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RAPID AND ACCURATE DETERMINATION OF CLOPIDOGREL IN TABLETS BY USING SPECTROPHOTOMETRIC AND CHROMATOGRAPHIC TECHNIQUES*

SAADET DERMİŞ ** and EKREM AYDOĞAN

***Department of Analytical Chemistry, Faculty of Pharmacy, University of Ankara, 06100 Tandoğan, Ankara – TURKEY*
(dermis@pharmacy.ankara.edu.tr)

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ABSTRACT

Quantitative determination of clopidogrel (CLP) was carried out by using zero-order and derivative ultraviolet (UV) spectroscopy. Calibration curves for CLP in 0.1N HCl between 0.1-0.8 mM (42-336 µg/mL) concentration range were obtained by the measurements at 267.5, 271.5 and 279.3 nm for first derivative, at 269.5, 273.4, 277.3 nm for second derivative and at 268.1, 271.3, 275.6 nm for third derivative UV spectrophotometry, respectively. In application, 270 nm in zero-order UV spectrophotometry, 279.3 nm in first derivative UV spectrophotometry, 269.5 nm in second derivative UV spectrophotometry and 275.6 nm in third derivative UV spectrophotometry were selected by their lowest relative standard deviation values in the validation studies. Mean recoveries and the relative standard deviations of the methods were found as 98.7 % and 1.90 % in zero-order UV spectrophotometry, 97.5 % and 0.76 % in first derivative UV spectrophotometry, 99.6 % and 1.25 % in second derivative UV spectrophotometry, 99.5 % and 1.15 % in third derivative UV spectrophotometry, respectively. In this study, the values of limit of detection and limit of quantitation were calculated at selected λ values as 0.01mM and 0.03 mM for zero-order (λ_{270} nm), 0.02 mM and 0.07 mM for first derivative ($\lambda_{279.3}$ nm), second derivative ($\lambda_{269.5}$ nm), third derivative ($\lambda_{275.6}$ nm) UV spectrophotometry, respectively. The proposed methods were applied to the determination of CLP in bulk and tablets. The results were compared statistically with each other and high performance liquid chromatography (HPLC) procedure. The differences were not significant. In HPLC method, an isocratic system consisted of a Nova-Pak® C18 analytical column and a mobile phase composed of pH 8 phosphate buffer : acetonitrile (30:70, v/v) at a flow rate 0.8 mL/min was used for the optimal chromatographic separation using UV detection at 210 nm. Applying HPLC method, linearity was observed in the concentration range from 1.26 to 7.55 µg/mL for CLP with a correlation coefficient 0.999. The values of the limit of detection and limit of quantitation were calculated as 0.29 µg/mL and 0.96 µg/mL, respectively. Synthetic samples were analyzed and mean recoveries and the relative standard deviations were found as 99.95 % and 0.40 % in HPLC. The spectrophotometric methods presented in this study can be used accurate, simple, economic and practical in the determination of CLP in tablets. The procedures do not require any separation step. The mean recoveries were found satisfactory in the methods. These methods also give repeatable results. Proposed methods could usefully be applied to routine quality control of tablets containing CLP. No interference was observed from common excipients in pharmaceutical formulations.

KEYWORDS: clopidogrel bisulfate, spectrophotometry, HPLC, determination, tablet.

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1. INTRODUCTION

Clopidogrel hydrogen sulfate (methyl (+)-(S)-α-(o-clorophenyl)-6,7-dihydrothieno (3,2-c) pyridin-5(4H)- acetate hydrogen sulfate) , whose structure is shown in Figure 1, is a new thienopyridine compound structurally related to ticlopidine [1]. It is indicated for the reduction of atherosclerotic events in patients with atherosclerosis documented by recent stroke, recent myocardial infraction or cardiovascular disease. It is an analogue of ticlopidine and acts by inhibiting adenosine diphosphate-mediated platelet aggregation. Clopidogrel inhibits platelet aggregation by selective preventing of the binding adenosine diphosphate (ADP) to its platelet reseptor. It is a potent antiplatelet drug used in thromboembolitic disorders [2-4].

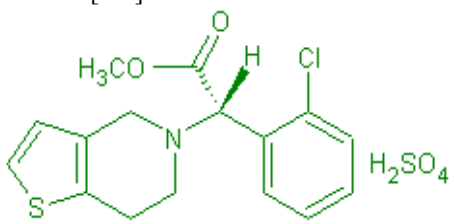

Figure 1. *Structure of clopidogrel bisulfate*

For this drug the literature reveals a variety of analytical methods such as spectrophotometry [5-8], HPLC [9-13], HPTLC [14], LC-MS [15,16], LC-MS-MS [17,18], voltammetry [19], electrophoresis [20]. Chromatographic procedures for the determination of CLP have been described, but these methods were all used for the analysis of CLP and its major metabolite in biological fluids. For example, LC-MS-MS method have been used to quantite CLP and its chief metabolite in plasma [18, 21]. A spectrophotometric method [6] was reported recently in literature for simultaneously analyzing aspirin and clopidogrel where the analysis was done after hydrolyzing the drugs. Spectrophotometric methods have already proved to be very useful in the field of drug analysis due to their simplicity, low cost and relatively short analysis time when compared with other techniques. The described methods are direct methods for analysis of CLP, and do not need any expensive equipment.
The methods can be easily applied in routine practices made in any laboratory possesing a spectrophotometer with a derivative accessory.

