TETRACYCLINE HYDROCHLORIDE (TETRACLCLINI HYDROCHLORIDUM)

Draft revision for The International Pharmacopoeia

(February 2019)

DRAFT FOR COMMENT

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In order to speed up the process for receiving draft monographs and for sending comments, please send your email address to <u>jonessi@who.int</u> and we will add it to our electronic mailing list. Please specify if you wish to receive monographs.

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SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/17.740: 50 Draft revision for The International Pharmacopoeia TETRACYCLINE HYDROCHLORIDE 52 (TETRACYCLINI HYDROCHLORIDUM) 53

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Description	Date
First draft received from collaborating laboratory	September 2017
Presentation to WHO Expert Committee on Specifications for Pharmaceutical Preparations	October 2017
Presentation at the consultation on screening technology, sampling and specifications for medicines	2-4 May 2018
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Draft revision sent out for public consultation	February – April 2019
Discussion at the informal consultation on screening technologies, laboratory tools and pharmacopoeial specifications for medicines	02 – 03 May 2019
Further follow-up action as required	

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> [Note from the Secretariat. It is proposed to revise the monograph on Tetracycline hydrochloride as follows:

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to use an LC method to the test for related substances (instead of the described TLC method),

62 63 to use an LC method for assay (instead of the described microbiological method), to update the style of the monograph.

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Changes from the current monograph are indicated in the text by insert or delete.]

66 67 68 TETRACYCLINE HYDROCHLORIDE 69 (TETRACYCLINI HYDROCHLORIDUM)

70 **Molecular formula.** C₂₂H₂₄N₂O₈,HCl

71 **Relative molecular mass.** 480.9

72 **Graphic formula.**

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74 **Chemical** name. $(4S,4aS,5aS,6S,12\alpha S)-4$ -Dimethylamino-1,4,4a,5,5a,6,11,12a-

75 octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide

76 monohydrochloride; $[4S-(4\alpha,4\alpha\alpha,5\alpha\alpha,6\beta,12\alpha\alpha)]-4-(dimethylamino)-1,4,4\alpha,5,5\alpha,6,11,12\alpha-1$

octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide

78 monohydrochloride; CAS Reg. No. 64-75-5.

- 79 **Description.** A yellow, crystalline powder.
- 80 **Solubility.** Soluble in water R, slightly soluble in ethanol (~750 g/l) TS; practically
- 81 insoluble in acetone R. It dissolves in solutions of alkali hydroxides and carbonates.
- 82 Solutions in water R become turbid on standing, owing to the precipitation of tetracycline.
- 83 **Category.** Antibiotic.
- 84 **Storage**. Tetracycline hydrochloride should be kept in a tightly closed container, protected
- 85 from light.
- 86 **Additional information.** Tetracycline hydrochloride decomposes rapidly in solutions
- 87 below pH 2, and less rapidly in solutions above pH 7. Even in the absence of light,
- 88 Tetracycline hydrochloride is gradually degraded on exposure to a humid atmosphere, the

Working document QAS/17.740 page 4

- 89 decomposition being faster at higher temperatures. Tetracycline hydrochloride is a semi-
- 90 <u>synthetic product derived from a fermentation product.</u>

91 **Requirements**

- 92 **Definition.** Tetracycline hydrochloride contains not less than 95.0% and not more than
- 93 <u>102.0% of C₂₂H₂₄N₂O₈,HCl, calculated with reference to the dried substance.</u>

