ISOMETAMIDIUM

IDENTITY

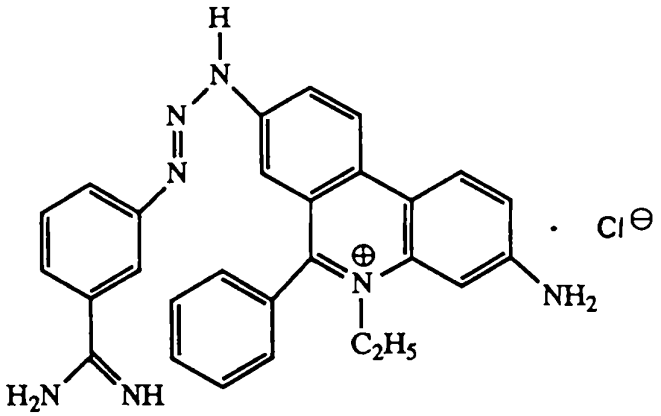
Chemical names: 3-Amino-8-[3-[3-(aminoiminomethyl)phenyl]-1-triazenyl]-5-ethyl-6-phenylphenanthridinium chloride

8-[3-(m-Amidinophenyl)-2-triazeno]-3-amino-5-ethyl-6-phenylphenanthridinium chloride

7-m-Amidinophenyldiazoamino-2-amino-10-ethyl-9-phenylphenanthridinium chloride

Synonyms: Samorin and Trypamidium

Structural formula:



Molecular formula: $C_{28}H_{26}N_7Cl$

Molecular weight: 496.04

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient

Appearance: Red crystals from aqueous methanol

Melting Point: Decomposes at 244-245°C

Technical Active Ingredients

The commercially available products, Samorin and Trypamidium, have isometamidium chloride as the principle component (~60%) with the remaining fraction comprising of two isomers, two analogs of a bis-species and homidium. Homidium is present at less than 1% and usually 0.5% of the drug. Isometamidium is presented as a dark reddish-brown powder with a solubility in water of 6% (w/v) at 20°C.

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Isometamidium is an antitrypanosomal agent. It is used for the treatment and prevention of animal trypanosomiasis principally in cattle but also in sheep, goats, buffalo, donkeys, horses, camels and dogs. Activity has been demonstrated against *Trypanosoma congolense*, *T.vivax*, *T.brucei*, and *T.evansi*. (Touratier, 1981)

The predominant conditions of use are as follows:

> In an average infected region: 2 to 4 treatments at 0.5 mg/kg (intramuscular route) every year
>
> In a heavily infected region: 4 to 6 treatments at 0.5 mg/kg (intramuscular route) every year or 2 to 4 treatments at 1 mg/kg (intramuscular route) every year. Concerning this latter dosage, the authors state that in practice it is only used in Republic of Central Africa.

Dosages

Isometamidium is prepared as a 1%, 2% or 4% (w/v) injectable aqueous suspension to be administered intramuscularly at a dose rate of 0.5 or 1.0 mg/kg body weight. Occasionally it is administered by intravenous injection (Dowler et al., in press). For intravenous use in cattle, isometamidium is used as a 1% aqueous solution to be administered at 0.6 mg/kg.

METABOLISM

Pharmacokinetics and bioavailability

The distribution and elimination of isometamidium was examined in lactating dairy cattle following intramuscular injection, 1.0 mg/kg, of ^{14}C-labeled material (Bridge et al, 1982). Peak concentrations of radiolabeled products were detected in plasma at 24 hrs (0.027 μg/ml) post dose and steadily declined to the limit of detection (0.01 μg/ml) by 29 days.

Kinabo and Bogan (1988b) investigated the absorption and distribution of isometamidium and its effect on tissues in cattle following intramuscular injection at 0.5 mg/kg body weight. The drug was rapidly detectable in serum at a mean concentration of only 0.020 μg/ml and declined to concentrations of lower than 0.010 μg/ml within two hours. After 120 hours, serum levels of isometamidium were below the limit of detection.

The distribution of isometamidium was examined in three lactating cows that were administered an IM injection of 1 mg [6-^{14}C]-Samorin/kg body weight in a 2% aqueous solution (Hawkins et al., 1991). Plasma concentrations of radioactivity peaked within 1 hour after dosing (0.051 - 0.160 μg/ml) and then decreased to 0.01 - 0.021 μg/ml at 12 hours. There was no significant decrease in plasma concentrations of radioactivity after 12 hours. The limit of detection was 0.006 μg/ml.

