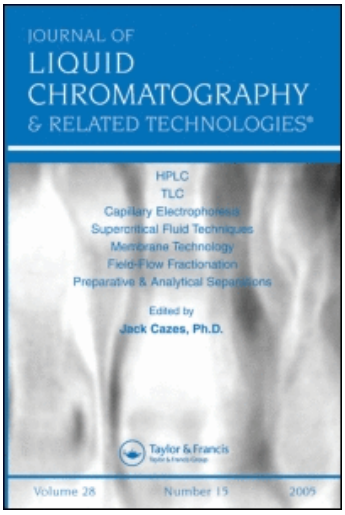

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W. P. Tong[a]; H. I. Scher[b]; D. P. Petrylak[b]; A. Dnistrian[c]; T. Curley[b]; Justin Vasquez[a]
[a] Program of Developmental Therapy and Clinical Investigation, [b] Division of Solid Tumor Oncology Department of Medicine, Genitourinary Oncology Service, [c] Department of Clinical Chemistry, Memorial Sloan-Kettering Cancer Center York Avenue New York, New York 10021 Department of Medicine Cornell University Medical College, New York, New York

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A RAPID ASSAY OF SURAMIN IN PLASMA

W. P. TONG[1], H. I. SCHER, [2]
D. P. PETRYLAK[2], A. DNISTRIAN[3],
T. CURLEY[2], AND JUSTIN VASQUEZ[1]
[1]*Program of Developmental Therapy and Clinical Investigation*
[2]*Genitourinary Oncology Service, Division of Solid Tumor Oncology*
Department of Medicine
[3]*Department of Clinical Chemistry*
Memorial Sloan-Kettering Cancer Center
1275 York Avenue
New York, New York 10021
Department of Medicine
Cornell University Medical College
New York, New York

ABSTRACT

The polysulfonated napthylurea suramin is currently undergoing extensive clinical trials. A narrow therapeutic window requires frequent dose adjustment to minimize toxicity. A simple, rapid and reproducible assay was developed for measuring suramin levels in plasma. Plasma samples are injected directly onto the column and eluted isocratically with acetonitrile and an ion pairing reagent. Retention time is approximately two minutes, recovery greater than 95% throughout the range of 50-300 ug/ml, with sensitivities to concentrations of 5 ug. A small sample size (0.5 ul) gave similar results allowing assays of finger-stick samples. Equal accuracy was

obtained using trypan blue as an internal standard. Careful monitoring, mandatory for safe administration of the agent, can be accomplished rapidly and accurately.

INTRODUCTION

Suramin is a polysulfonated napthylurea that was was used extensively to treat onchocerciasis and trypanasomiasis for many years (1). Following the demonstration that suramin inhibits reverse transcriptase (2), a clinical trial in patients with AIDS was performed (3). Subsequently, the agent was shown to inhibit binding Of a variety of polypeptide growth factors to their receptors including basic fibroblast growth factor (bFGF), transforming growth factor-beta (TGF-b), platelet derived growth factor (PDGF) and epidermal growth factor (EGF) (4-7). A phase I trial in patients with cancer showed antitumor activity against adrenal and renal cancer, and adult T-cell lymphoma (8). Intense interest was generated by the report of significant antitumor activity in patients with hormone resistant prostate cancer, a disease traditionally refractory to chemotherapy (9). Toxicities from the clinical trials included myelosuppression, anticoagulation and a peripheral neuropathy that can progress to a Guillain-Barre Syndrome (9,10).

Of greater import is the narrow therapeutic window of the drug both in-vitro and in-vivo and the variable kinetics in individual patients. Growth factor inhibition is clearly dose

dependent in a variety of cell lines (4-6). In a phase I/II clinical trial no responses were seen below serum suramin levels of 200 ug/ml, while the incidence of neurotoxicity increased dramatically above 300 ug/ml. Further, using a dose rate of 350 mg/m^2/day by continuous infusion, serum levels at 7 days ranged from 50-350 ug/ml (10). Published data on the pharmacokinetics of suramin in patients with HIV infection showed extensive protein binding, a terminal half-life of 44-54 days, with serum concentrations greater than 100 ug/ml for several weeks after discontinuation of therapy (11). The observed toxicities at serum concentrations above 300 ug/ml, the variable kinetics of loading in individual patients ant the long terminal half-life make frequent serum concentration monitoring a requisite component of all clinical trials with this agent.

Assays for suramin have been available for many years (12,13). The most widely used is a high pressure liquid chromatography (HPLC) method published by Klecker and Collins (14). The method involves three extractions of each sample with caution recommended to generate appropriate standard curves when levels above 100 and 200 ug/ml are expected. As our ongoing and future clinical trials with suramin require frequent dose adjustments, a rapid, reproducible and inexpensive assay is of major importance. Previous studies of methotrexate levels in rabbit plasma showed that measurements were greatly facilitated with equivalent accuracy by injection of the plasma sample

directly into the HPLC (15). The success with this approach prompted similar investigations using suramin.

