HPLC-UV METHOD TRANSFER FOR UPLC IN BIOANALYTICAL ANALYSIS: DETERMINATION OF TERBINAFINE FROM HUMAN PLASMA

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SUMMARY

The analytical laboratory has to analyze a great number of samples and must reduce the time. A solution of this problem is to develop new rapid and efficient qualitative and quantitative analysis procedure. Liquid chromatography (LC) is most suitable for the quantitative determination of drugs and related substances. However, conventional High Performance Liquid chromatography (HPLC) analyses usually need between 10 and 45 min. The use of Ultra Performance Liquid Chromatography (UPLC) system is an innovative approach because of its speed and sensitivity. This technique is gaining considerable attention in recent years for pharmaceutical and biomedical analysis. Transferring HPLC method to UPLC is common practice that allows pharmaceutical industry to minimize analysis time for drug development and quality control process.

A rapid and reliable analytical method for the determination of Terbinafine from human plasma were developed by using HPLC and transferred to UPLC system as a model for method transfer. A first approach, using Zorbax SB-Aq C18 column and optimized HPLC instrumentation was applied for the determination of Terbinafine from human plasma with mobile phase % 50 H₃PO₄ solution/ACN (40/60) v/v) at the flow rate 0.8 ml/min. Retention time of Terbinafine and Propyl Paraben as Internal Standard were 17.43 min and 7.27 min respectively. The developed method transferred to UPLC by using similar column packing, Waters Acquity BEH C18 1.7 μm 2.1 x 50 mm column, with mobile phase 50% H₃PO₄ solution/ACN (50/50,

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v/v) and the flow rate was 0.3 ml/min. The retention time of Terbinafine and Internal Standard were 2.98 min and 0.92 min.

Terbinafine was analyzed only 2.98 min allowing a reduction of the analysis time by a factor of 6 in comparison to a conventional HPLC method with a similar peak capacity.

Key words: HPLC, UPLC, method transfer, terbinafine, plasma

ÖZET


Terbinafinin insan plasmasından analizi model olarak alınarak, geliştirilen hızlı ve güvenilir YBSK yöntemi UPSK’ne metod transferi yapılmıştır. İlk analiz yönteminde Tebingin plasmanalı analizi HPLC sisteminde Zorbax SB-Aq C18 kolon kullanılmıştır. Mobil faz, 50 % H₃PO₄ çözeltisi/Asetonitril (40/60) v/v, 0.8 ml/dak akış hızında çalışılmıştır. Terbinafin ve propil parabenin alınkama zamanları sırasıyla, 17.43 ve 7.27 dakika bulunmaktadır. Geliştirilen bu metod benzer kolon dolgu materyali kullanılarak UPSK sistemine transfer edilmiştir. Waters Acquity BEH C18 1.7 μm 2.1 x 50 mm kolon 50 % H₃PO₄ çözeltisi/ACN (50/50, v/v) mobil faz olarak 0.3 ml/dakika akış hızında kullanılmıştır. Terbinafin ve propil parabenin alınkoma zamanları sırasıyla, 2.98 ve 0.92 dakika elde edilmiştir.

Terbinafinin UPSK ile analiz zamanı UPSK ne oranla 6 kat daha azdır ve benzer pik özelliklerinde, 2.98 dakika da elue edilmiştir.
INTRODUCTION

The development of a drug product is a long and tedious task, taking about 10–15 years from the synthesis of a lead compound to its commercialization. One of the main objectives of the pharmaceutical industry is to reduce this time period by using high-throughput discovery and screening methods (1). Therefore, the analytical laboratory has to manage a great number of samples and must reduce the time response delivery at each step during drug development. A solution for this problem is to develop new rapid and efficient procedures for qualitative and quantitative analysis.

Liquid Chromatography (LC) is considered to be the gold standard in pharmaceutical analysis. Liquid chromatography is most suitable for the quantitative determination of drugs and related substances. However, drug analyses usually need between 10 and 45 min. Today, there is a need for fast and ultra-fast methods with good efficiency and resolution for achieving separations in few minutes or even seconds. The present work describes a simple methodology for performing a successful method transfer from conventional LC to Ultra-Performance LC (2).

