YOUDEN’S TEST FOR CHROMATOGRAPHIC DETERMINATION OF ENALAPRIL IN PHARMACEUTICALS

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Background. Robustness tests were firstly introduced for avoiding problems in interlaboratory studies and identifying the factors potentially responsible. A robustness test performing in late validation procedure involves the possibility that when the method is established not robust, it should be redeveloped and optimized. At this stage much effort has been made and money spent for optimization and validation, and therefore avoiding this would be great.

Objective. The aim of the study was to consider the robustness of HPLC determination of enalapril (in tablets) by the Youden’s test.

Methods. Youden’s test was chosen as an efficient method to assess the robustness among all analytical methods that is by means of an experiment design, which involved seven analytical parameters combined in eight tests. In previous studies, we evaluated the chromatographic method robustness to quantify enalapril (in tablets) by Youden’s test.

Results. According to the Youden’s test criteria, HPLC method proved to be greatly robust regarding the enalapril content in introduction of variation of seven analytic parameters. The lowest variation in enalapril content was 0.91 %, when Grace Platinumр C8 EPS column (4.6 mm i.d. X 250 mm, 5 μm) was used. A holistic approach concerning simultaneous innovations in particle technology and instrument design was endeavored for the first time to meet and tackle the analytical laboratory issues. This was aimed at promoting success of analytical scientists as well as profitability and productiveness of business.

Conclusion. The Youden’s test has been proved to be an efficient and useful tool for evaluation of robustness of enalapril HPLC assay.

KEY WORD: enalapril; high-performance liquid chromatography; robustness; quantitative analysis; Youden’s test.

Introduction
Recently, Robustness testing is best known and most commonly used in the pharmaceutics because of the stringent regulations in the domain set by regulatory authorities that requires extensively validated methods. Therefore most definitions and existing methodologies, e.g. those from the ICH, are found in the field, as stated before. Though, this has no implications for robustness testing of analytical methods in other domains and therefore this guideline is not confined to pharmaceutical methods [1].

Evaluation of robustness of chromatographic method is a laborious, complex and straining process, taking into account a great number of analytical parameters considered while carrying out the test. Some authors consider specific analytical parameters presenting small variations in the nominal conditions; statistical analysis is made using the Student’s t-test or ANOVA test. Other alternative for evaluation of robustness of analytical methods is the Youden’s test. This test assesses not only the robustness of the method but also determines the each analytical parameter effect on final results. The main idea of the Youden’s test is not studying one alteration at time but introducing several changes all together in this way that the effects of individual changes can be determined [2, 3, 4].

Enalapril maleate is a maleate salt of enalapril, the ethyl ester of a long-acting angiotensin converting enzyme inhibitor, enalaprilat. Enalapril maleate is chemically defined as (S)-1-[N-[1-(ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]-L-proline, (Z)-2-butenedioate salt (1:1). Enalapril, after hydrolysis to enalaprilat, inhibits angiotensin-converting enzyme (ACE) in humans and animals. ACE is a peptidyl dipeptidase that catalyzes the conversion of angiotensin I to the
vasoconstrictor substance, angiotensin II. Angiotensin II also stimulates aldosterone secretion by the adrenal cortex. Enalapril in hypertension and heart failure beneficially effects primarily from suppression of the renin-angiotensin-aldosterone system. Inhibition of ACE leads to decrease of plasma angiotensin II that results in decrease of vasopressor activity and decrease of aldosterone secretion [5].

The aim of the research was to determine the robustness of HPLC (High-Performance Liquid Chromatography) method for evaluation of enalapril by means of Youden’s test, and define the analytical parameters that have greater influence on the final analysis.

Methods
Enalapril maleate was presented by Farmak pharmaceuticals (Kiev, Ukraine). HPLC grade acetonitrile, sodium dihydrophosphate dihydrate, phosphoric acid were got from Merck pharamaceuticals.

Instrumentation and chromatographic conditions
Agilent 1260, Grace Platinum® C8 EPS column (4.6 mm i.d. X 250 mm, 5 μm). Chromatographic separation was carried out at ambient temperature (22-25 °C). The compound was separated isocratically with a mobile phase consisting of acetonitrile and buffer solution pH 2.2 (25/75, v/v), at a flow rate 2.0 mL/min with injection volume 50 μL. Column temperature was 50 °C. The effluent was monitored spectrophotometrically at a wavelength 215 nm.

