgram/ml, 10 microgram/ml, 15 microgram/ml, 20 microgram/ml, 25 microgram/ml and 30 microgram/ml respectively for famotidine.
microgram /ml, 25 microgram /ml, 37.5 microgram /ml, 50 microgram /ml, 62.5 microgram /ml and 75 microgram /ml accordingly for diclofenac potassium and 62.5 µicrogm /ml, 125 µicrogm /ml, 187.5 µicrogm /ml, 250 microgram /ml, 312.5 microgram /ml and 375 microgram /ml respectively for chlorzoxazone.

3.1.6.5 Sample preparation

Twenty samples were selected from the lot which was previously weighed and grated. Powder which is equal to weight of five samples which is transferred into a conical flask and dissolved in 250 ml solvent, sonicated for 20 min and filtered with PVDF 0.45 µ filter. From the filtrate, 0.5 ml was pipetted furthermore, moved into a 10ml volumetric flask and the arrangement was make up to the volume with solvents.

3.1.6.6 Validation Method

Validation criterias like system suitability, linearity, accuracy, and preciseness, detection limit and quantification limit, robustness and solution stability are performing with ICH protocol.

3.1.6.6.1 Systems suitability tests:

To guarantee the purpose and reproducibility of the HPLC framework was sufficient for the investigation, a framework appropriateness test was recognized. 6 injection of 10 µL of the standard solutions of Paracetamol, Famotidine, Diclofenac potassium and Chlorzoxazone were injected for the assessment of the framework appropriateness parameters like tailing element, the quantity of hypothetical plates, maintenance time and resolution factor.

3.1.6.6.2 Specificity:

The specificity of the technique was performed by infusing blank solution, placebo solution and standard solutions of Paracetamol, Famotidine, Diclofenac potassium and Chlorzoxazone, separately.

3.1.6.6.3 Linearity:

By proper equates of standard Paracetamol, famotidine, diclofenac potassium and chlorzoxazone arrangements with the portable stage, six working arrangements going between 81.25-487.5 µg/mL, 5-30 µg/mL, 12.5-75.5 µg/mL and 62.5-375 µg/mL was prepared. Respectively experiment expansion point were achieved in triplicate as indicated by enhanced chromatographic conditions. The crest territories of the chromatograms was
plotted across the concentration of Paracetamol, Famotidine, Diclofenac potassium and Chlorzoxazone toobtainthecalibrationcurve.

3.1.6.6.4 Accuracy:

Recuperation ponders by the standard expansion technique were finished with a perspective to legitimize the precision of the proposed strategy. Already examined samples of Paracetamol, Famotidine, Diclofenac potassium and Chlorzoxazone to which known amountsof standard Paracetamol, Famotidine, Diclofenac potassium and Chlorzoxazone relating to half, 100% and 150% of target focus were included. The precision was communicated as the rate of analyte recuperated by the proposed technique.

3.1.6.6.5 Precision:

Accuracy was resolved as repeatability and middle of the intermediate precisions, as per ICH rules. Therepeatability andintermediateprecision isdeterminingbyexaminingthesamplesof Paracetamol, Famotidine, Diclofenac potassium and Chlorzoxazone.

3.1.6.6.6 Limitof detectionandthelimitof quantification:

Limitofdetectionandlimitof quantificationof Paracetamol, famotidine, diclofenac potassium and chlorzoxazone were resolute by calibration curve method. Solutions of Paracetamol, famotidine, diclofenac potassium and chlorzoxazone were set up in linearity extend and infused in triplicate. Normal crest region of three examinations was plotted alongside concentration. LOD and LOQ are calculating by following equation. LOD = (3.3 ×syx)/b, LOQ= (10.0×Syx)/b, s yx = residual variance; b =slope.

3.1.6.6.7 Robustness:

The robustness ofthetechniquewasdoneby purposely altering the chromatographic circumstances. The parameters included small variation in organic phase percentage in moving phase, stream rate and temperature of column.