MATERIALS AND METHODS

Reagents: Suramin was obtained from Dr. Klecker at the Developmental Therapy Branch of the National Cancer Institute. HPLC grade acetonitrile (Baker) was purchased from VWR, (Piscataway, New Jersey), HPLC grade ammonium acetate from Fisher Scientific (Springfield, New Jersey), trypan blue and and tetrabutyl ammonium dihydrogen phosphate (TBAP) 1M solution from Aldrich Chemical (Milwaukee, Wisconsin). Control plasma was obtained from specimens from volunteer blood donors at MSKCC.

Chromatography System: The modular chromatograph consists of a Rheodyne 7520 injection value, SP8810 isocratic pump, SP8450 Variable wavelength UV/VIS detector and WINner chromatographic station from Spectra-Physics (San Jose, California). The column was a Reliance Cyano cartridge column 4 x 80 by DuPont (Wilmington, Delaware). The mobile phase consisted of 35% acetonitrile and 25 mM ammonium acetate at ph 5.5 with 10 mM TBAP at a flow rate of 1 ml/min. The eluent is monitored at 320 nm at 0.01 AUFS. Suramin standards in water and plasma were centrifuged in an Eppendorf microfuge model 5412 for 5 minutes before injection. No additional sample preparation was performed. Five microliters of plasma was used per injection to ensure a complete flush and fill the injection

loop of 0.5 ul. Subsequently, plasma samples obtained by venipuncture and finger-stick were compared. For the internal standard method, 100 ul of 0.5% trypan blue aqueous solution was added to 900 ul of plasma and analyzed as described above.

RESULTS

After screening various types of columns and conditions, the Reliance cyano column 4x80 mm was used. This established appropriate isocratic conditions with satisfactory peak shapes for suramin analysis.

Standard curves for plasma and water in the concentration range of 50 to 300 ug/ml are illustrated in Figures 1A and 1B. The assay was linear throughout this range both in water ($y = 1.144x - 1.5$, $R^2 = 0.9998$) and in plasma ($y = 1.126x - 1.7$, $R^2 = 0.9996$). Plasma recovery is greater than 95% with a standard deviation of less than 2%. No interference from control plasma was observed. The retention time of suramin was 2 minutes. The limit of sensitivity was 0.5 ul of 5 ug/ml (0.25 ng, signal to noise ratio > 3 at 0.02 AUFS). As the therapeutic range is from 250-300 ug/ml, sensitivities were not pursued further.

Table 1 shows the results as peak area from aqueous standards and spiked plasma standards from 50 ug/ml to 300 ug/ml assayed over three Consecutive days. Recovery exceeded 96% in all cases, and was greater than 97% in the range of 200 to 300

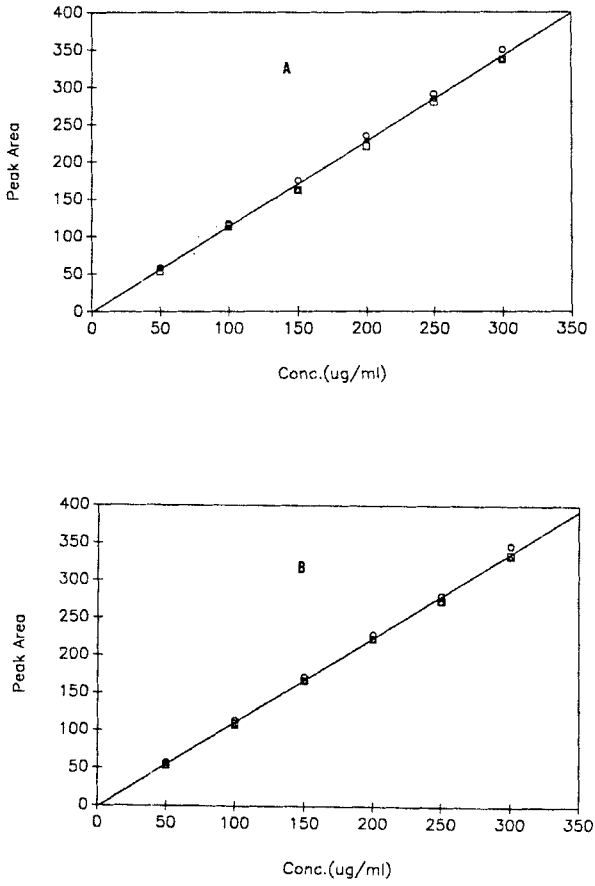

Figure 1: **Standard curve of suramin. A) Water. B) Spiked plasma. Symbols represent the same samples run on 3 separate days.**

Table 1: Peak areas of suramin in water and spiked plasma, and percent recovery over three different days: mean (standard deviation).

