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Development and validation of an improved HPLC method for the control of potentially counterfeit isometamidium products

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Abstract

Isometamidium, a mixture of related substances of which 8-(3-*m*-amidinophenyl-2-triazeno)-3-amino-5-ethyl-6-phenylphenanthridinium chloride hydrochloride (M&B4180A) is the principal active component, is the only chemical agent available for prophylaxis of veterinary trypanosomiasis. A method for the simultaneous quantitation of the major constituents M&B4180A, 3-(3-*m*-amidinophenyl-2-triazeno)-8-amino-5-ethyl-6-phenylphenanthridinium chloride hydrochloride (M&B38897), 7-(*m*-amidinophenyldiazo)-3,8-diamino-5-ethyl-6-phenylphenanthridinium chloride hydrochloride (M&B4250) and 3,8-di(3-*m*-amidinophenyltriazeno)-5-ethyl-6-phenylphenanthridinium chloride dihydrochloride (M&B4596) is described. The related substances are resolved on a Gemini C18 column (150 mm × 4.6 mm, 5 μm) using a mobile phase composed of a mixture of acetonitrile and 50 mM ammonium formate buffer pH 2.8 (25:75 v/v) at a flow rate of 1 ml/min with UV detection at 320 nm. The method is compatible with electrospray ionisation mass spectrometry and provides a tool for the control of substandard and counterfeit commercial preparations of isometamidium.

Keywords: Isometamidium; HPLC; LC–MS; Counterfeit medicines; Related substances

1. Introduction

The counterfeiting of medicines is a global problem, which has been on the ascendancy in recent years, and the incidence is usually significant in regions with weak drug regulatory and legal infrastructures. The international medical products anti-counterfeiting task force (IMPACT) estimates that more than 30% of the medicines on sale in some areas in Africa, Asia and Latin America may be counterfeit [1–3]. Whilst the focus of counterfeiting and substandard medication has been on human medicines, the veterinary market, which is relatively less regulated in developing economies, provides an unfortunate haven for counterfeit and substandard products [4].

Veterinary trypanosomiasis is arguably the most important constraint to cattle production in affected areas in sub-Saharan Africa [5]. The disease is mainly controlled by the use of chemical agents such as isometamidium chloride hydrochloride and diminazene aceturate [6]. Approximately 35 million doses of trypanocides are administered to domestic ruminants annually at an estimated cost of 35–40 million US dollars (*source*: Food and Agricultural Organisation of the United Nations (FAO), Rome). Isometamidium, the only agent available for chemoprophylaxis, is a mixture of related substances (Fig. 1) synthesized by the coupling of diazotised *m*-aminobenzamidine monohydrochloride with 3,8-diamino-5-ethyl-6-phenylphenanthridinium chloride to give a mixture of isomers [7–9]. The pharmacological action is primarily attributed to the most abundant component 8-[(*m*-amidinophenylazo)-amino]-3-amino-5-ethyl-6-phenyl phenanthridinium chloride hydrochloride (M&B4180A). The benzene diazonium ion is a weak electrophile, so the presence of the electron-withdrawing amidino substituent moiety greatly extends the range of coupling [10]. The nature and extent of these coupling reactions are influenced by the temperature and pH of the reaction medium. The correlation between the latter and the formation of M&B4180 and the related substances has been previously described [10]. The translation of these syntheses issues into the production of chemically non-equivalent commercial preparations was demonstrated with the only two commer-

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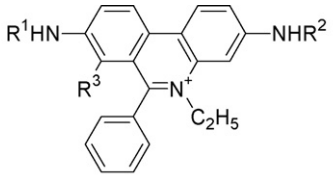

	R1	R2	R3
M&B 4180A	X	H	H
M&B 38897	H	X	H
M&B 4250	H	H	X
M&B 4596	X	X	H
Ethidium	H	H	H

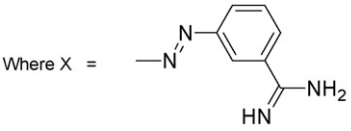

Fig. 1. Chemical structures of M&B4180A and the related substances resulting from the coupling of diazotised *m*-aminobenzamidine (X) with 3,8-diamino-5-ethyl-6-phenylphenanthridinium chloride (ethidium).

cially available products: the innovator product Samorin® and Veridium® in 1998 [11]. These products varied in terms of the amounts of the active component, related substances and the inorganic impurity ammonium chloride.