94 **Identity tests**

- Either tests A, E or tests B, D and E or tests C, D and E may be applied.
- 96 A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared
- 97 region. The infrared absorption spectrum is concordant with the spectrum obtained
- from tetracycline hydrochloride RS or with the reference spectrum of tetracycline
- 99 hydrochloride RS.
- B. Carry out the test as described under 1.14.1 Thin-layer chromatography using silica
- gel R6 as the coating substance and a mixture of acetonitrile R, methanol R and a 63
- g/L solution of oxalic acid R previously adjusted to pH 2 with ammonia (~260 g/L)
- TS (20:20:60 V/V/V) as the mobile phase. Apply separately to the plate 1 µL of each
- of the following 3 solutions in methanol R containing (A) 0.5 mg of the test substance
- per mL, (B) 0.5 mg of tetracycline hydrochloride RS per mL and (C) 0.5 mg of
- tetracycline hydrochloride RS, 0.5 mg of demeclocycline hydrochloride R and 0.5 mg
- oxytetracycline hydrochloride R per mL. Develop the plate for a distance of 15 cm.
- After removing the plate from the chromatographic chamber allow it to dry in air or in
- a current of air. Examine the chromatogram under ultraviolet light (254 nm). The test
- is not valid unless the chromatogram obtained with solution (C) shows three clearly
- separated spots. The principal spot in the chromatogram obtained with solution (A)
- 112 corresponds in position, appearance and intensity with the spot due to tetracycline in
- the chromatogram obtained with solution (B).

114	C. Carry out the test as described under 1.14.4 High-performance liquid chromatography
115	using the conditions given under "Assay". The retention time of the principal peak in
116	the chromatogram obtained with solution (1) corresponds to the retention time of the
117	tetracycline peak in the chromatogram obtained with solution (2).
118	D. To about 1 mg of the test substance add 2 mL of sulfuric acid (~1760 g/l) TS; a red-
119	violet colour is produced which on the addition of 0.1 mL of water R changes to
120	<u>yellow.</u>
121	E: A 0.05 g/mL solution yields reaction B described under 2.1 General identification tests
122	as characteristic of chlorides.
123	Specific optical rotation (1.4). Use a 10 mg/mL solution in hydrochloric acid (0.01 mol/L)
124	VS; $\left[\alpha\right]_{D}^{20} = -240$ to -255 with reference to the dried substance.
125	Loss on drying. Dry at 60 °C under reduced pressure (not exceeding 0.6 kPa or about 5
126	mm of mercury) over phosphorus pentoxide R for 3 hours; it loses not more than 20 mg/g.
120	mini of increary) over phosphorus pentoxide K for 5 nours, it foses not more than 20 mg/g.
127	pH value (1.13). pH of a 10 mg/mL solution, 1.8-2.8.
128	Sulfated ash (2.3). Not more than 5.0 mg/g.
129	Related substances. Carry out the test as described under 1.14.4 High-performance liquid
130	chromatography, using the conditions given below under "Assay".
131	Use solution (1) as described under "Assay". Prepare the following additional solutions
132	using mobile phase A as diluent. For solution (3) dissolve 12.5 mg of anhydrotetracycline
133	hydrochloride RS and dilute to 50.0 mL. For solution (4) dissolve 12.5 mg of 4-
134	epitetracycline hydrochloride RS and dilute to 50.0 ml. For solution (5) dissolve 12.5 mg
135	of 4-epianhydrotetracycline hydrochloride RS and dilute to 50.0 ml. For solution (6)
136	transfer 10.0 mL of solution (1) and 5.0 mL each of solution (3), (4) and (5) to a 50 mL
137	volumetric flask mix and dilute to volume. For solution (7) dilute 1 volume of solution (3)

