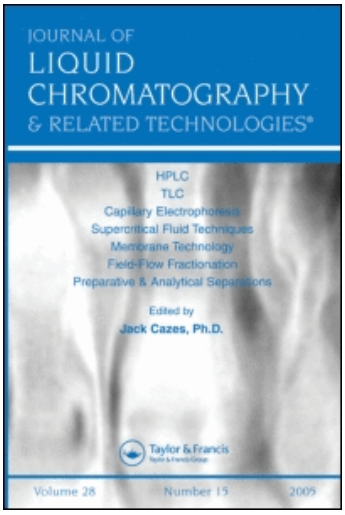

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Reversed Phase Liquid Chromatographic Method for the High-Throughput Analysis of Clopidogrel in Pharmaceutical Formulations Using a Monolithic Silica Column

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Abstract: A high-throughput, high performance liquid chromatographic method was developed and validated for the determination of clopidogrel in pharmaceutical dosage forms. The analysis was performed at room temperature using a reversed phase monolithic silica column Chromolith Performance 18e (100 mm × 4.6 mm I.D.). The mobile phase consisted of acetonitrile:phosphate buffer (50 : 50 *v*/*v*, pH 3.0) at a flow rate of 4.0 mL/min. The photodiode array detector was set at 235 nm. The developed method showed a good linear relationship in the concentration range from 1.0 to 40.0 μg/mL with a correlation coefficient of 0.999. The limit of detection and limit of quantification were 0.97 μg/mL and 3.52 μg/mL, respectively.

Keywords: HPLC, Monolithic column, Clopidogrel, Pharmaceutical analysis, High performance liquid chromatography

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INTRODUCTION

Clopidogrel, chemically known as (α,*S*)-α-(2-chlorophenyl)-6,7-dihydrothienol[3,2-*c*]pyridine-5(4*H*)-acetic acid methyl ester (Figure 1), is an analogue of ticlodipine (Ticlid) in structure and pharmaceutical function. Both drugs inhibit ADP-induced platelet aggregation.[1] However, clopidogrel therapy has fewer side effects and is at least as effective as a treatment with ticlopidine.[2] For patients with atherosclerotic vascular disease manifested as either recent ischemic stroke, recent myocardial infarction, or symptomatic peripheral arterial disease clopidogrel has been shown to be more effective than aspirin.[3]

Only the *S*-enantiomer of clopidogrel is used in pharmaceutical preparation. The parent compound itself is not active, only the cytochrome p450 derived metabolite exerts the effect on the ADP-induced platelet inhibition;[4] whereas the *R*-enantiomer has no antithrombotic function, but leads, at high doses, to convulsions in animal studies.[5] Clopidogrel is metabolized very rapidly in the liver,[5] thus, it is not detectable in the bloodstream.

There have been few methods reported about the analysis of clopidogrel. One study employed reversed-phase high performance liquid chromatography with a semi-micro column for quality control of pharmaceutical preparations.[6] The required run time of the chromatogram for the analysis of clopidogrel was least 7.5 min.[6] A more recent study analyzed the purity of 19 different tablets containing clopidogrel using chiral HPLC separation with an average run time of at least 17 min.[7]

The fastest possible run time is preferred for routine analysis, because it allows a high sample throughput and cost-effective analysis. In order to decrease the run times reported previously, a monolithic silica column was used. Contrary to conventional columns used for reversed-phase HPLC, monolithic silica columns are not packed, but consist of one single piece of porous silica, called "silica rod". Apart from a greater robustness in day-to-day handling, monolithic silica columns exhibit higher separation efficiency and higher permeability than conventional columns. It is, therefore, possible to use monolithic silica columns at much higher flow rates without compromising sample separation or interfering back pressure (for detailed reviews see references [8 and 9]). For example, it has been reported that by using monolithic

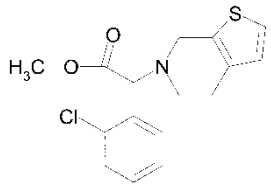

Figure 1. Chemical structure of Clopidogrel.

silica columns existing HPLC methods can be shortened; Van Nederkassel et al.[10] demonstrated, for three different methods, a reduction in run time from 15–30 min to 48 seconds, 1.8 min, or 3 min, using a Chromolith column.

The purpose of the present work is to establish a validated high-throughput method to determine clopidogrel hydrogen sulfate in pharmaceutical preparations by reversed phase liquid chromatography (HPLC), using a monolithic column.

