Journal of Pharmaceutical & Biomedical Analysis
Vol. 11, No. 11/12, pp. 1157–1160, 1993
Printed in Great Britain

0731–7085/93 $6.00 + 0.00
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Analysis of ticlopidine and related impurities by capillary electrophoresis*

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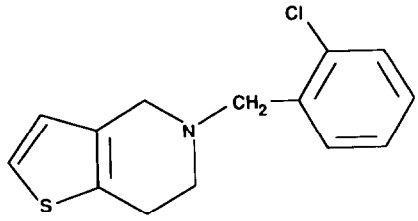
Abstract: Capillary electrophoresis has been used to analyse ticlopidine and its main impurities in bulk material. The minimum detectable amount of impurities was determined and the electrophoretic conditions used were discussed. The proposed method is also suitable to anlayse the drug in plasma.

Keywords: *Ticlopidine; capillary electrophoresis; impurity determination.*

Introduction

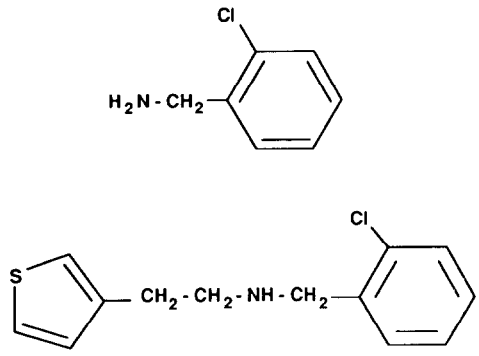
Ticlopidine hydrochloride (TP·HCl), thieno-[3,2-c]pyridine,5-[(2-chlorophenyl)methyl]-4,5,6,7-tetrahydrohydrochloride (**I**) (Scheme 1), is one of the more potent antithrombotic agents [1–4]. Recent studies on the therapeutic activity of this drug has showed its efficacy in the prevention of stroke and myocardial infarction in high risk patients [5–6].



Scheme 1

Two impurities, related to the synthesis, can remain in the active compound: 2-chlorobenzylamine (**II**) and N-[β-(2-thienyl)ethyl]-2-chlorbenzylamine (**III**) (Scheme 2).

Until now relatively few analytical methods regarding ticlopidine are reported and generally they were related to the pharmacokinetic studies [1–5]. Therefore the development of specific and sensitive analytical methods suitable for the quality control of ticlopidine hydrochloride could be useful.



Scheme 2

The predominant analytical technique in the quality control of drugs in recent years has been high-performance liquid chromatography (HPLC). Few applications of capillary electrophoretic (CE) technique in pharmaceutical analysis are present in the literature.

This paper describes a capillary electrophoresis method to determine ticlopidine hydrochloride and its two impurities in about 5 min. The analyses were carried out in some bulk material samples by using an uncoated capillary and on-line multiwavelength UV detector.

Experimental

Apparatus

The electrophoretic analyses were carried

*Presented at the 'Fourth International Symposium on Pharmaceutical and Biomedical Analysis', April 1993, Baltimore, MD, USA.

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out with a SpectraPHORESIS 1000 apparatus (Spectra Physics, San José, CA, USA) equipped with a multiwavelength UV–vis detector SpectraFocus with deuterium lamp (190–800 nm) and cooling-air circulation by Peltier effect system (15–60°C). The electrophoretic separations were performed using Supelco HPE fused silica capillaries (36 cm × 50 μm i.d. or 70 cm × 50 μm i.d., uncoated). Instrument control and data collection were performed by a computer (Spectra 386E).

On-column UV detection was used at the wavelengths of 200, 210 and 225 nm. The temperature of capillary was always 25°C and the applied voltage was 15 or 20 kV. The samples were injected by hydrodynamic mode for 2 s which is equivalent to an introduction volume of about 7 nl of sample.

Chemicals

Redistilled water was used for the preparation of the electrolyte and compound solutions. Sodium dihydrogen phosphate, phosphoric acid and tris(hydroxymethyl)-aminomethane were purchased from Merck (Darmstadt, Germany). Ticlopidine hydrochloride bulk materials, its standard and the standards of 2-chlorobenzylamine and N-[β-(2-theinyl)-ethyl]-2-chlorobenzylamine were kindly provided by Aschinpharma (Milan, Italy).

