Journal of Food and Drug Analysis

Volume 16 | Issue 2 Article 2

2008

Liquid chromatographic and potentiometric methods for deteminations of clopidogrel

Journal of Food and Drug Analysis, Vol. 16, No. 2, 2008, Pages 11-18 藥物食品分析 第十六卷 第二期

Liquid Chromatographic and Potentiometric Methods for Determinations of Clopidogrel

AMR LOTFY SABER*[1], MOHMED ALAA ELMOSALLAMY[1], ALAA ALSIDE AMIN[2] AND HAMADA MOHMED AHMED KILLA[1]

[1.] *Department of Chemistry, Faculty of Science, Zagazig University, Zagazig, Egypt*
[2.] *Department of Chemistry, Faculty of Science, Banha University, Banha, Egypt*

(Received: April 19, 2007; Accepted: July 12, 2007)

ABSTRACT

Two different techniques are developed for the determination of clopidogrel in pharmaceutical preparations. HPLC method has been developed where chromatographic analysis is performed on Nova-Pak® C_{18} column (3.9 mm × 150 mm, 5 μm) with an ammonium formate buffer adjusted with formic acid to pH 4.0, acetonitrile (40:60, v/v) as mobile phase, and detection at 225 nm. Good linearity (0.9993, r), accuracy (≥ 99.20 %), and precision (≤ 0.6 RSD) were obtained. Potentiometric measurments are based on tetrakis (p-chlorophenyl) borate-clopidogrel ion-pair as an electroactive material incorporating a plasticized PVC membrane with o-nitrophenyl octyl ether or dioctyl phthalate. The sensor is conditioned for at least two days in 0.1 M drug solution before use. It exhibits fast and stable Nernstian response for clopidogrel over the concentration range of $1.0 \times 10^{-5} \sim 1.0 \times 10^{-2}$ M and pH range of 1.5 - 4.0. Results with an average recovery of 100.6% and a mean standard deviation of 0.86% of the nominal were obtained. The sensor shows reasonable selectivity towards clopidogrel hydrogen sulphate in presence of many cations. No significant interferences are caused by drug excipients and diluents.

Key words: HPLC, potentiometry, pharmaceutical analysis, clopidogrel determination

INTRODUCTION

Clopidogrel hydrogen sulphate ($CG\text{-}H_2SO_4$), methyl(+)-(S)-(*o*-chlorophenyl)-6,7 dihydrothien[3,2-c]pyridine-5(4H)-acetate hydrogen sulphate (Scheme 1) is a new thienopyridine derivative. Clopidogrel is similar to ticlopidine in chemical structure but different in the side effect of reduction of white cells. Clopidogrel is indicated for the reduction of atherosclerotic events (myocardial infraction, stroke, and vascular death) in patients with atherosclerosis documented by recent stroke, recent myocardial infraction, or established peripheral arterial disease. Active metabolite of clopidogrel selectively inhibits adenylate cyclase, ADP-induced platelet aggregation by direct inhibition of ADP binding to its receptor, which further leads to the inhibition of subsequent ADP-mediated activation of the GPIIb-IIa complex[(1)].

Clopidogrel is inactive *in vitro*, and hepatic biotransformation via cytochrome P450 pathway, primarily by CYP3A4 and CYP3A5[(2)], is essential for its *in vivo* antiplatelet activity. The active metabolite[(3)], a thiol compound, is formed by the oxidation of clopidogrel to 2-oxoclopidogrel and subsequent hydrolysis. The active metabolite is highly labile and remains undetected in plasma. It was isolated *in vitro* from rat microsomes and the structure elucidation[(3)] was performed on a stabilized acrylonitrile derivative. Following an oral administration in humans, plasma levels of clopidogrel are very low due to extensive metabolism and difficulties of quantification. The metabolite (the carboxylic acid derivative) is pharmacologically inactive and represents 85% of circulating metabolites in human plasma. Since neither the parent drug nor the active metabolite is detected in plasma, measurement of pharmacodynamic effect by estimating platelet aggregation[(4)] is believed to be a better measure for *in vivo* estimations. The major circulating compound is the inactive carboxylic acid derivative, which is formed by hydrolysis of the ester function by carboxyl esterase[(5)]. Few methods for the determination of clopidogrel have been reported in literature. Recently, the nonenzymatic and enzymatic chiral inversion of clopidogrel has been investigated *in vitro* using 1H-NMR and a chiral HPLC procedure[(6)]. Moreover, a nonstereospecific HPLC assay method was also used to monitor the hydrolysis of clopidogrel.