The aim of this work was to develop sensitive, selective and validated stability indicating method for determination of CLP in presence of dosage forms using different spectroscopic methods. For single component preparations, the simplest assay method involves the direct measurement of UV absorption at the maximum, CLP is relatively weak . UV absorbance measurements at low concentration (dissolution testing) will be unreliable. Fortunately, the derivative transformation of spectral data has been proved to be valuable procedure for the identification and quantitation of several drugs [22].

In this study four spectrophotometric methods, direct absorbance measurement, first, second and third derivative method were subject to

quantitative analysis of CLP in single dosage form and synthetic samples tablet calibration graphs obtained were valid by using synthetic samples. The assay results were statistically compared with HPLC method. These four methods were being proved to be suitable for routine analysis for the commercial pharmaceutical preparation selected.The assay results of commercial pharmaceutical formulation of all methods proposed were in agreement with each other.

2. MATERIAL AND METHODS

2.1. Experimental

2.1.1. Apparatus

The spectrophotometric analysis were performed on a Shimadzu UV-1601 PC double beam spectrophotometer using 1cm quartz cells over the range 250-290 nm.
This spectrophotometer connected to a computer loaded with Shimadzu UVPC software was used for all the spectrophotometric measurements and treatment of data.

An Agilent 1100 liquid chromatograph equipped with a G13 79A degasser, 613 11A quarternary pump, G13 13A auto sampler, and 61315B DAD detector was used for chromatographic measurements. The chromatograms were recorded and the peaks were quantitated using the automatic integrator.
The chromatograms were carried out at a temperature of 40 ^{o}C on a Nova-Pak® C18 column of 3.9x150 mm (4 μm particle size).

2.1.2. Materials and reagents

Clopidogrel hydrogen sulfate was purchased from Sanovel. Clopidogrel pure sample was used as received ; (purity 99.9 %) Karum® tablet containing (75 mg) was obtained local pharmacies. All reagents and solvent used were of analytical grade and the solutions were prepared with doubly distilled water. All working solutions were prepared freshly everyday.

2.1.3. Solution Preparation

Stock solutions of concentrations 0.04 - 1.0 mM and $3x10^{-3}$ – $1.8x10^{-2}$ mM were prepared in 0.1N HCl and pH 8 phosphate buffer : acetonitrile (1:1, v/v) stored in dark bottles at +4 ^{o}C. The working solutions for spectrophotometric and chromatographic investigations were prepared by dilution of the stock solution. Phosphate buffer was prepared according to the USP pharmacopoeial procedure. The CLP concentration does not change with time. All working solutions were prepared freshly every day.

2.1.4. Procedures

Karum® tablet preparates given to humans contain Clopidogrel hydrogen sulfate and 79 mg of Clopidogrel hydrogen sulfate is equivalent to 75 mg free base (Clopidogrel). Twenty tablets were accurately weighed (the content of one tablet is 75 mg Clopidogrel) and finally powdered. The correct amount of

powder was dissolved in the 0.1N HCl and by stirring this solution for about 10 min, a stock solution was prepared. All the test solutions were obtained by diluting this stock solution with the 0.1N HCl. Zero-order spectra and derivative curves were recorded.

UV Spectrophotometry

Absorption spectra of CLP in 0.1N HCl were determined by zero-order spectrophotometry of this drug in tablet forms. For determination of CLP measurement of the peak-zero amplitude in the zero order spectra at 270 nm was used.

HPLC

The mobile phase was pH 8 phosphate buffer: acetonitrile (30:70,v/v). The flow rate was set at 0.8 mL/min with 10 µL as injection volume and the wavelenght of detection was 210 nm for chromatographic measurements. The chromatogram of the CLP was given in Figure 6. All the calculations concerning the quantitative analysis were performed with external standardization by the measurement of peak area.

2.1.5. Analysis of tablets:

2.1.5.1. Spectrophotometric method

Twenty tablets of CLP were weighed and thoroughly powdered. The average of one tablet was weighed out, transferred into a 50 mL volumetric flask and dissolved in 0.1N HCl; the flask was left in an ultrasonic bath for about 5 min. After 5 min of ultrasonic shaking, 5 mL of this solution was filtered thorough a 0.45 µm membrane filter. The volume was made up to 50 mL with 0.1 N HCl . All the test solutions were obtained by diluting this stock solution with 0.1 N HCl and UV spectra were then recorded.

2.1.5.2. Chromatographic method

Twenty tablets of CLP were weighed and thoroughly powdered. The average of one tablet was weighed out, transferred into a 100 mL volumetric flask and was dissolved in the mobile phase pH 8 phosphate buffer : acetonitrile (30:70, v/v). The flask was left in a ultrasonic bath for 10 min. After 10 min of ultrasonic shaking, 1 mL of this solution was filtered thorough a 0.45 µm membrane filter. The volume was made up to 100 mL with the mobile phase. All the test solutions were obtained by diluting this stock solution with the mobile phase and chromatograms were recorded.

