ELSEVIER

International Journal of Pharmaceutics 155 (1997) 27–34

international journal of pharmaceutics

Degradation of suramin in aqueous solutions

J.J. Kettenes-van den Bosch *, E. Hop, W. Overbeek, W.J.M. Underberg, J.H. Beijnen, A. Bult

Department of Pharmaceutical Analysis, Faculty of Pharmacy, Universiteit Utrecht, P.O. Box 80082, 3508 TB Utrecht, The Netherlands

Received 10 March 1997; received in revised form 8 May 1997; accepted 9 May 1997

Abstract

The stability of suramin, a symmetrical urea derivative with a broad pharmacological profile, was investigated in aqueous solution in the pH/H_0 range -1–13, with a validated stability-indicating high-performance liquid chromatographic assay. Between pH 3 and 9, degradation of suramin is spontaneous water-catalyzed and the main degradation product results from loss of the urea carbonyl. The structure of this degradation product was confirmed by mass spectrometry and proton magnetic resonance. Below pH 3 and above pH 9, degradation takes place by hydrolysis of the various amide bonds. A number of degradation products were identified by their liquid chromatographic retention times and ultraviolet spectra. The half-life of suramin at 25°C and pH 7, as calculated from the Arrhenius plot, is 5 years; t_{90} is 0.8 years under the same conditions. © 1997 Elsevier Science B.V.

Keywords: Suramin; Stability; Degradation products

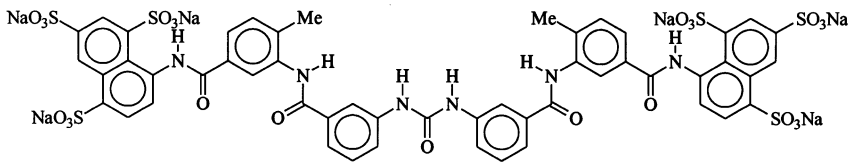
1. Introduction

Suramin (Fig. 1A) is a symmetrical urea derivative, with a broad pharmacological profile. Introduced in 1920 as a drug in the treatment of the parasitic disease trypanosomiasis, it was also shown to be effective against onchocerciasis (Hawking, 1978). In addition, suramin shows an inhibitive effect, in vitro, on reverse transcriptase, the DNA polymerase of retroviruses, and it blocks receptors for tumor growth factors (Mitsuya et al., 1984; Stein et al., 1989). This led to clinical trials with suramin in AIDS patients and in patients suffering from advanced prostate cancer (Beijnen et al., 1990a).

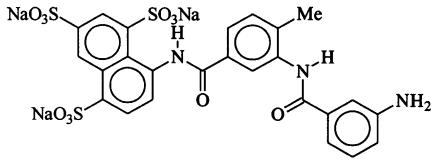
Although quantitative stability data on suramin in commonly used infusion fluids are available (Beijnen et al., 1990b), no systematic stability study has been reported to date.

* Corresponding author. Tel.: + 31 30 2536978; fax: + 31 30 2515114; e-mail: J.J.KETTENES@far.ruu.nl

PII S0378-5173(97)00166-X



A



B

Fig. 1. Structure of suramin (A) and its major degradation product (B).

In the present paper, the results are presented of a systematic stability study of suramin in aqueous solution, the objective being to optimize the pharmaceutical formulation of the drug.

2. Materials and methods

2.1. Materials

Suramin hexasodium salt (Germanin®, purity > 99% by HPLC) was obtained from Bayer Nederland BV, Mijdrecht, The Netherlands. 3-Amino-4-methylbenzoic acid and 1-aminonaphthalene-4,6,8-sulfonic acid (purity > 99% by HPLC) were from Bayer AG, Leverkusen, Germany. 3-Aminobenzoic acid (purity > 99%) was from Acros, Geel, Belgium. All other chemicals and the solvents were analytical grade and deionized water was used throughout.

