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Short Communication

Rapid high-performance liquid chromatographic determination of suramin in plasma of patients with acquired immune deficiency syndrome (AIDS) or AIDS-related complex (ARC)

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Introduction

Suramin, a drug used for the treatment of human trypanosomiasis and onchocerciasis [1], inhibits *in vitro* potency and blocks the cytopathic effect of human T-cell lymphotropic virus type III (HTLV-III) and lymphadenopathy associated virus (LAV), the etiologic agent of AIDS [2]. Studies on a limited number of AIDS or ARC-patients suggest that suramin also is capable of suppressing *in vivo* virus replication [3, 4]. In view of the potential toxicity of suramin there is a need to dose patients individually and to monitor the resulting plasma levels of suramin.

The methods available for analysis of suramin in plasma were until recently based on acid hydrolysis of suramin followed by coupling of the free amino groups with a chromophore to produce a compound which could be measured spectrophotometrically [5–7]. Apart from being time-consuming, these methods lack the specificity and selectivity of modern analytical techniques. Recently a procedure based on HPLC was published [8]. However, this procedure has several disadvantages:

(i) the use of a gradient elution system which involves prolongation of the analysis time;
(ii) the presence of the AIDS virus in analysed samples which is a possible hazard to laboratory personnel;
(iii) the use of an internal standard, trypan blue, which may contain interfering impurities that cause problems.

A similar HPLC method, with a rather time-consuming extraction procedure, was published shortly afterwards [9].

The present paper reports a rapid and sensitive method for the determination of suramin in plasma of AIDS patients by HPLC, after decontamination of the samples by incubation at 56°C for 30 min as described by Spire *et al.* [10].

Experimental

Materials and reagents

Suramin sodium (Germanin®, Bayer, Leverkusen, GFR) was kept under nitrogen in the dark at 4°C. All other solvents and chemicals used were of the highest purity available.

Sample preparation

Before analysis the samples were decontaminated by incubation at 56°C for 30 min.

100 μl of water and 700 μl of a mixture of methanol–acetonitrile–0.01 M tetrabutylammonium phosphate in water (46:5:19, v/v/v) were added to 200 μl decontaminated plasma in a disposable Eppendorf® tube. The mixture was vortexed for 30 s and centrifuged at 4000 rpm for 4 min in a cooling centrifuge at 2°C. The supernatant was filtered through a Millex-HV filter (0.45 nm) (Millipore, Brussels, Belgium). A 10 μl volume of the clear filtrate was injected into the HPLC system. Samples of blank plasma which had been spiked with an aqueous solution of suramin (1 μg μl^{-1}), to give plasma concentrations ranging from 2 to 200 μg ml^{-1}, were processed similarly.

Liquid chromatography

A model SP8000 liquid chromatograph (Spectra Physics, Eindhoven, The Netherlands) equipped with a model SP8400 variable wavelength UV-detector set at 238 nm, interfaced with a recording integrator (HP3390A, Hewlett–Packard, Brussels, Belgium) was used. A 250 × 4.6 mm Spherisorb 5-ODS column (Chrompack, Merksem, Belgium) was used throughout. The mobile phase consisted of a mixture of 46% methanol, 5% acetonitrile and 49% of a 0.005 M tetrabutylammonium phosphate (Low UV Pic A, Waters Associates, MA, USA) solution in 0.05 M phosphate buffer (pH:6.5). The flow rate was 1.0 ml min^{-1} and the column was kept at 40°C. During the night the column was flushed with a mixture of methanol–water (50:50, v/v) at a flow rate of 0.5 ml min^{-1}.

Evaluation of the stability of suramin at 56°C

Plasma samples spiked with 50 μg ml^{-1} suramin, were kept for 0, 0.5, 1.0, 2.0 and 3.0 h at 56°C and then stored at −20°C until analysis. Each time point was determined in triplicate.

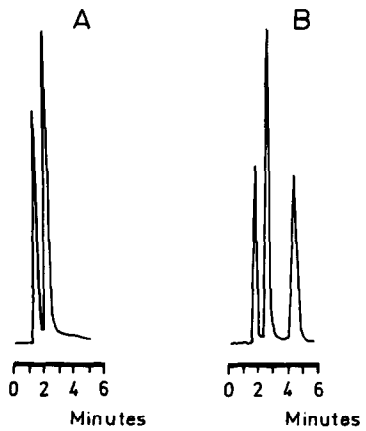

Figure 1
Chromatograms of plasma processed as described in the text. (A) Plasma of an AIDS patient before initiation of suramin therapy; (B) plasma of the same patient, after initiation of suramin therapy, containing 17.6 μg ml^{-1} of suramin (t_R = 4.20 min).

Results and Discussion

Figure 1 shows typical chromatograms obtained after extraction of plasma of an AIDS patient, before and after initiation of suramin treatment. The peak area of suramin increased linearly with concentration over the evaluated range of 2–200 μg ml^{-1}, with a mean (±SD) correlation coefficient of 0.9991 ± 0.0007 (N = 17). The detection limit, for a signal to noise ratio of 3:1, was approximately 1 μg ml^{-1}. Results illustrating accuracy and precision of the assay are shown in Table 1. As it was reported that incubation of plasma for 30 min at 56°C virtually clears the HTLV-III-LAV virus from plasma [10], the stability of suramin in plasma kept at 56°C was studied. No degradation of the product could be observed; the results expressed as percentage remaining of the concentration at time zero, were: 98% after 30 min, 104% after 1 h, 99% after 2 h and 105% after 3 h.

The presented analytical method can be used for the determination of suramin levels in AIDS patients. Figure 2 shows the levels in one AIDS patient, treated with suramin for a period of 152 days.

Table 1
Within- and between-run accuracy and precision for the determination of suramin in plasma

Concentration in plasma (μg ml^{-1})	Relative error (%)	R.S.D. (%)
Within-run (N = 5)		
10	+6.6	5.8
50	−0.2	4.4
150	+0.9	3.0
Between-run (N = 16)*		
50	+1.2	5.0

*Assayed over a period of three months.

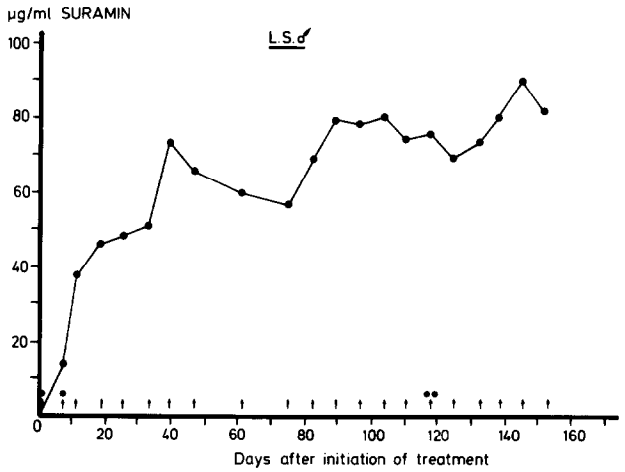

Figure 2
Concentration of suramin in the plasma of a patient undergoing therapy. The treatment was initiated with two consecutive intravenous infusions of 850 mg on days 0 and 7 (*), followed by weekly intravenous infusions of 500 mg, exceptionally, 250 mg (**), of suramin. The concentrations shown are the levels in plasma before the start of the infusion.

The method is simple, rapid and sensitive enough for the determination of suramin levels in patients treated with the usual dosage regime of suramin. The whole analytical procedure takes no longer than 10 min per sample. Moreover the method can be used in a safe manner, as the plasma samples can be decontaminated without affecting suramin levels.

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