

Coloured disinfectant preparation with a content of octenidine dihydrochloride

The present invention relates to a disinfectant preparation comprising a) octenidine dihydrochloride, b) isopropanol, c) annatto dye and d) water. In addition, the invention relates to a method for preparing the disinfectant preparations. Furthermore, the invention relates to said preparation for use in a method for disinfecting skin. The invention also relates to the use of the preparation for disinfecting skin or for staining skin.

In certain applications of antiseptic disinfectants, a colour marking of the treated surface (such as skin) is desirable, for example prior to a surgical intervention or during an operation. In addition to compositions based on the active ingredient PVP-iodine, which are already coloured due to the colour of the active ingredient (brown-red), there are coloured skin antiseptics on the market, wherein the active ingredients are non-ionic for example (e.g. Kodan[®] Tincture forte coloured, with 45% by weight 2-propanol, 10% by weight 1-propanol, 0.2% by weight 2-biphenylol, H₂O₂, E104, E110 and E151).

The group of cationic antiseptic active ingredients for skin antiseptics also includes chlorhexidine gluconate ("chlorhexidine" below), which has been put on the US market in formulations with 70% by volume 2-propanol (e.g. the product ChloroPrep[®] from Carefusion). On account of the high active ingredient concentrations in these products, a potential loss of efficacy on addition of small amounts of dyes does not matter in antiseptics based on chlorhexidine. In principle however, there are concerns about chlorhexidine-containing antiseptics, specifically on account of the potential formation of p-chloroaniline (carcinogen, toxic) as a potential degradation product on storage. In addition, owing to the comparatively low efficacy of chlorhexidine, relatively high active ingredient concentrations in the product are necessary.

WO2007/130981 A2 describes aqueous solutions of chlorhexidine and a cationic dye. EP 2 499 913 A1 is also concerned with antiseptic solutions containing chlorhexidine, and anionic dyes are proposed for the staining thereof.

WO2016/059653 A1 discloses blue-coloured chlorhexidine solutions for disinfecting skin.

WO97/46622 A1 describes the use of natural or nature-identical synthetic dyes for marking or staining materials. By way of example, the brief marking of operation areas on the skin by means of disinfectant solutions containing dye or colour pens is mentioned. The fact that certain active ingredients of disinfectants have a remanence effect, which must not be influenced by the dye used, is not addressed in WO97/46622 A1.

DE 40 33 690 A1 discloses adducts of norbixin with water-soluble or water-dispersible proteins or branched-chain or cyclic polysaccharides. Norbixin is a carotenoid having two carboxylic acid groups obtained from annatto seeds. Bixin is the monomethyl ester of norbixin. The adducts are used for colouring milk for example. The staining of skin by coloured disinfectants is not addressed.

The medicament and surgical skin antiseptic octeniderm® is approved as a colourless product (Schülke & Mayr GmbH, Norderstedt, Federal Republic of Germany). It contains 0.1% by weight octenidine dihydrochloride ("octenidine" for short below) as remanent active ingredient (and 30% by weight 1-propanol, 45% by weight 2-propanol, remainder: demineralized water). Octenidine is a quaternary ammonium compound of the imine type (cation) with chloride as anion and its antiseptic effectiveness may be inhibited by the vast majority of anionic dyes. Since octeniderm® contains only 0.1% octenidine, dyes added at relatively high concentration (up to 0.5%, such as in products containing chlorhexidine) for staining the skin may lead to a long-term (up to 24 h) distinct decrease in the remanent effect of octenidine.

It has been found that the triphenylmethane dye Patent Blue V, despite its anionic character, exhibits no restriction of the efficacy of octenidine in octeniderm®. However, blue dyes are not accepted on the market on account of the poor visibility of vessels under the skin.

DE 10 2014 107 412 A1 discloses non-solid disinfectant preparations which comprise a) one or more bispyridinium alkanes and b) one or more dyes selected from xanthene dyes, azo dyes and polyterpene compounds, wherein the preparation is free of fluorescein and salts thereof. When an aliphatic alcohol is used alone, the use of n-propanol is preferred over the use of isopropanol. An example preparation comprises 30% by weight n-propanol and 45% by weight isopropanol in addition to octenidine dihydrochloride and annatto dye.

