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ANALYSIS

HPLC METHOD FOR IDENTIFICATION AND QUANTIFICATION OF THREE ACTIVE SUBSTANCES IN A DERMATOLOGICAL PREPARATION – VIOSEPT OINTMENT

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Abstract: The study was aimed at developing a HPLC method to identify and quantify domiphen bromide, tripelennamine hydrochloride and clioquinol in Viosept ointment. The tested substances were successfully separated using Inertsil ODS-3 (250 × 4.6 mm, 5 μm) as a stationary phase and a gradient elution. Detection at 310 nm wavelength was applied for tripelennamine hydrochloride and clioquinol, and at 215 nm wavelength for domiphen bromide. Methods of extraction of the tested substances were developed: domiphen bromide and clioquinol were extracted with acetone from heated solutions, and tripelennamine hydrochloride was extracted in a hexane-water system. Validation procedure confirmed the method to be sufficiently selective, precise and accurate. Correlation coefficients of calibration curves pointed out that they were linear within the examined concentration range.

Keywords: domiphen bromide, tripelennamine hydrochloride, clioquinol, HPLC method

Human body is covered with skin, which provides main protection against external factors. Skin is exposed to mechanical and thermal damage as well as to adverse effects of radiation, chemicals, bacteria, viruses or fungi. These factors can cause a variety of diseases, many of which require long-term and complex treatment. A topical therapy with multi-component extended-spectrum preparations, as the Viosept ointment, typically provides sufficient therapeutic response. The drug contains three active substances: domiphen bromide, tripelennamine hydrochloride, and clioquinol. Structural formulas of the substances are shown in Figure 1.

Tripelennamine hydrochloride is an antihistamine and topical anesthetic agent, whereas clioquinol (quinoline compound) and domiphen bromide (quaternary ammonium compound from a group of cationic emulsifiers) have antibacterial and antifungal properties

Qualitative requirements and methods of quantification for domiphen bromide, tripelennamine hydrochloride, and clioquinol are described in pharmacopoeial monographs. Clioquinol monograph is included in European Pharmacopoeia (Ph. Eur.) and United States Pharmacopoeia (USP). British Pharmacopoeia (BP) contains a monograph for domiphen bromide, and USP features a tripelennamine hydrochloride monograph.

According to USP, purity and assay of clioquinol substance can be tested using gas chromatography (GC). USP also provides a detailed description of selected pharmaceutical forms containing clioquinol, i.e.: cream, suspension and skin powder containing clioquinol along with zinc oxide, lactic acid and lactose. Clioquinol concentrations in ointment and suspension have been determined with gas chromatography after transforming the substance into a silanol derivative, whereas clioquinol concentration in skin powder with a spectrophotometric method. An isocratic HPLC method for purity test of clioquinol is described in Ph. Eur.

Tripelennamine hydrochloride monograph in USP contains a detailed specification of purity and concentration tests using HPLC method with ion pair chromatography. HPLC has also been used in

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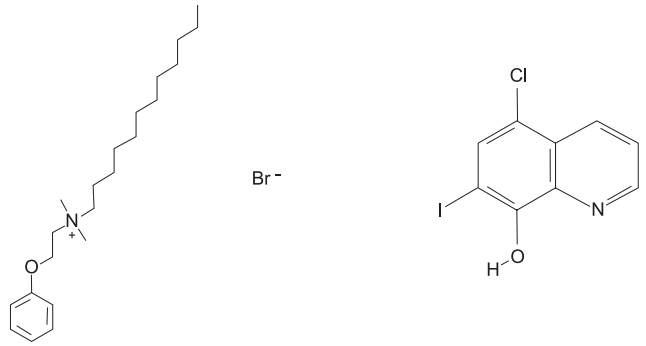
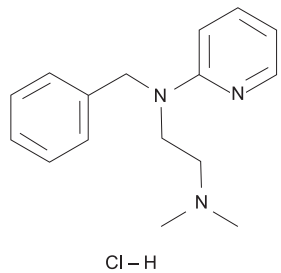


DOMIPHEN BROMIDE CLIOQUINOL



TRIPELENNAMINE HYDROCHLORIDE

Figure 1. Structures of the studied compounds

determining the drug substance concentration in solutions for injections.