Possible *in vivo* chiral inversion of carboxylic acid metabolite of clopidogrel in rats was also studied using (S)-(1-naphthyl)ethyl amine as a derivatisation reagent and a HPLC method with spectrofluorimetric detec-

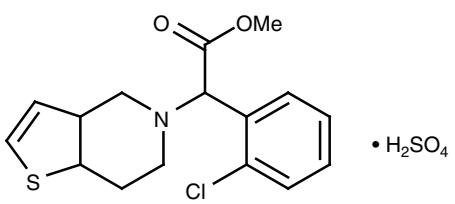
* Author for correspondence. Tel: +20121530134;
E-mail: amrlotfy2000@yahoo.com

tion[6]. Reversed phase HPLC methods for the determination of clopidogrel in pharmaceutical preparations have also been reported[7,8]. For the determination of carboxylic acid metabolite of clopidogrel in plasma and serum, a GC-MS[9] and LC-ESI-MS[10] methods have also been reported. GC method was used for determination of clopidogrel in tablets[11]. Estimation of carboxylic acid metabolite of clopidogrel in wistar rat plasma by HPLC and its application to a previous pharmacokinetic study[12]. Stability indicating HPTLC determination of clopidogrel hydrogen sulphate as bulk drug and in pharmaceutical dosage form has also been reported[13]. Focus of the present study was to develop an accurate, precise and robust liquid chromatographic method for the determination of clopidogrel hydrogen sulphate in tablets. The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines[14]. No sensor or potentiometric method for determination of clopidogrel has been reported in the literature to date. This study also includes development of a new potentiometric sensor based on tetrakis (p-chlorophenyl) borate-clopidogrel ion pair as a novel electroactive material incorporating in poly(vinylchloride) matrix membrane plasticized with either o-nitrophenyl octyl ether or dioctyl phthalate for determination of clopidogrel.

MATERIALS AND METHODS

I. *Materials*

(CG-H_2SO_4) was received from Pharma Company, 6 October City, Cairo, Egypt. HPLC grade acetonitrile was obtained from SDS (de Valdonne, France). Analytical grade ammonium formate was purchased from Sigma-Aldrich, Germany. Formic acid was obtained from E. Merck (Darmstadt, Germany). Plavix tablets were purchased from the market. Each plavix tablet consists of 75 mg of (CG-H_2SO^4). Grade 1 water was obtained from a Milli-Q ultrapure water purification system (Millipore, Bedford, MA, USA). Tetrakis (p-chlorophenyl) borate (T*p*ClPB) and tetrahydrofuran (THF) were obtained from Aldrich Chemical Co. (Milwaukee, USA). PVC (Breon S 110/0P) was obtained from BP Chemicals International (Barry, UK), o-nitrophenyl octyl ether (*o*-NPOE) was purchased from Fluka (Buchs, Switzerland) and dioctyl phthalate (DOP) from BDH (Poole, England).



Scheme 1. Chemical structure of clopidogrel hydrogen sulphate.

II. *High Performance LC Method*

(I) *Instrumentation*

The LC system consisted of a Waters model 481 UV detector, a Shimadzu LC-6A pump, and a model 7125 injector (Rheodyne, Berkeley California, USA) with 20 µL sample loop. The output signals were monitored and integrated using Perkin-Elmer TotalChrom software (version 6.2.1.).

(II) *Chromatographic Conditions*

The elution was isocratic. The mobile phase consisted of a mixture of aqueous 0.05 M ammonium formate adjusted with formic acid to pH 4.0 and acetonitrile (40:60, v/v). Ammonium formate buffer pH 4.0 (0.05 M) was prepared as follows[15]: 3.15 g of ammonium formate was dissolved in 950 mL of water, pH was adjusted to value 4.0 ± 0.1 with formic acid (diluted with water in ratio 1:5), and buffer was diluted to 1000 mL with water and then was filtered through a 0.45-µm (HVLP, Germany) membrane filter. The mobile phase also was filtered through a 0.45-µm (HVLP) filter prior to use. A symmetry C18 analytical column (3.9 mm × 150 mm, 5 µm particle size) (Waters, USA) was used for determination of the drug under investigation. The flow rate of mobile phase was 1.0 mL min-1 and the column was operated at ambient temperature (~25°C). Sample injection volume was 20 µL and UV detector was set at a wavelength of 225 nm.

