A SIMPLE AND SENSITIVE TLC METHOD FOR DETERMINATION OF CLOPIDOGREL AND ITS IMPURITY SR 26334 IN PHARMACEUTICAL PRODUCTS

D. Antić, S. Filipić, and D. Agbaba*

Institute of Pharmaceutical Chemistry and Drug Analysis, Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, 11000 Belgrade, Serbia

SUMMARY

A simple, conventional TLC method has been established for separation of clopidogrel and its hydrolytic product SR 26334, the main impurity in clopidogrel. Use of aluminium foil-backed silica gel 60F<sub>254</sub> plates with n-heptane–tetrahydrofuran, 1:1 (v/v), as mobile phase, enabled successful separation of clopidogrel in high excess from the impurity. The separated compounds were detected at 230 nm. Regression coefficients ($r \geq 0.999$), recovery (94.5–107.1%), and limit of quantification (0.047 mg mL<sup>-1</sup>) were evaluated and found to be satisfactory. The method is convenient for quantitative analysis and purity control of clopidogrel both in raw material and in dosage forms.

INTRODUCTION

Clopidogrel, methyl (+)-(S)-α-(o-chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine 5(4H)-acetate, is an antiplatelet agent widely used in the prevention of ischaemic stroke, myocardial infarction, and stroke [1,2]. Its carboxylic acid derivative (+)-(S)-(o-chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-acetic acid (SR 26334), which that can arise by hydrolysis of the ester group both in vitro, as a result of the action of humidity and temperature in combination, and in vivo, as a result of the action of carboxylesterase, is the main degradation product. Chemical structures of clopidogrel and SR 26334 are depicted in Fig. 1.

The literature contains reports of the determination of clopidogrel and its inactive metabolite SR 26334 in human plasma by HPLC [3–7] and GC [8]. Clopidogrel has also been assayed alone [9,10] and in the presence of SR 26334 and a chiral impurity [11] in dosage forms by HPLC. A sta-
Fig. 1
The chemical structures of clopidogrel and its impurity SR 26334

...bility-indicating HPTLC method has been established for determination of clopidogrel in the bulk drug and in pharmaceutical dosage forms [12]; it has been used for clopidogrel assay and for detection and quantification of impurities (without structure elucidation).

The latest edition of the US Pharmacopeia [13] contains monographs on clopidogrel as the bulk drug and on clopidogrel tablets, including the requirements for purity testing by HPLC as the official method.

Taking into consideration the advantages of instrumental planar chromatography, for example the possibility of rapid separation and determination of mixture components at low cost, and the lack of information about purity assay, the objective of this study was to develop a simple, rapid, and accurate TLC method for simultaneous determination of clopidogrel and its main impurity, SR 26334, and to determine the optimum chromatographic conditions.

EXPERIMENTAL

Chemicals and Reagents

Clopidogrel, methyl (+)-(S)-α-(o-chlorophenyl)-6,7-dihydrothieno-[3,2-c]pyridine-5(4H)-acetate hydrogen sulphate, and its impurity SR 26334, (+)-(S)-(o-chlorophenyl)-6,7-dihydrothiieno[3,2-c]pyridine-5(4H)-acetic acid were obtained from Sanofi Synthelabo Group, Paris, France. Plavix and Zyllt tablets were products of Sanofi Synthelabo Group, Paris, France and Krka, Novo Mesto, Slovenia, respectively. Other chemicals were of analytical-grade purity.
Stock solutions of clopidogrel standard substance (10 mg mL\(^{-1}\)) and SR 26334 standard substance (1.0 mg mL\(^{-1}\)) were prepared in methanol. Clopidogrel calibration solutions (0.6–1.4 mg mL\(^{-1}\)) and SR 26334 calibration solutions (0.05–0.2 mg mL\(^{-1}\)) were prepared by diluting the corresponding stock solutions. Aliquots of 1.0 µL were applied to the plate and subjected to chromatographic analysis.