In a monograph of tablets containing tripelennamine, a spectrophotometric method intended for testing the concentration of active substance and its quantities released in dissolution tests is also laid down.

According to a domiphen bromide monograph in BP, its concentration in active substances can be tested with a titration method. No pharmaceutical forms containing domiphen bromide are mentioned in any pharmacopoeia.

The present study was aimed at developing a HPLC method to identify and assay domiphen bromide, tripelennamine hydrochloride and clioquinol in the Viosept ointment.

Analysis of multi-component drugs such as ointments and creams containing active substances exhibiting different physicochemical properties presents some difficulties, e.g., isolation of individual substances.

Many papers refer to tripelennamine tested in pharmaceutical preparations (1–10) and biological materials (11–15). Different procedures were developed: chromatographic method such as liquid chromatography (HPLC) (1–6, 12), capillary electrophoresis (CE) (7, 8, 15), gas chromatography (GC) (11, 13). Other methods were also adopted like potentiometric methods (9), photometric (10) and spectrophotometric method (14). In almost all cases HPLC, GC, CE were used for the simultaneous determinations of antihistamine drug substances such as diphenhydramine, carbinoxamine, azatadine, chlorpheniramine, pheniramine, brompheniramine and others.

The available literature mentions a few reports related to the quantification of clioquinol in drugs. Determination of clioquinol in creams was carried out by spectrophotometric methods (16, 17). Liquid chromatography after precolumn derivatization was used for determination of clioquinol in the presence of metronidazole and tolnaftate (18). HPLC method

with electrochemical detection was developed for determination of clioquinol in plasma and tissues (19). Only one paper reporting an analysis of domiphen bromide by a HPLC method as a surfactant in bioprocess intermediates was found (20).

EXPERIMENTAL

Materials

Drug product: Viosept ointment containing tripelennamine hydrochloride 20 mg/g + clioquinol 20 mg/g + domiphen bromide 0.5 mg/g (batch number 10203; manufactured by: "Jelfa" S.A.)

Reference standards: tripelennamine hydrochloride (USP), clioquinol (CRS Ph. Eur.), domiphen bromide (RBH Ltd. UK), impurities A, B, C originating from clioquinol (CRS Ph. Eur.).

Reagents and instruments

Reagents: acetonitrile was HPLC grade, all other chemicals were of analytical grade.

Computer-controlled liquid chromatograph (Shimadzu) was fitted with UV-VIS SPD-10AV$_{VP}$ detector, DAD SPD-M10AV$_{VP}$, LC-10AT$_{VP}$ pump system, DGU-14A sample degassing unit, SCL-10A$_{VP}$ controller, and SIL-10AD$_{VP}$ automatic sample feeder.

Chromatographic column: Inertsil ODS-3, 250 × 4.6 mm, 5 mm, by HICHROM.

Standard solutions: clioquinol solution at 120 µg/mL concentration in acetone, domiphen bromide solution at 3 µg/mL concentration in acetone, tripelennamine hydrochloride solution at 120 µg/mL concentration in water obtained by extraction in the following procedure: 5.0 mL of aqueous solution of tripelennamine hydrochloride at 1.2 mg/mL concentration was transferred to a separator, with 30 mL of hexane and 15 mL of water added, and handled as described in the sample preparation procedure for concentration determination of tripelennamine hydrochloride. (see: content determination)

Development of the method

Chromatographic conditions

First, suitable wavelengths were determined based on the recorded spectra of the substances in acetone in the 200–350 nm range using DAD detector. The selected wavelengths were: λ = = 215 nm for domiphen bromide assay, and λ = 310 nm for tripelennamine hydrochloride and clioquinol. Next, a specific HPLC system has been sought, which would provide identification and sufficient separation of the three substances characterized by significantly different polarity.

Therefore, a number of chromatographic reversed-phase HPLC systems were tested, i.e., columns of various polarities (YMC-Pack C4, 5 µm, 150 × 4.6 mm by YMC Co. Ltd.; Spherimage-80 C6, 5 µm, 125 × 4.0 mm by Knauer; Symmetry C8, 5 µm, 250 × 4.6 mm by Waters; Supelcosil ABZ+PLUS, 5 µm, 250 × 4.6 mm by SUPELCO; CPS-2 Hypersil, 5 µm, 250 × 4.6 mm by Thermo; Inertsil ODS-3, 5 µm, 250 × 4.6 mm by HiChrom) and mobile phases of different compositions, containing the most popular solvents: methanol, acetonitrile and tetrahydrofuran. No system with isocratic elution was found, which would allow to separate and identify the three active substances within a relatively short time period. So, different variants of the gradient elution were tested.