(III) *Preparation of Stock and Working Standard Solutions*

The stock solution of CG-H_2SO_4 (500 µg mL^{-1}) was prepared by dissolving 0.05 g of CG-H_2SO_4 (99.78 %) in methanol in a 100-mL volumetric flask. Six different working/calibration solutions in mobile phase were prepared by appropriate dilution of the stock solution. The concentration range of the working standard solutions was 1.0-30.0 µg mL^{-1} as shown in the calibration curve (Figure 1).

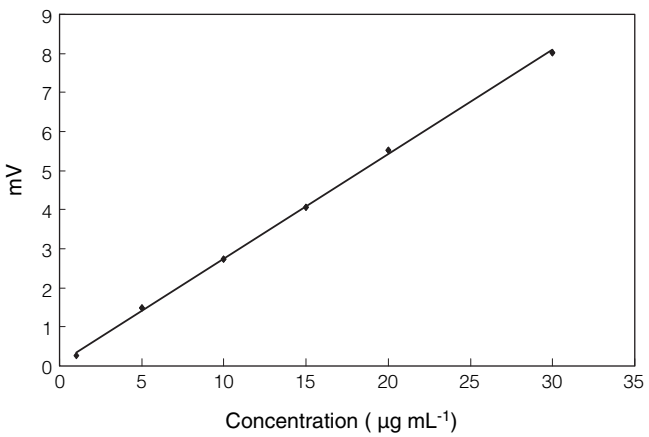


Figure 1. The calibration curve for clopidogrel hydrogen sulfate.

(IV) *Sample Preparation*

Ten tablets were weighed and finely powdered. A quantity of powder equivalent to one tablet (255.1 mg) containing 75 mg of (CG-H_2SO_4) was transferred in a 100-mL volumetric flask, dissolved in methanol, and filtered through 0.45-µm (HVLP, Germany) membrane filter. The filtrate (100, 200, 400 µL) was quantitatively transferred to three different 10-mL volumetric flasks to give three different concentrations (7.5, 15 and 30 µg mL^{-1}) respectively, and the solutions were diluted to the volume with mobile phase. A typical chromatogram of (CG-H_2SO_4) is shown in (Figure 2).

III. *Potentiometric Method*

(I) *Instrumentation*

Electrochemical measurements were made at room temperature (25 ± 1°C) with a PTI-15 digital pH meter using T*p*ClPB-clopidogrel membrane sensor in conjunction with an EIL type RJ 23 calomel reference electrode. A glass Ag-AgCl combination electrode (consort, S 210 B BB5) was used for pH measurements. The ISE internal reference solution was a silver-silver chloride in 0.1 M clopidogrel solution.