It is an object of the present invention to provide coloured disinfectant preparations in which the efficacy of octenidine is not inhibited and which are eligible for approval even with the added dye. It is also an object of the present invention to provide preparations of this kind which may be stored over a prolonged period and in so doing do not have a tendency to form precipitates that are disadvantageous when dosing, specifically without having to add additional auxiliaries (which merely prevent the formation of precipitates or the inhibition of the antiseptic effect and which contribute nothing to the antiseptic effect). In addition, it is an object of the invention to provide disinfectant preparations which, on application to the skin, do not adversely modify its natural pH. Furthermore, it is an object of the invention to further increase the stability of the dye.

It has now been proven that this object is achieved by a disinfectant preparation comprising

- a) 0.02 to 0.25% by weight octenidine dihydrochloride,
b) at least 50% by weight isopropanol,
c) annatto dye, wherein the amount of annatto dye is 0.005 to 0.05% by weight, specified as norbixin and based on the weight of the disinfectant preparation, and
5 d) water,
wherein the pH of the disinfectant preparation is 10.5 to 12.5, measured in the disinfectant preparation without components a) and b).

The invention is based, inter alia, on preparations which have a particularly good storage stability in the defined pH range. They are outstandingly effective due to the high isopropanol content, including a remanent effect due to the octenidine content. It was surprising, further-
10 more, that the preparation according to the invention is colour-stable, although a corresponding preparation formulated with chlorhexidine is not colour-stable. It was also surprising that the disinfectant preparations according to the invention can be formulated without adding an additional buffer system, which is why the natural pH of the skin is not adversely altered on appli-
15 cation.

Preparations according to the invention

a) Octenidine dihydrochloride

Component a), octenidine dihydrochloride, is present in preparations according to the in-
20 vention in an amount of 0.02 to 0.25% by weight, based on the weight of the preparation. The amount of octenidine dihydrochloride is preferably 0.03 to 0.225% by weight, more preferably 0.04 to 0.215% by weight, especially 0.05 to 0.2% by weight, such as about 0.05% by weight, or about 0.1% by weight, or about 0.2% by weight. The amount of octenidine dihydrochloride is particularly preferably 0.08 to 0.12% by weight, more preferably 0.09 to 0.11% by weight,
25 based in each case on the weight of the preparation.

b) Isopropanol

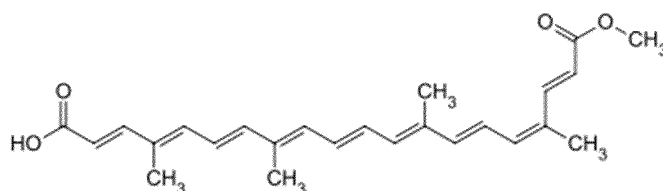
The preparation according to the invention comprises at least 50% by weight isopropanol, preferably 53 to 80% by weight isopropanol, more preferably 55 to 75% by weight isopropanol, in particular 58 to 70% by weight isopropanol, more preferably 60 to 65% by weight isopropa-
30 nol, such as about 63% by weight isopropanol.

c) Annatto dye

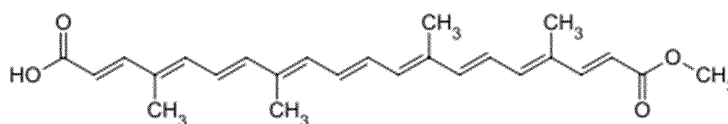
The preparation comprises annatto dye as component c), wherein the amount of annatto dye is 0.005 to 0.05% by weight, specified as norbixin and based on the weight of the disinfectant

preparation. The preparation preferably comprises 0.01 to 0.04% by weight annatto dye, in particular 0.015 to 0.025% by weight annatto dye, more preferably 0.017 to 0.022% by weight annatto dye, such as about 0.02% by weight annatto dye, specified in each case as norbixin and based on the weight of the disinfectant preparation.

