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A new high-performance liquid chromatography (HPLC) method for the analysis of halofantrine (HF) in pharmaceuticals

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Abstract

A simple, rapid and stability-indicating reverse-phase high-performance liquid chromatography (HPLC) method was developed for the assay of halofantrine (HF) base, 1,3-dichloro-α-[2-(dibutylamino)ethyl]-6-(trifluoromethyl)-9-phenanthrenemethanol. The HPLC method was validated for precision, accuracy, selectivity and linearity and range. It was used to assay for HF base in solid dispersions. Excellent linearity was observed between HF base concentration and the peak area ($R^2 = 0.9998$). The limit of detection was 1 ng (with a signal-to-noise ratio of 2:1), and the limit of quantitation was 10 ng (with a signal-to-noise ratio of 10:1). The method proved to be selective. Selectivity was validated by subjecting a stock solution of HF to acidic, basic, oxidative and thermal degradations. The peaks of the degradation products did not interfere with the peak of HF. Excipients present in the solid dispersions did not interfere with the analysis. © 2002 Published by Elsevier Science B.V.

Keywords: High-performance liquid chromatography; Liquid chromatography; Halofantrine; Malaria; Stability-indicating

1. Introduction

Halofantrine (HF) (Fig. 1), a phenanthrene methanol, is prescribed primarily as an alternative to treat acute malarial attacks caused by chloroquine-resistant and multidrug-resistant strains of *Plasmodium falciparum* [1]. In spite of the poor aqueous solubility of hydrochloride salt (1 μg/ml) [2], HF is marketed as the hydrochloride salt in three oral formulations: namely, suspension, tablets and capsules [3]. This, in turn, is responsible for the poor extent of absorption of HF after oral administration with wide intra- and intersubject variability. The resulting erratic plasma profiles may limit the therapeutic effectiveness and potentially stimulate the development of resistance [2]. Though marketed as the hydrochloride salt, many studies were conducted towards the use of more lipid-soluble drug systems to improve the bioavailability of the base form of HF. This is due to its high solubility in long-chain triglycerides and lymphatic transport being a major contributor in the transport of the base form [2].

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Table 1
SD of HF base with different carriers: quantities and proportions of HF base and excipient(s) used

Sample	PEG 8000 (g)	PVP K30 (g)	Gelucire® 44/14 (g)	Phosphatidyl choline (g)	HF (g) base	Ratio (carrier:HF)
SD1[a]	0.75	–	–	–	0.5	60:40
SD2	–	0.75	–	–	0.5	60:40
SD3	–	–	0.75	–	0.5	60:40
SD4	–	0.7425	–	0.0125 (1%)	0.495	60:40
SD5	–	0.5	–	–	0.5	50:50
SD6	–	2	–	–	0.5	80:20
SD7	–	4.5	–	–	0.5	90:10
SD8[a]	–	0.75	–	–	0.5	60:40

[a] SD1 and SD8 are two SDs containing the same type and proportion of carrier, but prepared by two different methods.

Several methods are reported in literature to assay for HF base [4–8]. The reported methods use a C8 or C18 column as a stationary phase to carry out the assays. No method for the assay of HF base in solid dosage forms is reported in literature.

In our studies, HF base was formulated in solid dispersions (SDs) [9]. An SD is a dispersion of one or more active ingredients in an inert carrier or matrix at solid state prepared by melting (fusion), solvent or melting–solvent method [10]. A simple, rapid, and stability-indicating high-performance liquid chromatography (HPLC) method was developed and validated for the analysis of HF base in SDs. This article will focus on the development and validation of the HPLC method.

