Determination of Piroctone Olamine (Octopirox) in Bulk by UV Spectrophotometric Method

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ABSTRACT: A simple and low-cost UV-spectrophotometric method has been developed and validated for the quantification of Octopirox in bulk. The linearity was found at 307 ± 1 nm in 10-50 µg/ml solution of ethanol-water (1:3, v:v) with $r^2 = 0.99$. The limit of detection was found to be 1.18 µg/ml, while the limit of quantification was 3.58 µg/ml. The method was validated for linearity, accuracy, precision, range, ruggedness and robustness.

Key words: Octopirox, validation, UV-spectrophotometry

INTRODUCTION

The piroctone olamine (CAS # 68890-66-4) is 1-hydroxy-4-methyl-6-(2,4,4-trimethyl)-pentyl-2(1*H*)-pyridone 2-aminoethanol salt (Figure 1).

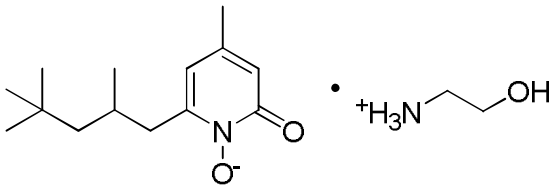

Figure 1. Structural formula of Octopirox.

Under the brand name Octopirox, it was used for the first time in July 1979 in the Seborin produced by Schwarzkopf & Henkel Düsseldorf (subsidiary of Hoechst).[1] Now-a-days, the compound is present in many cosmetic products for the treatment of dandruff (*Pityriasis simplex capillitii*), like shampoos, even for dogs and cats. It has fungicidal activity against all medically relevant dermatophytes, yeasts and mold fungi, due to penetration into the cell wall of yeast, fungi such as *Malassezia furfur* and complexation with iron (III) ions, which results in an inhibition of the energy metabolism in the mitochondria of the fungi.[1] The concentrations should be between 0.1 and 1.0%, depending on the type of final product, or should be further reduced for preparations that remain on the hair or the scalp. Minimum inhibition concentration for the most important species of fungi lies between 0.5 and 4.0 µg/ml.[2] In addition to the fungicidal activity, piroctone olamine also exhibits a bactericidal activity against gram-positive and gram-negative bacteria in 0.1-3.0% concentration in personal care compositions.[3] Moreover, *in vitro* model of experimental onychomycosis caused by *T. rubrum* pointed out a possible role of 0.5% octopirox nail lacquer as a preventive tool for subjects at risk or as a curative tool in the first stage of onychomycosis.[4] None of the *in vivo* studies gave any indication of its embryotoxic, teratogenic and mutagenic effect.[5]

Among methods of identification, there were reports of IR, UV spectra, TLC and melting point (133-136°C) determinations.[6-11] Considering UV data, absorption maximum of Octopirox was found at 317±2 nm and specific absorbance was calculated to be 214 to 236.[7] Moreover, for 0.1M methanolic

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sodium hydroxide solution in concentration 20 µg/ml it was reported at 230-360 nm.[8] The results showed that such solution was stable for 3 years according to Hoechst stability test and $E^{\%}_{cm}$ was 226-230 at 317 nm. Furthermore, Clariant GmbH proposed to determine Octopirox in shampoos in concentration of 0.1 to 5 g/100g by RP HPLC in methanol-water {95:5, v:v} after reduction by 15% solution of Ti(III) chloride with product UV detection at 300 nm.[9] Also RP HPLC method has been developed for simultaneous identification and quantitative determination of four anti-dandruff agents such as salicylic acid, ketoconazole, climbazole and octopirox in commercial anti-dandruff shampoo products in acetonitrile : water {60 : 40, v : v with potassium dihydrogen phosphate and orthophosphoric acid} with absorbances at 224 nm and 305 nm[10], or additionaly with 0.5 mM EDTA disodium salt - at 305 or 340 nm.[11] Besides, it was determined at 440 nm by yellow complexes formation with Fe^{2+} ions in 80% acetic acid.[2]

Hence, there there is lack of simple, fast, low-cost and accurate method for quantification of Octopirox in bulk. Thus, UV method has been developed, validated and proposed in this work.

MATERIALS AND METHODS

Instrumentation. The substance was weighed using analytical balances {Shimadzu AUX220 (10 mg - 220 g), Shimadzu Corporation, Shim Ukraine Ltd., Kyiv}. UV spectra were recorded on UV-VIS spectrophotometer UV-2600 (190-1100 nm) {Shimadzu Corporation, ShimUkraine Ltd., Kyiv}.