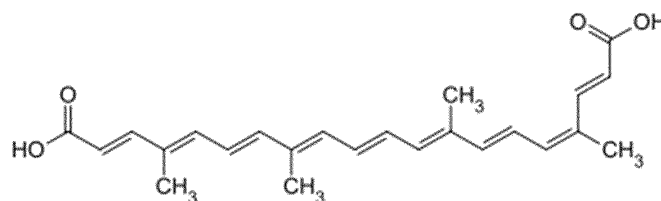
- 5 Norbixin (a xanthophyll) is a natural dye which is obtained by extracting annatto from the seeds of urucum or achiote plants (*Bixa orellana*). Norbixin may be obtained by removing the outer layer of the prepared seeds of the annatto shrub (*Bixa orellana*) by grinding the seeds in cold water and subsequent extraction with solvents such as acetone, methanol or hexane for example. The solution resulting therefrom is acidified bixin (specifically the methyl ester of the acid norbixin), which is then filtered, dried and the precipitate milled. The precipitate mainly
10 comprises cis- and trans-bixin, wherein the principle dye is cis-bixin. Norbixin can be obtained as the corresponding salt using alkaline sodium hydroxide solution, wherein cis-norbixin is again the principle dye.



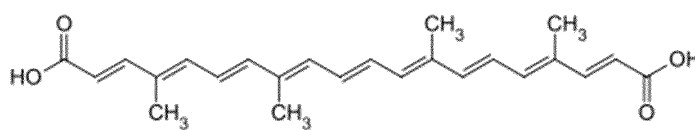
cis-bixin



trans-bixin



cis-norbixin



trans-norbixin

In preparations according to the invention, preferably neither fluorescein nor salts thereof
5 (such as the disodium salt, uranine) are present.

d) Water

Preparations according to the invention also contain d) water.

Preparations according to the invention may be formulated without a mandatory buffer sys-
tem and as a result do not unnecessarily influence the natural pH of (human) skin. Preferred
10 preparations according to the invention do not comprise any added buffer system.

Preferred preparations according to the invention comprise less than 35% by weight n-pro-
panol, preferably less than 25% by weight n-propanol, more preferably less than 15% by weight
n-propanol, especially less than 5% by weight n-propanol, wherein the preparation particularly
preferably does not comprise any n-propanol.

15 Preferred preparations according to the invention comprise less than 30% by weight etha-
nol, preferably less than 20% by weight ethanol, more preferably less than 10% by weight eth-
anol, especially less than 5% by weight ethanol, wherein the preparation particularly preferably
does not comprise any ethanol.

Preferred preparations according to the invention comprise less than 10% by weight aro-
20 matic alcohol, preferably less than 5% by weight aromatic alcohol, wherein the preparation par-
ticularly preferably does not comprise any aromatic alcohol.

Preferred preparations according to the invention do not comprise any glycerol, wherein the
preparation preferably does not comprise any polyol.

Preferred preparations according to the invention do not comprise any chloride, acetate or
25 gluconate salt of 1,1'-hexamethylenebis[5-(4-chlorophenyl)biguanide], preferably do not com-
prise any biguanide or salt thereof at all.

An example formulation of preparations according to the invention is specified below (figures in % by weight):

	Preferably/more preferably/especially about
Octenidine	0.03 to 0.225/0.04 to 0.215/0.05 to 0.2
2-Propanol	53 to 80/55 to 75/58 to 70
Annatto dye	0.01 to 0.04/0.015 to 0.025/0.017 to 0.022 ⁺

⁺ specified in each case as norbixin and based on the weight of the disinfectant preparation

5 Preferred preparations according to the invention comprise

a) 0.03 to 0.225% by weight octenidine dihydrochloride, preferably about 0.04 to 0.215% by weight octenidine dihydrochloride,

b) 53 to 80% by weight isopropanol, preferably 55 to 75% by weight isopropanol, especially 58 to 70% by weight isopropanol, such as about 63% by weight isopropanol,

10 c) annatto dye, wherein the amount of annatto dye is 0.01 to 0.04% by weight, specified as norbixin and based on the weight of the disinfectant preparation (especially 0.015 to 0.025, such as about 0.02% by weight, specified as norbixin and based on the weight of the disinfectant preparation), and

d) water,

15 wherein the pH of the disinfectant preparation is about 11 to 12, measured in the disinfectant preparation without components a) and b).

