CHAPTER – V
DEVELOPMENT OF UPLC METHODS FOR THE DETERMINATION OF DRUG PRODUCTS.
5.1 INTRODUCTION:

High efficiency and shorter run time are the basic requirements of high-speed chromatographic separations. To fulfill these requirements, a new separation technique i.e. ultra-performance liquid chromatography (UPLC), has been developed. The objective of the current study is to develop and validated reversed-phase UPLC method for the quantitative determination of drug product. First UPLC method is developed for the determination of mesalamine in the presence of degradation products and its process-related impurities in tablet dosage form and second UPLC method is developed to conduct in vitro dissolution studies of two different Antihypertensive formulations in tablet dosage form such as Dronedarone tablet and Telmisartan and Hydrochlorothiazide tablet.

Mesalamine (5-aminosalicylic acid, 5-ASA), the therapeutically active lead of sulfasalazine\(^1\)\(^-\)\(^3\), which is used as a gastrointestinal anti-inflammatory drug for the treatment of inflammatory bowel diseases\(^4\) and active ulcerative proctitis\(^5\)\(^-\)\(^6\). It is a tan to pink `crystalline powder, relatively insoluble in chloroform, ether, n hexane and ethyl acetate and freely soluble in dil. HCl and alkali hydroxides\(^7\)\(^-\)\(^8\). Mesalamine is available in tablet dosage forms (400 mg) and is an official drug of USP. Mesalamine protects against colorectal cancer in inflammatory bowel disease\(^9\). For safety and quality of drug product, related substances should be known. The new approach emphasizes on good amount of resolution between all related substances in the drug product analytical method. Hence there is need to develop stability indicating UPLC method for the determination of related substances of Mesalamine in tablet dosage form.

Dronedarone HCl mainly used for cardiac arrhythmias, it was recommended as an alternative to amiodarone for the treatment of atrial fibrillation and atrial flutter. Its chemical designation is N-(2-Butyl-3-(p-(3-(dibutylamino)propoxy)benzoyl)-5-benzofuranyl) methane sulfonamide. Dronedarone tablets were obtained from India market. Each tablet was labeled contain 400 mg of Dronedarone. Telmisartan is an angiotensin II receptor antagonist used in the management of hypertension. Its chemical design 4’-[(1, 4’- Dimethyl-2’-propyl-[2, 6’-bi-1H-benzimidazol]-1’-yl) methyl]-[1, 1’-biphenyl]-2-carboxylic acid. Hydrochlorothiazide is a thiazide type diuretic which reduces reabsorption of electrolytes from the renal tubules. Its chemical designation is 6-Chloro-3, 4-dihydro-2H-1, 2, 4-benzo-thiazidin-7-sulfonamide-1,1-
dioxide. Telmisartan and Hydrochlorothiazide are indicated for the treatment of hypertension. Telmisartan and Hydrochlorothiazide tablets were obtained from India market. Each tablet was labeled as Telmisartan 40 mg and Hydrochlorothiazide 12.5 mg, or Telmisartan 80 mg and hydrochlorothiazide 12.5 mg.

5.2. LITERATURE SURVEY:

In this section summarized some of the important analytical methods for the determination of mesalamine, Dronedarone HCl, Telmisartan and Hydrochlorothiazide as a individual component or in combination with another drug substance.

_Haney P.W., et. al. (1997)_

Haney P.W., et. al. reported liquid chromatographic method for the analysis of 5-aminosalicylic acid and its degradation product. The chromatographic separation was achieved on a reversed-phase, C₈ column with UV detection at 290 nm. This isocratic system was operated at ambient temperature and required 8 min of chromatographic time. The mobile phase consisted of methanol-phosphate buffer pH 7.4 (20:80, v/v). The flow-rate was maintained at 1.0 ml/min.

_Gotti R., et. al. (2001)_

Gotti R., reported electrokinetic chromatography with an ion-pair reagent for the determination of 5-aminosalicylic acid related impurities. The optimization of the experimental conditions was carried out considering some important requirements: resolution, reproducibility, detection limits of 0.1% (m/m) or less, short total analysis time. Preliminary investigations employing sodium dodecyl sulfate (SDS) as surfactant did not lead to the necessary resolution of the studied compounds; the addition of tetrabutyl ammonium bromide (TBAB) to the SDS micellar system resulted in the complete separation of all the compounds. The effects on the separation by several parameters such as TBAB concentration, SDS concentration, background electrolyte pH and concentration, were evaluated. Using a fused-silica capillary (8.5 cm effective length) complete analysis was obtained in a very short time. Under the optimised final conditions [120 mM 3-(cyclohexylamino)-2-hydroxy-1-propanesulfonic acid buffer, pH 10.20, 65 mM SDS in the presence of 55 mM TBAB and 5% methanol] the method was validated for specificity.
Development of UPLC methods for the determination of drug products.

Patil A.T. and Shaikh K.A.

precision, linearity, limits of detection and quantitation, and robustness: the 5-ASA related impurities can be quantified at least at the 0.1% (m/m) level.

Lee J., et. al. (2004)\textsuperscript{12}

Lee J., reported micellar electrokinetic capillary chromatography for the determination of impurities in the drug 5-aminosalicylic acid. Process impurities found in the drug substance 5-aminosalicylic acid were determined by micellar electrokinetic capillary chromatography (MECC). In order to enhance the detection of trace impurities, the parent drug was dissolved to an unusually high concentration of 5 mg/ml. To reduce the dispersive processes of electromigration dispersion and anti-stacking, both of which occur at high solute concentration, the electrolyte pH was adjusted to be close to the apparent isoelectric point (pI) of the zwitterionic drug. In this fashion, the net charge on the solute should approach zero thereby minimizing the aforementioned sources of band-broadening. Two additional developments are reported. Short sodium hydroxide washes were used to optimize the MECC reproducibility. The elimination of anti-stacking permitted the use of peak heights to quantities low level impurities with improved precision.

Nobilis M., et. al. (2006)\textsuperscript{13}

Nobilis M., et. al. reported High-performance liquid-chromatographic determination of 5-aminosalicylic acid and its metabolites in blood plasma. Chromatographic analysis were performed on a 250-4 mm column containing Purospher RP-18 e, 5 microm (Merck, Darmstadt, Germany) with a precolumn (4-4 mm). The column effluent was monitored using both UV photodiode-array (lambda = 313 nm) and fluorescence detectors (lambda (exc.) = 300 nm/lambda (emiss.) = 406 nm) in tandem. The identity of individual N-acyl-ASAs in the extracts from biomatrices was verified by characteristic UV-spectra and by HPLC/MS experiments.

Aparecida J.R., et. al. (2007)\textsuperscript{14}

Aparecida J.R., et. al. reported HPLC, DPPH and Nitrosation methods for Mesalamine determination in pharmaceutical dosage forms. HPLC with ultraviolet detection at 254 nm was carried out with a C18 column and a mobile phase constituted of 30 mmol/L monobasic
phosphate buffer (pH 7.0) and methanol (70:30; v/v), with 25% tetrabutylammonium hydrogen sulphate. The DPPH\(^*\) method was performed at 517 nm and using 100 mmol/L acetate buffer, pH 5.5, ethanol and 250 μmol/L ethanolic solution of DPPH\(^*\). The nitrosation method was accomplished by using a platinum electrode and standard 0.1 mol/L sodium nitrite as titrant solution.