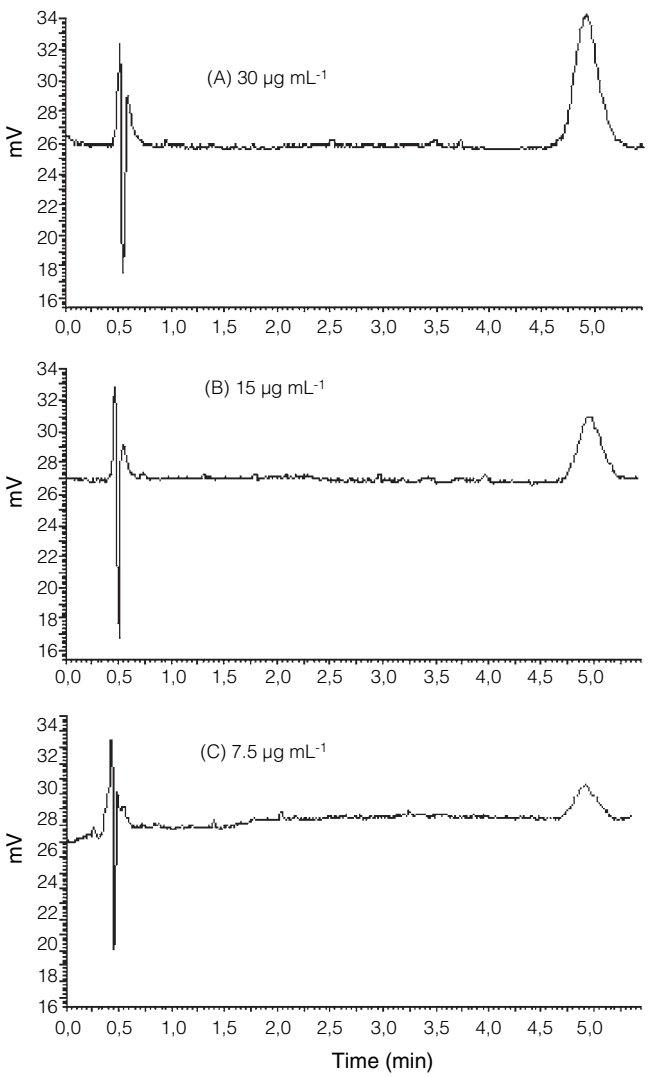


Figure 2. A typical chromatogram of clopidogrel at different concentrations.

(II) *Clopidogrel PVC Membrane Sensor*

Clopidogrel sensor was assembled as described previously[16,17]. T*p*ClPB (40 mg), *o*-nitrophenyl octyl ether or dioctyl phthalate (360 mg) and PVC (170 mg) were used. The sensor was conditioned by soaking in 0.1 M clopidogrel solution for at least two days before use and was stored in the same solution when not in use.

(III) *Sensor Calibration*

The sensor was calibrated by spiking with successive aliquots of standard solution into a 10^{-5} M solution of the calibrant. Alternatively, the calibration was carried out by immersing the sensor into a 50-mL beakers containing 20 mL of aliquots of standard 1.0×10^{-5} - 1.0×10^{-2} M clopidogrel solution starting from low to high concentrations. The electromotive force (e.m.f.) was plotted as a function of the logarithm of the clopidogrel concentration. The calibration graph was used for subsequent determination of unknown concentration of clopidogrel.

(IV) *Selectivity Coefficient*

Potentiometric selectivity coefficients $K_{CG.,B}$ were evaluated using the separate solution method according to the equation:

$$-\log k^{pot}_{CG.,B} = \frac{E_{CG.} - E_B}{S}$$

where $E_{CG.}$ and E_B are the response potentials of the sensor for clopidogrel ion and interferent B at 10^{-2} M respectively. S is the sensor slope (mV $decade^{-1}$).

(V) *Determination of Clopidogrel in Pharmaceutical Preparation*

Ten tablets were weighed and the average weight was calculated. The tablets were crushed to furnish a homogeneous powder and a quantity equivalent one tablet (0.2551 g) which contains 75 mg of (CG-H_2SO_4) was weighed in a 50-mL volumetric flask, dissolved in methanol, and filtered. The filtrate (4.1 mL) corresponding to 21 mg CG-H_2SO_4 was quantitatively transferred to a 50-mL volumetric flask and diluted to the mark with doubly distilled deionized water. After the pH was adjusted to 3.0, potential of the sensor was measured and compared with the calibration curve.

RESULTS AND DISCUSSION

I. *HPLC Method*

(I) *Method Development*

1. *Choice of Stationary Phase*

Chromatographic method was used on two batches of C_{18} columns provided by Waters (3.9 mm × 150 mm, 5 µm) and another manufacture to check the column performance. Due to difference in peak shape and in the retention time, the waters column gave the best results.

2. *Choice of Mobile Phase*

(CG-H_2SO_4) was eluted before 5 min with a mobile phase composed of an ammonium formate buffer pH 4.0 and acetonitrile (40:60, v/v) but by using a mobile phase composed of ammonium formate and acetonitrile (55:45, v/v) adjusted at the same pH value, (CG-H_2SO_4) was eluted at about 9 min. However, at the same composition and different pH, no significant difference in retention time was observed. Hence a mobile phase consisting of ammonium formate and acetonitrile (40:60, v/v) adjusted at pH 4.0 was chosen.

(II) *Method Validation*

Only (CG-H_2SO_4) was observed by analyzing the tablet solution samples, showing that the method was selective.

1. *Limits of Detection and Quantitation*

The limit of quantitation (LOQ) was established at a signal-to-noise ratio of 10. The LOQ of (CG-H_2SO_4) was determined by six injections of drug at the LOQ concentration and was found to be 1.0 µg mL^{-1} corresponding to 0.0034 mg of tablet.

