DEVELOPMENT AND VALIDATION OF HPLC-UV METHOD FOR THE ESTIMATION OF EPROSARTAN IN HUMAN PLASMA

MANISH THIMMA RAJU*, 1SRIKANTH GURRALA, 1J. VENKATESHWAR RAO, 2K.R.S. SAMBASIVA RAO

*Department of Pharmaceutical Analysis, Talla Padmavathi College of Pharmacy, Warangal, Andhra Pradesh, 1Head Department of Biotechnology, Acharya Nagarjuna University, Nagarjuna nagar Guntur, Andhra Pradesh

Email: manishcancer@gmail.com, steev.g99@gmail.com

Received: 03 Jan 2011, Revised and Accepted: 03 Feb 2011

ABSTRACT

A Simple, rapid, selective and sensitive HPLC method was developed and validated for the determination of eprosartan from human plasma. The drug was extracted with a mixture of 0.05M sodium hydroxide and ethyl acetate. Eprosartan was measured in plasma using a validated a HPLC method with UV detector at 235nm. Chromatographic peaks were separated on 5µm Inertsil, C18 column (4.6x250mmx5µm) using 60:40 v/v Phosphate buffer pH4, Acetonitrile as mobile phase at a flow rate of 1 ml/min. The chromatograms showed good resolution and no interference from plasma. The retention time of eporsartan and internal standard were approximately 5.3±0.05 min and 9.1±0.05 min respectively. The mean recovery from human plasma was found to be above 98%. The method was linear over the concentration range of 300 to 20,000ng/ml with coefficient of correlation (r²) 0.9983. Both intraday and interday accuracy and precision data showed good reproducibility. This method was successfully applied to pharmacokinetics studies.

Key words: Eprosartan, Liquid-Liquid Extraction, HPLC-UV Method, Validation.

INTRODUCTION

Eprosartan (fig-1) is an angiotensin II receptor antagonist used for the treatment of high blood pressure. It acts on the renin-angiotensin system in two ways to decrease total peripheral resistance. First, it blocks the binding of angiotensin II to AT1 receptors in vascular smooth muscle, causing vascular dilatation. Second, it inhibits sympathetic norepinephrine production, further reducing blood pressure. A recent advance in the treatment of hypertension and heart failure is the development of a new class of pharmacologic agents, angiotensin II receptor antagonists, which block the effects of the renin-angiotensin system directly at the angiotensin II receptor level. Angiotensin II, the primary mediator of the renin-angiotensin system, plays a major role in the regulation of fluid and electrolyte balance, blood pressure, and blood volume. Eprosartan (Teveten (SK&F 108566)), a new nonpeptide, non-biphenyl, non-tetrazole orally active angiotensin II receptor antagonist that is highly selective for the AT1 receptor subtype. Plasma samples were assayed for eporsartan concentrations using reversed-phase HPLC with UV detection. Three methods have been reported for the determination of eporsartan from plasma. The first two methods described by R.M. Alonso and coworkers was based on use of HPLC with solid phase extraction. These two methods were tedious and time consuming and having lot of solvent preparation steps. While the third method reported by Xue-ning li and coworkers was based on HPLC with mass spectrophotometric detection requires expertise. Ferreirós et al, a method for the quantitation of angiotensin II receptor antagonists (ARA-II) in human plasma. using liquid-chromatography (LC)-electrospray ionization tandem mass spectrometry (MS/MS). This method has been developed with respect to sample clean-up and investigation of ion suppression effects. HU Patel et al, reported simultaneous analysis of eprosartan and hydrochlorothiazide in tablets by HPTLC with ultraviolet absorption. Hence, an attempt was made to develop and optimize an alternative, simple, rigid and sensitive HPLC method for the estimation of Eprosartan from plasma which can be applied to pharmacokinetic study. The results of the analysis were validated by statistical method and recovery studies.

Fig. 1: Chemical structure of Eprosartan

MATERIALS AND METHODS

Eprosartan and losartan were purchased from Pei Li Pharmaceutical Ind.co. Ltd, Taiwan, HPLC grade acetonitrile, ethylacetate, methanol were purchased from SD fine chemicals, Mumbai, India. Analytical Grade Potassium Dihydrogen Phosphate and Sodium Hydroxide were purchased from SD fine chemicals, Mumbai, India. Pooled drug free expired human plasma was purchased from Red Cross Society, Warangal.

Chromatographic conditions

The HPLC system consisted of Alliance Waters 2695 with dual λ Absorbance UV detector. The wavelength of detection was set at 235nm. Separation was carried out on inertsil C18 column(n.6x250mmx5µm) using 60:40v/v phosphate buffer pH4, Acetonitrile as mobile phase at a flow rate of 1 ml/min. The mobile phase filtered through nylon millipore (0.2µm) membrane filter, purchased from Pall life sciences, Mumbai and degassed with ultrasonicator prior to use. Chromatography was carried out at room temperature 25ºC and maintains the column temperature at 32ºC.

Preparation of standard solutions

Stock solutions of eprosartan (0.5mg/ml) and losartan (1mg/ml) internal standard were prepared in methanol. Further dilutions were carried out in methanol. Calibration standards were prepared freshly by spiking drug free plasma with eporsartan stock solution to give the concentrations of 300,600,2000,3000,5000,10000,150000 and 20,000ng/ml.

Quality control standards

Lowest quality control standards, Median quality control standards and highest quality control standards were prepared by spiking drug free plasma with eprosartan to give solution containing 900, 10,000 and 18,000ng/ml respectively. They were stored at -20ºC till the time analyzed.

Sample preparation method

To 250µl of plasma, 50µl of losartan (50µg/ml) and 100µl buffer (0.05M NaOH, pH: 4) and vortexed. The drug was extracted with 2.5ml of ethyl acetate followed by centrifugation at 2000 rpm/min on a cooling centrifuge for 15 min at 4ºC. The organic phase was withdrawn and dried using lyophiliser. To the residue 300µl of mobile phase was added and respective samples were injected into column.