**Pastorini E., et. al. (2008)**\(^{15}\)

Pastorini E., et. al. reported HPLC-ESI-MS/MS method for the determination of 5-aminosalicylic acid and its major metabolite N-acetyl-5-aminosalicylic acid in human plasma. Plasma samples were analyzed after protein precipitation with methanol and the two analytes were separated using a C18 column with a mobile phase composed of 17.5 mmol/L acetic acid (pH 3.3):acetonitrile=85:15 (v/v) at 0.2 mL/min flow rate. 4-ASA and N-Ac-4-ASA were used as internal standards. Selective detection was performed by tandem mass spectrometry with electrospray source, operating in negative ionization mode and in multiple reaction monitoring acquisition (m/z 152-->108 for 5-ASA; m/z 194-->150 and 194-->107 for N-Ac-5-ASA).

**Patel K.M., et. al. (2010)**\(^{16}\)

Patel K.M., reported spectrophotometric method for the estimation of Mesalamine in Tablet Dosage Forms. It is based on Diazotization of Mesalamine with nitrous acid, to form diazotized Mesalamine, followed by its coupling with N-(1-naphthyl) ethylene-diamine dihydrochloride [Bratton-Marshall reagent] to form a violet colored chromogen with maximum absorption at 552 nm; it obeyed the Beer’s law in the concentration range of 2 – 30 μg/ml. It is based on the condensation of Mesalamine with p-dimethylaminobenzaldehyde to form the Schiff’s base that is a yellow colored chromogen and shows maximum absorbance at 440 nm; The Beer’s law is obeyed in the concentration range of 50 – 500μg/ml. Mesalamine has a phenolic group when made to react with Gibb’s reagent, in alkaline pH it forms a colored chromogen, exhibiting absorption maximum at 494 nm, and Beer’s law is obeyed in the concentration range of 5 – 60 μg/ml.
Moharana A. K., et. al. (2011)\textsuperscript{17}

Moharana A. K., reported visible spectroscopic method for the determination of Mesalamine in bulk and pharmaceutical formulation. When the drug reacts with 0.5N HCl and freshly prepared 0.4% w/v Ferric Nitrate solution the colour changes to brown. It shows absorption maximum at 432.6 nm and obeys Beer’s law in the concentration range 50–150μg mL\textsuperscript{-1}. The colouring reagent is stable when checked for its stability test; it is stable for 4 hours. The absorbance was found to increase linearly with increasing concentration of MSZ, which is corroborated by the calculated correlation coefficient value of 0.9995 (n=6).

Kanchana M. K., et. al. (2013)\textsuperscript{18}

Kanchana M. K. et. al. reported an open-label, randomized, crossover bioequivalence study of mesalamine 400 mg tablets in Indian healthy volunteers under fasting conditions. A validated liquid chromatography/tandem mass spectrometry (LC/MS/MS) method was used for determination of mesalamine/N-Acetyl mesalamine concentration in human plasma. Equipment used was a HPLC (1200 Series Agilent Technologies, Germany). MS/MS (ABI-SCIEX,Toronto, Canada) system. Column type used was a Thermo, HyPURITY C18, 150 x 4.6 mm, 5 μ, and the mobile phase used was 10mM Ammonium acetate:methanol (85:15 v/v) delivered at the flow rate of 0.6 mL/min, Injection volume was 10μL, Column temperature was 40°C with a isocratic elution system.

Gatkal S. H., et. al. (2013)\textsuperscript{19}

Gatkal S. H., et. al. reported a stability indicating assay method of Mesalamine by using different stress degradation conditions. Solution of mesalamine in distilled water shows maximum absorbance at 330 nm. Beer’s law was obeyed in the concentration range of 2-16 μg/ml with the slope, intercept, correlation coefficient, detection and quantitation limits were also calculated.

One more method reported for the spectrophotometric determination of Mesalamine in pharmaceutical formulation\textsuperscript{20}. Literatures survey revealed that the mesalamine drug substance is official in US Pharmacopoeia\textsuperscript{21} as well as in British Pharmacopoeia\textsuperscript{22}. Mesalamine extended
release capsules and mesalamine delayed-release tablets, formulation is also official in US Pharmacopoeia.


Momani F.A., et. al., reported HPLC method for the Determination of Hydrochlorothiazide and Enalapril Maleate in Tablet Formulations. The mobile phase consists of 3.0 mM Tetrabutyl ammonium hydrogen sulfate in Acetonitrile : water : Triethyl amine (14:85.6:0.4 V/V) solution, and the pH was adjusted to 4.1 by glacial acetic acid. The mobile phase was always filtered using 0.45 µm membrane filters and was degassed by vacuum prior to use. The sample solutions were also filtered using 0.45 µm membrane filters. The flow rate was 2 mL/min. The wavelength was 220nm.


Hertzog D.L., et. al. reported HPLC method for the simultaneous determination of losartan potassium, hydrochlorothiazide, and their degradation products. The analytical columns used to achieve chromatographic separation were Symmetry C8 columns (150×3.9 mm I.D., 5 µm particle size). The mobile phase for both the Losartan tablets and the Losartan/HCTZ tablets methods was made by first preparing a phosphate buffer solution of KH$_2$PO$_4$ and Na$_2$HPO$_4$ (pH 7.0, 0.02 M). This buffer solution was then mixed with acetonitrile in ratios of 85:15 (v/v) and 93:7 (v/v) buffer–acetonitrile to yield Mobile Phase A for Losartan tablets and Losartan/HCTZ tablets, respectively. Mobile Phase B is 100% acetonitrile for both methods. Mobile phases for pH robustness studies involved pH adjustment of the 0.02 M phosphate buffer with NaOH or H$_3$PO$_4$ prior to mixing with acetonitrile.

**Torrealday N., et. al., (2003)**

Torrealday N., et. al., reported a HPLC-fluorimetric method for the quantitation of the angiotensin II receptor antagonist telmisartan in urine. A high performance liquid chromatographic method with fluorimetric detection has been developed for the quantitation of the angiotensin II receptor antagonist (ARA II) 4-((2-n-propyl-4-methyl-6-(1-methylbenzimidazol-2-yl)-benzimidazol-1-yl)methyl)biphenyl-2-carboxylic acid (telmisartan).
in urine, using a Novapak C18 column 3.9 x 150 mm, 4 microm. The mobile phase consisted of a mixture acetonitrile-phosphate buffer (pH 6.0, 5 mM) (45:55, v/v) pumped at a flow rate of 0.5 ml/. Effluent was monitored at excitation and emission wavelengths of 305 and 365 nm, respectively. Separation was carried out at room temperature.

**Patel L. J., et. al. (2006)**

Patel L. J., et. al. reported HPLC method for the Simultaneous estimation of Bisoprolol fumarate and Hydrochlorothiazide in tablet dosage form. A Chromatographic conditions consists of Lichrospher 100 C-18, 5 µm column 20 cm × 4.6 mm in isocratic mode, with mobile phase containing water, acetonitrile and tetrahydrofuran in proportion of 80:20:5 v/v/v were used. The flow rate was 1 ml/min, and effluent was monitored at 225 nm. The retention time of Bisoprolol fumarate and Hydrochlorothiazide were 1.48 ± 0.02 and 4.72 ± 0.03 min respectively, and the resolution factor was 9.0.

**Wankhede S.B., et. al. (2007)**

Wankhede S.B., et. al. reported HPLC method for simultaneous estimation of Telmisartan and Hydrochlorothiazide in tablet dosage form. Chromatography was performed on a ODS Hypersil C18 (25 cm×4.6 mm I.D) column from thermo in isocratic mode with mobile phase containing acetonitrile:0.05 M KH₂PO₄ pH 3.0 (60:40). The flow rate was 1.0 ml/min and the eluent was monitored at 271 nm. The selected chromatographic conditions were found to effectively separate telmisartan (RT- 5.19 min) and hydrochlorothiazide (RT- 2.97 min).