- to 500 volumes. For solution (8) dilute 1 volume of solution (4) to 100 volumes. For
- solution (9) dilute 1 volume of solution (5) to 500 volumes.
- 140 <u>Inject alternately 10 μL each of solution (1), (6), (7), (8) and (9).</u>
- 141 The following peaks are eluted at the following relative retention with reference to
- tetracycline (retention time about 5 minutes): impurity A (4-epitetracycline) about 0.9;
- impurity B (2-acetyl-2-decarbamolyltetracycline) about 1.1; impurity D (4-
- epianhydrotetracycline) about 1.5; and impurity C (anhydrotetracycline) about 1.7.
- The assay is not valid unless in the chromatogram obtained with solution (6) the resolution
- between 4-epitetracycline and tetracycline is at least 2.5 and the resolution between 4-
- epianhydrotetracycline and anhydrotetracycline is at least 2.5.
- In the chromatogram obtained with solution (1):
- the area of any peak corresponding to impurity A is not greater than 1.5 times the
- area of the peak due to impurity A (4-epitetracycline) in the chromatogram obtained
- 151 with solution (8)(3.0%);
- the area of any peak corresponding to impurity B (2-acetyl-2-
- decarbamolyltetracycline) is not greater than 0.75 times the area of the peak due to
- impurity A (4-epitetracycline) in the chromatogram obtained with solution (8)
- 155 (1.5%).
- the area of any peak corresponding to impurity C is not greater than 1.25 times the
- area of the peak due to impurity C (anhydrotetracycline) in the chromatogram
- obtained with solution (7)(0.5%);
- the area of any peak corresponding to impurity D is not greater than 1.25 times the
- area of the peak due to impurity D (4-epianhydrotetracycline) in the chromatogram
- obtained with solution (9) (0.5%).
- 162 **Assay.** Carry out the test as described under 1.14.4 High-performance liquid
- 163 chromatography, using a stainless steel column (15 cm x 4.6 mm) packed with particles of

- base-deactivated silica gel, the surface of which has been modified with chemically-bonded
 octadecylsilyl groups (3 µm)¹.
- 166 <u>Use the following conditions for gradient elution:</u>
- 167 <u>Mobile phase A:</u> 1 volume of phosphoric acid (~1440g/L) TS in 1000 volumes

 168 of water R;
- 169 <u>Mobile phase B:</u> acetonitrile R.

	Mobile phase A	Mobile phase B	
Time (min)			<u>Comment</u>
	(% v/v)	(% v/v)	
<u>0-7.5</u>	<u>85 to 60</u>	15 to 40	Linear gradient
7.5-7.6	60 to 85	40 to 15	Return to initial
7.5 7.0	00 10 05	10 13	conditions
<u>7.6-10</u>	<u>85</u>	<u>15</u>	re-equilibration

- 170 Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet
- 171 spectrophotometer set at a wavelength of 280 nm. Maintain the column temperature at
- 172 <u>50 °C and the autosampler temperature at 10 °C.</u>
- 173 Prepare the following solutions using mobile phase A as diluent. For solution (1) dissolve
- 25.0 mg of the test substance and dilute to 200.0 mL. For solution (2) dissolve 25.0 mg of
- tetracycline hydrochloride RS and dilute to 200.0 mL.
- 176 <u>Inject alternately 10 μL each of solutions (1) and (2).</u>
- Measure the areas of the peak responses obtained in the chromatograms from solutions
- 178 (1) and (2), and calculate the percentage content of C₂₂H₂₄N₂O₈,HCl using the declared
- content of C₂₂H₂₄N₂O₈,HCl in tetracycline hydrochloride RS.

¹ A Prodigy ODS-3 column has been found suitable.

Working document QAS/17.740 page 8

- 180 **Bacterial endotoxins.** If intended for use in the manufacture of a parenteral dosage form
- without a further appropriate procedure for the removal of bacterial endotoxins, carry out
- the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.5
- 183 IU of endotoxin RS per mg of tetracycline hydrochloride.

Impurities

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186 A. (4R,4aS,5aS,6S,12aS)-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-

1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (4-

188 <u>epitetracycline</u>)

190 <u>B. (4S,4aS,5aS, 6S, 12aS)-2-acetyl-4-(dimethylamino)-3,6, 0,12,12a-pentahydroxy-6-</u>

methyl-4a,5a,6,12a-tetrahydrotetracene-1,11(4*H*,5*H*)-dione (2-acetyl-2-

decarbamolyltetracycline).

194 <u>C. (4*S*,4a*S*,12a*S*)-4-(dimethylamino)-3,10,11,12a-tetrahydroxy-6-methyl-1,12-dioxo-</u>

1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide (anhydrotetracycline)

D. (4*R*,4a*S*,12a*S*)-4-(dimethylamino)-3,10,11,12a-tetrahydroxy-6-methyl-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide (4-epianhydrotetracycline).

