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RESEARCH ARTICLE

Determination of Clopidogrel in Pharmaceutical Preparation by UV-Visible Spectrophotometry and High Performance Liquid Chromatography Methods

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HIGHLIGHTS

> Alternative UV-Visible Spectrophotometry and HPLC methods were successfully developed and validated for quantification of clopidogrel in standard solutions and pharmaceutical preparations.
> The results showed that these methods could be applicable on quality control studies of pharmaceutical.

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ABSTRACT

UV-Visible spectroscopy and high performance liquid chromatography (HPLC) methods were developed and validated for determination of clopidogrel in bulk and pharmaceutical formulations. In spectroscopy, clopidogrel spectrum at different solutions was taken and the best absorbance value was obtained at 202 nm wavelength in methanol-acetonitrile (50:50.h/h) mixture. In HPLC, parameters were optimized as follows: mobile phase was acetonitrile-methanol-water (45:45:10, v/v/v), C18 re-serve phase column was preferred, flow rate and injection volume were set as 0.9 mL/min and 10 µL, respectively. All measurements were performed at 230 nm wavelength. Meloxicam was used as an internal standard in HPLC. Linearity, analytic recovery, intra and inter-days precision and accuracy were investigated in order to per-form validation process, It was stated that HPLC measurements were linear at 0.25-30 µg/mL while UV-Visible method linear at 1.25-25 µg/mL. Intra and inter-days precision and accuracy values were 5.38% and 2.66% for HPLC method and 3.77% and 3.60% for UV-Vis. Mean analytic recovery value was found to be 99.5% for both methods. Developed and validated HPLC and UV-Vis methods were applied to pharmaceutical preparations including clopidogrel.

Contents

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1. Introduction

Clopidogrel is synthesized from "ticlopidine", an active substance known as adenosine diphosphate (ADP) inhibitor (Figure 1). This molecule is mainly used to reduce thrombotic events, in treatments of myocardial heart attacks, stroke and cardiovascular diseases [1]. Furthermore, it is reported that it decreases the frequency of stent thrombosis occurrence after percutaneous coronary intervention [2, 3]. Clopidogrel was listed formally in the United States Pharmacopoeia (USPA) in 2007 [4].

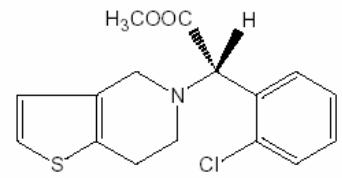

Figure 1 Chemical Structure of Clopidogrel

Ticlopidine and Clopidogrel is responsible for ADP receptor's non-recyclable inhibition. By this way, through ADP, thrombosis aggregation was inhibited. Abciximab is a monoclonal antibody which stops thrombosis aggregation by blocking the connection fibrinogen with glycoprotein IIb/IIIa receptors on thrombosis. Dipyridamole exhibits an anticoagulant effect by activating adenyl cyclase of thrombosis, and making potent antiagregant effect of endothelial prostacyclin [5].

A detailed literature survey stated that there is no accredited method for determination of clopidogrel in routine laboratory assays whereas there are various analysis methods [4].

In order to determine clopidogrel concentration in bulk and pharmaceutical formulations, some methods were specified in literature as voltammetry [6] liquid chromatography-mass spectroscopy (LC-MS, LC-MS-MS) [7–9], spectrophotometry, spectrodensitometer [10, 11], high performance liquid chromatography (HPLC) [4, 12–15], and electrophoresis [16, 17]. However, all these methods have several disadvantageous and drawbacks.

In this study; an alternative approach was revealed for determination of clopidogrel. HPLC and UV-Visible Spectroscopy Method were developed and validated ,and then, in application level, determination of clopidogrel in pharmaceutical preparation was carried out [18]. In proposed study, quantitative analysis was achieved without derivatisation. Furthermore, analytical recovery of proposed methods was quite acceptable.

2. Materials and Methods

2.1. Materials and Chemicals

Meloxicam (IS) and clopidogrel standards were obtained from Novagenix ARGE (R*D) Centre, Acetonitrile and methanol were kindly purchased from Merck (Germany). Instruments that were used along the analysis as follows: Scale (Bosh S 2000), Mixer Vortex (IKA), pH meter (Schott), Incubator (Memmert), Vacuum pump (Phenomenex), Spectrophotometer (Helios) and High Performance Liquıd Chromatography (HPLC) System (Agilent Technologies 1200 Series), Degazer (Agilent Technologies), Pump (Agilent Technologies), Column (C18, 5µm, 250x4,6mm) (Phenomenex Bondolone USA), Auto Simpler (Agilent Technologies) and Computer (HP).

