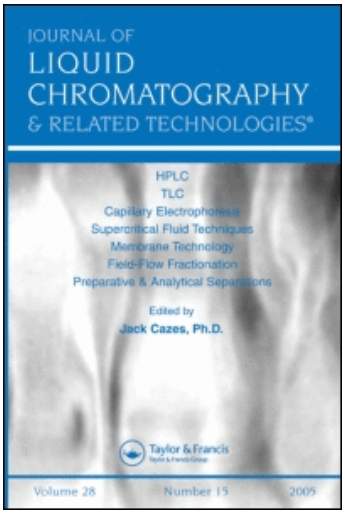

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Suramin Determination by Direct Serum Injection

L. L. Garcia[a]; Z. K. Shihabi[a]
[a] Department of Pathology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, North Carolina

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SURAMIN DETERMINATION BY DIRECT SERUM INJECTION

L. L. GARCIA AND Z. K. SHIHABI
Department of Pathology
Bowman Gray School of Medicine
Wake Forest University
Winston-Salem, North Carolina 27157-1072

ABSTRACT

Suramin is an anti-prostatic tumor drug. Due to its narrow therapeutic range and long half life, frequent monitoring is required to minimize toxicity. We describe here a rapid assay to measure suramin levels in serum. Diluted serum samples are injected directly onto an HPLC cartridge column packed with a wide pore packing material (300A) and eluted with 10 mmol/L phosphate buffer, pH 7.2 containing 15% acetonitrile with detection at 325 nm or 254 nm. The analysis is rapid (< 3 min), while the recovery is about 98% with no interference from common drugs.

INTRODUCTION

Suramin has been used for many years for the treatment of onchocerciasis and trypanasomiasis (1). Recently, reports of significant anti-prostatic tumor activity of suramin have renewed interest in the drug

(2) which has a narrow therapeutic window of about 200-300 mg/L. Below 200 mg/L, the drug is not effective, and above 300 mg/L the incidence of neurotoxicity increases dramatically (3). In addition to that, the drug has a long half-life of 44-54 days (4). Thus, continuous monitoring of the drug is necessary, supporting the need for a rapid, reproducible and inexpensive assay.

An HPLC method which involves solvent extractions of each sample and reversed-phase with ion-pairing has been described (5). Recently, Tong et. al. (3) reported a suramin assay, where plasma samples were injected directly onto a CN column and eluted isocratically with acetonitrile and an ion pairing reagent. However, the column did not last more than 50 injections.

Previously, we have shown that when injecting serum directly on a column, it is better to keep the acetonitrile concentration to a minimum to avoid protein precipitation (6). In addition to that, the use of protein wide-pore columns is more suited for direct serum injection (6). Under such conditions the column can be used for about 200 injections (7). Here we demonstrate that suramin can be determined using HPLC by direct serum injection on a wide-pore

column based on finding the optimum conditions for its elution.

MATERIALS AND METHODS

Reagents: Suramin was obtained from FBA Pharmaceuticals (West Haven, Connecticut).

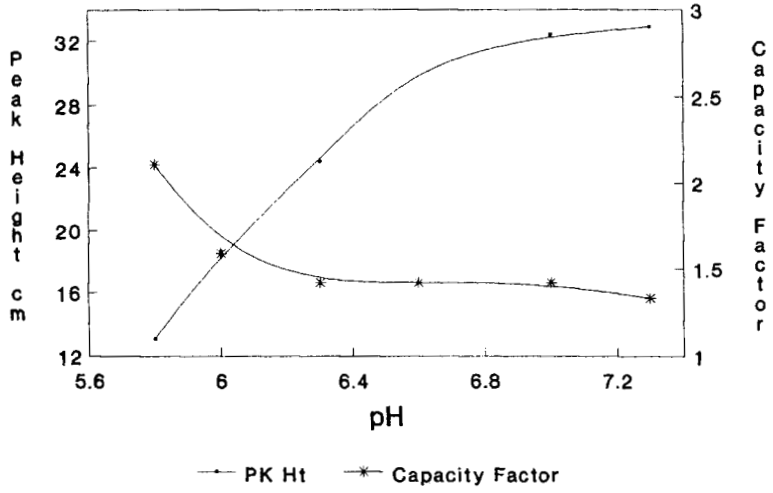
Equipment: A model 110A pump (Beckman Instruments, Fullerton, CA) was used to deliver the solvent at a flow rate of 1.2 mL/min through a 7µm, RP-300 , average pore size 300A, Aquapore cartridge column 100 X 4.6 mm (I.D.), (Applied Biosytems, San Jose, CA). The effluent was monitored at 325 nm, at 0.005 AUS with a variable wavelength detector (LKB Instruments, Bromma, Sweden).

Procedure: Serum, standards or controls (10 µL) were diluted with 1.0 mL of pump solvent and vortex-mixed for 5 sec before injection. An aliquot of 20 µL was injected on the column and eluted with 10 mmol/L phosphate buffer pH 7.2 containing 15% acetonitrile.