E. (4*S*,4a*S*,5a*S*, 6*S*, 12a*R*)-7-Chloro-4-(dimethylamino)-1,6,10,11,12a-pentahydroxy-6-methyl-3,12-dioxo-4,4a,5,5a-tetrahydrotetracene-2-carboxamide (chlortetracycline).

204 [Already established reference substances

205 Tetracycline hydrochloride ICRS

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Anhydrotetracycline hydrochloride ICRS

4-Epitetracycline hydrochloride ICRS

208 4-Epianhydrotetracycline hydrochloride ICRS

Reagents to be established

210 Demeclocycline hydrochloride R

211 Demeclocycline hydrochloride of a suitable quality should be used.

212 Oxytetracycline hydrochloride R

213 Oxytetracycline hydrochloride of a suitable quality should be used.]

Molecular formula. C22H24N2O8,HCl

216 **Relative molecular mass.** 480.9

217 Graphic formula.

Chemical name. (4S,4aS,5aS,6S,12αS) 4-Dimethylamino 1,4,4a,5,5a,6,11,12a
 octahydro 3,6,10,12,12a pentahydroxy 6 methyl 1,11 dioxo 2 naphthacenecarboxamide
 monohydrochloride; [4S (4α,4aα,5aα,6β, 12aα)] 4 (dimethylamino) 1,4,4a,5,5a,6,11,12a-

222	octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide
223	monohydrochloride; CAS Reg. No. 64-75-5.
224	Description. A yellow, crystalline powder; odourless.
225	Solubility. Soluble in 10 parts of water and in 100 parts of ethanol (~750 g/l) TS;
226	practically insoluble in acetone R and ether R.
227	Category. Antibiotic.
228	Storage. Tetracycline hydrochloride should be kept in a tightly closed container,
229	protected from light.
230	Labelling. The designation sterile Tetracycline hydrochloride indicates that the substance
231	complies with the additional requirements for sterile Tetracycline hydrochloride and may
232	be used for parenteral administration or for other sterile preparations.
233	Additional information. Tetracycline hydrochloride decomposes rapidly in solutions
234	below pH 2, and less rapidly in solutions above pH 7. Even in the absence of light,
235	Tetracycline hydrochloride is gradually degraded on exposure to a humid atmosphere, the
236	decomposition being faster at higher temperatures.
237	Requirements
238	Definition. Tetracycline hydrochloride contains when tested according to assay A not
239	less than 96.0% and not more than 102.0% of C22H24N2O8,HCl, and when tested
240	according to assay B not less than 950 International Units per mg, both calculated with
241	reference to the dried substance.
242	Identity tests
243	A. Carry out the test as described under 1.14.1 Thin layer chromatography, but using an
244	unlined chamber and a cellulose coating prepared as follows: To 0.275 g of carbomer R
245	add 120 mL of water, let the mixture stand for 1 hour while shaking it from time to time;