2.2. HPLC Conditions

Optimized chromatographic method parameters for clopidogrel were monitored in Table 1.

Table 1 HPLC method conditions for clopidogrel

Method Condition	Clopidogrel
Column	C18 (250x4,6mm,5 µm)
Dedector	UV
Wavelength	230 nm
Mobile Phase	Methanol:Acetonitrile:Deionized Water (45:45:10, V/V/V)
Column Temperature	Not controlled
Flow Rate	0.9 mL/min.
Injection volume	10 µL

2.3. Spectrophotometry Method Conditions and Optimization

Maximum absorbance was observed at 202 nm wavelength and measurements were taken under these conditions. Applied conditions in this study were given in Table 2.

Table 2 Spectrophotometry conditions for clopidogrel.

Active Substance	Clopidogrel
Cuvvette Type	Quartz
Dedector	UV-Visible
Wavelength	202nm
Solvent	Methanol:Acetonitrile: (50:50,v/v/v)

2.4. Preparations of HPLC Standard Solutions

Clopidogrel stock solution was prepared by dissolving the standard sample in volumetric flask via methanol-acetonitrile (1:1 v/v) mixture. Clopidogrel stock solution were prepared as 1 mg/mL concentration and diluting by 0.25, 0.50, 1, 2.5, 5, 7.5, 10, 12.5, 15, 17.5, 20, 22.5, 25, 30 µg/mL concentrations.

2.5. Preparation of Spectrophotometry Standard Solutions

Different concentrations 1.25, 2.50, 5, 7.5, 10, 12.5, 15, 20, 25 µg/mL, were prepared from stock solution of clopidogrel.

2.6. Preparation of Tablets

Application of the method into real samples were carried out by using 3 commercial tablets Atervix, Plavix and Karum

that contains clopidogrel. In this study, 8 tablets from each formulation were weigh, mean value of them were recorded. Then, these tablets were grinded and blend until they became powder. A certain amount of this mixture was taken to be clopidogrel weighing 1 tablet and put into 100 mL volumetric flask. It was solved in acetonitrile for analytic purity. Its volume was completed to 100 mL. Final solutions were filtered, the new solutions at the concentrations to be used in study were prepared and their concentration were determined.

3. Results and Discussion

3.1. Validation

Validation of each method were maintained in accordance with The International Conference in Harmonization (ICH) guideline.

3.2. Selectivity

While studying chromatograms obtained from standard solutions, retention time of clopidogrel and meloxicam were 3.1 and 1.2 minute (Figure 2). In spectrophotometry method, it was determined that clopidogrel standard solutions which were prepared in 50/50 (v/v) methanol:acetonitrile solvent mixture give max. absorbance at 202 wavelength (Figure 3). No interference was encountered in blank measurements for both methods.

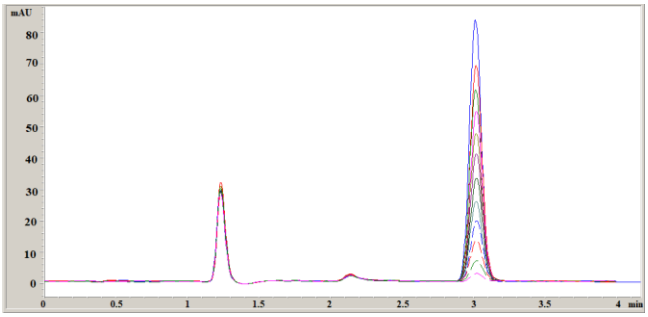

Figure 2 HPLC Chromatograms of Increasing Concentration of Clopidogrel Standard Solutions (0.25, 0.5, 1, 2.5, 5, 7.5, 10, 12.5, 15, 17.5, 20, 22.5, 25, 30 µg/mL)

