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Narrow-Bore Liquid Chromatography Coupled to Chemiluminescence Detection for the Analysis of Pharmaceutical Preparations Containing Hydrochlorothiazide and Captopril

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INTRODUCTION

Although reduction of the inner diameter of HPLC columns has a number of distinct advantages, one of the important limitations of miniaturization in flowing streams is the reduced detecting power of classical detectors and detection systems. The development of sensitive detection techniques that can be easily applied to miniaturized HPLC set-ups has been considered an important challenge in liquid chromatographic research of recent years. Amongst the cited detection techniques, chemiluminescence (CL)-based methods are focused on due to their high sensitivities together with the relatively small sample size per injection and low cost of instrumental equipment.

In the present study, a narrow-bore HPLC set-up is coupled to CL detection for the analysis of hydrochlorothiazide and the two-component mixture of hydrochlorothiazide and captopril in a tablet formulation based on the reaction of the drugs with cerium (IV) in acidic medium. Rhodamine 6G is suggested as a sensitizer. Pre-column derivatizing procedures are excluded, making the method more simple and rapid. A significant reduction in the flow-rate of mobile phase using narrow-bore LC together with the relatively high sensitivities using CL detection make the method more suitable for routine analysis.

PROCEDURE

The employed HPLC set-up coupled to CL detection for the determination of hydrochlorothiazide and captopril is shown in Fig. 1.

The separation was initially optimized on a 4.0 mm ID analytical HPLC column (RP C18, 125 × 4 mm, Merck, Darmstadt, Germany). As size reduction of a liquid chromatographic column offers a number of theoretical and practical advantages, a narrow-bore HPLC column (Ecocart, 125 × 3 mm, Merck, Darmstadt, Germany) was coupled to the CL detector (Chemlab Instruments, The Netherlands) for the analysis of the two compounds. This narrow-bore column offered a 40% solvent saving and is fully compatible with common HPLC systems. Figure 2 shows the chromatogram for the separation of a standard mixture of captopril and hydrochlorothiazide. Tiopronin was used as the internal standard. The mobile phase was an aqueous solution with 25 mmol octanesulphonic acid sodium salt (OSA) - 50 mmol/L acetic buffer (pH 3.25) containing 10% methanol (v/v) in a flow-rate of 0.6 mL/min. CL detection was carried out based on the reaction

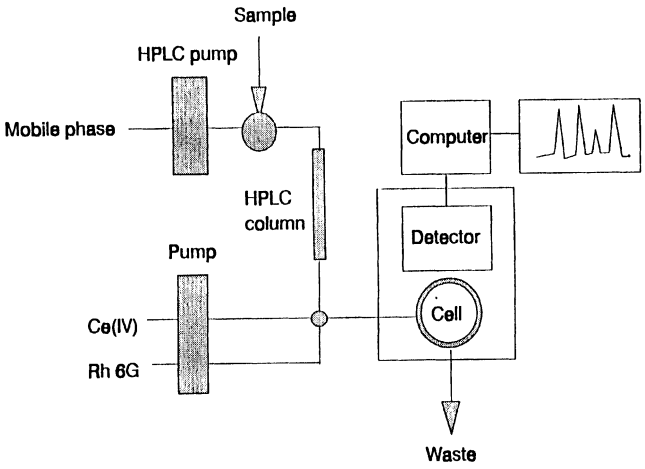

Figure 1. Schematic diagram of the HPLC set-up coupled to a CL-flow injection detector.

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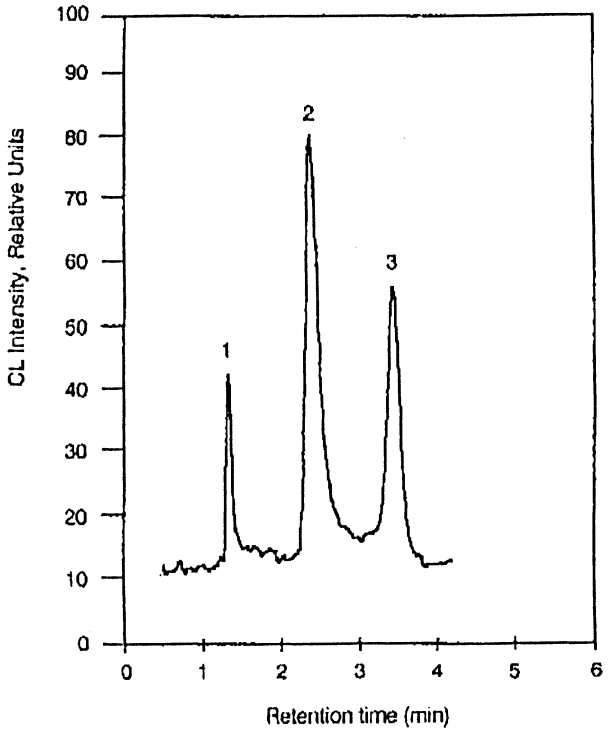

Figure 2. Chromatogram of an aqueous solution of a mixture containing (1) 7.7 nmol tiopronin (internal standard) (2) 0.83 nmol hydrochlorothiazide and (3) 2.3 nmol captopril using a narrow-bore column (Ecocart, 125 × 3 mm, 5 μm particle size) with 25 mmol/L OSA and 50 mmol/L acetic buffer as mobile phase; loop size: 25 μL.

of diuretics with Ce(IV) in sulphuric acid medium, sensitized by rhodamine 6G. Both 10 mmol/L Ce(IV) solution and 0.1 mmol/L rhodamine 6G solution in 0.1 mol/L H_2SO_4 medium were delivered in a flow-rate of 1.0 mL/min by a two-channel Gilson peristaltic pump (Minipuls 2).

RESULTS

The CL response in the detector was linearly related to the concentrations in the ranges 0.6–200 μmol/L (R = 0.9992) for hydrochlorothiazide and 8–300 μmol/L (R = 0.9989) for captopril. The detection limits (3s blank) are 5 pmol per injection for hydrochlorothiazide and 67 pmol per injection for captopril, with relative standard deviations below 4%.

The present method was applied to the analysis of two types of tablets: (1) Dichlotride® (50 mg/tablet hydrochlorothiazide) and Zestoretic® (12.5 mg/tablet hydrochlorothiazide) and (2) a tablet containing the mixture of hydrochlorothiazide (50 mg) and captopril (100 mg) (University Hospital of Ghent). The results showed that the method allows the determination of both drugs as such or in the mixture without significant interferences.

CONCLUSION

Narrow-bore LC coupled to CL detection offers the advantage of easy coupling of CL detection to HPLC systems. A significant reduction of the required volume of mobile phase using narrow-bore LC makes the method attractive for routine analysis. The detection limits are acceptable for the analysis of the two compounds in tablet formulations without the need of pre-column derivatization (as captopril shows no significant UV absorption). Tiopronin proved to be a suitable internal standard for this separation.

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