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Animal trypanosomosis: making quality control of trypanocidal drugs possible

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Summary

African animal trypanosomosis is arguably the most important animal disease impairing livestock agricultural development in sub-Saharan Africa. In addition to vector control, the use of trypanocidal drugs is important in controlling the impact of the disease on animal health and production in most sub-Saharan countries. However, there are no internationally agreed standards (pharmacopoeia-type monographs or documented product specifications) for the quality control of these compounds. This means that it is impossible to establish independent quality control and quality assurance standards for these agents. An international alliance between the Food and Agriculture Organization of the United Nations, the International Federation for Animal Health, the Global Alliance for Livestock Veterinary Medicines, the University of Strathclyde and the International Atomic Energy Agency (with critical support from the World Organisation for Animal Health) was established to develop quality control and quality assurance standards for trypanocidal drugs, with the aim of transferring these methodologies to two control laboratories in sub-Saharan Africa that will serve as reference institutions for their respective regions. The work of the international alliance will allow development of control measures against sub-standard or counterfeit trypanocidal drugs for treatment of trypanosome infection. Monographs on diminazene aceturate (synonym: diminazene diaceturate), isometamidium chloride hydrochloride, homidium chloride and bromide salts and their relevant veterinary formulations for these agents are given in the annex to this paper. However, the authors do not recommend use of homidium bromide and chloride, because of their proven mutagenic properties in some animal test models and their suspected carcinogenic properties.

Keywords

African animal trypanosomosis – Diminazene – Homidium – Isometamidium – Monograph – Pharmacopoeia – Quality assurance – Quality control – Trypanocidal drug – Trypanocide.

Introduction

Animal trypanosomosis, caused by trypanosomes of several species belonging to the genus *Trypanosoma*, is present in South America, Africa and part of Asia and affects a wide range of vertebrate animals. In sub-Saharan African countries, African animal trypanosomosis (AAT) is one of the most important animal diseases, with 50 million cattle exposed to the bites of infective tsetse flies (*Trypanosoma congolense, T. brucei brucei)* or to infective tsetse flies and biting flies *(T. vivax)* (1). In these zones, *T. congolense, T. vivax* and *T. brucei brucei* are the main pathogenic species encountered. The degree of disease severity in domestic ruminants depends on the species of the infecting trypanosomes, with *T. congolense* having a more severe impact than *T. vivax* and *T. brucei* on animal health and production (2). Nevertheless, trypanosomosis is generally recognised as a serious disease that may be fatal if untreated. This is particularly true in susceptible animals such as zebu cattle (3) but also in trypano-tolerant animals such as N'Dama cattle, which may suffer from pathological stress caused by trypanosomosis, as manifested by development of anaemia and reduced growth (3, 4, 5).

The Food and Agriculture Organization of the United Nations (FAO) estimates that more than three million cattle die of trypanosomosis every year (6); in addition, there are significant indirect losses in the livestock sector as a consequence of abortion, infertility, weight loss, reduced draught power and reduced milk production (7), all of which have an impact on people's livelihoods as well as on animal welfare. Treatment with trypanocidal drugs of proven quality, administered intramuscularly and at the recommended dosage, reduces both the direct and indirect losses caused by trypanosome infections in susceptible and in tolerant cattle (3, 5). Direct annual losses from AAT are currently some US$1.5 billion and, overall, have the effect of limiting Africa's agricultural income to approximately US$4.5 billion a year below its potential level (8). The financial benefit of controlling this disease has been calculated to range from below US$500 per km^2 to well over US$5,000 per km^2 (9). Given the importance of the livestock sector in sub-Saharan Africa, the consequence of AAT is that the lives of millions of individuals, families and rural communities who rely on cattle for their livelihood are devastated.

Apart from vector control, chemotherapy with trypanocidal drugs (also called trypanocides) is the most widely used method to combat AAT in most of sub-Saharan Africa. Internationally agreed standards for trypanocides, either as documented product specifications or as pharmacopoeia-type monographs, are lacking in the public domain, making quality control (QC) and compliance in the use of veterinary trypanocides in sub-Saharan Africa difficult and impossible to regulate.

Trypanocidal drugs in sub-Saharan Africa

Chemotherapy of AAT is undertaken using veterinary formulations that contain one of the currently available trypanocides. At present, only three compounds belonging to two chemical classes are widely available to treat trypanosomosis: diminazene diaceturate, belonging to the class of aromatic diamidines, and isometamidium chloride hydrochloride and homidium (chloride and bromide salts), which belong to the phenanthridinium class of trypanocidal agents. Diminazene diaceturate is indicated in the treatment of cattle trypanosomosis, whereas the phenanthridinium class of compounds is used for both treatment and prevention. Isometamidium chloride hydrochloride is mainly used as a prophylactic drug (10).

Homidium chloride and bromide are known to have mutagenic activity after metabolic activation in *Salmonella* Typhimurium *in vitro* (11). This activity has been confirmed in some *in vivo* systems but not in others (12). Additional studies indicate that homidium bromide can be toxic at high concentrations (13). Nevertheless, the antiparasitic activity of homidium has been shown, and veterinary products containing homidium salts are registered and used in some African countries. For comprehensiveness and clarity of information, pharmacopoeia-type monographs on homidium salts are therefore included in the annex to this paper. However, the authors recommend that use of homidium salts for prevention and treatment of trypanosomosis should be actively discouraged and replaced with agents with a more favourable toxicity profile (10).

The trypanocidal products described in the annex have been on the market for several decades and parasite resistance to all three available trypanocides is increasing (14, 15). Although efforts are ongoing to discover and develop new trypanocides by public–private partnerships such as the Global Alliance for Livestock Veterinary Medicines (GALVmed), among others (16, 17), no new therapeutic or prophylactic drugs are expected in the next few years as costs are prohibitive (18). Further, when new drugs do arrive, a better regulated market is required to ensure drug quality compliance.

Unfortunately, the abundance of poor-quality products, together with their incorrect usage, inappropriate storage, incorrect estimation of the weight of animals to be treated and use of contaminated syringes, can lead to under-dosage and treatment failure and contribute to resistance in trypanosome populations. In addition, the failure to use sanative pairs of drugs (i.e. use of only one trypanocidal drug instead of two) can lead to treatment failure and emergence and spread of parasite-resistant strains (10, 13, 15). It is therefore necessary that quality trypanocides be stored and administered correctly (10). The proper use of trypanocides (10, 14) has been shown to provide effective treatment and reduce the risk of increased drug resistance. Currently, however, there are no internationally agreed standards for QC of these compounds to ensure the quality of active ingredients, their concentration or their content in the finished veterinary medicinal products.

It has been estimated that every year approximately 50 million single-treatment doses of trypanocides, costing in the range of US$0.50 to $3.00 per dose (approximately equivalent to more than US$90 million per year), are administered to domestic ruminants in sub-Saharan Africa (1).

In tsetse-infested areas/countries of the sub-Sahara, trypanocidal drugs represent 40% to 50% of the total animal health market, providing a large and attractive target for the sale of sub-standard or counterfeit products (Merial, unpublished report).

The findings of market surveys and studies (19, 20, 21) on the quality of trypanocidal pharmaceutical formulations sold in markets in sub-Saharan Africa have shown that a substantial proportion were either registered products of poor quality, or were counterfeit products of poor quality or contained no active substance to treat trypanosomosis. In particular, market surveys in Benin, Cameroon, Chad, Mali, Mauritania, Senegal and Togo showed that more than 40% (range 43% to 93%) of the ivermectin, diminazene, oxytetracycline and albendazole formulations analysed were of sub-standard quality or counterfeit (22, 23, 24). Very limited published information is available regarding surveys on the quality of veterinary drugs in East Africa (25), but anecdotal evidence suggests that the situation is similar to that in western and Central Africa (21, 23, 24).