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then add gradually while stirring a sufficient volume of sodium hydroxide (~80 g/l) TS to adjust to pH 7.0. To this mixture add 30 g of cellulose R1 and a sufficient quantity of water (usually 60-80 mL) to obtain a coating substance of suitable consistency. Coat the plates with a layer 0.4 mm thick, and allow them to dry at room temperature. The plates thus coated are used after a suitable treatment both for the identity test and the test of "related substances". For the identity test spray the plate with phosphate/citrate buffer pH 252 4.5, TS, until traces of moisture appear. Dry the plate at 50°C for 30 minutes. 253 Prepare the following solutions immediately before use while protected from bright light: 254 Dissolve 5.0 mg of the test substance, 5.0 mg of chlortetracycline hydrochloride RS, 5.0 mg of oxytetracycline hydrochloride RS, and 5.0 mg of tetracycline hydrochloride RS in sufficient methanol R to produce 10 mL; this constitutes solution A. Dissolve 5.0 mg of 257 chlortetracycline hydrochloride RS and 5.0 mg of oxytetracycline hydrochloride RS in sufficient methanol R to produce 10 mL; this constitutes solution B. Dissolve 5.0 mg of chlortetracycline hydrochloride RS, 5.0 mg of oxytetracycline hydrochloride RS, and 5.0 mg of tetracycline hydrochloride RS in sufficient methanol R to produce 10 mL; this constitutes solution C. Apply separately to the plate 1 µl of each of solutions A, B and C, and spray it very finely and uniformly with trimethylpyridine (50 g/l) TS until traces of humidity appear (about 8 mL). Pour the mobile phase consisting of a mixture of 60 volumes of ethyl acetate R, 30 volumes of acetone R, and 6 volumes of water in the unlined chromatographic chamber. Place the plate in the chamber in such a manner that it is not in contact with the mobile phase. Allow the plate to become impregnated with the vapours for 1 hour. Then dip the plate into the mobile phase and allow the chromatogram to develop to a distance of 15 270 cm. After removing the plate from the chromatographic chamber, allow it to dry in air, expose it to the vapour of ammonia (~260 g/l) TS, and examine the chromatogram 272 immediately in ultraviolet light (365 nm). Three principal clearly separated spots are 273 obtained with solution A corresponding in position, appearance, and intensity with those

2/4	obtained with solution C, two or which correspond with the spots obtained with solution
275	B.
276	B. To about 1 mg add 2 mL of sulfuric acid (~1760 g/l) TS; a red-violet colour is
277	produced which on the addition of 0.1 mL of water changes to yellow.
278	C. A 0.05 g/mL solution yields reaction B described under 2.1 General identification tests
279	as characteristic of chlorides.
280	Specific optical rotation. Use a 10 mg/mL solution in hydrochloric acid (0.01 mol/l) VS
281	and calculate with reference to the dried substance; [α] _D ^{20°C} = 239° to 258°.
282	Loss on drying. Dry at 60°C under reduced pressure (not exceeding 0.6 kPa or about 5
283	mm of mercury) for 3 hours; it loses not more than 20 mg/g.
284	pH value. pH of a 10 mg/mL solution, 1.8-2.8.
285	Related substances. Carry out the test as described under 1.14.1 Thin-layer
286	chromatography, using a plate as prepared under the identity test A. To a sufficient
287	volume of disodium edetate (0.1 mol/l) VS add sodium hydroxide (~80 g/l) TS to adjust
288	to pH 7.0, and use this solution to spray the plate uniformly until traces of moisture
289	appear. Dry the plate at 50°C for 30 minutes.
290	Prepare the following solutions immediately before use, protecting them from bright
291	light: Dissolve 0.10 g of the test substance in sufficient methanol R to produce 10 mL;
292	this constitutes solution A. Dilute 2.5 mL of solution A to 10.0 mL with methanol R; this
293	constitutes solution B. Dissolve 5.0 mg of 4-epianhydrotetracycline hydrochloride RS
294	insufficient methanol R to produce 20 mL; this constitutes solution K. Dilute 2 mL of
295	solution K to 10 mL with methanol R; this constitutes solution C. Dissolve 5.0 mg of 4-
296	epitetracycline hydrochloride RS in sufficient methanol R to produce 8 mL; this
297	constitutes solution L. Dilute 2 mL of solution L to 10 mL with methanol R; this
298	constitutes solution D. Dissolve 5.0 mg of anhydrotetracycline hydrochloride RS in
299	sufficient methanol R to produce 20 mL: this constitutes solution M. Dilute 2 mL of