Dosage forms of the preparation

20 The disinfectant preparation according to the invention is a liquid aqueous-alcoholic preparation. The preparation according to the invention is preferably transparent. This facilitates the observation of disinfected areas.

Method for preparing the preparation according to the invention

25 The preparation according to the invention cannot be prepared by dissolving octenidine in an aqueous phase, independently of whether the aqueous phase has already been adjusted to the desired pH or already comprises the dye. Instead, it is necessary to formulate octenidine in the aqueous-alcoholic phase. It is also not possible to formulate the annatto dye in an already formulated aqueous-alcoholic phase. In accordance with the invention, therefore, the procedure is such that the dye is dissolved in the aqueous solution at the desired pH, in the range of 10.5 to
30 12.5, isopropanol is then added and finally the active ingredient octenidine is added. It is thus

possible in accordance with the invention, surprisingly, to prepare the preparations specified which - optionally after filtration following addition of the octenidine active ingredient - are storage-stable even over a prolonged period.

The invention therefore also relates to a method in which

- i. an aqueous alkaline solution is initially charged,
- ii. the annatto dye is added,
- iii. the pH of the solution is adjusted to the desired value in the range of 10.5 to 12.5,
- iv. isopropanol is added and then
- v. octenidine dihydrochloride is added.

Application

The disinfectant preparations according to the invention are used on the skin in a customary manner, particularly on human skin.

For the avoidance of doubt, it is pointed out that the skin on which the preparation is used is an animate skin.

In accordance with the present invention, the preparation is preferably used for skin anti-sepsis of undamaged skin. Preparations according to the invention are preferably applied to the skin in customary manner using swabs.

In this aspect, the invention also relates to a disinfectant preparation for use in a method for disinfecting skin, especially in humans, wherein the preparation comprises

- a) 0.02 to 0.25% by weight octenidine dihydrochloride, such as about 0.1% by weight octenidine dihydrochloride,
- b) at least 50% by weight isopropanol,
- c) annatto dye, wherein the amount of annatto dye is 0.005 to 0.05% by weight, specified as norbixin and based on the weight of the disinfectant preparation, and
- d) water,

and wherein the pH of the disinfectant preparation is 10.5 to 12.5, measured in the disinfectant preparation without components a) and b).

The preparation is preferably employed for use in a method in which the method is a i) pre-operative measure, ii) a measure prior to puncture or iii) a measure prior to injection. By way of example, punctures or injections are (cf. hygiene requirements in punctures and injections - recommendation of the commission on hospital hygiene and prevention of infection at the Robert Koch Institute (RKI), Federal Law Gazette 2011, 54:1135-1144, DOI 10.1007/s00103-011-1352-8, Springer-Verlag 2011, Tab. I therein :

- s.c. injection,
- s.c. injection with catheter system for s.c. infusion,
- i.m. injection,
- diagnostic lumbar puncture,
- 5 - lumbar puncture with single injection,
- peridural/intraspinal anaesthesia,
- joint puncture,
- intravitreal injection,
- kidney biopsy,
- 10 - amniocentesis chorionic villus biopsy,
- percutaneous endoscopic gastrostomy (PEG) and
- external ventricular drainage.

The invention also relates to the use of the preparation for disinfecting (preferably human) skin.

- 15 The invention also relates to the use of the preparation for staining (preferably human) skin.

The advantages of the invention are particularly apparent from the following examples. Amounts refer to weight unless otherwise stated.

Examples

- 20 Materials used:

- (purified) water
- aqueous potassium hydroxide solution (10%, 45%)
- montebixin 401 (pulverulent product having an 80% norbixin content, produced by Frutarom Peru S.A., Lima, Peru)
- 25 - hydrochloric acid (10%)
- isopropanol
- octenidine dihydrochloride (octenidine)
- chlorhexidine digluconate (CHX), used as aqueous solution (20% (m/V))

30 **Example 1: Preparations with octenidine dihydrochloride**

To prepare preparations 1A to 1F, purified water was initially charged in each case and then montebixin and potassium hydroxide solution were added and dissolved with stirring (ca. 2h).

