DIMINAZENE

First draft prepared by
Dr. R.J. Heitzman
Compton, Newbury
Berkshire, United Kingdom

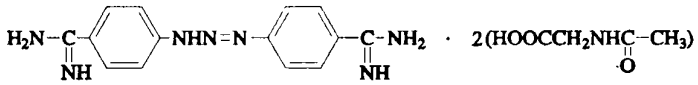
IDENTITY

Chemical names: 4,4'-(Diazoamino)dibenzamidine diaceturate

1,3-bis(*p*-Amidinophenyl)triazene bis(N-acetylglycinate)

Synonyms: Berenil

Structural formula:



Molecular formula: $C_{22}H_{29}N_9O_6$

Molecular weight: 515.54

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient: Diminazene aceturate.

Appearance: Yellow solid

Melting point: dec. 217°C

Solubility: Soluble in 14 parts water at 20°C, slightly soluble in alcohol, very slightly soluble in ether and chloroform.

UV_{max}: 369 nm?

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Diminazene aceturate is an antitrypanosomal agent for ruminants.

Dosages

Recommended therapeutic dose is either 3.5 mg/kg body weight administered intramuscularly.

METABOLISM

Summary of information provided for the 34th meeting of JECFA

The general metabolism, including pharmacokinetics, metabolic profiles and a radiometric study in cattle were evaluated at 34th meeting. The results indicated extensive metabolism in laboratory animals and cattle.

Pharmacokinetics

Studies were presented at the 34th meeting for the pharmacokinetics of diminazene aceturate in several laboratory species and for cattle and sheep. The drug has a short half-life in blood. The drug is excreted mostly in the urine of cattle and about 80% of the dose was excreted within 20 days after dosing.

Metabolism in Food Animals

After intra-muscular injection (i.m.) of 3.5 mg/kg to cattle the results indicated extensive metabolism. The parent drug and two metabolites (*p*-aminobenzamidine [22% of total residue] and *p*-amino-benzamide [4% of the radioactivity] were identified in urine of treated cattle. (Kellner et al., 1985 , 34th meeting).

TISSUE RESIDUE DEPLETION STUDIES.

Radiolabeled Residue Depletion Studies

Farm Species

One study was submitted to the 34th meeting for a radiolabeled study in cattle (Kellner et al., 1985). Cattle were administered 3.5 mg of diminazene aceturate by i.m. injection and total residues measured as parent drug equivalents at 7 and 20 days after dosing. The results are shown in Table 1. The residues were highest in liver and kidney but at easily measured levels in muscle and low in fat. The drug was well absorbed from the injection site.

Table 1. Diminazene aceturate residues in calves (mg/kg equivalents)

Tissue	7 days	20 days
Liver	75.5	24.4
Kidney	54.7	12.1
Spleen	2.51	1.00
Muscle	0.52	0.26
Injection site	0.69	0.64
Fat	0.20	<0.18

The data presented do not indicate the choice of a marker compound or marker tissue.

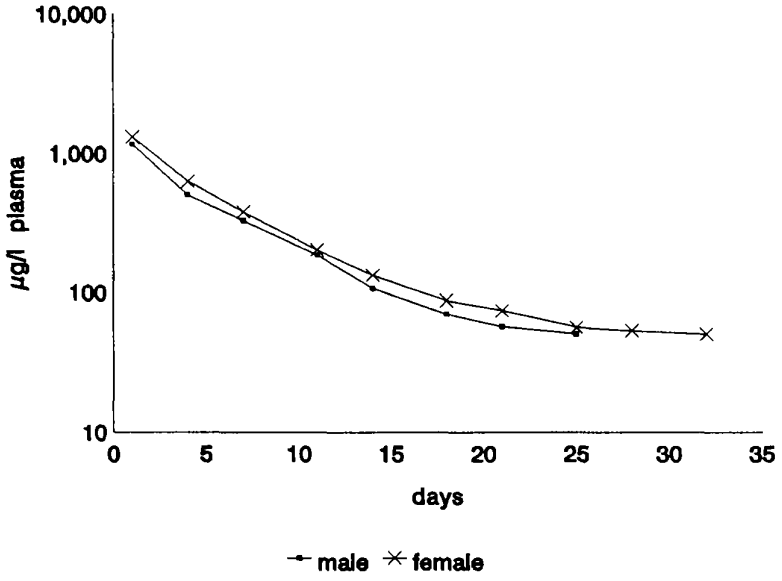
Other Residue Depletion Studies (with unlabeled drug)

Cattle

Two new studies are submitted in which residues of parent drug are determined in tissues and in milk.

