Chapter VI

Development and validation of an UPLC method for fast, sensitive and simultaneous determination of sartans in active pharmaceutical ingredients and its residues on stainless steel surface in formulation area
Introduction

Angiotensin II receptor blockers (ARBs) represent a class of effective and well tolerated orally active antihypertensive drugs\cite{1,2}. AT\textsubscript{1}-receptor antagonists or sartans, are a group of pharmaceuticals which modulate the renin-angiotensin-aldosterone system. Their main uses are in the treatment of hypertension (high blood pressure), diabetic nephropathy (kidney damage due to diabetes) and congestive heart failure. Angiotensin II receptor blockers are primarily used for the treatment of hypertension where the patient is intolerant of ACE inhibitor therapy. They do not inhibit the breakdown of bradykinin or other kinins, and are thus only rarely associated with the persistent dry cough and/or angioedema that limit ACE inhibitor therapy. More recently, they have been used for the treatment of heart failure in patients intolerant of ACE inhibitor therapy, particularly Candesartan. Irbesartan and Losartan have trial data showing benefit in hypertensive patients with type II diabetes, and may delay the progression of diabetic nephropathy. Candesartan is used experimentally in preventive treatment of migraine\cite{3,4}. Lisinopril has been found less often effective than Candesartan at preventing migraine.\cite{5} Activation of AT\textsubscript{1} receptors leads to vasoconstriction, stimulation of the release of catecholamines and antidiuretic hormone and promote growth of vascular and cardiac muscle\cite{2}. AT\textsubscript{1} receptor blockers antagonize all those effects.

Losartan was the first drug of this class marketed, shortly followed by Valsartan, Irbesartan, Telmisartan, Candesartan and others\cite{2}. Most of the ARBs are tetrazole derivatives that exclude telmisartan which contains two imidazole rings (Fig: 6.1). ARBS are generally comes under class II drug of BCS classification (low solubility and high permeability). But Valsartan belongs to the BCS class III drug classified as low permeability and high solubility drug. Due to its poor solubility, most of the sartans are difficult to remove from production equipment.

In the pharmaceutical industry, an important step in the manufacture of pharmaceutical products is the cleaning of equipment and surface\cite{3}. Inadequate cleaning of a pharmaceutical manufacturing plant or inadequate purging of the individual pieces of equipment used in multi-product manufacturing or equipment not dedicated to individual
products may lead to contamination of the next batch of pharmaceutics manufactured using the same equipment. Challenges for cleaning validation are encountered especially when developing sensitive analytical methods capable of detecting traces of active pharmaceutical ingredients that are likely to remain on the surface of the pharmaceutical equipment after cleaning. A method's inability to detect some residuals could mean that either the method is not sensitive enough to the residue in question or the sampling procedure is inadequate\textsuperscript{[4]}. To validate the cleaning procedure there must be sensitive and reproducible analytical method. The aim of this study was to demonstrate the applicability of UPLC by developing a simple, accurate, precise method to determine the residues of four sartans in support of cleaning validation.

**Valsartan**

![Chemical structure of Valsartan](image)

Chemical Formula: $\text{C}_{24}\text{H}_{29}\text{N}_{5}\text{O}_{3}$

CAS number : 137862-53-4

Molecular weight : 435.519

IUPAC Name : $(S)$-3-methyl-2-$(N\cdot[N\cdot[2\cdot(2H\cdot1,2,3,4-tetrazol\cdot5\cdotyl)biphenyl\cdot4\cdot yl]methyl]pentanamido)butanoic$ acid
Candesartan

Chemical Formula: \( \text{C}_{24}\text{H}_{20}\text{N}_{6}\text{O}_{3} \)

CAS number: 139481-59-7

Molecular weight: 440.45

IUPAC Name: 2-ethoxy-1-((4-[2-(2H-1,2,3,4-tetrazol-5-yl)phenyl]phenyl)methyl)-1H-1,3-benzodiazole-6-carboxylic acid

Irbesartan

Chemical Formula: \( \text{C}_{25}\text{H}_{28}\text{N}_{6}\text{O} \)

CAS number: 138402-11-6

Molecular weight: 428.53

IUPAC Name: 2-butyl-3-((4-[2-(2H-1,2,3,4-tetrazol-5-yl)phenyl]phenyl)methyl)-1,3-diazaspiro[4.4]non-1-en-4-one
Telmisartan

Chemical Formula: \( \text{C}_{33}\text{H}_{30}\text{N}_{4}\text{O}_{2} \)

CAS number : 144701-48-4

Molecular weight : 514.617

IUPAC Name : 2-(4-\{[4-methyl-6-(1-methyl-1\text{-}H-1,3-benzodiazo-2-yl)-2-propyl-1\text{-}H-1,3-benzodiazo-1-yl]methyl\}phenyl)benzoic acid

Fig: 6.1 Structure of sartans

Literature review

Several reported methods are available for determinations of assay of both these drugs individually or simultaneously with other drugs but have not come across any simultaneous determination method for determination of all four sartans. This experiment aims to achieve very short run times which have not yet been reported.