The pH was then adjusted to the desired value with potassium hydroxide solution or hydrochloric acid ("xxx" = was used). Isopropanol was then added and dissolved with stirring and finally octenidine was added and dissolved with stirring to give preparations 1A to 1F (Table 1).

5 **Table 1. Constituents of preparations 1A to 1F**

No.	Constituent, parts by weight	1A	1B	1C	1D	1E	1F
1	Water	36.5 75	36.5 75	36.5 75	36.5 75	36.5 75	36.3 15
2	KOH 10%	0.20 0	0.20 0	0.20 0	0.20 0	0.20 0	---
	KOH 45%	---	---	---	---	---	0.46 0
3	Montebixin 401	0.02 5	0.02 5	0.02 5	0.02 5	0.02 5	0.02 5
4	KOH 10%	---	---	---	---	xxx	---
	KOH 45%	---	---	---	---	---	xxx
5	Hydrochloric acid 10%	xxx	xxx	xxx	xxx	---	---
6	Isopropanol	63.1 00	63.1 00	63.1 00	63.1 00	63.1 00	63.1 00
7	Octenidine	0.10 0	0.10 0	0.10 0	0.10 0	0.10 0	0.10 0
	Total	100. 000	100. 000	100. 000	100. 000	100. 000	100. 000
	pH (after addition No. 4/5):	8.0	9.0	10.0	11.0	12.0	13.0

The results of the investigations of the stability of preparations 1A to 1F with octenidine are reported below in Tables 3 to 8 (n.c. = not conducted. S = solution P = precipitate). These results show that preparations with an octenidine and annatto dye content are only sufficiently storage-stable in the narrow pH range of 10.5 to 12.5.

5

These stability results after 6 months therefore show a higher annatto stability when the pH in the aqueous phase (water + annatto) is adjusted to a narrow highly alkaline pH range. These formulations were then admixed with the required amount of alcohol, applied to the skin and the skin pH measured before and after application. The skin pH was modified and influenced relatively little.

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Example 2: Preparations with 2.0% chlorhexidine digluconate (m/v)

To prepare preparations 2A to 2F, the procedure as in Example 1 was followed, with the difference that the active ingredient chlorhexidine gluconate was added in the final step instead of octenidine, to give preparations 2A to 2F, the composition of which is reported in Table 2.

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Table 2. Constituents of preparations 2A to 2F

No.	Constituent, parts by weight	2A	2B	2C	2D	2E	2F
1	Water	24.5 7	24.5 7	24.5 7	24.5 7	24.5 7	24.3 1
2	KOH 10%	0.20	0.20	0.20	0.20	0.20	---
	KOH 45%	---	---	---	---	---	0.46
3	Montebixin	0.02	0.02	0.02	0.02	0.02	0.02
	401	5	5	5	5	5	5
4	KOH 10%	---	---	---	---	xxx	---
	KOH 45%	---	---	---	---	---	xxx
5	Hydrochloric acid 10%	xxx	xxx	xxx	xxx	---	---
6	Isopropanol	63.1 00	63.1 00	63.1 00	63.1 00	63.1 00	63.1 00
7	CHX 20% (m/V)	12.1 05	12.1 05	12.1 05	12.1 05	12.1 05	12.1 05
	Total	100. 000	100. 000	100. 000	100. 000	100. 000	100. 000
	pH (after addition No. 4/5):	8.0	9.0	10.0	11.0	12.0	13.0

The results of the investigations of the stability of preparations 2A to 2F with a chlorhexidine gluconate content are reported below in Tables 9 to 14 (n.c. = not conducted. S = solution
5 P = precipitate). It follows that preparations with a chlorhexidine gluconate and annatto dye content are not storage-stable, even in the pH range of 10.5 to 12.5, whereas this pH has proven to be particularly advantageous for preparations according to the invention with an octenidine and annatto dye content.