3. RESULTS AND DISCUSSION

Figure 2 indicates the UV absorption spectra of CLP in 0.1N HCl. The standart series of CLP in the concentration (0.04-1.0 mM) 16.8 - 420 µg/mL were prepared in the above solvent mixture. The absorption spectra of the prepared standard series solutions were plotted in the wavelenght range of 250-290 nm. The recorded absorption spectra were used for calibration graphs. In present study, the direct absorbance measurement method was developed subject drugs in samples. The aim of the application of three alternative method was testing direct absorbance measurement method. The interference of excipients in tablet was not observed according to the obtained results.

3.1. Direct Absorbance Measurement Method:

The absorbance of standard series of CLP in the above concentration range were measured the peak amplitude corresponding to the maximum wavelenght, 270 nm (Figure 2). The measured absorbance values were plotted versus concentration and a straight line was obtained. Linearity was observed in the concentration range from 16.8 to 420 µg/mL (0.04 - 1.0 mM) with a correlation coefficient 0.999. The calculated calibration equation Y = 663.15xC – 0.007 was used for the determination of CLP in bulk and tablets. The limit of detection and quantification were 1.39 and 4.62 µg/mL for the CLP, respectively.

3.2 First Derivative Method:

In this method, the first derivative spectra were calculated with a $\Delta\lambda$=2 nm interval from the stored zero-order absorption spectra of the prepared samples in 0.1 N HCl (Figure 3). For the determination of CLP in the bulk and tablets, the calibration graphs were used, which obtained by measuring the $dA / d\lambda$ values at 267.5 nm, 271.5 nm and at 279.3 nm. Three calibration graphs were tested for synthetic mixtures. Regression equations, correlation coefficients and relative standard errors in the methods were shown in table 1. This method was successfully applied to the tablets selected and the results were illustrated in table 3,6.

3.3. Second Derivative Method:

The second derivative spectra were plotted with a $\Delta\lambda$ =2 nm interval from the stored zero-order absorption spectra of the prepared samples in 0.1N HCl (Figure 4). Linear calibration graphs were obtained by measurement of the $d^2A / d\lambda^2$ values at 269.5 nm, 273.4 nm and 277.3 nm for this drug in bulk and tablets, respectively. Three calibration graphs were tested for synthetic mixtures. Statistical analysis were done and indicated in table 1,4.

3.4. Third Derivative Method:

There are three maxima (268.1, 271.3, 275.6 nm) in the third derivative spectra of the solution of CLP in 0.1N HCl in the range of 250-290 nm (Figure 5). The third derivative spectra were plotted with a $\Delta\lambda$=2 nm interval from the stored zero-order absorption spectra of the prepared samples in 0.1N HCl. The determination of CLP can simply be made by reading absorbances at the three

maxima and two minima in the third derivative spectra and by measuring $d^3A / d\lambda^3$ values in third derivative spectra of its solution at 268.1 nm, 271.3 nm, 275.6 nm.
In this method, the mean recoveries and relative standard deviations calculated for synthetic mixtures prepared in our laboratory are illustrated in table 5. Recovery results of this method was found satisfactory.

3.5. Analysis of Tablets:

Four rapid, simple and very cheap spectrophotometric methods were successfully applied for the quantitative analysis of CLP in tablets. The results of tablet analysis obtained from these four methods were summarized in table 6. For a comparison, t-test was subject to evaluate the results. A significant difference between four methods was not observed according to the test results.

3.5.1. Spectrophotometric method

Proposed methods were applied to the determination of CLP in tablets. Each pharmaceutical formulation was analyzed by performing six independent determinations.

In application, 270 nm in direct absorbance UV spectrophotometry, 279.3 nm in first derivative spectrophotometry, 269.5 nm in second derivative spectrophotometry and 275.6 nm in third derivative spectrophotometry were selected by their lowest relative standard deviation values in the validation studies, table 2-5. Statistical results were obtained for CLP and were found to be in agreement with the label claims (Table 6).

3.5.2. Chromatographic method

Applying HPLC method, linearity was observed in the concentration range from 1.26 to 7.55 µg/mL for CLP with a correlation coefficient 0.999. The values of the limit of detection (LOD) and limit of quantitation (LOQ) were calculated as 0.29 µg/mL and 0.96 µg/mL, respectively. Synthetic samples were analyzed and mean recoveries and the relative standard deviations were found as 99.95 % and 0.40 % in HPLC.

4. CONCLUSION

In conclusion, these spectrophotometric methods were proposed for simultaneous determination of CLP in bulk and tablets. The validation of results obtained in these derivative spectral methods were realized by using the zero-order spectrophotometric method.
These methods can be used in routine analysis of CLP for the bulk and for the pharmaceutical preparations containing this drug. UV spectrophotometry is an advantageous method by the elimination of possible interferences from the other materials placed in the commercial formulations. As seen in the table 1, in assay results LOD (3 x standard deviation /slope of analytical curve) and LOQ (10 x standard deviation /slope of analytical curve) of the direct UV absorption method was smaller than those obtained by using derivative methods. LOD and LOQ were calculated according to

USP guidelines [23-25]. Also, these methods are very easy due to not requiring any separation and extraction steps.
The assay results obtained using these methods for CLP were also compared with UV absorption spectrophotometric method. This method is used as a reference method due to absence of an official method for this drug. Summary of the assay results for commercial preparation was shown in table 1. There was no significant difference between each three methods.

The described methods are direct methods for analysis of CLP, and do not need any expensive equipment. The methods can be easily applied to routine practices made in any laboratory possesing a spectrophotometer with a derivative accessory.