Concentration (ug/ml)	Peak Area in Water	Peak Area Spiked Plasma	% Recovery
50	56.3 (2.4)	54.6 (1.4)	97
100	114 (2.1)	110 (2.9)	96
150	167 (5.9)	169 (2.2)	101
200	228 (5.7)	225 (2.6)	99
250	285 (4.5)	276 (3.3)	97
300	342 (5.9)	338 (6.2)	99

ug/ml. The Rheodyne 7520 injection valve has high precision for submicroliter samples which allows use of the external standard method.

Table 2 shows the peak of area of the suramin standards and trypan blue using the internal standard method. With a slight decrease in the amount of acetonitrile, the retention times for trypan blue and suramin were 1.5 and 2.8 minutes respectively.

Table 2: Peak areas of suramin standards and trypan blue using the internal standard technique.

Concentration (ug/ml)	Suramin Peak Area	Trypan Blue Peak Area
50	46	20.6
100	88	20.2
150	129	19.9
200	176	18.9
250	224	18.4
300	277	18.5

The standard curve with the internal standard is $y = 0.513x-0.71$ with an R^2 value of 0.996.

As plasma contains many materials that can limit the efficacy of a column, we next investigated the capacity of the column to handle multiple samples without deterioration. Figure 1 shows the results for control, samples 1, 30 and 60 applied to the same column. The suramin peak was observed to broaden after injection number 50. By injection 60, illustrated, the peak was quite broad. However, the integrated peak area was essentially

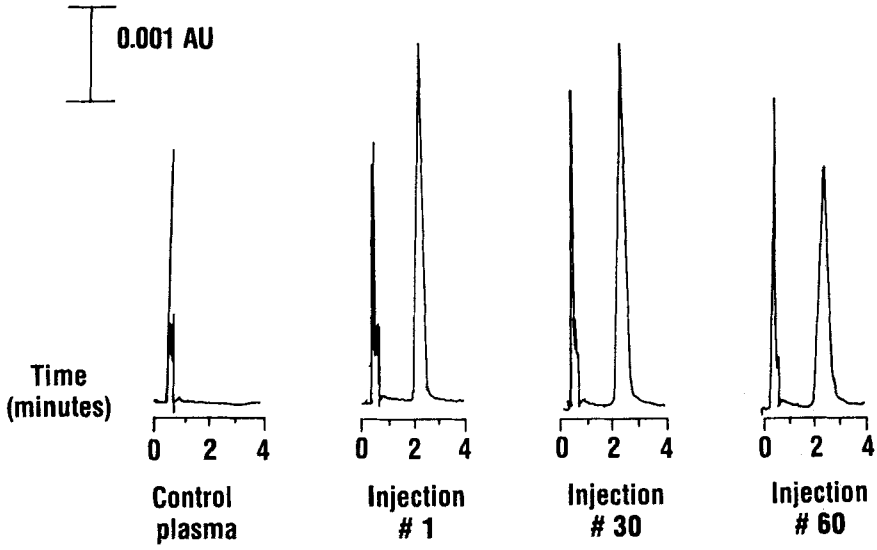

Figure 2. **Control plasma and multiple injections of spiked plasma. Calculated peak areas were similar (see text).**

unchanged from that observed for the first sample. As peak height and peak shapes may vary, peak areas were used for calculating plasma concentrations. Attempts to regenerate the column were not successful, but, as the column is inexpensive, no further attempts to clean or guard the column were made.

Figure 3 shows a representative comparison of a venipuncture (3A) and finger stick (3B) sample. The actual sample size analyzed was 0.5 ul, making it possible to perform the assay with only 10 ul (for duplicate determinations) of plasma. As

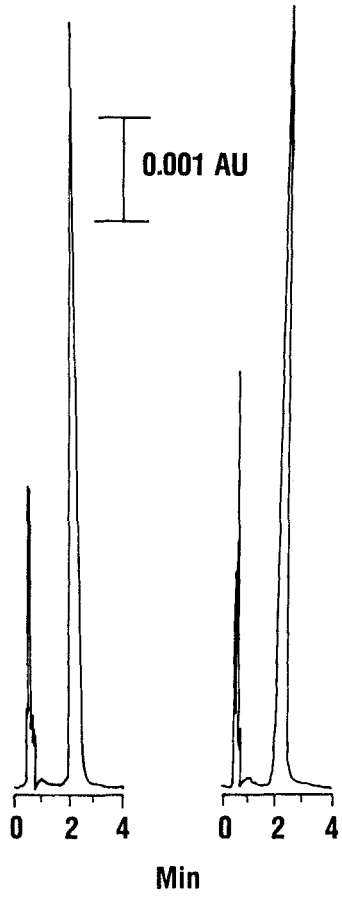

Figure 3. **Comparison of venipuncture (left) and finger stick (right) specimens. As noted in text, peak areas were virtually identical.**