Capillary electrophoresis procedure

The analyses were performed by using two different analytical conditions: (1) uncoated capillary (36 cm × 50 μm i.d.) filled with 60 mM phosphate buffer (pH 3); (2) uncoated capillary (70 cm × 50 μm i.d.) filled with 40 mM tris(hydroxymethyl)-aminomethane–70 mM phosphate buffer (pH 3). Each day, at the beginning of the analytical process, the capillary was washed with 0.1 M sodium hydroxide for 30 min. The subsequent conditioning cycles were: washing with 0.1 M sodium hydroxide for 2 min, buffer for 3 min and sampling of standards or samples by hydrodynamic mode for 2 s.

Analysis of ticlopidine hydrochloride

Standards and working standard. Pure standards of each compound were used to study the correct electrolyte, pH, applied voltage, current value and working temperature. Different working standard solutions were prepared by solubilizing 1 mg ml^{-1} of ticlopidine hydrochloride in the buffer. These solutions were used to obtain: (a) the electrophoretic profile by adding 1×10^{-2} mg ml^{-1} of impurities **II** and **III**; (b) to verify the linearity of calibration curves by adding nor-ephedrine as internal standard, and different amount of two impurities.

Assay of bulk material samples. The analyses were carried out on samples prepared from six batches of ticlopidine bulk material. About 100 mg of each ticlopidine bulk sample, exactly weighed, were solubilized with 30 ml of the electrolyte and quantitatively diluted to 100 ml. This solution, added with a convenient amount of the internal standard, was filtered by 0.45 μm Millipore filter. An aliquot of about 1.5 ml was transferred in a vial and used for the quantitative analysis.

Results and Discussion

The structures of ticlopidine and its impurities suggested that they could be analysed as cations. Preliminary experiments using a short uncoated capillary confirmed the cathodic movement of ticlopidine and compounds **II** and **III**. When an uncoated fused-silica capillary is used to analyse charged compounds, the separation happens principally through electroendosmotic flow. Therefore the analysis of cations generally require little time. The electropherogram (Fig. 1) shows that ticlopidine, the internal standard and its impurities were resolved in about 5 min. The identity of impurities found in the bulk material was confirmed: (1) by the increase of respective peak heights when the bulk material solution was alternatively enriched with a fixed amount of the standard solution of the impurity **II** or **III**; (2) by the comparison of the UV spectra obtained during the run with those obtained from the standard solutions of drug and impurities (Fig. 2).

As shown in the electropherogram (Fig. 1), ticlopidine (migration time 4.49 min) is very well resolved from the impurity **II** (migration time 3.53 min) and impurity **III** (migration time 4.62 min) although the difference in migration times is very short. These migration times provide very rapid qualitative analysis, but for quantitative determinations the necessary increase of the active compound concentration reduces the resolution of peaks related to ticlopidine and impurity **III**. In order to increase the resolution between these two

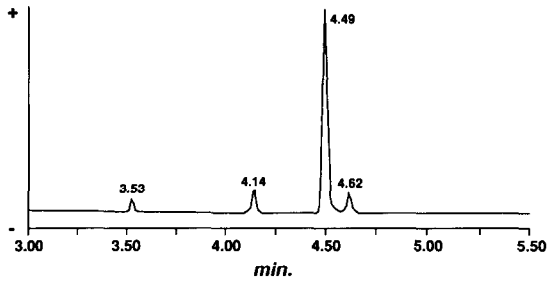

Figure 1
Electropherogram of a working standard mixture containing ticlopidine, impurities and internal standard, using 60 mM phosphate buffer (pH 3) as electrolyte. Capillary 36 cm × 50 μm i.d.; applied voltage 20 kV, 60 μA, temperature 25°C; sampling by hydrodynamic mode for 2 s. Migration time: ticlopidine 4.49 min, nor-ephedrine hydrochloride (internal standard) 4.14 min, impurity **II** 3.63 min and impurity **III** 4.62 min.