2. Materials and methods

2.1. Chemicals and reagents

HF base was supplied by SmithKline Beecham Pharmaceuticals (Essex, UK). Acetonitrile (HPLC grade), monobasic potassium phosphate (HPLC grade) and hydrochloric acid (HCL), 1 N, were purchased from Fisher Scientific (Fair Lawn, NJ). Sodium hydroxide (NaOH) (USP grade) was purchased from Spectrum Quality Products (New Brunswick, NJ), Kollidone (PVP K30) was purchased from Amend Drug & Chemical Co., Inc. (Irvington, NJ) and PEG 8000 and type XVI-E L-α-phosphatidyl choline (L-α-lecithin) from fresh egg yolk were purchased from Sigma chemical Co. (St. Louis, MO). Gelucire® 44/14 was obtained from Gattefosse (Cedex, France). All chemicals were used as received and were not purified further. All glassware, prior to their use in the assay, were silanized with AquaSil™ fluid. AquaSil™ was purchased from Pierce (Rockford, IL).

2.2. Apparatus

A Hewlett Packard Series 1100 (HP 1100) system equipped with a multiple-wavelength UV detector and an HP 3395 integrator was used. The liquid chromatography (LC) column was a Zorbax® SB-CN (Cyano) column (5 μ, 150 × 4.6 mm internal diameter, Mac Mod Analytical Inc., Chadds Ford, PA).

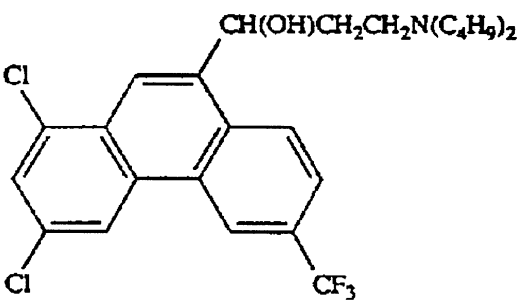

Fig. 1. Molecular structure of HF base.

