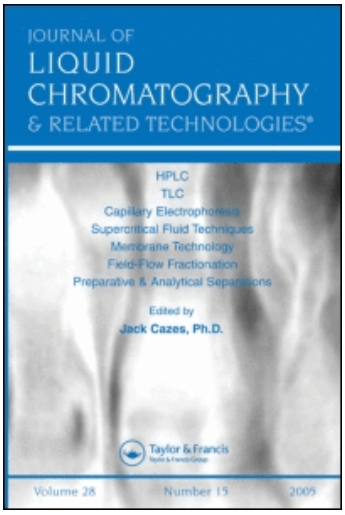

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SURAMIN DETERMINATION BY CAPILLARY ELECTROPHORESIS

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ABSTRACT

Suramin is an anti-prostate-tumor drug, which has a narrow therapeutic range and long half-life. For these reasons, frequent monitoring of the drug is required to minimize toxicity. A rapid assay to measure suramin levels in serum by CE is described here. Serum samples deproteinized with acetonitrile (containing 3-isobutyl-1-methylxanthine as internal standard) were electrophoresed for 2.5 min at 15 KV in a CAPSO buffer (63 mmol/L) pH 9.7 with detection at 254 nm. The test by area under the peak is linear between 50-500 mg/L. The effects of several factors such as voltage, buffer pH and ionic strength on peak height and migration time were studied. This method demonstrates the potential of CE in the clinical chemistry laboratory.

INTRODUCTION

Suramin is a polysulfonated naphthylurea drug that has been used for the treatment of African Sleeping

Sickness (1). Recent reports of significant anti-prostate-tumor activity of suramin have renewed interest in the drug (1). Suramin has a narrow therapeutic range of about 200-300 mg/L; below 200 mg/L, it is not effective, while above 300 mg/L the incidence of neurotoxicity increases dramatically (2). The drug also has a very long half-life of 44-54 days (3). For these reasons, suramin needs to be continuously monitored. Thus, a rapid, reproducible and inexpensive assay is needed.

A lengthy HPLC method which involves three solvent extractions of each sample and reversed-phase with ion-pairing has been described (4). Recently, another HPLC suramin assay, in which plasma samples are injected directly onto a CN column and eluted isocratically with acetoritrile and an ion pairing reagent, has been reported (2). However, the column did not last more than 50 injections.

Here, a simple and rapid method for assaying suramin based on capillary electrophoresis (CE) is described. Using CE for separating and quantifying various compounds is relatively new (5-7), especially for clinical chemistry applications (8). This method is an example of the potential of CE for fast and simple assays of drugs and small molecules in serum.

MATERIALS AND METHODS

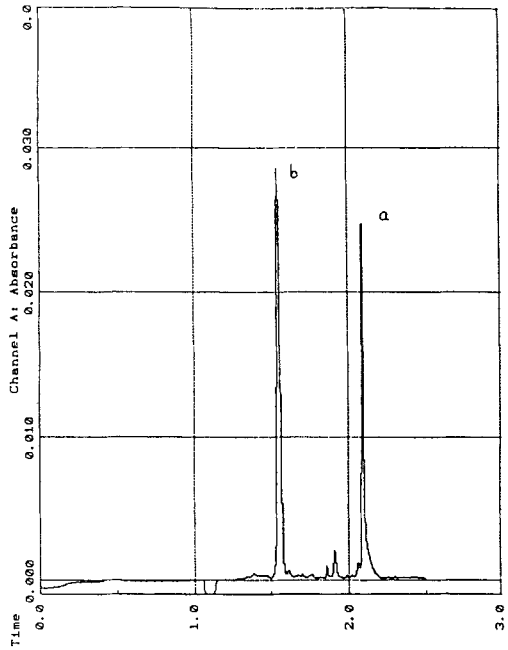
REAGENTS: Suramin was obtained from FBA Pharmaceuticals (West Haven, CT), and 3-isobutyl-1-methylxanthine from Aldrich (Milwaukee, WI).

INSTRUMENT: An automated capillary electrophoresis instrument (Beckman Instruments, Palo Alto, CA) was set at 15 KV, 24° C, and 254 nm. The capillary was 25 cm X 50 µm (i.d.). The electrophoresis buffer was CAPSO (63 mmol/L) pH 9.7 containing 0.2 % polyethelyne glycol 8000. Samples were introduced by pressure injection for 5 sec.

PROCEDURE: Serum or standard samples (100 µL) were deproteinized by mixing for 30 sec with 150 µL of acetonitrile containing (3-isobutyl-1-methylxanthine, 300 mg/L as an internal standard) and then centrifuged for 1 min at 14,000 x *g*. The supernatant was introduced into the capillary by pressure injection and electrophoresed for 2.5 min. After each sample the capillary was washed with NaOH (2 mol/L, 0.7 min), phosphoric acid (50 mmol/L, 0.7 min), and electrophoresis buffer (1 min).