ABSTRACT

A Simple, rapid, selective and sensitive HPLC method was developed and validated for the determination of eprosartan from human plasma. The drug was extracted with a mixture of 0.05M sodium hydroxide and ethyl acetate. Eprosartan was measured in plasma using a validated HPLC method with UV detector at 235nm. Chromatographic peaks were separated on 5µm Inertsil, C18 column (4.6x250mmx5µm) using 60:40 v/v Phosphate buffer pH4, Acetonitrile as mobile phase at a flow rate of 1 ml/min. The chromatograms showed good resolution and no interference from plasma. The retention time of eporsartan and internal standard were approximately 5.3±0.05 min and 9.1±0.05 min respectively. The mean recovery from human plasma was found to be above 98%. The method was linear over the concentration range of 300 to 20,000ng/ml with coefficient of correlation (r²) 0.9983. Both intraday and interday accuracy and precision data showed good reproducibility. This method was successfully applied to pharmacokinetics studies.

Key words: Eprosartan, Liquid-Liquid Extraction, HPLC-UV Method, Validation.

MATERIALS AND METHODS

Eprosartan and losartan were purchased from Pei Li Pharmaceutical Ind.co. Ltd, Taiwan, HPLC grade acetonitrile, ethylacetate, methanol were purchased from SD fine chemicals, Mumbai, India. Analytical Grade Potassium Dihydrogen Phosphate and Sodium Hydroxide were purchased from SD fine chemicals, Mumbai, India. Pooled drug free expired human plasma was purchased from Red Cross Society, Warangal.

Chromatographic conditions

The HPLC system consisted of Alliance waters 2695 with dual λ Absorbance UV detector. The wavelength of detection was set at 235nm. Separation was carried out on inertsil C18 column(n.6x250mmx5µm) using 60:40v/v phosphate buffer pH4, Acetonitrile as mobile phase at a flow rate of 1 ml/min. The mobile phase filtered through nylon millipore (0.2µm) membrane filter, purchased from Pall life sciences, Mumbai and degassed with ultrasonicator prior to use. Chromatography was carried out at room temperature 25ºC and maintains the column temperature at 32ºC.

Preparation of standard solutions

Stock solutions of eprosartan (0.5mg/ml) and losartan (1mg/ml) internal standard were prepared in methanol. Further dilutions were carried out in methanol. Calibration standards were prepared freshly by spiking drug free plasma with eprosartan stock solution to give the concentrations of 300,600,2000,3000,5000,10000,150000 and 20,000ng/ml.

Quality control standards

Lowest quality control standards, Median quality control standards and highest quality control standards were prepared by spiking drug free plasma with eprosartan to give solution containing 900, 10,000 and 18,000ng/ml respectively. They were stored at -20ºC till the time analyzed.

Sample preparation method

To 250µl of plasma, 50µl of losartan (50µg/ml) and 100µl buffer (0.05M NaOH, pH: 4) and vortexed. The drug was extracted with 2.5ml of ethyl acetate followed by centrifugation at 2000 rpm/min on a cooling centrifuge for 15 min at 4ºC. The organic phase was withdrawn and dried using lyophiliser. To the residue 300µl of mobile phase was added and respective samples were injected into column.
Validation

Specificity

A solution containing 300ng/ml was injected on to the column under optimized chromatographic conditions to show the separation of eprosartan from the impurities from the plasma. The specificity of the method was checked for the interference from plasma.

Linearity

Spiked concentrations were plotted against peak area ratios of eprosartan to internal stds and the best fit line was calculated. Wide range calibration was determined by solutions containing 300ng/ml to 20,000ng/ml.

Recovery studies

The % mean recoveries were determined by measuring the responses of the extracted plasma Quality control samples at HQC, MQC and LQC against unextracted Quality control samples at HQC, MQC and LQC.

Limit of quantification

To estimate the LOQ, a drug free blank plasma sample was extracted and injected ten times as described under optimized chromatographic conditions. The noise level was then determined, the limit of quantification for eprosartan was determined,(signal to noise ratio=10).

Precision and accuracy

Intraday precision and accuracy was determined by analyzing quality control standards (900,1000,18000ng/ml) and LLOQ. Quality control standards (300ng/ml) five times a day randomly, interday precision and accuracy was determined from the analysis of each quality control stds (900,10000,18000ng/ml) and LLOQC standards(300ng/ml) once on each of five different days.

Stability studies

The stability of eprosartan was determined by measuring concentration change in control sample overtime. The plasma control samples were stored in eppendorff tubes at -20°C. Stability was tested by subjecting the plasma controls to three freeze thaw cycles and stored for 24hrs at room temperature.

RESULTS AND DISCUSSION

Under the chromatographic conditions employed, the sample showed sharp peaks of drug & internal standard with good resolution. The retention time of the drug was found to be 5.3 ± 0.05 min and the retention time of internal standard was 9.1 ± 0.03 min (figure-2). The method developed was validated for specificity, accuracy & precision, linearity and stability as per USFDA guidance. The results of validating parameters are given below.

Specificity of the method was proven by the absence of the peaks near the reaction time of drug as well as the internal standard (figure-3). The calibration function (peak area ratio Vs Concentration) was linear over working range of 300 to 20,000ng/ml with eight point calibration used for quantification by linear regression. The regression equation for the analysis was Y=5.32e-0.05x-4.82e-0.003 with coefficient of correction (r²) = 0.9979 (figure-4). The % mean recovery for eprosartan in LQC, MQC and HQC was 85.69%, 80.56% and 81.58% respectively (Table-1).
Fig. 4: Spiked concentrations (300 to 20,000 ng/ml) were plotted against peak area ratio vs concentration with eight point calibration used for quantification by linear regression.