300 solution M to 10 mL with methanol R; this constitutes solution E. Dissolve 20 mg of 301 chlortetracycline hydrochloride RS in sufficient methanol R to produce 20 mL; this 302 constitutes solution N. Dilute 2 mL of solution N to 10 mL with methanol R; this 303 constitutes solution F. Dissolve 10 mg of tetracycline hydrochloride RS in sufficient 304 methanol R to produce 20 mL; this constitutes solution P. Mix together 0.5 mL of each of 305 the following solutions K, L, M, N and P; this constitutes solution G. 306 Apply separately to the plate 1 µl of each of solutions A, B, C, D, E, F, and G, and spray 307 it very finely and uniformly with trimethylpyridine (50 g/l) TS until traces of humidity 308 appear (about 8 mL). 309 As the mobile phase, use a mixture of 60 volumes of ethyl acetate R, 30 volumes of 310 acetone R, and 6 volumes of water. Allow the chromatogram to develop to a distance of 311 15 cm. After removing the plate from the chromatographic chamber, allow it to dry in air, 312 expose it to the vapour of ammonia (~260 g/l) TS, and examine the chromatogram 313 immediately in ultraviolet light (365 nm). The spot corresponding to 4-epitetracycline 314 hydrochloride obtained with solution B is not more intense than that obtained with 315 solution D (5% of 4-epitetracycline hydrochloride). The spots corresponding to 4-316 epianhydrotetracycline hydrochloride, anhydrotetracycline hydrochloride, and 317 chlortetracycline hydrochloride obtained with solution A are not more intense than those 318 obtained with solution C (0.5% of 4-epianhydrotetracycline hydrochloride), solution E 319 (0.5% of anhydrotetracycline hydrochloride), and solution F (2% of chlortetracycline 320 hydrochloride). The test is not valid unless the chromatogram obtained with solution G 321 shows 5 clearly separated spots. 322 Anhydroderivatives. Dissolve about 0.2 g, accurately weighed, in sufficient 323 hydrochloric acid (0.02 mol/l) VS to produce 50 mL. Place 10.0 mL in a separator, add 324 10 mL of chloroform R and 10 mL of citrate buffer, pH 5.4 TS, and shake for 2 minutes. 325 Separate the chloroform layer and measure the absorbance at 437 nm against a solvent 326 cell containing chloroform R; not more than 0.18 (preferably use 2-cm cells for the 327 measurement and calculate the absorbance of a 1-cm layer).

328	Assay
329	A. Dissolve about 0.25 g, accurately weighed and previously dried at 60°C under reduced
330	pressure, in 5 mL of formic acid (~1080 g/l) TS and 10 mL of glacial acetic acid R1, add
331	10 mL of dioxan R, 5 mL of mercuric acetate/acetic acid TS, and titrate with perchloric
332	acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration. Method A. Each mL of
333	perchloric acid (0.1 mol/l) VS is equivalent to 48.09 mg of C ₂₂ H ₂₄ N ₂ O ₈ ,HCl.
334	B. Carry out the assay as described under 3.1 Microbiological assay of antibiotics, using
335	either (a) Bacillus pumilus (NCTC 8241 or ATCC 14884) as the test organism, culture
336	medium Cm1 with a final pH of 6.5-6.6, sterile phosphate buffer, pH 4.5 TS, an
337	appropriate concentration of tetracycline (usually between 2 and 20 IU), and an
338	incubation temperature of 37-39°C, or (b) Bacillus cereus (ATCC 11778) as the test
339	organism, culture medium Cm1 with a final pH of 5.9-6.0, sterile phosphate buffer, pH
340	4.5 TS, an appropriate concentration of tetracycline (usually between 0.5 and 2 IU), and
341	an incubation temperature of 30-33°C. The precision of the assay is such that the fiducial
342	limits of error of the estimated potency $(P = 0.95)$ are not less than 95% and not more
343	than 105% of the estimated potency. The upper fiducial limit of error of the estimated
344	potency $(P = 0.95)$ is not less than 950 IU per mg, calculated with reference to the dried
345	substance.
346	Additional Requirements for Tetracycline Hydrochloride for sterile use
347	Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial
348	endotoxins; contains not more than 0.5 IU of endotoxin RS per mg.
349	Sterility. Complies with 3.2 Test for sterility.
350	***