EXPERIMENTAL

Chemicals and Material

HPLC-grade methanol, acetonitrile, and *o*-phosphoric acid 85% were purchased from Fisher Scientific, Fair Lawn, New Jersey, USA, monosodium-dihydrogen-phosphate was obtained from BDH Ltd, Poole, Dorset, UK. Clopidogrel hydrogen sulfate was purchased from Sigma Chemical Co., St Louis, MO, USA. Plavix® tablets (Sanofi Synthelabo, Ambares, France) were supplied by King Faisal Specialist Hospital and Research Centre, Pharmacy Services. Acrodisc 4CR syringe filters, PTFE, 4 mm diameter, 0.45 μm pore size was obtained from Aldrich Chemical Company, Inc., Milwaukee, Wisconsin, USA.

Instrumentation

The chromatographic system consisted of a Waters Solvent delivery system 600, consisting of a tertiary pump and a controller 600, 717plus Autosampler, and a Waters photo diode array detector PDA 996 (Waters, Milford, Massachusetts, USA). The column was a Chromolith Performance 18e, 100 mm × 4.6 mm I.D. (Merck KGaA, Darmstadt, Germany). The whole system was controlled by Waters Millenium software, which was also used for the integration. The Millipore Milli-Q Plus System (Bedford, Massachusetts, USA) was used for deionised water.

Preparation of Standard Solutions

Standards were prepared from a 2 mg/mL stock solution in methanol. The range was between 1.0 and 40 μg/mL. A 50 mM phosphate buffer was prepared for the mobile phase.

Analysis of the Pharmaceutical Formulation

The content of clopidogrel per tablet was, according to the label 75 mg, which is equivalent to 97.875 mg clopidogrel hydrogen sulfate per tablet. Four

tablets were weighed (average weight 258.8 mg), powdered, and an aliquot equivalent to one tablet (258.8 mg) was weighed accurately and transferred to a 100 mL volumetric flask. Methanol was used for extraction. The solution was sonicated for 1 h and the volume was adjusted to 100 mL with methanol. After centrifugation (2500 rpm, 10 min), the supernatant was filtered.

Optimization

Mobile phases consisting of methanol:water (or phosphate buffer), as well as acetonitrile:phosphate buffer of various ratios, were used at a variety of flow rates and pH values to gain optimum chromatographic conditions.

Method Validation

Precision and Accuracy

A standard solution of 1:100 diluted sample solution was chosen and analyzed ten times.

Linearity

A stock solution of clopidogrel hydrogen sulfate was prepared in methanol. A series of standard curves were prepared over a concentration range from 1.0 to 40.0 μg/mL. The data of peak area versus concentration was treated by linear least square regression analysis, using B.E.N. version 2.0, a program for calculating analytical limits of calibrations according to DIN 32645, Institute of Legal Medicine and Traffic Medicine, Heidelberg, Germany.

Limit of Detection and Limit of Quantification

LOD and LOQ were determined from the calibration curve, using the BEN version 2.0 software.

Stability of the Analyte

The dissolved and filtered analyte was analyzed at 0, 24, and 96 hours; the sample was stored at room temperature. The relative standard deviation shows an estimate of the stability of the analyte.

RESULTS AND DISCUSSION

Optimisation

Methanol:water (50:50, *v*/*v*) was used as mobile phase, however, this mobile phase gave unsatisfactory results at different pH values, therefore, a mobile phase composed of acetonitrile and 50 mM phosphate buffer was used.

The effect of different pH values of the mobile phase was studied. Acetonitrile:50 mM phosphate buffer (50:50, *v*/*v*) was used at pH 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, and 5.5. It was noticed that more acidic pH values lead to shorter retention times and better peak shape, i.e., the peaks displayed less tailing. Therefore, pH 3.0 was chosen for this study. Its peak shape was symmetrical and the sample peak well separated from the solvent front.

The effect of different ratios of the mobile phase constituents on the separation, i.e., acetonitrile:phosphate buffer at 70:30, 60:40, 50:50, 40:60, and 30:70 (all *v*/*v*, pH 3.0) was investigated. Figure 2 shows the effect of the acetonitrile concentration on the capacity factor (*k*). The higher the ratio of buffer in the mobile phase, the longer is the retention time and the broader is the peak. Therefore, the ratio of 50:50 (*v*/*v*) acetonitrile:phosphate buffer (pH 3.0) was used for the further study.

Finally, the effect of the flow rate on the separation was studied. A standard solution was run at flow rates between 1.0 and 4.0 mL/min. The results are shown in Figure 3. A flow rate of 4.0 mL/min was chosen, since it resulted in a fast and well resolved separation.