**Yan T., et. al. (2008)**

Yan T., et. al. reported Liquid chromatographic-tandem mass spectrometric method for the simultaneous quantitation of Telmisartan and Hydrochlorothiazide in human plasma. Sample preparation involved liquid-liquid extraction with diethyl ether-dichloromethane (60:40, v/v). The analytes and internal standard, probenecid, were separated on a Venusil XBP-C(8) column using gradient elution with acetonitrile-10 mM ammonium acetate-formic acid at a flow rate of 1.2 mL/min. Detection was by electrospray negative ionization mass spectrometry using multiple
reaction monitoring of the transitions at m/z 513.0→469.4 for telmisartan, m/z 295.9→268.9 for hydrochlorothiazide and m/z 283.9→239.9 for probenecid.

**Kurade V.P., et. al., (2009)**

Kurade V.P., et. al., reported RP-HPLC method for estimation of Ramipril and Telmisartan in tablets, A Genesis C18 column having dimensions of 4.6×250 mm and particle size of 5 µm in isocratic mode, with mobile phase containing a mixture of 0.01 M potassium dihydrogen phosphate buffer (adjusted to pH 3.4 using orthophosphoric acid): methanol : acetonitrile (15:15:70 v/v/v) was used. The mobile phase was pumped at a flow rate of 1.0 ml/min and the eluents were monitored at 210 nm. The selected chromatographic conditions were found to effectively separate Ramipril (R_t : 3.68 min) and Telmisartan (R_t : 4.98 min) having a resolution of 3.84.

**Vijayamirtharaj R., et.al. (2010)**

Vijayamirtharaj R., et. al. reported RP-HPLC method for the simultaneous estimation of Telmisartan and Atorvastatin calcium in tablet dosage forms. Chromatographic conditions consisted of mobile phase Acetonitrile: Buffer (0.01M Potassium dihydrogen phosphate) 65:35 PH 4.00 (adjusted with Orthophosphoric acid) and the wavelength selected was 250nm. The flow rate was kept at 2.0 ml/min, and the injection volume was 10µl. The separation was performed at ambient temperature. Retention time of Telmisartan and Atorvastatin calcium was found to be 3.72 and 6.14 minutes respectively.

**Gangola R., et. al. (2011)**

Gangola R., reported Spectrophotometric method for the Simultaneous Determination of Hydrochlorothiazide and Telmisartan in Combined Dosage Form. This method is based on the principle that absorbance difference between two points on mixture spectra is directly proportional to concentration of component of interest and independent of interfering component. Set of two wavelengths λ1 (258nm) and λ2 (299nm) for estimation of Hydrochlorothiazide and λ3 (316nm) and λ4 (326nm) for estimation of Telmisartan were selected on above principle and overline spectra. Determined absorbance difference (A1-A2) and
(A3-A4) values and plotted calibration curves between absorbance difference values and concentration of drug.

**Patel A., et. al. (2012)**

Patel A., et. al. reported RP-HPLC method development and validation of Dronedarone HCl in its Pure form and tablet dosage form. The method was carried out using Hypersil ODS 3V 250×4.6 mm, 5µm column with mobile phase comprised of Buffer: Acetonitrile (42:58%v/v). Buffer use is Potassium dihydrogen phosphate buffer with pH 3.0. The flow rate was set at 1.1 ml/min and effluent was detected at 220nm. The retention time of Dronedarone was found to be 4 minute.

**Landge S. B., et. al., (2013)**

Landge S. B., reported Stability Indicating RP-HPLC Method for the Determination of Dronedarone Hydrochloride and Its Potential Process-Related Impurities in Bulk Drug and Pharmaceutical Dosage form. A Waters HPLC (Milford, MA, USA) equipped with Alliance 2695 separations module and 2996 photodiode array detector was used for method development, forced degradation studies, and method validation. The column Ascentis® Express C18, 10 cm × 4.6 mm, 2.7 µm (SUP-ELCO Analytical, USA), thermostated at 35°C was used for the analysis/study. The mobile phase-A consisting a mixture of buffer (0.05M Ammonium dihydrogen ortho-phosphate) and methanol in the ratio of 80:20 v/v and mobile phase-B consisting a mixture of acetonitrile, me- thanol and water in the ratio of 45:45:10 v/v. The flow rate and injection volumes were 1.2 mL·min⁻¹ and 10 µl respectively. The analysis was carried out under gradient conditions as follows, time (min)/A (v/v): B (v/v); T0.01/ 65:35, T7.0/45:55, T17.0/45:55, T24.0/30:70, T28.0/30:70, T29.0/65:35, T35.0/65:35. The photodiode array detector was used in the scan mode from 200 nm to 400 nm.

Other than above mentioned methods few more methods are also reported for the quantification of Dronedarone in pharmaceutical formulation by HPLC**36-37** and HPTLC**38** . Spectrophotometric methods**39-40** and HPLC methods**41-44** are reported for the quantification of Telmisartan and Hydrochlorothiazide in tablet dosage form. HPLC methods**45-47** for Simultaneous estimation of Telmisartan in combination with another drug substance are also reported. Few
HPLC methods^{48-50} are reported for the simultaneous estimation of Hydrochlorothiazide in combination with another drug substance. Quantification of related substances of Telmisartan and Hydrochlorothiazide in tablet dosage form by HPLC method^{51} is reported.

5.3 STABILITY-INDICATING UPLC METHOD FOR THE DETERMINATION OF MESALAMINE RELATED IMPURITIES IN TABLET DOSAGE FORM.

The aim of the present work was to develop and validate a simple, precise, accurate, short runtime and specific method for the quantification and separation of process related impurities/degradation product by reversed-phase UPLC method, in mesalamine tablet formulation. Chromatographic separation has been achieved on an UPLC CSH C18 150 mm x 2.1 mm, 1.7 µm column. Mobile phase consisting solvent A 0.05 molar (M) dipotassium hydrogen phosphate and 0.5% octane sulphonic acid (pH 2.0 buffer). Solvent B 0.05 molar (M) dipotassium hydrogen phosphate and 0.5% octane sulphonic acid (pH 5.5 buffer) : methanol : acetonitrile in the ratio of 900:80:20 v/v. delivered at flow rate of 0.15 mL min^{-1} and the detection wavelength 230 nm. Forced degradation studies carried for mesalamine drug product to demonstrate the stability-indicating power of the UPLC method. The drug product was subjected oxidative, acid, base hydrolysis, thermal and photolytic stress conditions. Mesalamine was found to degrade significantly in base hydrolysis and Peroxide stress conditions compare to acid hydrolysis, thermal and photolytic degradation conditions. The degradation products were well resolved from main peak and its impurities, mass balance is more than 99%, thus proved the stability indicating power of the method. The developed method was validated as per ICH guidelines with respect to specificity, linearity, limit of detection, limit of quantification, accuracy, precision and robustness.

5.3.1 DRUG PROFILE:

5.3.1.1 Mesalamine
1. Chemical Name: 5-amino-2-hydroxybenzoic acid
2. Chemical Structure:
3. **Molecular Formula**: $C_7H_7NO_3$

4. **Molecular Weight**: 153.14

5. **Description**: White colored powder

6. **Solubility**: Mesalamine is listed as soluble in HCl, slightly soluble in cold water and alcohol, and more soluble in hot water.

