Hydroxychloroquine MEDITOP 200 mg film-coated tablets

Analytical methods

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A kiadványt 2020-2.1.1-ED-2020-00023 projekt támogatja.
A Projekt az Innovációs és Technológiai Minisztérium Nemzeti Kutatási, Fejlesztési és Innovációs Alapból nyújtott támogatásával, a Nemzeti Kutatási, Fejlesztési és Innovációs Hivatallal kötött támogatási szerződés alapján valósult meg.

2.1.P.5.1. Specification

Hydroxychloroquine sulphate 200 mg film-coated tablet is tested according to the specification detailed below. The specification is for release and shelf-life.

Tests	*Methods*	*Acceptance criteria*	*Frequency*
Appearance	visual	White or almost-white, round, biconvex, film-coated tablets	every batch
Identification[1] (Hydroxychloroquine sulphate)	*HPLC*	Identical	every batch
Identification[1] (Hydroxychloroquine sulphate)	*IR*	Identical	every batch
Diameter		9.1 ± 0.2 mm	every batch
Thickness		3.8 – 4.8 mm	every batch
Average mass	*Ph.Eur. [2.9.5]*	320.0 mg ± 5 %	every batch
Uniformity of Mass	*Ph.Eur. [2.9.5]*	Average mass ± 5 % (18/20) Average mass ± 10 % (20/20)	every batch
Disintegration	*Ph.Eur. [2.9.1]*	NMT* 15 min	every batch
Dissolution	*Ph.Eur. [2.9.3]*	NLT 80% (Q) in 15 min	every batch
Assay:	*HPLC*	200.0 mg ± 5.0 %/film-coated tablet	every batch
Uniformity of dosage units[1]	*Ph.Eur. [2.9.40]*	AV≤15[§]	every batch

Related substances *HPLC*			every batch
- Impurity B		NMT*0.15 %	
- Impurity C		NMT*0.4 %	
- Any individual unspecified degradation product		NMT*0.10 %	
- Total impurities		NMT*0.6 %	
Loss on Drying		NMT 6.0 %	every batch
Microbiological purity	*Ph.Eur.* *[2.9.12]* *[2.9.13]*	TAMC ≤ 10^3 CFU/g TYMC ≤ 10^2 CFU/g Absence of Escherichia coli/g	every batch

*NMT: Not more than, NLT: Not less than

§ The acceptance value of the first 10 dosage units is less than L1. If the acceptance value is greater than L1, test 20 additional dosage units and calculate the acceptance value. The final acceptance value is less than L1, and no individual content of the dosage unit is less than (1-L2x0.01)M, or more than (1+L2x0.01)M. L1 is 15, L2 is 25.

[1] Test is not included in shelf-life specification.

2.1.P.5.2 Analytical Procedures

1 Appearance

Procedure: Checked by visual examination.

Requirement: White or almost-white, round, biconvex, film-coated tablets

2 Identification-Hydroxychloroquine sulphate

HPLC

Procedure:

As described in the assay test. The retention time of the main peak of the sample chromatogram is similar in retention time (± 0.5 min) to that of the main peak of the standard chromatogram.

Requirement: Identical

IR

Procedure:

Powder at least 10 film-coated tablets. The spectrum of the sample is similar to the spectrum of the hydroxychloroquine sulphate reference material (correlation is not less than 95 %)

Requirement: Identical

3 Average mass and uniformity of mass

Method: Ph.Eur.2.9.5

Test:

Weigh individually 20 film-coated tablets taken at random.

Evaluation:

Not more than 2 of the individual masses deviate from the average mass by more than ± 5 % and none deviates more than ± 10 %.

Requirement: Average mass: 310.0 mg ± 5 %
Uniformity of mass: Average mass ± 5.0 % (18/20)
Average mass ± 10.0 % (20/20)

4 Dimensions

Procedure:

Test 20 film-coated tablets by tablet tester instrument.

Requirement:	Diameter: 9.1 ± 0.2 mm Thickness: 3.8 – 4.8 mm

5 Disintegration

Method:	Ph.Eur.2.9.1

Test:

Determine the disintegration individually 6 film-coated tablets by a suitable disintegration tester with no disks, using 37 ± 0.5 °C water as medium.

