Short communication

Validated assay for the determination of celiprolol in plasma using high-performance liquid chromatography and a silanol deactivated reversed-phase support

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Abstract

Previously reported methods for the determination of celiprolol in plasma could not be satisfactorily employed due to interference from plasma components. Thus, an improved, convenient and efficient method for the determination of the plasma concentration of celiprolol was developed using a simple solvent extraction step followed by high-performance liquid chromatography on a silanol deactivated C_{18} column with fluorescence detection. The plasma interference was resolved from celiprolol and the typical trailing of basic compounds on reversed-phase HPLC was eliminated. The peak-area ratio versus plasma concentration was linear over the range of 5–1000 ng/ml and the detection limit was 5 ng/ml.

Keywords: Celiprolol

1. Introduction

Celiprolol, 3-[3-acetyl-4-(3-*tert.*-butylamino-2-hydroxypropoxy)-phenyl]-1,1-diethylurea, is a cardioselective β_1-adrenoceptor antagonist with vasodilator properties and is mainly used in the treatment of hypertension and angina pectoris [1]. Although β-blockers were formerly considered inappropriate in the treatment of heart failure because of their negative inotropic action, several studies have recently shown symptomatic benefit [2]. Although metoprolol has been used in these patients, β-blockers with reduced cardiodepressant activity and vasodilator properties might be more advantageous [3]. In low

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doses (25 mg daily) celiprolol causes a significant fall in natriuretic factors and a small rise in cardiac output in patients with heart failure [4]. Thus, initial dosage must be low (one-eighth standard dose). Since the bioavailability of low dose celiprolol in heart failure Chinese patients is not known, this prompted us to develop a sensitive and precise assay which could follow the pharmacokinetics of low dose celiprolol in human plasma.

Initial attempts to determine celiprolol in human plasma (from healthy Chinese volunteers) using previously reported methods [5,6] failed due to co-elution of a persistent interfering peak. Traditional methods of separation failed so a new assay method employing minimal solvent extraction steps with the use of silanol deactivated C_{18} column (Waters Puresil) was developed. No interference from plasma

was observed and plasma concentrations of celiprolol as low as 5 ng/ml could be detected.

2. Conditions

2.1. Materials

Celiprolol and the internal standard, propranolol, were obtained as the hydrochloride salt from Rhone Poulenc (Hong Kong) and Zeneca (Hong Kong) respectively. HPLC grade methanol, ethyl acetate and methyl-tert.-butyl ether were purchased from Mallinckrodt (USA). Analytical grade triethylamine was purchased from Riedel-de Haen (Germany) and phosphoric acid, sodium hydroxide and acetic acid were from BDH (UK). All reagents were used without further purification. Stock solutions of celiprolol and propranolol were prepared in water at 0.5 mg/ml and stored at 4°C. The working solutions for plasma spiking (celiprolol at 50, 100, 250, 500, 1000, 2500, 5000 and 10 000 ng/ml and propranolol at 1000 ng/ml) were freshly prepared. Blank Chinese human plasma samples were obtained from the Red Cross (Hong Kong) and stored at −20°C until used. Nova-Pak C_{18} radial compression cartridge column (100×8 mm, 4 μm) and Puresil C_{18} column (150× 4.6 mm, 5 μm, 120 Å) were purchased from Waters (USA).

2.2. Chromatography

A Hewlett-Packard series 1050 HPLC system equipped with an HP 1046A programmable fluorescence detector and operating under ChemStation software control was used (Hewlett-Packard, USA). The HPLC mobile phase was composed of 45% methanol in a triethylamine–phosphate buffer (1% TEA adjusted to pH 3 with phosphoric acid). Chromatographic runs were performed at a flow-rate of 1 ml/min on a Waters Puresil C_{18} high carbon loading end-capped column. The fluorescence of the eluent was monitored at 240 nm (excitation) and 478 nm (emission) for celiprolol and switched to 240 nm (excitation) and 342 nm (emission) at 7 min for propranolol.