### Table 1: Recovery - Eprosartan

<table>
<thead>
<tr>
<th>ID</th>
<th>(area ratio) Unextracted</th>
<th>(area ratio) Extracted</th>
<th>%Recovery Unextracted</th>
<th>MOI</th>
<th>(area ratio) Unextracted</th>
<th>(area ratio) Extracted</th>
<th>%Recovery</th>
<th>(area ratio) Unextracted</th>
<th>(area ratio) Extracted</th>
<th>%Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.117</td>
<td>0.093</td>
<td>79.49</td>
<td>1.5</td>
<td>1.314</td>
<td>2.644</td>
<td>81.73</td>
<td>2.173</td>
<td>82.19</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.116</td>
<td>0.097</td>
<td>83.62</td>
<td>1.494</td>
<td>1.193</td>
<td>79.85</td>
<td>2.628</td>
<td>2.143</td>
<td>81.54</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.123</td>
<td>0.109</td>
<td>88.62</td>
<td>1.48</td>
<td>1.135</td>
<td>76.69</td>
<td>2.619</td>
<td>2.139</td>
<td>81.67</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.124</td>
<td>0.106</td>
<td>85.48</td>
<td>1.486</td>
<td>1.162</td>
<td>78.20</td>
<td>2.627</td>
<td>2.144</td>
<td>81.61</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.122</td>
<td>0.107</td>
<td>87.70</td>
<td>1.475</td>
<td>1.182</td>
<td>80.14</td>
<td>2.634</td>
<td>2.132</td>
<td>80.94</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.121</td>
<td>0.108</td>
<td>89.26</td>
<td>1.478</td>
<td>1.197</td>
<td>80.92</td>
<td>2.627</td>
<td>2.142</td>
<td>81.54</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.121</td>
<td>0.103</td>
<td>85.69</td>
<td>1.486</td>
<td>1.197</td>
<td>80.56</td>
<td>2.630</td>
<td>2.146</td>
<td>81.58</td>
<td></td>
</tr>
<tr>
<td>±SD</td>
<td>0.003</td>
<td>0.007</td>
<td>0.010</td>
<td>0.062</td>
<td>0.008</td>
<td>0.014</td>
<td>0.32</td>
<td>0.66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>%CV</td>
<td>2.71</td>
<td>6.44</td>
<td>5.15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The intraday and interday precision and accuracy of the method was found to be 0.66 to 6.44% and 87.84 to 100.21% respectively for the quality control samples. This is within the acceptance limits of precision is 15% and accuracy is 85 to 115% (Table-2). The limit of Quantification was found to be 300 ng/ml at such concentration the inter day precision was 2.38 and the accuracy was 100.7%. Which are within the acceptance limits of precision is 20% and accuracy is 80 to 120% (Table-3).

### Table 2: Precision and accuracy of quality control standards

<table>
<thead>
<tr>
<th>Batch ID</th>
<th>QC ID</th>
<th>LQC Actual conc.(ng/mL)</th>
<th>MQC Actual conc.(ng/mL)</th>
<th>HQC Actual conc.(ng/mL)</th>
<th>Intraday ± SD</th>
<th>Interday ± SD</th>
<th>Mean %CV</th>
<th>Mean %Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>944.815</td>
<td>9983.803</td>
<td>18165.52</td>
<td>0.03</td>
<td>0.03</td>
<td>3.22</td>
<td>95.43</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>866.674</td>
<td>9914.829</td>
<td>17842.11</td>
<td>0.13</td>
<td>0.13</td>
<td>1.44</td>
<td>100.21</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>854.908</td>
<td>9980.013</td>
<td>17731.98</td>
<td>0.03</td>
<td>0.03</td>
<td>1.44</td>
<td>100.21</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>842.138</td>
<td>9751.858</td>
<td>18068.13</td>
<td>0.04</td>
<td>0.04</td>
<td>1.44</td>
<td>100.21</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>844.796</td>
<td>9795.174</td>
<td>18385.31</td>
<td>0.05</td>
<td>0.05</td>
<td>1.44</td>
<td>100.21</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.04</td>
<td>0.04</td>
<td>1.44</td>
<td>100.21</td>
</tr>
<tr>
<td>%CV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.22</td>
<td>3.22</td>
<td>3.22</td>
<td>3.22</td>
</tr>
<tr>
<td>%Accuracy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>95.43</td>
<td>95.43</td>
<td>95.43</td>
<td>95.43</td>
</tr>
</tbody>
</table>

### Table 3: Precision and accuracy of LLOQC standard

<table>
<thead>
<tr>
<th>Parameters</th>
<th>LLOQC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actual conc.(ng/mL)</td>
<td>300</td>
</tr>
<tr>
<td>1</td>
<td>299.796</td>
</tr>
<tr>
<td>2</td>
<td>314.703</td>
</tr>
<tr>
<td>3</td>
<td>298.651</td>
</tr>
<tr>
<td>4</td>
<td>299.378</td>
</tr>
<tr>
<td>5</td>
<td>297.353</td>
</tr>
<tr>
<td>Mean</td>
<td>301.9762</td>
</tr>
<tr>
<td>±SD</td>
<td>7.175</td>
</tr>
<tr>
<td>%CV</td>
<td>2.38</td>
</tr>
<tr>
<td>%Accuracy</td>
<td>100.7</td>
</tr>
</tbody>
</table>
Stability was assessed by comparing against the freshly thawed quality control samples. The mean stability for HQC and LQC were 103.29 and 99.69 respectively, which is within the acceptance limits of 85 to 115%. Plasma Quality control samples of Eprosartan were found to be stable for at least one month (Table 4).

Eprosartan is soluble in methanol. Hence standard solutions were prepared in methanol. The proportion of acetonitrile in the mobile phase was optimized to 40% and 60% mobile phase was made up of with phosphate buffer (pH: 4). A slight increase and decrease in concentration of acetonitrile and pH by 2% does not affect the reaction times. The extraction of eprosartan was based on liquid-liquid extraction technique. Various solvent systems were tried for recovery studies. The maximum recovery was obtained with a mixture of phosphate buffer (pH 4) and ethyl acetate. Five drugs were attempted for selection as internal standard. The other drugs tried were found to be overlapping with reaction time of eprosartan under the optimized chromatographic conditions.

ACKNOWLEDGEMENT

I am very much thankful to Talla Padmavathi College of Pharmacy, Warangal, for giving permission to carry out my research work. I am very much thank full to Professor and Principal J.Venkateswar Rao, Talla Padmavathi College of Pharmacy, Warangal, for his guidance, kind help and constant encouragement at every step during the progress of my work without which successful completion of this work would not have been possible.