Table 3. Stability of formulation 1A (pH 8.0)

Test parameter	Method	Blank value	3 months at		6 months at	
			-5°C	25°C	-5°C	25°C
Appearance of the solution	organoleptic	clear, orange-red	clear, vibrant orange + fine flakes in S.	clear, orange	clear, vibrant orange + fine flakes in S.	clear, orange
Isopropanol (%)	GC	63.2	n.c.	n.c.	62.6	63.1
Octenidine (%)	HPLC	0.100	n.c.	n.c.	0.101	0.101
Dye absorption maximum		449				
Dye extinction §	UV/Vis	1.10	0.72	0.43	0.53	0.33
Dye recovery (%) (based on blank value)	spectrometry	---	65	39	48	30
L*a*b* - values	L*	78.3	79.0	81.5	81.6	83.0
	a*	27.5	25.2	21.9	21.6	19.3
	b*	130.5	131.2	134.6	134.2	136.0

§ = 500 mg of the formulation were diluted to 25 ml with 96% ethanol by volume and subsequently measured at the absorption maximum.

Table 4. Stability of formulation 1B (pH 9.0)

Test parameter	Method	Blank value	3 months at		6 months at	
			-5°C	25°C	-5°C	25°C
Appearance of the solution	organoleptic	clear, orange-red	clear, vibrant orange + fine flakes in S.	clear, orange	clear, vibrant orange + fine flakes in S.	clear, orange
Isopropanol (%)	GC	63.2	n.c.	n.c.	63.1	62.3
Octenidine (%)	HPLC	0.101	n.c.	n.c.	0.102	0.103
Dye absorption maximum §	UV/Vis spectrometry	449				
Dye extinction (calculated on the basis of 500 mg/25 ml)		1.00	0.87	0.42	0.79	0.31
Dye recovery (%) (based on blank value)		---	87	42	79	31
L*a*b* - values	M0041-01					
		L*	79.5	81.7	79.8	83.5
		a*	26.2	21.7	24.9	18.3
		b*	132.1	134.7	132.1	136.4

§ = 500 mg of the formulation were diluted to 25 ml with 96% ethanol by volume and subsequently measured at the absorption maximum.

Table 5. Stability of formulation 1C (pH 10.0)

Test parameter	Method	Blank value	3 months		6 months	
			-5°C	25°C	-5°C	25°C
Appearance of the solution	organoleptic	clear, orange-red	clear, vibrant orange + fine flakes in S.	clear, orange	clear, vibrant orange + fine flakes in S.	clear, orange
Isopropanol (%)	GC	63.2	n.c.	n.c.	62.6	62.8
Octenidine (%)	HPLC	0.101	n.c.	n.c.	0.102	0.102
Dye absorption maximum	UV/Vis spectrometry	448				
Dye extinction §		1.17	0.73	0.38	0.51	0.26
Dye recovery (%) (based on blank value)		---	62	32	44	22
L*a*b* - values	L*	78.6	79.7	83.2	82.9	85.3
	a*	27.0	23.7	17.2	17.6	13.4
	b*	130.8	131.9	135.4	134.7	137.1

§ = 500 mg of the formulation were diluted to 25 ml with 96% ethanol by volume and subsequently measured at the absorption maximum.

Table 6. Stability of formulation 1D (pH 11.0)

Test parameter	Method	Blank value	3 months		6 months	
			-5°C	25°C	-5°C	25°C
Appearance of the solution	organoleptic	clear, orange-red	clear, orange-red	clear, orange-red	clear, orange-red	clear, orange-red
Isopropanol (%)	GC	63.4	n.c.	n.c.	62.5	62.9
Octenidine (%)	HPLC	0.100	n.c.	n.c.	0.102	0.101
Dye absorption maximum		448				
Dye extinction §	UV/Vis spectrometry	1.40	1.40	1.33	1.18	1.28
Dye recovery (%) (based on blank value)		---	100	95	84	91
L*a*b* - values	M0041-01	76.5	76.0	77.3	77.6	78.0
		30.4	30.7	28.7	27.9	27.7
		127.9	127.1	128.7	129.1	129.6

§ = 500 mg of the formulation were diluted to 25 ml with 96% ethanol by volume and subsequently measured at the absorption maximum.