The absorption of ^{14}C-isometamidium was investigated in female rats following a single 1 mg/kg oral dose (Smith et al., 1981). Minimal absorption of the dose was observed. By day 7 after dosing, all tissues contained less than 0.010 mg/kg, at which time 99% of the administered dose had been voided in the feces.

The relay bioavailability of isometamidium residues in bovine tissues was examined in rats. Sixteen adult male rats were fed for 7 and 21 days with a standard diet containing lyophilized liver and kidney from a calf dosed IM with a combination of 45 mg of ^{14}C-isometamidium and 73 mg of cold isometamidium at 1 mg/kg body weight. Intake of ^{14}C-isometamidium in residues through the feed was estimated to be 8.64 μg/rat/day. Another six rats were given a single dose of 2.245 mg of ^{14}C-isometamidium/kg body weight by oral gavage as an aqueous solution. No radioactivity was detected (<0.02 ng ^{14}C-isometamidium residues/g wet tissue) in any of the samples from urine, serum, blood, kidney, liver, spleen, muscle, stomach, and small intestine. Cumulative excretion of radioactivity in feces was 89.85% for the 7 day group, 90.48% for the 21 day group, and 92.69% for the oral gavage group. No statistically significant differences were found between the groups. No clinical or pathological lesions were found. The results indicate that isometamidium residues in tissues are not bioavailable to any significant extent. The authors suggest that the poor bioavailability of the residues may be due to the cationic nature and high affinity binding of the drug to macromolecules. (Kinabo et al., 1989)

Metabolism Studies

Studies with rats (Philips et al., 1967) and cattle (Kinabo and Bogan, 1988b) have indicated that isometamidium metabolites could not be found in the blood. The latter study indicated the injection site was the primary depot for prophylaxis. The presence of active metabolites would have been suspected if isometamidium concentrations at the injection site were as transient and low as those in serum.

Cattle

Samples from three lactating cows that were treated with IM injection of 1 mg [6-^{14}C]-Samorin/kg body weight in a 2% aqueous solution were analyzed for patterns of radiolabelled metabolites using the HPLC procedure as described by Mignot et al. (1991b). Qualitatively, it was shown that compounds in liver, kidney, injection site, plasma, and urine were the same as those present in the parent drug. Metabolic profiles in milk and muscle could not be determined due to low levels of radioactivity. Methodology problems occurred in the analysis of plasma, liver, and kidney. Thirty-one percent of total activity was lost when depositing the plasma sample on the C_{18} cartridge for liquid/solid extraction. For liver and kidney, the first step of total radioactivity extraction was unsuccessful, causing the quantity of total radioactivity injected in the HPLC to be variable and yielding unclear chromatograms. Isometamidium was the main component found in all samples except in liver.

For pooled plasma at 1 hour after treatment, final extraction of total radioactivity was 63.4%. After chromatography, isometamidium represented 47.8% with 38.6% for purple isomer and/or bis-compound, 10.8% for pseudo-isometamidium and 2.7% for unknown metabolites. For pooled urine, at day 2 after treatment, final extraction of total radioactivity was 35.6%. After chromatography, isometamidium represented 71.5% of the chromatogram with 13.9% for purple isomer and/or bis-compound, 7.4% for pseudo-isometamidium and 7.1% for unknown metabolites.

For liver, on days 3, 10, and 30, final extraction of total radioactivity was 54.2%, 42.2%, and 37.0%, respectively. After chromatography, the proportion of isometamidium decreased over time (47.8%, 27.7% and 27.2%) so that by day 30, its level was exceeded by purple isomer. Unknown metabolites represented 24.1%, 33.5%, and 24.6%. For kidney, on days 3, 10, and 30, final extraction of total radioactivity was 49.4%, 41.3%, and 55.8% respectively. After chromatography, the proportion of isometamidium fell with time (38.4%, 36.1%, and 21.7%) while purple isomer and pseudo-isometamidium increased with the latter predominating. Unknown metabolites were less than 12%. For injection site, on day 30, final extraction of total radioactivity was 81.5%. After chromatography, isometamidium represented 73.9% of the chromatogram and unknown metabolites represented 5.1%.