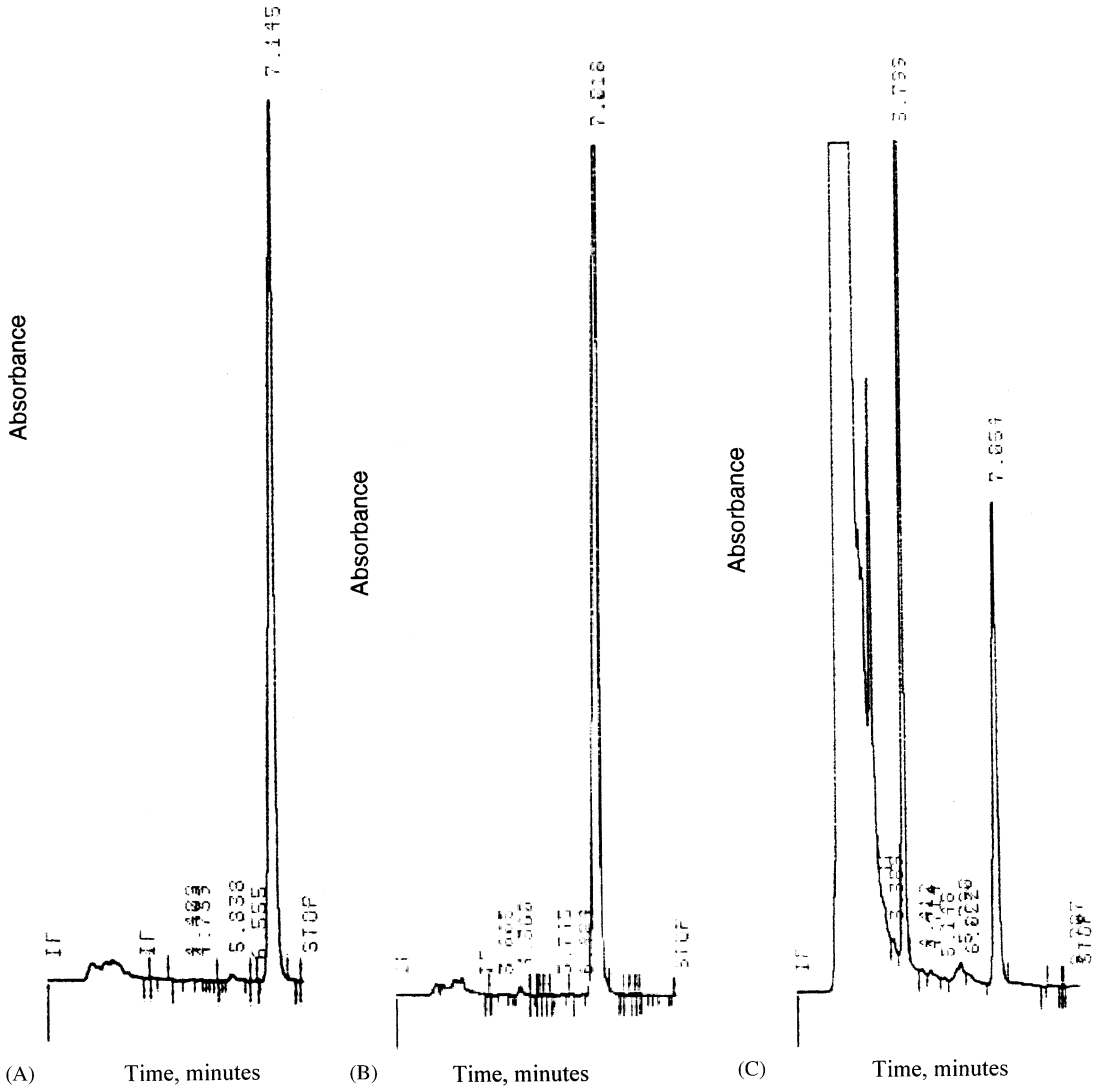

Fig. 2. Sample chromatograms: chromatogram A is from HF base standard solution (retention time of 7.145 min for HF base); chromatogram B is from a solution subjected to thermal degradation (retention time of 7.018 min for HF base); chromatogram C is from a solution subjected to oxidation (retention time of 7.054 min for HF base); chromatogram D is from a solution subjected to acid degradation (retention time of 7.338 min for HF base); chromatogram E is from a solution subjected to alkaline degradation (retention time of 7.320 min for HF base).

2.3. Chromatographic conditions

The mobile phase consisted of 80:20 (v/v) acetonitrile—0.025 M potassium phosphate monobasic aqueous buffer (pH 5.0 adjusted with 0.1 N NaOH solution). The flow rate was 1 ml/min. The wavelength for detection was 259 nm, the chart speed was 0.5 cm/min, the column temperature was ambient and the injection volume was 100 μl.

2.4. Procedures

2.4.1. Preparation of stock and standard solution

Three stock solutions of HF base were prepared by dissolving 5.0 mg in 100.0 ml of mobile phase to yield a concentration of 50 μg/ml, each. These solutions were diluted with mobile phase as needed to prepare different standard solutions. Each standard solution was injected into LC. The mean peak areas of all the tested concentrations were used to construct a standard calibration curve to test the linearity and regression coefficient (R^2) of the HPLC method. The variation between three successive injections ($n = 3$) of each standard solution was tested (intra-day variation). Precision of the method was tested by injecting a standard solution of each of 0.01 and 0.1 μg/ml of HF base eight consecutive times. A test for ruggedness was performed by evaluating the inter-day variation between peak areas by injecting a 1