A few methods have been reported for determination of Candesartan- Ganesh, et al. reported - RP- HPLC method development and validation of Candesartan Cilexetil in Bulk and Their Pharmaceutical dosage forms\(^8\). Kamalakkannan, et al. reported - Analytical method development and validation for Candesartan Cilexetil as bulk drug and in pharmaceutical dosage forms by HPLC\(^9\). Subba Rao, et al. reported - A stability-indicating LC method for Candesartan cilexetil"\(^10\). Naseem AC, et al. reported - Determination of Candesartan cilexetil in tablet dosage forms and dissolution testing samples by first derivative UV spectrophotometric method\(^11\). İncilay Süslü, et al. reported - Square-wave adsorptive
stripping voltammetric determination of candesartan cilexetil in pharmaceutical formulations\textsuperscript{[12]}. Süslü I, et al. reported - Voltammetric determination of candesartan cilexetil in its Cu (II) complex and application to pharmaceutical formulations\textsuperscript{[13]}.

A few methods have been reported for determination of Irbesartan- Kamepall, et al. reported - RP-HPLC-DAD Method for Determination of Irbesartan in Bulk and Tablets Exposed to Forced Conditions\textsuperscript{[14]}. Kishanta, et al. reported - Method Development, Validation and Stability Study of Irbesartan in Bulk and Pharmaceutical Dosage Form by UV-Spectrophotometric Method\textsuperscript{[15]}. Gupta, et al. reported - Electrochemical determination of antihypertensive drug Irbesartan in pharmaceuticals\textsuperscript{[16]}. Hanaa, et al. reported - Voltammetry of Irbesartan Drug in Pharmaceutical Formulations and Human Blood, Quantification and Pharmacokinetic Studies\textsuperscript{[17]}. Dhanawade, et al. reported - Derivative Spectrophotometric Method for Estimation of Irbesartan in Bulk Drug and Dosage form\textsuperscript{[18]}.

A few methods have been reported for determination of Telmisartan-Sujana K, et al. reported - Stability indicating RP HPLC method for the determination of Telmisartan in pure and pharmaceutical formulation\textsuperscript{[19]}. Phani Kishore, et al. reported - Development and validation of stability indicating HPLC method for the estimation of Telmisartan related substances in tablets formulation\textsuperscript{[20]}. Sahu, et al. reported - Comparative Study of Forced Degradation Behavior of Telmisartan by UPLC and HPLC and Development of Validated Stability Indicating Assay Method According to ICH Guidelines\textsuperscript{[21]}. Sagar, et al reported - Development of UV Spectrophotometric Method of Telmisartan in Tablet Formulation\textsuperscript{[22]}. Ajit Pandey, et al reported - UV-Spectrophotometric Method for estimation of Telmisartan in Bulk and Tablet Dosage Form\textsuperscript{[23]}. MS Palled, et al reported - Difference spectrophotometric determination of telmisartan in tablet dosage forms\textsuperscript{[24]}.

A few methods have been reported for determination of Valsartan - Bhatia M. et al. reported - Determination and validation of valsartan and its degradation products by isocratic HPLC\textsuperscript{[25]}. Vinzuda D.U., et al. reported - RP-HPLC Method for Determination of Valsartan in Tablet Dosage Form\textsuperscript{[26]}. G Thanusha, et al. reported - Validated RP-
HPLC Method for the Quantitative Estimation of Valsartan in Bulk and a Pharmaceutical Dosage Forms\textsuperscript{[27]}. K.R.Gupta, et al. reported - UV-Spectrophotometric methods for estimation of Valsartan in bulk and tablet dosage form\textsuperscript{[28]}. Habib IH, et al. reported - Stripping voltammetric determination of valsartan in bulk and pharmaceutical products\textsuperscript{[29]}.

To the best of the author’s knowledge no method is available in the literature for simultaneous determination of sartans (Candesartan, Telmisartan, Irbesartan and Valsartan) and their impurities by UPLC. The current chapter thus describes a unique and novel method for simultaneous determination of these drugs using UPLC.