Milk concentrations of radioactivity peaked at Day 2 post-injection for cow 1 (0.0068 μg/ml), at Day 3 for cow 2 and 3 (0.0044 and 0.0062 μg/ml). Cow 3 which was the last animal to be sacrificed had milk concentrations of radioactivity that ranged from 0.0014 - 0.0030 μg/ml from Day 5 to Day 31. Concentrations of radioactivity in tissues were highest at the injection site: 422 mg/kg at 72 hours, 233 mg/kg at 240 hours, and 87 mg/kg at 720 hours with an apparent half-life of approximately 12 days. Liver (maximum 4.60 mg/kg) and kidney (maximum 3.39 mg/kg) had the next highest concentrations. Concentrations of radioactivity in skeletal muscle ranged from 0.012-0.017 mg/kg at different sacrifice times. Table 1 shows a summary of total ^{14}C-residues in each tissue assayed and the amount of unchanged isometamidium. (Mignot et al., 1991a; Hawkins et al., 1991)

Table 1. Concentration (mg/kg) of Total Residue (TR) and Unchanged Isometamidium (I) in Tissues of Dairy Cows Treated with 1 mg/kg ^{14}C-Samorin Intramuscularly

Withdrawal Time (days)	Liver TR	Liver I	Kidney TR	Kidney I	Muscle* TR	Fat* TR	Inj. Site TR	Inj. Site I
3	4.72	1.22	3.38	0.64	0.013	0.017	422	NA
10	2.53	0.30	2.53	0.38	0.017	0.007	233	NA
30	2.26	0.23	2.02	0.24	0.012	0.011	96	58

* - Unchanged isometamidium was not determined in these tissues.
NA - Not Analyzed

Rat

Metabolism in rat plasma was determined by dosing thirty-eight female rats with 2 mg/kg Trypamidium by IV. Blood was sampled at 10 and 30 minutes, and 2, 5, 20 and 24 hours after treatment. Another 126 female rats received either 12.5, 50 or 200 mg/kg/day for 21 days by oral gavage. Blood samples were drawn at 30 minutes and 3 hours after treatments on days 0, 13 and 20. After a solid/liquid extraction, plasma extracts were analyzed by HPLC with UV detection. The limit of quantification was 0.01 μg/ml. Following the single IV dose, isometamidium was only measurable at time 10 minutes (0.1772 μg/ml) and 30 minutes (0.0862 μg/ml) after treatment. These results indicate that the rate of elimination is very high. Following the 21 day oral dose, isometamidium could not be detected even at the highest dosage. (Mignot and Lefebvre, 1991)

TISSUE RESIDUE DEPLETION STUDIES

Cattle

In the pharmacokinetic study described previously, Bridge et al. (1982) found the highest concentration of radiolabeled products, 73.5 mg/kg, was located at the injection site 72 hours post-injection. The half-life of the injection site residues was calculated to be 39 days. The liver and kidney tissues were the other main sites of radioactivity localization. The peak concentrations of isometamidium equivalents were 7.1 and 5.8 mg/kg at 72 hours with elimination half-lives of 25 and 35 days for the liver and kidney tissues respectively. In addition to tissues, milk samples were collected and analyzed during the 90 day post injection period. Most of the samples had levels of isometamidium which were below the limit of detection (0.01 μg/ml). However, some cows did produce positive samples (0.0138 - 0.0174 μg/ml) on single occasions from 5 to 70 days post-injection.

Isometamidium residues in calves have been reported using a sensitive HPLC method. In this study the calves were administered isometamidium at 0.5 mg/kg by IM injection. Isometamidium was only detectable in the serum up to 2 hours after injection, at a mean maximum concentration of 0.02 μg/ml. The highest concentration of isometamidium was detected at the injection site at 7, 21 and 42 days. The results of the various tissue assays have been tabulated in Table 2. (Kinabo and Bogan, 1988b)

Table 2. Isometamidium Residues in Calf Tissues (mg/kg) in Mean ± SD after IM Injection of 0.5 mg/kg

Tissue	Days Post-injection 7	21	42
Injection site	1270 ± 272	315 ± 173	208 ± 94
Liver	4.80 ± 0.84	4.07 ± 0.35	0.75 ± 1.41
Kidney	5.21 ± 3.36	2.98 ± 0.64	0.70 ± 0.11
Muscle	1.00 ± 0.02	0.87 ± 0.01	0.59 ± 0.12