Tissues. 14 German Black Pied young cattle (7 M, 7 F) weighing between 247 and 264 kg, were treated with an i.m. injection of 3.56 mg drug /kg body weight. Blood plasma levels were measured for a 35 day period (see Figure 1), and edible tissues and the injection site were sampled at slaughter 21, 28 and 35 days post-treatment. The concentration of parent drug was measured using a new HPLC method (see below).

Figure 1. Plasma concentrations of diminazene diaceturate in young cattle.



The results for the concentrations of parent drug in tissues from Report No.:K1 90/2 are given in Table 2. The concentrations were higher in liver and kidney than in muscle. The half lives of the drug in liver, kidney and at the injection site are between 6 and 8 days. It was not possible to estimate the half life in muscle because the concentrations of the residues were very close to the limit of detection for the analytical method.

Table 2. **Residues of Diminazene diaceturate in calf tissues after a single i.m. dose of 3.56 mg per kg body weight**

Days	Liver (μg/kg)	Kidney (μg/kg)	Muscle (μg/kg)	Inj. site (μg/kg)
21	10725	1509	<100	6723
21	5367	3924	312	8328
21	4908	2458	465	12362
21	6056	2587	367	9936
28	3363	2437	113	3827
28	777	1136	104	4030
28	4316	1577	175	7712
28	6571	2505	241	7265
35	1247	903	140	2482
35	805	724	115	2533
35	1355	636	156	388
35	2092	586	164	5226
$T_{½}$ (d)	6.1	7.7	-	6.2
r	-0.774	-0.845	-	-0.723

The half life ($T_{½}$) and regression coefficient (r) were calculated using log regression over the 21-35 day time period.

Milk. Four cows were administered diminazene aceturate by an i.m. injection at a dose of 3.56 mg/kg body weight. Milk was collected twice daily for 21 days post dosing. The residues of parent drug were monitored using an HPLC method (see below) with a detection limit of 0.05 mg/l. No residues were found.

Bound Residues and Bioavailability

No data is available on the nature of the total residues. As the drug undergoes extensive metabolism and there are one or maybe two long elimination phases (34th meeting) it could be expected that there are considerable amount of bound residues.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES.

The methods submitted to the 34th meeting were not considered satisfactory. New methods were requested and the sponsor has submitted two new HPLC methods, one for edible tissues and plasma and the other for milk.

Edible tissues (muscle, liver and kidney) method. Internal standard (Carba-Berenil, Hoechst 15 030 A) was added to 1 g tissue and the mixture homogenised. After the addition of 2 ml ethanol, mixing and centrifugation, the upper layer was transferred to a tube and 6 ml buffer, pH 9.0 added. The sample was extracted (clean-up phase) on a C18 Bond Elut column. The volume of the eluate was reduced and an aliquot of the sample run on an HPLC column (Nucleosil 120-5 C-18, 5 μm) with 0.05*M* tetraethylammonium phosphate buffer, pH 2.8

: Acetonitrile (92:8;w/v) as mobile phase. Detection was by UV at 369 nm.

The method was linear over the range 500-20000 μg/kg. (r = 0.9998). The method is accurate and recoveries of drug at spikes of 2500 μg/kg were 28.2 ± 1.6%, 38.5 ± 7.5% and 39.6 ± 5.5% for liver, kidney and muscle respectively. The limit of detection is about 100 μg/kg based on a signal ratio of 3:1, and the limit of quantitation 300 μg/kg.

Diminazene acetate was stable for 6 hours at room temperature and over two freeze-thaw cycles. Repeatability CVs were 1.34 - 12.63% over the range 500-20000 μg/kg and reproducibility was CV = 1.34% for a 20 mg/kg spike measured daily for 6 days.