Since the introduction of isometamidium in 1958 [12], internationally agreed standards and monographs are either lacking or inaccurate. With the recent influx of different brands of isometamidium from several manufacturers onto the sub-Saharan African market and the world-wide problem of counterfeiting of medicines, it has become necessary to define a suitable method for controlling the major constituents of commercial samples. This study describes the development and validation of a simple HPLC method for the quantification of M&B4180A and the major related substances (M&B4250, M&B38897 and M&B4596) in commercial samples. The method, unlike others reported previously, is compatible with electrospray ionisation mass spectrometry and therefore enables the confirmation or otherwise of M&B4180A and related substances in potentially counterfeit commercial products. The method has been applied to a number of commercially available samples of isometamidium to investigate pharmaceutical and/or chemical equivalence and also highlight, for the first time, the incidence of definite fake versions of isometamidium in Africa. This method should provide the basis for the establishment of international specifications for isometamidium.

2. Experimental

2.1. Materials and reagents

HPLC-grade acetonitrile was obtained from VWR International Ltd. (Poole, UK). Analytical reagent grade glacial acetic acid (99.9%) was obtained from Sigma–Aldrich (Dorset, UK) and ammonium acetate was obtained from BDH Laboratory Supplies (Poole, UK). Authentic standards and a secondary reference mixture of isometamidium and related substances containing 8-(3-*m*-amidinophenyl-2-triazeno)-3-amino-5-ethyl-6-phenylphenanthridiniumchloride hydrochloride (ISM, 58.6%, w/w), 3-(3-*m*-amidinophenyl-2-triazeno)-8-amino-5-ethyl-6-phenylphenanthridinium chloride hydrochloride (M&B38897, 13.3%, w/w), 7-(*m*-amidinophenyldiazo)-3,8-diamino-5-ethyl-6-phenylphenanthridinium chloride hydrochloride (M&B4250, 13.7%, w/w) and 3,8-di(3-*m*-amidinophenyltriazeno)-5-ethyl-6-phenylphenanthridinium chloride dihydrochloride (M&B4596, 8.1%) was a kind gift from Merial Limited (Toulouse, France). Commercial preparations of isometamidium were obtained from the open market in West-Africa.

2.2. Preparation of solutions

2.2.1. Standard solutions

For HPLC analysis, 21.4 mg of the mixture of standards was accurately weighed using a Mettler AT20 analytical balance (readability 0.01 mg). The weighed sample was dissolved in, and made up to 25 ml with a 25% (v/v) solution of acetonitrile in water to produce a stock solution containing 0.05% (w/v) M&B4180A, 0.007% (w/v) M&B4596, 0.01% (w/v) M&B38897 and 0.01% (w/v) M&B4250. Aliquots of the stock solution were suitably diluted with 25% (v/v) acetonitrile in water to produce calibration solutions of concentrations described in Section 2.3.

2.2.2. Sample preparation

Samples (85 mg) of different commercial samples of isometamidium were weighed accurately, dissolved in and made up to 100 ml with a solution of 25% (v/v) acetonitrile in water. The resultant solution was diluted 10-fold with 25% (v/v) acetonitrile in water prior to analysis.

2.2.3. Ammonium acetate buffer (100 mM, pH 4.0)

Ammonium acetate (7.71 g) was accurately weighed, dissolved in approximately 800 ml of water and the pH adjusted to a value of 4.0 with acetic acid. The resulting solution was made up to 1 l with water.

2.2.4. Ammonium formate buffer (50 mM, pH 2.8)

Ammonium formate (3.15 g) was accurately weighed, dissolved in approximately 800 ml of water and the pH adjusted to a value of 2.8 with formic acid. The resulting solution was made up to 1 l with water.