The use of sub-standard or counterfeit trypanocides has severe implications for animal health, public health and the local economy. Ineffective treatment with sub-curative doses of these drugs increases the risk of emergence of drug resistance in trypanosome populations (14, 24). Trypanocidal drug resistance in animals has now been reported in 17 African countries (21). Moreover, food safety is also compromised by allowing unspecified and potentially harmful chemicals and/or their residues to enter the food chain. Careful examination of the packaging of trypanocides to check the quality of the carton, printing, sealing and hologram, and the indicated shelf life, may on occasion provide grounds to suspect that the drug is of poor quality. However, it is necessary to use standardised chemical analytical procedures to identify sub-standard or counterfeit drugs with certainty.

Currently there are no authoritative pharmacopoeia-type monographs or product specifications for these widely used trypanocidal drugs. The only monographs available were published more than 35 years ago and cover diminazene diaceturate, in the British Pharmacopoeia (Vet) Codex 1968, and homidium bromide, in the British Pharmacopoeia (Vet) 1977. The methodologies and technologies described are no longer considered to be feasible, reliable or valid.

Without updated and accurate quality reference protocols or standard procedures, it is not possible to provide independent QC and QA for veterinary trypanocides available throughout sub-Saharan Africa. There is an urgent need to disseminate QC reference standards and procedures for diminazene diaceturate, isometamidium chloride hydrochloride and homidium (chloride and bromide) and to develop veterinary formulations that contain these active pharmaceutical ingredients.

International alliance for quality control of veterinary trypanocidal drugs

In line with the recommendation of the World Organisation for Animal Health (OIE) Conference on Veterinary Medicinal Products in Africa (Dakar, Senegal, 2008) (20, 24), and in consultation with African institutions, an international alliance was created to help overcome the problem of uncontrolled, sub-standard, falsified or fake trypanocidal drugs. This alliance comprises the FAO, through the Programme Against African Trypanosomosis (PAAT), the International Federation for Animal Health (IFAH, a global representative body of companies engaged in research, development, manufacturing and commercialisation of animal health products), the University of Strathclyde, GALVmed, and the International Atomic Energy Agency (IAEA), through its Joint FAO/IAEA Agriculture and Biotechnology Laboratories. The initiative is critically supported by the OIE.

The aim of this alliance is to establish international reference standards in the form of pharmacopoeia-type monographs and standard operating procedures for the QC and QA of trypanocidal products. Such standards would benefit all the stakeholders (Veterinary Services, livestock keepers, drug producers) involved in regulation and control of veterinary medicines as they would enable independent QC and support QA of these products. To this end, and on the basis of previous analytical studies of isometamidium chloride hydrochloride (18, 26, 27), diminazene diaceturate (28), homidium chloride (Merial, internal unpublished data) and homidium bromide (Laprovet, internal unpublished data), pharmacopoeia-type monographs have been prepared and are included in the annex to this article.

The United Nations Industrial Development Organization is an associated partner to this initiative, together with the United Nations Office on Drugs and Crime, and has agreed to provide support, particularly in the framework of the recent resolution regarding the marketing of fraudulent drugs (29). The initiative also received financial support from the United Kingdom Department for International Development, through GALVmed.

The OIE is one of three international standard-setting bodies recognised by the World Trade Organization (WTO), under the Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement), and is the main such body for animal health matters. In this alliance, the OIE is providing institutional support and a framework for promotion of the monographs on the trypanocidal drugs and their formulations. In an effort to enlarge the alliance and promote a strong Africa-based ownership, regional research centres such as the Centre International de Recherche-Développement sur l'Élevage en Zone Subhumide (CIRDES), based in Burkina Faso, and the International Trypanotolerance Centre, based in the Gambia, will also be included.

The knowledge and analytical procedures developed for the QC and QA of trypanocidal drugs have now been established in two existing African-based laboratories (one in eastern Africa, a second in western Africa). This followed a public call for proposals and an external audit to select two laboratories, namely the Laboratoire de Contrôle des Médicaments Vétérinaires de l'EISMV (LACOMEV) in Dakar, Senegal (serving West and Central Africa), and the Tanzania Food and Drugs Authority (TFDA) in Dar es Salaam, Tanzania (serving eastern and southern Africa). Through the alliance, these two laboratories have been provided with the necessary analytical equipment to carry out QC of these agents, and laboratory technical staff have received the necessary training to use the developed analytical QC methods. This initiative is expected to enhance the sustainability of the two laboratories by enlarging their existing mandate for QC of veterinary drugs at a local and regional level. The laboratories will enable reliable QC results to be used by drug registration authorities and to serve as an arbiter if there is a legal dispute regarding a falsified or fake product. However, although laboratory analysis can reveal deficiencies, some falsified drugs can be identified by checking the quality of the packaging before laboratory analysis.

The monographs on diminazene diaceturate, isometamidium chloride hydrochloride, and homidium chloride and bromide (together with the active pure substances and their respective pharmaceutical formulations) that were developed through this alliance are given in the annex to this article. The monographs are expected to enable laboratories in Africa, Asia and South America, as well as those of veterinary pharmaceutical companies, to carry out QC of the described trypanocidal drugs on a common platform.

Conclusions

The problem of poor-quality and/or falsified or fake drugs is particularly pronounced in developing countries. For anti-infectives (30), including veterinary trypanocides, the use of poor-quality products is important in the development of drug-resistant strains of the relevant pathogen. In a climate of increasing reports of parasite resistance (31), it is essential to safeguard the quality of existing medicines. Poor-quality products have severe implications for both animal health (sub-standard or falsified products leave infections untreated or ineffectively treated, with consequences for animal welfare and resistance development) and food safety, as they create problems of unspecified, unwanted and/or unknown chemicals and their residues in the food chain.

Proper use of the drugs is also recommended. Indeed, poor storage of an effective and genuine drug, misuse (contaminated syringes), under-dosing (inappropriate storage or incorrect weight estimation of the animal), and/or failure to use sanative pairs of drugs (use of only one trypanocidal drug instead of two) may also lead to ineffective treatment and, eventually, to the emergence and spread of resistant strains. It is therefore important to highlight the correct procedures for storage and use of trypanocides, including accurate weight estimation of the animal (10).

In sub-Saharan Africa trypanocidal drugs represent about 40% of the total animal health market and the costs of these drugs are mainly borne by small-scale livestock holders. Ineffective treatment can result in the death of animals and a significant reduction in the livestock productivity on which livestock keepers depend. The international alliance has enabled development of monographs on diminazene aceturate, isometamidium chloride hydrochloride, homidium chloride and bromide and their formulations. These monographs will serve as international points of reference and assist in QC of trypanocidal drugs available in sub-Saharan markets.

The establishment and support of two analytical control laboratories in sub-Saharan Africa (LACOMEV and TFDA) that serve as reference laboratories within their respective regions will enable regulatory authorities and other concerned stakeholders to control the quality of these much needed and widely used products. In relation to stakeholders concerned with animal health and production, these may include, but are not limited to, registration authorities, inspectors, Veterinary Services, farmers and farmers' associations/cooperatives, veterinarians and pharmacists. As a result of these tools, it is now possible to start building effective measures for the control of trypanocidal medicines.

Registered drugs that are quality controlled in accordance with international reference documents are needed so that the responsible authorities and the farmers are able to create sustainable and reliable farming sectors through the proper use of quality medicines. This will also translate into improved animal health, increased productivity and safe food for consumers.