Table 7. Stability of formulation 1E (pH 12.0)

Test parameter	Method	Blank value	3 months		6 months	
			-5°C	25°C	-5°C	25°C
Appearance of the solution	organoleptic	clear, orange-red	clear, orange-red	clear, orange-red	clear, orange-red	clear, orange-red
Isopropanol (%)	GC	63.2	n.c.	n.c.	63.1	63.5
Octenidine (%)	HPLC	0.100	n.c.	n.c.	0.101	0.100
Dye absorption maximum		447				
Dye extinction §	UV/Vis spectrometry	1.59	1.56	1.49	1.64	1.47
Dye recovery (%) (based on blank value)		---	98	94	103	92
L*a*b* - values	M0041-01	76.0	75.8	77.0	76.2	77.9
		30.9	31.2	29.2	30.5	27.9
		127.2	126.9	128.6	127.2	129.4

§ = 500 mg of the formulation were diluted to 25 ml with 96% ethanol by volume and subsequently measured at the absorption maximum.

Table 8. Stability of formulation 1F (pH 13.0)

Test parameter	Method	Blank value	3 months		6 months	
			-5°C	25°C	-5°C	25°C
Appearance of the solution	organoleptic	clear, orange-red	clear, orange-red	clear, orange-red	clear, orange-red	clear, orange-red
Isopropanol (%)	GC	63.5	n.c.	n.c.	62.2	62.4
Octenidine (%)	HPLC	0.098	n.c.	n.c.	0.095 #	0.087 #
Dye absorption maximum	UV/Vis spectrometry	447				
Dye extinction §		1.52	1.52	1.41	1.50	1.37
Dye recovery (%) (based on blank value)		---	100	93	99	90
L*a*b* - values	M0041-01	77.0	76.6	78.1	77.3	79.2
		29.1	29.6	26.8	28.8	25.3
		128.6	127.9	129.8	128.7	131.1

= a peak occurs underneath the internal standard, the samples were therefore calculated using external calibration

§ = 500 mg of the formulation were diluted to 25 ml with 96% ethanol by volume and subsequently measured at the absorption maximum.

Table 9. Stability of formulation 2A (pH 8.0)

Test parameter	Method	Blank value	3 months		6 months	
			-5°C	25°C	-5°C	25°C
Appearance of the solution	organoleptic	clear, orange-red	Clear, vibrant orange	Clear, vibrant orange	Clear, vibrant orange	clear, orange
Isopropanol (%)	GC	63.2	n.c.	n.c.	62.8	63.2
CHX (%)	HPLC	2.33	n.c.	n.c.	2.30	2.27
Dye absorption maximum	UV/Vis spectrometry	450				
Dye extinction §		1.05	0.46	0.33	0.31	0.22
Dye recovery (%) (based on blank value)		---	44	31	30	21
L*a*b* - values	M0041-01	L*	80.0	83.0	84.2	86.1
		a*	22.2	17.3	14.7	10.4
		b*	132.4	135.3	136.2	136.4

§ = 500 mg of the formulation were diluted to 25 ml with 96% ethanol by volume and subsequently measured at the absorption maximum.

Table 10. Stability of formulation 2B (pH 9.0)

Test parameter	Method	Blank value	3 months		6 months	
			-5°C	25°C	-5°C	25°C
Appearance of the solution	organoleptic	clear, orange-red	Clear, vibrant orange	Clear, vibrant orange	Clear, vibrant orange	clear, orange
Isopropanol (%)	GC	63.3	n.c.	n.c.	62.8	62.2
CHX (%)	HPLC	2.33	n.c.	n.c.	2.29	2.28
Dye absorption maximum	UV/Vis spectrometry	450				
Dye extinction §		1.12	0.55	0.38	0.34	0.25
Dye recovery (%) (based on blank value)		---	49	34	30	22
L*a*b* - values	M0041-01	L*	80.1	82.9	84.1	86.0
		a*	21.8	17.5	14.8	10.7
		b*	132.1	135.4	136.0	136.5

§ = 500 mg of the formulation were diluted to 25 ml with 96% ethanol by volume and subsequently measured at the absorption maximum.