2.2. Instrumentation

2.2.1. High performance liquid chromatography (HPLC)

The HPLC system consisted of a U6K injector, a Model 6000A pump, an automated Gradient Controller Model 680, and a UV Model 441 (all Waters Associates, Milford, MA, USA) or a photodiode array (PDA) model 1040A (Hewlett-Packard, Palo Alto, CA) detector; a Model SP4270 integrator (Spectra-Physics, Santa Clara, CA) was used for quantitation of the peak areas. The column used was a 50942 LiChroCart 125 × 4 mm Lichrospher 100 RP-8 (Merck, Darmstadt, Germany), with a particle size of 5 μm.

2.2.2. Proton magnetic resonance (^1H NMR)

^1H NMR spectra (1D-^1H and 2D-$^1H^1H$ (COSY) spectra) of suramin and its major degradation product were recorded in D_2O (99.8%, Merck) with a Gemini 300 MHz spectrometer

(Varian Associates, NMR Instruments, Palo Alto, CA, USA), at 23°C, and with HDO at 4.8 ppm as the reference line. The concentration was approximately 5 mg in 0.8 ml D_2O.

2.2.3. Mass spectrometry (MS)

Negative ion spectra of the major degradation product were obtained with a JMS-SX/SX102A tandem mass spectrometer (BEBE; JEOL, Tokyo, Japan) operating at an accelerating voltage of 10 kV. A xenon beam with an energy of about 6 keV was applied for obtaining fast atom bombardment (FAB) mass spectra. *m*-Nitrobenzyl alcohol was used as the matrix. In addition, a negative ion electrospray (ES) mass spectrum was obtained with a Fisons VG Platform Benchtop LC-MS (Micromass, Altricham, UK). The nebulizing gas had a flow of 25 l/h, and the drying gas 300 l/h. The cone voltage was 48 kV.

Degradation experiments at elevated temperatures were carried out with a Thermoplus 1450/Thermomix 1440 water bath (S. Braun, Melsungen AG, Germany).

2.3. Methods

2.3.1. Mobile phases

For isocratic elution, a solvent system consisting of methanol:acetonitrile:0.05 M phosphate buffer pH 6.5, 390:50:560 (w/w/w), was used, the phosphate buffer containing 0.005 M tetrabutylammonium bromide (TBABr) (Beijnen et al., 1990a). For gradient elution, solution A consisted of methanol:acetonitrile:buffer pH 6.5 (0.01 M triethylamine, the pH adjusted to 6.5 with trifluoroacetic acid, 0.01 M ammonium acetate) 10:2:88 (w/w/w), and solution B 46:5:49 (w/w/w) of the same components. The following linear gradient was used: 2 min isocratic elution with A, A to B in 6 min, 4 min isocratic elution with B, and back to A in 1 min.

2.3.2. Buffer solutions

For pH 1 and 2, perchloric acid solutions were used (0.1 and 0.01 M respectively); for pH 3, 4, and 5 0.01 M acetate buffers; for pH 6 and 7 0.01 M phosphate buffers; for pH 8 through 11 0.01 M borate buffers, and for pH 12.5 and 13 sodium hydroxide solutions. The ionic strength (μ) of all solutions was brought to 0.3 with sodium chloride, except for the solutions where $[H^+]$ or $[OH^-]$ exceeded 0.3 M.

2.3.3. Degradation experiments

The buffer solutions were spiked with suramin to obtain an initial suramin concentration of 0.001 M. The solutions were divided over a number of vials (one for each point of the degradation curves) and the vials placed in a water bath at 80.0 ± 0.2°C. Vials were removed from the water bath at selected time intervals, immediately cooled in ice to quench the reaction, and kept in the freezer until HPLC analysis. Prior to HPLC analysis the solutions were diluted 1:10 with phosphate buffer pH 7.