2.3. Validation studies

The rectilinear relationship between concentrations of the analytes and the UV detector response was evaluated. The concentrations used were 0.002, 0.004, 0.006, 0.008 and 0.01% (w/v) M&B4180A; 0.0003, 0.0006, 0.0008, 0.0011 and 0.0014% (w/v) M&B4596; 0.0005, 0.0009, 0.0014, 0.0018, 0.0023% (w/v) M&B38897 and 0.0005, 0.0010, 0.0014, 0.0019, 0.0024% (w/v) M&B4250.

The repeatability of the method was assessed over the specified linear ranges described above at three different concentrations (M&B4180A: 0.002, 0.005 and 0.01% (w/v); M&B4596: 0.0003, 0.0007 and 0.0014% (w/v); M&B: 38897 0.0005, 0.0012 and 0.0025% (w/v); M&B4250: 0.0005, 0.0012 and 0.0025% w/w). Three different preparations of the analytical standard were analysed in triplicate on the same day for the determination of intra-day assay precision. These determinations were repeated using freshly prepared standard solutions on three separate days to determine inter-day precision of analysis.

The analysis, in triplicate, of a commercial sample using freshly prepared solutions (as described in Section 2.2.2) on 3 separate days was used to compute the inter-day ($n = 3$ separate determinations) and intra-day precision of the method.

The stability of the analytical solutions was determined for M&B4180A, M&B4596, M&B38897 and M&B4250 at the concentrations described above for the assessment of repeatability. Analytical solutions were injected repeatedly ($n = 30$) over a 60 h period and R.S.D.s % computed for the peak areas due to the respective analytes.

The performance characteristics of the method were based on the resolution between the critical pair (M&B4180A and M&B38897) and the robustness of the method as a function of small changes in the pH (between 2.8 and 3.5), buffer strength (25 and 50 mM ammonium formate) of the mobile phase, stability of analytical solutions and the effect of temperature (20–40 °C) on resolution. The limits of detection and quantitation for each analyte were determined as S/N of three and R.S.D. % ≤ 5%, respectively.

2.4. HPLC/HPLC–MS analysis

An Agilent HP1100 series quaternary pump with ChemStation® software version 10.02 for data acquisition equipped with a HP1100 series UV–vis detector was used. The HPLC was also coupled to an Agilent MSD SL single-quadrupole mass spectrometer via an electrospray ionisation source. M&B4180A and the related substances were separated at ambient temperature on a Gemini C18 column (150 mm × 4.6 mm i.d., 5 μm particle size, 110A°, Phenomenex, Cheshire, UK,) coupled with a Phenomenex C18 Securigard® column. The mobile phase composed of a mixture of acetonitrile and 50 mM ammonium formate buffer pH 2.8 (25:75 v/v) was delivered at a flow rate of 1 ml/min with a split ratio of 1 in 50. For mass spectrometric analysis, compounds were detected using the following conditions: nebulising gas pressure, 10 psi; drying gas flow rate, 7 l/min; drying gas temperature, 300 °C; capillary voltage, 4000 V; positive ion mode; gain: 1; threshold: 150; step size: 0.10; peak width: 0.10 min; cycle time: 1.02 s/cycle. Data was acquired in full scan mode (m/z 100–650) at a fragmentor voltage of 70 V.

3. Results and discussion

Isometamidium and its major related substances contain highly basic amidino and quaternary nitrogen moieties which interact avidly with residual silanols on silica-based columns. Although Kinabo and Bogan [13] proposed the use of ion-pairing reagents to overcome these interactions, the method failed to achieve separation between the related substances, especially the only two positional isomers (M&B4180A and M&B38897). Tettey et al. [14] described a RP-HPLC method based on the use of phosphate buffer (20 mM, pH 3) as the aqueous component of the mobile phase. The method that was validated with respect to the principal component (M&B4180A) was successful in achieving baseline separation of all the known constituents in commercial isometamidium. However, the method did not facilitate absolute quantification of the major manufacturing impurities M&B38897 and M&B4250 which can vary significantly in the manufacturing process [7,10]. This was due to the unavailability of authentic reference standards at the time. Boibessot et al. [15] reported an MS compatible method involving the use of formic acid in the analysis of the putative metabolites of the single component M&B4180. However, the method was neither designed nor validated for the known manufacturing impurities. In the present study, using a well-characterised analytical standard of M&B4180A and the known impurities, the suitability of NH_4^+ containing mobile phases, which provide a competing ion to reduce analyte–silanol interactions, has been investigated.