Table 11. Stability of formulation 2C (pH 10.0)

Test parameter	Method	Blank value	3 months		6 months	
			-5°C	25°C	-5°C	25°C
Appearance of the solution	organoleptic	clear, orange-red	Clear, vibrant orange	Clear, vibrant orange	Clear, vibrant orange	clear, orange
Isopropanol (%)	GC	63.1	n.c.	n.c.	62.9	62.7
CHX (%)	HPLC	2.32	n.c.	n.c.	2.31	2.28
Dye absorption maximum	UV/Vis spectrometry	450				
Dye extinction §		1.16	0.49	0.39	0.34	0.26
Dye recovery (%) (based on blank value)		---	42	34	29	22
L*a*b* - values	M0041-01	L*	80.1	82.9	84.2	85.9
		a*	21.0	17.7	14.0	10.9
		b*	132.1	135.4	135.7	136.4

§ = 500 mg of the formulation were diluted to 25 ml with 96% ethanol by volume and subsequently measured at the absorption maximum.

Table 12. Stability of formulation 2D (pH 11.0)

Test parameter	Method	Blank value	3 months		6 months	
			-5°C	25°C	-5°C	25°C
Appearance of the solution	organoleptic	clear, orange-red	Clear, vibrant orange (somewhat darker)	Clear, vibrant orange	Clear, vibrant orange	Clear, vibrant orange
Isopropanol (%)	GC	63.2	n.c.	n.c.	62.2	63.0
CHX (%)	HPLC	2.34	n.c.	n.c.	2.32	2.28
Dye absorption maximum	UV/Vis spectrometry	450				
Dye extinction §		1.29	0.85	0.54	0.34	0.26
Dye recovery (%) (based on blank value)		---	66	42	26	20
L* a* b* - values	M0041-01	77.1	77.3	82.2	82.1	84.9
		30.6	27.4	19.9	19.8	13.5
		129.2	129.0	135.0	134.6	136.4

§ = 500 mg of the formulation were diluted to 25 ml with 96% ethanol by volume and subsequently measured at the absorption maximum.

Table 13. Stability of formulation 2E (pH 12.0)

Test parameter	Method	Blank value	3 months		6 months	
			-5°C	25°C	-5°C	25°C
Appearance of the solution	organoleptic	clear, orange-red	clear, orange	clear, orange-red	clear, orange	clear, orange-red
Isopropanol (%)	GC	63.5	n.c.	n.c.	63.0	63.0
CHX (%)	HPLC	2.34	n.c.	n.c.	2.30	2.24 #
Dye absorption maximum	UV/Vis spectrometry	449				
Dye extinction §		1.42	1.31	1.12	0.56	0.36
Dye recovery (%) (based on blank value)		---	92	79	39	25
L*a*b* - values	M0041-01	75.5	73.6	77.4	76.0	79.6
		33.0	31.8	29.2	30.8	25.6
		126.9	123.5	129.4	127.3	132.0

= additional peaks in the chromatogram => degradation products

§ = 500 mg of the formulation were diluted to 25 ml with 96% ethanol by volume and subsequently measured at the absorption maximum.

Table 14. Stability of formulation 2F (pH 13.0)

Test parameter	Method	Blank value	3 months		6 months	
			-5°C	25°C	-5°C	25°C
Appearance of the solution	organoleptic	clear, orange-red	cloudy, red-orange + fine P. and needle-like crystals	clear, orange-red + crystals	cloudy, red-orange + fine P. and needle-like crystals	clear, orange-red + crystals
Isopropanol (%)	GC	63.4	n.c.	n.c.	62.9	63.1
CHX (%)	HPLC	1.77	n.c.	n.c.	1.26 [#]	1.04 [#]
Dye absorption maximum	UV/Vis spectrometry	448				
Dye extinction [§]		1.45	1.40	1.25	1.34	1.15
Dye recovery (%) (based on blank value)		---	97	86	92	79
L*a*b* - values	M0041-01	L*	68.9	77.4	75.7	78.9
		a*	28.7	27.4	27.9	24.1
		b*	115.0	129.0	126.3	130.6

[#] = additional peaks in the chromatogram => degradation products[§] = 500 mg of the formulation were diluted to 25 ml with 96% ethanol by volume and subsequently measured at the absorption maximum.