The pH of the buffer solutions (pH 2–13) was measured at room temperature and at the temperature of the experiment with a Consort P514 pH meter (Salm and Kipp BV, Breukelen, The Netherlands) and a Slim electrode (Hamilton, Dundee, UK). The pH of the solutions was measured before and after degradation, and did not change during the experiments. pH/H_0 values 0, -1 and -2 were calculated according to Hammett and Deyrup. Degradation was monitored over at least three half-lives. Experiments were carried out in duplicate.

2.3.4. Isolation of the major degradation product

Fifteen milligrams of suramin were dissolved in 3 ml MilliQ water and kept at 100°C for 6 h. The solution was cooled to room temperature and lyophilized. The residue was dissolved in acetonitrile:water 10:3 and chromatographed on a 180 × 20 mm Lichroprep Si-60 15–25 μM column with a mobile phase consisting of acetonitrile:water 10:3, the water containing 1% trifluoroacetic acid. Two milliliter fractions were collected. Fractions 30–33, containing the major degradation product (purity $>98\%$), were combined and the solvent removed in a stream of nitrogen. Fractions 34–40 contained the same product in a purity of over 90%; also these fractions were combined and the solvent removed.

2.3.5. Analysis

For measuring the degradation curves, the isocratic ion-pair HPLC method was used, whereas the analysis of degradation products was carried out with the gradient system and PDA detector. The detection wavelength was 254 nm.

3. Results and discussion

3.1. Validation of the method

The peak area changed linearly with the concentration over at least three decades (concentration range 10^{-4}–10^{-7} M; $r = 0.9999$). The detection limit for suramin, defined as the amount giving a signal-to-noise ratio of 3, was 4.2×10^{-8} M (6.0×10^{-10} g). There are no indications that, under the experimental conditions, degradation products coelute with suramin: the UV spectrum obtained with an on-line PDA detector did not change during elution of the suramin peak in a partly degraded sample. Only the UV spectrum of the major degradation product (Fig. 1B) is similar to that of suramin; however, this compound has a smaller capacity factor k'.

3.2. pH profile

The pH of the solutions did not change during the degradation experiments.

The degradation of suramin in dilute solutions at various pH values follows pseudo-first order kinetics, as indicated by the linearity of the ln[suramin] versus time plots. In Table 1 observed rate constants, k_{obs}, are presented for the various pH values. The log k_{obs} − pH profile of suramin over the pH range −1–12 is presented in Fig. 2. Between pH 3–9, the degradation of suramin appears to be independent of the pH and spontaneous water-catalyzed. Below pH 3 the degradation is specific acid-catalyzed, above pH 9 specific base catalysis starts to contribute. The pK_a of 1-naphthalenesulfonic acid is 0.2. Therefore, at pH < 1 protonation of the sulfonic acid groups probably influences the profile and results in a slope smaller than 1, indicating that the protonated sulfonic acid degrades with a lower velocity than the deprotonated compound.

Table 1
k_{obs} and correlation coefficients for various pH values

pH	k_{obs} (s^{-1})	r
−1.03	1.130×10^{-4}	0.996
0.99	2.02×10^{-5}	0.993
2.07 (2.00)	1.20×10^{-5}	0.998
3.06 (3.00)	9.40×10^{-6}	0.996
3.98 (4.01)	9.47×10^{-6}	1.00
5.01 (4.98)	9.13×10^{-6}	0.995
5.89 (6.02)	1.01×10^{-5}	0.999
6.72 (6.98)	9.36×10^{-6}	0.993
7.58 (8.06)	1.06×10^{-5}	1.00
8.58 (8.75)	9.98×10^{-6}	0.994
9.12 (9.97)	1.15×10^{-5}	0.998
10.20 (10.97)	1.40×10^{-5}	0.997
10.85 (12.50)	1.93×10^{-5}	0.999
11.35 (13.00)	4.21×10^{-5}	0.999

pH at the temperature of the experiment are shown without brackets; pH values shown between brackets correspond to pH at room temperature.