Generally, the increase in the ionic strength of either the ammonium formate or ammonium acetate buffers up to concentrations of 50 and 100 mM, respectively resulted in an improvement in the chromatographic peak shapes. This observation is consistent with competition between the highly basic moieties in these phenanthridinium salts and the ammonium ions for electrostatic interaction with residual silanols. Optimal separation of the analytes (Fig. 2), as judged by the resolution between the critical pair M&B4180A and M&B38897, was achieved with mobile phases containing either ammonium acetate (100 mM, pH 4.0, Fig. 2a) or ammonium formate (50 mM, pH 2.8, Fig. 2b). Although the ammonium acetate buffer produced an R_s value of 2.5 for the critical pair, the asymmetric nature of the peak due to M&B4596 ($A_s = 4.1$) precluded its use (Fig. 2a). The presence of two highly basic amidino moieties in addition to the quaternary nitrogen in M&B4596 makes it particularly susceptible to peak tailing due to interactions with residual silanols. Ammonium formate, in contrast to ammonium acetate, produced a better peak shape for M&B4596 with a peak symmetry factor of approximately 2.3 and an improved R_s value of 3.0 for the critical pair at a pH of 2.8 (Fig. 2b). This was achieved at a reduced buffer concentration of 50 mM compared with 100 mM for ammonium acetate. Due to the instability of some silica-based columns at pH values of below 2.8, the method was further evaluated using ammonium formate buffer at a pH of 3.5 (Fig. 2c). Although the altered conditions produced an increase in the retention times of the analytes, the R_s value for the critical pair was ≥2.5 and illustrates a wider flexibility of the proposed conditions in terms of choice of pH within the effective buffering range of ammonium formate (pH 2.8–4.8). An assessment of the effect of temperature over a range of 20–40 °C on the separation of the analytes showed a gradual, albeit insignificant,

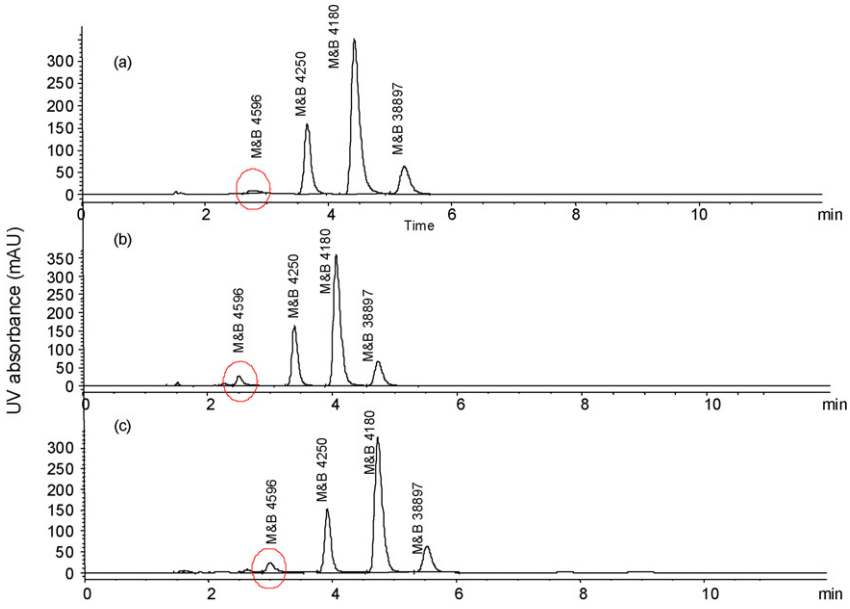

Fig. 2. Representative chromatogram of the separation of the major components of isometamidium using the conditions described in Section 2.3 with a mobile phase composed of: (a) mixture of acetonitrile and ammonium acetate buffer (100 mM, pH 3.8) (25:75 v/v), (b) mixture of acetonitrile and ammonium formate buffer (50 mM, pH 2.8) (25:75 v/v) and (c) mixture of acetonitrile and ammonium formate buffer (50 mM, pH 3.5) (25:75 v/v). The peak due to the highly basic bis-amidino compound M&B4596 is highlighted to illustrate the effect of the analytical conditions on peak shape.