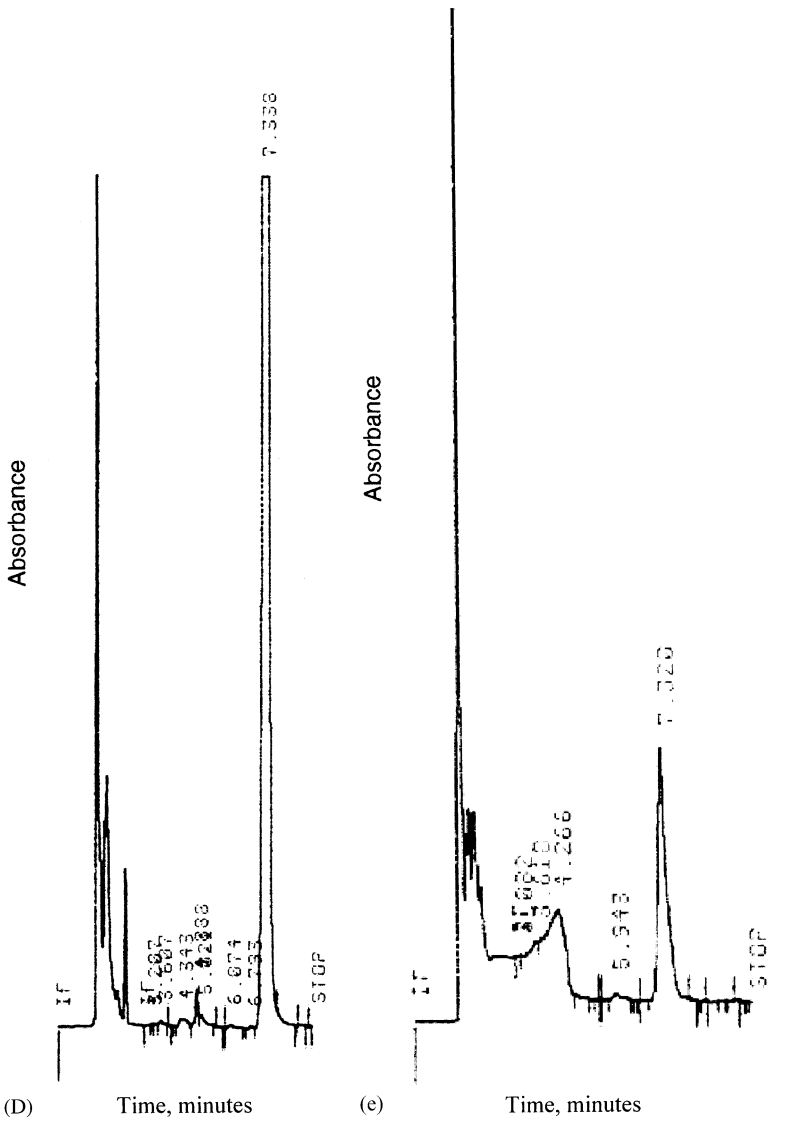

Fig. 2. (*Continued*)

Table 2
Assay of HF base in SDs

Sample	% w/w HF base of formula (theoretical value)	Total mass of formula (mg)	Total HF base (mg)	% w/w HF base of formula (actual)	% recovery HF base ± standard deviation
SD1	40	8.0	3.1	38.7	96.7 ± 0.31
SD2	40	19.2	8.1	42.2	105.5 ± 0.11
SD3	40	21.6	10.2	47.3	118.3 ± 0.13
SD4	39.6	13.9	5.4	39.1	98.8 ± 0.22
SD5	50	14.0	7.0	49.8	99.7 ± 0.26
SD6	20	15.9	3.0	18.8	94.0 ± 0.33
SD7	10	9.8	1.1	10.7	107.2 ± 0.24
SD8	40	14.9	5.7	38.4	96.1 ± 0.32

μg/ml standard solution of HF base on 5 consecutive days.

2.4.2. Oxidation of HF

A 0.8 ml of the stock solution of HF base (50 μg/ml) was transferred to a 10 ml volumetric flask and the volume was made up to 10 ml with 3% hydrogen peroxide (H_2O_2) solution. The mixture was heated and kept at 80 °C for about 1 h, cooled to room temperature (25 °C), the volume readjusted with 3% H_2O_2 and the solution filtered through a 0.45 μ syringe filter and injected into LC to detect the peaks of oxidation.

2.4.3. Thermal degradation of HF

A 0.8 ml of the stock solution of HF base (50 μg/ml) was transferred to a 10 ml volumetric flask and the volume was made up to 10 ml with mobile phase. The mixture was heated and kept at 80 °C for 1 h, cooled to room temperature, the volume readjusted with mobile phase and the solution filtered through a 0.45 μ syringe filter and injected into LC to detect peaks of thermal degradation.