The limit of detection (LOD) was calculated according to Reference 15.

$$LOD = \frac{3.3.\ \sigma}{S}$$

where σ is noise peak-to-peak of baseline in the chromatogram of placebo solution, S is slope of the regression line acquired by measuring of the linearity. The limit of detection (LOD) of (CG-H_2SO_4) was 0.3 µg mL^{-1}.

2. *Linearity*

Linearity was evaluated by analysis of working standard solutions of (CG-H_2SO_4) at six different concentrations[14]. Signals and concentrations of the drug were subjected to regression analysis to calculate the calibration equation and correlation coefficients. The regression equation obtained for the pharmaceutical preparations was $y = 0.267x + 0.07$ ($r = 0.9993$, $n = 6$). The range of linearity was 1.0 – 30.0 µg mL^{-1}.

3. *Accuracy*

Accuracy of the method was determined by analyzing the solution which equivalent to one tablet. Three solutions of concentration levels at 7.5, 15 and 30 µg mL^{-1} were made in triplicate from this sample and analyzed for 3 consecutive days. Solutions for the calibration curves were prepared fresh every day. The assay accuracy variation shown in terms of relative mean error (RME) and % recovery are tabulated in Table 1[14]. The RME values are below ± 1.0% for the intra-day assay experiments. Due to the good accuracy, no internal standard was needed.

4. *Precision*

Precision of the method for the determination of (CG-H_2SO_4) was studied using the parameters repeatability, intermediate precision, and robustness.

Table 1. Accuracy of determination of (CG-H_2SO_4) in tablets using HPLC

Day of analysis	Theoretical concentrations (µg mL^{-1})	Actual concentrations (µg mL^{-1}) (n = 3)	Recovery (%)	RME (%)
Day 1	7.5	7.45	99.40	-0.67
	15	14.98	99.88	-0.13
	30	29.85	99.50	-0.50
Day 2	7.5	7.44	99.20	-0.80
	15	14.92	99.46	-0.53
	30	29.89	99.63	-0.37
Day 3	7.5	7.44	99.20	-0.80
	15	14.90	99.33	-0.67
	30	29.89	99.63	-0.37

Journal of Food and Drug Analysis, Vol. 16, No. 2, 2008

Table 2. Inter- and Intra-day assay variation of (CG-H_2SO_4) using HPLC method. The inter-day data were obtained by randomly choosing one replicate for each day

Day of analysis	Theoretical concentrations (μg mL^{-1})	Actual concentrations (μg mL^{-1}) (n = 3)	SD	RSD (%)
Intra-day:				
Day 1	7.5	7.45	0.04	0.47
	15	14.98	0.01	0.09
	30	29.85	0.11	0.36
Day 2	7.5	7.44	0.04	0.57
	15	14.92	0.06	0.38
	30	29.89	0.08	0.26
Day 3	7.5	7.44	0.04	0.57
	15	14.90	0.07	0.48
	30	29.89	0.08	0.26
Inter-day:	7.5	7.46	0.03	0.38
	15	14.96	0.03	0.19
	30	29.92	0.06	0.19

Repeatability in the intra-day variations in assay obtained at different concentration levels is expressed in terms of RSD values calculated from the data of each day for 3 days. RSD values of assay were found to be below 0.5% (Table 2).

Intermediate precision, which is the inter-day variation at the same concentration level, was determined on successive days. The intermediate precision for assay of (CG-H_2SO_4) was found to be below 1.0% RSD (Table 2.). Robustness of a method is a measure of its capacity to remain unaffected by small variations in method conditions. The robustness of the proposed method was evaluated by altering pH of the mobile phase. The results (not shown) indicated lack of significant differences between the conditions of the method developed. The method is satisfactory for determination of (CG-H_2SO_4) in tablets. These results also show that the filtration of the samples can be performed without loss of analyte.

5. *Stability of Standard Solutions*

Stability of standard and sample solutions was determined by monitoring the solutions of standard (CG-H_2SO_4) and of a tablet solution sample over a period of two weeks[14]. The results showed that the retention times and signals were almost unchanged (RSD % <1.0) and that no significant degradation was observed within the given period, indicating the solutions were stable for at least two weeks.

