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K. Ye. Peleshok, D. B. Koval
I. HORBACHEVSKY TERNOPIL NATIONAL MEDICAL UNIVERSITY

YOUDEN'S TEST OF THE CHROMATOGRAPHIC DETERMINATION OF ATENOLOL IN PHARMACEUTICALS

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

The aim of the study *– to evaluate the rubustness of HPLC determination of atenolol in tablets using Youden's test.*

Research 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 enalapril in tablets using Youden's test.*

Results and Discussion. *By using the Youden's test criteria, HPLC method showed to be greatly robust concerning atenolol content, at the introduction of variation in seven analytic parameters. The lowest variation in atenolol content was 0.96 %, when was used column Zorbax C8 (4.6 mm i.d. ×150 mm, 5 μm). 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 in order to make analytical scientists more successful and businesses more profitable and productive.*

Conclusion. *Youden's test proved to be an efficient and helpful tool for the robustness evaluation for assay of atenolol by HPLC. Youden's test can be applied successfully for the robustness evaluation in validation process of analytical methods.*

KEY WORDS: **atenolol; high-performance liquid chromatography; robustness; quantitative analysis; Youden's test.**

INTRODUCTION. Analytical method validation is a vital step following method development for ensuring reliable and accurate method performance. Among examined figures of merit, robustness/ruggedness study allows us to test performance characteristics of the analytical process when operating conditions are altered either deliberately or not. The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. This study yields useful information, being a fundamental part of method validation. Since many experiments are required, this step is high demanding in time and consumables. In order to avoid the difficult task of performing too many experiments the Youden test which makes use of fractional factorial designs and has been proved to be a very effective approach. The main advantage of Youden test is the fact that it keeps the required time and effort to a minimum, since only a limited number of determinations have to be made, using combinations of the chosen investigated factors [1].

Atenolol (Fig.) is a synthetic isopropylaminopropanol derivative used as an antihypertensive, hypotensive and antiarrhythmic. Atenolol is chemically known as 2-[4-[2-hydroxy-3-(propan-2-ylamino)propoxy]phenyl]acetamide. Atenolol acts as a peripheral, cardioselective beta blocker specific for beta-1 adrenergic receptors, without intrinsic sympathomimetic effects. It reduces exercise heart rates and delays atrioventricular conduction, with overall oxygen requirements decreasing [2, 3].

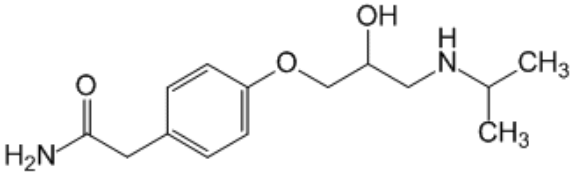

Figure. Chemical structure of atenolol.

The aim of the study was to evaluate the robustness of HPLC (High-Performance Liquid Chromatography) method for the quantitation of atenolol, using Youden's test, and determine the analytical parameters that present greater influence in the final results of the analysis.

RESEARCH METHODS. Atenolol was supplied by Refik Saydam National Public Health Agency. The methanol used in experiments was HPLC gradient grade and potassium dihydrogen phosphate was of Ph.Eur.reagent grade and purchased from Merck Darmstdat, Germany. Analytical Balance Mettler Toledo MPC227, pH-metter Metrohm 827, demineralized water from TKA Micro system, with final conductivity less than 0.05µS/cm. IKA orbital shaker KS4000i was used for sample agitation. The nylon and regenerated cellulose RC 0.45um syringe filters were purchased from Agilent Technologies.

Dionex Ultimate 3000 UHPLC system controlled by Chromeleon version 6.80, composed of quaternary LPG pump ultimate 3000, autosampler ultimate 3000, ultimate 3000 column compartment, four channel UV-Vis detector ultimate 3000 RS. Shimadzu Nexera XR UPLC system with LPG Quaternary Pump LC-20AD with degasser DGU-20A5R, Autosempler SIL-20AC, PDA detector M20-A, Column Oven and Controller CBM-20A controlled by Lab Solutions version 5.97. The used column Zorbax C8 (4.6 mm i.d. ×150 mm, 5 µm), purchased from Sigma-Aldrich Supelco. The optimum mobile phase composition was composed of methanol and 25 mM solution potassium dihydrogen phosphate pH 7.3 (55:45, V/V), pumped with 1.0 mL/min at 40 °C set temperature of column oven, with UV detector set to 225 nm wavelength. Analyses performed on column Zorbax C8 (4.6 mm i.d. ×150 mm, 5 µm) [4].