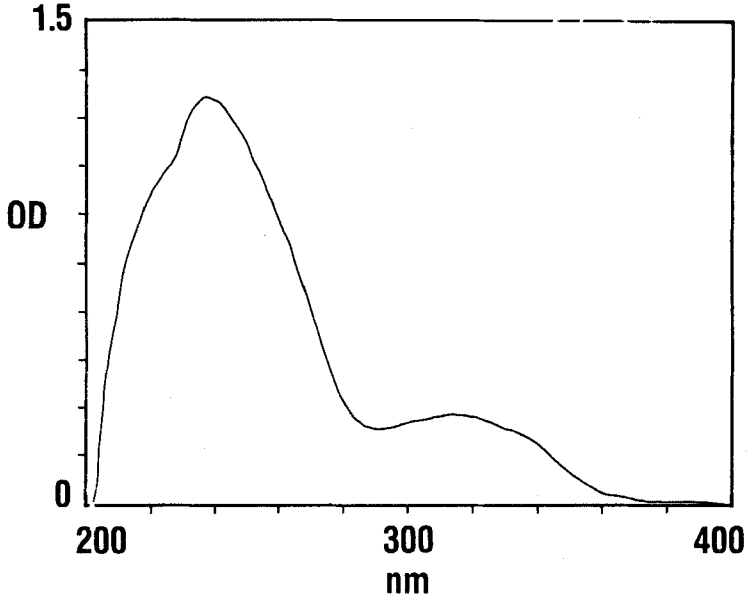

Figure 4. UV spectrum of suramin in mobile phase.

shown in the Figure 4, comparable results were obtained using the finger-stick sample.

DISCUSSION

Suramin is a novel anticancer agent with a unique putative mechanism of action. As serum levels vary widely among individuals, and the therapeutic window is narrow, frequent dose adjustments are necessary. The present assay allows rapid and reproducible determinations on small volume samples. The assay technique bypasses the need for multiple extractions and

gradient elution, reducing variation and the time required for assay.

The system was derived from previous work using methotrexate which showed little plasma interference when monitoring at 315 nM. Suramin has UV absorption (Figure 4) in this range allowing determinations at this wavelength. The amount of acetonitrile was important. In practice, small increments of 1% ACN were added until the desired retention time was reached. The results obtained were reproducible on the same samples on three consecutive days using independent runs. The Rheodyne 7520 syringe-loading micro injector is designed to inject submicroliter samples with high precision and low dispersion (16). With this injection valve and a filled loop injection procedure, an external method of assay was possible. A guard column was not used due to cost and time considerations. As an alternative, a solid phase extraction (SPE) procedure can be used to extend the life of the column. However, the cost of 50 extractions exceeds the cost of a new analytical column. Although some broadening of the suramin peak was observed (vide supra), peak areas were unchanged, allowing stable assays for at least 50 determinations. The applicability of the technique is further enhanced by its adaptability to small sample volumes. Indeed, assays from finger stick and venipuncture samples gave similar results.

The filled loop method on which the assay is based, allows an external standard to be used with high precision. Alternatively, trypan blue can be incorporated as an internal standard. This method allows monitoring if the injection is performed smoothly, particularly when the autosampler is used. It does, however, require larger sample sizes with good pipetting technique.

Suramin is currently under active investigation in a variety of tumor types. Its actions are complex, and may not be limited to growth factor inhibition. The drug also inhibits adrenal steroidogensis (17), glycolysis (18), and iduronate synthetase leading to an accumulation of glycosaminoglycans producing a Hurler's syndrome (19). Of greater import is the observation that suramin is not cytotoxic in many in-vitro systems (4,6) suggesting the need for maintenance of serum levels in the therapeutic range. This is quite narrow, as response correlates with levels above 200 ug/ml (p=.034) and neurotoxicity increased from 14% above 300, 50% above 350, to 100% above 400 ug/ml. The time required to achieve therapeutic levels varied with extent of disease. Patients with more extensive disease, required longer time periods for loading (10). While these were attributed to changes in volumes of distribution, the exact etiology was unclear. The interaction of suramin with other drugs is largely unknown. Further, if the action of suramin proves to be tumoristatic as opposed to tumoricidal, maintenance

of therapeutic levels for longer durations may be required. In this setting, close serum suramin level monitoring will be even more critical. The assay system described will greatly facilitate safe administration of this novel compound.

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