2.3. Sample preparation

The plasma samples (1 ml) were treated in glass centrifuge tubes with 50 μl of the internal standard solution, followed by sodium hydroxide (0.5 M, 0.5 ml) and methyl-tert.-butyl ether (5 ml). The mixture was vortexed (5×10 s), centrifuged (1000 g, 5 min) and the separated organic layer was transferred to clean centrifuge tubes and evaporated to dryness using a vacuum concentrator (Centrivap, Labconco, Missouri, USA). The samples were reconstituted in the HPLC mobile phase (200 μl) and aliquots (80 μl) were analysed by HPLC. The concentration of celiprolol was quantified by peak-area ratio with respect to the internal standard. Weighting factors were not used in the regression analysis.

The standard samples for celiprolol were prepared by spiking blank plasma with 100 μl of the working solution and analysed employing the same procedure. The calibration curve was obtained from spiked plasma samples in duplicates over three independent runs. The precision of the assay was assessed by analysing replicates of spiked plasma samples (5×) at high and low celiprolol concentrations (300 and 30 ng/ml) on three separate days. The intra-day and inter-day variations were calculated.

3. Results and discussion

3.1. Chromatography

Initial results using previously reported methods showed a significant interference peak co-eluting with celiprolol (Fig. 1). In addition, the percentage recovery using ethyl acetate extraction was not acceptable (Table 1). Employment of a solid-phase extraction procedure in the sample preparation improved the recovery and reduced the interference from plasma, however the single-use solid-phase extraction cartridges are relatively expensive.

The use of the silanol deactivated reversed-phase column improved the peak shape and eliminated the poor trailing characteristics typical of basic compounds compared to other more commonly used reversed-phase media such as Nova-pak. The plasma component that co-eluted with celiprolol previously was well separated on the Waters Puresil column.
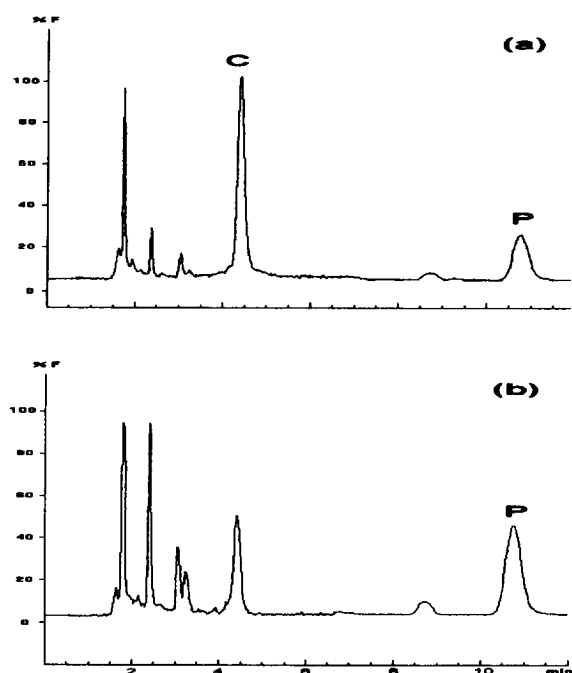
Fig. 1. Chromatogram of plasma samples on Waters RCM Nova-Pak C_{18} column using 60% methanol in 1% triethylamine–phosphate buffer, pH 3, at 1.5 ml/min. (a) Plasma blank spiked with celiprolol at 250 ng/ml and propranolol at 50 ng/ml. (b) Plasma blank spiked with propranolol at 50 ng/ml. Peaks: C=celiprolol; P=propranolol.
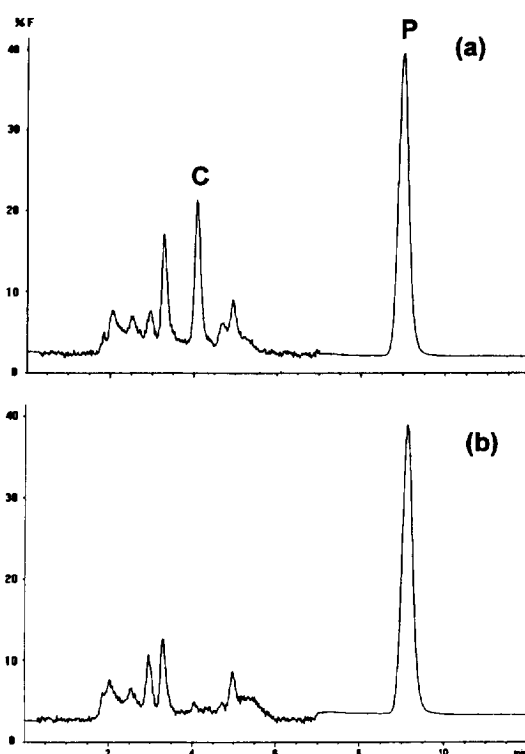