Requirement:	not more than 15 min

6 Dissolution

Test:

Apparatus:	App. 2 (paddle), Ph. Eur. / USP
Temperature:	37 ± 0.5 °C
Rotation speed:	50 rpm
Sample amount:	1 film-coated tablet per vessel
Dissolution medium:	900 ml of 0.1 M hydrochloric acid
Detection:	343 nm
Sample amount:	10 ml

Sample solution:

Place one film-coated tablet into each of the six vessels before starting rotation of the blade.

After 15 minutes of agitation withdraw 10 ml sample. A portion of the solution is filtered through a 0.45µm CA syringe filter, discharging the first 1-2 ml. Dilute 5.0 ml of the filtrate to 100.0 ml with the diluent. *(hydroxychloroquine sulphate concentration: 0.011 mg/ml*)

Standard solution:

Weight accurately approx. 22.2 mg of hydroxychloroquine sulphate standard into a 100.00 ml volumetric flask, dissolve in 80.0 ml of dissolution medium, and dilute to volume with dissolution media. Prior to measurement filter a portion of the solution through a 0.45µm CA syringe filter, discharging the first 1-2 ml. Dilute 5.00 ml of this solution to 100.00 ml with diluent. *(hydroxychloroquine sulphate concentration: 0.011 mg/ml*)

Measurement:

Measure the absorbances of the solutions at 343 nm using dissolution medium as blank solution.

Calculation (hydroxychloroquine sulphate):

Hydroxychloroquine sulphate (%) = $\frac{A_S}{A_{std}} \times \frac{W_{std}}{100 \times 20} \times P_{std} \times \frac{900 \times 20}{200}$

Where:
A_S: absorbance of the sample solution
A_{std}: absorbance of the standard solution
W_S: amount of sample taken (mg)
W_{std}: amount of hydroxychloroquine sulphate standard material taken (mg)
P_{std}: potency of the standard material, (%)

Requirement: Not less than 80 % (Q) of the label claim is dissolved in 15 minutes.

7 Assay

Preparation of phosphate buffer:

Dissolve 1.36 g of KH_2PO_4 in 900 ml of purified water, add 0.15 g of sodium haxanesulfonate and adjust the pH to 7.0 ± 0.05 with tryethilamine, dilute to 1000 ml with purified water, and mix well by stirring for 10 min. Check pH and degas.

Diluent:

Mix 500 ml of methanol, 500 ml of purified water and 4 ml 10 % (V/V) sulfuric acid.

Chromatographic conditions:

Mobile phase A:	Methanol – phosphate buffer (10:90 V/V)
Mobile phase B:	Phosphate buffer-methanol (15:85 V/V)
Flow:	0.35 ml/min
Column:	AQUITY C18 BEH, 5x2.1 mm, 1.7 µm
Injection volume:	4 µl
Detection:	254 nm
Autosampler temperature:	25°C
Column temperature:	40°C
Run time:	12 min

Gradient profile:

Time (min)	Mobil phase A (%)	Mobile phase B (%)
0	100	0

1	100	0
11	0	100
12	100	0

Hydroxychloroquine sulphate standard solution:

Weight accurately approx. 25 mg of hydroxychloroquine sulphate standard material into a 50.0 ml volumetric flask, dissolve in 40.0 ml of diluent, and dilute to volume with diluent. Dilute 1.0 ml of the solution to 10.0 ml with diluent *(hydroxychloroquine sulphate concentration: 0.05 mg/ml*)

Sample solution:

Finely powder at least 10 tablets. Weight accurately approx. 320 mg material into a 100.00 ml volumetric flask, add 80.0 ml of diluent, sonicate for 10 min and dilute to volume with diluent. Dilute 1.00 ml of this solution to 50.00 ml with diluent. Filter a portion of the solution through a 0.45µm CA syringe filter, discharging the first 1-2 ml. *(hydroxychloroquine sulphate concentration: 0.05 mg/ml)*

System suitability:

Apply 5 consecutive injections of the hydroxychloroquine sulphate standard solution. The RSD of the hydroxychloroquine sulphate peak area is not more than 2 %, the USP tailing is between 0.8 – 1.5 and the theoretical plate number is not less than 5000.