It is my pleasure to express my sincere thanks to Dr. Sanath Kumar Medical Officer, Red Cross Society of India, Warangal, for providing expired human plasma. I am also grateful to my scholars and my friends for their kind help from time to time at each and every step of my project work.

REFERENCES

Development and validation of HPLC-UV method for the estimation of oxaprozin in human plasma

**ABSTRACT**

A simple, rapid, selective and sensitive HPLC method was developed and validated for the determination of oxaprozin from human plasma. The drug was extracted with ethyl tertiary butyl methyl ether. Oxaprozin was measured in plasma using a validated HPLC method with UV detector at 240nm chromatographic peaks were separated on 5µm inertsil, C18 column (4.6x250mmx5µm) using 40:60 v/v Phosphate buffer pH3, Acetonitrile as mobile phase at a flow rate of 1 ml/min. The chromatograms showed good resolution and no interference from plasma. The retention time of oxaprozin and internal standard (Nevirapine) were approximately 7.7±0.05 min and 3.4±0.03 min respectively. The mean recovery from human plasma was found to be above 50%. The method was linear over the concentration range of 0.78 to 100 µg/ml with coefficient of correlation (r^2) 0.9983. Both intraday and interday accuracy and precision data showed good reproducibility. This method was successfully applied to pharmacokinetics studies.

**Key words**: oxaprozin, bioanalytical method, LLE, HPLC

**INTRODUCTION**: Oxaprozin (4, 5-diphenyl-2-oxazole propionic acid) is a non-steroidal anti-inflammatory drug used for treatment of pain, inflammation, and rheumatic conditions. A simple HPLC method was developed and validated for the determination of oxaprozin in tablet formulations and bulk drugs. Although Validated HPLC methods for analysis of oxaprozin in biological fluids have been reported. So far to our present knowledge there is no economical method for fenofibrate is available in the literature for analyzing biological samples. It is felt necessary to develop a simple rapid economical HPLC method for the quantitative determination of fenofibrate. The work also includes the validation of the developed method. The devised method was found to be selective and reliable, and faster and more straightforward than other reported bioanalytical methods.

**MATERIALS AND METHODS**: Oxaprozin and nevirapine were obtained from CIPLA Pharmaceuticals, Mumbai. HPLC grade acetonitrile, ethylacetate, methanol were purchased from SD fine chemicals, Mumbai, India. Analytical Grade Potassium Dihydrogen Phosphate and Sodium Hydroxide were purchased from SD fine chemicals, Mumbai, India. Pooled drug free expired human plasma was purchased from Red Cross Society, Warangal.

**Chromatographic Conditions**: The HPLC system consisted of Alliance waters 2695 with dual absorbance UV detector. The wavelength of detection was set as 240nm. Separation was carried out on inertsil C18 column (4.6x250mmx5µm) using 40:60 v/v phosphate buffer pH3, Acetonitrile as mobile phase at a flow rate of 1 ml/min. The mobile phase filtered through nylon mills pore (0.2µm) membrane filter, purchased from pall life sciences, Mumbai and degassed with Ultrasonicator prior to use. Chromatography was carried out at room temperature 25°C and maintains the column temperature at 32°C.

**Preparation of Standard Solutions**: Stock solutions of Oxaprozin (0.5mg/ml) and Nevirapine (1mg/ml) internal standard were prepared in methanol. Further dilutions were carried out in methanol. Calibration standards were prepared freshly by spiking drug free plasma with Oxaprozin stock solution to give the concentrations of 0.7, 1.5, 3, 6, 12, 25, 50 and 100 µg/ml.

**Quality Control Standards**: Median quality control standards and highest quality control standards were prepared by spiking drug free plasma with Oxaprozin to give solution containing 2, 4 and 8µg/ml respectively. They were stored at -20°C till the time analyzed.

**Sample Preparation Method**: To 250µl of plasma, 25µl of nevirapine (100µg/ml) was added and vortexed. The drug was extracted with 2.5ml of tertiary butyl methyl ether followed by centrifugation at 2000 rpm/min on a cooling centrifuge for 15min at 4°C. The organic phase was withdrawn and dried using lyophiliser. To the residue 300µl of mobile phase was added and respective samples were injected into column.

**VALIDATION**: Specificity: A solution containing 0.7µg/ml was injected on to the column under optimized chromatographic conditions to show the separation of oxaprozin from the impurities from the plasma. The specificity of the method was checked for the interference from plasma.

**Linearity**: Spiked concentrations were plotted against peak area ratios of oxaprozin to internal stds and the best fit line was calculated. Wide range calibration was determined by solutions containing 0.7 µg/ml to 100µg/ml.

**Recovery studies**: The % mean recoveries were determined by measuring the responses of the extracted plasma Quality control samples at HQC, MQC and LQC against unextracted Quality control samples at HQC, MQC and LQC.

**Limit of quantification**: To estimate the LOQ, a drug free blank plasma sample was extracted and injected ten times and analyzed as described under optimized chromatographic conditions. The noise level was then determined, the limit of quantification for oxaprozin was determined.(signal to noise ratio=10).

**Precision and accuracy**: Intraday precision and accuracy was determined by analyzing quality control standards (2, 4 and 8µg/ml) and LLOQ Quality control standards (0.7µg/ml) five times a day randomly, interday precision and accuracy was determined from the analysis of each quality control stds (2, 4 and 8µg/ml) and LLOQC standards (0.1µg/ml) once on each of five different days.

**Stability studies**: The stability of oxaprozin was determined by measuring concentration change in control sample overtime. The plasma control samples were stored in
RESULTS & DISCUSSION:
Under the chromatographic conditions employed, the sample showed sharp peaks of drug & internal standard with good resolution. The retention time of the drug was found to be 7.0 + 0.05 min and the retention time of internal standard was 3.4±0.03 min (figure-2). The method developed was validated for specificity, accuracy & precision, linearity and stability as per USFDA guidance. The results of validating parameters are given below.

The % mean recovery for 0xaprozin in LQC, MQC and HQC was 63%, 66% and 62% respectively (Table-1).