II. *Potentiometric Method*

Plasticized PVC membrane sensor incorporating T*p*ClPB-clopidogril ion pair was prepared with suitable solvent mediators and electrochemically evaluated as membrane sensor for clopidogrel under static mode of operations according to IUPAC recommendations[18]. The membrane was prepared using a casting solution of the composition 7:63:30 wt % of KTpClPB, o-nitrophenyl octyl ether or dioctyl phthalate and PVC, respectively. The two plasticizers have different dielectric constants. The sensor was soaked in drug solution and tested as clopidogrel sensor. Table 3 summarizes the potentiometric response characteristics of the sensor. It was found that the clopidogrel sensor plasticized with the two different plasticizers have almost the same characteristics. The sensor showed Nernstian response over the concentration range of 1.0×10^{-5} - 5.0×10^{-2} mol L^{-1} clopidogrel with cationic slopes of 61.7 and 59.3 mV $decade^{-1}$ for *o*-NPOE and DOP based sensor, respectively. The detection limit was 1.0×10^{-5} M with both plasticizers. Least squares

Table 3. Response characteristics of TpClPB-clopidogrel PVC membrane sensor

Parameter	Value	
	o-NPOE	DOP
Slope, (mV $decade^{-1}$)	61.7	59.3
Intercept, (mV)	218.5	201.4
Correlation coefficient, (r)	0.9874	0.9993
Lower limit of detection, (M)	1.0×10^{-5}	1.0×10^{-5}
Lower limit of linear range, (M)	5.0×10^{-5}	4.0×10^{-5}
Working pH range	1.5 - 4.0	1.5 - 4.0
Life span/week	11	12

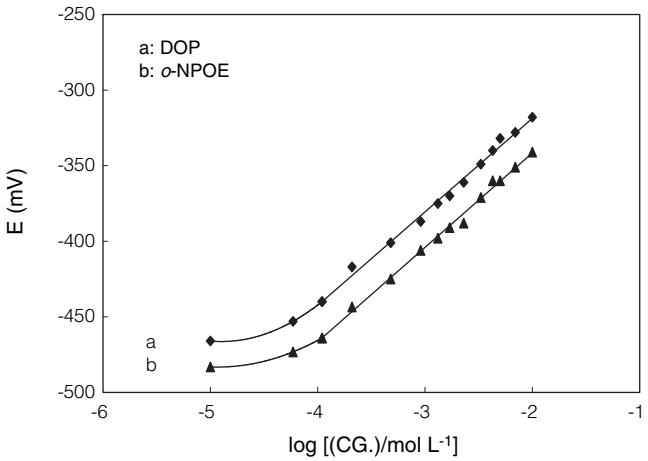


Figure 3. Potentiometric response of T*p*ClPB – clopidogrel PVC membrane sensor.

analysis of the data gave the relationships: E(mV) = 61.7 log(CG) - 218.5 and E(mV) = 59.3 log(CG) - 201.4 for *o*-NPOE and DOP based sensor, respectively. A typical calibration plot of the sensor is shown in Figure 4. The sensor displayed constant and stable potential readings within 0.2 mV from day to day and the calibration slope did not change by more than 1.0 mV decade-1 over a period of 11 weeks. The sensor was useful for 11 and 12 weeks for o-NPOE and DOP based sensor, respectively.

(I) *Effect of pH*

Influence of pH on the potentiometric response of the sensor was studied using 10^{-4}, 10^{-3} and 10^{-2} M of clopidogrel solutions (Figure 4). From pH-potential profiles, it is evident that the potential readings are constant over the pH range 1.5 - 4.0. Within this acidic range, clopidogril is completely soluble, dissociated and sensed as a monovalent charged ion. At pH values lower than 1.5, the potential readings decreased due to interference by H^+ ions. At higher pH values (>4.0), progressive precipitation of the drug was observed.

(II) *Effect of Foreign Ions*

The potentiometric response of T*p*ClPB-clopidogrel PVC membrane sensor was tested in the presence of several inorganic cations. Potentiometric selectivity coefficients $k^{pot}_{CG.,B}$ were used to evaluate the degree of interference. The data given in Table 4 were obtained using the separate solutions method[16,18] at 10^{-2} M clopidogrel. It is clear that the sensor is highly selective for clopidogrel ions compared with some common cations. Pharmaceutical excipients and diluents (e.g., glucose, maltose, manitol, starch, talc powder and magnesium stearate) at concentration as high as 400-fold molar excess over clopidogril did not interfere. The *o*-NPOE based sensor was generally more selective than the DOP based sensor.

