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Robustness evaluation of the chromatographic determination of nifedipine in pharmaceuticals

Topicality. Robustness tests were originally introduced to avoid problems in interlaboratory studies and to identify the potentially responsible factors.

The **aim** of this study was the rubustness evaluation of the chromatographic determination of nifedipine in medicines using Youden's test.

Materials and methods. Youden's test is a reliable method to evaluate the robustness of analytical methods, by means of an experiment design which involves seven analytical parameters combined in eight tests. In the present study, we assessed the robustness of a chromatographic method to quantify nifedipine using Youden's test. Youden's test showed to be a simple and feasible procedure to evaluate the robustness of chromatographic methods.

Results and discussion. Using the criteria of Youden's test, the chromatographic method showed to be highly robust regarding of nifedipine content, when variations in seven analytical parameters were introduced. The highest variation in nifedipine content was 0.28 %, when the concentration of trifluoroacetic acid in the mobile phase was altered; a value considerably low and not significant in routine analyses.

Conclusions. Youden's test showed to be a reliable and useful tool for the robustness evaluation of the chromatographic method for assay of nifedipine. Therefore, Youden's test can be successfully applied for the robustness evaluation in validation process of analytical methods by HPLC.

***Key words**: nifedipine; validation; robustness; chromatography; quantitative analysis; Youden's test*

Л. С. Логойда

Аналіз робастності хроматографічного визначення ніфедипіну в лікарських засобах

Актуальність. Робастність – здатність аналітичної методики не зазнавати впливу малих, заданих аналітиком змін в умовах виконання методики, є показником надійності методики при її використанні в заданих умовах.

Метою даного дослідження був аналіз робастності хроматографічного визначення ніфедипіну з використанням Юден тесту.

Матеріали та методи. Випробування Юден тесту є надійним методом аналізу робастності аналітичних методів за допомогою планування експерименту, який включає сім аналітичних показників, об'єднаних у вісім випробувань. У цьому дослідженні ми оцінювали робастність хроматографічного методу для кількісного визначення ніфедипіну з використанням Юден тесту. Юден тест показав, що він є простим і доступним у процедурі оцінки робастності хроматографічних методів.

Результати та їх обговорення. Використовуючи критерії Юден тесту, хроматографічний метод показав високу оцінку робастності щодо місту ніфедипіну, коли були введені зміни в семи аналітичних параметрів. Найбільша варіація в вмісті ніфедипіну була 0,28 %, коли була змінена концентрація трифлорацетатної кислоти в рухомій фазі.

Висновки. Юден тест виявився надійним і корисним для оцінки надійності хроматографічного методу кількісного визначення ніфедипіну. Таким чином, Юден тест може бути успішно застосований для оцінки робастності в процесі валідації аналітичних методів за допомогою високоефективної рідинної хроматографії.

***Ключові слова:** ніфедипін; валідація; робастність; хроматографія; кількісний аналіз; Юден тест*

Л. С. Логойда

Анализ робастности хроматографического определения нифедипина в лекарственных средствах

Актуальность. Робастность – это способность аналитической методики не подвергаться влиянию малых, задаваемых аналитиком изменений в условиях выполнения методики и быть показателем надежности методики при ее использовании в указанных условиях.

Целью данного исследования был анализ робастности хроматографического определения нифедипина с использованием Юден теста.

Материалы и методы. Испытание Юден теста является надежным методом анализа робастности аналитических методов с помощью планирования эксперимента, который включает семь аналитических показателей, объединенных в восемь испытаний. В этом исследовании мы оценивали робастность хроматографического метода для количественного определения нифедипина с использованием Юден теста. Юден тест показал, что он является простым и доступным в процедуре оценки робастности хроматографических методов.

Результаты и их обсуждение. Используя критерии Юден теста, хроматографический метод показал высокую оценку в содержании робастности нифедипина, когда были введены изменения в семи аналитических параметрах. Наибольшая вариация в содержании нифедипина составляла 0,28 %, когда была изменена концентрация трифлорацетатной кислоты в подвижной фазе.

Выводы. Юден тест оказался надежным и полезным инструментом для оценки надежности хроматографического метода для нифедипина. Таким образом, Юден тест может быть успешно применен для оценки робастности в процессе валидации аналитических методов с помощью высокоэффективной жидкостной хроматографии.