2.4.4. Degradation of HF by acid

A 0.8 ml of the stock solution of HF base (50 μg/ml) was transferred to a 10 ml volumetric flask and the volume was made up to 10 ml with 1 N HCL. The mixture was heated and kept at 80 °C for 1 h, then cooled to room temperature and the volume readjusted with 1 N HCl. The pH of the solution was adjusted to neutrality by adding 1 N NaOH, the solution was filtered through a 0.45 μ syringe filter and injected into LC to detect peaks of degradation products.

2.4.5. Degradation of HF by alkali

A 0.8 ml of the stock solution of HF base (50 μg/ml) was transferred to a 10 ml volumetric flask and the volume was made up to 10 ml with 1 N NaOH. The mixture was heated and kept at 80 °C for 1 h, then cooled to room temperature and the volume readjusted with 1 N NaOH. The pH of the solution was adjusted to neutrality by adding 1 N HCl, the solution was filtered through a 0.45 μ syringe filter and injected into LC to detect peaks of degradation products.

2.4.6. Assay of HF in SDs

HF base content of SDs was assayed by HPLC. 10 mg of the dispersion was accurately weighed and dissolved in 50 ml of the mobile phase. 0.2 ml of this solution was diluted with 20 ml of the mobile phase, and the diluted sample was analyzed by HPLC. The drug content of each batch was determined as follows:

$$\text{Concentration of the sample} = \frac{A \times B}{C},$$

where, A is the concentration of standard solution, B the mean peak area of the sample, and C the mean peak area of the standard solution.

Table 1 summarizes the contents of each SD and gives the percentage weight of each ingredient.

2.4.7. Assay procedure and calculations

A 100 μl quantity of the assay solution was injected into LC using the conditions described. For comparison, an identical volume of the standard solution was injected. Since the ratio of peak areas was related to the concentrations of the drug, the results were calculated using the following equation:

$$\text{Percent of label claim found} = \frac{(R_{\text{par}})_a}{(R_{\text{par}})_s} \times 100,$$

where $(R_{\text{par}})_a$ is the peak area of the drug and $(R_{\text{par}})_s$ is the peak area of the standard solution.

3. Results and discussion

The proportions of the organic and aqueous phases were adjusted to obtain a rapid and simple assay method for HF base with a reasonable run time, suitable retention time and sharpness of