Sample Preparation

Powdered dosage form containing 50 mg clopidogrel was extracted with methanol (10 mL). For determination of SR 26334 this solution (10 µL) was applied to the TLC plate. For determination of clopidogrel 2 mL of the solution was diluted to 10 mL with methanol and 1.0 µL was applied to the plate.

Chromatography

TLC was performed on aluminium-backed silica gel 60F\(_{254}\) plates from Merck (Darmstadt, Germany). Samples were applied to the plates by means of a Camag (Muttenz, Switzerland) Linomat IV sample applicator. Ascending chromatography was performed with n-heptane–tetrahydrofuran, 1:1 (v/v), as mobile phase in a twin-trough TLC chamber previously saturated with mobile phase vapour for 15 min. The development time was 20 min. The plates were then air-dried and the spots were detected under UV light at 254 nm. For quantitative analysis chromatograms were scanned at 230 nm with a Camag TLC Scanner II, with computer system and CATS software V.3.12 (Camag), in linear reflectance/absorbance mode. Peak areas were used for quantification.

To check its suitability as an official approach for drug and purity assays by responsible pharmaceutical authorities, the method was validated for linearity, accuracy, reproducibility, limit of detection (LOD), limit of quantification (LOQ), and robustness.

To avoid systematic error, the effects on the peak shape and resolution of the impurity of application of a larger amount of drug should be determined. The accuracy of the method was therefore checked by determining SR 26334 in the presence of clopidogrel. For this purpose, clopidogrel solutions (5 mg mL\(^{-1}\)) containing no detectable amount of the impurity were spiked with SR 26334 solutions of different concentration (0.005, 0.01, and 0.02 mg mL\(^{-1}\)), corresponding to 0.1, 0.2, and 0.4% SR 26334, and analysed as described above.
The accuracy of the method was also assessed by determining clopidogrel in tablets prepared in the laboratory from the excipient mixture spiked with 800, 1000, or 1200 ng clopidogrel.

The intra-assay precision of the method was assessed by replicate \((n = 6)\) chromatographic analysis of clopidogrel and SR 26334 at three different concentrations.

The limits of detection (LOD) and quantification (LOQ) for both clopidogrel and SR 26334 were defined as the amounts for which the experimental signal-to-noise ratios were 3:1 and 10:1, respectively.

To evaluate the robustness of the method the composition of the mobile phase was varied slightly and the effects on the \(R_F\) values of the compounds were determined.

**RESULTS AND DISCUSSION**

Because of the different polarity of the two substances, satisfactory resolution without peak tailing was achieved by use of \(n\)-heptane–tetrahydrofuran, 1:1 \((v/v)\), as mobile phase. The \(R_F\) values of clopidogrel and SR 26334 were 0.74 and 0.36, respectively.

The relationship between peak area and amount of substance applied was linear over the concentration ranges tested \((0.8–1.2 \mu g\) per spot for clopidogrel and \(0.05–0.2 \mu g\) per spot for SR 26334). The regression equations were \(y = (1.09 \pm 0.02)x + (45.8 \pm 20.13)\) for clopidogrel and \(y = (2.40 \pm 0.04)x + (199.5 \pm 5.21)\) for SR 26334, where \(x\) is in ng. Correlation coefficients, \(r\), were \(\geq 0.999\).

Densitometric profiles of clopidogrel samples spiked with SR 26334 during assessment of the accuracy of the method are presented in Fig. 2. Calculated recoveries were plotted against expected values (corresponding to SR 26334 standard without clopidogrel). Recoveries and relative standard deviations \((RSD)\) were 94.5 \(\pm 3.5\)%, 106.6 \(\pm 2.8\)%\), and 107.1 \(\pm 1.2\)% for impurity levels of 0.1, 0.2, and 0.4%, respectively. Acceptance criteria for accuracy of drug impurity testing depend on impurity levels. For levels below 0.5% and above 0.5%, respectively, recovery of 80–120% and 90–110% and \(RSDs\) of 10 and 5%, respectively, are acceptable [11].