Milk. Internal standard (Carba-Berenil, Hoechst 15 030 A) and a buffer solution pH 9.0 were added to 1 ml cow milk. After mixing and centrifugation and the removal of fat the sample was extracted (clean-up phase) on a C18 Bond Elut column. The volume of the eluate was reduced and an aliquot of the sample run on an HPLC column (Nucleosil 120-5 C-18, 5 μm) with 0.05*M* tetraethylammoniumphosphate buffer, pH 2.8 : Acetonitrile (92:8;w/v) as mobile phase. Detection was by UV at 369 nm.

The method was linear over the range 100-1000 μg/l. (r = 0.9998). The method is accurate and recoveries of drug at spikes of 500 μg/l were 75.7 ± 5.1%. The limit of detection is about 50 μg/l based on a signal ratio of 3:1. The limit of quantitation is about 150 μg/l. Diminazene acetate was stable for 6 hours at room temperature and over two freeze-thaw cycles. Repeatability CVs were 2.03 - 9.80% over the range 100-1000 μg/kg and reproducibility was CV = 2.03% for a 1 mg/kg spike measured daily for 4 days.

APPRAISAL

The new information provides two suitable methods for measuring residues of diminazene aceturate in milk and edible tissues.

After a single i.m. injection of 3.56 mg/kg body weight (recommended dose) no residues were detected in milk samples and data on residues was provided for muscle, liver and kidney tissues and the injection site.

There is still no satisfactory evidence for:

1. A suitable marker compound - it appears to be assumed that parent drug is satisfactory. The ratio of metabolites to total residues is not known.

2. Information on bound residues. As the drug undergoes extensive metabolism and there are one or maybe two long elimination phases (34th meeting) it could be expected that there is a considerable amount of bound residues. Although the measurements are made in two different studies the values for total residues at 20 days post dosing (Table 1) and the concentration of parent drug at 21 days (Table 2) give an indication that parent drug is about 27% and 22% of the residues in liver and kidney respectively and that parent drug accounts for most of the residues in muscle and milk.

3. The drug is also recommended for intravenous injection at a 2.0 mg/kg body weight dose in a different formulation from the one used for i.m. injection. No data is submitted on residues using the intravenous injection.

Calculation of MRLs using ADI of 100 μg/kg of body weight (6000 μg per person per day).

The following assumptions were made:

- Combining the data in Table 1 at 20 days with the data in Table 2 at 21 days, 27% and 22% of the total residues are parent drug in liver and kidney, respectively, and 100% in muscle and milk.

The Committee recommends MRLs for cattle of 12000 μg/kg for liver, 6000 μg/kg for kidney, 500 μg/kg for

muscle, and 150 μg/l for milk. The milk value is set at the limit of quantitation of the analytical method.

The theoretical ingested residue of parent drug equivalents, if in the unlikely event, all of the MRL was consumed is:

Tissue	MRL (μg/kg)	Daily intake (kg)	μg[1]	%TR[1]	EQ (μg)
Muscle	500	0.300	150	100	150
Liver	12000	0.100	1200	27	4444
Kidney	6000	0.050	300	22	1364
Milk	150	1.5	225	100	225
	Total		**1875**		**6183**

[1]Parent drug

REFERENCES

Kellner, H.M., Eckert, H.G. and Volz, M.H. (1985). Studies in cattle on the disposition of the anti-trypanosomal drug, diminazene diaceturate (Berenil). Trop. Med. Parasit. 36, 199-204.

Klatt, P and Schmidt, D. (1991). Report on blood plasma level determinations and residue studies in organs of cattle after one intramuscular administration of the commercial preparation Berenil for veterinary use. Hoechst report Nº K1 90/2

Klatt, P and Schmidt, D. (1991). Report on the excretion of diminazene aceturate in the milk of cows after one intramuscular administration of the commercial preparation Berenil for veterinary use. Hoechst report Nº K1 90/1

Schmidt, D and Albrecht, A. (1992). Determination of Berenil (diminazene diaceturate) in cow milk by high pressure liquid chromatography (HPLC) Hoechst report Nº 01-L42-0637-92.

Schmidt, D and Albrecht, A. (1992). Determination of Berenil (diminazene diaceturate) in bovine tissues by high pressure liquid chromatography (HPLC) Hoechst report Nº 01-L42-0638-92.