The effect of the ionic strength was determined at pH 6; at this pH the degradation was independent of the ionic strength ($0.1 < \mu < 0.9$). The influence of buffer components was not investigated. However, the small fluctuations in k_{obs}, over the pH range 2–9 suggest that buffer components do not significantly affect the degradation.

3.3. Arrhenius plot

The Arrhenius plot at pH 7 is presented in Fig. 3. The activation energy E_a, was calculated as 123

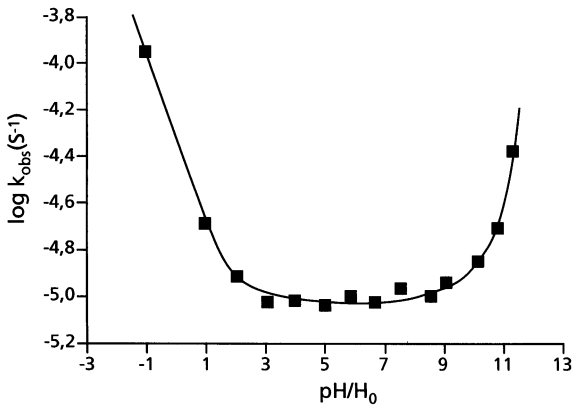


Fig. 2. pH profile of suramin, measured at 80°C.

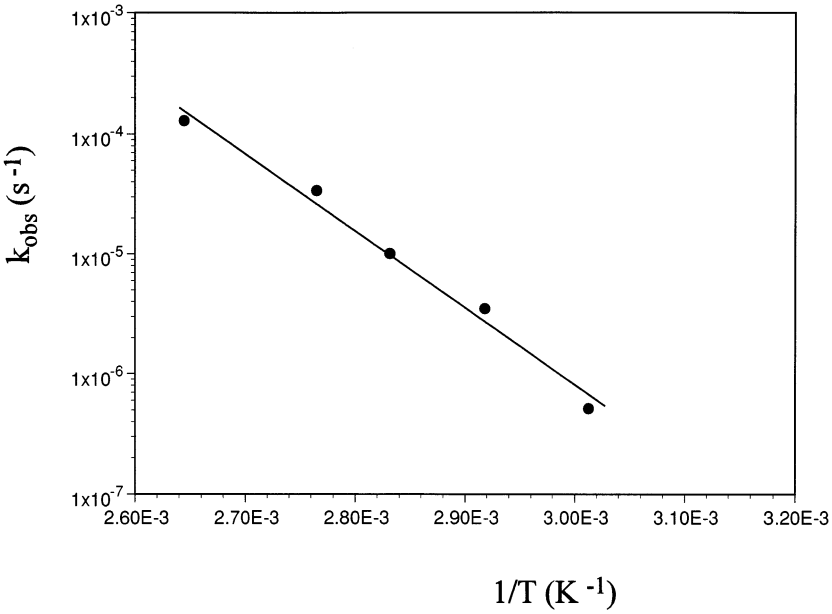


Fig. 3. Arrhenius plot of suramin at pH 7; temperature interval 332–378 K, $r = 0.994$.

kJ/mol, the frequency factor is 1.7×10^{13} s^{-1}. The Arrhenius plot was obtained by plotting ln k_{obs} against $1/T$, after determining k_{obs} at five temperatures between 332–378 K. Half-lives at these temperatures varied from 1.6–376 h. Extrapolating to 25°C results in a calculated half-life of 5 years at room temperature, and a t_{90} of 0.8 years.