Calculation:

Hydroxychloroquine sulphate (% of LC) = $\frac{A_s}{A_{std}} \times \frac{W_{std}}{25 \times 10} \times P_{std} \times \frac{100 \times 20}{W_s} \times \frac{T}{200}$

Where:
A_s: area of the hydroxychloroquine sulphate peak in the sample solution
A_{std}: area of the hydroxychloroquine sulphate peak in the standard solution
W_s: amount of sample taken (mg)
W_{std}: amount of hydroxychloroquine sulphate standard material taken (mg)
P_{std}: potency of the standard material, (%)
T: average filling mass of the film-coated tablets

Requirement: 95.0 – 105.0 % of LC (200.0 mg ± 5.0 %/ film-coated tablet)

8 Uniformity of dosage units

Test:

Select not less than 30 film-coated tablets taken at random. Accurately weigh 10 film-coated tablets individually (w_1, w_2, …w_{10}).

Express the individual contents (x_i) as percentage of the label claim, and calculate the acceptance value (AV):

$$x_i = \frac{w_i}{W} \times A$$

Where:

A: content of the active substance (hydroxychloroquine sulphate), expressed as percentage of the label claim, determined by the assay method.

W: mean of individual masses (w_1, w_2, …w_n)

w_i: individual film-coated tablet mass

Variable	Definition	Conditions	Value
T	Target content per dosage unit at time of manufacture, expressed as a percentage of label claim. T=100 % in our case.		
n	Sample size. (Number of the film-coated tablets in the sample)		
k	Acceptability constant	If n = 10	2.4
		If n = 30	2.0
s	Sample standard deviation		$\left[\frac{\sum_{i=1}^{n}\left(x_i - \overline{X}\right)^2}{n-1}\right]^{1/2}$
RSD	Relative standard deviation		$\frac{100 \times s}{\overline{X}}$
M If T ≤ 101.5 %	Standard value	If 98.5 % ≤ $\overline{X}$ ≤ 101.5%	M = $\overline{X}$ (AV = ks)
		If $\overline{X}$ ≤ 98.5 %	M = 98.5 % (AV = 98.5- $\overline{X}$ + ks)
		If $\overline{X}$ > 101.5 %	M =101.5 % (AV = $\overline{X}$ - 101.5+ ks)
M If T > 101.5 %	Standard value	If 98.5 % ≤ $\overline{X}$ ≤ T	M = $\overline{X}$ (AV = ks)
		If $\overline{X}$ ≤ 98.5 %	M = 98.5 % (AV = 98.5- $\overline{X}$ + ks)
		If $\overline{X}$ > T	M =T % (AV = $\overline{X}$ - T+ ks)
AV	Acceptance value		AV = $\left\lvert M - \overline{X} \right\rvert + k \times s$
L1	Maximum allowed acceptance value		L1 = 15.0
L2	Maximum allowed range for deviation of each dosage unit tested from the calculated value of M	On the low side, no dosage unit result can be less than 0.75M while on the high side, no dosage unit result can be greater than 1.25 M.	L2 = 25.0

Evaluation:

The acceptance value of the first 10 dosage units is less than L1. If the acceptance value is greater than L1, test 20 additional dosage units and calculate the acceptance value. The final acceptance value is less than L1, and no individual content of the dosage unit is less than (1-L2x0.01)M, or more than (1+L2x0.01)M. L1 is 15, L2 is 25.

Acceptance criteria: AV ≤ 15.0

9 Related substances

Preparation of phosphate buffer:

Dissolve 1.36 g of KH_2PO_4 in 900 ml of purified water, add 0.15 g of sodium haxanesulfonate and adjust the pH to 7.0 ± 0.05 with tryethilamine, dilute to 1000 ml with purified water, and mix well by stirring for 10 min. Check pH and degas.

Diluent:

Mix 500 ml of methanol, 500 ml of purified water and 4 ml 10 % (V/V) sulfuric acid.

Chromatographic conditions:

Mobile phase A:	Methanol – phosphate buffer (10:90 V/V)
Mobile phase B:	Phosphate buffer-methanol (15:85 V/V)
Flow:	0.35 ml/min
Column:	AQUITY C18 BEH, 5x2.1 mm, 1.7 µm
Injection volume:	4 µl
Detection:	254 nm
Autosampler temperature:	25°C
Column temperature:	40°C
Run time:	12 min

Gradient profile:

Time (min)	Mobil phase A (%)	Mobile phase B (%)
0	100	0
1	100	0
11	0	100
12	100	0

Hydroxychloroquine sulphate standard stock solution:

Weight accurately approx. 25 mg of hydroxychloroquine sulphate standard material into a 25.00 ml volumetric flask, dissolve in 20.0 ml of diluent, and dilute to volume with diluent. *(hydroxychloroquine sulphate concentration: 1.0 mg/ml*)