Fig. 2. Chromatogram of plasma samples on Waters Puresil C_{18} column using 45% methanol in 1% triethylamine–phosphate buffer, pH 3, at 1 ml/min. (a) Plasma blank spiked with celiprolol at 50 ng/ml and propranolol at 50 ng/ml. (b) Plasma blank spiked with propranolol at 50 ng/ml. Peaks: C=celiprolol; P=propranolol.

Celiprolol and propranolol were eluted at 4.1 and 9.1 min respectively (Fig. 2). The interference from plasma background peaks was insignificant. The small peak that elutes at about the retention time of celiprolol corresponds to less than 2 ng/ml celiprolol in terms of peak area.

3.2. Linearity and quantification limit

Since peak plasma levels of 236 and 679 ng/ml for single oral doses of 100 and 200 mg respectively were reported, a calibration curve covering the range

of 5–1000 ng/ml was selected [7]. A linear calibration curve was obtained for the peak-area ratio of celiprolol versus propranolol. The linear regression equation was

$$y = 0.0103(\pm 0.00006)x + 0.11(\pm 0.03) \quad ,r^2 = 0.9986$$

The detection limit of celiprolol is about 5 ng/ml with a signal-to-noise ratio of 3:1.

3.3. Recovery

The extraction recovery of the analyte and internal standard were determined by comparing the respective peak areas of the chromatogram of the extracted spiked plasma sample relative to the untreated standards containing an equivalent amount of the compounds. Methyl-tert.-butyl ether was found to

Table 1
Recovery of celiprolol and propranolol from plasma with solvent extraction

Solvent	Recovery (%)	
	Celiprolol	Propranolol
Methyl-tert.-butyl ether	85	100
Ethyl acetate	38	33

provide better extraction efficiency compared to ethyl acetate. The recoveries using methyl-*tert.*-butyl ether extraction for celiprolol and propranolol are shown in Table 1.

3.4. Precision and accuracy

The precision of the assay was estimated by performing the analysis of replicates (5×) of spiked plasma samples at high and low celiprolol concentrations on 3 different days. The within-day variations were 5 to 14% at 30 ng/ml and 1 to 5% at 300 ng/ml. The inter-day variations were 4% at 30 ng/ml and 3% at 300 ng/ml.

4. Conclusion

The method described above allows the separation of celiprolol from the interfering plasma peak, which was not feasible using previously reported methods. This unknown peak has not been reported in other studies employing human plasma samples. As it appeared in three different batches of plasma obtained from healthy Chinese volunteers, this unknown interfering peak is probably a naturally occurring component of human plasma. The identity of this component was not further investigated, nor was it determined if plasma freshly collected from patients showed such an interfering peak.

The advantage of this assay is in its simplicity. It requires a single solvent extraction using methyl-*tert.*-butyl ether from the alkalinised plasma. The precision is satisfactory at both the high and low concentration range. The detection limit is about 5 ng/ml with a signal-to-noise ratio of 3. The use of propranolol as the internal standard is satisfactory. The use of the Puresil C_{18} column gave a good separation between the celiprolol peak and the plasma components, thus eliminating the need to employ more elaborate sample preparation procedures.

Acknowledgments

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