3.4. Degradation products

The LC chromatograms of suramin after about 20 h at 80°C and pH 1, 7 and pH 13, and that of a mixture of the reference compounds 3-aminobenzoic acid, 3-amino-4-methylbenzoic acid, 1-aminonaphthalene-4,6,8-trisulfonic acid, and suramin are presented in Fig. 4, the UV spectra of a number of relevant peaks and reference compounds in Fig. 5. The chromatograms were obtained with gradient elution. Between pH 1–10, the chromatograms of the degradation mixtures show the presence of one major degradation product (peak 7, $k' = 7.4$) and only traces of other components. Its retention time is shorter than that of suramin (peak 10, $k' = 8.7$), indicating that the compound is slightly more polar. Its UV spectrum has absorption maxima at the same wavelengths as suramin itself and, therefore, must have the same chromophores. The product (Fig. 1B) resulting from loss of the urea carbonyl from the symmetrical suramin molecule satisfies these requirements, and explains why only one degradation product is obtained. Beijnen et al. (1990b) observed that the sum of the absorbances of suramin and its degradation product remain constant over time, from which they concluded that the two compounds have the same molar absorptivity and probably the same chromophores.

The ^{1}H and COSY spectra of suramin (A) and its degradation product (B) show the same aromatic spin systems to be present in both compounds. The naphthalene moiety shows two protons in the ortho position (A: 8.29 and 7.80 ppm, $J = 8.2$ Hz; B: 8.33 and 7.96 ppm, $J = 8.1$ Hz) and two in meta position (A: 9.43 and 8.67 ppm, $J = 1.8$ Hz; B: 9.42 and 8.71 ppm, $J = 1.7$ Hz). The 1,2,4-substituted benzene shows a singlet (broad through long range coupling; A: 7.93 ppm; B: 7.90 ppm) and two protons in ortho position (A: 7.21 and 7.73 ppm, $J = 8.1$ Hz; B: 7.42 and 7.87 ppm, $J = 7$ Hz). The 1,3-substituted benzene ring shows a singlet (broad through long range coupling; A: 7.87 ppm; B: 7.70 ppm), a triplet