Hydroxychloroquine sulphate standard solution (1%):

Dilute 1.00 ml of hydroxychloroquine sulphate standard stock solution to 100.00 ml with diluent. Filter a portion of the solution through a 0.45µm CA syringe filter, discharging the first 1-2 ml *(hydroxychloroquine sulphate concentration: 0.01 mg/ml*)

Hydroxychloroquine sulphate standard solution (0.2%):

Dilute 2.00 ml of hydroxychloroquine sulphate standard solution (1%) to 10.00 ml with diluent. *(hydroxychloroquine sulphate concentration: 0.002 mg/ml*)

Sample solution:

Finely powder the content of at least 10 film-coated tablets. Weight accurately approx. 160 mg material into a 100.00 ml volumetric flask, add 80.0 ml of diluent, sonicate for 10 min and dilute to volume with diluent. Filter a portion of the solution through a 0.45µm CA syringe filter, discharging the first 1-2 ml. *(hydroxychloroquine sulphate concentration: 1.0 mg/ml)*

System suitability:

Apply 5 consecutive injections of the hydroxychloroquine sulphate standard solution (0.05%). The RSD of the hydroxychloroquine sulphate peak area is not more than 2 %, the USP tailing is between 0.8 – 1.5 and the theoretical plate number is not less than 5000.

Peak identification:

	retention time (RT)	relative retention time (RRT)
Hydroxychloroquine sulphate	6	------
Impurity B	4.8	0.8
Impurity C	5.4	0.9

Calculation:

$$\text{Impurity (\%)} = \frac{A_S \times CF}{A_{std}} \times \frac{W_{std}}{25 \times 100 \times 5} \times P_{std} \times \frac{100}{W_s} \times \frac{T}{AC}$$

Where:
A_s: area of an impurity peak in the sample solution
$A_{std:}$ area of the hydroxychloroquine sulphate peak in the standard solution
$W_{s:}$ amount of sample taken (mg)
W_{std}: amount of hydroxychloroquine sulphate standard material taken (mg)
P_{std}: potency of the standard material, (%)
AC: assay result (mg of hydroxychloroquine sulphate/film-coated tablet)
T: average mass of film-coated tablets (mg)
CF: correction factor for impurity B = 1.6; other peaks =1.0

Reporting threshold: 0.05%.

Acceptance criteria:

Impurity B	NMT 0.15 %
Impurity C	NMT 0.4 %
Any individual unspecified degradation product	NMT 0.10 %
Total impurities	NMT 0.6 %

10 Loss on Drying

Test:

Finely powder the content of at least 10 film-coated tablets. Dry 2.0 g of the powder to constant mass in a automated dryer.

Acceptance criteria: NMT 6.0 %

11 Microbiological purity

Procedure:

According to Ph. Eur. [2.6.12.; 2.6.13.]. Detailed test conditions are available separately.

Requirement:

TAMC ≤ 10^3 CFU/g
TYMC ≤ 10^2 CFU/g
Absence of *Escherichia coli*/g

3.2.P.5.6 Justification of Specifications

1 Description

For the description of the Hydroxychloroquine sulphate tablets the following characteristics have been selected:

- Color: checked by visual examination, as the color of the product has not changed during the stability studies no quantitative method has been considered.
- Shape: checked by visual examination.
- Size: checked by certified tools, the dimensions of the product are strictly controlled during manufacture.

2 Identification of the active ingredient

To identify the pharmaceutically active ingredient in the product we selected the HPLC assay method. The identification is based on comparison of the key characteristics (retention time, peak shape and peak size) and the diode array spectrums of the standard and the sample solutions. They are able to discriminate between compounds that are present in the product. A second identification is based on the comparison of IR spectrum of the sample and the reference material. A correlation of 95 % has been accepted as the requirement.

3 Uniformity of mass

The test is carried out as described in the European Pharmacopoeia *[2.9.5. Uniformity of Single-Dose preparations]* for tablets. The percentage of deviation requirement is determined by tablet mass, which is more than 250 mg.

4 Hardness

The test is carried out as described in the European Pharmacopoeia *[2.9.8. Resistance to Crushing of Tablets]* using a suitable instrument, able to measure the force in newton (N). The results are expressed as the mean, minimum and maximum values registered.