As it was explained in the text, direct UV spectrophotometric method exist maxima at 270 nm for CLP giving opportunity for its determination by reading absorption values at this wavelenght. The determination of CLP can simply be made by reading absorbances at 267.5 nm and 271.5 nm and 279.3 nm in the first derivative spectra and by measuring $d^2A / d\lambda^2$ values in second derivative spectra of its solution at 269.5 nm, 273.4 nm, 277.3 nm and by measuring $d^3A / d\lambda^3$ values in third derivative spectra of its solution at 268.1 nm, 271.3 nm, 275.6 nm.

In application, 270 nm in zero-order UV spectrophotometry, 279.3 nm in first derivative UV spectrophotometry, 269.5 nm in second derivative UV spectrophotometry and 275.6 nm in third derivative UV spectrophotometry were selected by their lowest relative standard deviation values in the validation studies (Table 2-5). Mean recoveries and the relative standard deviations of the methods were found as 98.7 % and 1.90 % in zero-order UV spectrophotometry, 97.5 % and 0.76 % in first derivative UV spectrophotometry, 99.6 % and 1.25 % in second derivative UV spectrophotometry, 99.5 % and 1.15 % in third derivative UV spectrophotometry, respectively.

In this study, the values of LOD and LOQ were calculated at selected λ values as 0.01 mM and 0.03 mM for zero-order(λ270 nm), 0.02 mM and 0.07 mM for first derivative (λ279.3 nm), second derivative (λ269.5 nm), third derivative (λ275.6 nm), UV spectrophotometry, respectively.
In the methods, the mean recoveries and relative standard deviations calculated for synthetic mixtures prepared in our laboratory are illustrated in table 6. Recovery results of these methods were found satisfactory.

These methods were being proved to be suitable for routine analysis for the commercial pharmaceutical preparation selected. The assay results of commercial pharmaceutical formulation of all methods proposed were in agreement with each other.

The statistical parameters and recovery study data clearly indicate the reproducibility and accuracy of the methods. Analysis of the pharmaceutical formulations containing CLP showed no interference from the common excipients and additives.

In order to show the accuracy of developed derivative spectrophotometric techniques, this analysis methods which also applicable to tablet dosage form of drug, were compared to drug analysis results, performed by high pressure liquid chromatography method. Necessary statistical studies demonstrated that there is no difference was for sensitivity between methods in the drug analysis (Table 6).

The applicability of the proposed methods to the assay of simple dosage forms were examined by analysing tablets. The spectrophotometric results were compared with the chromatographic results by means of Student's t-test at a 95% confidence level, and no significant difference was found between them (Table 6). Analysis results in this study demonstrated that the active substance amount in the drug is within determined limits in the pharmacopoeia.

The proposed methods were developed as an alternative substitute to the chromatographic method, and the results obtained were promising.

It was demonstrated that developed method could be applied as easily, accurately and sensitively to pharmaceutic dosage form. The difference found unsignificant according to t values that was obtained with comparison of methods were more lower than table t value (2.57), (Table 6). It is seen that obtained results are compatible within confidence interval. These results exhibit the accuracy of developed proposed techniques. These methods may be recommended for routine and quality control analysis of the drug investigated in pharmaceutical dosage forms. Hence, this approach could be considered for the determination of CLP in quality control laboratories. Thus, these methods can be adopted as an alternative to the existing methods.

ÖZET

Klopidogrel (CLP)'in sıfır derece ve türev ultraviole (UV) spektroskopisi yöntemi kullanarak kantitatif tayini yapılmıştır. 0.1N HCl içerisinde CLP için 0.1-0.8 mM konsantrasyon aralığında, kalibrasyon eğrileri sırasıyla 267.5, 271.5 ve 279.3 nm de birinci türev, 269.5, 273.4, 277.3 nm de ikinci türev ve 268.1, 271.3, 275.6 nm dalga boylarında üçüncü türev UV spektrofotometrisi için yapılan ölçümlerden elde edilmiştir. Uygulamada, validasyon çalışmalarında en düşük bağıl standart sapma değerlerinin elde edildiği sıfırıncı derece UV spektrofotometrisinde 270 nm, birinci türev UV spektrofotometrisinde 279.3 nm, ikinci türev UV spektrofotometrisinde 269.5 nm ve üçüncü türev UV spektrofotometrisinde 275.6 nm dalga boyları seçilmiştir. Metodların ortalama geri kazanım ve bağıl standart sapma değerleri sırasıyla sıfır derece UV spektrofotometrisinde % 98.7 ve % 1.90, birinci türev UV spektrofotometrisinde % 97.5 ve % 0.76, ikinci türev UV spektrofotometrisinde % 99.6 ve % 1.25, üçüncü türev UV spektrofotometrisinde % 99.5 ve % 1.15 olarak bulunmuştur. Bu çalışmada, tayin alt sınırı ve teşhis alt sınırı değerleri seçilmiş dalga boylarında sırasıyla; sıfır derece (λ_{270} nm) için 0.01mM ve 0.03 mM, birinci türev ($\lambda_{279.3}$ nm), ikinci türev ($\lambda_{269.5}$ nm), üçüncü türev ($\lambda_{275.6}$ nm) UV spektrofotometrisi için 0.02 mM ve 0.07 mM olarak hesaplanmıştır. Önerilen metodlar klopidogrel'in etkin madde ve tabletlerindeki miktar tayinine uygulanmıştır. Sonuçlar istatistiksel olarak kendi aralarında ve yüksek performans sıvı kromatografisi (YPSK) yöntemiyle karşılaştırılmıştır. Sonuçlar arasındaki farklılıklar önemsizdir. YPSK yönteminde, Nova-Pak C18 kolonu, mobil faz olarak, 0.8 mL/dak akış hızında pH 8 fosfat tamponu : asetonitril (30:70, h/h) dan oluşan izokratik sistem ve 210 nm de deteksiyon optimal kromatografik koşullar olarak belirlenmiştir. Uygulanan YPSK yönteminde klopidogrel için lineerlik 1.26-7.55 µg/mL konsantrasyon aralığında 0.999 korelasyon katsayısıyla elde edilmiştir. Tayin ve teşhis alt sınırı değerleri sırasıyla 0.29 µg/mL ve 0.96 µg/mL olarak hesaplanmıştır. YPSK yönteminde, ortalama geri kazanım ve bağıl standart sapma değerleri sırasıyla % 99.9 ve % 0.4 olarak bulunmuştur. Bu çalışma, spektrofotometrik metodların klopidogrel'in tabletlerdeki miktar tayininde doğru, basit, ekonomik ve pratik olarak kullanılabileceğini göstermiştir. Bu metodlar tekraredilebilir sonuçlar vermektedir. Önerilen metodlar klopidogrel içeren tabletlerin rutin kalite kontrolüne uygulanabilir. Farmasötik formülasyonlardaki genel katkı maddelerinden hiçbir şekilde etkilenme görülmemektedir.