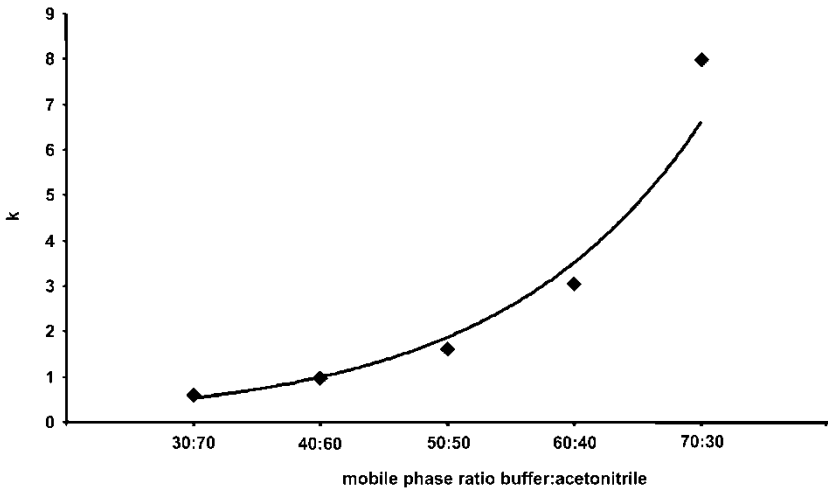

Figure 2. The effect of the mobile phase ratio on the capacity factor *k*. All ratios are given as *v*/*v*.

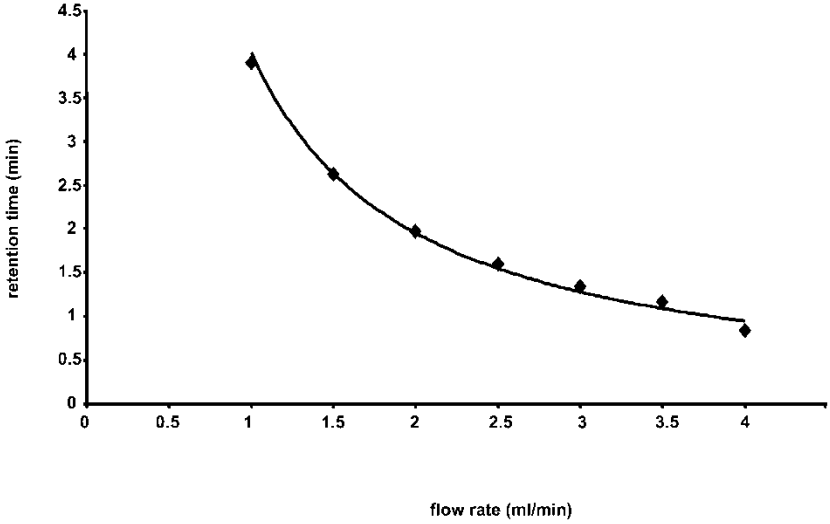

Figure 3. The effect of the flow rate on retention time of the clopidogrel peak.

Accuracy

Injecting the sample ten times showed an accuracy of 98.2% for clopidogrel hydrogen sulfate.

Reproducibility and Stability

The sample was injected at 0, 24, and 96 hours; the resulting recoveries were 98.9, 99.1, and 99.1%, respectively. This resulted in an average recovery of 98.8 $\pm$ 0.58% ($\pm$ standard deviation).

Linearity

The regression analysis of the calibration for clopidogrel hydrogen sulfate showed good linearity over the concentration range of 1.0 to 40.0 μg/mL. The coefficient of correlation (R) was 0.999 with slope and intercept values of 4665 and 2086, respectively. A typical standard curve is shown in Figure 4.

Limit of Detection and Limit of Quantification

The minimum detectable amount is 0.97 μg/mL and the limit of quantification is 3.52 μg/mL.