Sample preparation

Twelve tablets of each preparation were studied to obtain statistically significant results. The tablets with declared contents of 80 mg valsartan were purchased from local drug store, pharmacy. The tablets were put in 100 mL measuring flasks and dissolved in 50 mL 50 % v/v methanol, ultrasound crushed and treated for 2 minutes and shaked 15min with orbital shaker. After that measuring flasks were filled to mark for 100 mL, the final concentration was 1mg/ml for atenolol. Samples were filtered with RC 0.45um syringe filters and injected.

RESULTS AND DISCUSSION. The robustness evaluation of HPLC method for atenolol 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 [5–7].

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 chro-

Table 1 – **Analytical parameters and variations for the robustness evaluation of HPLC method for atenolol quantitation**

Parameter		Nominal condition			Variation		
A/a	Methanol in mobile phase	55	-	A	45	-	a
B/b	25 mM solution potassium dihydrogen phosphate pH 7.3 in mobile phase	45	-	B	55	-	b
C/c	pH of solution potassium dihydrogen phosphate in mobile phase	7.3	-	C	7.0	-	c
D/d	Column temperature, °C	40	-	D	30	-	d
E/e	Mobile phase flow rate, ml/min	1.0	-	E	0.7	-	e
F/f	Column supplier	Zorbax C8	-	F	Grace Platinump C8 EPS	-	f
G/g	Chromatograph model	Shimadzu Nexera XR UPLC system	-	G	Agilent 1260 Infinity II system	-	g

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
25 mM solution potassium dihydrogen phosphate pH 7.3 in mobile phase	B	B	b	b	B	B	b	b
pH of solution potassium dihydrogen phosphate 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

matographic 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 (Rt), 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:

Effect C/c = (s + u + w + y) / 4 – (t + v + x + z) / 4Eq. (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.

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 3 and 4 min, along with good resolution. In both equipments (Shimadzu Nexera XR UPLC system and Agilent 1260 Infinity II system), were carried out simultaneously the assays for the robustness evaluation of the chromatographic method. The results obtained in the eight runs to enalapril sample and standard solutions.

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

By using the criteria of Youden's test, HPLC method proved to be greatly robust regarding content of atenolol, when variations in seven analytical parameters were introduced. The lowest variation in atenolol content was 0.96 %, when was used column Zorbax C8 (4.6 mm i.d. ×150 mm, 5 µm).

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 in order to make analytical scientists more successful and businesses more profitable and productive.

Table 3 – **Effects of the analytical parameters in content and retention time (Rt) for atenolol HPLC quantitation**

Effect	Content (%)	Rt (min)
Methanol in mobile phase	0.12	-0.56
25 mM solution potassium dihydrogen phosphate pH 7.3 in mobile phase	0.16	-0.29
pH of solution potassium dihydrogen phosphate in mobile phase	0.13	0.09
Column temperature	-0.09	0.07
Mobile phase flow rate	-0.07	0.08
Column supplier	0.96	-2.85
Chromatograph model	-0.03	0.06

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

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К. Є. Пелешок, Д. Б. Коваль

ТЕРНОПІЛЬСЬКИЙ НАЦІОНАЛЬНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ ІМЕНІ І. Я. ГОРБАЧЕВСЬКОГО МОЗ УКРАЇНИ

ЮДЕН ТЕСТ ХРОМАТОГРАФІЧНОГО ВИЗНАЧЕННЯ АТЕНОЛОЛУ В ЛІКАРСЬКИХ ЗАСОБАХ

Резюме

Вступ. *Випробування на робасність спочатку було введено для того, щоб уникнути проблем під час виконання міжлабораторних досліджень та виявити потенційно відповідальні фактори. Здійснення перевірки надійності в кінці процедури валідації передбачає ризик того, що, коли з'ясується, що метод не є надійним, його слід переробити й оптимізувати. На даному етапі вже було докладено багато зусиль і витрачено багато коштів на оптимізацію та перевірку, тому хочеться цього уникнути.*