3.1.6.6.8 Stability:

The sample and standard arrangements infused at zero hour (correlation test) and a short time later 24 hours (strength test) by keeping at encompassing room temperature. Soundness was unaltering by deciding %RSD for test and standard arrangements.
3.1.7 Results and Discussion

Method Development

For the optimization the procedure for drug assay in pharmaceutical dosage forms, primary examination are directed to choose the best and ideal circumstances. Criteria like an perfect versatile stage and their proportions at ideal pH were studied in detail so as to accomplish a sensible level of detachment of analytes.

Many eluents are testing by using varied proportions of solvents like acetonitrile, methanol, water and buffer at various pH conditions. But, the best results were obtained by phosphate buffer (20 Mm) pH 6.6 balanced with weakened ortho phosphoric acid and acetonitrile taken in gradient program shown in table 3.1.7.1 at stream rate of 1ml/min followed by identification at 270 nm. Fig. 3.1.7.1 shows the chromatogram obtained from standard mixture by using the above optimized method.

Method Validation

Suitability of the system

This tests are performing to ensure the validity of analytical procedure. Information from six infusions of 10 µL of the working standard arrangements of PARA, FTD, DLF and CLZ are used for the interpretation of system suitability. From the result obtained the %RSD of all the six injections was within the limit. Theoretical plates were found to be more than 5000 and the peak tailing was less than 1.2 which shows that it is within the range. Purity angle for the drug peaks was less than the purity threshold which implies that no impedance was there in the retention time of main peak. Results of all these parameters were shown in the table 3.1.7.2.

Linearity

By appropriate aliquots of the standard PARA, FTD, DLF and CLZ solutions with the versatile stage, six working solutions running between 81.25 - 487.5 µg/mL, 5-30 µg/mL, 12.5 - 75.5 µg/mL and 62.5 - 375 µg/mL were prepared and injected (n = 3). The peak areas were plotted beside the concentration of PARA, FTD, DLF and CLZ together and the results are shown Figures. The linearity were signified by a straight regression equation as follows: y (PARA) = 14041. x - 6955.4 ($r^2$=0.998), y (FTD) = 2565. x -111.36($r^2$ = 0.999), y (DLF) = 1590.9. x - 1029.5 ($r^2$ = 0.999) and y (CLZ) =
8144.5x + 1157.9 (r² = 0.999). Figures show the calibration curves of PARA, FTD, DLF and CLZ.

Table 3.1.7.1: Gradient program of optimized method

<table>
<thead>
<tr>
<th>Time</th>
<th>Flow</th>
<th>%Buffer</th>
<th>Acetonitrile</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>3.5</td>
<td>1</td>
<td>90</td>
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<td>50</td>
<td>50</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>11</td>
<td>1</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>14</td>
<td>1</td>
<td>90</td>
<td>10</td>
</tr>
</tbody>
</table>

Figure 3.1.7.1: HPLC Chromatogram of simultaneous determination of Paracetamol, Famotidine, Diclofenac potassium and Chlorzoxazone.
Table 3.1.7.2: System suitability of PARA, FTD, DLF and CLZ

<table>
<thead>
<tr>
<th>Std. Solution</th>
<th>Parameters (n=6)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R_t$</td>
<td>Resolution</td>
<td>Tailing</td>
<td>Theoretical Plates</td>
</tr>
<tr>
<td>PARA</td>
<td>4.8</td>
<td>-</td>
<td>1.18</td>
<td>6533</td>
</tr>
<tr>
<td>FTD</td>
<td>6.6</td>
<td>8.99</td>
<td>1.04</td>
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<tr>
<td>DLF</td>
<td>7.7</td>
<td>7.70</td>
<td>1.08</td>
<td>76940</td>
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<tr>
<td>CLZ</td>
<td>8.8</td>
<td>8.88</td>
<td>1.09</td>
<td>58627</td>
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</table>