decrease in retention times with increase in temperature which did not compromise resolution of the critical pair. Subsequently all analyses were performed at ambient temperature (~20 °C). The stability of analytical solutions assessed at three different concentrations for each component of isometamidium as described in Section 2.3 produced R.S.D.% values ($n = 36$ injections over 60 h) for peak areas which were typically less than 3.0, 1.7 and 0.7% for the low, intermediate and high concentrations (Section 2.3), respectively. Thus solutions are sufficiently stable to justify the analysis of freshly prepared samples over a 24 h period.

The chromatographic peak areas showed a rectilinear relationship to analyte concentration within the specified ranges (Table 1) which are consistent with the expected concentrations on dilution of the innovator product Samorin®. Linear regression analysis showed that the correlation coefficients (R^2) of all calibration curves were ≥0.995 with minimal variation in the slopes and intercepts (Table 1).

The performance characteristics and validation data for the method using the mobile phase containing ammonium formate buffer (50 mM, pH 2.8) are summarised in Table 2. The intra-day assay precision (R.S.D. %) of peak areas for M&B4180A at the working concentration of 100 μg/ml (0.46) was comparable to the value of 0.51 previously reported by Tettey et al. [14]. With the exception of M&B4596 which produced a high R.S.D. % for intra-day precision (3.47%, Table 2) at the proposed working concentrations, the other components demonstrated a repeatability of ≤1.7.

Table 1
Regression analyses of calibration curves generated from the analysis of M&B4180 and related substances

Analyte	Range (μg/ml)	n	Slope	Intercept	Correlation coefficient (R^2)
M&B4596	3–15	4	34.7 ± 0.3	41.1 ± 4.6	0.9998 ± 0.0001
M&B4250	5–25	4	89.6 ± 0.1	18.4 ± 1.6	1.0000 ± 0.0000
M&B4180	20–100	4	56.3 ± 0.1	74.7 ± 4.2	1.0000 ± 0.0001
M&B38897	5–25	4	52.2 ± 0.1	14.3 ± 2.1	1.0000 ± 0.0001

The method was compatible with mass spectrometric detection and the compounds showed the following distinct signals in the spectra: $m/z = 460$ and 230.5 for the $[M]^+$ and $[M+H]^{2+}$ ions due to M&B4180A ($t_R = 5.1$ min), M&B4250 ($t_R = 4.2$ min) and M&B38897 ($t_R = 5.9$). The structural similarity of the related substances makes it difficult to distinguish between M&B4180A, M&B4250 and M&B38897 based on MS data alone. The refinement of the MS data with chromatographic retention times enables unambiguous identification of these compounds. However, distinct MS data was obtained for the precursor/degradation product ethidium ($t_R = 9.5$, $m/z = 314$ for $[M]^+$) and the bis-amidino substituted analogue, M&B4596 ($t_R = 2.9$, $m/z = 606$, 303.5 and 202.7 for $[M]^+$, $[M+H]^{2+}$ and $[M+H]^{3+}$, respectively). The LC–MS method subsequently provides selectivity in analysis with respect to the degradation product ethidium, which is also well resolved from the other analytes.