Мета дослідження *– визначити робасність хроматографічного визначення атенололу в таблетках з використанням Юден тесту.*

Методи дослідження. *Ефективним є метод оцінки надійності аналітичних методів за допомогою Юден тесту шляхом розробки експерименту, який включає сім аналітичних параметрів, об'єднаних у восьми тестах. Під час досліджень було оцінено надійність хроматографічного методу для кількісного визначення еналаприлу в таблетках з використанням Юден тесту.*

Результати й обговорення. *При застосуванні критеріїв випробування Юдена метод високоефективної рідинної хроматографії показав високу надійність щодо вмісту атенололу за умов введення варіації семи аналітичних параметрів. Найменша зміна вмісту еналаприлу становила 0,96 %, коли використовували колонку Zorbax C8 (4,6 мм i.d. ×150 мм, 5 мкм). Уперше розроблено цілісний підхід, що передбачає одночасне впровадження інновацій у технології частинок та проектування приладів. Це необхідно для того, щоб зробити вчених-аналітиків успішнішими, а підприємства – більш прибутковими та продуктивними.*

Висновки. *Юден тест виявився ефективним і корисним інструментом для оцінки робасності під час аналізу атенололу методом високоефективної рідинної хроматографії. Його можна успішно застосовувати для оцінки робасності в процесі валідації аналітичних методик.*

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

Е. Е. Пелешок, Д. Б. Коваль
ТЕРНОПОЛЬСКИЙ НАЦИОНАЛЬНЫЙ МЕДИЦИНСКИЙ УНИВЕРСИТЕТ ИМЕНИ И. Я. ГОРБАЧЕВСКОГО МОЗ УКРАИНЫ

ЮДЕН ТЕСТ ХРОМАТОГРАФИЧЕСКОГО ОПРЕДЕЛЕНИЯ АТЕНОЛОЛА В ЛЕКАРСТВЕННЫХ СРЕДСТВАХ

Резюме

Вступление. *Испытания на робасность изначально было введено для того, чтобы избежать проблем во время выполнения межлабораторных исследований и выявить потенциально ответственные факторы. Осуществление проверки надежности в конце процедуры валидации предусматривает риск того, что, когда окажется, что метод не надежен, его следует переработать и оптимизировать. На данном этапе уже было приложено много усилий и потрачено много средств на оптимизацию и проверку, поэтому хочется этого избежать.*

Цель исследования *– определить робасность хроматографического определения атенолола в таблетках с использованием Юден теста.*

Методы исследования. *Эффективным является метод оценки надежности аналитических методов с помощью Юден теста путем разработки эксперимента, который включает семь аналитических параметров, объединенных в восьми тестах. Во время исследований было оценено надежность хроматографического метода для количественного определения эналаприла в таблетках с использованием Юден теста.*

Результаты и обсуждение. *При применении критериев испытания Юдена метод высокоэффективной жидкостной хроматографии показал высокую надежность в отношении содержания атенолола в условиях введения вариации семи аналитических параметров. Наименьшее изменение содержания эналаприла составляло 0,96 %, когда использовали колонку Zorbax C8 (4,6 мм i.d. ×150 мм, 5 мкм). Впервые разработан целостный подход, предусматривающий одновременное внедрение инноваций в технологии частиц и проектирование приборов. Это необходимо для того, чтобы сделать ученых-аналитиков успешнее, а предприятия – более прибыльными и продуктивными.*

Выводы. *Юден тест оказался эффективным и полезным инструментом для оценки робасности во время анализа атенолола методом высокоэффективной жидкостной хроматографии. Его можно успешно применять для оценки робасности в процессе валидации аналитических методик.*

КЛЮЧЕВЫЕ СЛОВА: **атенолол; высокоэффективная жидкостная хроматография; робасность; количественный анализ; Юден тест.**

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Address for correspondence: *K. Ye. Peleshok, I. Horbachevsky Ternopil National Medical University, Maidan Voli 1, Ternopil, 46001, Ukraine, e-mail: peleshok@tdmu.edu.ua.*