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Trypanosomose animale : perspectives de mise en œuvre du contrôle qualité pour les médicaments trypanocides

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Résumé

De toutes les maladies animales, la trypanosomose animale africaine est certainement le principal obstacle au développement de l'élevage en Afrique subsaharienne. Outre les mesures de lutte contre les vecteurs, le recours aux médicaments trypanocides joue un rôle important pour maîtriser l'impact de cette maladie sur la santé et la production animales dans la plupart des pays de la région. Néanmoins, il n'existe pour l'instant aucune norme acceptée au plan international pour le contrôle qualité de ces molécules (monographies de type pharmacopée ou spécifications argumentées sur les produits). De ce fait, il est impossible de mettre en place une procédure indépendante de contrôle de la qualité et des normes d'assurance qualité pour ces produits. Un partenariat international a été mis en place, formé par l'Organisation des Nations unies pour l'alimentation et l'agriculture, la Fédération internationale pour la santé animale, l'Alliance mondiale pour les médicaments vétérinaires destinés à l'élevage (GALVmed), l'Université de Strathclyde et l'Agence internationale de l'énergie atomique (avec le soutien essentiel de l'Organisation mondiale de la santé animale) afin d'élaborer des normes applicables au contrôle de la qualité et à l'assurance qualité pour les médicaments trypanocides, dans le but de transférer ces méthodologies à deux laboratoires de contrôle d'Afrique subsaharienne qui feront office d'institutions de référence en la matière dans leurs régions respectives. Les travaux de ce partenariat international permettront de mettre en œuvre des mesures visant à lutter contre les médicaments trypanocides non conformes ou contrefaits pour le traitement de la trypanosomose. Les auteurs fournissent, en annexe du présent article, des monographies sur l'acéturate de diminazène (synonyme : diacéturate de diminazène), le chlorhydrate de chlorure d'isométamidium, le chlorure et le bromure d'homidium ainsi que sur la formulation de ces composants pour un usage vétérinaire. Néanmoins, les auteurs déconseillent de recourir au bromure ou au chlorure d'homidium en raison de leur caractère mutagène, qui a été démontré chez certains modèles animaux, et de leurs propriétés carcinogènes suspectées.

Mots-clés
Assurance qualité – Contrôle de la qualité – Diminazène – Homidium – Isométamidium – Médicament trypanocide – Monographie – Pharmacopée – Trypanocide – Trypanosomose animale africaine.

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Tripanosomosis animal: cómo hacer posible el control de calidad de los medicamentos tripanocidas

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Resumen
Posiblemente, la tripanosomosis animal africana es la patología animal que en mayor medida lastra el desarrollo agropecuario en el África subsahariana. Para combatir los efectos de la enfermedad en la sanidad y la producción animales en la mayoría de los países subsaharianos, además de luchar contra el vector, es importante utilizar medicamentos tripanocidas. Sin embargo, no hay normas internacionalmente acordadas (monografías del estilo de farmacopeas o especificaciones de los productos bien documentadas) con respecto al control de calidad de esos compuestos, por lo que resulta imposible establecer normas para procesos independientes de control y garantía de calidad de esos fármacos. De ahí que se creara una alianza internacional formada por la Organización de las Naciones Unidas para la Alimentación y la Agricultura (FAO), la Federación Internacional de Sanidad Animal, la Global Alliance for Livestock Veterinary Medicines, la Universidad de Strathclyde y el Organismo Internacional de Energía Atómica (con apoyo esencial de la Organización Mundial de Sanidad Animal) destinada a elaborar normas de control y garantía de la calidad de los medicamentos tripanocidas, con el objetivo de transferir estos métodos a dos laboratorios de control del África subsahariana que pudieran ejercer de establecimientos de referencia para sus respectivas regiones. La labor de esta alianza internacional permitirá definir medidas para controlar los medicamentos tripanocidas falsificados o de calidad deficiente para el tratamiento de la infestación por tripanosomas. En el anexo a este artículo se ofrecen monografías sobre las sales aceturato de diminazeno (sinónimo: diaceturato de diminazeno), cloruro de clorhidrato de isometamidio y bromuro y cloruro de homidio y sus correspondientes formulaciones veterinarias. Los autores, sin embargo, desaconsejan el uso de bromuro y cloruro de homidio por sus demostradas propiedades mutagénicas en algunos modelos animales experimentales y sus presuntas propiedades cancerígenas.

Palabras clave
Control de calidad – Diminazeno – Farmacopea – Garantía de calidad – Homidio – Isometamidio – Medicamento tripanocida – Monografía – Tripanocida – Tripanosomosis animal africana.

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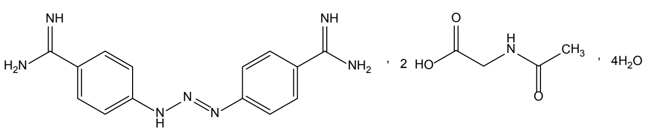
Annex

Monograph: Pharmaceutical substances: diminazene diaceturate tetrahydrate



$C_{14}H_{15}N_7.2C_4H_7NO_3.4H_2O$

Relative molecular mass: 587.6.

Chemical name: 4,4′-diamidinodiazoaminobenzene diaceturate tetrahydrate. CAS RN: 908-54-3.

Description: yellow to orange powder.

Solubility: dissolve 1 g diminazene diaceturate tetrahydrate in water R and make up to 14 ml with the same solvent. The powder dissolves completely to give a yellow solution free from particulate matter. Diminazene diaceturate tetrahydrate is slightly soluble in ethanol and very slightly soluble in diethyl ether and in chloroform.

Category: trypanocidal and babesicidal agent.

Storage: store in a closed container excluding light and humidity.

Requirements: diminazene diaceturate tetrahydrate contains not less than 98% and not more than the equivalent of 102% of $C_{14}H_{15}N_7.2C_4H_7NO_3$ calculated with reference to the dried substance.

Identity tests: use either tests A and B or tests B and C.

A. Carry out the examination as described in Section 1.7 (Spectrophotometry in the infrared region) in the International Pharmacopoeia[1]. The infrared absorption spectrum is concordant with the spectrum obtained from diminazene diaceturate tetrahydrate RS or with the reference spectrum of diminazene diaceturate tetrahydrate.

B. Carry out the examination as described in Section 1.6 (Spectrophotometry in the visible and ultraviolet regions) in the International Pharmacopoeia[1]. The light absorption, in the range 230 nm to 400 nm, of a 0.001% w/v solution in water R exhibits a maximum at 370 nm. The absorbance at 370 nm is about 0.65.

C. Examine the chromatograms obtained for the related substances test.

Melting temperature: determine as described in Section 1.2 (Determination of melting temperature, melting range, congealing point, boiling point, and boiling range) in the International Pharmacopoeia[1]. Melting temperature is 203°C to 217°C.

pH value: determine as described in Section 1.13 (Determination of pH) in the International Pharmacopoeia[1]. The pH of a solution of 1% w/v diminazene diaceturate tetrahydrate in carbon-dioxide-free water R at 20°C is between 5.2 and 6.5.

Loss on drying: dry 1 g diminazene diaceturate tetrahydrate at 70°C under reduced pressure to a constant weight; it loses not more than 13%.

Water: determine as described in Section 2.8 (Determination of water by the Karl Fischer method, Method A) in the International Pharmacopoeia[1], using 0.1 g of the substance; the water content is not more than 13% w/w.

Sulfated ash: determine as described in Section 2.3 (Sulfated ash) in the International Pharmacopoeia[1]. Determined in 1 g of diminazene diaceturate tetrahydrate, it does not exceed 0.1% w/w.

Related substances: carry out the test described in Section 1.14.4 (High-performance liquid chromatography) in the International Pharmacopoeia[1] using a stainless steel column (150 mm × 4.6 mm) packed with microparticles of octadecyl silica (5 µm). Mobile phase: mix 10 volumes of acetonitrile R and 10 volumes of methanol R with 80 volumes of a 20 mM solution of ammonium formate R in water R (1.26 g/l) previously adjusted to pH 4 with formic acid R.

Prepare the following solutions in water R immediately before use: solution (a), diminazene diaceturate tetrahydrate 1 mg/ml; use solution (a) to prepare solution (b), diminazene diaceturate tetrahydrate 10 µg/ml.