RESULTS AND DISCUSSION

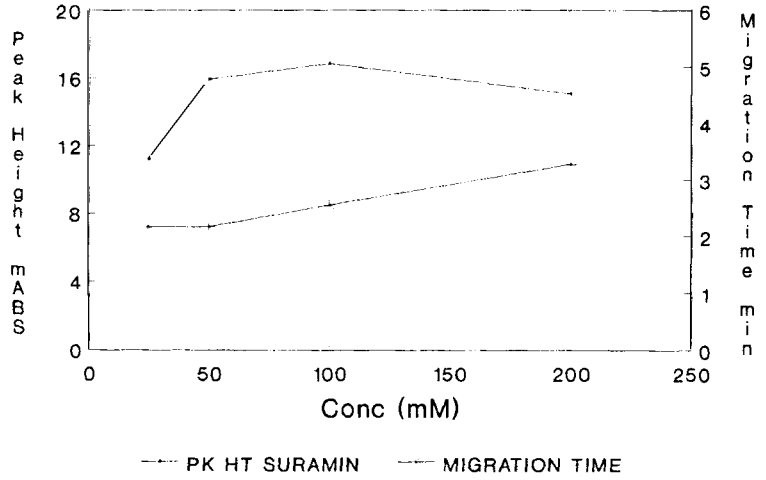
Acetonitrile deproteinization has been previously shown to be a suitable method for removal of serum proteins for drug and small molecules determination by



1. Electropherogram from a patient receiving suramin Peak (a) is suramin at a concentration of 74 mg/L. Peak (b) is the internal standard. (Time scale is in minutes).

CE (9). The electropherogram from a patient receiving suramin, Fig. 1 is relatively clean and free from interferences from serum proteins, common drugs and endogenous substances.

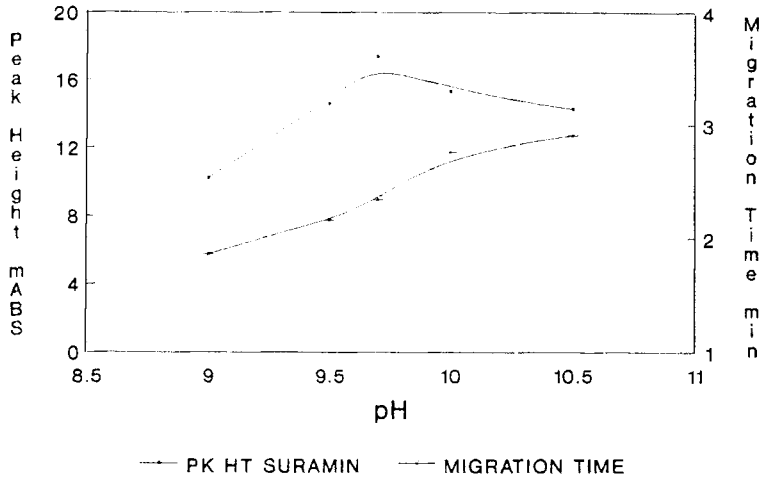
The effect of several factors which might affect the separation of this drug were investigated. The effect of the buffer concentration on peak height (PKHT) and migration time is illustrated on Fig. 2. As the buffer ionic strength increased, the PKHT increased, plateaued



2. Effect of the buffer concentration on peak height and migration time of suramin.

and then decreased. Meanwhile, the migration time increased with increasing buffer concentration. A 63 mmol/L buffer concentration was chosen for routine use, since at this concentration the PKHT was high and the migration time short i.e. fast separation. A pH of 9.7 was chosen because at this pH, the PKHT was the highest with a reasonable migration time (about 2 min), Fig. 3.

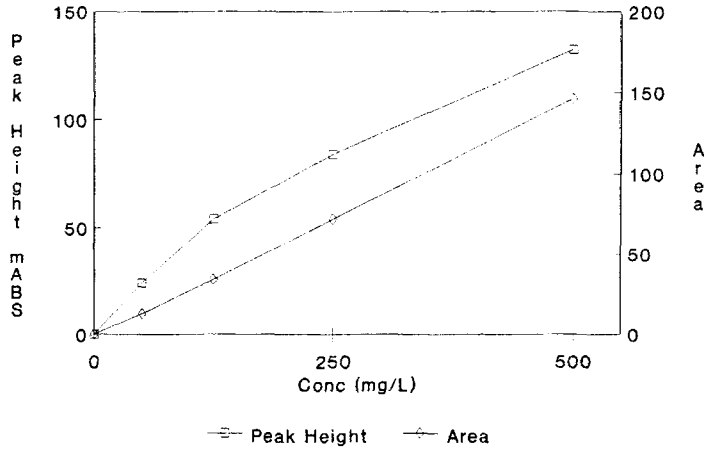
In CE, it is important to find the optimal voltage that gives maximal separation, while producing enough heat that can be dispersed rapidly through the capillary walls. Based on the plot of KV vs μA, a voltage of 15 KV was used for subsequent experiments. It has been shown that in CE, the sample size affects



3. Effect of buffer pH on peak height and migration time of suramin.

the plate number (10). PKHT increased as the amount of sample injected increased up to a maximum at an injection of 8 sec and then it decreased, while the RT remained practically constant.

A standard serum curve in the range of 50 to 500 mg/L of suramin, Fig. 4, shows linearity throughout this range when the peak area is considered. However, unlike other compounds previously studied (9), deviation from linearity is encountered when the peak height is used. It was apparent that to obtain a good reproducibility, it was necessary to wash the capillary with NAOH and phosphoric acid after each sample run. Table I shows the effect of washing the capillary with



4. Linearity of the suramin assay by peak height and peak area.

TABLE I

The Effect of Washing the Capillary on the Precision (CV %) of the Assay

Wash	S H	S R	H/H	R/R
Phos acid	5.26	4.45	2.56	1.23
NaOH	2.47	1.65	2.68	.66
Phos acid + NaOH	2.30	1.24	2.56	.32

Where S H and S R are the % CV of the height and area of the suramin peak, and H/H and R/R are the % CV ratios of the heights and areas of the suramin and internal standard peaks, respectively (internal standard data not shown).

acid, base or both on reproducibility. No interference from common drugs was observed. Recovery was 93% (n=4). This method demonstrates that CE can be used for a rapid determination of drugs in serum such as suramin.

ACKNOWLEDGMENTS

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