REFERENCES

[1] Budavari, S., Eds., In: The Merck Index, 13th ed., Merck & Co., Inc., Whitehouse Station, NJ, (2001), p.856.

[2] Herbert, J. M., Frehel, D., Vallee, E., Kieffer, G., Gouy, D., Berger, Y., Necciari, J., Defreyn, G., and Maffrand, J.P., Cardiovascular Drug Reviews, 11 (2): (1993), 180 - 198.

[3] Physicians Desk Reference (PDR®) (2007), 61st ed., pp. 2926-2929.

[4] Sweetman, S.C., Martindale: The Complete Drug Reference (2007), The Pharmaceutical Press (London), (35): 1424-1425.

[5] Mishra, P., Dolly, A., Indian Journal of Pharmaceutical Sciences, 67(4): (2005), 491- 493.

[6] Mishra, P., Dolly, A., Indian Journal of Pharmaceutical Sciences, 68(3): (2006), 365 - 368.

[7] The United States Pharmacopoeia, Thirtieth Revision, and The National Formulary, 25th ed., Rockville, USA, pp.1802 - 1805.

[8] Zaazaa, H.E., Abbas, S.S., Abdelkawy, M., Abdelrahman, M.M., Talanta, 78: (2009), 874 - 884.

[9] Mitakos A., Panderi, I., J. Pharm. Biomed. Anal., 28(3-4): (2002), 431 - 438.

[10] Singhs, S.S., Sharma,K., Barot, D., Mohan, P.R., Lohray V.B., J. Chromatogr. B. Analyt Technol Biomed. Life Sci., 821(2): (2005), 173 - 180.

[11] Souri, E., Jalalizadeh, H., Kebriaee-Zadeh, A., Shekarchi, M., Dalvandi, A., Biomed. Chromatogr., 20(12): (2006), 1309-1314.

[12] Gandhimati, M., Ravi, TK., Indian Journal of Pharmaceutical Sciences , 69(1): (2007), 123 - 125.

[13] Nirogi , R.V.S., Kandikere , V.N., Shukla, M., Mudigonda, K., Maurya S., Boosi, R., Rapid Commun Mass Spectrom., 20: (2006), 1695 - 1700.

[14] Agrawal, H., Kaul, N., Paradkar, A.R., Mahadik, K.R., Talanta, 61(5): (2003), 581 - 589.

[15] Mitakos, A., Panderi, I., Anal. Chim. Acta, 505: (2004), 107- 114.

[16] Ksycinska, H., Rudzki, P., Bukowska-Kiliszek, M., J. Pharm. Biomed. Anal., 41(2): (2006), 533 - 539.

[17] Lagorce, P., Perez, Y., Ortiz, J., Necciari, J., Bressole, F., J.Chromatogr B. Biomed Sci Appl., 720(1-2): (1998), 107-117.

[18] Takahashi, M., Pang, H., Kawabata, K., Farid, A.N., Kurihara, A., J. Pharm. Biomed. Anal., 48(4): (2008), 1219-1224.

[19] Dermiş, S., Aydoğan, E., (2009), Die-Pharmazie, (In Press). Reference No. P 9123/ DOI:10.1691/ph.2009.9123

[20] Fayed, A.S., Weshahy, S.A., Shehata, M.A., Hassan, N.Y., Pauwels, J., Hoogmartens, J., Van Schepdael A., J Pharm Biomed Anal., 49: (2009), 193-200.

[21] Robinson, A., Hillis, J., Neal, C., Leary, A.C., J. Chromatogr. B Analyt. Technol. Biomed. Life Sci., 842(2): (2007), 344-354.