Figure 3.1.7.2: Blank chromatogram of PARA, FTD, DLF and CLZ
Figure 3.1.7.3: Placebo chromatogram of PARA, FTD, DLF and CLZ
Figure 3.1.7.3: A System suitability chromatogram

Figure 3.1.7.4: Linearity 25% chromatogram of PARA, FTD, DLF and CLZ
Figure 3.1.7.5: Linearity 50% chromatogram of PARA, FTD, DLF and CLZ

Figure 3.1.7.6: Linearity 75% chromatogram of PARA, FTD, DLF and CLZ
Figure 3.1.7.7: Linearity 100% chromatogram of PARA, FTD, DLF and CLZ

Figure 3.1.7.8: Linearity 125% chromatogram of PARA, FTD, DLF and CLZ
Figure 3.1.7.9: Linearity 150% chromatogram of PARA, FTD, DLF and CLZ

Figure 3.1.7.10: Calibration curve for PARA (A), FTD (B), DLF (C) and CLZ (D)
Table 3.1.7.3: Accuracy studies of PARA, FTD, DLF and CLZ

<table>
<thead>
<tr>
<th>Recovery level (%)</th>
<th>Standard added</th>
<th>Amount added (mg)</th>
<th>Mean recovery (mg) (n=3)</th>
<th>Mean % Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>PARA</td>
<td>162.5</td>
<td>160.95</td>
<td>99.05</td>
</tr>
<tr>
<td></td>
<td>FTD</td>
<td>10</td>
<td>10.17</td>
<td>101.73</td>
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<tr>
<td></td>
<td>DLF</td>
<td>25</td>
<td>25.45</td>
<td>101.83</td>
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<tr>
<td></td>
<td>CLZ</td>
<td>125</td>
<td>125.17</td>
<td>100.14</td>
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### Table 3.1.7.3

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Active Ingredient</th>
<th>Concentration</th>
<th>Measured Value</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>PARA</td>
<td>325</td>
<td>325.97</td>
<td>100.30</td>
</tr>
<tr>
<td></td>
<td>FTD</td>
<td>20</td>
<td>20.03</td>
<td>100.16</td>
</tr>
<tr>
<td></td>
<td>DLF</td>
<td>50</td>
<td>50.53</td>
<td>101.07</td>
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<td></td>
<td>CLZ</td>
<td>250</td>
<td>252.92</td>
<td>101.17</td>
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<td>150</td>
<td>PARA</td>
<td>487.5</td>
<td>491.59</td>
<td>100.84</td>
</tr>
<tr>
<td></td>
<td>FTD</td>
<td>30</td>
<td>30.21</td>
<td>100.70</td>
</tr>
<tr>
<td></td>
<td>DLF</td>
<td>75</td>
<td>75.12</td>
<td>100.17</td>
</tr>
<tr>
<td></td>
<td>CLZ</td>
<td>375</td>
<td>376.12</td>
<td>100.30</td>
</tr>
</tbody>
</table>

**Accuracy**

The precision of a logical strategy is the recognition of results acquired by that technique to the genuine worth for the example. It is communicated as %recovery. In the present study standard addition method was followed to determine the % recovery. And it is determined by.

Accuracy was assessed by spiking the active ingredients at different concentrations 50%, 100% and 150% each of the labelled claim and injected in developed chromatographic conditions in triplicate. The recovery was found to be between 99.05 – 101.84 for all the four drugs and it is shown in table 3.1.7.3.

**Precision**

Portability furthermore transitional accuracy were determined in harmony with ICH rules. The samples (n=6) were assayed on the day of analysis and also the consequent day. Six replicate injections in similar concentration are
analysed on 2 disparate days with various investigator and section for checking the variety in the exactness and the % RSD for PARA, FTD, DLF and CLZ were found to be within acceptable limit of ≤2.

Both the repeatability and intermediate precision were carried out with three different concentrations. There is no major difference seen in the accuracy results did between two back to back days and the outcomes were mentioned in following table 3.1.7.4.