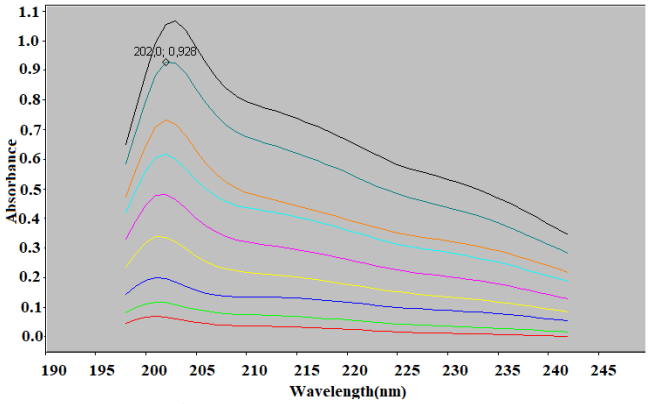

Figure 3 Spectrum of Clopidogrel Standard Solutions at 1.25, 2.5, 5, 7.5, 10, 12.5, 15, 20 and 25 µg/mL Concentrations.

3.3. Linearity and Working Range

The linearity of HPLC was determined with 14 standard solutions at 0.25-30 µg/mL concentration and of spectroscopy method with 9 standard solutions at 1.25-25µg/mL concentration. In HPLC peak area rates (clopidogrel peak area/IS peak area) obtained against solution concentration at stated concentration rates; and in Spectroscopy, absorbance values against clopidogrel concentration were added to graph and calibration curves were derived (n=6) (Figure 4). Correlation coefficients were obtained by making regression analysis of calibration curves (Table 3).

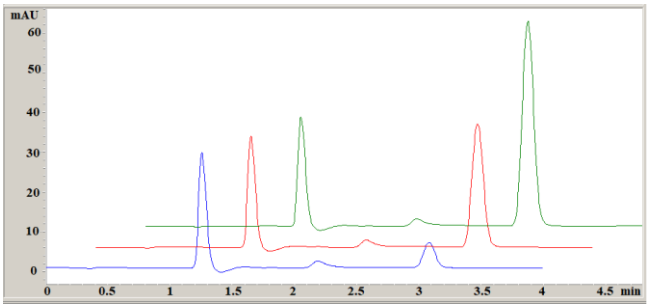

Figure 4 Chromatogram of the clopidogrel quality control solutions.

Table 3 Statistical Analysis Values of Calibration Curves Derived from Clopidogrel Standard Studying Solution.

Method	HPLC	UV-Vis
Concentration (mg/mL)	0.25-30	1.25-25
λ(νμ)	230	202
LRa	0.1331x – 0.0251	0.0443x + 0.0229
Sa	$66.8x10^{-4}$	0.0002
Sb	$1.5x10^{-3}$	0.0034
R	00.9999	0.991

λ: wavelength, [a]: 6 calibration curves, LR: linear regression, Sa: standard deviation of the slope in the regression curve, Sb: standard deviation of the slope in the regression curve, r: correlation coefficient.

3.4. Precision and Accuracy

Quality control solutions were prepared at 3 different concentrations (2.5, 12.5 and 20 µg/mL) in clopidogrel calibration curves. Intra-day (six times in a day with the same methods and conditions) and inter day (6 times in different 3 days with the same method) analyses of these solutions were performed (Figure 5). Average of the analysis results and standard deviation were determined. The accuracy was given by relative error (RE = (found-added)/added) x 100) and the precision by relative standard deviation (%RSD = (SD/average) x 100) Analytic recovery was calculated by standard addition method (Table 4).

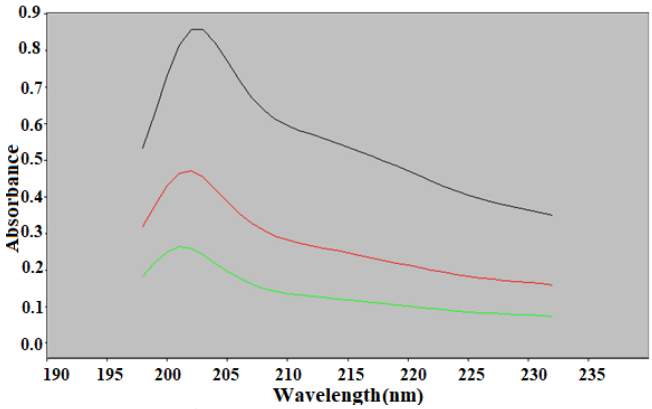

Figure 5 Spectrums of clopidogrel quality control solutions at 2.5, 12.5 and 20 µg/mL concentrations.