Materials and methods

Reagents and Chemicals

HPLC gradient grade ACN and methanol from Merck (Mumbai, India) has been used. Potassium dihydrogen phosphate (AR grade), ortho phosphoric acid and triethylamine Solution from Merck have been used. Demineralized water was further purified in the laboratory by filtering through an ultrapure Milli-Q (Millipore, Milford, MA, USA). The drug substances, standards and impurities required for this work were obtained from Dr Reddy’s laboratories ltd.

Instrumentation and liquid chromatographic conditions

Chromatographic separation was carried out on a Waters Aquity UPLC with photodiode array detector. The output signal was monitored and processed using Empower 2 software. The mobile phase buffer consisted of 0.01M of potassium dihydrogen phosphate pH adjusted to 2 with diluted ortho phosphoric acid as mobile phase A and aceotnitrile as mobile phase B. The chromatographic separation was performed in gradient mode (min/%B, 0/30, 1.2/46, 2.2/80, 2.5/90, 2.7/30, 3.0/30) The chromatographic separation was carried out in used Kinetex XB C18 (50 mm X 2.1 mm, and 1.7 µm particle size) at flow rate of volume 0.8 ml/min with 1.0 µl injection volume.
The column temperature was at 25°C. UV detection was performed at $\lambda_{\text{max}}$ 225 nm. All the glassware used for the following experimentation is of class A grade to obtain maximum precision.

**Standard preparation for assay for active pharmaceutical ingredient**

Telmisartan standard stock solution: Accurately weighed and transferred Telmisartan working std in 20 ml volumetric flask, dissolved in 10 ml of 0.1N NaOH, added 5ml of acetonitrile and sonicated for 5 minutes and e made up to the volume with water to get final concentration of 3000µg/mL.

Accurately weighed and transferred Candesartan, Irbesartan, Valsartan working standard in 100ml volumetric flask, dissolved in 60 ml of acetonitrile, to this added 10 ml of Telmisartan standard stock solution, and sonicated for 5 minutes and made up to volume with water to get final concentration of 100µg/mL of Candesartan, Irbesartan, Valsartan and 300µg/mL of Telmisartan and filtered through 0.22 µm filter.

**Sample preparation for assay for active pharmaceutical ingredient**

Accurately weighed and transferred Candesartan, Irbesartan, Valsartan and Telmisartan active pharmaceutical ingredient in 100ml volumetric flask, dissolved in 60 ml of acetonitrile and sonicated for 5 minutes made up to volume with water to get final concentration of 100µg/mL of Candesartan, Irbesartan, Valsartan and 300µg/mL of Telmisartan and filtered through 0.22 µm filter.

**Cleaning standard and sample preparation**

The stock solution of standard was prepared by accurately weighing Candesartan, Irbesartan, Valsartan and Telmisartan working standard and transferring to a 100 mL volumetric flask, dissolved in 60 ml of acetonitrile and volume made up to the mark with water to get final concentration of 20µg/mL. The selected surfaces (10 cm×10 cm) of stainless steel, previously cleaned and dried, were sprayed with 1ml of cleaning stock solution, for the positive swab control at all concentration levels, and the solvent was
allowed to evaporate. The surface was wiped in one direction with wet tex swab soaked with extraction solution (4 ml water and 6 ml of methanol) was pipette into swab tube. The background control sample was prepared from the extraction solvent. The negative swab control was prepared in the same way as the samples, using swabs, which had not been in contact with the test surface. Subsequently, the tubes were placed in an ultrasonic bath for 10 min and the solutions were analyzed by UPLC.

Results and Discussion

Acceptance limit calculation

Careful examination of the vessel for trace residues is vital to the pharmaceutical manufacturing process as residues can contaminate subsequent products. The maximum allowable carryover (MACO) is the acceptable transferred amount from the previous to the following product. The MACO is determined based on the therapeutic dose, toxicity and generally 10 ppm criterion. Once the maximum allowable residue limit in the subsequent product was determined, the next step was the determination of the residue limit in terms of the contamination level of active ingredient per surface area of equipment. The total surface area of the equipment in direct contact with the product was accounted for in the calculation. The limit per surface area was calculated from the equipment surface area and the most stringent maximum allowable carryover. The 0.1% dose limit criterion is justified by the principle that an active pharmaceutical ingredient (API) at a concentration of 1/1000 of its lowest therapeutic dose will not produce any adverse effects on human health. The calculated limits per surface area (LSA) in the case of sartans were 2 µg/swab pro 100 cm². A stainless steel surface area of 10 cm×10 cm was chosen for practical reasons.