Robustness

This was done by modifying the remarkable chromatographic positions like change in the percent natural quality (± 5%), section temperature (± 50c) and the stream rate (± 0.1mL) did not acquire any vital changes the chromatographic plan and the % RSD were additionally inside as far as possible, It demonstrates performed method is hearty and result was shown as taking after 3.1.7.5.

Samplesolution stability

The sample was infused following 24 hours and it didn't demonstrate any apparent change (table 3.1.7.6). The standard variance and relative standard variance are less than two which shows that the sample solution is stable.

<table>
<thead>
<tr>
<th>Concentration µg/ml</th>
<th>Mean measured concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Repeatability (n=6)</td>
</tr>
<tr>
<td>PARA</td>
<td></td>
</tr>
<tr>
<td>162.5</td>
<td>161.80</td>
</tr>
<tr>
<td>243.75</td>
<td>244.77</td>
</tr>
<tr>
<td>325</td>
<td>327.17</td>
</tr>
<tr>
<td></td>
<td>FTD</td>
</tr>
<tr>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>10</td>
<td>10.09</td>
</tr>
<tr>
<td>15</td>
<td>15.14</td>
</tr>
<tr>
<td>20</td>
<td>20.19</td>
</tr>
<tr>
<td></td>
<td>DLF</td>
</tr>
<tr>
<td>25</td>
<td>25.34</td>
</tr>
<tr>
<td>37.5</td>
<td>36.88</td>
</tr>
<tr>
<td>50</td>
<td>49.06</td>
</tr>
<tr>
<td></td>
<td>CLZ</td>
</tr>
<tr>
<td>125</td>
<td>126</td>
</tr>
<tr>
<td>187.5</td>
<td>188.71</td>
</tr>
<tr>
<td>250</td>
<td>251.65</td>
</tr>
</tbody>
</table>

Figure 3.1.7.14: Repeatability chromatogram
Figure 3.1.7.15: Intermediate precision

Robustness
The robustness depicted in table 3.1.7.5.

Table 3.1.7.5: Robustness studies of PARA, FTD, DLF and CLZ

<table>
<thead>
<tr>
<th>Parameters</th>
<th>%RSD of peak area response</th>
<th>Mean tailing factor</th>
<th>Mean retention time in min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PAR A</td>
<td>FTD</td>
<td>DLF</td>
</tr>
<tr>
<td>flow rate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td>1.3</td>
<td>1.4</td>
<td>1.8</td>
</tr>
<tr>
<td>std</td>
<td>1.1</td>
<td>0.9</td>
<td>0.8</td>
</tr>
<tr>
<td>-0.2</td>
<td>0.8</td>
<td>1.3</td>
<td>0.2</td>
</tr>
<tr>
<td>% Organic phase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+5</td>
<td>0.8</td>
<td>0.1</td>
<td>0.6</td>
</tr>
<tr>
<td>std</td>
<td>1.1</td>
<td>0.9</td>
<td>0.8</td>
</tr>
<tr>
<td>-5</td>
<td>0.6</td>
<td>0.1</td>
<td>1.4</td>
</tr>
<tr>
<td>Column Temp.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+5</td>
<td>0.3</td>
<td>0.1</td>
<td>0.9</td>
</tr>
<tr>
<td>std</td>
<td>1.1</td>
<td>0.9</td>
<td>0.8</td>
</tr>
<tr>
<td>-5</td>
<td>0.1</td>
<td>1.0</td>
<td>0.5</td>
</tr>
</tbody>
</table>

*n=6 for each parameter
Figure 3.1.7.13: Robustness (flow minus) chromatogram of PARA, FTD, DLF and CLZ

![Chromatogram of PARA, FTD, DLF and CLZ](image1)

Figure 3.1.7.14: Robustness (flow plus) chromatogram of PARA, FTD, DLF and CLZ

![Chromatogram of PARA, FTD, DLF and CLZ](image2)
Figure 3.1.7.15: Robustness (mobile phase minus) chromatogram of PARA, FTD, DLF and CLZ