Isometamidium residues were determined by treating 19 young bulls with a single gluteal IM injection of 1 mg/kg. Blood samples were drawn 10 times between 0.25 and 48 hours after dosing, on days 3, 5, 9, and at approximately weekly intervals until sacrifice. Tissue samples were collected at 1, 3, and 6 months after treatment. Samples were analyzed using the method of Mignot et al. (1991b). For bulls slaughtered one month post-treatment, the plasma concentration of isometamidium (mean = 30.82 ng/ml) peaked at one hour post-dosing. Levels declined rapidly thereafter so that in two out of five animals at 48 hours and in five out of five at 72 hours, they were below the limit of quantification (0.008 μg/ml). Isometamidium levels in muscle and fat from all animals were below the limit of quantification at all sacrifice times (0.1 mg/kg). In liver, the mean concentration of isometamidium was 0.251 mg/kg at one month post-treatment. At three months post-treatment, isometamidium was detected in only one out of five livers (0.132 mg/kg). At six months post-treatment, no isometamidium was detected in any of the livers. In kidney at one month post-treatment, isometamidium was detected in all animals. At three and six months post-treatment, no isometamidium was detected in any kidney. At the injection site one month post-treatment, mean isometamidium concentration was 129.5 mg/kg. By 3 months, the mean level dropped to 38.7 mg/kg. At 6 months, the mean level was 1.35 mg/kg and two bulls had values below the limit of quantification. Table 3 shows the concentrations of isometamidium in bull tissues at 1, 3, and 6 months following a single injection. (Mignot et al., 1991c; Bosc et al., 1991)

Table 3. Isometamidium Concentrations (mg/kg) in Mean ± SD after IM Injection of 1 mg/kg in Young Bulls

Withdrawal Time (months)	Fat	Muscle	Liver	Kidney	Inj. Site
1	BQ	BQ	0.251 ± 0.03	0.386± 0.13	129.5 ± 145.9
3	BQ	BQ	BQ	BQ	38.7 ± 37.7
6	BQ	BQ	BQ	BQ	1.346 ± 0.7

BQ: Below the limit of quantification (0.1 μg/ml)

Goats

Isometamidium residues in goats treated by intramuscular and intravenous injection at a level of 0.5 mg/kg were evaluated using a spectrophotometric method (Braide and Eghianruwa, 1980). Table 4 shows the concentrations of isometamidium found in tissues 4 and 12 weeks after a single dose.

Table 4. Isometamidium Residues (mg/kg) in Goat Tissues

Tissue	Time (weeks)	Route of Administration	
		Intramuscular	Intravenous
Liver	4	5.52 ± 0.38	11.39 ± 0.61
	12	ND	6.78 ± 0.29
Kidney	4	2.51 ± 0.16	9.29 ± 0.52
	12	<1.25	3.26 ± 0.20
Muscle	4	ND	ND
	12	NA	NA
Fat	4	ND	ND
	12	NA	NA
Injection Site	4	2.51 ± 0.21	ND
	12	ND	NA

ND - Not detected

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

Earlier analytical procedures lacked sensitivity and specificity such as the spectrophotometric method (Philips et al., 1967) which could not detect isometamidium concentrations less that 1 μg/ml in the plasma. The HPLC method developed by Perschke and Vollner (1985) was indirect in that isometamidium was converted to homidium before assay and therefore not specific.

Most of the methods reported for isometamidium, or isometamidium isomers and analogues, are for determination in plasma or serum. Kinabo and Bogan (1988a) developed an analytical procedure using solid-phase extraction and ion-pair reverse phase HPLC with fluorescence detection for isometamidium in bovine serum and tissues. Although the assay could detect levels of isometamidium down to 0.010 μg/ml in serum, the sensitivity was limited to 0.50 mg/kg in the tissue. The authors suggest that this may be due to strong binding of isometamidium to mucopolysaccharides, nucleic acid and lipids.

An improved method to assay isometamidium in body fluids and tissues has been developed (Mignot et al., 1991c). Isometamidium is separated from endogenous compounds with a C_{18} cartridge (solid-liquid extraction) and the sample is injected into an HPLC. The method is capable of separating isometamidium from other Trypamidium compounds. In this study, only isometamidium was measured quantitatively. The percent recoveries from fortified matrices were as follows: plasma 76%, fat 68%, kidney 68%, muscle 61%, liver 56%, milk 89%, and urine 89%. The calibration curves are linear for concentrations ranging from 0 to 160 ng/ml for plasma and from 0 to 1000 ng/ml (or μg/kg) for milk and tissues. The limits of quantification are 8 ng/ml for plasma, 10 ng/ml for urine, and 100 μg/kg (or ng/ml) for fat, muscle, kidney, liver, and milk. Specificity was demonstrated by the lack of chromatographic peaks from interfering biological compounds.