7. **Melting Point**: 283°C

8. **Category**: Anti inflammatory agent

5.3.1.2 *Mesalmine related impurities*:

<table>
<thead>
<tr>
<th>Impurity A: - Sulfanilic acid</th>
<th>Impurity B: - 3-Amino benzoic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Impurity A" /></td>
<td><img src="image2" alt="Impurity B" /></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Impurity C: - 3-Amino salicylic acid</th>
<th>Impurity D: - 2,5-dihydroxy benzoic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image3" alt="Impurity C" /></td>
<td><img src="image4" alt="Impurity D" /></td>
</tr>
</tbody>
</table>
Development of UPLC methods for the determination of drug products.

Impurity E: - 3-Amino phenol

\[
\text{OH} \\
\text{NH}_2
\]

Impurity F: - Salicylic acid

\[
\text{O} \quad \text{C} \quad \text{OH} \\
\text{HO}
\]

5.3.2 EXPERIMENTAL:

5.3.2.1 Working standards:

The working standard was procured from India market having following batch number and potency.

<table>
<thead>
<tr>
<th>Working Standard</th>
<th>Batch Number</th>
<th>Potency (on as is basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesalamine</td>
<td>ME004321</td>
<td>99.4 % w/w</td>
</tr>
</tbody>
</table>

5.3.2.2 Sample:

The Samples was procured from Indian Market. The test sample bears following details,

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Sun Pharmaceuticals Ltd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Product Name</td>
<td>Mesacol</td>
</tr>
<tr>
<td>Label Claim</td>
<td>800 mg and 400 mg</td>
</tr>
</tbody>
</table>

5.3.2.3 Instrument / Apparatus Used:

i) All the glassware used for the experiment were certified ‘A’ grade manufactured by SCHOTT Glass India Pvt. Ltd. Mumbai, India

ii) A calibrated UPLC Waters ACQUITY UPLC series comprised of degasser, quaternary pump, auto injector, column compartment, PDA detector.

iii) Sanyo, Leicestershire, UK, Photo stability chamber.

iv) dry air oven Cintex, Mumbai, India.

v) A calibrated digital pH meter, manufactured by Mettler-Toledo Inc, Columbus, OH.

vi) A calibrated analytical balance, manufactured by Sartorius, Germany.

vii) A sonicator, manufactured by Amrut Enterprises, Pune, India.
5.3.2.4 Reagents and chemicals:

All reagents and chemicals were used from Merck chemicals. Octane sulphonic acid, Hydrochloric acid and Dipotassium hydrogen phosphate were used as GR grade. Acetonitrile and Water were used as HPLC grade. 0.1N Hydrochloric acid were used as diluent.

5.3.2.5 Preparation of standard solutions

A stock solution of mesalamine (500 μg mL⁻¹) was prepared by dissolving an appropriate amount in diluent. Standard solutions containing 5 μg mL⁻¹ were prepared from this stock solution.

5.3.2.6 Preparation of sample solution

Tablet powder equivalent to 50 mg drug was dissolved in diluent with rotary shaking for 10 min and sonication for 10 min to give a solution containing 500 μg mL⁻¹. This solution was filtered through a 0.45 μm pore size Nylon 66 membrane filter. Typical chromatogram of sample solution spiked with impurities is as shown in figure 5.1.

![Typical chromatogram of sample solution spiked with impurities.](image-url)
5.3.2.7 Chromatographic conditions:

```
mobile phase A: 0.05 molar (M) dipotassium hydrogen phosphate and 0.5% octane sulphonic acid (pH 2.0 buffer).
mobile phase B: 0.05 molar (M) dipotassium hydrogen phosphate and 0.5% octane sulphonic acid (pH 5.5 buffer) : methanol : acetonitrile in the ratio of 900:80:20 v/v.

Column: UPLC CSH C18 150 mm x 2.1 mm, 1.7 µm.
Column oven temperature: 30°C
Flow: 0.15 mL min\(^{-1}\)
Wavelength: 230 nm
Injection volume: 2 µL
Runtime: 36 minutes.
Mobile phase gradient: Table 1.
```

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Flow (mL min(^{-1}))</th>
<th>Mobile phase A (%)</th>
<th>Mobile phase B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.2</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>1.2</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>1.2</td>
<td>85</td>
<td>15</td>
</tr>
<tr>
<td>27</td>
<td>1.2</td>
<td>80</td>
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<td>0</td>
</tr>
<tr>
<td>36</td>
<td>1.2</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

5.3.2.8 Procedure:

HPLC system was set up as described under chromatographic conditions. Standard and sample solution was prepared according to 4.3.5 and made single injection of each of solvent mixture as a blank, standard solution (six injections), placebo solution and sample solution in to the chromatographic system. Recorded the chromatograms at 230 nm and measure the peak area counts for all eluting peaks. Examined the blank and placebo chromatogram for any extraneous peaks and disregard corresponding peaks observed in the chromatogram of the sample solution.
Table 5.1: Chromatographic performance data.

<table>
<thead>
<tr>
<th>Compound</th>
<th>RT (Min)</th>
<th>RRT</th>
<th>Tailing factor NMT 2.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imp. A</td>
<td>2.6</td>
<td>0.2</td>
<td>1.00</td>
</tr>
<tr>
<td>Imp. B</td>
<td>4.2</td>
<td>0.6</td>
<td>1.04</td>
</tr>
<tr>
<td>Mesalamine</td>
<td>7.0</td>
<td>1.0</td>
<td>1.03</td>
</tr>
<tr>
<td>Imp. C</td>
<td>9.2</td>
<td>1.3</td>
<td>1.01</td>
</tr>
<tr>
<td>Imp. D</td>
<td>11.7</td>
<td>1.7</td>
<td>1.10</td>
</tr>
<tr>
<td>Imp. E</td>
<td>15.6</td>
<td>2.2</td>
<td>1.05</td>
</tr>
<tr>
<td>Imp. F</td>
<td>21.0</td>
<td>3.0</td>
<td>1.02</td>
</tr>
</tbody>
</table>

5.3.2.9 Calculations:

\[
\text{Known Impurity } \% (\text{w/w}) = \frac{\text{AT WS DT P AW}}{\text{AS DS WT}100 \text{ LC}} \\
\text{Unknown Impurity } \% (\text{w/w}) = \frac{\text{AT}_1 \text{ WS DT P AW}}{\text{AS DS WT}100 \text{ LC}}
\]

Total Impurities = Sum of all known impurities and unknown impurities

Where,
- AT : Area of Known impurity peak in sample solution.
- AT<sub>1</sub> : Area of Unknown impurity peak in sample solution.
- AS : Average area of Mesalamine peak in standard solution.
- WS : Weight of Mesalamine standard in mg.
- DS : Dilution of Mesalamine standard in ml.
- DT : Dilution of Mesalamine sample solution.
- WT : Weight of Mesalamine sample in mg.
- P : Potency of Mesalamine working standard on as is basis.
- LC : Label claim of Mesalamine in mg per tablet.
- AW : Average weight of tablet in mg.
5.3.3 RESULTS AND DISCUSSION:

The main objective of development of RP-UPLC method was separation of Mesalamine related impurities in tablet dosage form. As the method should be able to determine all impurities of the drug product in single run with the good amount of resolution. Method should be accurate, reproducible, robust, stability indicating, free from interference (blank/placebo/other unknown degradation product) and straight forward enough for routine use in quality control laboratory.\(^{52-55}\) Initial method development was started with Isocratic mobile phase. Different combination of buffer : acetonitrile in the range of 90:10 to 10:90 v/v has been tried, it has been observed that Sulfanlic acid impurity is most polar in nature. Increase in organic concentration more than 5% in the buffer (95:5 buffer:acetonitrile) leads elution of sulfanlic acid impurity in the void volume. So Switch to gradient mobile phase where Solvent A contains 0.05 molar (M) dipotassium hydrogen phosphate and 0.5% octane sulphonic acid (pH 2.0 buffer) and Solvent B 0.05 molar (M) dipotassium hydrogen phosphate and 0.5% octane sulphonic acid (pH 5.5 buffer) : methanol : acetonitrile in the ratio of 900:80:20 v/v. Different gradient programs has been tried to improves the run time less than 60 minutes with good retention of Sulfanlic acid on the column. During the optimization of chromatographic condition, Solvent A contains 0.05 molar (M) dipotassium hydrogen phosphate and 0.5% octane sulphonic acid (pH 2.0 buffer). Solvent B 0.05 molar (M) dipotassium hydrogen phosphate and 0.5% octane sulphonic acid (pH 5.5 buffer) : methanol : acetonitrile in the ratio of 900:80:20 v/v. UPLC gradient program was set as time (min) / % solvent B: 0/0, 9/0, 15/15, 27/20, 30/75, 31/0 and 36/0. The column temperature was maintained at 30 °C and the detection was monitored at a wavelength 230 nm. Flow rate of mobile phase was 0.15 mL min\(^{-1}\).

5.3.3.1 Validation of method:

The optimized chromatographic conditions was validated by evaluating specificity, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), robustness and system suitability, in accordance with ICH guidelines Q2 (R1)\(^{56-58}\).
5.3.3.1.1 Specificity:

The specificity of a method is its suitability for analysis of a substance in the presence of potential impurities. Stress testing of a drug substance can help to identify likely degradation products, which can help to establish degradation pathways and the intrinsic stability of the molecule. It can also be used to validate the stability-indicating power of the analytical procedures used. The specificity of the LC method for Mesalamine has been determined in the presence of six impurities.

5.3.3.1.2 Forced degradation study of drug product:

Stress studies of the drug product was utilized for the identification of the possible degradation products and for the validation of the stability-indicating analytical procedures. It is the ability of the analytical method to measure the analyte response in the presence of its degradation products\(^{59-60}\). The result obtained from the forced degradation studies are summarized in Table 5.2.

**Table 5.2: Stress testing (forced degradation) data.**

<table>
<thead>
<tr>
<th>Stress Condition</th>
<th>% Net Degradation</th>
<th>Purity Angle</th>
<th>Purity threshold</th>
<th>Purity flag</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid Hydrolysis</td>
<td>0.5</td>
<td>0.782</td>
<td>0.871</td>
<td>No</td>
</tr>
<tr>
<td>Base Hydrolysis</td>
<td>6.8</td>
<td>0.141</td>
<td>0.241</td>
<td>No</td>
</tr>
<tr>
<td>Peroxide Oxidation</td>
<td>5.2</td>
<td>0.140</td>
<td>0.412</td>
<td>No</td>
</tr>
<tr>
<td>Photolytic-sunlight</td>
<td>0.2</td>
<td>0.314</td>
<td>0.547</td>
<td>No</td>
</tr>
<tr>
<td>Heat Stress</td>
<td>0.8</td>
<td>0.224</td>
<td>0.548</td>
<td>No</td>
</tr>
<tr>
<td>Humidity Stress</td>
<td>0.2</td>
<td>0.142</td>
<td>0.458</td>
<td>No</td>
</tr>
</tbody>
</table>

Peak purity has been checked for the Mesalamine peak by using PDA detector in stress samples. Assay of stressed samples has been performed by comparison with reference standard and the mass balance (\(\%\) assay + \(\%\) impurities + \(\%\) degradation products) were calculated. The mass balance for the stressed samples was close to 99% . There was no peak found at the
retention time of mesalamine and its all six impurities in blank and placebo blend chromatograms proves no interference from blank and placebo. During the forced degradation study, the peak of mesalamine and all known impurities was found to be pure and no considerable degradation of drug product was observed in acidic, thermal, and photolytic conditions and considerable degradation observed in basic and oxidative stress conditions. The chromatograms were checked for the appearance of any extra or overlapping peaks. The purity of the principle and other chromatographic peaks was found to be satisfactory. This study confirmed the stability indicating power of the UPLC method. (Figure: 5.2, 5.3, 5.4).

**Fig. 5.2: Typical chromatogram of acid stressed sample solution.**
5.3.3.1.3 Limits of detection and quantification:

The LOD and LOQ was determined at a signal to noise ratio of 3:1 and 10:1 respectively, by injecting a series of dilute test solutions of known concentrations. Precision study was also carried out at the LOQ level by injecting six preparations and calculating the percentage of R.S.D of the area as shown in Table 5.3.

5.3.3.1.4 Linearity:

Solutions for testing the linearity of the related substances were prepared by diluting the impurity stock solution to six different concentrations from the LOQ to 150% of the permitted maximum level of the impurity (i.e. the LOQ to 0.6% for Mesalamine and all impurities for an
analyte concentration of 500 μg mL⁻¹. The correlation coefficients, slopes, and y-intercepts of the calibration plots are reported. Calibration plots for the six related substances were linear over the ranges tested. The correlation coefficients was >0.999 for all of the components (Table 3). These results show that there was an excellent correlation between the peak area and concentration for the six impurities.

5.3.3.1.5 Precision:

The precision of the method was verified by repeatability and intermediate precision. Repeatability was checked by injecting six individual preparations of the real sample of Mesalamine spiked with 0.30% of its six impurities. The intermediate precision of the method was also evaluated using a different analyst and a different instrument, and performing the analysis on different day. %RSD of area for each impurity was calculated for both precision as well as intermediate precision, and was found to be within 2%. These results confirmed the precision and ruggedness of the method as shown in Table 5.3.

**Table 5.3: Regression and precision data.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>LOQ μg/ml</th>
<th>LOD μg/ml</th>
<th>Regression equation (y)</th>
<th>Correlation coefficient</th>
<th>Precision (% RSD)</th>
<th>Precision LOQ (% RSD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Slope (b)</td>
<td>Intercept (a)</td>
<td>Intra day</td>
<td>Inter day</td>
</tr>
<tr>
<td>Mesalamine</td>
<td>0.20</td>
<td>0.07</td>
<td>24781</td>
<td>+1471</td>
<td>0.9998</td>
<td>0.54</td>
</tr>
<tr>
<td>Imp. A</td>
<td>0.23</td>
<td>0.08</td>
<td>21456</td>
<td>+1025</td>
<td>0.9997</td>
<td>0.25</td>
</tr>
<tr>
<td>Imp. B</td>
<td>0.17</td>
<td>0.05</td>
<td>39124</td>
<td>+1054</td>
<td>0.9999</td>
<td>0.36</td>
</tr>
<tr>
<td>Imp. C</td>
<td>0.19</td>
<td>0.06</td>
<td>32154</td>
<td>-953</td>
<td>0.9996</td>
<td>0.41</td>
</tr>
<tr>
<td>Imp. D</td>
<td>0.15</td>
<td>0.05</td>
<td>50124</td>
<td>+1247</td>
<td>0.9997</td>
<td>0.41</td>
</tr>
<tr>
<td>Imp. E</td>
<td>0.17</td>
<td>0.08</td>
<td>41021</td>
<td>-597</td>
<td>0.9998</td>
<td>0.45</td>
</tr>
<tr>
<td>Imp. F</td>
<td>0.21</td>
<td>0.07</td>
<td>21756</td>
<td>+998</td>
<td>0.9996</td>
<td>0.23</td>
</tr>
</tbody>
</table>
5.3.3.1.6 Accuracy:

For the impurities, recovery was determined in triplicate for LOQ, 0.15, 0.30, and 0.45% of the analyte concentration (500 μg mL⁻¹) for Mesalamine, and then recovery of the impurities was also calculated as shown in Table 5.4.