Table 4 Intra and inter-days accuracy and precision values of clopidogrel standard solution (n=6)

Method	λ (nm)	Added (µg/mL)	Intra-day			Inter-days		
			Average±SD (µg/mL)	Accuracy %RE	Precision %RSD	Average±SD (µg/mL)	Accuracy %RE	Precision % RSD
UV-Vis	202	2.5	2.39±0,05	-4.40	2.09	2.51±0.11	0.40	4.38
		12.5	12.16±0.11	-2.72	0.91	12.28±0.20	-1.76	1.63
		20	20.17±0.12	0.85	0.60	19.73±0.39	-1.35	1.98
HPLC	230	2.5	2.49±0.09	-0.40	3.61	2,52±0,08	0.80	3.18
		12.5	12.36±0.15	-1.12	1.21	12.28±0.20	-1.76	1.63
		20	20.17±0.44	0.85	2.18	19.73±0.39	-1.35	1.98

λ: wavelength, SD: standard deviation (n=6), RE: relative error, RSD: relative standard deviation

3.5. Limit of Detection (LOD) and Quantification (LOQ)

In HPLC, the lowest concentrations assayed where the signal /noise ratio was at least 3, this concentration was described as LOD. The LOQ was defined as a signal/noise ratio of 10. Under these chromatographic conditions, LOD and LOQ values were calculated as 0.25 and 0.09 µg/mL.

In spectroscopy, a series of solutions at concentrations have smaller value than the smallest value of calibration curves, were prepared and their absorbance were monitored by six times. Their %RSD were determined. It was stated that concentration value whose %RSD values is lower than 20% was LOD and LOQ was defined as %RSD value is lower than 10%. LOQ of the proposed study was 1.25 while LOD was 0.45 µg/mL.

3.6. Analytic Recovery

Analytic recovery from pharmaceutical preparation was carried with standard addition method. For this purpose, 8 tablets from Plavix, Karum and Atervix pharmaceutical preparation contain 75 mg clopidogrel was grinded and mixed homogeneously. A sample weighing 1 tablet was taken and dissolved in acetonitrile. 5 µg/mL concentration of pharmaceutical were obtained and measurements were taken for both methods. Then, 3 different standard solutions at 2, 12.5 and 20 µg/mL concentration were added separately and measured again. According to total peak area values for HPLC and to absorbance values for Spectrophotometry; the formula was ((the value of total solution-the value of added standard solution)/the value of pharmaceutical preparation solution x 100) (Table 5).

Table 5 The value of analytic recovery of pharmaceutical preparation

Commercial preparation	λ(nm) Method	Added (µg/mL)	Average±SS (µg/mL)	Recovery	%RSD
Plavix	202 UV- Vis	2.5	7.45±0.08	99.0	1.07
		12.5	17.52±0.21	100.4	1.20
		20	24.82±0.31	96.4	1.25
Karum	202 UV- Vis	2.5	7.53±0.07	100.6	0.93
		12.5	17.55±0.09	101.0	0.51
		20	24.90±0.40	98.0	1.61
Atervix	202 UV- Vis	2.5	7.52±0.08	100.4	1.06
		12.5	17.37±0.07	97.4	0.41
		20	24.92±0.48	98.4	1.93
Plavix	230 HPLC	2.5	7.49±0.07	99.8	0.93
		12.5	17.47±0.08	99.4	0.46
		20	25.08±0.11	101.6	0.44
Karum	230 HPLC	2.5	7.53±0.09	100.6	1.20
		12.5	17.54±0.13	100.8	0.74
		20	24.94±0.12	98.8	0.48
Atervix	230 HPLC	2.5	7.49±0.08	99.8	1.07
		12.5	17.47±0.09	99.4	0.52
		20	24.95±0.71	99.0	2.85

λ: wavelength, SD: standard deviation (n=6), RE: relative error, RSD: relative standard deviation

UV-Visible Absorption Spectroscopy Method is accepted as one of the preferred techniques at quantification of pharmaceuticals in terms of high sensitivity, medium and high selectivity, high accuracy, certainty, facility, and comfortability [19]. In this technique, UV spectra is obtained through absorbance values of the light on the substances against the wavelength in graph. Thus, A=f (λ) function [20]. At 1.25-25 µg/mL concentration range in which the method was linear, absorbance values against the concentration of a series standard (1.25, 2.50, 5, 7.5, 10, 12.5, 15, 20 and 25 µg/mL) were plotted and calibration curve was obtained. In regression analysis of calibration curve; it was determined that regression equation is A=0.0443x+0.0229 (A: Absorbance; V: Clopidogrel concentration), correlation coefficient was 0.9991, LOD was 0.45 µg/mL, LOQ was 1.25 µg/mL. Studies in intra and inter-days precision and accuracy showed that relative standard deviation (%RSD) and relative error (%RE) was lower than 4.5% and recovery value was 99.1%.