***Ключевые слова:** нифедипин; валидация; робастность; хроматография; количественный анализ; Юден тест*

INTRODUCTION

Both the ICH and the USP guidelines define the robustness of an analytical procedure as a measure of its capacity to remain unaffected by small but deliberate variations in procedural parameters listed in the documentation, providing an indication of the method's or procedure's suitability and reliability during normal use. But while robustness shows up in both guidelines, interestingly enough, it is not in the list of suggested or typical analytical characteristics used to validate a method (again, this apparent discrepancy is changing in recently proposed revisions to USP chapter 1225. Robustness tests were originally introduced to avoid problems in interlaboratory studies and to identify the potentially responsible factors. This means that a robustness test was performed at a late stage in the method validation since interlaboratory studies are performed in the final stage. Thus the robustness test was considered a part of method validation related to the precision (reproducibility) determination of the method. However, performing a robustness test late in the validation procedure involves the risk that when a method is found not to be robust, it should be redeveloped and optimised. At this stage much effort and money have already been spent in the optimisation and validation, and therefore one wants to avoid this. Therefore the performance of a robustness test has been shifting to earlier points of time in the life of the method [1].

The evaluation of the robustness of chromatographic methods often is complex and laborious, taking into account the large number of analytical parameters that should be considered to carry out the test. Some authors select specific analytical parameters to be evaluated, introducing small variations in the nominal conditions and the statistical interpretation is performed by means of Student's *t*-test or ANOVA test. Other wider alternative to determine the robustness of analytical methods is the Youden's test. This test allows not only evaluating the method robustness but also pointing out the influence of each analytical parameter in the final results. The basic idea of Youden's test is not to study one alteration at time but to introduce several changes at once, in such a manner that the effects of individual changes can be ascertained [2, 3].

The **aim** of the work was to evaluate the robustness of the chromatographic method for the quantitation of nifedipine, using Youden's test, and determine the analytical parameters that present higher influence in the final results of the analysis.

MATERIALS AND METHODS

The objects of the study were tablets "Fenigidin Zdorovja" (Ukraine). The chromatographic analysis of nifedipine performed on liquid chromatographs Agilent 1290 and HP 1100 systems. The columns used Nucleosil C18 (4.6 × 150 mm with a particle size of 5 microns) and Ascentis Express C18 (column size 4.6 × 150 mm with a particle size of 5 microns). The column temperature was 35 °C. The mobile phase consisted of methanol R and 0.1 % solution of trifluoroacetic acid R (55 : 45), at a flow rate of 1.5 ml/min. The detection was performed at 265 nm.

Standard solution. 20.0 mg of nifedipine SPhU dissolve in *methanol R* and dilute with the same solvent to 20.0 ml volume. 2.0 ml of the resulting solution adjusted to 20.0 ml of solvent.

Sample solution. To 200.0 mg powder pounded tablets, add 10 ml of *methanol R*, shake in ultrasonic bath for 10 min and add *methanol R* to the volume of 20.0 ml. Filter through a membrane filter with a pore size of 0.45 microns, discarding the first 5 ml of filtrate. 2.0 ml of the resulting filtrate adjusted to 20.0 ml of solvent.

The robustness evaluation of the chromatographic method for nifedipine quantitation was performed using the method proposed by Youdene Steiner (1975). Seven analytical parameters were selected and small variations were induced in the nominal values of the method. Then, eight runs were performed aiming to determine the influence of each parameter in the final result. The seven analytical parameters employed, as well as the introduced variations are demonstrated at Tab. 1. The analytical conditions at the nominal values are represented by capital letters and the conditions with the small variation are represented by lowercase letters.

The seven parameters and its respective variations were combined in eight assays or chromatographic runs, performed in a random order. Tab. 2 demonstrates the

Table 1

ANALYTICAL PARAMETERS AND VARIATIONS FOR THE ROBUSTNESS EVALUATION OF THE CHROMATOGRAPHIC METHOD FOR NIFEDIPINE QUANTITATION

Parameter	Nominal condition	Variation					
A/a	Methanol in mobile phase	55	–	A	50	–	a
B/b	0.1 % solution of trifluoroacetic acid in mobile phase	45	–	B	50	–	b
C/c	Concentration of trifluoroacetic acid in mobile phase, %	0.1	–	C	0.05	–	c
D/d	Column temperature, °C	35	–	D	30	–	d
E/e	Mobile phase flow rate, ml/min	1.5	–	E	1.0	–	e
F/f	Column supplier	Ascentis Express C18	–	F	Nucleosil C18	–	f
G/g	Chromatograph model	Agilent 1290	–	G	HP 1100	–	g

