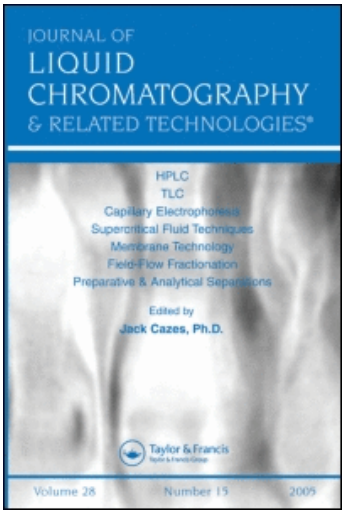

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Application of Flow Programming in the Analysis of Drugs and Their Metabolites in Biological Fluids

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APPLICATION OF FLOW PROGRAMMING IN THE ANALYSIS OF DRUGS AND THEIR METABOLITES IN BIOLOGICAL FLUIDS

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ABSTRACT

An HPLC procedure using flow programming under isocratic elution conditions for determination of drugs and their metabolites in biological fluids is discussed. This technique was used in the analysis of triamterene and its metabolites in urine, chlorothiazide in plasma and hydrochlorothiazide in urine. Advantages of flow programming over conventional procedures such as isocratic elution and gradient elution are discussed.

INTRODUCTION

HPLC analysis employing isocratic elution conditions is the most common procedure used for the analysis of drugs and their metabolites in biological samples. Occasionally, a gradient elution technique is employed for complex samples that consist

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of components with a wide range of distribution coefficients. Gradient elution refers to the technique of increasing the solvent strength as the separation proceeds. It is used to decrease analysis time and optimize separations with respect to time and resolution. Flow programming, which involves changes in flow rate under isocratic conditions, even though known in principle, is not widely used. Application of flow programming under isocratic conditions for separation of drugs and their metabolites in biological fluids is briefly discussed here. The biological samples used were part of in-vivo bioavailability studies (1,2). The methods are described in brief, primarily to illustrate the use of flow programming.

EXPERIMENTAL

Chromatography was performed using Varian Model 5000 LC systems equipped with a Perkin-Elmer fluorescence detector or Varichrom variable-wavelength uv detector. Chromatographic separations were carried out using Varian MicroPak MCH type (reverse-phase) columns. All solvents used were HPLC grade. Biological samples used were part of in-vivo bioavailability studies (1,2).

Triamterene and triamterene sulfate in urine:

The urine sample was spiked with internal standard (3,5-dibromosalicylic acid) and injected directly onto an HPLC system equipped with a reverse-phase column and fluorescence detector.

The mobile phase consisted of 45% methanol in 0.1% aqueous potassium dihydrogen phosphate. The flow rate was increased from 1 ml/min to 3.5 ml/min over the first 10-minute period, and then lowered to 1.0 ml/min over the next 2-minute period. Under these conditions, the retention times for triamterene sulfate, 3,5-dibromosalicylic acid and triamterene were 2.8, 4.5 and 8.8 minutes, respectively.

Hydrochlorothiazide in urine:

The urine sample was spiked with internal standard (sulfadiazine) and injected onto an HPLC system equipped with a reverse-phase column and fixed-wavelength, 254 nm uv detector. The mobile phase consisted of 5% acetonitrile in 0.1% aqueous potassium dihydrogen phosphate. The flow rate was increased from 1 to 1.9 ml/min over the first 27 minutes and then lowered to 1 ml/min over the next 3 minutes. Under these conditions, the retention times for hydrochlorothiazide and sulfadiazine were 18 and 21 minutes, respectively.

Chlorothiazide in plasma

The plasma sample was spiked with internal standard (sulfathiazole) and mixed with 2 ml of acetonitrile. After centrifugation, the supernatant was separated, evaporated to dryness, reconstituted in 50 mcl of methanol, and 30 mcl was injected onto an HPLC system equipped with a reverse-phase column and a variable-wavelength uv detector set at 280 nm. The mobile phase was 4% methanol in 0.2% acetic acid. The flow rate

was increased from 1 ml to 3.4 ml/min over the first 21 minutes, then further increased to 5 ml/min over the next 2 minutes, and maintained at that flow rate for an additional 12 minutes. At the end of the run (35 minutes), the flow rate was returned to 1 ml/min. Under these conditions, the retention times for chlorothiazide and sulfathiazole were 13.5 and 26 minutes, respectively.

RESULTS AND DISCUSSION

In gradient elution, the column is subjected to the varying polarity of the eluent. This alters the behavior of the column, and at times, it does not return to its original condition at the beginning of the next sample analysis. This affects the reproducibility of the sample analysis. Flow programming under isocratic conditions, a new technique for separation of drugs and their metabolites in biological fluids, was developed to overcome some of the drawbacks of gradient elution. In flow programming, the flow of the isocratic solvent is increased (or decreased) over a period of time. Both the initial and final flow rates and the time interval are selected. The change in flow rate occurs linearly over the selected time interval. The column is always equilibrated with the solvent, thus giving more reproducible sample analysis.

It has been reported that for HPLC analysis of triamterene and its metabolites, the urine samples need to be analyzed once

for the parent drug and then a second time for the metabolites (3). Using flow programming, as described above, it has been possible to analyze for both triamterene and its major metabolite in a single injection.

Similarly, flow programming under isocratic conditions has been used successfully in the analysis of hydrochlorothiazide in urine and chlorothiazide in plasma.

Flow programming under isocratic conditions offers the following advantages:

a) drugs and metabolites which otherwise might require different analytical conditions and more than one sample injection can be analyzed in a single injection; b) the column is not exposed to different solvent conditions as in the gradient elution technique, and is thereby not subjected to 'shocks' during a run; c) the column is always equilibrated with the solvent and therefore no column regeneration is required; d) the chromatograms are more reproducible; e) the life of the column is extended; f) it is easy to operate; and g) it is suitable for use with all detectors, including refractive index detectors.

Thus, flow programming under isocratic conditions opens a new avenue for obtaining effective separation using HPLC.

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