3.1. Application to real samples

The challenges involved in the commercial production of a consistent mixture of components in isometamidium led to the incidence of non-chemically equivalent products in the past (Tettey et al. [14]). Samorin®/Trypamidium®, currently the only World Health Organization (WHO)-FAO Joint Expert Committee on Food Additives (JECFA) approved product, recognizes these challenges and provides a workable range for the contents (%, w/w) of M&B4180A (55–65%), M&B4596 (5–10%), M&B38897 (10–20%) and M&B4250 (10–20%) in commercial

Table 2
Repeatability and sensitivity of HPLC method

	M&B4180A	M&B38897	M&B4250	M&B4596
Inter-day precision				
Day 1 ($n=3$)	*20 μg/ml*, 1306 ± 14.8 (1.13%)	*5 μg/ml*, 317 ± 18.0 (5.69%)	*5 μg/ml*, 542 ± 2.9 (0.54%)	*3 μg/ml*, 138 ± 8.9 (6.30%)
Day 2 ($n=3$)	*50 μg/ml*, 3283 ± 22.7 (0.69%)	*12 μg/ml*, 767 ± 6.7 (0.87%)	*12 μg/ml*, 1303 ± 25.0 (1.92%)	*7 μg/ml*, 346 ± 3.9 (1.13%)
Day 3 ($n=3$)	*100 μg/ml*, 6586 ± 79.7 (1.21%)	*25 μg/ml*, 1567 ± 9.7 (0.62%)	*25 μg/ml*, 2693 ± 15.1 (0.56%)	*14 μg/ml*, 679 ± 6.4 (0.95%)
Intra-day precision				
Solution 1	*20 μg/ml*, 1304 ± 9.8 (0.75%)	*5 μg/ml*, 325 ± 20.4 (6.27%)	*5 μg/ml*, 545 ± 6.65 (1.22%)	*3 μg/ml*, 126 ± 16.6 (13.16%)
Solution 2	*50 μg/ml*, 3277 ± 17.4 (0.53%)	*12 μg/ml*, 748 ± 19.7 (2.64%)	*12 μg/ml*, 1297 ± 6.1 (0.47%)	*7 μg/ml*, 318 ± 29.0 (1.15%)
Solution 3	*100 μg/ml*, 6546 ± 30.1 (0.46%)	*25 μg/ml*, 1547 ± 25.7 (1.66%)	*25 μg/ml*, 2685 ± 12.9 (0.48%)	*14 μg/ml*, 678 ± 23.5 (3.47%)
Limit of detection				
S/N = 3 (μg/mL)	0.04	0.05	0.02	1.40
Limit of quantitation				
R.S.D. % ≤ 5 (μg/mL)	0.20	0.10	0.05	2.80

All precision data are mean ± S.D. ($n=3$) with R.S.D. % values in parenthesis. Precision data represents peak areas for analytes corrected for quantities of analytical standards materials used in the preparation of solutions. The concentrations in μg/ml (italicized) represent the levels at which precision have been assessed.

samples (*source*: Merial SAS, Lyon, France). The validation of the repeatability (inter- and intra-day precision) of the proposed HPLC method using the JEFCA approved product (Table 3) yielded R.S.D. % values typically less than 2% with the exception of that of the highly basic M&B4596 which contains two amidino moieties in addition to a quaternary nitrogen.

The reproducibility of the method, as assessed by comparative analysis of the same five commercial products in two different laboratories (University of Strathclyde and International Atomic Energy Agency Laboratory, Seibersdorf) showed no significant difference ($p<0.05$) using a two-sample paired comparison test.

The lack of consistency in the amounts of additives/excipients added to commercial products, which is reflected in the actual weight of contents (Table 4), makes it difficult to assess equivalence in terms of % w/w. Rather a useful comparison is obtained based on the amounts of the analytes per 125 mg or 1 g sachet of product. The data (Table 4) shows a significant variation in profiles of these products with respect to the content of the known chemical entities and excipients/additives. The WHO recognizes counterfeit medicines as part of a broader phenomenon of substandard pharmaceuticals (WHO Fact sheet no. 275). The WHO definition of substandard medicines as ones "which are manufactured below established standards of quality and therefore dangerous to patient's health and ineffective for the treatment of diseases" is difficult to apply to isometamidium in the absence of an internationally established specification or standard of quality for the product. Therefore, it is not immediately apparent if the lack of chemical and pharmaceutical similarity between the innovator and some commercially available product results from the manufacture of substandard products.