UV method was successfully applied to 3 commercial pharmaceutical preparation for determination of clopidogrel. Chromatography is a whole of methods which are used

commonly for reserving, knowing and determining of chemical synthesis of which quantity is unknown and in which there are some other substances [21]. Between these methods; HPLC has more advantages like accuracy, precision, repeatability, selectivity, sensitivity, recovery, having opportunity for analysis with low volume sample and getting results rapidly. For these reasons, HPLC has been used commonly in drug industry for quantification of pharmaceutical preparation or the analysis of active substances in biological fluids [22]. In this study, a new HPLC method developed for determination of clopidogrel.

In HPLC study, some parameters like temperature, column, mobile phase components and percentages may affect the run time. So, optimization of chromatographic conditions was needed in order to improve the distinction and acceptable results. In this study, the reverse phase C18 column (5 µm, 250x4.6 mm) was used and different mobile phase mixtures as (water-methanol rate: 70:30, 80:20 and 90:10 h/h) tested. According to results obtained, the value in which acetonitrile-methanol-water rate was 45:45:10 h/h/h was determined as optimum. Column temperature was uncontrolled, mobile phase flow rate was 0.9, wavelength was 230 and injection volume was 10 µL. While determining these parameters, firstly data in literature were examined and according to these data, the optimal ranges were determined. In this study; good linearity was obtained without derivative agents used in some other methods. Based on recovery values as well; it was observed that the study has satisfactory results.

It was determined that the method was linear at 0.25-30 µg/ml concentration. A clopidogrel standard solution at 0.25, 0.50, 1, 2.5, 5, 7.5, 10, 12.5, 15, 17.5, 20, 22.5, 25 and 30 µg/mL were prepared. Meloxicam standard solution at 10 µg/mL concentration was added as internal standard. Against each solution concentration, the rates clopidogrel peak area to meloxicam was plotted and calibration curve was obtained. It was determined that the regression equation was y=0.1331x-0.0251 (y: the rate of clopidogrel peak area to IS (meloxicam) peak area, x: clopidogrel concentration); correlation coefficient for clopidogrel (r) was 0.9999, LOD and LOQ were 0.09 and 0.25 µg/mL. Intra and inter days %RSD and %RE were lower than 3.7% and 2.0% respectively. It is claimed that HPLC is applicable, for clopidogrel quantification in 3 pharmaceutical preparation. Average recovery value from tablet was 99.9% and %RSD is lower than 3%. The analysis time of our method is shorter than the others in literature and It is a big superiority.

Wavelength for UV-Vis technique were 202 nm. This wavelength is not appropriate for IS. So, we have changed the wavelength for a better chromatographic separation. Our pre-studies exhibited that there is no significant difference between 202 and 230 nm for HPLC studies in our concentration range.

4. Conclusion

In this study, alternative UV and HPLC methods were successfully developed and validated for quantification of clopidogrel in standard solutions and pharmaceutical preparations. The results showed that these methods could be applicable on quality control studies of pharmaceutical; because of their sensitivity, selectivity, accuracy and precision values. It has been though that these data obtained from the proposed work will be guiding for further works.

References

[1] Moshfegh K, Redondo M, Julmy F, Wuillemin WA, Gebauer MU, Haeberli A, et al. Antiplatelet effects of clopidogrel compared with aspirin after myocardial infarction: enhanced inhibitory effects of combination therapy. *Journal of the American College of Cardiology* (2000) **36**(3):699–705.

[2] Steinhubl SR, Berger PB, Mann III JT, Fry ETA, DeLago A, Wilmer C, et al. Early and sustained dual oral antiplatelet therapy following percutaneous coronary intervention: a randomized controlled trial. *Jama* (2002) **288**(19):2411–2420.