Operate at flow rate 0.7 ml/min with an ultraviolet spectrophotometer detector set at wavelength 254 nm.

Inject 20 µl each of solutions (a) and (b) and allow 20 min analysis time for each sample.

Measure the areas of the peak responses in the chromatograms for solutions (a) and (b) and calculate the content of related substances as a percentage. In the chromatogram of solution (a), the area of any peak, other than the principal peaks, is not greater than that obtained with solution (a) (1%). The sum of the areas of all peaks, other than the principal peak, is not greater than twice the area of the principal peak obtained with solution (b) (2%). Disregard any peak with an area less than 0.05 times the area of the principal peak in the chromatogram of solution (b). The test is not valid unless the relative standard deviation of the retention time of the principal peak, in replicate injections for a system suitability test, is not greater than 2 and the resolution between adjacent peaks is not less than 1.5.

Assay: determine as described in Section 1.14.4 (High-performance liquid chromatography) in the International Pharmacopoeia[1] using a stainless steel column (150 mm × 4.6 mm) packed with microparticles of octadecyl silica (5 µm). Mobile phase: mix 10 volumes of acetonitrile R and 10 volumes of methanol R with 80 volumes of a 20 mM solution of ammonium formate R in water R (1.26 g/l) previously adjusted to pH 4 with formic acid R. Prepare the following solutions in water R immediately before use: solution (a) diminazene diaceturate tetrahydrate 0.1 mg/ml; solution (b) diminazene diaceturate tetrahydrate RS 0.1 mg/ml. Operate at flow rate 0.7 ml/min with an ultraviolet spectrophotometer detector set at wavelength 254 nm.

Inject 20 µl each of solutions (a) and (b) and allow 20 min analysis time for each sample. The test is not valid unless the relative standard deviation of the retention time of the principal peak, in replicate injections for a system suitability test, is not greater than 2 and the resolution between adjacent peaks is not less than 1.5.

Measure the areas of the peak responses in the chromatograms of solutions (a) and (b) and calculate the content of $C_{14}H_{15}N_7.2C_4H_7NO_3$, taking into account the titre of diminazene diaceturate tetrahydrate RS.

Monograph: Dosage forms: Specific monographs: diminazene diaceturate preparation for injection

Description: granules.

Solubility: dissolve 2 g of diminazene diaceturate preparation for injection in water R and make up to 15 ml with the same solvent. The diminazene diaceturate preparation dissolves completely to give a yellow solution free from particulate matter within 5 min.

Category: trypanocidal and babesicidal agent.

Storage: store in a closed container excluding light and humidity.

Labelling: the designation diminazene diaceturate preparation for injection indicates that the substance complies with the general monograph regarding parenteral preparations in the International Pharmacopoeia[1] and may be used for parenteral administration. Expiry date.

Additional information: diminazene diaceturate preparation for injection contains a mixture of diminazene diaceturate tetrahydrate ($C_{14}H_{15}N_7.2C_4H_7NO_3.4H_2O$) and antipyrine ($C_{11}H_{12}N_2O$, phenazone). If vitamin(s) are present they must not interfere with any of the tests.

Requirements: the content of $C_{14}H_{15}N_7.2C_4H_7NO_3.4H_2O$ and $C_{11}H_{12}N_2O$ should be 95% to 105% of the stated amount.

Identity tests: tests A and B must be used.

A. Carry out the examination as described in Section 1.6 (Spectrophotometry in the visible and ultraviolet regions) in the International Pharmacopoeia[1]. The light absorption in the range 230 nm to 400 nm of a 0.001% w/v solution in water R exhibits maxima at 370 nm and 242 nm. The absorbance at 370 nm (diminazene diaceturate tetrahydrate) is about 0.65.

B. In the assay, the retention times of the peaks in the chromatogram of the test solution are the same as those in the chromatogram of the reference solution (c).

Assay: determine as described in Section 1.14.4 (High-performance liquid chromatography) in the International Pharmacopoeia[1] using a stainless steel column (150 mm × 4.6 mm) packed with microparticles of octadecyl silica (5 µm). Mobile phase: mix 10 volumes of acetonitrile R and 10 volumes of methanol R with 80 volumes of a 20 mM solution of ammonium formate R in water R (1.26 g/l) previously adjusted to pH 4 with formic acid R.

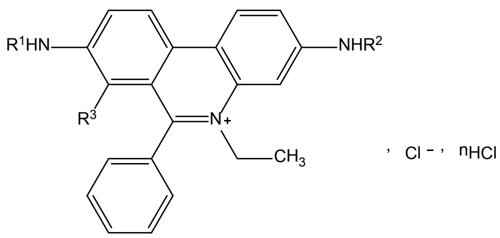
Prepare the following solutions in water R immediately before use: solution (a), diminazene diaceturate tetrahydrate preparation 0.1 mg/ml; solution (b), diminazene diaceturate tetrahydrate RS 0.1 mg/ml; solution (c), antipyrine RS (phenazone EP) 0.1 mg/ml.

Operate at flow rate 0.7 ml/min with an ultraviolet spectrophotometer detector set at wavelength 254 nm.

Inject 20 µl each of solutions (a), (b) and (c) and allow 20 min analysis time for each sample. The test is not valid unless the relative standard deviation of the retention time of the principal peak, in replicate injections for a system suitability test, is not greater than 2 and the resolution between the peaks is not less than 1.5.

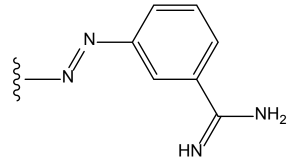
Measure the areas of the peak responses obtained in the chromatograms of solutions (a), (b) and (c) and calculate the content of $C_{14}H_{15}N_7.2C_4H_7NO_3.4H_2O$ and $C_{11}H_{12}N_2O$ in the sample.

Monograph: Pharmaceutical substances: isometamidium chloride hydrochloride



	R^1	R^2	R^3	n
I	X	H	H	1
II	H	X	H	1
III	H	H	X	1
IV	X	X	H	2

Where X =



Chemical name: isometamidium chloride hydrochloride is a mixture of 8-(*m*-amidinophenyldiazoamino)-3-amino-5-ethyl-6-phenylphenanthridinium chloride hydrochloride (I, principal component), its positional isomer 3-(*m*-amidinophenyldiazoamino)-8-amino-5-ethyl-6-phenylphenanthridinium chloride hydrochloride (II), 7-(*m*-amidinophenyldiazo)-3,8-diamino-5-ethyl-6-phenylphenanthridinium chloride hydrochloride (III) and 3,8-di-(3-*m*-amidinophenyltriazeno)-5-ethyl-6-phenylphenanthridinium chloride dihydrochloride (IV).

Description: dark purple to brown powder.

Solubility: dissolve 1 g isometamidium chloride hydrochloride in water R and make up to 20 ml with the same solvent. The isometamidium chloride hydrochloride dissolves completely to give a red solution free from particulate matter within 4 min. Isometamidium chloride hydrochloride is also soluble in dilute acetic acid.

Category: trypanocidal agent.

Storage: store in a closed container excluding light and humidity.

Requirements: the content is expressed as the sum of the three isomers 8-(*m*-amidinophenyldiazoamino)-3-amino-5-ethyl-6-phenylphenanthridinium chloride hydrochloride (I, principal component), 3-(*m*-amidinophenyldiazoamino)-8-amino-5-ethyl-6-phenylphenanthridinium chloride hydrochloride (II) and 7-(*m*-amidinophenyldiazo)-3,8-diamino-5-ethyl-6-phenylphenanthridinium chloride hydrochloride (III), together with the structurally related compound 3,8-di-(3-*m*-amidinophenyltriazeno)-5-ethyl-6-phenylphenanthridinium chloride dihydrochloride (IV). The material must contain all four components and isomer I must account for at least 55%, with the other components accounting for not more than 40% and an overall minimum content of 90% for the four components.