(III) *Determination of Clopidogrel*

Validity of the clopidogrel PVC membrane sensor for the determination of clopidogrel was assessed by determining 4.2 µg - 4.2 mg mL^{-1} standard clopidogrel hydrogen sulphate using the calibration graph method. The results obtained showed an average recovery of 100.65% and a mean standard deviation of 0.86% (n = 5) using both solvent mediators based sensors. Clopidogrel (as anticoagulants) in different pharmaceutical preparations was also determined (Table 5). Average recoveries

Table 4. Potentiometric selectivity coefficients of T*p*ClPB-clopidogrel PVC membrane sensor

Interferent, B	$k^{pot}_{CG.,B}$	
	o-NPOE	DOP
Glucose	3.1×10^{-4}	4.1×10^{-4}
Maltose	2.3×10^{-4}	3.4×10^{-4}
Urea	2.5×10^{-3}	1.1×10^{-2}
Ba^{+2}	2.6×10^{-3}	7.7×10^{-3}
Mg^{+2}	2.1×10^{-3}	6.9×10^{-3}
Na^{+}	2.6×10^{-3}	6.9×10^{-3}
NH^{4+}	5.8×10^{-3}	2.5×10^{-2}
K^{+}	9.0×10^{-4}	6.4×10^{-3}
Ca^{+2}	1.2×10^{-3}	4.8×10^{-3}
Fe^{+2}	9.3×10^{-3}	3.3×10^{-2}

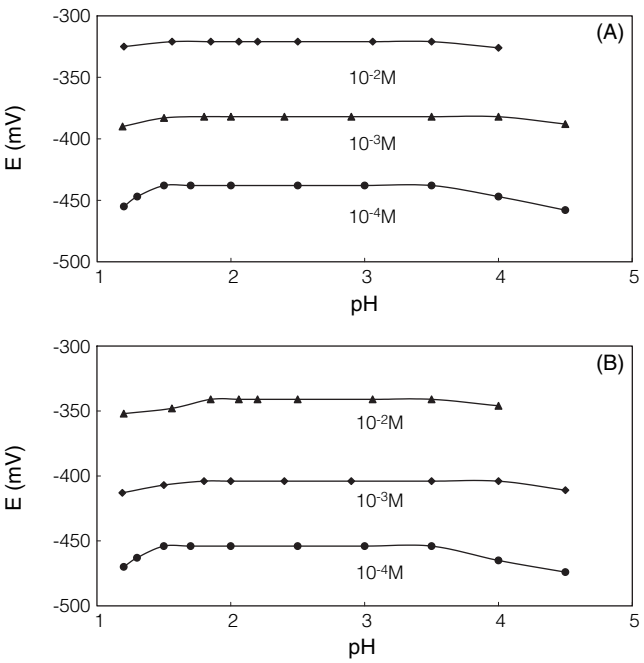


Figure 4. pH – potential profile of T*p*ClPB-clopidogrel PVC based membrane sensor. (A) DOP and (B) *o*-NPOE.

Journal of Food and Drug Analysis, Vol. 16, No. 2, 2008

Table 5. Determination of clopidogrel in pharmaceutical preparations using TpClPB-clopidogrel PVC membrane sensor

Trade name and source	Nominal content (mg/tab)	Recovery* (%)	
		o-NPOE	DOP
Plavix "Pharma Company, 6 October City, Cairo, Egypt"	75 mg / tab	100.8 ± 0.2	100.5 ± 0.2

*Average of 5 measurements.

of 100.8% and 100.5% of the nominal and mean standard deviation of 0.84% and 0.89% for the *o*-NPOE and DOP based sensors were obtained, respectively.

CONCLUSIONS

From the present work we can conclude that there are different advantages where the sensor technique more simple and cheaper than HPLC techniques. On the other hand, HPLC gave a rapid (less than 5 min) analysis, higher accuracy and precision, and lower LOD than sensor method and because of the good accuracy, no internal standard was needed. The methods can be used for determination of (CG-H_2SO_4) in tablets without any interferences.

ACKNOWLEDGEMENTS

Dr. Amr L. Saber wishes to thank Prof. Dr. Greibrokk, Prof. Dr. Lundanes, their group and International Scholarship Section for supporting the HPLC-technique. He would also like to thank Chemistry Department, Oslo University, for their cooperation and encouragement in carrying out the HPLC work.