Preparation of mobile phase
To prepare buffer solution pH 2.2.: 3.59 g of sodium dihydrophosphate dihydrate was dissolved in 1800 ml of water, the pH of the solution was fixed with phosphoric acid to the value (2.2±0.05), and then the volume of the solution with water R to 2000.0 ml was added and mixed.

Stock standard solutions
20 mg of the standard sample of enalapril maleate was dissolved in a solvent, added 0.5 ml of a solution of enalaprilat with a concentration of 0.4 mg/ml and 2.0 ml of enalapril diketopiperazine solution at a concentration of 0.4 mg/ml was adjusted to a volume of 100.0 ml with the same solvent.

Procedures
The standard solutions were prepared by dilution of the stock standard solution of mobile phase. Triplicate 50.0 μL injections were made for each concentration and chromatographed under the conditions described above. The peak area of each concentration was plotted against the corresponding concentration to obtain the calibration graph and regression equation was computed [6].

Results
The robustness assessment of HPLC method for enalapril quantitation was performed by the method suggested by YoudenSteiner. For the nominal values of the method, seven analytical parameters were chosen and minor variations were induced. After, eight runs were completed in order to determine the effect of each parameter on the final result. The seven analytical parameters as well as the variations are presented in Table 1. The analytical circumstances of the nominal values are defined by capital letters and of the small variation – by lowercase letters.

The seven parameters and their respective variations were joined into eight assays or chromatographic runs randomly performed. The factorial combination of parameters for the Youden’s test is presented in Table 2. The results of the analyses are defined by the letters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Nominal condition</th>
<th>Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/a</td>
<td>Acetonitrile in mobile phase</td>
<td>25 – A</td>
</tr>
<tr>
<td>B/b</td>
<td>Buffer solution pH 2.2 in mobile phase</td>
<td>75 – B</td>
</tr>
<tr>
<td>C/c</td>
<td>pH of buffer solution in mobile phase</td>
<td>2.2 – C</td>
</tr>
<tr>
<td>D/d</td>
<td>Column temperature, °C</td>
<td>50 – D</td>
</tr>
<tr>
<td>E/e</td>
<td>Mobile phase flow rate, ml/min</td>
<td>2.0 – E</td>
</tr>
<tr>
<td>F/f</td>
<td>Column supplier</td>
<td>Grace Platinum® C8 EPS – F</td>
</tr>
<tr>
<td>G/g</td>
<td>Chromatograph model</td>
<td>Agilent 1290 – G</td>
</tr>
</tbody>
</table>
from s to z. Hence, when combination 1 was assessed, the result was s, for combination 2 the result was t, and so on.

Three injections of each sample and standard solutions at the normal concentration were administered for each combination. A 30-minute pause for system stabilization took place after alteration of chromatographic column or mobile phase composition. In each combination the assessed results were for a peak area, retention time (Rt), tailing factor (T), theoretical plates number (N) and captopril content.

The following equation was used for evaluation of the effect of the column temperature on the final analyses results:

\[
\text{Effect C/c} = \frac{(s+u+w+y)}{4} - \frac{(t+v+x+z)}{4}
\]

The Youden’s test allows definite establishing of the parameters, which have a greater influence on the results of the analyses, and control more rigorously the eventual variations of these parameters that may arise during a routine analysis.

**Discussion**

In this research, the first trials were aimed to find optimal chromatographic conditions. The objective of the chromatographic method development was achievement of a peak tailing factor <1.5, retention time of between 4 and 5 minutes in consort with well resolution [7-17]. In both equipment (Agilent 1290 and HP1100), the analyses of the robustness evaluation of chromatographic method were carried out simultaneously. The results were attained in eight runs to enalapril sample and standard solutions.

The effects of the parameter variations on the analysis results are presented in Table 3.

By means of the Youden’s test criteria, HPLC method proved to be significantly robust as regards the content of enalapril in case of introduced variations of seven analytical parameters [18]. The lowest variation in enalapril content was 0.91 %, when column Grace Platinumр C8 EPS column (4.6 mm i.d.×250 mm, 5 μm) was used.