Identity tests: either tests A and B or tests B and C may be used.

A. Carry out the test as described in Section 1.14.1 (Thin layer chromatography) in the International Pharmacopoeia[1] using a silica gel G (0.25 mm) and a mixture of 6 volumes pyridine R, 6 volumes acetonitrile R, 4 volumes 1-butanol R and 1 volume formic acid R as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions in methanol R: solution (a), isometamidium chloride hydrochloride RS 1 mg/ml; solution (b), isometamidium chloride hydrochloride test sample 1 mg/ml; solution (c), homidium chloride RS 1 mg/ml. Allow the solvent front to ascend 10 cm above the line of application. After removal of the plate, allow it to air dry for 10 min and examine the chromatogram under white light. For solution (a), four well-resolved coloured spots are visible under white light. From the point of application the colours of the spots should be yellow (IV), purple (III), orange (I) and orange (II). The chromatogram for solution (b) should match the chromatogram for solution (a); disregard a purple spot near the origin. For solution (c) there should only be one red spot with an Rf value greater than all the constituents in solutions (a) and (b).

B. Carry out the examination as described in Section 1.6 (Spectrophotometry in the visible and ultraviolet regions) in the International Pharmacopoeia[1]. The light absorption, in the range 250 nm to 450 nm, of a 0.0001% w/v solution in water R exhibits maxima at 276 nm, 312 nm and 379 nm. The absorbance at 379 nm is about 1.11 and the absorbance ratios $A_{276/379}$ and $A_{312/379}$ are about 0.9 and 0.9 respectively.

C. In the assay, the retention times of the peaks in the chromatogram of solution (b) are the same as those in the chromatogram of solution (a).

Determination of pH: determine as described in Section 1.13 (Determination of pH) in the International Pharmacopoeia[1]. The pH of a 1% w/v isometamidium chloride hydrochloride solution in carbon-dioxide-free water R at 20°C is between 4 and 7.

Loss on drying: dry 1 g isometamidium chloride hydrochloride to constant weight at 105°C under reduced pressure; it loses not more than 8% w/w.

Water: determine as described in Section 2.8 (Determination of water by the Karl Fischer method, Method A) in the International Pharmacopoeia[1] using 0.100 g of the substance; the water content is not more than 8% w/w.

Sulfated ash: determine as described in Section 2.3 (Sulfated ash) in the International Pharmacopoeia[1]. Determined in 0.5 g isometamidium chloride hydrochloride, it does not exceed 1% w/w.

Chlorides: determine as described in Section 2.2.1 (Limit test for chlorides) in the International Pharmacopoeia[1]. The chloride content expressed in terms of the dried substance should not be more than 14% w/w.

Related substances: carry out the test described in Section 1.14.4 (High-performance liquid chromatography) in the International Pharmacopoeia[1] using a stainless steel column (150 mm × 4.6 mm) packed with microparticles of octadecyl silica (5 µm). Mobile phase: mix 25 volumes of acetonitrile R with 75 volumes of a 50 mM solution of ammonium formate R in water R (3.15 g/l) previously adjusted to pH 2.8 with formic acid R. Prepare the following solutions in 25% v/v acetonitrile R in water R immediately before use: (a) isometamidium chloride hydrochloride 0.1 mg/ml; (b) isometamidium chloride hydrochloride RS 0.1 mg/ml; (c) homidium chloride RS 0.10 mg/ml.

Operate at flow rate 0.7 ml/min with an ultraviolet spectrophotometer detector set at wavelength 254 nm.

Inject 20 µl each of solutions (a), (b) and (c). The run time should be 5 min longer than the principal peak obtained in the chromatogram of solution (c).

Identification of components. Use the chromatogram supplied with isometamidium chloride hydrochloride RS for peak identification.

Relative retention. With reference to the principal isomer 8-(*m*-amidinophenyldiazoamino)-3-amino-5-ethyl-6-phenylphenanthridinium chloride hydrochloride (I, retention time about 6 minutes): 3-(*m*-amidinophenyldiazoamino)-8-amino-5-ethyl-6-phenylphenanthridinium chloride hydrochloride (II, about 1.2); 7-(*m*-amidinophenyldiazo)-3,8-diamino-5-ethyl-6-phenylphenanthridinium chloride hydrochloride (III, about 0.82); 3,8-di-(3-*m*-amidino-phenyltriazeno)-5-ethyl-6-phenylphenanthridinium chloride dihydrochloride (IV, about 0.56); impurity A (homidium, about 2).

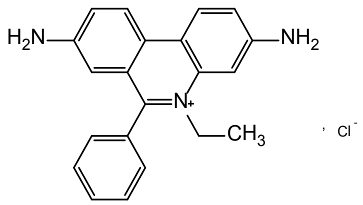
System suitability. The resolution between the two isomers 8-(*m*-amidinophenyldiazoamino)-3-amino-5-ethyl-6-phenylphenanthridinium chloride hydrochloride (I, principal component) and 3-(*m*-amidinophenyldiazoamino)-8-amino-5-ethyl-6-phenylphenanthridinium chloride hydrochloride (II) should be at least 2.5.

Limit for impurities: the content of impurity A should not be more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (≤1% w/w).

Assay: measure the areas of the peak responses obtained in the chromatograms of solutions (a) and (b) in the related substances test and calculate the percentage content of the components of isometamidium chloride hydrochloride from the declared content of isometamidium chloride hydrochloride RS.

Impurities:

Impurity A



A: 3,8-diamino-5-ethyl-6-phenylphenanthridinium chloride (homidium chloride).

Monograph: Dosage forms: Specific monograph: isometamidium chloride hydrochloride veterinary powder

Description: dark purple to brown powder.

Solubility: dissolve 1 g isometamidium chloride hydrochloride in water R and make up to 20 ml with the same solvent. The isometamidium chloride hydrochloride dissolves completely to give a red solution free from particulate matter within 4 min.

Category: trypanocidal agent.

Storage: store in a closed container excluding light and humidity.

Labelling: the designation isometamidium chloride hydrochloride veterinary powder indicates that the substance complies with the general monograph regarding parenteral preparations in the International Pharmacopoeia[1] and may be used for parenteral administration. Expiry date.

Additional information: isometamidium chloride hydrochloride is a mixture of 8-(*m*-amidinophenyldiazoamino)-3-amino-5-ethyl-6-phenylphenanthridinium chloride hydrochloride (principal component), its positional isomer 3-(*m*-amidinophenyldiazoamino)-8-amino-5-ethyl-6-phenylphenanthridinium chloride hydrochloride, 7-(*m*-amidinophenyldiazo)-3,8-diamino-5-ethyl-6-

phenylphenanthridinium chloride hydrochloride and 3,8-di-(3-*m*-amidinophenyltriazeno)-5-ethyl-6-phenylphenanthridinium chloride dihydrochloride.

Definition: isometamidium chloride hydrochloride veterinary powder (may contain antipyrine as an excipient) must contain all four components and the principal component must account for not less than 55% of the stated content of isometamidium chloride hydrochloride, with the sum of the other components accounting for not more than 40%. The total content of isometamidium chloride hydrochloride should be 95% to 102% with respect to the stated content.

Identity tests: either tests A and B or tests B and C may be used.

A. Carry out the test as described in Section 1.14.1 (Thin layer chromatography) in the International Pharmacopoeia[1] using a silica gel G (0.25 mm) and a mixture of 6 volumes pyridine R, 6 volumes acetonitrile R, 4 volumes 1-butanol R and 1 volume formic acid R as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions in methanol R: solution (a), isometamidium chloride hydrochloride RS 1 mg/ml; solution (b), isometamidium chloride hydrochloride 1 mg/ml; solution (c), homidium chloride RS 1 mg/ml. Allow the solvent front to ascend 10 cm above the line of application. After removal of the plate, allow it to air dry for 10 min and examine the chromatogram under white light. For solution (a), four well-resolved coloured spots are visible under white light. From the point of application the colours of the spots should be yellow, purple, orange and orange. The chromatogram for solution (b) should match the chromatogram for solution (a); disregard a purple spot near the origin. For solution (c) there should only be one red spot with an Rf value greater than all the constituents in solutions (a) and (b).