Low polarity columns (C18) were found eligible to obtain a satisfactory separation of the tested substances with a suitable gradient elution.

The following chromatographic system was finally selected: Inertsil ODS-3 chromatographic column, 250 × 4.6 mm, 5 µm; temperature: 40°C; mobile phase A: 1.0 mL H_3PO_4 in 1.0 mL of water; mobile phase B: acetonitrile

Gradient elution:

time (min)	mobile phase B (%)
0 – 5	10
5 – 12	10 → 85
12 – 20	85
20 – 22	85 → 10
22 – 25	10

Mobile phase flow rate of 1.5 mL/minute and injection volume 50 µL were applied.

Sample solutions

The following solvents were tried for an efficient isolation of drug substances from the ointment: methanol, ethanol, acetonitrile, tetrahydrofuran, dimethylformamide and acetone.

All active substances: tripelennamine hydrochloride, domiphen bromide and clioquinol were well soluble in acetone, which was used to extract them as follows:

– in a one step without heating, or with heating up to 60°C, or with heating up to 80°C.

The ointment was weighted and transferred to a 50 mL volumetric flask, approximately 40 mL of acetone was added, then agitated on mechanical shaker for approximately 30 min or heated in a water bath for approximately 30 min. The heater temperature was 60 or 80°C. The solutions were cooled to room temperature, made up to 50 mL volume and filtered through cellulose filter (84 g/m^2).

– A triple procedure with heating up to 40°.

Table 1. Effect of applied extraction methods on determination of active substances.

Substance determined [declared content]	Solvent: acetone			
	Single extraction without heating	Triple extraction 30 min/ 40°C	Single extraction 30 min/ 60°	Single extraction 30 min/ 80°
	Found amount of active substance [mg/g]	Found amount of active substance [mg/g]	Found amount of active substance [mg/g]	Found amount of active substance [mg/g]
Tripelennamine hydrochloride [20 mg/g]	13.14 16.74 16.81 16.00	18.44 17.84 17.54 18.33	18.64 19.08 17.69 17.23	15.28 16.44 19.26 18.87
	Mean = 15.67 mg	Mean = 18.04 mg	Mean = 18.16 mg	Mean = 17.46 mg
	RSD = 11.02%	RSD = 2.34%	RSD = 4.68%	RSD = 10.98%
Clioquinol [20 mg/g]	19.61 20.68 19.39 19.90	18.89 19.18 19.30 19.22	19.35 19.78 19.44 19.77	19.68 19.35 19.23 19.77
	Mean = 19.90 mg	Mean = 19.15 mg	Mean = 19.59 mg	Mean = 19.51 mg
	RSD = 2.83%	*Mean* = 0.93%	RSD = 1.14%	RSD = 1.33%
Domiphen bromide [0.5 mg/g]	0.48 0.44 0.42 0.42	0.49 0.47 0.47 0.46	0.48 0.46 0.43 0.47	0.49 0.50 0.48 0.49
	Mean = 0.44 mg	Mean = 0.47 mg	Mean = 0.46 mg	Mean = 0.49 mg
	RSD = 6.43%	RSD = 2.41%	RSD = 4.70%	RSD = 2.04%

The ointment was weighted and transferred to a conical flask, approximately 15 mL of acetone was added and heated in a water bath at 40°C for 30 min. The solution was cooled during a few minutes in refrigerator until excipients were precipitated; after that the supernatant was filtered to a 50 mL volumetric flask through cellulose filter (84 g/m^2). The operation was repeated twice and then the volumetric flask was made up to 50 mL with acetone.

RESULTS AND DISCUSSION

The results shown in Table 1 proved that acetone was a suitable solvent for extraction of clioquinol and domiphen bromide from the Viosept ointment, preferably at 80°C. However, the results for tripelennamine hydrochloride assay after extraction in acetone were unrepeatable and too low (Table 1). Therefore, acetone was not suitable for the content determination of tripelennamine. For this compound a different method was needed. An extraction hexane-water was found to be the most suitable one (see: content determination). The obtained results are shown in Table 2.