Ultra Performance Liquid Chromatography (UPLC) is a relatively new technique giving new possibilities in liquid chromatography, especially concerning decrease of time and solvent consumption (3,4).

Two important parameters, the injection volume and the mobile phase flow rate, have to be modified for transferring an isocratic method in a column packed with identical stationary phase but different particle size.

The UPLC system allows shortening analysis time up to nine times comparing to the conventional system using 5 μm particle packed analytical columns. In comparison with 3 μm particle packed analytical columns analysis should be shortened about three times. The negative effect of particle decrease is back-pressure increase about nine times (versus 5 μm) or three times (versus 3 μm), respectively. The separation on UPLC is performed under very high pressures (up to 100 MPa is possible in UPLC system), but it has no negative influence on analytical column or other components of chromatographic system (5). Separation efficiency remains maintained or is even improved.

As it is very well known from Van Deemter equations, the efficiency of
chromatographic process is proportional to particle size decrease. According to his model describing band broadening, which describes the relationship between height equivalent of theoretical plate (HETP) and linear velocity, one of the terms (path dependent term), is dependent on a diameter of particle packed into the analytical column. Smaller particle diameter can significantly reduce HETP which results in higher efficiency and the flatter profile of Van Deemter curve (Figure 1). Consequently, the mobile phase flow-rate increase does not have negative influence to the efficiency as it could be observed at 10 or 5 μm particles and the negative aspect of small particle packed columns used in HPLC is, however, high back-pressure generating (10).

![Molecular structure of terbinafine.](image)

Terbinafin, ((E)-N,6,6-trimethyl-N-(naphthalen-1-ylmethyl)hept-2-en-4-yn-1-amine) (Figure 2). Terbinafine hydrochloride is a synthetic allylamine antifungal. It is highly lipophilic in nature and tends to accumulate in skin, nails, and fatty tissues (6). Terbinafin is used for treatment of dermal affections in the form of creams, gels, tablets and solutions. Mostly, terbinafin has been determined using HPLC method in different biological materials (7, 8). A rapid and reliable analytical method for the determination of Terbinafine from human plasma were developed by using HPLC and transferred to UPLC system as a model for method transfer because of the long retention at the HPLC system.
Figure 1. Van Deemter curves for different particle sizes (10, 5, 3, 1.7 μm)

MATERIAL AND METHOD

Chemicals and reagents
Terbinafin and propyl paraben (IS) were obtained from Cipla and Marmara Ecza Coop. respectively. Acetonitrile, Ortho-phosphoric acid, triethylamine and n-hexan were purchased from Merck (Darmstadt, Germany). All other chemicals were analytical grade. HPLC grade solvents were used. Drug free plasma sample was obtained from Turkish Blood Centre. HPLC grade water was used throught the study (Millipore, Bedford, USA).

Instrumentation
The HPLC system of Agilent 1100 series with UV detector and Waters Acquity UPLC system were used for analyse terbinafin.

Chromatographic Conditions
Plasma sample processing
Terbinafin was extracted from plasma samples by using liquid-liquid extraction. 1 ml plasma samples in polypropylene tubes were spiked with 200 ml (100mg/ml) Internal standart (IS) solution and 0.1 M 100 ml \( \text{H}_3\text{PO}_4 \) and vortexed for 20 seconds. 5 ml extraction solvent, n-hexane were added to each tube and vortexed 1 minute to extract terbinafin. The tubes were centrifuged at 5000 rpm, 5°C for 10 min. The supernatants were transferred into clean tubes and evaporated to dryness under nitrogen atmosphere. After
evaporation samples were reconstituted and injected to chromatographic systems.

RESULTS AND DISCUSSION

Transferring methods between instruments and laboratory are common practice that allows analytical scientists to maximize equipment and method usage. Method transfer can be difficult task. When methods are transferred between different systems such as HPLC to UPLC, differences in the systems, separation power and selectivity of the column are important parameters.