B. Carry out the examination as described in Section 1.6 (Spectrophotometry in the visible and ultraviolet regions) in the International Pharmacopoeia[1]. The light absorption in the range 250 nm to 450 nm of a 0.0001% w/v solution in water R exhibits maxima at 276 nm, 312 nm and 379 nm. The absorbance at 379 nm is about 1.11 and the absorbance ratios $A_{276/379}$ and $A_{312/379}$ are about 0.90 and 0.90 respectively. When antipyrine is present in the formulation the absorbance ratio ($A_{312/379}$) should be used.

C. In the assay, the retention times of the peaks in the chromatogram of solution (b) are the same as those in the chromatogram of solution (a).

Determination of pH: determine as described in Section 1.13 (Determination of pH) in the International Pharmacopoeia[1]. The pH of 1% w/v isometamidium chloride hydrochloride solution in carbon-dioxide-free water R at 20°C is between 4 and 7.

Loss on drying: dry 1 g isometamidium chloride hydrochloride to constant weight at 105°C under reduced pressure; it loses not more than 8% w/w.

Water: determine as described in Section 2.8 (Determination of water by the Karl Fischer method, Method A) in the International Pharmacopoeia[1] using 0.100 g of the substance; the water content is not more than 8% w/w.

Sulfated ash: determine as described in Section 2.3 (Sulfated ash) in the International Pharmacopoeia[1]. Determined in 0.5 g isometamidium chloride hydrochloride, it does not exceed 1% w/w.

Chlorides: determine as described in Section 2.2.1 (Limit test for chlorides) in the International Pharmacopoeia[1]. The chloride content expressed in terms of the dried substance should not be more than 14% w/w.

Assay: carry out the test described in Section 1.14.4 (High-performance liquid chromatography) in the International Pharmacopoeia[1] using a stainless steel column (150 mm × 4.6 mm) packed with microparticles of octadecyl silica (5 µm). Mobile phase: mix 25 volumes of acetonitrile R with 75 volumes of a 50 mM solution of ammonium formate R in water R (3.15 g/l) previously adjusted to pH 2.8 with formic acid R. Prepare the following solutions in 25% v/v acetonitrile R in water R immediately before use: solution (a), place an accurately weighed quantity of the formulation equivalent to about 100 mg of isometamidium chloride hydrochloride in a 100 ml volumetric flask and make up to volume; solution (b), transfer 10 ml of solution (a) to a 100 ml volumetric flask and dilute to volume; solution (c), isometamidium chloride hydrochloride RS 0.1 mg/ml.

Operate at flow rate 0.7 ml/min with an ultraviolet spectrophotometer detector set at wavelength 254 nm. If antipyrine is present in the preparation, the concentration of acetonitrile R should be adjusted to ensure that it separates from the first eluting compound in solution (b).

Inject 20 µl each of solutions (b) and (c). The run time should be 15 min.

Identification of components. Use the chromatogram supplied with isometamidium chloride hydrochloride RS for peak identification.

Relative retention. With reference to the principal isomer 8-(*m*-amidinophenyldiazoamino)-3-amino-5-ethyl-6-phenylphenanthridinium chloride hydrochloride (retention time about 6 minutes): 3-(*m*-amidinophenyldiazoamino)-8-amino-5-ethyl-6-phenylphenanthridinium chloride hydrochloride (about 1.2); 7-(*m*-amidinophenyldiazo)-3,8-diamino-5-ethyl-

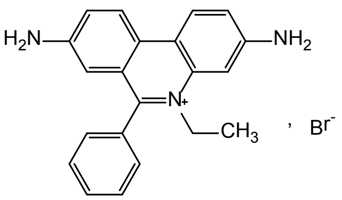
6-phenylphenanthridinium chloride hydrochloride (about 0.82); 3,8-di-(3-*m*-amidinophenyltriazeno) -5-ethyl-6-phenylphenanthridinium chloride dihydrochloride (about 0.56).

System suitability. Reference solution (c).

Resolution: minimum 2.5 between the two isomers presenting a relative retention time of about 1.2.

Measure the areas of the peak responses in the chromatograms from solutions (a) and (b) and calculate the percentage content of the components with reference to the dried substance.

Monograph: Pharmaceutical substances: homidium bromide



$C_{21}H_{20}N_3Br$

Relative molecular mass: 394.3

Chemical names: homidium bromide; ethidium bromide; 3,8-diamino-5-ethyl-6-phenylphenanthridinium bromide. CAS RN: 1239-45-8.

Description: dark red crystalline or amorphous powder.

Solubility: dissolve 1 g homidium bromide in water R and make up to 20 ml with the same solvent. Homidium bromide dissolves completely to give a red solution free from particulate matter. Homidium bromide is also slightly soluble in chloroform R.

Category: trypanocidal agent.

Storage: store in a closed container excluding light and humidity.

Requirements: homidium bromide contains not less than 98% and not more than the equivalent of 102% of $C_{21}H_{20}N_3Br$ calculated with reference to the dried substance.

Identity tests: either A and C or B and C may be used.

A. Carry out the examination as described in Section 1.7 (Spectrophotometry in the infrared region) in the International Pharmacopoeia[1]. The infrared absorption spectrum is concordant with the spectrum obtained from homidium bromide RS or with the reference spectrum of homidium bromide.

B. Carry out the examination as described in Section 1.6 (Spectrophotometry in the visible and ultraviolet regions) in the International Pharmacopoeia[1]. The light absorption in the range 250 nm to 450 nm of a 0.001% w/v solution in water R exhibits a maximum at 283 nm.

C. In the assay, the retention time of the peak in the chromatogram of solution (b) is the same as that in the chromatogram of solution (a).

Determination of pH: determine as described in Section 1.13 (Determination of pH) in the International Pharmacopoeia[1]. The pH of solution of a 2% w/v of homidium bromide in carbon-dioxide-free water R at 20°C is between 4 and 7.

Loss on drying: dry 1 g homidium bromide to constant weight at 130°C under reduced pressure; it loses not more than 10% w/w.

Water: determine as described in Section 2.8 (Determination of water by the Karl Fischer method, Method A) in the International Pharmacopoeia[1] using 0.1 g of the substance; the water content is not more than 2% w/w.

Sulfated ash: determine as described in Section 2.3 (Sulfated ash) in the International Pharmacopoeia[1]. Determined in 0.5 g homidium bromide, it does not exceed 0.2% w/w.

Iron: determine as described in Section 2.2.4 (Limit test for iron) in the International Pharmacopoeia[1]. Fuse the residue from the sulfated ash test (see above) with 1 g anhydrous sodium carbonate and allow to cool. Add 10 ml 10% (m/m) hydrochloric acid and after the vigorous reaction has subsided, heat on a steam bath for 10 min. Then transfer to a 50 ml flask. In a second 50 ml flask, add 10 ml water R and 2 ml standard iron solution (Fe III solution, 20 ppm). To both flasks, add 2 ml 20% (m/v) citric acid solution and 1 ml 10% (m/v) thioglycollic acid solution, make just alkaline to litmus paper with 10% (m/m) ammonia and add 1 ml in excess. Dilute to 50 ml with water R, mix and set aside for 5 min. Any colour produced in the sample solution should not exceed that in the standard.