Method validation

Specificity

To verify the specificity of the method, the solvent (acetone), the solutions of standard active substances, and of potential impurities originating from clioquinol (A, B, C acc. to Ph. Eur.) were injected onto the column. Figure 2 shows a chromatogram of the standards' mixture in acetone (approximately 0.1 mg/mL) at 215 nm and 310 nm. The peaks of the solvent (acetone), impurities and placebo do not interfere with the peaks of the actives substances.

Linearity

Linearity was tested within a concentration range: 5–160 μg/ml for clioquinol (in acetone, λ = 310 nm); 0.4–6 μg/mL for domiphen bromide (in acetone, λ = 215 nm), 2–200 μg/mL for tripelennamine hydrochloride (in water, λ = 310 nm). Table

Table 2. Effect of applied extraction method on determination of tripelennamine hydrochloride (triple extraction; hexane-water).

Substance determined	Declared content [mg/g]	Found amount of active substance [mg/g]	Mean of found amount of active substance [mg/g]	RSD [%]
Tripelennamine hydrochloride	20.0	20.83 21.19 20.78 21.13	20.98	0.99

Table 3. Calibration curve parameters.

	λ = 310 nm		λ = 215 nm
	Clioquinol	Tripelennamine hydrochloride	Domiphen bromide
Line equation (n = 6)	y =36436.3x + 69143.5	y = 95918.7x - 2536.5	y = 63212.2x - 8610.6
Standard error of the estimate S_y	17381	19614	2322
Correlation coefficient (r)	0.9999	0.9999	0.9996
Limit of detection (LOD)	1.6 μg/mL	0.7 μg/mL	0.1 μg/mL
Limit of quantitation (LOQ)	4.8 μg/mL	2.0 μg/mL	0.4 μg/mL

3 shows the linear regression parameters with correlation coefficients, which are very close to unity.

Limit of detection and limit of quantification

Limit of detection (LOD) and limit of quantification (LOQ) were established based on the calibration curve parameters: LOD = $3.3 \cdot S_y/a$ and LOQ = $10 \cdot S_y/a$, where S_y = standard error of estimation, a = slope of a straight line. Results are presented in Table 3.

Validation of the chromatographic system

Precision

Standard solutions: acetone solution of domiphen bromide (3 μg/mL), clioquinol (120 μg/mL) and aqueous solution of tripelennamine hydrochloride (120 μg/mL) were injected onto the column six times. The RSD of the peak areas ranged from 0.2% (tripelennamine), 0.4% (clioquinol) to 1.5% (domiphen bromide). RSD ≤ 2.0% was chosen as an acceptance criterion.

Accuracy

Weighted portions of standards of the active substances (80, 100 and 120% of the declared content) were added to approx. 0.3 g of placebo. The samples were analyzed for content determination. Recovery of 98.0–102.0% (Table 4) confirmed accuracy of the method.

Robustness

The effect of the mobile phase flow ratio (± 0.2 mL/min), column temperature (± 5°C), composition of the mobile phase A (± 0.2 mL H_3PO_4) and of the mobile phase B (ACN or ACN : H_2O 9 : 1, v/v), and column type on retention times, symmetry factor (A_S) and resolution (R_S) were evaluated for the standard mix solutions (approximately 0.1 mg/mL). The results are presented in Table 5.

Stability of solutions

Stability studies were performed for tripelennamine hydrochloride and clioquinol standard solu-

Table 4. Results of recovery in the Viosept ointment.