APPRAISAL

An evaluation of new residue data for isometamidium has been completed. In the metabolism study in lactating cows injected with ^{14}C-Samorin, it was shown qualitatively that the metabolites found in plasma, urine, injection site, liver and kidney were the same as those present in the parent drug but no homidium was detected. Metabolic profiles in milk and muscle could not be determined due to low levels of radioactivity. Isometamidium was the main component found in kidney and the injection site, but not liver. It was determined that parent isometamidium comprises only 20 and 16% of the total residue in liver and kidney.

The bioavailability of isometamidium residues was measured by refeeding lyophilized calf tissues containing incurred residues of ^{14}C-Samorin to rats and measuring the amount of radioactivity absorbed and excreted by the rats. The results of this assessment showed that there were no detectable residues in the urine, serum, blood, kidney, liver, spleen, muscle, stomach, or small intestine of the rats. Cumulative excretion of radioactivity in the rat feces was approximately 90% after oral dosing with calf tissues containing incurred isometamidium residues and 93% after oral dosing of drug in an aqueous solution. The results indicate that isometamidium residues in tissues are not bioavailable to any significant extent. The poor bioavailability of the residues may be due to the cationic nature and high affinity binding of the drug to macromolecules.

In the study of isometamidium residues in young bulls, plasma levels dropped to below the limit of quantification by 72 hours after treatment. Tissue samples were collected at 1, 3, and 6 months after treatment. Muscle and fat had isometamidium levels that were below the limit of quantification at all three sacrifice times. At three months post-treatment, isometamidium was detected in only one out of five livers. At six months post-treatment, no isometamidium was detected in any of the livers. By three months, isometamidium concentration in kidney had dropped below the limit of quantification. At six months, two out of five injection sites had isometamidium levels below the limit of quantification.

In lactating cows, isometamidium levels in milk peaked on day 2 (6.8 μg/L) and remained below 3 μg/L after day 7.

An improved HPLC method for measuring isometamidium residues in milk and tissue has been developed. The sponsor has validated the method to 0.1 mg/kg, and the percent recoveries, linearity, intraday precision and accuracy, and limit of quantification reported are acceptable.

Based on the ADI of 0-100 μg/kg established by JECFA, the permitted daily intake of isometamidium would be 6 mg of total drug-related residue contributed by 500 g of food animal meat plus 1.5 L of the milk in the diet of a 60-kg person. At 30 days of withdrawal, the intake of residues of isometamidium is well below the ADI. Based on the data from the study in bulls administered isometamidium at 1 mg/kg intramuscularly, JECFA recommended an MRL of 100 μg/kg for parent isometamidium in muscle and fat, 500 μg/kg in liver, 1000 μg/kg in kidney, and 100 μg/L in milk (see Table 5).

Table 5. Recommended MRLs for Isometamidium in Cattle

Tissue	Concentration at Day 30 Withdrawal, mg/kg 1 mg/kg IM	Total Residue Consumed mg(a)	Recommended MRL μg/kg parent	Theoretical Maximum Daily Intake mg(a)
Muscle	<0.1	0.03	100	0.03
Liver	0.25(1.25)b	0.12	500(2500)b	0.25
Kidney	0.39(2.44)c	0.12	1000(6300)c	0.31
Fat	<0.1	0.01	100	0.01
Milk	0.0068(d)	0.01	100(e)	0.15
Total		0.29		0.75

a) Based on a daily intake of 0.3 kg muscle, 0.1 kg liver, 0.05 kg kidney and fat, and 1.5 L milk
b) Adjusted observed value by 20% to estimate total residues
c) Adjusted observed value by 16% to estimate total residues
d) This value represents the highest concentration of total isometamidium residues found in milk. It occurs 2 days after dosing
e) This MRL μg/L in milk is based on the limit of quantitation of the analytical method

The injection site concentrations at 30 days withdrawal averaged 96 mg/kg; however, it was determined that this does not adversely impact human food safety for the following reasons:

1) isometamidium residues in tissues are not bioavailable to any significant extent,
2) consumption of an injection site would be extremely rare, and
3) the maximum theoretical intake of residues from muscle, liver, kidney, fat and milk of isometamidium at 30 days withdrawal is well below the ADI.