Table 5.4. Evaluation of accuracy.

<table>
<thead>
<tr>
<th>Amount spiked</th>
<th>LOQ</th>
<th>50%</th>
<th>100%</th>
<th>150%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesalamine</td>
<td>98.1 ± 0.52</td>
<td>100.1 ± 0.41</td>
<td>99.9 ± 0.58</td>
<td>100.1 ± 0.14</td>
</tr>
<tr>
<td>Imp. A</td>
<td>99.1 ± 0.45</td>
<td>99.2 ± 0.52</td>
<td>97.5 ± 0.64</td>
<td>98.9 ± 0.28</td>
</tr>
<tr>
<td>Imp. B</td>
<td>99.5 ± 0.74</td>
<td>99.6 ± 0.71</td>
<td>98.8 ± 0.28</td>
<td>98.9 ± 0.14</td>
</tr>
<tr>
<td>Imp. C</td>
<td>97.5 ± 0.46</td>
<td>99.0 ± 0.84</td>
<td>100.1 ± 0.45</td>
<td>98.1 ± 0.58</td>
</tr>
<tr>
<td>Imp. D</td>
<td>97.9 ± 0.85</td>
<td>99.7 ± 0.54</td>
<td>99.9 ± 0.12</td>
<td>99.5 ± 0.18</td>
</tr>
<tr>
<td>Imp. E</td>
<td>98.9 ± 0.74</td>
<td>100.5 ± 0.54</td>
<td>98.2 ± 0.58</td>
<td>98.8 ± 0.38</td>
</tr>
<tr>
<td>Imp. F</td>
<td>99.9 ± 0.63</td>
<td>99.4 ± 0.68</td>
<td>98.6 ± 0.88</td>
<td>98.7 ± 0.28</td>
</tr>
</tbody>
</table>

5.3.3.1.7 Robustness:

The purpose of robustness test is to check whether the chromatographic performance is affected by small deliberate changes in operating conditions. In order to study the effect of flow rate on resolution, it was changed to 0.12 and 0.17 mL min⁻¹. The effect of pH was studied at pH 1.8 and 2.2 for solvent A and pH 5.8 and 6.2 for solvent B. The effect of column temperature was studied at 25 and 35 °C. In all of these experiments the mobile phase components were not changed. The effect of the percent organic strength on resolution was studied by varying acetonitrile by −10% to +10%, while other mobile phase components were held constant. In all of the deliberately varied chromatographic conditions, the selectivity as well as the performance of the method were unchanged, which proves the robustness of the method.

5.3.3.1.8 Stability in solution and in the mobile phase:

The solution stability of Mesalamine test preparation and standard preparation was carried out upto 48 hrs at room temperature. The chromatograms of these solutions were
recorded separately with an interval of 1 h up to 24 h and the peak responses was compared. It has been observed that the standard and test solution of mesalamine is stable upto 24 hrs at room temperature. The mobile phase stability was carried out by analyzing the freshly prepared test sample and standard preparation. The same mobile phase was used throughout the experiment. A system suitability parameters for all results were compared and it has been observed that the mobile phase is stable upto 48 hrs.

5.3.4. CONCLUSION:

A gradient RP-UPLC method was successfully developed for the estimation of mesalamine related impurities in pharmaceutical dosage form. Method is precise, accurate, linear, robust, rugged, and specific. A satisfactory result was obtained from validation of the method. Exposure of mesalamine drug product to stress conditions indicates that the drug is susceptible to acid, base hydrolysis; oxidation, photolysis and heat degradation with maximum degradation observed in base and oxidative conditions. A stability-indicating method was developed, which separates all the degradation products formed under variety of conditions.

5.4. VALIDATED UPLC METHODS FOR IN VITRO DISSOLUTION STUDIES OF TWO DIFFERENT ANTIHYPERTENSIVE FORMULATIONS IN TABLET DOSAGE FORM.

For in vitro dissolution study of any drug product, numbers of analyzed samples are large in number, So there is need of lesser run time method for the analysis of these samples. To the best of our knowledge reported analytical methods in the literature survey are having run time more than five minutes. So to overcome this problem UPLC method is best choice for reducing the run time and increase the output of analysis. Literature survey also reveals that there is no one UPLC method is reported for in vitro dissolution study of these two formulations. The present work describes analytical parameters aimed to achieve an alternative for the quantification of Dronedarone HCl tablet formulation and Telmisartan and hydrochlorothiazide tablet formulation by UPLC method with shorter run time i.e. 1 minutes and 3 minutes respectively for in vitro dissolution study in tablet dosage form.
A simple, rapid, and robust RP-UPLC methods has been developed and validated for Dronedarone HCl tablet and Telmisartan and Hydrochlorothiazide tablet as two different formulations at 290nm and 265nm wavelength respectively, in order to assess in vitro drug release profile of drug product. Acquity UPLC BEH C18 50 x 2.1mm, 1.7µm column is used for both the formulation. Purified water adjusted pH 2.5 with orthophosphoric acid is used as solution A and acetonitrile is used as solution B. For Dronedarone HCl tablets 45:55 v/v solution A and solution B is used as mobile phase, where as for Telmisartan and Hydrochlorothiazide tablets mobile phase gradient program was set as time (min) / % solution B: 0/10, 1/10, 2.0/90, 2.5/10 and 3.0/10. The flow rate was 0.5 mL min⁻¹, The column temperature was maintained at 40 ºC. The UPLC method and dissolution test condition were validated to meet requirement of ICH guideline and this validation inferred from specificity, precision, accuracy, linearity and robustness. In addition filter suitability, standard and sample solution stability was demonstrated. All results were acceptable and this confirmed that the method is suitable for its intended use in routine in vitro dissolution study of drug products.

5.4.1. DRUG PROFILE:

5.4.1.1 Dronedarone HCl:

1. Chemical Name:
   N-{2-butyl-3-[4-(3-dibutylaminopropoxy)benzoyl]benzofuran-5-yl}methanesulfonamide, hydrochloride

2. Chemical Structure:
3. Molecular formula: $C_{31}H_{44}N_2O_5S$
4. Molecular Weight: 556.76
5. Description: white fine powder
7. Melting Point: 141.2 °C
8. Category: Cardiac arrhythmias

5.4.1.2 Telmisartan:
1. Chemical Name: 4’-[(1, 4’-Dimethyl-2’-propyl-[2, 6’-bi-1H-benzimidazol]-1’y1) methyl]-[1, 1’-biphenyl]-2-carboxylic acid
2. Chemical Structure:

![Chemical Structure](image)

3. Molecular Formula: $C_{33}H_{30}N_4O_2$
4. Molecular Weight: 514.63
5. Description: Fine, white powder
7. Melting Point: 261.263 °C
8. Category: Hypertension
5.4.1.3 *Hydrochlorothiazide*:

1. Chemical Name: 6-Chloro-3, 4-dihydro-2H-1, 2, 4-benzothiazidin-7-sulfonamide-1,1-dioxide
2. Chemical Structure:

3. Molecular Formula: C$_7$H$_8$ClN$_3$O$_4$S$_2$
4. Molecular Weight: 297.74
5. Description: White crystalline powder
7. Melting Point: 274 °C
8. Category: Hypertension

5.4.2. **EXPERIMENTAL:**

5.4.2.1 *Working standards*:

The working standard was procured from India market having following batch number and potency.