5 Disintegration

The test is carried out as described in the European Pharmacopoeia *[2.9.1. Disintegration of Tablets and Capsules – Test A; Tablets and capsules of normal size]* using a suitable apparatus without disks. The apparatus is operated for the prescribed time (15 min) and the condition of the tablets in the tubes checked visually.

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6 Uniformity of Dosage Units

The test is carried out as described in the European Pharmacopoeia *[2.9.40. Uniformity of Dosage Units]*.
As the product contains more than 25 mg of the active substance which is also comprises more than 25 % of the total mass, the Mass Variation Test of European Pharmacopoeia had been selected. The calculation of acceptance value (AV) and the acceptance criteria is applied as described in the Pharmacopoeia.

7 Assay

For the reliable determination of the API contents we have developed an HPLC method, based on the *European Pharmacopoeial Monograph of Hydroxychloroquine Sulphate*. The selectivity of the method had been verified. After that, the method has been validated.

8 Related substances

A selective HPLC method had been developed to detect the related substances of the tablets. The method is based on the *European Pharmacopoeial Monograph of Hydroxychloroquine Sulphate*. The applicability of the method had been verified by analysing stressed samples. The method performance characteristics had been checked during the validation process.

9 Dissolution

A selective dissolution method has been developed to determine the dissolved amount for the active ingredient. The method is based on the *United States Pharmacopoeial Monograph of Hydroxychloroquine Sulphate Tablet*. The method has appropriate discrimination power, and the dissolved active ingredients are detected by selective HPLC method. In the used media the sink conditions were established and maintained during the tests.

With the infinity time-point determination we demonstrated that with this method 100% of the APIs could be dissolved. The sample and standard solutions are stable in these conditions. The sampling is done manually, carried out as described US Pharmacopoeia. Prior to the HPLC measurements, the sample and standard solutions are filtered through a 0.45 μm MCE filter. No significant absorbance of the APIs was observed. As our tablet is an immediate release dosage form a single-point measurement was accepted. The method was found discriminatory when tablets with different compositions and manufacturing process had been tested, and stability indicating as testing tablets stored at different storage conditions. Finally, the method had been validated.

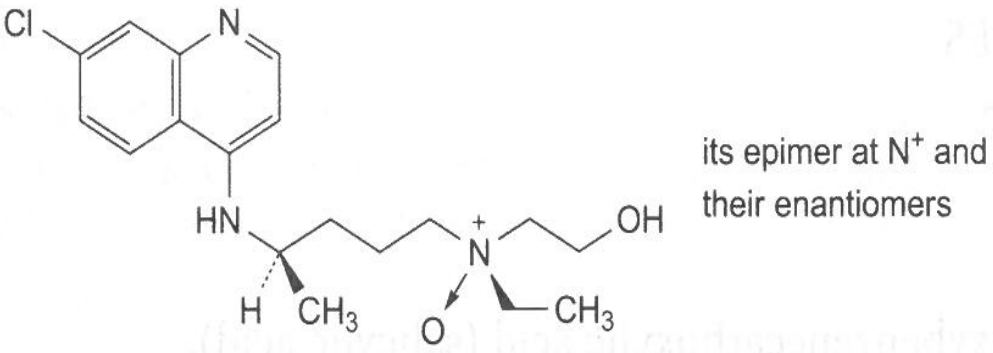
10 Microbiological Purity

The microbiological testing of the product is carried out as described in the current European Pharmacopoeia [*2.6.12 Microbiological Examination of Non-Sterile Products: Microbiological Enumeration Tests* and *2.6.13 Microbiological Examination of Non-Sterile Products: Test for Specified Micro-Organisms]*. Acceptance criteria for total aerobic microbial count (TAMC), total combined yeast/mould count (TYMC) and growth of colonies of Escherichia coli has been established.

