ORIGINAL ARTICLE

Youden's test of the chromatographic determination of captopril in pharmaceuticals

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Abstract

Introduction: 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 optimized. At this stage, much effort and money have already been spent in the optimization and validation, and therefore one wants to avoid this. The aim of this study was to evaluate the rubustness of ultra-high-performance liquid chromatography (UHPLC) determination of captopril in tablets using Youden's test. **Materials and Methods:** An efficient method to assess the robustness of analytical methods is by Youden's test, by means of an experiment design which involves seven analytical parameters combined in eight tests. In the recent studies, we assessed the robustness of a chromatographic method to quantify captopril in tablets using Youden's test. **Results:** Using the Youden's test criteria, UHPLC method showed to be greatly robust concerning captopril content, at the introduction of variation in seven analytic parameters. The highest variation in captopril content was 0.96%, when was used column Nucleosil C18 (4.6 mm × 150 mm with a particle size of 5 µ). Using column Ascentis Express C18 Fused-Core® technology (column size 4.6 mm × 150 mm with a particle size of 5 µ) reduces the retention time of captopril more than 2 times. **Conclusion:** Youden's test proved to be an efficient and helpful tool for the robustness evaluation for assay of captopril by UHPLC.

Key words: Captopril, quantitative analysis, robustness, ultra-high-performance liquid chromatography, Youden's test

INTRODUCTION

Robustness testing is in recent time best known and most commonly only used in the pharmaceutical world because of the stringent regulations in that domain set by regulatory authorities which require extensively validated methods. Therefore, most definitions and existing methodologies, for example, those from the ICH, can be found in that field, as one can observe from the above. Although this has no implications for robustness testing of analytical methods in other domains and this guideline is therefore not confined to pharmaceutical methods.[1]

It is a laborious, complex, and straining process to evaluate the robustness of chromatographic method, taking into account the large number of analytical parameters that should be considered to carry out the test. Some authors select

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specific analytical parameters to be considered, 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 evaluates not only the method robustness but also highlights the effect 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 way that the effects of individual changes can be ascertained.[2-4]

Captopril, 1-[(2S)-3-mercapto-2-merhyl-1-oxopropyl]-L-proline, (2S)-1-[(2S)-2-Methyl-3-sulphanylpropanoyl] pyrrolidine-2-carboxylic acid, is a sulfhydryl-containing analog of proline with antihypertensive activity and potential antineoplastic activity.

The aim of the work was to evaluate the robustness of ultra-high-performance liquid chromatography (UHPLC) method for the quantitation of captopril, using Youden's test, and determine the analytical parameters that present greater influence in the final results of the analysis.

MATERIALS AND METHODS

USP grade CPT and USP grade HCT were kindly donated by Darnitsa, Kiev, Ukraine. Solvents purchased from Merck, Darmstadt, Germany high-performance liquid chromatography (HPLC) grade. Tablets captopril was kindly provided by Ternofarm, Ternopil, Ukraine. Tablets captopres was kindly provided by Darnitsa, Kiev, Ukraine.

The chromatographic analysis of captopril performed on liquid chromatographs Agilent 1290 and HP 1100 systems. The columns used Nucleosil C18 (4.6 mm × 150 mm with a particle size of 5 µ) and Ascentis Express C18 Fused-Core® technology (column size 4.6 mm × 150 mm with a particle size of 5 µ). The compounds were separated isocratically with a mobile phase consisting of methanol and 0.1% solution of trifluoroacetic acid (40/60, v/v) at a flow rate 1.2 mL/min with injection volume 2 µL. The effluent was monitored spectrophotometrically at wavelength 220 nm. Column temperature was 35°C.

Standard Solution

Primary stock solutions of captopril were prepared daily separately by dissolving 25 mg of each in 25 mL volumetric flasks (1.0 mg/mL) in mobile phase.

Sample Solution

The contents of 20 tablets labeled to contain 25 mg of captopril were weighed individually, mixed, and powdered. Amount of the powder equivalent to two tablets content was weighed accurately, then transferred into 50 ml volumetric flask and diluted with mobile phase. Filtering of the sample solution was then carried out using 0.45 µm filters (Millipore, Milford, MA).

RESULTS

The robustness evaluation of UHPLC method for captopril quantitation was performed using the method proposed by Youdene Steiner. Seven analytical parameters were selected and small variations were induced in the nominal values of the method. Then, eight runs were performed with an aim to determine the effect 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 alteration of chromatographic column or mobile phase composition, there was a waiting of 30 min for system stabilization. The evaluated results in each combination were peak area, retention time (R_t), tailing factor (T), theoretical plates number (N), and captopril content.

For evaluating the effect of the column temperature in the final result of the analyses, the following equation was used:

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

Through the use 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.

DISCUSSION

In this study, our first trials were directed to find optimal chromatographic conditions, our objective of the chromatographic method development was to achieve a peak tailing factor <1.5, retention time in between 1 and 3 min, along with good resolution.[5-15] In both equipments (Agilent 1290 and HP1100) were carried out simultaneously, the

Table 1: Analytical parameters and variations for the robustness evaluation of UHPLC method for captopril quantitation

Parameter		Nominal condition			Variation		
A/a	Methanol 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	35	-	D	30	-	d
E/e	Mobile phase flow rate, ml/min	1.2	-	E	1.5	-	e
F/f	Column supplier	Ascentis Express C18 Fused-Core® technology	-	F	Nucleosil C18	-	f
G/g	Chromatograph model	Agilent 1290	-	G	HP 1100	-	g

UHPLC: Ultra-high-performance liquid chromatography

Table 2: Factorial combination of the analytical parameters for robustness evaluation

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

Table 3: Effects of the analytical parameters in content and retention time (R_t) for captopril UHPLC quantitation

Effect	Content (%)	R_t (min)
Methanol 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	0.12	0.05
Column temperature	–0.05	0.05
Mobile phase flow rate	–0.03	0.05
Column supplier	–0.96	–2.05
Chromatograph model	–0.04	0.11

UHPLC: Ultra-high-performance liquid chromatography

assays for the robustness evaluation of the chromatographic method. The results obtained in the eight runs to captopril sample and standard solutions.

In Table 3, the effects of the parameter variations in the analysis results presented.

Using the criteria of Youden's test, UHPLC method proved to be greatly robust regarding content of captopril, when variations in seven analytical parameters were introduced.[16,17] The highest variation in captopril content was 0.96% when was used column Nucleosil C18 (4.6 mm × 150 mm with a particle size of 5 µ). Relevant advancement in instrumentation and column technology were made to realize dramatic increases in resolution, speed and sensitivity of UHPLC/HPLC.

For the first time, a holistic approach involving simultaneous innovations in particle technology and instrument design was endeavored to meet and tackle the issues of the analytical laboratory. This was done to make analytical scientists more successful and businesses more profitable and productive.

Ascentis Express columns, based on Fused-Core particle technology, provide more than twice the speed and efficiency of traditional columns at half the backpressure of sub-2-µm

columns. Using column Ascentis Express C18 Fused-Core® technology (column size 4.6 mm × 150 mm with a particle size of 5 μ) decrease the retention time of captopril more than 2 times.

CONCLUSION

Youden's test proved to be an efficient and helpful tool for the robustness evaluation of UHPLC method for assay of captopril in pharmaceuticals. Therefore, Youden's test can be applied successfully for the robustness evaluation in validation process of analytical methods.

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