Optimization of the sample treatment for cleaning method validation

The stainless steel plates were spiked with required quantities of Candesartan, Irbesartan, Telmisartan, valsartan and surface was wiped with tex swab soaked with of different volume of water and methanol as extraction solvent placed into tubes and the tubes were sonicated for different times (5 and 20 min) and the solutions were analyzed by UPLC.
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The optimum conditions were achieved with 40% water and 60% methanol as the extracting solvent and sonication time of 10 min. This technique was applied in the subsequent work.

Method development

The main objective was to develop a single UPLC method that can be used for the estimation of Candesartan, Irbesartan, Telmisartan, Valsartan in active pharmaceutical ingredient, in cleaning samples and in dissolution samples.

For analysis, the combination of 0.1% ortho phosphoric acid, 0.01M KH₂PO₄ (pH 2.5), 0.01M KH₂PO₄ (pH 2.0) with acetonitrile is tried as the mobile phase. The amount of buffer was varied from 30% to 70% and flow rate varied from 0.3 ml min⁻¹ to 1.0 ml min⁻¹.

The sufficient separation, tailing factor and plate number were achieved with the proposed mobile phase (acetonitrile and 0.01M KH₂PO₄ (pH 2.0)) at flow rate 0.8 ml min⁻¹. Wavelength 225nm was selected for detection because the all drug have a sufficient absorption and low quantities can be detected correctly. Furthermore, the calibration curve obtained at 225nm showed good linearity. Regarding the chromatographic procedure, different columns, BEH C18, (50X2.1mm, 1.7µ) Zorbax SB C8 (50x4.6mm, 1.8µ) Kinetix XB-C-18 (50 x 2.1, 1.7µ) were evaluated but the Kinetix XB-C-18 (50 x 2.1, 1.7µ) was preferred to improve the peak symmetry, plate number and resolution. The column temperature was varied from 25 to 40°C but the analysis at 25°C was preferred to improve the peak symmetry, plate number and resolution. The injection volume varied between 1µl to 3 µl, at 1 µl peak symmetry was good with good plate count and resolution. On the basis of solubility of all sartans as well as stability, difluent was selected.

Method robustness evaluation Factorial design

As the final method is selected against method attributes, it is highly likely that the selected method is reliable and will remain operational over the lifetime
of product. Therefore, the evaluation of method robustness is mainly for the method verification and finalization. A risk-based approach based on the QbD principles set out in ICH Q8 and Q9 was applied to the evaluation of method robustness and ruggedness. Structured methodologies for risk assessment, such as Design of experiment can be implemented to identify the potential risk of the due to a small change of method parameters column temperature (25 and 30°C), Mobile phase Buffer pH and flow rate were simultaneously evaluated to assess the effects of these parameters on each of the four response variables. Optimization of the analytical method was tested applying $2^3$ full factorial designs. The experimental domain of the selected factors is shown in Table: 6.1.

**Table: 6.1** Chromatographic conditions and the range investigated during method optimization

<table>
<thead>
<tr>
<th>Factors (chromatographic variables)</th>
<th>Low</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column temperature</td>
<td>25°C</td>
<td>30°C</td>
</tr>
<tr>
<td>Mobile phase buffer pH</td>
<td>2.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.6 ml/min</td>
<td>0.8 ml/min</td>
</tr>
</tbody>
</table>

Resolution (Rs1) between Telmisartan and Irbesartan was chosen as the response parameters. The design matrix of $2^3$ full factorial design methodology shows 12 treatment combinations of a low (−) and high (+) level of the factors Table: 6.2.
Table: 6.2 Model matrix for $2^3$ full factorial design methodologies with response data

<table>
<thead>
<tr>
<th>Runs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
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<td>5</td>
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<td>6</td>
</tr>
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<td>7</td>
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<td>8</td>
</tr>
<tr>
<td>9</td>
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<tr>
<td>10</td>
</tr>
<tr>
<td>11</td>
</tr>
<tr>
<td>12</td>
</tr>
</tbody>
</table>

Based on the results of these experiments, the following statistical parameters and ANOVA equations with model graph that Resolution (Rs1) between Telmisartan and Irbesartan was chosen as the response parameters with the most important chromatographic conditions was derived using Design Expert 8.0.