Substance determined	Amount added [declared content]	Recovery [%]	Mean value of recovery [%]	RSD [%]
Tripelennamine hydrochloride	96 μg/mL 80%	99.32 98.75 99.02	99.03	0.29
	120 μg/mL 100%	99.48 98.72 101.12	99.77	1.23
	144 μg/mL 120%	99.22 98.56 100.10	99.63	0.45
Clioquinol	96 μg/mL 80%	100.70 100.33 100.30	100.44	0.22
	120 μg/mL 100%	100.19 100.63 100.91	100.58	0.36
	144 μg/mL 120%	99.80 100.48 101.09	100.46	0.65
Domiphen bromide	2.4 μg/mL 80%	98.29 100.84 99.43	99.52	1.28
	3 μg/mL 100%	102.00 99.30 102.00	101.10	1.54
	3.6 μg/mL 120%	102.00 99.30 102.00	100.87	1.37

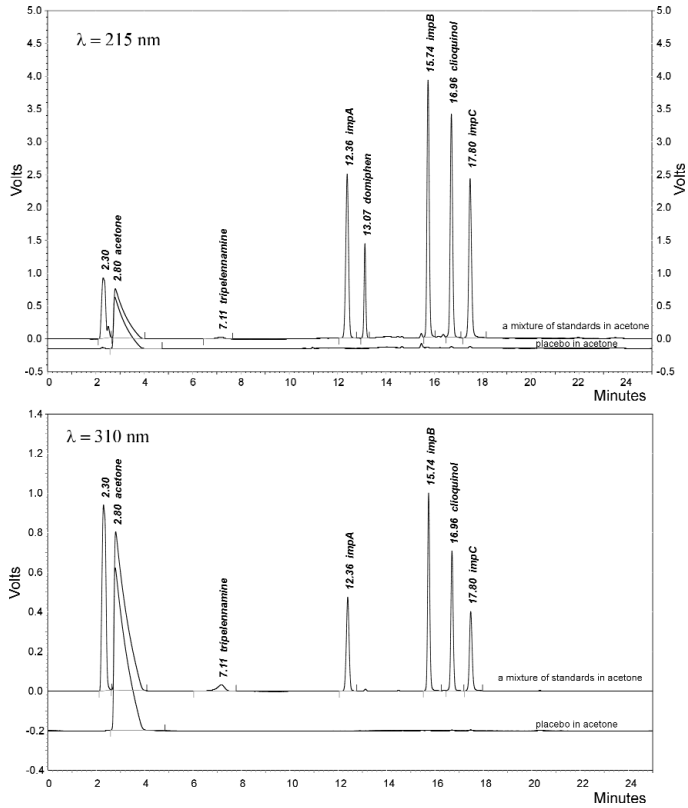


Figure 2. Chromatograms of a mixture of standards: tripelennamine, clioquinol, domiphen, impurities originating from clioquinol (approximately 0.1 mg/mL) and placebo in acetone, at λ = 215 nm and λ = 310 nm

tions. Determination of impurities originating from domiphen was omitted due to its stability and low dose in the product (0.5 mg/g).The aqueous and acetone solutions of tripelennamine hydrochloride (120 µg/mL) stored at ambient temperature were assayed after 12 and 48 h. The RSD of the assay of tripelennamine were within 1% (n = 6). The stability experiments showed that no significant changes in the content of impurities were observed in acetone or aqueous solution either. In both cases the total impurities percent was lower than the trace level (< 0.05%). This confirms that the standard aqueous solutions of tripelennamine used during the assay were stable for at least 48 h at room temperature.

The effect of the heating process of acetone solutions (up to 80°C for 30 min in a water bath) was also analyzed to make sure that the potential degradation products of tripelennamine did not interfere with determination of the two other actives substances. Similar results (< 0.05% of total impurities) were observed.

Standard solutions of clioquinol (120 µg/mL) in acetone and acetone heated up to 80°C for 30 min in a water bath were also tested after 12 and 48 h. The solutions were protected from light and stored at ambient temperature. The following results, for the solutions heated, or not were obtained: impurity A 1.0%, impurity B 0.6%, impurity C 0.5%, and other impurities in trace quantities. Additionally, stability of clioquinol solutions in methanol, which is used in a method for purity described in the monograph of clioquinol in Ph. Eur., were investigated. No significant effect on the quantity of degradation products was observed.