Table 2

FACTORIAL COMBINATION OF THE ANALYTICAL PARAMETERS FOR ROBUSTNESS EVALUATION BY YOUDEN'S TEST

Analytical parameter	Factorial combination							
Methanol in mobile phase	A	A	A	A	a	a	a	a
0.1 % solution of trifluoroacetic acid in mobile phase	B	B	b	b	B	B	b	b
Concentration of trifluoroacetic acid in mobile phase	C	c	C	c	C	c	C	c
Column temperature	D	D	d	d	d	d	D	D
Mobile phase flow rate	E	e	E	e	e	E	e	E
Column supplier	F	f	f	F	F	f	f	F
Chromatograph model	G	g	g	G	g	G	G	g
Result	s	t	u	v	w	x	y	z

factorial combination of the parameters for the Youden's test. The analyses results are shown by letters from *s* to *z*. Hence, when combination 1 was assayed, the obtained result was *s*. When combination 2 was assayed, the obtained result was *t*, and so successively.

In each combination, three injections of each sample and standard solutions were carried out, at the work concentration. After the change of chromatographic column or mobile phase composition, 30 min were awaited for system stabilization. The evaluated results in each combination were peak area, retention time (Rt), tailing factor (T), theoretical plates number (N) and verapamil hyrdochloride content.

To determine the influence of variations of each parameter in the final result, the mean of the four values corresponding to the capital letters (nominal conditions) was compared to the mean of the four values corresponding to the lowercase letters (altered conditions). For example, to evaluate the effect of the column temperature in the final result of the analyses, the following equation was employed:

$$Effect\ C/c = (s + u + w + y) / 4 - (t + v + x + z) / 4 \quad Eq.\ (1)$$

Thus, the influence of the seven analytical parameters regarding the peak area, retention time (Rt), tailing factor (T), theoretical plates number (N) and nifedipine content were evaluated. By means of Youden's test, it is possible to establish certainly the parameters which present higher influence in the final result of the analyses and perform a more rigorous control in the eventual variations of these parameters that may occur during a routine analysis.

Table 3

EFFECTS OF THE ANALYTICAL PARAMETERS IN CONTENT AND RETENTION TIME (Rt) OF THE CHROMATOGRAPHIC METHOD FOR NIFEDIPINE QUANTITATION

Effect	Content (%)	Rt (min)
Methanol in mobile phase	0.2	-0.56
0.1 % solution of trifluoroacetic acid in mobile phase	0.14	-0.33
Concentration of trifluoroacetic acid in mobile phase	0.28	-1.12
Column temperature	-0.04	0.03
Mobile phase flow rate	-0.01	0.05
Column supplier	-0.01	-0.12
Chromatograph model	-0.05	0.15

RESULTS AND DISCUSSION

The assays for the robustness evaluation of the chromatographic method were carried out simultaneously in both equipments, Agilent 1290 and HP1100. The results obtained in the eight runs to nifedipine sample and standard solutions [4, 5, 6, 7].

To evaluate the effect of each parameter, the average of the four values corresponding to altered conditions was subtracted from the average of the four values obtained at the nominal conditions, as demonstrated in *Eq. (1)*. The effects of the parameter variations in the analysis results are presented in Tab. 3.

Using the criteria of Youden's test, the chromatographic method showed to be highly robust regarding nifedipine content, when variations in seven analytical parameters were introduced. The highest variation in nifedipine content was 0.28 %, when the concentration of trifluoroacetic acid in the mobile phase was altered; a value considerably low and not significant in routine analyses. The retention time of nifedipine peak was more considerably influenced by three analytical parameters. Some parameters such as column temperature, mobile phase flow rate, column supplier and chromatograph model presented low influence in the evaluated factors of the chromatographic method.

CONCLUSIONS

Youden's test showed to be a reliable and useful tool for the robustness evaluation of the chromatographic method for assay of nifedipine. Therefore, Youden's test can be successfully applied for the robustness evaluation in validation process of analytical methods by HPLC.

Conflict of Interests: authors have no conflict of interests to declare.

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