<table>
<thead>
<tr>
<th>Working Standard</th>
<th>Batch Number</th>
<th>% Potency (on as is basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dronedarone HCl</td>
<td>DR00258</td>
<td>99.2</td>
</tr>
<tr>
<td>Telmisartan HCl</td>
<td>TL00132</td>
<td>99.5</td>
</tr>
<tr>
<td>Hydrochlorothiazide</td>
<td>HZ00132</td>
<td>99.1</td>
</tr>
</tbody>
</table>
5.4.2.2 Sample:
The sample was procured from India market. The test sample bears following details.

<table>
<thead>
<tr>
<th>Sample details</th>
<th>Dronedarone HCl tablet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufacturer</td>
<td>Emcure pharmaceuticals Ltd.</td>
</tr>
<tr>
<td>Product Name</td>
<td>Dilsave</td>
</tr>
<tr>
<td>Label Claim</td>
<td>400 mg</td>
</tr>
<tr>
<td>Sample details</td>
<td>Telmisartan and Hydrochlorothiazide tablet</td>
</tr>
<tr>
<td>Manufacturer</td>
<td>Dr. Reddys Ltd.</td>
</tr>
<tr>
<td>Product Name</td>
<td>Telsartan H</td>
</tr>
<tr>
<td>Label Claim</td>
<td>40mg/12.5 mg, 80 mg /12.5 mg,</td>
</tr>
</tbody>
</table>

5.4.2.3 Instrument / Apparatus Used:
I) All the glassware used for the experiment were certified ‘A’ grade manufactured by SCHOTT Glass India Pvt. Ltd. Mumbai, India
II) waters Acquity series comprised of degasser, quaternary pump, auto injector, column compartment,
III) A calibrated digital pH meter, manufactured by Mettler-Toledo Inc. Columbus, OH. Private Limited Mumbai, India.
IV) A calibrated analytical balance, manufactured by Sartorius, Germany.
V) A sonicator, manufactured by Amrut Enterprises, Pune, India.
VI) A vacuum oven manufactured by Quality instruments and equipments, Mumbai, India.

5.4.2.4 Reagents and Chemicals:
All reagents and chemicals were used from Merck chemicals. Orthophosphoric acid were used as GR grade. Acetonitrile and Water were used as HPLC grade.

5.4.2.5 Solution preparation:
A stock solution of Dronedarone HCl (1000 μg mL⁻¹) was prepared by dissolving an appropriate amount of working standard in solvent mixture. Standard solutions containing 400 μg mL⁻¹ were prepared from this stock solution in dissolution medium. Two different stock
solution of Telmisartan (880 µg mL$^{-1}$) and Hydrochlorothiazide (250 µg mL$^{-1}$) was prepared by dissolving an appropriate amount of working standard in methanol. One Standard solution containing Telmisartan (44 µg mL$^{-1}$) and Hydrochlorothiazide (12.5 µg mL$^{-1}$) as prepared from the stock solution in dissolution medium. Another Standard solutions containing Telmisartan (88 µg mL$^{-1}$) and Hydrochlorothiazide (12.5 µg mL$^{-1}$) was prepared from stock solution in dissolution medium. UPLC chromatogram of Dronedardone tablet is as shown in figure 5.5, where as Telmisartan and Hydrochlorothiazide tablet is as shown in figure 5.6.

![Fig. 5.5: Typical UPLC chromatogram of Dronedarone sample solution.](image-url)

![Fig.5.6: Chromatogram of Telmisartan and Hydrochlorothiazide sample solution.](image-url)
5.4.2.6 Chromatographic conditions for UPLC method:

Chromatographic parameters for in vitro dissolution study of Dronedarone tablets was chromatographic column used was an Acquity UPLC BEH C18 50 x 2.1mm, 1.7µm. The flow rate was 0.5 mL min⁻¹ and column temperature was maintained at 40 ºC. Mobile phase consists of Solution A as purified water adjusted pH 2.5 with Orthophosphoric acid and Solution B is Acetonitrile. The detection was monitored at a wavelength of 290 nm. The injection volume was 0.50 µL. A mixture of water and acetonitrile in the proportion of 50:50 (v/v) used as a solvent or diluent. Similary Chromatographic parameters for in vitro dissolution study of Telmisartan and Hydrochlorothiazide tablets was chromatographic column Acquity UPLC BEH C18 50 x 2.1mm, 1.7µm. The flow rate was 0.5 mL min⁻¹ and column temperature was maintained at 40 ºC. Mobile phase consists of Solution A as purified water adjusted pH 2.5 with Orthophosphoric acid and Solution B is acetonitrile the detection was monitored at a wavelength 265 nm. The injection volume was 0.50 µL.

5.4.2.7 Procedure:

UPLC system was set up as described under chromatographic conditions. Standard and sample solution was prepared according to 4.3.5 and made single injection of each of solvent mixture as a blank, standard solution (six injections), placebo solution and sample solution in to the chromatographic system.

- Recorded the chromatograms at 265 nm and measure the peak area counts for all eluting peaks.
- Examined the blank and placebo chromatogram for any extraneous peaks and disregard corresponding peaks observed in the chromatogram of the sample solution.

5.4.2.8 Calculations:

A) Calculation for Dronedarone HCl:

\[
\% \text{ Drug Release of Dronedarone HCl} = \frac{\text{AT} \times \text{WS} \times \text{DT} \times \text{P} \times 100}{\text{AS} \times \text{DS} \times 1 \times 100 \times \text{LC}}
\]
Where,

- \( AT \): Area of Dronedarone peak in sample solution.
- \( AS \): Average area of Dronedarone peak in standard solution.
- \( WS \): Weight Dronedarone standard in mg.
- \( DS \): Dilution of Dronedarone standard in ml.
- \( DT \): Dilution of Dronedarone sample solution.
- \( P \): Potency of Dronedarone HCl working standard on as is basis.
- \( LC \): Label claim of Dronedarone in mg per tablet.

**B) Calculation for Telmisartan:**

\[
\% \text{ Drug Release of Telmisartan} = \frac{AT_T \times WS_T \times DT_T \times P_T}{100} \times \frac{1}{AS_T \times DS_T \times LC_T}
\]

Where,

- \( AT_T \): Area of Telmisartan peak in sample solution.
- \( AS_T \): Average area of Telmisartan peak in standard solution.
- \( WS_T \): Weight Telmisartan standard in mg.
- \( DS_T \): Dilution of Telmisartan standard in ml.
- \( DT_T \): Dilution of Telmisartan sample solution.
- \( P_T \): Potency of Telmisartan HCl working standard on as is basis.
- \( LC_T \): Label claim of Telmisartan in mg per tablet.

**C) Calculation for Hydrochlorothiazide:**

\[
\% \text{ Drug Release of Hydrochlorothiazide} = \frac{AT_H \times WS_H \times DT_H \times P_H}{100} \times \frac{1}{AS_H \times DS_H \times LC_H}
\]

Where,

- \( AT_H \): Area of Hydrochlorothiazide peak in sample solution.
- \( AS_H \): Average area of Hydrochlorothiazide peak in standard solution.
Development of UPLC methods for the determination of drug products.