Content determination

Clioquinol and domiphen bromide

The solutions were prepared in brown volumetric flasks. Approx. 0.3 g of the ointment was weighted and transferred to a 50 mL volumetric flask, then 40 mL of acetone was added and heated in a water bath up to 80°C for approximately 30 min. The solution was cooled to room temperature, made

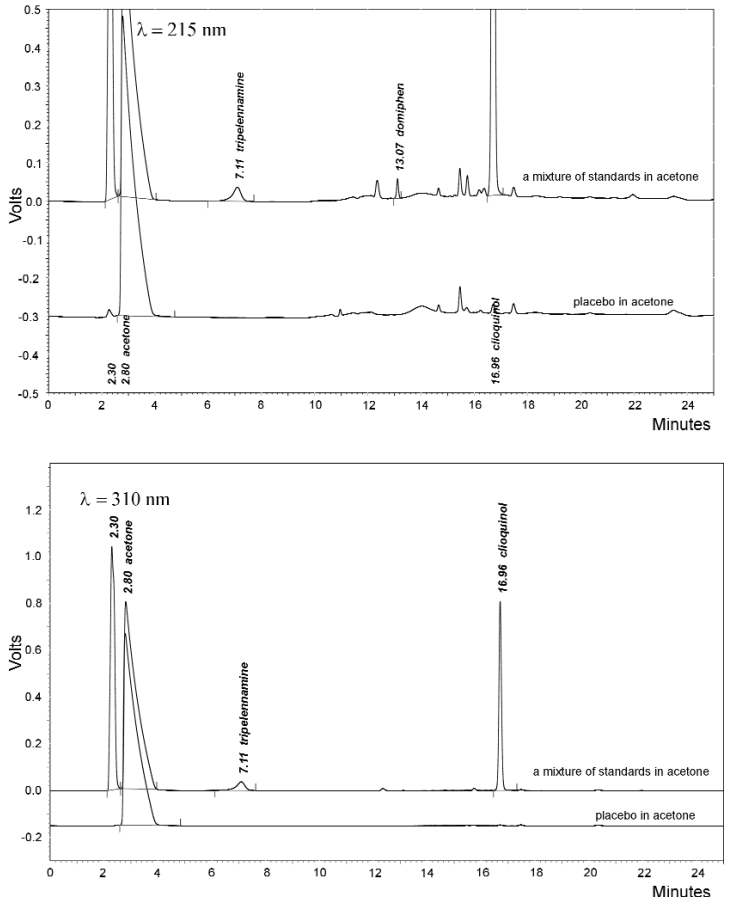


Figure 3. Chromatograms of a Viosept ointment and placebo extracted to acetone at ë = 215 nm (for determination of domiphen) and λ = 310 nm (for determination of clioquinol)

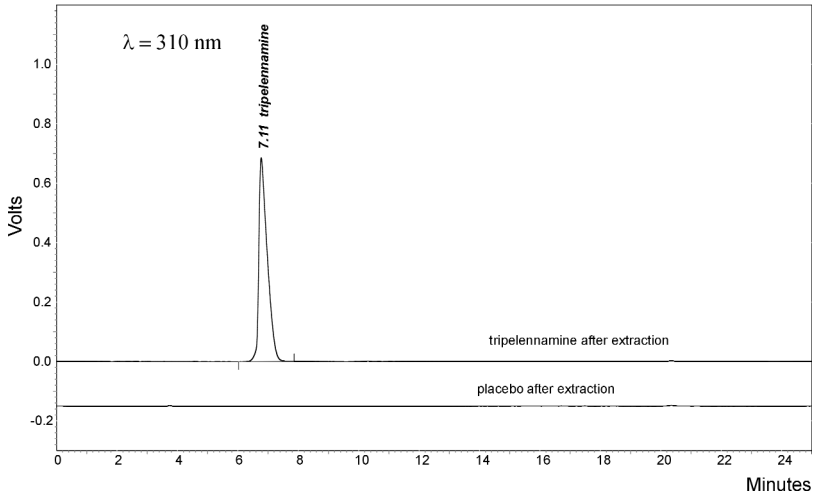


Figure 4. Chromatograms of a Viosept ointment and placebo after extraction with hexane-water, at λ = 310 nm (for determination of tripelennamine)

Table 5. Robustness of the proposed HPLC method.