The intraday and interday precision and accuracy of the method was found to be 0.8 to 2.8% and 105 to 109 respectively for the quality control samples. This is within the acceptance limits of precision is 15% and accuracy is 85 to 115% (Table-2).

The % mean recovery for 0xaprozin in LQC, MQC and HQC was 63%, 66% and 62% respectively (Table-1).

Stability was assessed by comparing against the freshly thawed quality control samples. The %mean stability for HQC and LQC were 111.6 and 106.7 respectively, which is within the acceptance limits of 85 to 115%. Plasma Quality control samples of 0xaprozin were found to be stable for at least one month (Table-4).
0xaprozin is soluble in methanol. Hence standard solutions were prepared in methanol. The proportion of acetonitrile in the mobile phase was optimized to 60% and 40% mobile phase was made up of with phosphate buffer (pH: 3). A slight increase and decrease in concentration of acetonitrile and pH by 2% does not affect the reaction times. The extraction of 0xaprozin was based on liquid-liquid extraction technique. Various solvent systems were tried for recovery studies. The maximum recovery was obtained with ethyl acetate. Five drugs were attempted for selection as internal standard. The other drugs tried were found to be overlapping with reaction time of 0xaprozin under the optimized chromatographic conditions.

CONCLUSION:
The analytical method developed and validated for the quantitative determination of 0xaprozin from plasma was simple, rapid, specific, sensitive, accurate and precise. Hence, the method is quite suitable to detect the drug from plasma samples of human volunteers.

ACKNOWLEDGEMENT
I am very much thankful to Talla Padmavathi College of Pharmacy, Warangal, for giving permission to carry out my research work. I am very much thank full to Professor and Principal J.Venkateshwar Rao, Talla Padmavathi College of Pharmacy, Warangal, for his guidance, kind help and constant encouragement at every step during the progress of my work without which successful completion of this work would not have been possible.

It is my pleasure to express my sincere thanks to Dr. Sanath Kumar Medical Officer, Red Cross Society of India, Warangal, for providing expired human plasma. I am also grateful to my scholars and my friends for their kind help from time to time at each and every step of my project work.

REFERENCES:

Source of support: Nil, Conflict of interest: None Declared
INTRODUCTION:
Fenofibrate is a fibric acid derivative and chemically, 2-[4-(4-chlorobenzoyl) phenoxy]-2-methyl propanoic acid-1-methyl ester. An oral fibrate lipid lowering agent could markedly reduce elevated plasma concentrations of triglycerides. It also decreases elevated plasma concentrations of LDL and total cholesterol. A simple UV-Spectrophotometric and HPLC methods for the estimation of fenofibrate in bulk drugs, HPLC and TLC-Video densitometry were developed and validated for the estimation of fenofibrate in biological fluid such as plasma, serum and urine. So far to our present knowledge there is no economical method for fenofibrate is available in the literature for analyzing biological samples. It is felt necessary to develop a simple rapid economical HPLC method for the quantitative determination of fenofibrate. The work also includes the validation of the developed method. The devised method was found to be selective and reliable, and faster and more straightforward than other reported bioanalytical methods.

MATERIALS AND METHODS:
Fenofibrate and nevirapine were obtained from CIPLA Pharmaceuticals, Mumbai. HPLC grade acetonitrile, ethylacetate, methanol were purchased from SD fine chemicals, Mumbai, India. Analytical Grade ammonium acetate buffer, purchased from pall life sciences, Mumbai and degassed with nitrogen. HPLC grade acetonitrile, ethylacetate, methanol were purchased from SD fine chemicals, Mumbai, India. Analytical Grade ammonium acetate buffer, purchased from pall life sciences, Mumbai and degassed with nitrogen. Ammonium acetate buffer, Acetonitrile as mobile phase at a flow rate of 1 ml/min. The chromatograms showed good resolution and no interference from plasma. The retention time of fenofibrate and internal standard (Nevirapine) were approximately 6.6±0.05 min and 5.2±0.03 min respectively. The mean recovery from human plasma was found to be above 62%. The method was linear over the concentration range of 0.3 to 20 µg/ml with coefficient of correlation (r²) 0.9983. Both intraday and interday accuracy and precision data showed good reproducibility. This method was successfully applied to pharmacokinetics studies.

PREPARATION OF STANDARD SOLUTIONS:
Stock solutions of fenofibrate (0.5 mg/ml) and Nevirapine (1 mg/ml) internal standard were prepared in methanol. Further dilutions were carried out in 60% acetonitrile. Calibration standards were prepared freshly by spiking drug free plasma with fenofibrate stock solution to give the concentrations of 0.3, 0.6, 1.2, 2.5, 5.0, 10, and 20 µg/ml.

CHROMATOGRAPHIC CONDITIONS:
The HPLC system consisted of Alliance waters 2695 with dual absorbance detectors. The wavelength of detection as set at 295nm. The chromatograms showed no interference from plasma. The retention time of fenofibrate and internal standard (Nevirapine) were 6.6±0.05 min and 5.2±0.03 min respectively. The mean recovery from human plasma was found to be above 62%. The method was linear over the concentration range of 0.3 to 20 µg/ml with coefficient of correlation (r²) 0.9983. Both intraday and interday accuracy and precision data showed good reproducibility. This method was successfully applied to pharmacokinetics studies.

Received on: 12-06-2011; Revised on: 08-07-2011; Accepted on:16-10-2011

ABSTRACT
A simple, rapid, selective and sensitive HPLC method was developed and validated for the determination of fenofibrate from human plasma. The drug was extracted with ethyl acetate. Fenofibrate was measured in plasma using a validated HPLC method with UV detector at 295nm chromatographic peaks were separated on 5µm intensil, C18 column (4.6x250mmx5µm) using 60:40 v/v 20mM ammonium acetate buffer with initial PH 3.5 and total cholesterol. A simple UV-Spectrophotometric and HPLC methods for the estimation of fenofibrate in biological fluid such as plasma, serum and urine. So far to our present knowledge there is no economical method for fenofibrate is available in the literature for analyzing biological samples. It is felt necessary to develop a simple rapid economical HPLC method for the quantitative determination of fenofibrate. The work also includes the validation of the developed method. The devised method was found to be selective and reliable, and faster and more straightforward than other reported bioanalytical methods.