Related substances: carry out the test as described in Section 1.14.4 (High-performance liquid chromatography) in the International Pharmacopoeia[1] using a stainless steel column (150 mm × 4.6 mm) packed with microparticles of octadecyl silica (5 µm). Mobile phase: mix 35 volumes of acetonitrile R with 65 volumes of a 20 mM solution of ammonium formate R in water R (1.26 g/l) previously adjusted to pH 2.8 with formic acid R. Prepare the following solutions in 35% v/v acetonitrile R in water R immediately before use: solution (a), homidium bromide 1 mg/ml; solution (b) homidium bromide 0.1 mg/ml.

Operate at flow rate 1 ml/min with an ultraviolet spectrophotometer detector set at wavelength 290 nm.

Inject 20 µl each of solutions (a) and (b) and allow 10 min analysis time for each sample. Measure the areas of the peak responses obtained in the chromatograms from solutions (a) and (b) and calculate the related substances as a percentage. In the chromatogram for solution (a), the area of any peak, other than the principal peak, is not greater than that obtained with solution (b). The sum of the areas of all peaks, other than the principal peak, is not greater than twice the area of that obtained with solution (b). Disregard any peak with an area less than 0.1% of the principal peak in the chromatogram obtained with solution (b). The test is not valid unless the relative standard deviation of the retention time of the principal peak is not greater than 1.

Assay: carry out the test as described in Section 1.14.4 (High-performance liquid chromatography) in the International Pharmacopoeia[1] using a stainless steel column (150 mm × 4.6 mm) packed with microparticles of octadecyl silica (5 µm). Mobile phase: mix 35 volumes of acetonitrile R with 65 volumes of a 20 mM solution of ammonium formate R in water R (1.26 g/l) previously adjusted to pH 2.8 with formic acid R. Prepare the following solutions in 35% v/v acetonitrile R in water R immediately before use: solution (a) homidium bromide 0.1 mg/ml; solution (b) homidium bromide RS 0.1 mg/ml.

Operate at flow rate 1 ml/min with an ultraviolet spectrophotometer detector set at wavelength 290 nm.

Inject 20 µl each of solutions (a) and (b) and allow 10 min analysis time for each sample. Measure the areas of the peak responses obtained in the chromatograms from solutions (a) and (b) and calculate the content of $C_{21}H_{20}N_3Br$.

Monograph: Dosage forms: Specific monograph: homidium bromide tablets

Category: trypanocidal agent

Storage: store in a closed container excluding light and humidity.

Requirements: complies with the general monograph regarding tablets in the International Pharmacopoeia[1]. Homidium bromide tablets contain not less than 90% and not more than 110% of the amount of $C_{21}H_{20}N_3Br$ stated on the label.

Labelling: the designation homidium bromide tablets for injection indicates that the substance complies with the general monographs regarding tablets and parenteral preparations in the International Pharmacopoeia[1] and may be used for intramuscular administration. Expiry date.

Additional information: homidium bromide tablets contain principally homidium bromide ($C_{21}H_{20}N_3Br$).

Identity tests: either A and C or B and C may be used.

Extract a quantity of the powdered tablets containing 0.1 g of homidium bromide with 10 ml of absolute ethanol, filter and evaporate the filtrate to dryness. The residue complies with the following test(s):

A. Carry out the examination as described in Section 1.7 (Spectrophotometry in the infrared region) in the International Pharmacopoeia[1]. The infrared absorption spectrum is concordant with the spectrum obtained from homidium bromide RS or with the reference spectrum of homidium bromide.

B. Carry out the examination as described in Section 1.6 (Spectrophotometry in the visible and ultraviolet regions) in the International Pharmacopoeia[1]. The light absorption in the range 230 nm to 450 nm of a 0.001% w/v solution exhibits a maximum at 283 nm.

C. In the assay, the retention time of the peak in the chromatogram of solution (b) is the same as that in the chromatogram of solution (a).

Assay: carry out the test as described in Section 1.14.4 (High-performance liquid chromatography) in the International Pharmacopoeia[1] using a stainless steel column (150 mm × 4.6 mm) packed with microparticles of octadecyl silica (5 µm). Mobile phase: mix 35 volumes of acetonitrile R with 65 volumes of a 20 mM solution of ammonium formate R in water R (1.26 g/l) previously adjusted to pH 2.8 with formic acid R. Weigh and powder 20 homidium bromide tablets. Prepare the following solutions immediately before use: solution (a), a quantity of tablet powder equivalent to about 0.1 g of homidium bromide in 100 ml water R; solution (b), dilute solution (a) 1:5 with acetonitrile R:water R (35:65); solution (c), homidium bromide RS 0.5 mg/ml.

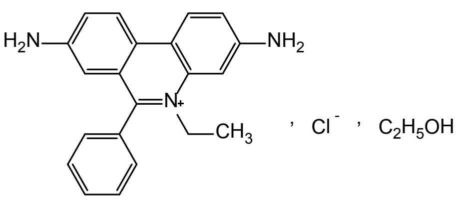
Operate at flow rate 1 ml/min with an ultraviolet spectrophotometer detector set at wavelength 290 nm.

Inject 20 µl each of solutions (b) and (c) and allow 10 min analysis time for each sample. Measure the areas of the peak responses obtained in the chromatograms from solutions (b) and (c) and calculate the content of $C_{21}H_{20}N_3Br$.

Dissolution: carry out the examination as described in Section 5.3 (Disintegration test for tablets and capsules) in the International Pharmacopoeia[1]. Place one tablet of homidium bromide in a 100 ml flask and add 10 ml of purified water R at 20°C. Mix vigorously with an electromagnetic agitator. The homidium bromide tablet dissolves completely to give a red solution free from particulate matter within 10 min.

Uniformity of mass: carry out the examination as described in Section 5.2 (Uniformity of mass for single-dose preparations) in the International Pharmacopoeia[1]. Not more than 2 of 20 individual masses deviate from average mass by more than 5% and none deviates by more than 10%. If film-coated tablets fail this test it may be because of variability in the thickness (mass) of the coatings. In such a case, the test in Section 5.1 (Uniformity of content for single-dose preparations) in the International Pharmacopoeia[1] should be used; if the tablets meet the requirement of this test, they can be considered acceptable.

Monograph: Pharmaceutical substances: homidium chloride ethanolate



$C_{21}H_{20}N_3Cl.C_2H_5OH$

Relative molecular mass: 395.9.

Chemical name: homidium chloride; ethidium chloride; 3,8-diamino-5-ethyl-6-phenyl-phenanthridinium chloride ethanolate. CAS RN: 602-52-8.

Description: dark red crystalline powder.

Solubility: dissolve 1.25 g homidium chloride ethanolate in water R and make up to 50 ml with the same solvent. The homidium chloride ethanolate dissolves completely to give a red solution free from particulate matter.

Category: trypanocidal agent.

Storage: store in a closed container excluding light and humidity.

Requirements: homidium chloride ethanolate contains not less than 98% and not more than the equivalent of 102% of $C_{21}H_{20}N_3Cl.C_2H_5OH$ calculated with reference to the dried substance.

Identity tests: either A and C or B and C may be used.

A. Carry out the examination as described in Section 1.7 (Spectrophotometry in the infrared region) in the International Pharmacopoeia[1]. The infrared absorption spectrum is concordant with the spectrum obtained from homidium chloride ethanolate RS or with the reference spectrum of homidium chloride ethanolate.

B. Carry out the examination as described in Section 1.6 (Spectrophotometry in the visible and ultraviolet region) in the International Pharmacopoeia[1]. The light absorption in the range 250 nm to 450 nm of a 0.001% w/v solution in water R exhibits a maximum at 283 nm.

C. In the assay, the retention time of the peak in the chromatogram of solution (b) is the same as that in the chromatogram of solution (a).

Determination of pH: determine as described in Section 1.13 (Determination of pH) in the International Pharmacopoeia[1]. The pH of solution of a 1% w/v of homidium chloride ethanolate in carbon dioxide-free water R at 20°C is between 4 and 7.