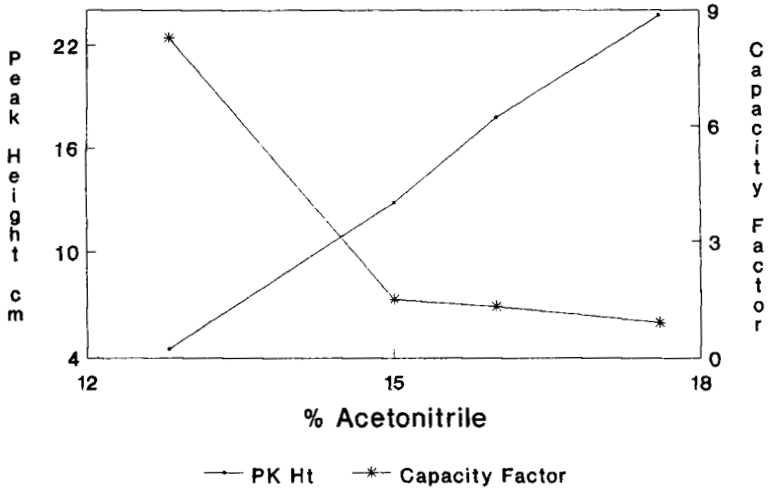
RESULTS AND DISCUSSION

A high concentration of acetonitrile in the mobile phase causes serum protein precipitation and consequently an increase in column pressure and loss

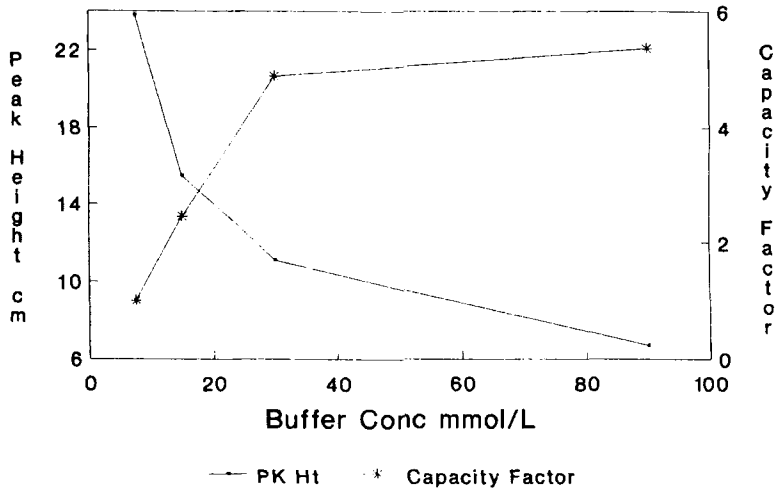
of resolution . The use of a minimum concentration of acetonitrile together with a wide pore column (protein column) decreases protein precipitation. Thus, the column can be used for a longer period of time. For this reason, we investigated the optimum conditions to keep the acetonitrile concentration to the minimum and at the same time keeping the capacity factor (K′) low to achieve rapid separation. The effect of the buffer pH on peak height (PKHT) and (k′) is illustrated in Fig. 1. PKHT increased with increasing pH up to about pH 6.6. The k′ did not change between pH 7.3 to 6.3 but increased at lower pH. We chose a pH of 7.2 since at this pH the K′ is low i.e. the separation is fast without the need to increase the acetonitrile concentration. As the percentage of acetonitrile in the mobile phase increased, the PKHT increased linearly, while the k′ decreased , Fig. 2. A concentration of 15% acetonitrile was chosen because it was the minimum concentration of acetonitrile that gave an acceptable k′ with a good peak height. As the ionic concentration of the mobile phase is increased from 7.5 to 90 mmol/L the PKHT decreased while the k′ increased as shown in Fig. 3. We decided to use a 10 mmol/L buffer for routine use since at this concentration the K′ is small.



1. Effect of buffer pH on peak height and capacity factor (K′) of suramin.



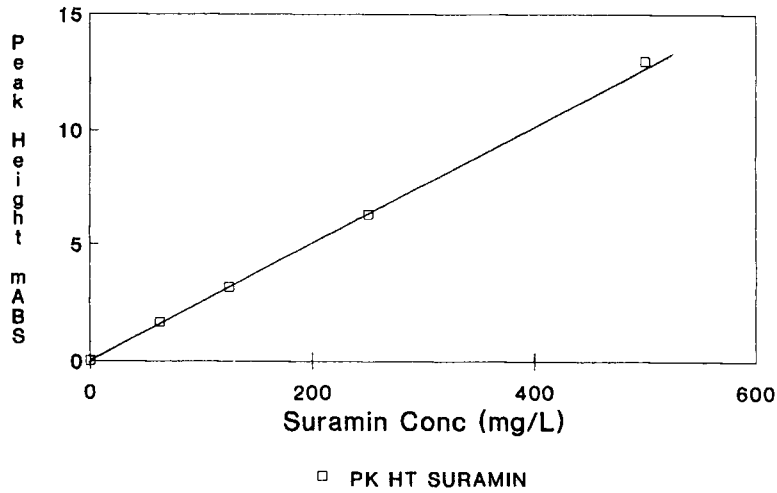
2. Effect of the percentage of acetonitrile used in the mobile phase on peak height and capacity factor (k′) of suramin.