		Tripelennamine	**Imp. A**	**Domiphen**	**R_S imp. A / domiphen**	**Imp. B**	**Clioquinol**	**Imp. C**	**R_S clioquinol /imp. C**
		RT [min] (A_S)	**RT** [min] (A_S)	**RT** [min] (A_S)		**RT** [min] (A_S)	**RT** [min] (A_S)	**RT** [min] (A_S)	
Flow rate	1.7 mL/min	**6.38** (1.42)	**11.66** (1.10)	**12.67** (1.11)	**5.16**	**15.12** (1.06)	**16.14** (1.09)	**16.98** (1.42)	**3.51**
	1.5 mL/min	**7.11** (1.39)	**12.36** (1.07)	**13.07** (1.08)	**4.89**	**15.74** (1.06)	**16.96** (1.07)	**17.80** (1.42)	**4.08**
	1.3 mL/min	**8.32** (1.48)	**13.13** (1.08)	**13.59** (1.10)	**3.44**	**16.51** (1.06)	**17.75** (1.07)	**18.79** (1.42)	**3.90**
Temperature	45°C	**7.33** (1.43)	**12.40** (1.07)	**13.04** (1.10)	**4.64**	**15.62** (1.08)	**16.67** (1.05)	**17.54** (1.34)	**3.85**
	40°C	**7.11** (1.39)	**12.36** (1.07)	**13.07** (1.08)	**4.89**	**15.74** (1.06)	**16.96** (1.07)	**17.80** (1.42)	**4.08**
	35°C	**6.99** (1.40)	**12.25** (1.10)	**13.10** (1.10)	**5.88**	**15.84** (1.05)	**17.03** (1.06)	**18.02** (1.24)	**3.89**
Mobile phase A	1.2 mL H_3PO_4	**7.06** (1.42)	**12.06** (1.07)	**13.22** (1.08)	**6.48**	**15.72** (1.07)	**16.86** (1.05)	**17.80** (1.34)	**4.38**
	1.0 mL H_3PO_4	**7.11** (1.39)	**12.36** (1.07)	**13.07** (1.08)	**4.89**	**15.74** (1.06)	**16.96** (1.07)	**17.80** (1.39)	**4.08**
	0.8 mL H_3PO_4	**7.84** (1.40)	**12.66** (1.11)	**13.05** (1.11)	**2.78**	**15.78** (1.08)	**16.89** (1.07)	**17.84** (1.34)	**3.82**
Mobile phase B	**ACN**	**7.11** (1.39)	**12.36** (1.07)	**13.07** (1.08)	**4.89**	**15.74** (1.06)	**16.96** (1.07)	**17.80** (1.39)	**4.08**
	ACN:H_2O (9 : 1)	**7.76** (1.40)	**12.59** (1.09]	**13.33** (1.08)	**5.09**	**16.23** (1.13)	**17.67** (1.11)	**18.91** (1.44)	**4.06**
Columns	**s: 2850**	**7.11** (1.39)	**12.36** (1.07)	**13.07** (1.08)	**4.89**	**15.74** (1.06)	**16.96** (1.07)	**17.80** (1.42)	**4.08**
	s:17212722	**6.32** (1.44)	**12.08** (1.19)	**12.94** (1.09)	**6.09**	**15.59** (1.13)	**16.66** (1.16)	**17.55** (1.60)	**3.38**

RT – retention time; **A_s –** symmetry factor; **R_s** – resolution

Table 6. Results and statistic evaluation of assay in the Viosept ointment.

Active substances	Declared amount of active substances [mg/g]	Found amount of active substances [mg/g]	
		Mean X ± ΔX (PU = 95%, n = 6)	RSD [%]
Tripelennamine hydrochloride	20.0	21.04 ± 0.22	0.99
Clioquinol	20.0	19.55 ± 0.27	1.33
Domiphen bromide	0.5	0.46 ± 0.008	1.66

up to 50 mL volume and filtered through a cellulose filter (84 g/m^2).

Aliquots of 50 µL of the prepared standard and sample solutions were injected onto the column. Chromatograms were recorded at two different wavelengths: 310 nm for determination of clioquinol and 215 nm for determination of domiphen. Figure 3 shows chromatograms of sample solutions and placebo in acetone. Table 6 shows the results of determination.

Tripelennamine hydrochloride

Approx. 0.3 g of the ointment was weighted and transferred to a separatory funnel; 30 mL of hexane was added and agitated for approx. 2 min until the base of ointment was dissolved. Next, 15 mL of water was added. The funnel was then agitated for approximately 2 min and left until the layers have separated. The water layer was transferred to a 50 mL volumetric flask. The extraction was then repeated twice, and the water layers were collected in the flask, made up to volume and filtered through a cellulose filter (84 g/m^2).