[22] Ojeda, C.B., Rojas, F.S. and Pavon, J.M.C., Talanta, 42: (1995), 1195 - 1214.

[23] Miller, J.C., Miller, J.N., Statistics for Analytical Chemistry, Second ed., Ellis Harwood Limited, London, (1992).

[24] ICH Draft Guidelines on Validation of Analytical Procedures: Definitions and Terminology, Federal Register, Volume 60, IFPMA, Switzerland, (1995), 11260 - 11268.

[25] The United States Pharmacopoeia, 25th. Revision, The National Formulary, United States Pharmacopoeial Convention, Inc., Rockville, MD (2002), 2256 - 2259.

Figure 2. *Original spectra of a)25.2 µg/mL b) 33.6 µg/mL c) 42µg/mL d) 84µg/mL e)168µg/mL f) 252 µg/mL g) 336 µg/mL solution of CLP in 0.1N HCl.*

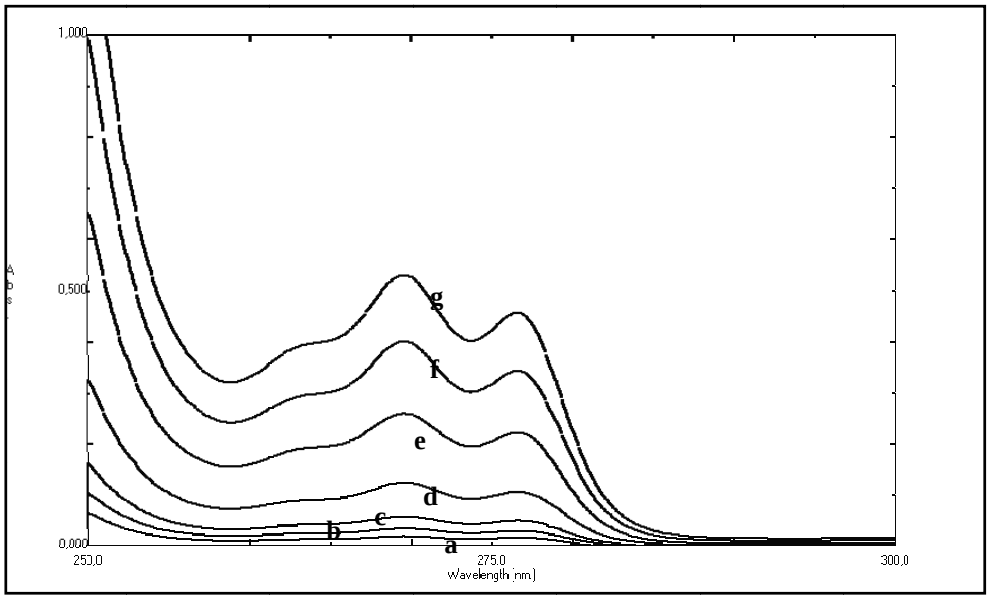

Figure 3. *First derivative spectra of a) 42µg/mL b)84µg/mL c)168µg/mL d)252µg/mL e)336µg/mL solution of CLP in 0.1N HCl.*

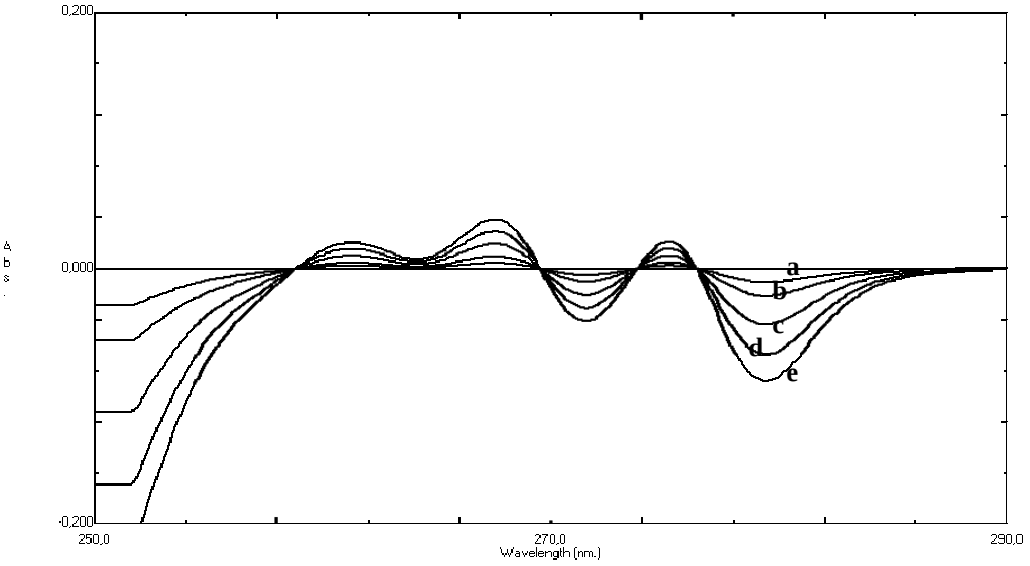

Figure 4. *Second derivative spectra of a) 42µg/mL b)84µg/mL c)168µg/mL d)252µg/mL e)336µg/mL solution of CLP in 0.1N HCl.*