In experiments with laboratory prepared tablets containing excipients and 800, 1000, or 1200 ng clopidogrel recoveries were 98.5, 99.2, and 100.3%, respectively. The respective \(RSD\) values were 2.95, 2.5, and 2.1%, respectively.
Densitograms obtained from a reference clopidogrel sample (1); 100, 50, and 200 ng SR 26334 standard (2, 4, and 6, respectively); and samples of clopidogrel spiked with 0.2, 0.1, and 0.4% SR 26334 (3, 5, and 7, respectively)

Results from determination of the intra-assay precision of the method, with related statistical data, are listed in Table I.

Table I
Results from determination of the intra-assay precision of the method

<table>
<thead>
<tr>
<th>Substance</th>
<th>Conc. taken (µg mL⁻¹)</th>
<th>Peak area ± RSD (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clopidogrel</td>
<td>800</td>
<td>986.7 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>1167.7 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>1200</td>
<td>1268.6 ± 1.1</td>
</tr>
<tr>
<td>SR 26334</td>
<td>100</td>
<td>496.05 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>175</td>
<td>805.9 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>849.4 ± 1.6</td>
</tr>
</tbody>
</table>

LOD for clopidogrel and SR 26334 were 0.024 and 0.014 mg mL⁻¹, respectively (corresponding to an SR 26334 impurity level of 0.028%). LOQ for the compounds were 0.079 and 0.047 mg mL⁻¹, respectively, corres-
ponding to SR 26334 impurity level of 0.095%. These LOD and LOQ values for clopidogrel, equivalent to 24 and 79 ng per spot, respectively, are better than those reported by Agrawal et al. (40 and 120 ng per spot, respectively [12]), who developed an HPTLC method for determination of clopidogrel. In contrast, LOD and LOQ values for SR 26334 obtained by Gomez et al., who used a HPLC method, were 0.5 and 1.5 ng per spot, respectively [11], much lower than those recorded in our study.

The results obtained from evaluation of the robustness of the method are summarized in Table II. These clearly show that the variations tested had no effect on the resolution of the two substances.

**Table II**

Effect of variation of the composition of the mobile phase on $R_\text{f}$ values

<table>
<thead>
<tr>
<th>Mobile phase composition ($v/v$)</th>
<th>$R_\text{f}$</th>
<th>Clopidogrel</th>
<th>SR 26334</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$-Heptane</td>
<td>6.0</td>
<td>0.82</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>5.5</td>
<td>0.78</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>4.5</td>
<td>0.81</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>0.88</td>
<td>0.37</td>
</tr>
</tbody>
</table>

$a$ Mean values ($n = 3$)

The method was used to screen commercial dosage forms; the results obtained are presented in Table III and Fig. 3. $RSD$ values obtained for clopidogrel (0.6 and 2.1%) confirmed the precision of the method. No traces of SR 26334 impurity were detected in the samples tested; this is in agreement with the manufacturer’s claim, and conforms with the USP 29 requirement that levels should be below 0.2% [13].

**Table III**

Results from assay of clopidogrel and its impurity SR 26334 in pharmaceutical dosage forms

<table>
<thead>
<tr>
<th>Sample</th>
<th>Clopidogrel</th>
<th>SR 26334</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Expected</td>
<td>Found$^a$</td>
</tr>
<tr>
<td>Plavix</td>
<td>100</td>
<td>98.1 ± 1.06</td>
</tr>
<tr>
<td>Zyllt</td>
<td>100</td>
<td>103.6 ± 2.06</td>
</tr>
</tbody>
</table>

$a$ Mean ± relative standard deviation ($n = 5$)

$^b$ Not detected – concentration below the limit of detection of the method
This densitometric TLC method enables accurate, reproducible, and selective identification and quantification of clopidogrel and its impurity SR 26334. The method can be successfully used not only for determination of trace amounts of the impurity in pharmaceutical dosage forms but also for study of the stability of the active drug component.

ACKNOWLEDGEMENT

This work was supported by the Ministry for Science, Technology, and Environmental Protection of Serbia, contract No. 142071.

REFERENCES