Aliquots of 50 µL of the prepared standard and sample solutions were injected onto the column. Figure 4 shows chromatograms of the sample and placebo solutions after extraction with hexane-water. Table 6 shows the results of determination.

CONCLUSIONS

A simple HPLC method for the quantitative determination of three active substances: domiphen bromide, tripelennamine hydrochloride, and clioquinol in the Viosept ointment was optimized and validated. A C18 reversed-phase column and a gradient elution were used.

The developed method was selective, precise and accurate, and complied with the acceptance criteria, including symmetry factor ($A_S < 1.5$) and resolution ($R_S > 2.0$) between all peaks. The specificity was confirmed as the peaks originating from the solvent, placebo, impurities and drug substances did not interfere. Calibration curves of the three active substances were linear ($r \geq 0.999$). Values of recovery of the substances from the ointment were within the range 98.0–102.0%.

REFERENCES

1. Kelly M.T., Smyth M.R., Dadgar D.: Analyst 114, 1377 (1989).
2. Dolan J.W, Snyder L.R., Djordjevic N.M., Hill D.W., Waeghe T.J.: J. Chromatogr. A 857, 1 (1999).
3. Gil-Agusti M., Monferrer-Pons L., Esteve-Romero J., Garcia-Alvarez-Coque M.C.: J. AOAC Int. 84, 1687 (2001).
4. Capella-Peiro M.E., Gil-Agusti M., Monferrer-Pons L., Esteve-Romero J.: Anal. Chim. Acta 454 125 (2002).
5. Bose D., Durgbanshi A., B Capella-Peiro M.E., Gil-Agusti M., Carda-Broch S., Esteve-Romero. J.: Indian J. Chem. 43, 9 (2004).
6. Martínez-Algaba C., Bermúdez-Saldaña J.M., Villanueva-Camañas R.M., Sagrado S., Medina-Hernández M.J.: J. Pharm. Biomed. Anal. 40, 312 (2006).
7. Leung G.N.W., Tang H.P.O., Tso T.S.C., Wan T.S.M.: J. Chromatogr. A 738, 141 (1996).
8. Rambla-Alegre M., Paris-Vincente J., Esteve-Romero J., Capella Peiró M.E., Bose D.: Anal. Chim. Acta 666, 102 (2010).
9. Lima J.L.F.C., Montenegro M.C.B.S.M., Sales M.G.F.: J. Pharm. Biomed. Anal. 14, 931 (1996).
10. San Andres M.P., Sicilia D., Rubio S., Perez Bendito D.: Analyst 114, 1079 (1989).

11. Mackell M.A., Poklis A.: J. Chromatogr. 235, 445 (1982).
12. Thomson H.C. Jr., Holder C.L., Althaus J.R.: Talanta 30, 251 (1983).
13. Chen X.H., Franke J.P., Wijsbeek J., De Zeeuw R.A.: J. Anal. Toxicol. 18, 150 (1994).
14. Ragno G., Ioele G., Risoli A.: Anal. Chim. Acta 512, 173 (2004).
15. Martínez-Gómez M.A., Carril-Avilés M.M., Sagrado S., Vilanueva-Camañas R.M., Medina Hernández M.J.: J. Chromatogr. A 1147, 261 (2007).
16. Gimenez-Izquierdo J., Guiteras J., Izquierdo A, Prat M.D.: Fresenius J. Anal. Chem. 341, 638 (1991).
17. Van De Vaart F.J., Hulshoff A., Indemans A.W.M.: J. Pharm. Biomed. Anal. 1, 507 (1983).
18. Rizk M., Belal F., Ibrahim S., Ahmed S., Sheribah Z.A.: J. Pharm. Biomed. Anal. 27, 813 (2002).
19. Bondiolotti G.P.,Pollera C., Pirola R., Bareggi S.R.: J. Chromatogr. B 837, 87 (2006).
20. Chen L.H., Price C.E., Goerke A., Lee A.L., DePhillips P.E.: J. Pharm. Biomed. Anal. 40, 964 (2006).

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