Counterfeit isometamidium products, which are deliberately and fraudulently mislabelled with respect to source or identity, may however be assigned as substandard without ambiguity. For example, the analysis of a counterfeit version of Samorin® (Fig. 3) found on the West-African market by the HPLC and HPLC–MS methods described in this study showed the complete absence of M&B4180A, M&B4596, M&B38897, M&B4250 and the putative starting material/degradation product ethidium. This was in addition to incomplete labelling instructions on the counterfeit version.

Eisler et al. [4] speculated that the absence of protective levels of isometamidium in cattle following treatment under field conditions was probably attributable to the use of adulterated or counterfeit drugs. This study confirms that assertion and also demonstrates marked differences in chemical composition and formulation of current commercially available isometamidium products. These observations have significant implications with respect to treatment failures and toxicity of unknown additives/adulterants. Isometamidium remains the only chemical agent for the prophylaxis and treatment of veterinary trypanosomiasis. It is obvious that the difficulties in its synthesis, the absence of regulatory standards and drug counterfeiting have resulted in the marketing of products, which bear no chemical or pharmaceutical similarity to each other, or the innovator product. The application of this proposed validated method which

Table 3
Precision of HPLC analysis of isometamidium and related substances in a commercial sample

	M&B4180A	M&B38897	M&B4250	M&B4596
Inter-day precision				
Mean ± S.D. ($n=3$) (% w/w)	62.9% ± 0.54	8.0% ± 0.02	7.6% ± 0.09	5.8% ± 0.15
R.S.D. %	0.87%	0.25%	1.17%	2.67%
Intra-day precision				
Mean ± S.D. ($n=3$) (% w/w)	62.3% ± 1.13	8.0% ± 0.12	7.5% ± 0.11	5.5% ± 0.06
R.S.D. %	1.82%	1.49%	1.53%	1.15%

Fig. 3. Front and rear images of sachets of a genuine (A, top) and counterfeit (B, bottom) versions of Samorin®. The counterfeit version was found on the open market in West-Africa. Note the absence of the warning 'keep out of the reach of children' on the front of the counterfeit version.

Table 4
Results from HPLC analysis of various commercial isometamidium products

	Manufacturer	Wt. of contents of sachet	M&B4596	M&B38897	M&B4250	M&B4180A
Innovator Specifications (mg) for 125 mg sachet[a]			6.25–12.50	12.5–25.0	12.5–25.0	68.75–81.3
Trypamidium®–Samorin®	Merial, Lyon, France	125 mg	11.3 mg	16.4 mg	17.6 mg	74.0 mg
Kelamidium (batch 8898)	Kela, Hoogstraten, Belgium	3000 mg	5.6 mg	9.1 mg	15.8 mg	64.4 mg
Innovator Specifications (mg) for 1g sachet[a]			50–100	100–200	100–200	550–650
Inomidium	Inouko Generics, Paris, France	1.04 g	60.3	65.5	99.0	653.5
Lobidium	LOBS International Health, Nuilly sur Seine, France	1.04 g	55.7	79.3	125.4	602.6
Isometamidium	PKM International	1.30 g	92.4	145.6	143.6	773.8
Veridium® (lot 93A1)	Ceva Sante Animale, Libourne, France	1.08 g	50.8	99.5	103.1	640.2
Veridium® (lot 101A1)	Ceva Sante Animale, Libourne, France	1.08 g	62.6	86.4	82.1	679.3
Kelamidium (batch 7937)	Kela, Hoogstraten, Belgium	2.15 g	79.2	37.4	15.3	419.0
Kelamidium (batch 9536)	Kela, Hoogstraten, Belgium	1.86 g	72.8	114.3	127.3	608.7

[a] Based on the innovator specification of M&B4180A (55–65%), M&B4596 (5–10%), M&B38897 (10–20%) and M&B4250 (10–20%).

allows for absolute quantitation of the four major components and LC–MS screening of the authenticity of products should facilitate the control of the quality of these products in international commerce.

Acknowledgments

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