KEY words: fenofibrate, bioanalytical method, LLE, HPLC

Figure-1. Structure of Fenofibrate

PREPARATION OF STANDARD SOLUTIONS:
Stock solutions of fenofibrate (0.5 mg/ml) and Nevirapine (1 mg/ml) internal standard were prepared in methanol. Further dilutions were carried out in 60% acetonitrile. Calibration standards were prepared freshly by spiking drug free plasma with fenofibrate stock solution to give the concentrations of 0.3, 0.6, 1.2, 2.5, 5.0, 10, and 20 µg/ml.

QUALITY CONTROL STANDARDS:
Lower quality control standards, Median quality control standards and highest quality control standards were prepared by spiking drug free plasma with fenofibrate to give solution containing 1, 8 and 16µg/ml respectively. They were stored at -20°C till the time analyzed.

SAMPLE PREPARATION METHOD:
To 500µl of plasma, 50µl of nevirapine (50µg/ml) was added and vortexed. The drug was extracted with 3ml of ethyl acetate followed by centrifugation at 2000 rpm/min on a cooling centrifuge for 15min at 4°C. The organic phase was withdrawn and dried using lyophiliser. To the residue 250µl of mobile phase was added and respective samples were injected into column.

VALIDATION:
Specificity: A solution containing 0.3µg/ml was injected on to the column under optimized chromatographic conditions to show the separation of fenofibrate from the impurities from the plasma. The specificity of the method was checked for the interference from plasma.

Linearity: Spiked concentrations were plotted against peak area ratios of fenofibrate to internal stds and the best fit line was calculated. Wide range calibration was determined by solutions containing 0.3µg/ml to 20µg/ml.

RECOVERY STUDIES:
The % mean recoveries were determined by measuring the responses of the extracted plasma Quality control samples at HQC, MQC and LQC against unextracted Quality control samples at HQC, MQC and LQC.

LIMIT OF QUANTIFICATION:
To estimate the LOQ, a drug free blank plasma sample was extracted and injected ten times and analyzed as described under optimized chromatographic conditions. The noise level was then determined, the limit of quantification for fenofibrate was determined.(signal to noise ratio=10).

PRECISION AND ACCURACY:
Intraday precision and accuracy was determined by analyzing quality control standards (1, 8 and 16µg/ml) and LLOQ Quality control standards (0.15µg/ml) five times a day randomly, interday precision and accuracy was determined from the analysis of each quality control stds (1, 8 and 16µg/ml) and LLOQ Standard (0.3µg/ml) once on each of five different days.
Stability studies:
The stability of fenofibrate was determined by measuring concentration change in control sample overtime. The plasma control samples were stored in eppendorff tubes at -20°C. Stability was tested by subjecting the plasma controls to three freeze thaw cycles and stored for 24hrs at room temperature.

RESULTS & DISCUSSION:
Under the chromatographic conditions employed, the sample showed sharp peaks of drug & internal standard with good resolution. The retention time of the drug was found to be 6.6±0.05 min and the retention time of internal standard was 5.2±0.03 min (figure-2). The method developed was validated for specificity, accuracy & precision, linearity and stability as per USFDA guidance. The results of validating parameters are given below.

The intraday and interday precision and Accuracy of the method was found to be 0.25 to 4.85% and 103.12 to 107.3 respectively for the quality control samples. This is within the acceptance limits of precision is 15% and accuracy is 85 to 115% (Table-2). The limit of Quantification was found to be 0.3µg/ml at such concentration the inter day precision was 2.85 and the accuracy was 102.3%. Which are within the acceptance limits of precision is 20% and accuracy is 80 to 120% (Table-3).

Stability was assessed by comparing against the freshly thawed quality control samples. The % mean stability for HQC and LQC were 109.6 and 97.6 respectively, which is within the acceptance limits of 85 to 115%. Plasma Quality control samples of Fenofibrate were found to be stable for at least one month (Table-4).

Fenofibrate is soluble in methanol. Hence standard solutions were prepared in methanol. The proportion of acetonitrile in the mobile phase was optimized to 60% and 40% mobile phase was made up of with ammonium acetate buffer (pH 4). A slight increase and decrease in concentration of acetonitrile and pH by 2%
does not affect the reaction times. The extraction of fenofibrate was based on liquid-liquid extraction technique. Various solvent systems were tried for recovery studies. The maximum recovery was obtained with ethyl acetate. Five drugs were attempted for selection as internal standard. The other drugs tried were found to be overlapping with reaction time of fenofibrate under the optimized chromatographic conditions.

CONCLUSION:
The analytical method developed and validated for the quantitative determination of Fenofibrate from plasma was simple, rapid, specific, sensitive, accurate and precise. Hence, the method is quite suitable to detect the drug from plasma samples of human volunteers.

ACKNOWLEDGEMENT
I am very much thankful to Talla Padmavathi College of Pharmacy, Warangal, for giving permission to carry out my research work. I am very much thank full to Professor and Principal J. Venkateshwar Rao, Talla Padmavathi College of Pharmacy, Warangal, for his guidance, kind help and constant encouragement at every step during the progress of my work without which successful completion of this work would not have been possible.

It is my pleasure to express my sincere thanks to Dr. Sanath Kumar Medical Officer, Red Cross Society of India, Warangal, for providing expired human plasma. I am also grateful to my scholars and my friends for their kind help from time to time at each and every step of my project work.

REFERENCES:

Source of support: Nil, Conflict of interest: None Declared
Development and validation of HPLC-UV method for the estimation of zaltoprofen in human plasma

T. Manish Kumar a, Gurrala Srikanth b, Vandana Pamulaparthi c, Dr. J. Venkateshwar Rao c, Prof. KRS. Sambasiva Rao c

a Central Analytical Lab, Balaji Institute of Pharmaceutical Sciences, Laknepal, Narsampet, Warangal-506331, Andhra Pradesh, India
b Department of Pharmaceutical Chemistry, Gland Institute of Pharmaceutical Sciences, Kothapet, Narsapur, Medak, Andhra Pradesh, India
c Department of Pharmaceutical Analysis, Telha Padmavathi College of Pharmacy, Warangal, Andhra Pradesh, India

∗Corresponding author.