Table: 6.3 Analysis of variance table for Resolution (Rs1) between Telmisartan and Irbesartan

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>p-value</th>
<th>Prob &gt; F</th>
</tr>
</thead>
</table>

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<table>
<thead>
<tr>
<th>Model</th>
<th>A-Column Temperature</th>
<th>B-Mobile phase buffer pH</th>
<th>C-Flow rate</th>
<th>AB</th>
<th>AC</th>
<th>BC</th>
<th>ABC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.84</td>
<td>0.12</td>
<td>9.71</td>
<td>0.036</td>
<td>0.036</td>
<td>2.93</td>
<td>0.1621</td>
</tr>
<tr>
<td>A-Column Temperature</td>
<td>0.036</td>
<td>0.036</td>
<td>2.93</td>
<td>0.21</td>
<td>0.21</td>
<td>17.22</td>
<td>0.0143</td>
</tr>
<tr>
<td>B-Mobile phase buffer pH</td>
<td>0.21</td>
<td>0.21</td>
<td>17.22</td>
<td>4.500E-004</td>
<td>4.500E-004</td>
<td>0.036</td>
<td>0.8581</td>
</tr>
<tr>
<td>C-Flow rate</td>
<td>4.500E-004</td>
<td>4.500E-004</td>
<td>0.036</td>
<td>0.036</td>
<td>0.036</td>
<td>2.93</td>
<td>0.1621</td>
</tr>
<tr>
<td>AB</td>
<td>0.013</td>
<td>0.013</td>
<td>1.03</td>
<td>0.013</td>
<td>0.013</td>
<td>1.03</td>
<td>0.3669</td>
</tr>
<tr>
<td>AC</td>
<td>0.54</td>
<td>0.54</td>
<td>43.64</td>
<td>0.54</td>
<td>0.54</td>
<td>43.64</td>
<td>0.0027</td>
</tr>
<tr>
<td>BC</td>
<td>2.450E-003</td>
<td>2.450E-003</td>
<td>0.20</td>
<td>2.450E-003</td>
<td>2.450E-003</td>
<td>0.20</td>
<td>0.6796</td>
</tr>
</tbody>
</table>

The Model F-value of 9.71 implies the model is significant. There is only a 2.21% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant.

ANOVA equation

\[ Rs1 = +2.93 +0.055 \times A -0.13 \times B -7.500E-003 \times C +0.055 \times A \times B +0.040 \times A \times C+0.26 \times B \times -0.018 \times A \times B \times C \]

3D surface plots:

In order to better estimate how the variables affect the response over the whole experimental domain including any interaction, 3D surface plots for resolution between impurity C and impurity B were constructed using Design expert 8.0.

The resolution between Telmisartan and Irbesartan (Rs1) predicted from 3D surface plots also coincide with observed resolution value which is listed in Table: 6.2. Moreover from the above 3D surface plots, where one parameter kept constant and other parameter can be changed and we can predict resolution by clicking at any point of the contour plot. If we extrapolate to other two axis, it will give corresponding values of the other two variable factors.
Factor C (Flow rate) kept constant:

\[ X_1 = A: \text{Column temperature} \]
\[ X_2 = B: \text{Mobile phase buffer pH} \]
Actual Factor
C: Flow rate = 0.80

Factor B (mobile phase Buffer) kept constant:

\[ X_1 = A: \text{Column temperature} \]
\[ X_2 = C: \text{Flow rate} \]
Actual Factor
B: Mobile phase buffer pH = 2.50
Factor A (column temperature) kept constant:

Fig: 6.2 3D Surface plots for Resolution (Rs1) between Telmisartan and Irbesartan (Rs1)

Cube plots are useful for representing the effects of three factors at a time. The resolution between Telmisartan and Irbesartan (Rs1) predicted from 3D surface plots also coincide with observed resolution value which is listed in Table: 6.2. Moreover from
the above cube plots, by clicking at any of given point, it will give statistical values such as confidence interval, probability interval, standard error mean and standard error prediction.

Method Validation

Validation is required for any new or amended method to ensure that it is capable of giving reproducible and reliable results. Once the chromatographic conditions had been selected, the method was validated, whereby attention was paid to the selectivity, linearity, limit of detection, limit of quantification, precision and accuracy.

Specificity

For Assay Method

Specificity is the ability of the method to accurately measure the analyte response in the presence of all potential sample components. Specificity is the ability of the method to measure the analyte response in presence of its process related impurities. The specificity of the developed HPLC method was performed by injecting blank solution and standard solution of each sartans separately. The chromatograms of sartans were compared with the blank chromatogram, to verify the blank interference. No peak was observed at the retention time of Sartans (Fig: 6.8) Hence the method is specific for the determination of sartans in active pharmaceutical ingredient.