[3] Mehta SR, Yusuf S, Peters RJG, Bertrand ME, Lewis BS, Natarajan MK, et al. Effects of pretreatment with clopidogrel and aspirin followed by long-term therapy in patients undergoing percutaneous coronary intervention: the PCI-CURE study. *The Lancet* (2001) **358**(9281):527–533.

[4] Akter R, Banik S, Ghosh A, Rashid MMO. Optimized and Validated RP-HPLC Method for the Determination of Clopidogrel in Bulk and Pharmaceutical Formulation. *Journal of Scientific Research* (2016) **8**(3):439–446.

[5] Cingi I, Erol K. *Farmakoloji [Pharmacology]*. Health Personnel Associate Programme. Eskişehir: Anadolu University (1996).

[6] Dermiş S, Aydoğan E. Electrochemical study of the antiplatelet agent clopidogrel and its determination using differential pulse voltammetry in bulk form and pharmaceutical preparations with a glassy carbon electrode. *Die Pharmazie-An International Journal of Pharmaceutical Sciences* (2010) **65**(3):175–181.

[7] Takahashi M, Pang H, Kawabata K, Farid NA, Kurihara A. Quantitative determination of clopidogrel active metabolite in human plasma by LC–MS/MS. *Journal of pharmaceutical and biomedical analysis* (2008) **48**(4):1219–1224.

[8] Mitakos A, Panderi I. Determination of the carboxylic acid metabolite of clopidogrel in human plasma by liquid chromatography–electrospray ionization mass spectrometry. *Analytica chimica acta* (2004) **505**(1):107–114.

[9] Ksycinska H, Rudzki P, Bukowska-Kiliszek M. Determination of clopidogrel metabolite (SR26334) in human plasma by LC–MS. *Journal of pharmaceutical and biomedical analysis* (2006) **41**(2):533–539.

[10] Chaudhari PB, Pawar PD, Narkhede KP. Stability indicating spectrophotometric method for determination and validation of Clopidogrel bisulfate in tablet dosage form. *Int J Res Ayur & Pharm* (2010) **1**(2):418–423.

[11] Zaazaa HE, Abbas SS, Abdelkawy M, Abdelrahman MM. Spectrophotometric and spectrodensitometric determination of Clopidogrel Bisulfate with kinetic study of its alkaline degradation. *Talanta* (2009) **78**(3):874–884.

[12] Mounika A, Sriram N. Method development and validation of clopidogrel bisulphate by reverse phase-HPLC in bulk and pharmaceutical dosage forms. *Int. J. Pharm. And anal. Res* (2012) **1**(1):1–7.

[13] Al-Khayat MA, Haidar S, Mando H. Development and vali-dation of RP-HPLC method for determination of clopidogrel in tablets. *Int. J. Pharm. Sci. Rev. Res* (2012) **14**:1–5.

[14] Mitakos A, Panderi I. A validated LC method for the determination of clopidogrel in pharmaceutical preparations. *Journal of pharmaceutical and biomedical analysis* (2002) **28**(3-4):431–438.

[15] Rao RN, Raju AN, Nagaraju D. Development and validation of a liquid chromatographic method for determination of enantiomeric purity of citalopram in bulk drugs and pharmaceuticals. *Journal of pharmaceutical and biomedical analysis* (2006) **41**(1):280–285.

[16] Serra H, do Rosário Bronze M, Simplício AL. Simultaneous determination of clopidogrel and its carboxylic acid metabolite by capillary electrophoresis. *Journal of Chromatography B* (2010) **878**(19):1480–1486.

[17] Fayed AS, Weshahy SA, Shehata MA, Hassan NY, Pauwels J, Hoogmartens J, et al. Separation and determination of clopidogrel and its impurities by capillary electrophoresis. *Journal of pharmaceutical and biomedical analysis* (2009) **49**(2):193–200.

[18] Huber L. *Validation and qualification in analytical laboratories*: CRC Press (2007).

[19] Sekaran CB, Rani AP. Development and validation of spectrophotometric method for the determination of DPP-4 inhibitor, sitagliptin, in its pharmaceutical preparations. *Eclética Química* (2010) **35**(3):45–53.

[20] Skoog DA, West D, Holler F. *Analitik Kimya Temelleri [Fundamentals of Analytic Chemistry]: Vol 2*. Ankara: Bilim Yayıncılık (1999).
[21] Smith I. *Chromatography*: Elsevier (2013).
[22] Brown P, Hartwick RA. High performance liquid chromatography (1988).