A holistic approach concerning simultaneous innovations in particle technology and instrument design was endeavored for the first time to meet and tackle the analytical laboratory issues. This was aimed at promoting success of analytical scientists as well as profitability and productiveness of business. The Platinum™ column advantage controlled silica exposure is the dissimilarity that makes Platinum™ columns unique. Instead of thorough covering of the silica with bonded phase to hide the silica, the exposure of the silica in Platinum™ columns is controlled to provide a dual mode separation

<table>
<thead>
<tr>
<th>Analytical parameter</th>
<th>Factorial combination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetonitrile in mobile phase</td>
<td>A A A A a a a a</td>
</tr>
<tr>
<td>Buffer solution pH 2.2 in mobile phase</td>
<td>B B b b B B b b</td>
</tr>
<tr>
<td>pH of buffer solution in mobile phase</td>
<td>C c C c C c C c</td>
</tr>
<tr>
<td>Column temperature</td>
<td>D D d d d d D D</td>
</tr>
<tr>
<td>Mobile phase flow rate</td>
<td>E e E e E e E e</td>
</tr>
<tr>
<td>Column supplier</td>
<td>F f f F F f f F</td>
</tr>
<tr>
<td>Chromatograph model</td>
<td>G g g G g G G g</td>
</tr>
</tbody>
</table>

**Table 3. Effects of the analytical parameters on content and retention time (Rt) for enalapril HPLC quantitation**

<table>
<thead>
<tr>
<th>Effect</th>
<th>Content (%)</th>
<th>Rt (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetonitrile in mobile phase</td>
<td>0.15</td>
<td>-0.26</td>
</tr>
<tr>
<td>Buffer solution pH 2.2 in mobile phase</td>
<td>0.16</td>
<td>-0.27</td>
</tr>
<tr>
<td>pH of buffer solution in mobile phase</td>
<td>0.12</td>
<td>0.05</td>
</tr>
<tr>
<td>Column temperature</td>
<td>-0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Mobile phase flow rate</td>
<td>-0.03</td>
<td>0.05</td>
</tr>
<tr>
<td>Column supplier</td>
<td>0.91</td>
<td>-2.05</td>
</tr>
<tr>
<td>Chromatograph model</td>
<td>-0.04</td>
<td>0.11</td>
</tr>
</tbody>
</table>
with both polar and non-polar sites exposed to the samples. This extends polar selectivity well beyond the other reversed-phase columns and gives separations that other columns cannot.

**Conclusion**
Youden’s test proved to be an efficient and useful tool for the robustness evaluation of HPLC method for assay of enalapril in pharmaceuticals. Therefore, Youden’s test can be successfully used for the robustness evaluation for validation process of analytical methods.

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This research received no external funding.

**Conflict of Interests**
The author declares no conflict of interest.

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**ЮДЕН ТЕСТ ХРОМАТОГРАФІЧНОГО ВИЗНАЧЕННЯ ЕНАЛАПРИЛУ В ЛІКАРСЬКИХ ЗАСОБАХ**

**Л.С. Логойда**

ТЕРНОПІЛЬСЬКИЙ НАЦІОНАЛЬНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ ІМЕНІ І. Я. ГОРБАЧЕВСЬКОГО, ТЕРНОПІЛЬ, УКРАЇНА

**Вступ.** Випробування на робасність спочатку були введені, щоб уникнути проблем у міжлабораторних дослідженнях та виявити потенційно відповідальні фактори. Виконання перевірки надійності в кінці процедури валідації передбачає ризик того, що, коли виявиться, що метод не є надійним, його слід переробити і оптимізувати. На цьому етапі вже було витрачено багато зусиль і грошей на оптимізацію і перевірку, і тому хочеться цього уникнути.

**Мета дослідження** – визначити робасність хроматографічного визначення еналаприлу в таблетках з використанням тесту Юдена.

**Методи дослідження.** Ефективний метод оцінки надійності аналітичних методів за допомогою тесту Юдена шляхом розробки експерименту, який включає сім аналітичних параметрів, об’єднаних у восьми тестах. У дослідженнях ми оцінювали надійність хроматографічного методу для кількісного визначення еналаприлу в таблетках з використанням тесту Юдена.

**Результати.** Використовуючи критерії випробування Юдена, метод ВЕРХ показав високу надійність щодо вмісту еналаприлу при введені варіації семи аналітичних параметрів. Найнижча зміна вмісту еналаприлу становила 0,91%, коли використовувалася колонка Grace Platinumr C8 EPS-колони (4,6 мм i.d. X 250 мм, 5 мкм). Вперше розроблено цілісний підхід, що передбачає одночасне провадження інновацій у технології частинок та проектування приладів. Це було зроблено для того, щоб зробити вченів-аналітиків більш успішними, а підприємства - більш прибутковими та продуктивними.

**Висновки.** Тест Юдена виявився ефективним і корисним інструментом для оцінки робастності для аналізу еналаприлу методом ВЕРХ.

**КЛЮЧОВІ СЛОВА:** еналаприл; високоефективна рідинна хроматографія; робасність; кількісний аналіз; Юден тест.

**Відомості про автора**

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References


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