To check the performance of the optimized LC method for the separation of degradation products, the drug was subjected to various stress conditions. The sample solution was employed for acidic, alkaline, thermal and oxidation degradation condition (in 1N HCl for 30 min at 60°C), (in 1 NaOH for 30 min at 60°C) (80°C for 24 h), and (in 10% H₂O₂ for 30 min at 60°C). The samples have been chromatographed according to the experimental method to demonstrate the resolution of the all four sartans from any unknown peaks. All four sartans has chromatographic resolution more than 1.5 from other peaks. The mains peaks were well separated from degradation products peaks;
purity angle of main peaks was more than purity threshold that proves method specificity. The results of degradation studies are shown in Table: 6.4.

**Table: 6.4 Results of degradation studies**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Candesartn</th>
<th>Irbesartan</th>
<th>Telmisartan</th>
<th>Valsartan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample as such</td>
<td>100.96</td>
<td>100.87</td>
<td>100.11</td>
<td>100</td>
</tr>
<tr>
<td>Thermal sample</td>
<td>100.58</td>
<td>95.04</td>
<td>96.29</td>
<td>94.88</td>
</tr>
<tr>
<td>80°C for 24 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acid sample 1N HCL 60°C ½hr</td>
<td>79.29</td>
<td>84.62</td>
<td>88.92</td>
<td>83.21</td>
</tr>
<tr>
<td>Base sample 1N NaOH 60°C ½ hr</td>
<td>0</td>
<td>87.22</td>
<td>87.79</td>
<td>85.53</td>
</tr>
<tr>
<td>10% H₂O₂ sample 60°C ½ hr</td>
<td>7.49</td>
<td>81.14</td>
<td>85.121</td>
<td>86.55</td>
</tr>
</tbody>
</table>

**For Cleaning Method**

The specificity of the cleaning method was checked by injecting standard solution, sample solution, the background control sample, the negative swab control, swabbed un-spiked stainless steel 10 cm×10 cm plate as described.
Fig: 6.5 Chromatogram obtained from Cleaning sample solution

Fig: 6.6 Chromatogram obtained from background control sample

Fig: 6.7 Chromatogram obtained from negative swab control sample
Fig: 6.8 Chromatogram obtained unspiked stainless steel sample

Peak purity has been verified for all of the impurities and for both main peaks. Peak purity shows that impurity peaks as well as main peaks are homogeneous under all the stress conditions. By the above-mentioned fact we can confirm that the method is a stability-indicating method. The chromatograms and purity plots of the stressed samples are shown in Fig: 6.9

Sample As such
Base degraded sample

Telmisartan

Irbesartan

Valsartan

Candesartan

Peroxide degraded sample

Telmisartan

Irbesartan
**Chapter VI**

**Valsartan**

**Candesartan**

**Thermal degraded sample**

**Telmisartan**

**Irbesartan**

**Fig: 6.9** Purity plots for sartans

**Precision**

The precision of the method was evaluated by performing six independent assays of formulation sample; cleaning sample and the % RSD was calculated. The %RSD values for all the four drugs found to be less than 2.

The intermediate precision of the method was investigated by repeating the precision studies on other days by different analyst on different system using reagents from different lot. The intermediate precision, expressed as the %RSD was found to be less than 2. The data obtained suggested that the method exhibited an excellent precision and intermediate precision. The results are given in Table: 6.5.
### Table: 6.5. Results of Precision and Intermediate Precision

<table>
<thead>
<tr>
<th>Name of the API</th>
<th>Precision</th>
<th>Intermediate Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% RSD (Assay sample)</td>
<td>% RSD (Cleaning sample)</td>
</tr>
<tr>
<td>Candesartan</td>
<td>0.68</td>
<td>0.73</td>
</tr>
<tr>
<td>Irbesartan</td>
<td>0.25</td>
<td>0.31</td>
</tr>
<tr>
<td>Telmisartan</td>
<td>0.21</td>
<td>0.47</td>
</tr>
<tr>
<td>Valsartan</td>
<td>0.20</td>
<td>0.56</td>
</tr>
</tbody>
</table>

**Fig: 6.10** Chromatogram obtained from Precision assay sample

**Fig: 6.11** Chromatogram obtained from Cleaning assay sample
Limit of Detection and Quantification

The limit of detection and limit of quantification were determined by means of signal to noise ratio method. Low concentrations of solutions were prepared using the working standards of drugs and injected into the system. The signal to noise ratios of the peaks was checked. The concentration of solution was lowered till the signal to noise ratio is between 2 and 3 for limit of detection (LOD) and signal to noise ration ratio is between 9.5-10.4 for limit of quantification (LOQ). The solution was in six replicates at LOQ concentration level and injected into the system. The % RSD of peak areas was calculated to prove method repeatability at LOQ.

