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Flow-injection Analysis of Hydrochlorothiazide Applying Sensitised Chemiluminescence Detection: optimisation in View of Narrow-Bore HPLC

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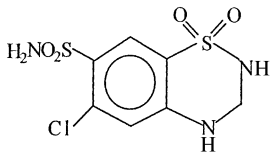
INTRODUCTION

Although reduction of the inner diameter of HPLC columns has a number of distinct advantages, one of the important limitations of miniaturisation 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 miniaturised 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 (Calokerinos *et al.*, 1995) are focussed at due to their high sensitivities together with the relatively small sample size per injection and low cost of instrumental equipment.



(**1**) Hydrochlorothiazide

In the present study, the results of a new flow-injection method for the determination of the diuretic hydrochlorothiazide (**1**) (6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide-1,1-dioxide) is proposed. The method is based on the CL reaction of hydrochlorothiazide with cerium(IV) in sulphuric acid medium, sensitised by the rhodamine 6G dye (Zhang *et al.*, 1995). The method was successfully applied to the determination of hydrochlorothiazide in pharmaceutical preparations containing, amongst others, lactose, maize starch, calcium phosphate, magnesium stearate, potassium chloride and the colorant E 110 (disodium-6-hydroxy-5-(4-sulphonatophenylazo) naphthalene-2-sulphonate) as the concomitant species. Apart from the single formulation, hydrochlorothiazide was determined as well in tablets combined with the antihypertensive lisinopril. The results proved that the detection method is quite selective for S-containing compounds.

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EXPERIMENTAL

The flow injection system used in the present study is shown in Fig. 1. Working standard solutions containing 0.33–130 μmol/L hydrochlorothiazide were prepared by diluting a concentrated fresh standard solution of hydrochlorothiazide with 20% (v/v) methanol solution. The CL signal was measured by injecting 50 μL of the working standard solution into the carrier stream, which then joined the reagent streams (10 mmol/L Ce(IV) and

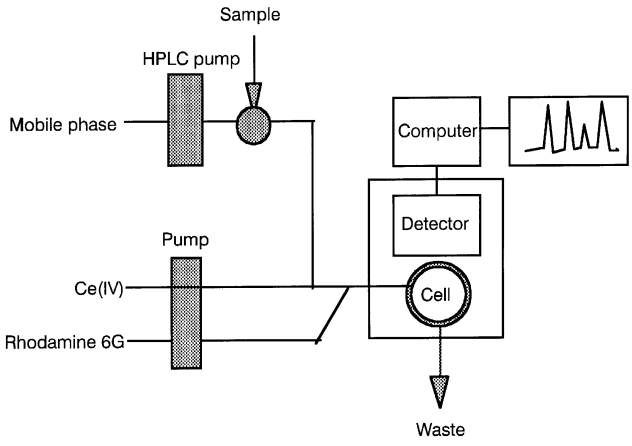

Figure 1. Flow injection manifold for the determination of hydrochlorothiazide.

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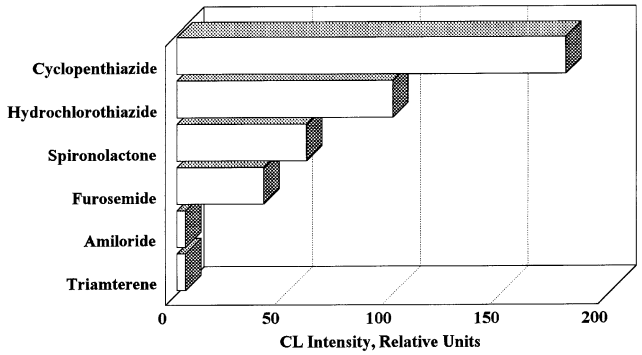

Figure 2. Relative CL intensities of some selected pharmaceutical compounds in acidic Ce(IV) - rhodamine 6G system.

0.1 mmol/L rhodamine 6G in 0.1 mol/L H_2SO_4 solutions, respectively). Peak heights of the CL light emission vs hydrochlorothiazide concentration were used for the calibration.

RESULTS AND DISCUSSION

The effects of Ce(IV), sulphuric acid, methanol, rhodamine and of the reagent flow-rates upon the CL emission were investigated, together with the effects of sample injection volume and micellar reagents. The optimum conditions are 10 mmol/L of Ce(IV) and 0.1 mmol/L of rhodamine 6G in 0.1 mol/L H_2SO_4 solution; 20% methanol was used for the preparation of samples. The total flow-rate of reagents was 0.7 mL/min.

Under the optimum conditions described above, the calibration graph was linear in the range 0.33–130 μmol/L of hydrochlorothiazide and the regression equation was $I = 1.8155C + 6.4564$, $r = 0.9993$ ($n = 10$, 50 μL sample per injection), where I is the relative peak intensity and C the concentration of hydrochlorothiazide. The relative standard deviation (RSD) for 10 μmol/L hydrochlorothiazide was 2.4% ($n = 10$). The detection limit, defined as three times the standard deviation of the reagent blank signal is 0.15 μmol/L hydrochlorothiazide (7.5 pmol per injection). The possibility of applying the proposed method to the determination of other S-containing pharmaceutical compounds was also explored (Fig. 2). The results proved that the detection method is selective for S-containing compounds.

The proposed method was successfully applied to the determination of hydrochlorothiazide as such or in a mixture formula containing 12.5 mg of hydrochlorothiazide and 20 mg of lisinopril (tablets formulation). Acceptable correlation is found between the label values and the results obtained by the proposed method. The method shows promise for routine control analysis of pharmaceutical preparations containing hydrochlorothiazide as such or in a mixture with lisinopril.

CONCLUSION

The proposed CL detection system is rapid, inexpensive and relatively sensitive for the determination of the pharmaceutical compounds. The interface between miniaturised HPLC systems and an appropriate CL detector is quite simple since the detection technique is particularly suitable for the detection of small sizes of samples. Further work on this subject is in progress, aiming at narrow-bore liquid chromatographic applications.

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