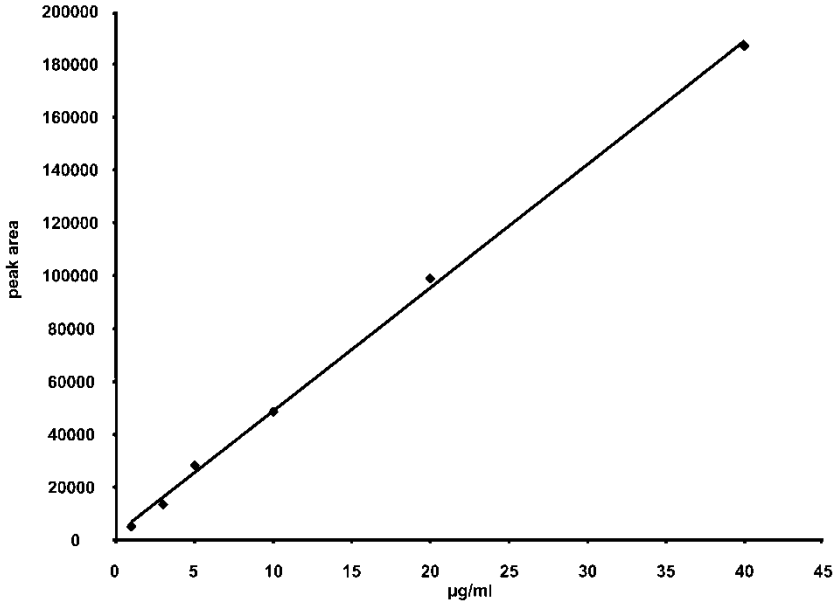

Figure 4. A typical calibration curve of clopidogrel hydrogen sulfate in methanol. Slope and intercept are 4665 and 2086, respectively. R^2 is 0.999.

Recovery

An aliquot of the finely powdered tablet equivalent to the weight of one tablet was dissolved in methanol. After centrifugation and filtration, a 99.1% recovery was obtained. A typical chromatogram of standard clopidogrel hydrogen sulfate is shown in Figure 5A as compared to the one obtained from the methanolic extract of the pharmaceutical formulation (Figure 5B). As can be seen, no interferences from the tablets excipients, which consisted of anhydrous lactose, hydrogenated castor oil, microcrystalline cellulose, polyethylene glycol 6000, and pregelatinized starch. The pink film coating the tablet contains ferric oxide, hydroxypropyl methylcellulose 2910, polyethylene glycol 6000, and titanium dioxide. The tablets are polished with carnauba wax. The analyte is eluted after less than 1.5 min, the overall run is completed after 3.5 min. The advantage of the proposed method for the determination of clopidogrel by HPLC is the short run time compared to the methods reported by Mitakos et al.[6] and Gomez et al.[7] Accordingly, the proposed method offers an accurate high-throughput alternative that enables time efficient quality control of pharmaceutical preparations containing clopidogrel. Furthermore, the calibration range reported here can detect smaller amounts than the one reported in the chiral method

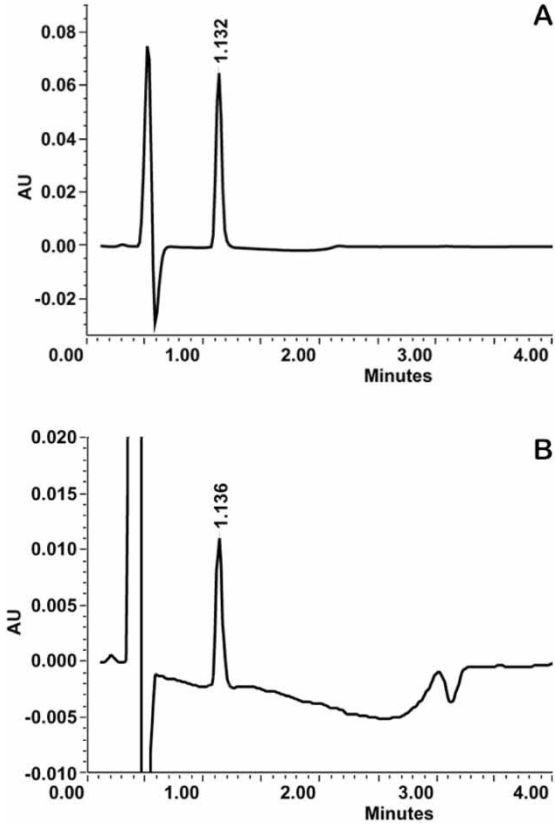

Figure 5. A typical chromatogram of A) clopidogrel hydrogen sulfate standard solution and B) a methanolic extract of clopidogrel hydrogen sulfate from its tablet formulation. Details for chromatographic conditions: see Experimental.

(60–140 μg/mL),[7] and it is not as restricted as reported by Mitakos et al.[6] (1.0–3.0 μg/mL).

CONCLUSION

This paper describes a validated method for the high-throughput analysis of clopidogrel hydrogen sulfate in pharmaceutical preparations. The reported analytical run times are the shortest so far described, which is due to the use of a Chromolith Performance 18e column under reversed phase UV detection. There were no interferences from the tablets excipients. The proposed method is suitable for the analysis of clopidogrel in bulk material and in pharmaceutical dosage forms for quality control purposes.

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