The LOD for Candesartan, Irbesartan, Telmisartan and Valsartan found to be 0.20 µg/ml, 0.15µg/ml, 0.30µg/ml and 0.15µg/ml respectively. The LOQ values found to be as 0.60 µg/ml, 0.40 µg/ml, 0.85µg/ml and 0.50 µg/ml for candesartan, irbesartan, telmisartan and valsartan respectively. % RSD at LOQ found to be 0.43, 0.74, 0.63 and 0.54 for Candesartan, Irbesartan, Telmisartan and Valsartan respectively.

Linearity

Linearity was demonstrated by injecting impurities at limit of quantification level, 25%, 50%, 100%, 150%, 200% and 300% with respect to the specification level of assay and cleaning method. Plotted the calibration curve by taking concentration on X-axis and peak area on Y-axis, calculated the correlation coefficient and % y-intercept (Table: 6.6).

Table: 6.6 Results of Linear regression analysis of sartans

<table>
<thead>
<tr>
<th>Impurity name/ Statistical parameter</th>
<th>Correlation coefficient</th>
<th>Slope</th>
<th>Intercept</th>
<th>Bias at 100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candesartan</td>
<td>0.9994</td>
<td>6320</td>
<td>517.8</td>
<td>0.8892</td>
</tr>
<tr>
<td>Irbesartan</td>
<td>0.9992</td>
<td>9225</td>
<td>174.4</td>
<td>0.4919</td>
</tr>
<tr>
<td>Telmisartan</td>
<td>1.0000</td>
<td>21725</td>
<td>881.4</td>
<td>1.2570</td>
</tr>
<tr>
<td>Valsartan</td>
<td>0.9986</td>
<td>7849</td>
<td>651</td>
<td>2.9548</td>
</tr>
</tbody>
</table>
Accuracy

The accuracy of the method was studied by recovery studies. The sample solution was prepared at six different concentration levels i.e. 25%, 50%, 100%, 150%, 200% & 300%, specified amounts of standard had been added to these solutions and assay of these solutions was performed. The added amounts were calculated in terms of recovery, which were found to be between 98 – 102% (Table: 6.7).

**Table: 6.7** Results of recovery studies of assay samples

<table>
<thead>
<tr>
<th>Level</th>
<th>Candesartan</th>
<th>Irbesartan</th>
<th>Telmisartan</th>
<th>Valsartan</th>
</tr>
</thead>
<tbody>
<tr>
<td>25%</td>
<td>100.97</td>
<td>99.56</td>
<td>100.58</td>
<td>100.48</td>
</tr>
<tr>
<td>50%</td>
<td>99.76</td>
<td>98.12</td>
<td>100.39</td>
<td>100.72</td>
</tr>
<tr>
<td>100%</td>
<td>99.99</td>
<td>101.05</td>
<td>100.71</td>
<td>101.20</td>
</tr>
<tr>
<td>150%</td>
<td>101.42</td>
<td>101.69</td>
<td>99.03</td>
<td>98.95</td>
</tr>
<tr>
<td>200%</td>
<td>99.11</td>
<td>98.40</td>
<td>98.09</td>
<td>99.57</td>
</tr>
<tr>
<td>300%</td>
<td>100.82</td>
<td>98.85</td>
<td>99.96</td>
<td>99.15</td>
</tr>
<tr>
<td>%RSD</td>
<td>0.86</td>
<td>1.47</td>
<td>1.03</td>
<td>0.92</td>
</tr>
</tbody>
</table>
Fig: 6.12 Chromatogram obtained from 50 % accuracy sample

Fig: 6.13 Chromatogram obtained from 75 % accuracy sample

Fig: 6.14 Chromatogram obtained from 100 % accuracy sample
The cleaning sample recovery studies were performed by spiking the cleaning sample with known concentrations of standard. The added amount was calculated in terms of recovery, the assay was performed in six replicates, %RSD was calculated.
which was found to be less than 2 for all the four drugs, that proves method accuracy (Table: 6.8).

Table: 6.8 Results of recovery studies of cleaning samples

<table>
<thead>
<tr>
<th>Level</th>
<th>Candesartan</th>
<th>Irbesartan</th>
<th>Telmisartan</th>
<th>Valsartan</th>
</tr>
</thead>
<tbody>
<tr>
<td>25%</td>
<td>99.02</td>
<td>101.82</td>
<td>101.47</td>
<td>99.71</td>
</tr>
<tr>
<td>50%</td>
<td>99.42</td>
<td>100.82</td>
<td>101.44</td>
<td>99.20</td>
</tr>
<tr>
<td>100%</td>
<td>99.38</td>
<td>99.05</td>
<td>100.62</td>
<td>98.53</td>
</tr>
<tr>
<td>150%</td>
<td>99.21</td>
<td>99.90</td>
<td>100.77</td>
<td>98.95</td>
</tr>
<tr>
<td>200%</td>
<td>97.64</td>
<td>101.64</td>
<td>100.81</td>
<td>99.7</td>
</tr>
<tr>
<td>300%</td>
<td>97.17</td>
<td>98.84</td>
<td>100.84</td>
<td>98.41</td>
</tr>
<tr>
<td>%RSD</td>
<td>0.99</td>
<td>1.28</td>
<td>0.36</td>
<td>0.56</td>
</tr>
</tbody>
</table>