REFERENCES

Bosc, F., Huet, A.M., Aumont, D., Bernardin, J.B., Longo, F., Rolland, M.L. and Weil, A. 1991. Determination of isometamidium concentrations in plasma and tissue samples of young bulls following intramuscular administration of trypamidium at a level of 1 mg/kg (Animal Phase). Unpublished report submitted to FAO by Rhone Merieux, Toulouse Cedex, France.

Braide, V. and Eghianruwa, K.I. 1980. Isometamidium Residues in Goat Tissues after Parenteral Administration. Res. Vet. Sci., **29**, 111-113.

Bridge, C.M., Smith, G.E., Smith, R., Templeton, R. and Yoxall, A.T. 1982. Samorin: Study in the lactating cow, of the distribution and excretion of radiolabelled drug-derived products, following the administration of a single intramuscular dose of [6-14C] Samorin. M & B Research Report R. Ph. 176.

Dowler, M.B.E., Schillinger, D. and Connor, R.J. (in press). Notes on the routine intravenous use of isometamidium in the control of bovine trypanosomiasis in the Kenya Coast. Trop Anim. Hlth Prod.

Hawkins, D.R., Elsom, L.F., Kane, T.J. and Cameron, D.M. 1991. The pharmacokinetics and metabolism of ^{14}C-Samorin in lactating cows. Unpublished report submitted to FAO by Rhone Merieux, Toulouse Cedex, France.

Kinabo, L.D.B. and Bogan, J.A. 1988a. Solid-phase extraction and ion-pair reversed-phase HPLC of isometamidium bovine serum and tissues. Acta Trop. (in press).

Kinabo, L.D.B. and Bogan, J.A. 1988b. Pharmacokinetic and histopathological investigation of isometamidium in cattle. Res. Vet. Sci., **44**, 267-269.

Kinabo, L.D.B., Bogan, J.A., McKellar, Q.A. and Murray, M. 1989. Relay bioavailability and toxicity of isometamidium residues: a model for human risk assessment. Vet. Hum. Tox., **31**, 417-421.

Mignot, A. and Lefebvre, M. 1991. Determination of isometamidium concentrations in plasma of rats after IV administration (2 $mg \cdot kg^{-1}$) and repeated oral administrations (12.5, 50, 200 $mg \cdot kg^{-1} \cdot day^{-1}$). Unpublished report submitted to FAO by Rhone Merieux, Toulouse Cedex, France.

Mignot, A., Lefebvre, M., Couerbe, P. and Vidal, R. 1991a. Study on the Metabolism following single intramuscular administration of ^{14}C-Samorin (1 $mg \cdot kg^{-1}$) to lactating cows. Unpublished report submitted to FAO by Rhone Merieux, Toulouse Cedex, France.

Mignot, A., Lefebvre, M. and Vidal, R. 1991b. Development and validation of a new high-performance liquid chromatography assay of isometamidium. Unpublished report submitted to FAO by Rhone Merieux, Toulouse Cedex, France.

Mignot, A., Lefebvre, M. and Vidal, R. 1991c. Determination of isometamidium concentration in plasma and tissue samples of young bulls after an intramuscular administration of trypamidium at a level of 1 $mg \cdot kg^{-1}$. Unpublished report submitted to FAO by Rhone Merieux, Toulouse Cedex, France.

Persche, M. and Vollner, L. 1985. Determination of the trypanocidal drugs Homidium, Isometamidium and Quinapyramine in bovine serum or plasma using HPLC. Acta Trop., **42**, 209-216.

Phillips, F.S., Sternberg, S.S., Cronin, A.P., Sodergen, J.E. and Vidal, P.M. 1967. Physiological disposition and intracellular localization of isometamidium. Cancer Research, **27**(1), 333-349.

Smith, J., Bridge, C.M. and Smith, G.E. 1981. Samorin: Study, in the female rat, of the distribution and excretion of radiolabelled drug-derived products after intramuscular or oral administration of a single dose of [6-14C] Samorin. M & B Research Report RES/3851.

Touratier, L. 1981. (The advantage of using isometamidium for the control at animal trypanosomiasis). International Scientific Council for Trypanosomiasis Research and Control. 16th Meeting, Yaounde, Cameroon, 1979. OAU/STRC. 308-316.