Received on: 12-06-2011; Revised on: 08-07-2011; Accepted on: 16-10-2011

ABSTRACT

A simple, rapid, selective and sensitive HPLC method was developed and validated for the determination of zaltoprofen from human plasma. The drug was extracted with ethyl acetate. Zaltoprofen was measured in plasma using a validated a HPLC method with UV detector at 254nm chromatographic peaks were separated on 5µm intensil, C18 column (4.6x250mmx5µm) using 40:60 v/v Phosphate buffer pH3, Acetonitrile as mobile phase at a flow rate of 1 ml/min. The chromatogram showed good resolution and no interference from plasma. The retention time of zaltoprofen and internal standard (Nevirapine) were approximately 4.0±0.05 min and 10.7±0.03 min respectively. The mean recovery from human plasma was found to be above 50%. The method was linear over the concentration range of 0.13 to 20µg/ml with coefficient of correlation (r²) 0.9983. Both intraday and interday accuracy and precision data showed good reproducibility. This method was successfully applied to pharmacokinetics studies.

Key words: zaltoprofen, bioanalytical method, LLE, HPLC

INTRODUCTION:

Zaltoprofen, 2-(10,11-dihydro-10-oxodibenzo[b,f]thiepin-2-yl)pro-pionic acid is a potent non-steroidal anti-inflammatory drug (NSAID). It has been used clinically for treatment of post-operative pain and low back pain for more than ten years, and has recently been reported to cause potent inhibition of cyclooxygenase-2 with fewer side effects on the gastro-intestinal tract and to induce apoptosis in a variety of cell lines. Zaltoprofen is a unique compound that also has anti-bradykinin activity. Its analgesic effects may be a result of inhibition of bradykinin B1 re-ceptor-mediated bradykinin responses not only of cyclooxygenases but also of bradykinin-induced 12-lipoxygenase inhibitors. A simple and rapid RPHPLC analysis method for direct determination of (+) and (-)zaltoprofen glucuronide in rat hepatic microsomes and from pharmaceutical bulk dosage forms were developed and validated. Heon-Woo Lee et al prepared a LC-ms/ms method for the determination of zaltoprofen in human plasma. So far to our present knowledge there is no economical method for ZLT determine. The work also includes the validation of the developed method. The devised method was found to be selective and reliable, and faster and more straightforward than other reported bioanalytical methods.

MATERIALS AND METHODS:

Zaltoprofen and nevirapine were obtained from CIPLA Pharmaceuticals, Mumbai, HPLC grade acetronilite, ethylacetate, methanol were purchased from SD finex chemicals, Mumbai, India. Analytical Grade Potassium Dihydrogen Phosphate and Sodium Hydroxide were purchased from SD fine chemicals, Mumbai, India. Pooled drug free expired human plasma was purchased from Red Cross Society, Warangal.

Chromatographic Conditions:

The HPLC system consisted of Alliance waters 2695 with dual absorbance UV detector. The wavelength of detection as set at 254nm. Separation was carried out on inertsil C18 column(4.6x250mmx5µm) using 40:60 v/v phosphate buffer pH3, Acetonitrile as mobile phase at a flow rate of 1 ml/min. The mobile phase filtered through nylon mill pore(0.2µm) membrane filter, purchased from pall life sciences, Mumbai and degassed with Ultrasorinicator prior to use. Chromatography was carried out at room temperature 25°c and maintained the column temperature at 32°c.

Preparation of Standard Solutions:

Stock solutions of zaltoprofen (0.5mg/ml) and Nevirapine (1mg/ml) internal standard were prepared in methanol. Further dilutions were carried out in methanol. Calibration standards were prepared freshly by spiking drug free plasma with zaltoprofen stock solution to give the concentrations of 0.15, 0.3, 0.6, 1.2, 2.5, 5.0, 10, and 20 µg/ml.

Quality Control Standards:

Lowest quality control standards, Median quality control standards and highest quality control standards were prepared by spiking drug free plasma with zaltoprofen to give solution containing 0.5, 9 and 18µg/ml respectively. They were stored at -20°C till the time analyzed.

Sample Preparation Method:

To 500µl of plasma, 50µl of nevirapine (60µg/ml) was added and vortexed. The drug was extracted with 5ml of ethyl acetate followed by centrifugation at 2000 rpm/min on a cooling centrifuge for 15min at 4°C. The organic phase was withdrawn and dried using lyophiliser. To the residue 300µl of mobile phase was added and respective samples were injected into column.

VALIDATION:

Specificity:

A solution containing 0.15µg/ml was injected on to the column under optimized chromatographic conditions to show the separation of zaltoprofen from the impurities from the plasma. The specificity of the method was checked for the interference from plasma.
Linearity: 
Spiked concentrations were plotted against peak area ratios of zaltoprofen to internal stds and the best fit line was calculated. Wide range calibration was determined by solutions containing 0.15 μg/ml to 20μg/ml.

Recovery studies: 
The % mean recoveries were determined by measuring the responses of the extracted plasma Quality control samples at HQC, MQC and LQC against unextracted Quality control samples at HQC, MQC and LQC.

Limit of quantification: 
To estimate the LOQ, a drug free blank plasma sample was extracted and injected ten times and analyzed as described under optimized chromatographic conditions. The noise level was then determined, the limit of quantification for zaltoprofen was determined (signal to noise ratio=10).

Precision and accuracy: 
Intraday precision and accuracy was determined by analyzing quality control standards (0.5, 9 and 18μg/ml) and LLOQ Quality control standards (0.15μg/ml) five times a day randomly. Interday precision and accuracy was determined from the analysis of each quality control stds (0.5, 9 and 18μg/ml) and LLOQC standards (0.15μg/ml) once on each of five different days.

Stability studies: 
The stability of zaltoprofen was determined by measuring concentration change in control sample overtime. The plasma control samples were stored in eppendorff tubes at -20°C. Stability was tested by subjecting the plasma controls to three freeze thaw cycles and stored for 24hrs at room temperature.