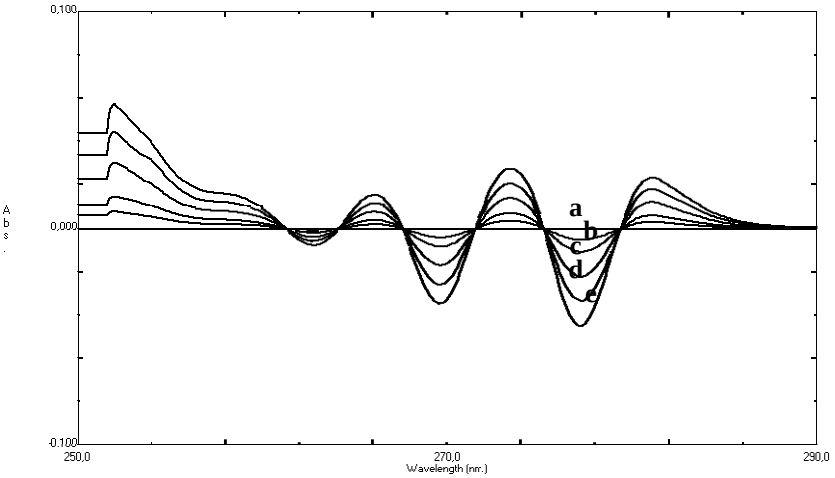

Figure 5. *Third derivative spectra of a) 42µg/mL b)84µg/mL c)168µg/mL d)252µg/mL e)336µg/mL solution of CLP in 0.1N HCl.*

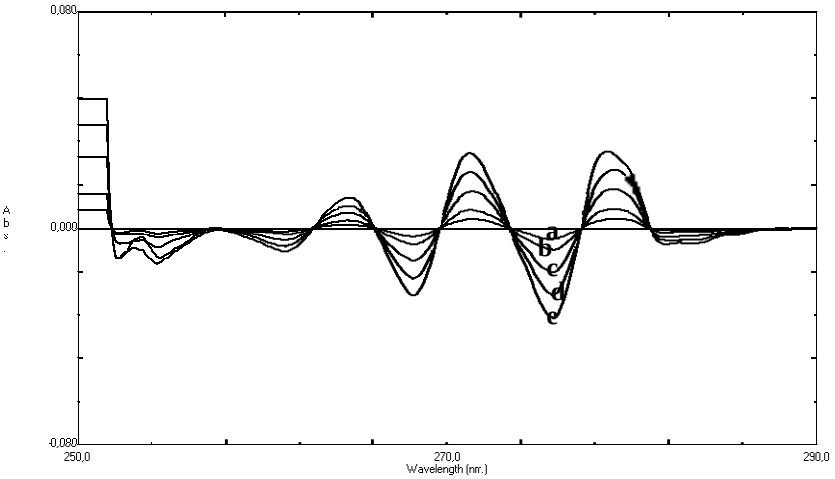

Figure 6. *Chromatogram of CLP in (pH 8) Phosphate buffer : Acetonitrile (30:70,v/v).*