REFERENCES

1. Plavixtm, RxMed: Pharmaceutical Information, Sanofi/ Bristal-Mayers Squibb.
2. Clarke, T.A. and Waskell, L.A. 2003. The metabolism of clopidogrel is catalyzed by human cytochrome P450 3A and is inhibited by atrovastatin. Drug Metabol. Dispos. 31: 53-59.
3. Perillo, J. M., Maftouh, M., Anrieu, A., Uzabiaga, M. F., Fedeli, O., Savi, P., Pascal, M., Herbert, J. M., Maffrand, J. P. and Picard, C. 2002. Structure and stereochemistry of the active metabolite of clopidogrel. Drug Metabol. Dispos. 30: 1288-1295.
4. Rao, T. R. K., Usha, P. R., Naidu, M. U. R., Gotay, J. A. and Meena, M. 2003. Bioequivalence and tolerability study of two brands of clopidogrel tablets, using inhibition of platelet aggregation and pharmacodynamic measures. Curr. Therap. Res. 64: 685-696.
5. Herbert, J. M., Frehel, D., Vallee, E., Kieffer, G., Gouy, D., Berger, Y., Necciati, J., Defreyn, G. and Maffrad, I. P. 1993. Clopidogrel, a novel antiplatelet and antithrombotic agent. Crdiovasc. Drug Rev. 11: 180-198.
6. Reist, M., Roy-de vos, M., Montseny, J. P., Mayer, J. M., Carrupt, P. A., Berger, Y. and Testa, B. 2000. Very slow chiral inversion of clopidogrel in rats: a pharmacokinetic and mechanistic investigation. Drug Metab. Dispos. 28: 1405-1410.
7. Enein, H. Y. A., Hoenen, H., Ghanem, A. and Koll, M. 2005. Reversed phase liquid chromatographic method for the high-throughput analysis of clopidogrel in pharmaceutical formulations using a monolithic silica column. J. Liq. Chromatogr. 28: 1357-1365.
8. Mitakos, A. and Panderi, I. 2002. A validated LC method for the determination of clopidogrel in pharmaceutical preparations. J. Pharm. Biomed. Anal. 28: 431-438.
9. Lagorce, P., Perez, Y., Ortiz, J., Necciari, J. and Bressol, F. 1998. Assay method for the carboxylic acid metabolite of clopidogrel in human plasma by gas chromatography - mass spectrometry, J. Chromatogr. Biomed. Appl. 720: 107-117.
10. Mikatos, A. and Panderi, I. 2004. Determination of the carboxylic acid metabolite of clopidogrel in human plasma by liquid chromatography-electrospray ionization mass spectrometry. Anal. Chim. Acta 505: 107-114.
11. Kamble, S. N. and Venkatachalam, A. 2005. Gas chromatographic determination of clopidogrel from tablet dosage forms. Indian J. Pharm. Sci. 67: 128-129.
12. Singh, S. S., Sharma, K., Barot, D., Mohan, P. R. and Lohray, V. B. 2005. Estimation of carboxylic acid metabolite of clopidogrel in Wistar rat plasma by HPLC and its application to a pharmacokinetic study. J. Chromatogr. B 821: 173-180.
13. Agrawal, H., Kaul, N., Paradkar, A. R. and Mahadik, K. R. 2003. Stability indicating HPTLC determination of clopidogrel bisulfate as bulk drug and in pharmaceutical dosage form. Tal. 61: 581-589.
14. ICH. 1996. Q213 Validation of Analytical Procedure: Methodology, International Conference on Harmonization, Geneva.
15. Jedlička, A., Klimeš, J. and Grafnetterova, T. 2004. Reversed-phase HPLC methods for purity test and assay of pioglitazone hydrochloride in tablets.

Pharmazie 59: 178-182.
16. Cragg, A., Moody, G. J. and Thomas, J. D. R. 1974. PVC Matrix membrane ion-selective electrodes. Construction and laboratory experiments. J. Chem. Educ. 51: 541-544.
17. Ma, T. S. and Hassan, S. S. M. 1982. Organic Analysis Using Ion-Selective Electrodes. Vol. 1. Belcher, R. and Anderson, D. M. W. Academic Press. London, U. K.
18. IUPAC. 1995. Analytical chemistry division commission on analytical nomenclature. Pure Appl. Chem. 67: 507-512.