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Robustness Evaluation of the Chromatographic Determination of Amlodipine in Tablet Dosage Form

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ABSTRACT

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

The aim of this study was robustness evaluation of the chromatographic determination of amlodipine in tablet dosage form using Youden's test.

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 amlodipine in tablets using Youden's test. Youden's test showed to be a simple and feasible procedure to evaluate the robustness of chromatographic methods.

Results: Using the criteria of Youden's test, the highest variation in amlodipine content was 0.44 %, when the concentration of trifluoroacetic acid in the mobile phase was altered; a value considerably low and not significant in routine analyses.

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

KEYWORDS

Amlodipine, Validation, Robustness, Chromatography, Quantitative analysis, Youden's test

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INTRODUCTION

Robustness can be described as the ability to reproduce the (analytical) method in different laboratories or under different circumstances without the occurrence of unexpected differences in the obtained result(s), and a robustness test as an experimental set-up to evaluate the robustness of a method. The term ruggedness is frequently used as a synonym. Robustness tests were originally introduced to avoid problems in interlaboratory studies and to identify the potentially responsible factors[1]. This means that a robustness test was performed at a later stage in the method validation since interlaboratory studies are performed in the final stage.

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].

AIMS AND OBJECTIVES

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

MATERIALS AND METHODS

The chromatographic analysis of amlodipine was performed on liquid chromatographs Agilent 1290 and HP 1100 systems. The columns used were 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 30 °C. The mobile phase consisted of acetonitrile R and 0.1% solution of trifluoroacetic acid R (40:60), at a flow rate of 1.0 ml / min. The detection was performed at 237 nm.

Preparation of Standard solution. 27.7 mg of amlodipine besylate SPhU was dissolved in a solvent (water R- acetonitrile R (1:1))

and diluted with the same solvent to about 50.0 ml. Aliquot of the resulting solution adjusted to 20.0 ml of solvent.

Preparation of Sample solution. To sample powder pounded tablets equivalent to 10 mg of amlodipine, was added 70 ml of solvent (water R- acetonitrile R (1:1)), shaked in ultrasonic bath for 15 minutes. The solution was cooled and adjusted to the volume of solvent 100.0 ml. Filter through a membrane filter with a pore size of 0.45 microns, discarding the first 5 ml of filtrate.

The robustness evaluation of the chromatographic method for amlodipine 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 table 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. Table 2 demonstrates the 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 amlodipine 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 *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 amlodipine 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 1 Analytical parameters and variations for the robustness evaluation of the chromatographic method for amlodipine quantitation

Parameter	Nominal condition			Variation			
A/a	Acetonitrile in mobile phase	40	-	A	45	-	a
B/b	0.1% solution of trifluoroacetic acid in mobile phase	60	-	B	55	-	b
C/c	Concentration of trifluoroacetic acid in mobile phase, %	0.1	-	C	0.05	-	c
D/d	Column temperature, °C	30	-	D	25	-	d
E/e	Mobile phase flow rate, ml/min	1.0	-	E	1.5	-	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							
Acetonitrile 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

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 amlodipine sample and standard solutions but without robustness study using Youden's test[4-8]. 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 table 3.

Table 3 Effects of the analytical parameters in content and retention time (Rt) of the chromatographic method for amlodipine quantitation

Effect	Content (%)	Rt (min)
Acetonitrile in mobile phase	**0.15**	**-0.26**
0.1% solution of trifluoroacetic acid in mobile phase	**0.16**	**-0.27**
Concentration of trifluoroacetic acid in mobile phase	**0.44**	**-1.05**
Column temperature	**-0.05**	**0.05**
Mobile phase flow rate	**-0.03**	**0.05**
Column supplier	**-0.01**	**-0.12**
Chromatograph model	**-0.04**	**0.11**

Using the criteria of Youden's test, the chromatographic method showed to be highly robust regarding amlodipine content, when variations in seven analytical parameters were introduced. The highest variation in amlodipine content was 0.44 %, 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 amlodipine 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.

CONCLUSION

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

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