Initial transfer of the HPLC method to UPLC was accomplished by applying different flow rate and sample injection volume (9). Chromatograms from this UPLC method had very narrow peaks and suitable resolution. The mobil phase flow rate was increased until limited by column back pressure. At the end reducing total run time by using UPLC method was more economical. A dramatic decrease in solvent consumption was also obtained. Table I shows comparison of the original HPLC method to UPLC conditions.

Table I. Chromatographic conditions used for analysis of Terbinafin with HPLC and UPLC system.

<table>
<thead>
<tr>
<th></th>
<th>HPLC assay</th>
<th>UPLC assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Columns</td>
<td>Zorbax Sb-Aq, 4.6x150mm, 5mm</td>
<td>Waters Aquity BEH, 2.1x50mm, 1.7mm</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>50% H$_3$PO$_4$ Solution/ACN (40/60) v/v</td>
<td>50% H$_3$PO$_4$ Solution/ACN (50/50, v/v)</td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.8 ml/min</td>
<td>0.3 ml/min</td>
</tr>
<tr>
<td>Injection volume</td>
<td>5 ml</td>
<td>20ml</td>
</tr>
<tr>
<td>UV detection</td>
<td>224 nm</td>
<td>224 nm</td>
</tr>
<tr>
<td>Run time</td>
<td>20 min</td>
<td>5 min</td>
</tr>
</tbody>
</table>

The selected sample used for transferring a method performed in a conventional HPLC system to UPLC system. The original separation was performed in approximately 20 min and efficiently transferred to UPLC with less than 5 min of analysis time (Figure 3). Sensitivity was not significantly affected between two systems. Linear range was found 50-2000 ng/ml with
LOQ 50 ng/ml for HPLC and UPLC conditions. System suitability tests are used to verify the resolution and reproducibility of chromatographic system are adequate for the analysis to be done. Accuracy was assessed by back calculation of the injection peak areas using the calibration curve to give the calculated concentration for each injection. Precision was evaluated by the peak area relative standard deviation of triplicate injections (RSD>2). The results for both HPLC and UPLC systems were similar and two methods were found to be accurate and precise. Tailing factor, capacity factor and resolution are the main component of chromatographic suitability.

![HPLC-UV Chromatogram of Terbinafin and IS from spiked human plasma](image1)

**Figure 3**: a) HPLC-UV Chromatogram of Terbinafin and IS from spiked human plasma

![UPLC-UV Chromatogram of Terbinafin and IS from spiked human plasma](image2)

**Figure 3**: b) UPLC-UV Chromatogram of Terbinafin and IS from spiked human plasma

System suitability test for both systems can be seen in Figure 4 and 5. System suitability parameters were acceptable for both systems. The acceptance criteria of suitability parameters were determined by FDA guidelines(11). The capacity factor ($k'$) and resolution (R) are acceptable when the result greater than 2, tailing factor is smaller than 2. There is no significantly
difference between two systems for tailing, capacity factor and resolution.

Figure 4: System suitability test for HPLC-UV system

Peak: THCL

System Suitability Separation Results

<table>
<thead>
<tr>
<th>Name</th>
<th>RT</th>
<th>USP Resolution</th>
<th>USP Tailing</th>
<th>K Prime</th>
<th>Width @ Tangent</th>
</tr>
</thead>
<tbody>
<tr>
<td>PP</td>
<td>0.951</td>
<td>1.020728</td>
<td>2.170860</td>
<td>0.068950</td>
<td></td>
</tr>
<tr>
<td>THCL</td>
<td>2.671</td>
<td>17.978986</td>
<td>0.899419</td>
<td>7.903579</td>
<td>0.119580</td>
</tr>
</tbody>
</table>

Figure 5: System suitability test for UPLC-UV system
CONCLUSION

A successful method transfer from conventional LC to Ultra-Performance LC was performed for the determination of Terbinafin from human plasma. The UPLC method was found to be capable of giving faster retention times than that of achieved with HPLC method. The method developed can be used for bioequivalence and pharmacokinetic studies.

Acknowledgement

The author wish to thank the colleagues who have provided technical support, Hüseyin Yalçınkaya and Elif Aksu from Yeditepe University GLP Laboratory in İstanbul.

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