RESULTS & DISCUSSION: 
Under the chromatographic conditions employed, the sample showed sharp peaks of drug & internal standard with good resolution. The retention time of the drug was found to be 4.0±0.05 min and the retention time of internal standard was 10.7±0.03 min (figure-2). The method developed was validated for specificity, accuracy & precision, linearity and stability as per USFDA guidance. The results of validating parameters are given below.

Figure-2: Retention times of Aqueous Mixture consists of Zaltoprofen (5μg/ml) and nevirapine (5μg/ml)

Specificity of the method was proven by the absence of the peaks near the reaction time of drug as well as the internal standard (figure-3).

Figure-3: Blank plasma sample showing no interference at the RT of zaltoprofen and nevirapine

The calibration function (peak area ratio Vs Concentration) was linear over working range of 0.15 to 20μg/ml with eight point calibration used for quantification by linear regression. The regression equation for the analysis was Y=5.32e-0.05x+4.82e-0.003 with coefficient of correction (r²) = 0.9979. (figure-4).

Figure-4: Spiked concentrations (0.15 to 20µg/ml) were plotted against peak area ratio Vs Concentration with eight point calibration used for quantification by linear regression.

The % mean recovery for zaltoprofen in LQC, MQC and HQC was 50.2%, 49.9% and 51.6% respectively (Table-1).
The intraday and interday precision and accuracy of the method was found to be 0.56 to 2.47% and 91.23 to 111.13 respectively for the quality control samples. This is within the acceptance limits of precision is 15% and accuracy is 85 to 115% (Table-2). The limit of Quantification was found to be 0.15µg/ml at such concentration the inter day precision was 1.66 and the accuracy was 98.7%. Which are within the acceptance limits of precision is 20% and accuracy is 80 to 120% (Table-3).

Stability was assessed by comparing against the freshly thawed quality control samples. The mean stability for HQC and LQC were 103.6 and 93.9 respectively, which is within the acceptance limits of 85 to 115%. Plasma Quality control samples of Zaltoprofen were found to be stable for at least one month (Table-4).

Table-4: Freeze-thaw Stability of Quality Control Standards

<table>
<thead>
<tr>
<th>Freeze – thaw III Cycles</th>
<th>QC ID</th>
<th>LQC</th>
<th>HQC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actual conc.(µg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.5</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>0.431</td>
<td>18.292</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.478</td>
<td>18.762</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.49</td>
<td>18.624</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.433</td>
<td>18.323</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.451</td>
<td>18.857</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.470</td>
<td>18.647</td>
<td></td>
</tr>
<tr>
<td>±SD</td>
<td>0.040</td>
<td>0.207</td>
<td></td>
</tr>
<tr>
<td>% CV</td>
<td>0.48</td>
<td>1.43</td>
<td></td>
</tr>
<tr>
<td>% Accuracy</td>
<td>93.93</td>
<td>103.60</td>
<td></td>
</tr>
</tbody>
</table>

Zaltoprofen is soluble in methanol. Hence standard solutions were prepared in methanol. The proportion of acetonitrile in the mobile phase was optimized to 60% and 40% mobile phase was made up of with phosphate buffer (pH: 4). A slight increase and decrease in concentration of acetonitrile and pH by 2% does not affect the reaction times. The extraction of zaltoprofen was based on liquid-liquid extraction technique. Various solvent systems were tried for recovery studies. The maximum recovery was obtained with ethyl acetate. Five drugs were attempted for selection as internal standard.

The other drugs tried were found to be overlapping with reaction time of zaltoprofen under the optimized chromatographic conditions.

CONCLUSION:

The analytical method developed and validated for the quantitative determination of Zaltoprofen from plasma was simple, rapid, specific, sensitive, accurate and precise. Hence, the method is quite suitable to detect the drug from plasma samples of human volunteers.

ACKNOWLEDGEMENT

I am very much thankful to Talla Padmavathi College of Pharmacy, Warangal, for giving permission to carry out my research work. I am very much thank full to Professor and Principal J.Venkateshwar Rao, Talla Padmavathi College of Pharmacy, Warangal, for his guidance, kind help and constant encouragement at every step during the progress of my work without which successful completion of this work would not have been possible.

It is my pleasure to express my sincere thanks to Dr. Sanath Kumar Medical Officer, Red Cross Society of India, Warangal, for providing expired human plasma. I am also grateful to my scholars and my friends for their kind help from time to time at each and every step of my project work.

REFERENCES:


Source of support: Nil, Conflict of interest: None Declared

T. Manish Kumar et al. / Journal of Pharmacy Research 2011,4(10),
DEVELOPMENT AND VALIDATION OF HPLC-UV METHOD FOR THE
ESTIMATION OF EPROSARTAN IN HUMAN PLASMA

Manish T, Srikanth G, Venkateshwar Rao J, Raghunandan N

Department of Pharmaceutical Analysis, Talla Padmavathi College of Pharmacy
Warangal, Andhra Pradesh.

Department of Pharmaceutical Chemistry, Balaji Institute of Pharmaceutical Sciences
Warangal, Andhra Pradesh.

A Simple, rapid, selective and sensitive HPLC method was developed and validated for the
determination of eprosartan from human plasma. The drug was extracted with a mixture of
0.05M sodium hydroxide and ethyl acetate. Eprosartan was measured in plasma using a
validated a HPLC method with UV detector at 235nm chromatographic peaks were separated on
5μm intensil, C18 column (4.6x250mmx5μm) using 60:40 v/v Phosphate buffer pH4,
Acetonitrile as mobile phase at a flow rate of 1 ml/min. The chromatograms showed good
resolution and no interference from plasma. The retention time of eprosartan and internal
standard were approximately 5.3±0.05 min and 9.1± 0.03 min respectively. The mean recovery
from human plasma was found to be above 88%. The method was linear over the concentration
range of 300 to 20,000ng/ml with coefficient of correlation (r²) 0.9983. Both intraday and
interday accuracy and precision data showed good reproducibility. This method was
successfully applied to pharmacokinetics studies.