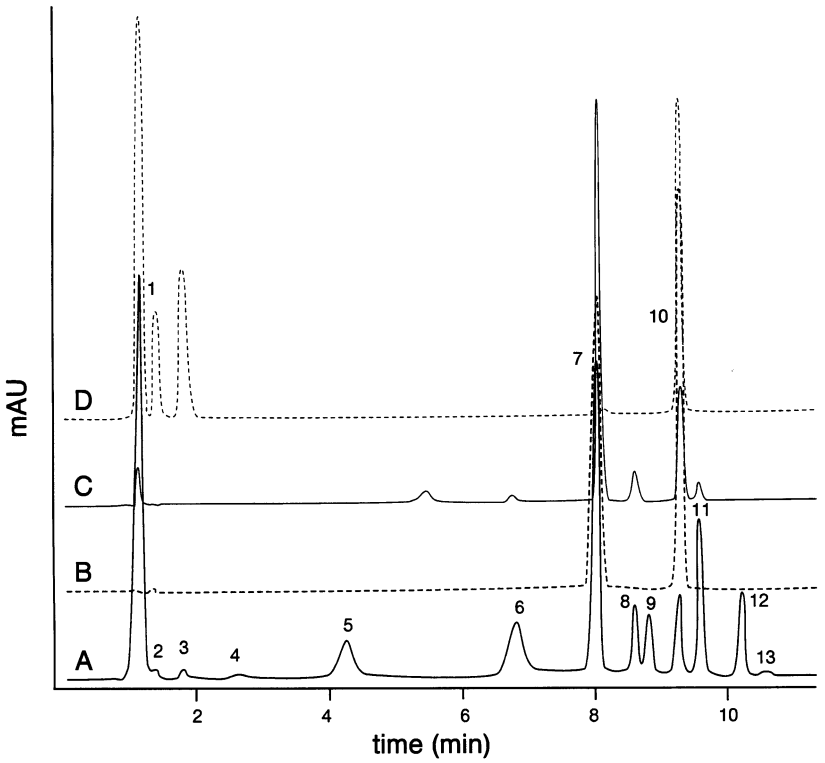


Fig. 4. Chromatograms of partly degraded suramin solutions at various pH values and degradation stages, obtained with gradient elution; A: pH 13, $3 \times t_{1/2}$; B: pH 7, $t_{1/2}$; C: pH 1, $2 \times t_{1/2}$; D: reference compounds. (1) 1-aminonaphthalene-4,6,8-trisulfonic acid; (2) 3-aminobenzoic acid; (3) 3-amino-4-methylbenzoic acid; (7) 1-[3-(3-aminobenzoyl)amino-4-methylbenzoyl]aminonaphthalene-4,6,8-trisulfonic acid (Fig. 1B); (10) suramin (Fig. 1A).

(double doublet, A; 7.29 ppm, $J = 7.7$ and 8.1 Hz; B: 7.52 ppm, $J = 8$ Hz) and two doublets (A: 7.23, $J = 8.1$ Hz, and 7.43 ppm, $J = 7.7$ Hz; B: 7.42 and 7.74 ppm, $J = 7$ Hz). The methyl singlet is at 2.10 ppm in A and at 2.23 ppm in B.

The negative ion FAB mass spectrum of B shows the $[M-Na]^-$ peak at m/z 678 consistent with the proposed structure. A minor peak at m/z 811 could originate from a matrix adduct with compound B. In the ES mass spectrum, the molecular ion peak appears at m/z 634 $[M - 3Na + 2H]^-$; smaller peaks occur at m/z 656 $[M - 2Na + H]^-$ and m/z 678 $[M - Na]^-$. Doubly charged ions occur at m/z 316.5 and m/z 327.5.

The chromatogram of the suramin degradation at pH 13 shows 13 peaks: those of the 4 reference compounds (peak 1, 2, 3 and suramin, peak 10) and 9 others. Several of these components are also present in the chromatogram of the degradation mixture obtained at pH 1, but at this pH compound 7 is the major component, even after 45 h.

From the structure of suramin, 11 degradation products can be expected, all arising from hydrolysis of the various amide bonds in the suramin molecule. In five of the possible degradation products, the aminonaphthalenetrisulfonic acid moiety is still present. This moiety will appear in the UV spectrum with absorption maxima at 260 and 320–360 nm. Maxima at these wavelengths occur in the UV spectra of peaks 1, 5, 7, 8 and 11. The remaining peaks can be visualized as originating from: (1) the condensation product of 3-aminobenzoic acid and 3-amino-4-methylbenzoic

acid; (2) suramin from which both aminonaphthalenetrisulfonic acid groups have been removed; and (3) products in which, in addition to the aminonaphthalenetrisulfonic acid groups, also one or both 3-amino-4-methylbenzoic acid groups have disappeared. The UV spectra of these products would be similar to those of mixtures of 3-aminobenzoic acid and 3-amino-4-methylbenzoic acid in various ratios. Peaks 6, 9 and 12 are likely candidates. No spectra were obtained from peaks 4 and 13, due to the low concentration in which they are present in the degradation mixture.