Application of the method to assay dissolution samples

The accurate determination of sartans in complex media such as dissolution requires selective and sensitive analytical methodologies. The dissolution samples of all four sartans were prepared according to current USP dissolution methods. The samples were injected into the system and assay of the solutions was performed. The results are shown in Table: 6.9.

Table: 6.9 Application of method to dissolution studies

<table>
<thead>
<tr>
<th>Dissolution media</th>
<th>Valsartan</th>
<th>Irbesartan</th>
<th>Telmisartan</th>
<th>Candesartan</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1N HCl</td>
<td>100.98</td>
<td>99.73</td>
<td>99.58</td>
<td>NA</td>
</tr>
<tr>
<td>pH 4.5 Acetate Buffer</td>
<td>103.32</td>
<td>95.44</td>
<td>98.02</td>
<td>99.70</td>
</tr>
<tr>
<td>pH 6.8 Phosphate buffer</td>
<td>102.13</td>
<td>100.56</td>
<td>100.33</td>
<td>NA</td>
</tr>
<tr>
<td>pH 7.5 phosphate buffer</td>
<td>NA</td>
<td>NA</td>
<td>98.81</td>
<td>NA</td>
</tr>
</tbody>
</table>
Chapter VI

Conclusion

A simple, accurate, precise LC method is developed for the determination of Candesartan, Irbesartan, Telmisartan and Valsartan. This method has also been validated as per ICH guidelines. Forced degradation studies are carried out by stressing at variety of conditions. All the degradant peaks are well separated from the principle peaks. The method is validated with respect to and found to be precise. The accuracy is carried out on 7 levels from LOQ to 300% of the specification limit and the recoveries of all the peaks are within acceptable limits. The linearity is carried out on 7 levels from LOQ to 300% of the specification limit. The correlation coefficient is found to be more than 0.998 for all the 8 peaks. Limit of detection (LOD) and Limit of quantification (LOQ) results demonstrated the extremely high sensitivity of the method. The method is found to be specific, precise, linear and accurate in the range of its intended application. The QbD based method optimization helped in generating a design space and operating space with knowledge of all method performance characteristics and limitations and successful method robustness within the operating space. A single method can be used for determination these drugs in cleaning swabs and suitable for assay of active pharmaceutical ingredient for these four drugs. This method can be applied to dissolution testing of all the four drugs.

On the basis of this study, it appears that the use of UPLC for the quantification of API residues in cleaning validation samples in product formulation area is practical. The time reducing and solvent saving characteristics of UPLC method are very advantageous, compared to the most widely used conventional HPLC technique. The concept of applying a generic method for several API residues for a product line is feasible and practical if the structure and properties of compounds to be determined are similar.
Chapter VI

References

Papers published:

1. Simultaneous determination of Omeprazole and Domperidone impurities in active pharmaceutical ingredients by UPLC. **(paper accepted on 04-03-13, Ref. M.S.No: 3200)** (International Journal of Pharma and Bio sciences)

2. Development and Validation of a Stability-Indicating RP-UPLC Method for Assay of 1-(4-Hydrozinophenyl) methyl-1,2,4-triazole Dihydrochloride and Estimation of its Related Compounds  

3. Identification and characterization of oligomers of amipilloc acids impurity in ampicillin sodium active pharmaceutical ingredient stability studies.  
   **(International Journal Of Chemical and Pharmaceutical Sciences)** 2012, Sep., Vol.3 (3)

Papers Communicated:

1. Development and validation of stability indicating method for the determination of related substances and assay of sparfloxacin by UPLC  
   **(Journal of liquid chromatography and liquid technologies)**

2. QBD approach to development and validation of HPLC method for the determination of related compounds of Nimesulide in drug substance  
   **(International Journal of Chemical and Analytical Science)**

Papers under Pipeline:

1. A Quality by Design based approach to stability indicating method for determination of related substances of Dexlansoprazole by UPLC

2. Simultaneous determination of Amlodipine Besylate and Benazepril HCl impurities in finished product by UPLC