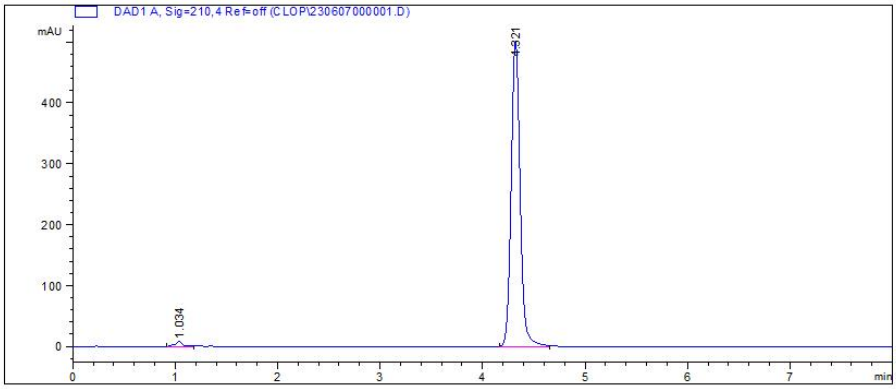

Table 1. *Determination of CLP by applying the proposed methods*

	Zero-order UV spec.	First-order UV spec.method			Second-order UV spec.method			Third-order UV spec.method		
	λ_{270nm}	$\lambda_{267.5nm}$	$\lambda_{271.6nm}$	$\lambda_{279.3nm}$	$\lambda_{269.6nm}$	$\lambda_{273.4nm}$	$\lambda_{277.3nm}$	$\lambda_{268.1nm}$	$\lambda_{271.3nm}$	$\lambda_{275.6nm}$
Calibration range*	0.1-0.8 mM (42-336 µgmL^{-1})	0.1-0.8 mM (42-336 µgmL^{-1})			0.1-0.8 mM (42-336 µgmL^{-1})			0.1-0.8 mM (42-336 µgmL^{-1})		
Regression equation										
• Slope	0.6735	0.049	0.050	0.11	0.0445	0.0343	0.0572	0.0312	0.0344	0.0412
• SE of slope	$4.20x10^{-3}$	$8.14x10^{-4}$	$1.1x10^{-3}$	$1.6x10^{-3}$	$1.73x10^{-4}$	$8.14x10^{-4}$	$4.72x10^{-4}$	$6.05x10^{-4}$	$9.45x10^{-4}$	$6.05x10^{-4}$
• Intercept	0.0106	$5.18x10^{-4}$	$3.23x10^{-4}$	$3.5x10^{-4}$	$6.95x10^{-4}$	$1.83x10^{-5}$	$6.22x10^{-4}$	$2.87x10^{-4}$	$2.44x10^{-4}$	$2.87x10^{-4}$
• SE of intercept	$2.06x10^{-3}$	$4.0x10^{-4}$	$5.41x10^{-4}$	$8.2x10^{-4}$	$3.52x10^{-4}$	$4.01x10^{-4}$	$2.32x10^{-4}$	$2.97x10^{-4}$	$4.65x10^{-4}$	$2.97x10^{-4}$
• Correlation coefficient	0.9998	0.9992	0.9981	0.9993	0.9998	0.9983	0.9997	0.9988	0.9977	0.9993
• SE of correlation coefficient	$6x10^{-3}$	$4.66x10^{-4}$	$6.3x10^{-4}$	$9.6x10^{-4}$	$2.02x10^{-4}$	$4.66x10^{-4}$	$2.71x10^{-4}$	$3.46x10^{-4}$	$5.41x10^{-4}$	$3.46x10^{-4}$
LOD (mM)	0.0101	0.0268	0.0356	0.0246	0.0246	0.0385	0.031	0.0315	0.0446	0.0238
LOQ (mM)	0.0303	0.0804	0.1067	0.0738	0.0738	0.1155	0.0402	0.0945	0.1337	0.0715

* All the calculations in the calibration were carried out by using molar concentration.

Table 2. *Recovery data obtained in standard solutions of CLP in 0.1N HCl using zero-order UV spectrophotometry*

No	Added (µg/mL)	270 nm	
		Found (µg/mL)	Recovery %
1	42	42.6	101.4
2	84	81.4	96.9
3	168	163.1	97.1
4	252	251.4	99.7
5	336	330.8	98.5
X			98.7
S.D.			1.88
R.S.D.			1.90

X=mean, S.D.=standard deviation, R.S.D.=relative standard deviation

Table 3. *Recovery data obtained for standard solutions of CLP in 0.1N HCl by first derivative UV spectrophotometry*

No	Added (µg/mL)	267.5 nm		271.5 nm		279.3 nm	
		Found (µg/mL)	Recovery %	Found (µg/mL)	Recovery %	Found (µg/mL)	Recovery %
1	42	43.2	102.8	40.5	96.5	40.73	96.97
2	84	81.2	96.7	82.5	98.2	81.8	97.5
3	168	168.7	100.4	162.3	96.6	163.3	97.2
4	252	256.2	101.7	254.7	101.1	249.0	98.8
5	336	328.5	97.7	330.3	98.3	326.2	97.1
X			99.8		98.1		97.5
S.D.			2.59		1.86		0.74
R.S.D.			2.60		1.89		0.76

Table 4. *Recovery data obtained in standard solutions of CLP in 0.1N HCl using second derivative UV spectrophotometry*

No	Added (µg/mL)	269.5 nm		273.4 nm		277.3 nm	
		Found (µg/mL)	Recovery %	Found (µg/mL)	Recovery %	Found (µg/mL)	Recovery %
1	42	42.4	101.0	41.85	99.6	41.3	98.3
2	84	82.1	97.7	87.16	103.7	85.3	101.6
3	168	167.0	99.4	165.5	98.5	166.1	98.8
4	252	251.9	99.9	257.4	102.1	246.8	97.9
5	336	336.8	100.3	330.8	98.5	327.6	97.5
X			99.6		100.5		98.8
S.D.			1.24		2.35		1.63
R.S.D.			1.25		2.33		1.65

Table 5. *Recovery data obtained in standard solutions of CLP in 0.1N HCl using third derivative UV spectrophotometry*

No	Added (µg/mL)	268.1 nm		271.3 nm		275.6 nm	
		Found (µg/mL)	Recovery %	Found (µg/mL)	Recovery %	Found (µg/mL)	Recovery %
1	42	41.5	98.9	42.1	100.1	41.6	99.2
2	84	84.6	100.7	86.0	102.4	84.5	100.5
3	168	165.4	98.5	167.8	99.8	166.0	98.8
4	252	246.2	97.7	247.0	98.1	247.6	98.2
5	336	340.4	101.3	332.6	98.9	339.3	100.9
X			99.4		99.8		99.5
S.D.			1.52		1.62		1.14
R.S.D.			1.53		1.63		1.15

Table 6*. Comparative studies for CLP formulations*

Analysis techniques	Zero-order UV Spec. (λ_{270nm})	First derivative Spec. method ($\lambda_{279.3nm}$)	Second derivative Spec. method ($\lambda_{269.5nm}$)	Third derivative Spec. method ($\lambda_{275.6nm}$)	HPLC
Formulation[a] (tablet)					
Mean (mg)[b]	74.88	74.65	74.72	74.54	75.24
RSD (%)	0.97	0.55	0.56	0.65	0.40
Calculated t value T, theoretical (n=6) (p=0.05)	0.23[c]	0.22[c]	0.21[c]	0.21[c]	t_{table}= 2.57

[a]Tablet, 75 mg per tablet
[b]Each value is the mean of five experiment
[c]NS, not significiant