3.2.P.5.5 Characterisation of Impurities

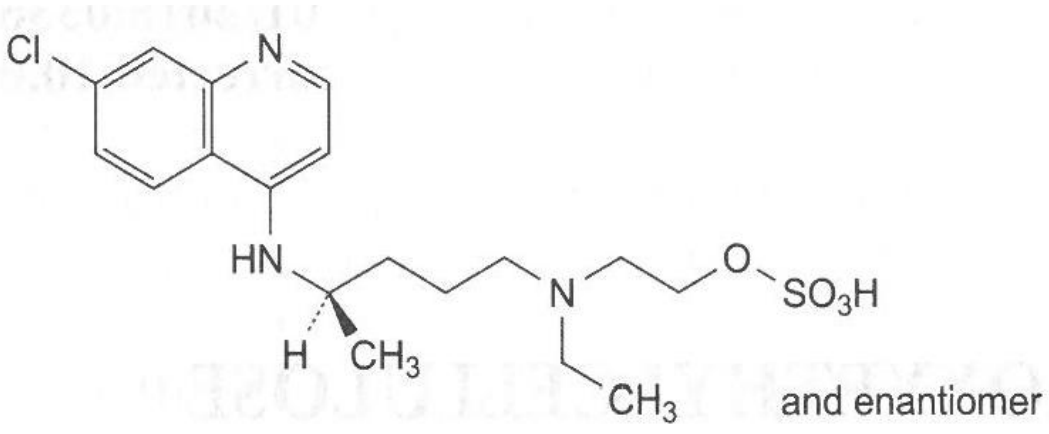
Potential organic impurities of hydroxychloroquine sulphate listed in the European Pharmacopoeial monograph are as follows:

Name: *impurity A*



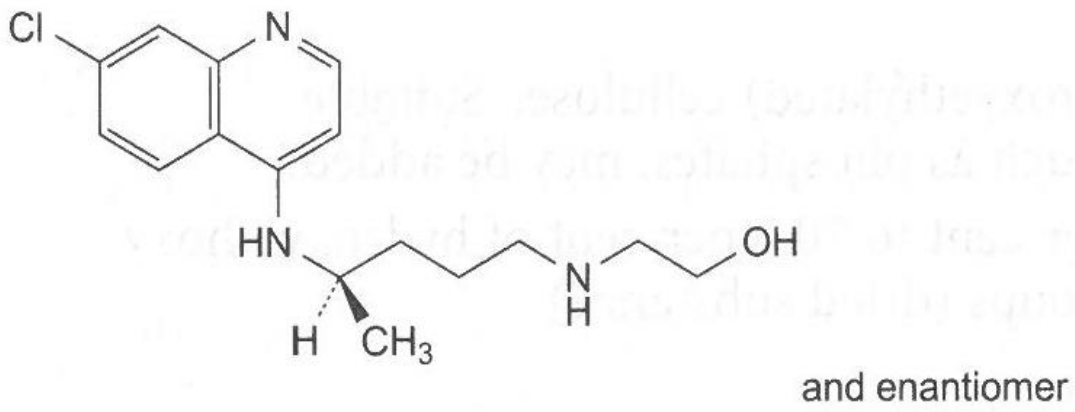
Synonym/chemical name: *mixture of diastereoisomers of 4-[(7-chloroquinolin-4-yl)amino]-N-(hydroxyethyl)penta-1-amine-N-oxide*

Name: *impurity B*



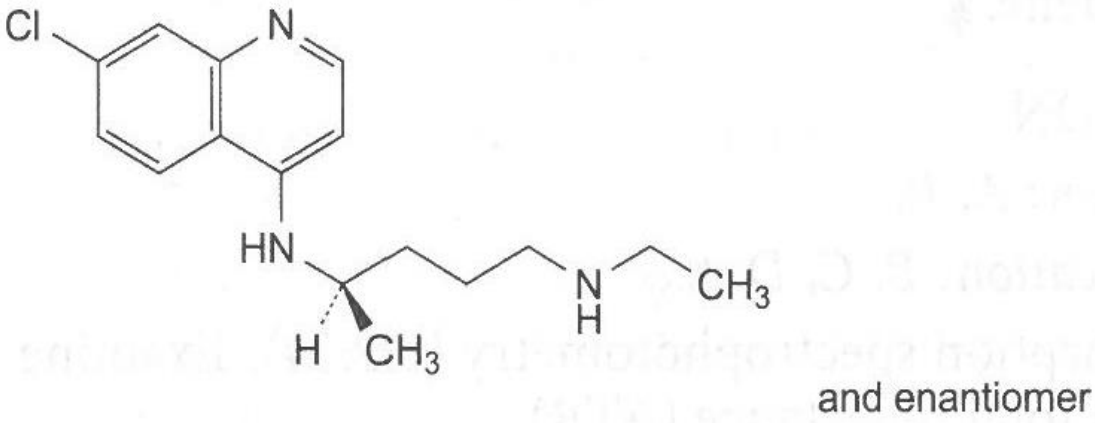
Synonym/chemical name: *2-[[(4RS)-4-[(7-chloroquinolin-4-yl)amino]pentyl]-(ethyl)amino]ethyl hydrogen sulphate*