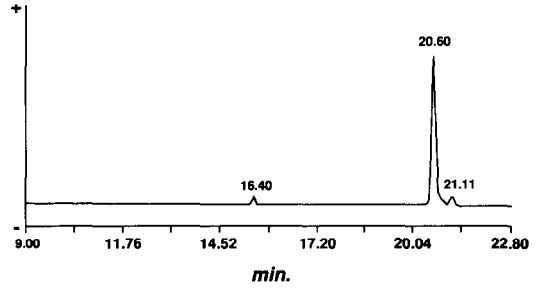

Figure 2
Electropherogram of a working standard mixture containing ticlopidine and impurities using 40 mM Tris–70 mM phosphate buffer (pH 3) as electrolyte. Capillary 70 cm × 50 μm i.d.; applied voltage 20 kV, 60 μA, temperature 25°C; sampling by hydrodynamic mode for 2 s. Migration time: ticlopidine 20.60 min, impurity **II** 16.40 min and impurity **III** 21.11 min.

peaks, several electrolyte systems were tested. Among them only Tris 40 mM–phosphate 70 mM buffer (pH 3), used with a longer capillary (70 cm × 50 μm i.d.), gave a slightly better resolution between ticlopidine (20.6 min) and impurity **III** (21.11 min). However, the analysis time was much longer (Fig. 3), the baseline noise increased and moreover the peak areas were not repeatable. The peak area differences were not found when the analyses were carried out using a short capillary filled with phosphate buffer. The increased capillary length and migration time could be the cause of small changes in the electroendosmotic flow produced unreliable quantitative data. Therefore, the quantitative analysis of ticlopine was carried out with short capillary using the first proposed electrolyte.

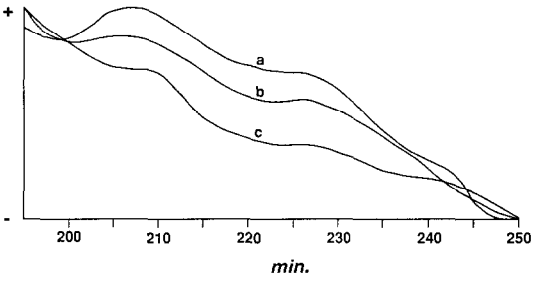

Figure 3
UV spectra of ticlopidine HCl (a), impurity **II** (c) and **III** (b) obtained from the electropherograms of Figs 1 and 2.

The relative standard deviation of the migration times were about 0.55% (n = 30) and 0.8 (n = 20) for intra-day and inter-day analyses, respectively. The linearity of the calibration graphs was tested in the ranges of 0.1–2 mg ml^{-1} for ticlopidine and 2–20 μg ml^{-1} for each impurity. The correlation coefficients and the relative standard deviations (eight determinations) were 0.999, ±1.3%; 0.989, ±2%; and 0.991, ±1.7% for ticlopidine, impurity **II** and impurity **III**, respectively. Complete resolution of ticlopidine and impurity **III**, and reliable integrations were obtained when the concentration of the active compound was not greater than 2 mg ml^{-1}. The lower detection limit was 2.1 μg ml^{-1} for impurity **II** and 1.7 μg ml^{-1} for impurity **III**. The impurities **II** and **III** were always present in the bulk material batches analysed with mean values of about 0.2% (w/w). Sometimes also an unknown impurity was present.

The analysis of ticlopidine hydrochloride, its volatile (2-chlorobenzylamine) and non volatile ([β-(2-thienyl)ethyl]-2-chlorbenzylamine) impurities, shows the usefulness of CE for the quality control of small molecule pharmaceuticals. The very short analysis time, the modest running costs and very high sensitivity make CE a technique potentially complementary to HPLC.

Acknowledgements — The authors thank the Italian agency of Spectra Physics who provided the possibility to use a SpectraPHORESIS 1000 apparatus. Dr Salvatore Fanali is thanked for his helpful suggestions. This research was supported by grants from Università 'La Sapienza' (MURST–Ricerca di Ateneo, quota 60%) and MURST 40%.

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[*Received for review 19 April 1993; revised manuscript received 7 July 1993*]