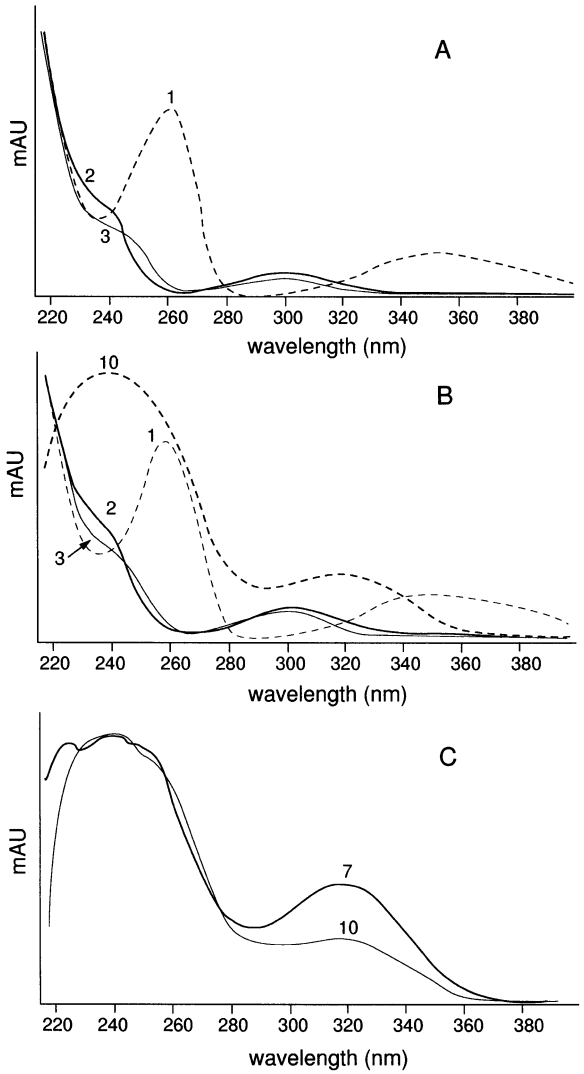


Fig. 5. UV spectra (PDA) of suramin and its degradation products; numbering is the same as in Fig. 4: (A), Spectra of peaks 1, 2, and 3 of degraded samples; (B), reference compounds 1, 2, 3, and 10; (C), peaks 7 and 10.

4. Conclusions

The degradation of suramin is spontaneous water-catalyzed over the pH range 3–9. At low pH values the degradation is not solely catalyzed by H^+; other factors, such as protonation of the sulfonic acid groups, seem to contribute to the degradation in this pH region as well. A major degradation step (and the only one at intermediate pH values) is loss of the urea carbonyl. In acidic and alkaline medium this is accompanied by hydrolysis of the various amide bonds, with or without concomitant loss of the urea carbonyl.

For clinical applications in which the pH will not be outside the range 3–10, only the major degradation product (B) is important. It will be of interest to know whether this product or one or more of the other hydrolysis products are present as metabolites in biological fluids.

The half-life at 298 K and pH 7 as calculated from the Arrhenius plot, is 5 years with a t_{90} of 0.8 years. The stability of suramin in aqueous solutions will, therefore, not cause serious problems in formulation and storage of the drug.

Acknowledgements

The authors thank Mr C. Versluis, Bijvoet Center for Biomolecular Research, Mass Spectrometry Group, University Utrecht, Faculty of Chemistry for running the FAB-MS spectra, and Dr H.-S. Möschler, Central Research, Bayer AG, Leverkusen (Germany) for generously providing samples of 3-amino-4-methylbenzoic acid and 1-aminonaphthalene-4,6,8-trisulfonic acid.

References

Beijnen, J.H., van Gijn, R., de Clippeleir, J.J.M., Vlasveld, L.Th., Horenblas, S., Underberg, W.J.M., 1990a. Rapid

determination of suramin in micro-volumes of plasma by using ion-pair high performance liquid chromatography. J. Drug Dev. 3 (1), 21–26.

Beijnen, J.H., van Gijn, R., Horenblas, S., Underberg, W.J.M., 1990b. Chemical stability of suramin in commonly used infusion fluids. DICP Ann. Pharmacother. 24, 1056–1058.

Hawking, F., 1978. Suramin: with special reference to onchocerciasis. Adv. Pharmacol. Chemother. 15, 289–322.

Mitsuya, H., Popovic, M., Yarchoan, R., Matshushita, S., Gallo, R.C., Broder, S., 1984. Suramin protection of T cells in vitro against infectivity and cytopathic effect of HTLV-III. Science 226, 172–174.

Stein, C.A., LaRocca, R.V., Thomas, R., McAtee, N., Myers, C.E., 1989. Suramin: an anticancer drug with a unique mechanism of action. J. Clin. Oncol. 17, 499.