Name: *impurity C*



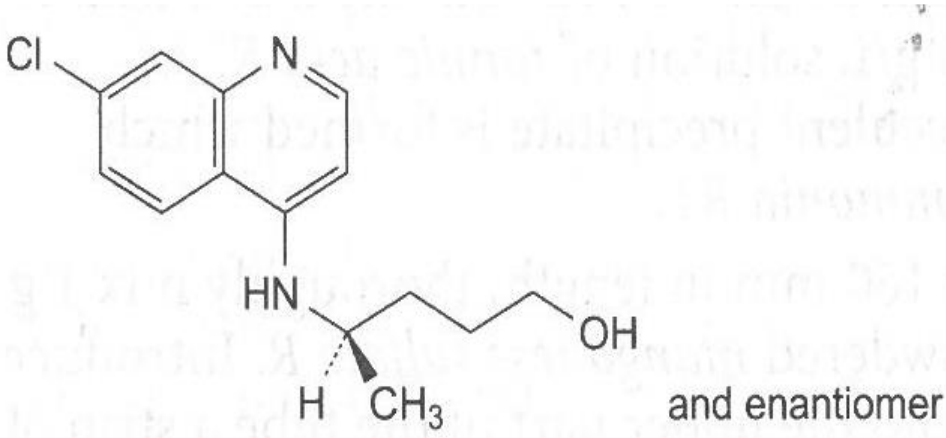
Synonym/chemical name: *2-[[(4RS)-4-[(7-chloroquinolin-4-yl)amino]pentyl]-(ethyl)amino]ethan-1-ol*

Name: *impurity D*



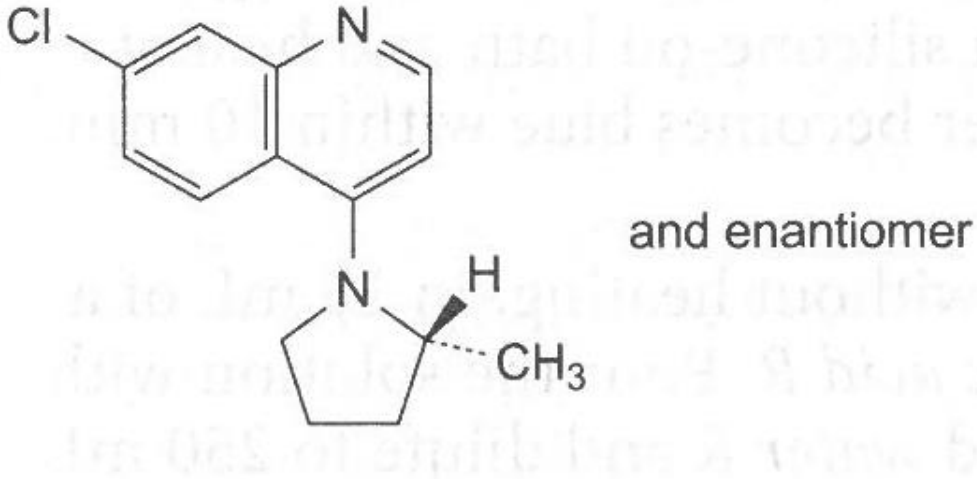
Synonym/chemical name: *(4RS)-N^4-(7-chloroquinolin-4-yl)-N^1-ethylpentane-1,4-diamine*

Name: *impurity E*



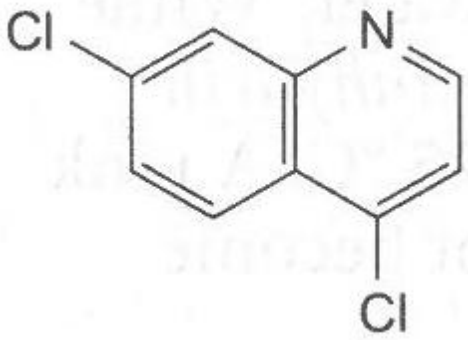
Synonym name: *(4RS)-4-[(7-chloroquinolin-4-yl)amino]pentane-1-ol*

Name: *impurity F*



Synonym name: *7-chloro-4-[(RS)-2-methylpyrrolidi-1-yl]quinoline*

Name: *impurity G*



Synonym name: *4,7-dichloroquinoline*