Loss on drying: dry 1 g homidium chloride ethanolate to constant weight at 130°C under reduced pressure; it loses not more than 10% w/w.

Water: determine as described in Section 2.8 (Determination of water by the Karl Fischer method, Method A) in the International Pharmacopoeia[1] using 0.1 g of the substance; the water content is not more than 2% w/w.

Sulfated ash: determine as described in Section 2.3 (Sulfated ash) in the International Pharmacopoeia[1]. Determined in 0.5 g homidium chloride ethanolate, it does not exceed 0.2% m/m.

Chlorides: determine as described in Section 2.2.1 (Limit test for chlorides) in the International Pharmacopoeia[1]. The chloride content expressed in terms of the dried substance should not be more than 10.4% m/m.

Iron: determine as described in Section 2.2.4 (Limit test for iron) in the International Pharmacopoeia[1]. Fuse the residue from the sulfated ash test (see above) with 1 g anhydrous sodium carbonate and allow to cool. Add 10 ml 10% (m/m) hydrochloric acid. After the vigorous reaction has subsided, heat on a steam bath for 10 min, then transfer to a 50 ml flask. In a second 50 ml flask, add 10 ml of water R and 2 ml of standard iron solution (Fe III solution, 20 ppm). To both flasks, add 2 ml 20% (m/v) citric acid solution and 1 ml 10% (m/v) thioglycollic acid solution, make just alkaline to litmus paper with 10% (m/m) ammonia and add 1 ml in excess. Dilute to 50 ml with water R, mix and set aside for 5 min. Any colour produced in the sample solution should not exceed that in the standard.

Related substances: carry out the test as described in Section 1.14.4 (High-performance liquid chromatography) in the International Pharmacopoeia[1] using a stainless steel column (150 mm × 4.6 mm) packed with microparticles of octadecyl silica (5 µm). Mobile phase: mix 35 volumes of acetonitrile R with 65 volumes of a 20 mM solution of ammonium formate R in water R (1.26 g/l) previously adjusted to pH 2.8 with formic acid R. Prepare the following solutions in 35% v/v acetonitrile R in water R immediately before use: solution (a), homidium chloride ethanolate 1 mg/ml; solution (b), homidium chloride ethanolate 0.1 mg/ml.

Operate at flow rate 1 ml/min with an ultraviolet spectrophotometer detector set at wavelength 290 nm.

Inject 20 µl each of solutions (a) and (b) and allow 10 min analysis time for each sample. Measure the areas of the peak responses obtained in the chromatograms from solutions (a) and (b) and calculate the related substances as a percentage. In the chromatogram of solution (a), the area of any peak, other than the principal peak, is not greater than that obtained with solution (b). The sum of the areas of all peaks, other than the principal peak, is not greater than twice the area of that obtained with solution (b). Disregard any peak with an area less than 0.1% of the principal peak in the chromatogram of solution (b). The test is not valid unless the relative standard deviation of the retention time of the principal peak is not greater than 1.

Assay: carry out the test as described in Section 1.14.4 (High-performance liquid chromatography) in the International Pharmacopoeia[1] using a stainless steel column (150 mm × 4.6 mm) packed with microparticles of octadecyl silica (5 µm). Mobile phase: mix 35 volumes of acetonitrile R with 65 volumes of a 20 mM solution of ammonium formate R in water R (1.26 g/l) previously adjusted to pH 2.8 with formic acid R. Prepare the following solutions in 35% v/v acetonitrile R in water R immediately before use: solution (a), homidium chloride ethanolate 0.1 mg/ml; solution (b), homidium chloride ethanolate RS 0.1 mg/ml.

Operate at flow rate 1 ml/min with an ultraviolet spectrophotometer detector set at wavelength 290 nm.

Inject 20 µl each of solutions (a) and (b) and allow 10 min analysis time for each sample. Measure the areas of the peak responses obtained in the chromatograms from solutions (a) and (b) and calculate the content of $C_{21}H_{20}N_3Cl.C_2H_5OH$.

Monograph: Dosage forms: Specific monograph: homidium chloride tablets

Category: trypanocidal agent.

Storage: store in a closed container excluding light and humidity.

Requirements: complies with the general monograph regarding tablets in the International Pharmacopoeia[1]. Homidium chloride tablets contain not less than 90% and not more than 110% of the amount of $C_{21}H_{20}N_3Cl.C_2H_5OH$ stated on the label.

Labelling: the designation homidium chloride tablets for injection indicates that the substance complies with the general monographs regarding tablets, and parenteral preparations in the International Pharmacopoeia[1] and may be used for intramuscular administration. Expiry date.

Additional information: homidium chloride tablets contain a mixture of homidium chloride ethanolate ($C_{21}H_{20}N_3Cl.C_2H_5OH$).

Identity tests: Either A and C or B and C may be used.

Extract a quantity of powdered tablets containing 0.1 g of homidium chloride ethanolate with 10 ml of absolute ethanol, filter and evaporate the filtrate to dryness. The residue complies with the following test(s):

A. Carry out the examination as described in Section 1.7 (Spectrophotometry in the infrared region) in the International Pharmacopoeia[1]. The infrared absorption spectrum is concordant with the spectrum obtained from homidium chloride ethanolate RS or with the reference spectrum of homidium chloride ethanolate.

B. Carry out the examination as described in Section 1.6 (Spectrophotometry in the visible and ultraviolet region) in the International Pharmacopoeia[1]. The light absorption in the range 230 nm to 450 nm of a 0.001% w/v solution exhibits a maximum at 283 nm.

C. In the assay, the retention time of the peak in the chromatogram of solution (b) is the same as that in the chromatogram of solution (a).

Assay: carry out the test as described in Section 1.14.4 (High-performance liquid chromatography) in the International Pharmacopoeia[1] using a stainless steel column (150 mm × 4.6 mm) packed with microparticles of octadecyl silica (5 µm). Mobile phase: mix 35 volumes of acetonitrile R with 65 volumes of a 20 mM solution of ammonium formate R in water R (1.26 g/l) previously adjusted to pH 2.8 with formic acid R. Weigh and powder 20 homidium chloride tablets. Prepare the following solutions immediately before use: solution (a), a quantity of tablet powder equivalent to about 0.1 g of homidium chloride ethanolate in 100 ml water R; solution (b), dilute solution (a) 1:5 with acetonitrile R:water R (35:65); solution (c), homidium chloride ethanolate RS 0.5 mg/ml.

Operate at flow rate 1 ml/min with an ultraviolet spectrophotometer detector set at wavelength 290 nm.

Inject 20 µl each of solutions (b) and (c) and allow 10 min analysis time for each sample. Measure the areas of the peak responses obtained in the chromatograms from solutions (b) and (c) and calculate the content of $C_{21}H_{20}N_3Cl.C_2H_5OH$.

Dissolution: carry out the examination as described in Section 5.3 (Disintegration test for tablets and capsules) in the International Pharmacopoeia[1]. Place one tablet of homidium chloride in a 100 ml flask and add 10 ml purified water R at 20°C. Mix vigorously with an electromagnetic agitator. The homidium chloride tablet dissolves completely to give a red solution free from particulate matter within 10 min.

Uniformity of mass: carry out the examination as described in Section 5.2 (Uniformity of mass for single-dose preparations) in the International Pharmacopoeia[1]. Not more than 2 of 20 individual masses deviate from average mass by more than 5% and none deviates by more than 10%. If film-coated tablets fail this test it may be because of variability in the thickness (mass) of the coatings. In such a case, the test in Section 5.1 (Uniformity of content for single-dose preparations) in the International Pharmacopoeia[1] should be used; if the tablets meet the requirement of this test, they can be considered acceptable.

References

1. World Health Organization. The International Pharmacopoeia. Available at: apps.who.int/phint/en/p/docf/ (accessed on 24 January 2012).