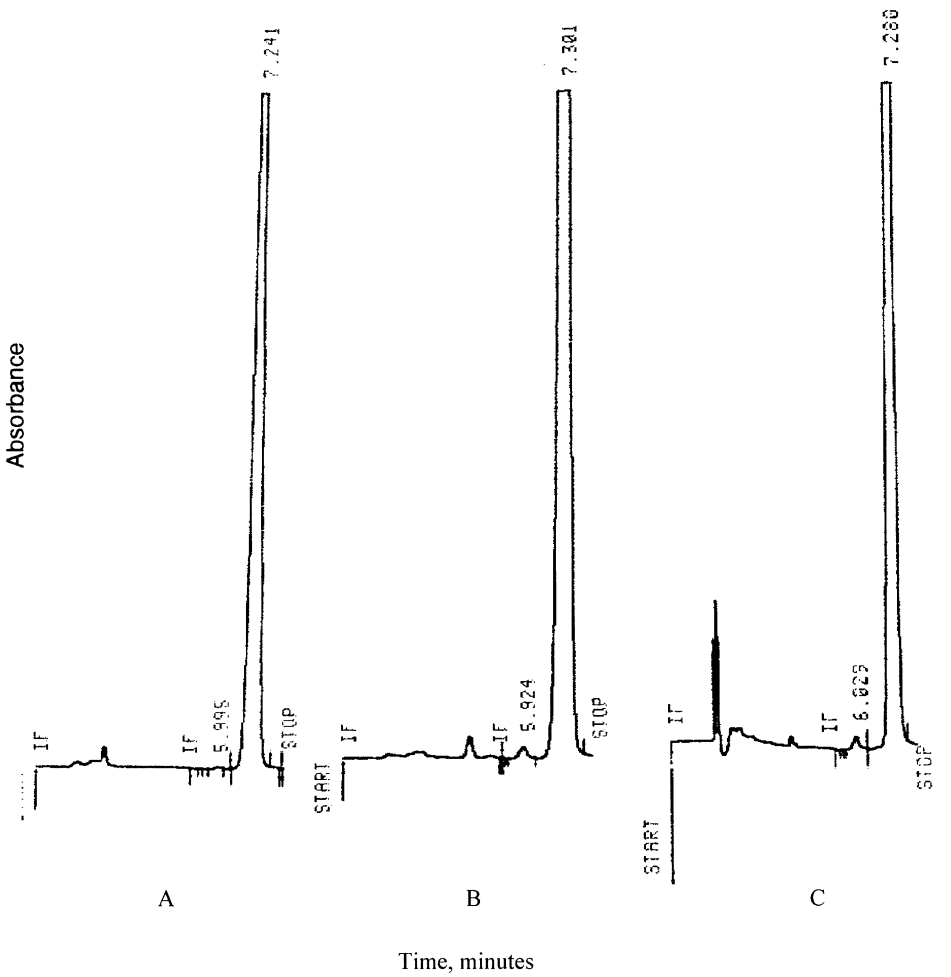

Fig. 3. Sample chromatograms: chromatogram A is from an SD of HF base with PEG 8000 (retention time of 7.241 min for HF base); chromatogram B is from an SD of HF base with PVP K30 (retention time of 7.301 min for HF base); chromatogram C is from an SD of HF base with Gelucire® 44/14 (retention time of 7.280 min for HF base).

peak (the tailing or asymmetry factor was measured to be 1–1.1). Under experimental conditions, the chromatogram of HF (Fig. 2A) showed a single peak for HF base around 7 min.

3.1. Linearity and range

The standard curves for HF base were linear over the investigated concentration range (0.1–5 μg/ml) with a percent relative standard deviation (%RSD) of not more than 0.6 (intra-day variability) based on three successive readings and a correlation coefficient of not less than 0.9998. %RSD for the inter-day reproducibility of the assay for a 1 μg/ml standard solution of HF injected on 5 consecutive days was found to be not more than 1.23.

3.2. Limit of quantitation and limit of detection

Under the developed HPLC conditions, the limit of quantitation was determined to be 10 ng/ml, while the limit of detection was determined to be 1 ng/ml. The publications previously referred to reach a limit of quantitation of 10 ng/ml for HF base, but none reached a limit of detection as low as 1 ng/ml. It is possible that the greater sample size (100 μl) in our studies, versus a sample size of 20 μl in the other reported assays, may have contributed to a greater sensitivity of the developed method.

3.3. Precision

Results for precision tests performed on each of a standard solution of HF of 0.01 and 0.1 μg/ml showed that the %RSD was not more than 2.29 for the former and 0.53 for the latter.

3.4. Selectivity

The method proved to be both selective as well as stability-indicating. There was little degradation of HF base thermally or by acid hydrolysis. HF base degraded by oxidation and by alkaline hydrolysis. The peaks of the degradation products were separate from the drug peak (Fig. 2B–E).

3.5. Accuracy

The results in Table 2 indicate that the developed method can be used to quantify HF base in SDs. The recovery of HF base from SDs was essentially quantitative, and there was no interference from the excipients present in the dosage forms (Fig. 3). There were, however, errors in the recovery of HF from SD3 and SD7, probably due to the adsorption of HF base onto glass or due to experimental errors during weighing (accounted for later on during the formulation studies). Percentage recovery was calculated from theoretical amount of HF added.

4. Conclusions

A rapid, simple, sensitive and stability-indicating HPLC method was developed and validated according to the USP XXIV guidelines [11] for the assay of HF base. It is the first reported method in literature to quantify HF base in solid dosage forms. The limit of detection was 1 ng/ml and the limit of quantitation was 10 ng/ml. The method proved to be selective. The peaks of the degradation products did not interfere with the peak of HF base. Excipients present in SDs also did not interfere with the analysis. The recovery of HF base from SDs was essentially quantitative.

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