3. Effect of the buffer concentration on peak height and capacity factor (K') of suramin.

It is evident from the previous figures that the optimal conditions for using a minimum amount of acetonitrile fall on the slope of a line. For this reason, it is important to prepare the mobile phase precisely, while keeping in mind that slight adjustments may be needed after prolonged use of the column.

Serum recovery of added suramin (200 mg/L) compared to aqueuos standards is 98.0 % (n=3). The standard serum curve in the range of 50 to 500 mg/L of suramin, Fig. 4 shows linearity throughout this range. The coefficient of variation is 4.6% at a



4. Linearity of the suramin assay.

mean of 125 mg/L (n=15). The minimum detectable level (3X baseline noise) is 13 mg/L . No interference was observed from the common drugs listed in Table 1.

We explored the possibility of detection at a wavelength of 254 nm, which is a more common wavelength for fixed wavelength detectors. At this wavelength, the detection is about four times more sensitive compared to that at 325 nm (i.e. samples can be diluted 400 fold, and thus the column can be used for a longer period of time); however, procainamide and theophylline eluted close to suramin at this wavelength. Since the suramin therapeutic

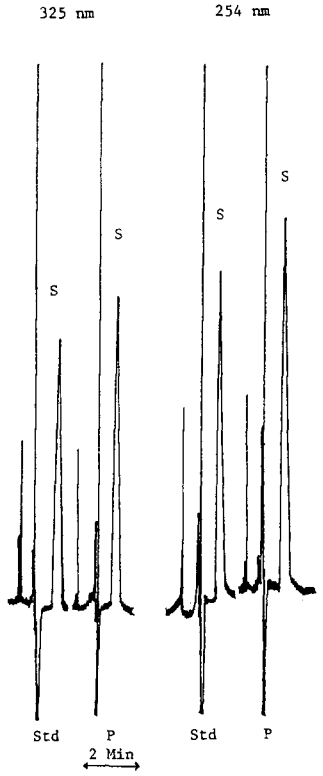
TABLE 1

List of The Drugs Which Did Not Interfere With Suramin Analysis.

Drug	mg/L
Theophylline	40
Phenytoin	30
Phenobarbital	50
Carbamazepine	13
Lidocaine	6
Procainamide	12
N-acetylprocainamide	10
Quinidine	5
Salicylic acid	100

range is about 15 times higher than that for procainamide or theophylline, the interference from these two drugs is negligible. Figure 5 demonstrates the chromatograph of a patient receiving suramin. The chromatographs are clean, and the retention time for suramin is about 2.5 min.

We have previously pointed out that sample extraction is the biggest problem preventing the widespread of HPLC in the clinical laboratories (6). In this method, sample extraction is avoided and serum is injected directly onto the column after dilution with the mobile phase. Our experience indicated that the protein column for samples diluted



5. Chromatographs of a standard (200 mg/L, Std) and a patient (P) receiving suramin (S) at 325 and 254 nm. (Samples were diluted 101 fold for the 325 nm and 401 fold for the 254 nm detection).

100 fold can be used for over 200 injections (7). When the pressure increases, the cartridge is reversed (6). The savings on labor, ease of operation and speed in this method greatly out-weigh the cost of the cartridge column.

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REFERENCES

1. Hawking F. Suramin: With Special Reference to Onchocerciasis. Adv. Pharmacol. Chemother. 15:289-322, 1978.

2. Myers C.E., Stein C., LaRocca R., Cooper M., Cassidy J. and McAtee N. Suramin: An Antagonist of Heparin-binding Tumor Growth Factors with Activity Against a Broad Spectrum of Human Tumors. Proc. Amer. Soc. Clin. Onc. 8(256):66, 1989.

3. Tong W.P., Scher H.I., Petrylak D.P., Dnistrian A., Curley T., and Vasquez J. A Rapid Assay of Suramin in Plasma. J. Liq. Chromatog. 13:2269-2284, 1990.

4. Collins J.M., Klecker R.W., Yarchoan R., Lane H.C., Fauci A.S., Redfield R.R., Broder S., and Myers C.E. Clinical Pharmacokinetics of Suramin in Patients with HTLV-III/LAV Infection. J. Clin. Pharmacol. 26:22-26, 1989.

5. Klecker R.W., Jr., and Collins J.M. Quantification of Suramin by Reverse-phase Ion-pairing High-performance Liquid Chromatography. J. Liq. Chromatog. 8:1685-1696, 1985.

6. Shihabi Z.K. Review of Drug Analysis with Direct Serum Injection on the HPLC Column. J. Liq. Chromatog. 11:1579-1593, 1988.

7. Shihabi, Z.K., and Dyer R.D. Serum Injection on the Column for Carbamazepine Assay. J. Liq. Chromatog. 10:2383-2391, 1987.

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