Figure 3.1.7.16: Robustness (mobile phase plus) chromatogram of PARA, FTD, DLF and CLZ
Figure 3.1.7.17: Robustness (temperature minus) chromatogram of PARA, FTD, DLF and CLZ

Figure 3.1.7.18: Robustness (temperature plus) chromatogram of PARA, FTD, DLF and CLZ
Table 3.1.7.6: Stability studies of PARA, FTD, DLF and CLZ

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amount of drug found at 0 hr*(mg)</th>
<th>Amount of drug found at 24hr*(mg)</th>
<th>Amount of Deviation(mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PARA</td>
<td>327.17</td>
<td>329.16</td>
<td>1.99</td>
</tr>
<tr>
<td>FTD</td>
<td>20.19</td>
<td>19.85</td>
<td>0.34</td>
</tr>
<tr>
<td>DLF</td>
<td>49.06</td>
<td>50.86</td>
<td>1.8</td>
</tr>
<tr>
<td>CLZ</td>
<td>251.65</td>
<td>245.42</td>
<td>6.22</td>
</tr>
</tbody>
</table>

*n=6 for each parameter

**LOD and LOQ**

Detection limit and quantification limit of PARA, FTD, DLF and CLZ was resolved by using calibration curve technique. Solutions of PARA, FTD, DLF and CLZ was prepared and injected in the linearity range (n=3). The graph plotted against average peak areas and concentration. The LOD and LOQ values was calculated with the equations as, LOD = (3.3 ×Syx)/b and LOQ= (10.0×Syx)/b, Where Syx is the remaining difference because of regression; b refers slope. LOD and LOQ of PARA, FTD, DLF and CLZ were performed by the use of calibration curve procedure and the performed results as shown in 3.1.7.7.

Table 3.1.7.7: LOD and LOQ values of PARA, FTD, DLF and CLZ

<table>
<thead>
<tr>
<th>Drug</th>
<th>LOD</th>
<th>LOQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>PARA</td>
<td>0.4321</td>
<td>1.2959</td>
</tr>
<tr>
<td>FTD</td>
<td>0.6213</td>
<td>1.8849</td>
</tr>
</tbody>
</table>
Table Analysis

Content of PARA, FTD, DLF, and CLZ was found in the pharmaceutical dosage forms as the proposed procedure and the results as shown in 3.1.7.8.

Table 3.1.7.8: Analysis of marketed formulation

<table>
<thead>
<tr>
<th>Marketed formulation</th>
<th>Ingredients</th>
<th>Labeled amount (mg)</th>
<th>Amount found (mg)</th>
<th>Found%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Andic – MR tablets</td>
<td>PARA</td>
<td>325</td>
<td>322.62</td>
<td>99.27</td>
</tr>
<tr>
<td></td>
<td>FTD</td>
<td>20</td>
<td>20.04</td>
<td>100.23</td>
</tr>
<tr>
<td></td>
<td>DLF</td>
<td>50</td>
<td>49.35</td>
<td>98.7</td>
</tr>
<tr>
<td></td>
<td>CLZ</td>
<td>250</td>
<td>252.77</td>
<td>101.11</td>
</tr>
</tbody>
</table>

Figure 3.1.7.19: Assay chromatogram of PARA, FTD, DLF and CLZ
A novel RP-HPLC-PDA procedure was carried out for the same time estimation of PARA, FTD, DLF and CLZ in bulk and tablet in which the active agents are present in variable concentrations. Because of the wide variability among the drugs, their polarities and also there concentrations in the dosage form it became a tough task to optimise the method which gave great determination for all the four medications with a short run time (15min). The created technique was approved according to ICH rules. The developed method was simple, specific as the excipients have no interference in the determination of main components, precise, accurate, and sensitive. The proposed new methods are using for daily analysis of PARA, FTD, DLF and CLZ in mixed dosage form which are present in variable concentrations. That is also applied in the QC of bulk manufacturing of presented API’s.