**WS_H**: Weight Hydrochlorothiazide standard in mg.

**DS_H**: Dilution of Hydrochlorothiazide standard in ml.

**DT_H**: Dilution of Hydrochlorothiazide sample solution.

**P_H**: Potency of Hydrochlorothiazide working standard on as is basis.

**LC_H**: Label claim of Hydrochlorothiazide in mg per tablet.

### 5.4.3. RESULT AND DISCUSSION:

#### 5.4.3.1 Method development and optimization:

As number of test samples in dissolution study are large in number, so the main objective of the present study is shorter run time UPLC method for determination of Dronedarone HCl with run time less than 2 minutes and for Telmisartan and Hydrochlorothiazide less than 4 minutes. To work on the cost effectiveness of the method it has been tried to use same column and mobile phase for the analysis of these two formulations. So different columns with different particle size has been tried i.e. 2.5µm, 2.0µm and 1.7 µm. It has been observed that 2.5µm, 2.0µm particle size column are unable to provide the shorter run time compare to 1.7 µm particle size column. Different type of stationary phases such as C8 and C18 has been tried. On C8 type of stationary phase Dronedarone is elutes in void volume where as in C18 type of stationary phase retention of Dronedarone has been observed. Buffer pH 2.0 to 4.5 has been tried it has observed that higher pH provides the more retention of Telmisartan compare to lower pH, so finally 2.5 pH gives expected result. Different mobile phase combination of buffer (pH 2.5) : acetonitrile in the range of 90:10 to 10: 90 v/v has been tried. Mobile phase ratio buffer : acetonitrile 45:55 v/v provides the expected retention time for Dronedarone. Whereas gradient mobile phase in the ratio of time (min) / % solution B: 0/10, 1/10, 2.0/90, 2.5/10 and 3.0/10 the expected retention time for Telmisartan and Hydrochlorothiazide.

During the optimization of the method the chromatographic column used was an Acquity UPLC BEH C18 50 x 2.1mm, 1.7µm. with Mobile phase used as purified water adjusted pH 2.5 with Orthophospheric acid (solution A )and Acetonitrile(solution B) in the ratio 45:55 (v/v) for dronedarone whereas gradient mobile phase in the ratio of time (min) / % solution B: 0/10, 1/10, 2.0/90, 2.5/10 and 3.0/10 for Telmisartan and Hydrochlorothiazide. The flow rate was
Development of UPLC methods for the determination of drug products.

0.5 mL min\(^{-1}\). The column temperature was maintained at 40 °C and the detection was monitored at a wavelength 290 nm for Dronedarone whereas 265 nm for Telmisartan and Hydrochlorothiazide.

5.4.3.2 Validation of method:

The optimized chromatographic conditions were validated by evaluating specificity, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), robustness and system suitability, in accordance with ICH guidelines Q2 (R1)\(^{56-58}\).

5.4.3.2.1 Specificity:

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Peak purity of analytes in the presence of blank and Placebo is checked where purity angle is less than the purity threshold is observed for all analytes.

5.4.3.2.2 Limits of detection and quantification:

LOD and LOQ determination for Dronedarone, Telmisartan and Hydrochlorothiazide was estimated by their respective methods, as the amounts for which the signal-to-noise ratio was 3:1 and 10:1 respectively, by injecting a series of dilute solutions of known concentrations. The results of LOD and LOQ determination is reported in table 5.5.

5.4.3.2.3 Linearity:

Linearity was established by analyzing six concentrations of Dronedarone HCl ranging between 10% to 150% of test concentration. By plotting the peak area ratio against the corresponding concentration. The correlation coefficients, slopes, and y-intercepts of the calibration plots are reported. The correlation coefficients were >0.999 (Table II). These results show there was an excellent correlation between the peak area and concentration for the Dronedarone HCl.
5.4.3.2.4 Precision:

Precision of the method was determined in relation to repeatability (intraday) and intermediate precision (interday). In order to evaluate the repeatability of the methods, six samples were determined during the same day. The intermediate precision of the method was evaluated using different analyst and different instrument, and performing the analysis on different day. % RSD of area was calculated for both precision as well as intermediate precision and was found within 2%. These results confirmed the precision and ruggedness of the method (Table 5.5).

**Table 5.5: Regression and precision data**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Precision %RSD</th>
<th>Linearity range µg/mL</th>
<th>Slope</th>
<th>LOD µg/mL</th>
<th>LOQ µg/mL</th>
<th>Intercept</th>
<th>Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dronedarone</td>
<td>0.21</td>
<td>40-600</td>
<td>441451</td>
<td>0.04</td>
<td>0.1</td>
<td>-215.15</td>
<td>0.9997</td>
</tr>
<tr>
<td>Telmisartan</td>
<td>0.15</td>
<td>8.8-133.2</td>
<td>514321</td>
<td>0.007</td>
<td>0.018</td>
<td>+315.56</td>
<td>0.9994</td>
</tr>
<tr>
<td>Hydrochlorothiazide</td>
<td>0.31</td>
<td>1.38-20.88</td>
<td>321457</td>
<td>0.002</td>
<td>0.006</td>
<td>-784.23</td>
<td>0.9996</td>
</tr>
</tbody>
</table>

5.4.3.2.5 Robustness:

The effect of pH was studied at pH 2.3 and 2.7. The effect of column temperature was studied at 35 and 45 °C. Effect of flow rate at 0.4 mL min⁻¹ and 0.6 mL min⁻¹ was studied. In all these experiments the mobile phase components were not changed. The effect change in organic strength on retention time of analyte was studied by varying concentration of acetonitrile by −10 to +10% while other mobile phase components were held constant. In all the deliberate varied chromatographic conditions the selectivity as well as the performance of the method were unchanged proves the robustness of the method.

5.4.3.2.6 Accuracy:

Recovery was determined in triplicate analysis of spiked sample for 50%, 100% and 150% of the analyte concentration (400 µg mL⁻¹) Dronedarone HCl, Telmisartan (88 µg mL⁻¹) and Hydrochlorothiazide (12.5 µg mL⁻¹). This recovery study is performed Respective dissolution media of analytes. recovery was calculated. (Table 5.6).
Table 5.6: Results of recovery study

<table>
<thead>
<tr>
<th>Compound</th>
<th>Wt. Spiked(mg)</th>
<th>Wt. Recovered (mg)</th>
<th>% Recovery</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dronedarone</td>
<td>200.52</td>
<td>200.20</td>
<td>99.8</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>400.21</td>
<td>400.30</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>600.33</td>
<td>600.12</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>Telmisartan</td>
<td>20.24</td>
<td>20.12</td>
<td>99.4</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>80.16</td>
<td>80.20</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>120.4</td>
<td>120.31</td>
<td>99.9</td>
<td></td>
</tr>
<tr>
<td>Hydrochlorothiazide</td>
<td>6.32</td>
<td>6.29</td>
<td>99.5</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>12.51</td>
<td>12.45</td>
<td>99.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>18.82</td>
<td>18.78</td>
<td>99.8</td>
<td></td>
</tr>
</tbody>
</table>

5.4.3.2.7 Stability in solution and in the mobile phase:

No significant change in the amounts of the analytes was observed during solution stability. The results from solution stability and mobile phase stability experiments confirmed that standard solutions and sample was stable for up to 24 h during determination of dissolution. The mobile phase was stable up to 48h.

5.4.4. CONCLUSION:

The RP-UPLC method developed for quantitative analysis of Dronedarone HCl in tablet dosage form, Telmisartan and Hydrochlorothiazide tablet dosage form is precise, accurate, linear, robust, rugged and specific. Satisfactory results were obtained from validation of the method.

5.5. REFERENCES:


Development of UPLC methods for the determination of drug products.


[13] High-performance liquid-chromatographic determination of 5-aminosalicylic acid and its...


Development of UPLC methods for the determination of drug products.


[52] On the Reproducibility of Column Performance in Liquid Chromatography and the Role
Development of UPLC methods for the determination of drug products.