Reagents and solutions. Ethanol was of 96% (v:v) concentration and medical purity ("Pharmasept", Lohvytsky alcohol factory, Chervonozavodske, Ukraine), distilled water was used throughout all experiments. Working substance of Octopirox was purchased from Clariant, Frankfurt, Germany.

Validation. *Calibration curve*. The initial standard solution (0.05%) was prepared by dissolving 0.0500 gm of the Octopirox in 100.0 ml flask with 25 ml of 96% ethanol, stirring for 5 min, addition of distilled water, stirring for 5 min additionally. Several aliquots of standard solution were taken to make the next series of working standard solutions in the 10-50 µg/ml (0.001-0.005%) range by dilution of the aliquots in the ethanol-water (1:3, v:v) solution. All solutions were stored at 18-22°C.

The calibration curve of Octopirox was constructed by UV-VIS spectrophotometric absorption data, monitored 5 times for each sample, at wavelength of maximum absorbance at 307±1 nm in 3 ml cuvette with 1 cm layer.

The regression equation was obtained by the method of least squares for n=5. Regression equation: Y = slope*x + intercept. Slope, intercept and regression coefficient were determined from the regression analysis calculations in Microsoft Excel 2007.[12]

Using this linear equation, regression coefficient (r^2) and the detection limits were determined. Accuracy: mean ± SD; Linearity (lowest – highest concentration while curve is linear); SE of intercept: √ of $\sum(y-y')/n$), where y - standard concentration, y' - found concentration; SD of intercept: SE of intercept*√n

The limit of detection (LOD): 3.3*(SD of intercept / slope); and the limit of quantitation (LOQ): 10*(SD of intercept / slope). The LOD was defined by the concentration with a signal-to-noise ratio of 3. The analyte peak in the LOQ sample should be identifiable, discrete, and reproducible with a precision of ±20% and accuracy within 80%–120%. The deviation of standards other than LOQ should be not more than ±15% of the nominal concentration.

Precision (repeatability of the method) was evaluated by repeated absorption detection and the results were expressed as the mean standard deviation (SD) and the percent relative standard deviation RSD (%) = SD / Mean, calculated for absorbances in all concentrations and recovery data. For intra-day analysis the samples were analyzed six times a day at 09:00 am, 11:00 am, 01:00 pm, 03:00 pm, 05:00 pm, and 07:00 pm, while for inter-day stability it was analyzed for 6 consecutive days at 09:00 am.

RESULTS AND DISCUSSION

According to Clariant product specifications, Octopirox is white to slightly yellowish-white crystalline powder with mild characteristic odor, very slightly soluble in water, and freely soluble in ethanol, chloroform and ether.[6] Its pKa is 7.4. The solubility of Octopirox is greatly dependent on the pH: it is greater in the neutral and weakly alkaline ranges than in the acidic one, due to formation of free acid form.

Considering our pharmacokinetics investigations of cosmetic and pharmaceutical dosage forms, it was decided to study Octopirox ethanol solutions; and to make the procedure cheaper, the smallest concentration of ethanol in water was studied to be appropriate for dilution. It was found that solution of 96% ethanol and distilled water in proportion 1 to 3 has made a stable and clear solution for weeks, especially, when 0.04-0.05 g of substance was taken for the first dilution in 100 ml flask.

Validation of the method was performed in accordance to the analytical methods validation parameters.[13] The precision of the method was checked by measuring absorption maxima of Octopirox standard (10-50 µg/ml) solutions five times. Standard deviations of each measured absorbencies were within 0.0030-0.0060 and RSD was 0.0048-0.0138%.

The linearity was evaluated by linear regression analysis, which was calculated by the least-square regression method measuring maximum absorbance of Octopirox standard solutions in the concentration range from 10 to 50 µg/ml. The corresponding specific absorbencies, accuracy and recovery data are given in Table 1.

Table 1. Concentration of standard Octopirox solutions, their absorbances, accuracy, recovery data.

Standard C., µg/mL	$\bar{A}$ of 5 means	SD	%RSD	$E^{\%}_{cm}$	[a]Found C. µg/ml	[b]Recovery
10	0.2151	0.0030	0.0138	215	10.1485	101.4851
20	0.4108	0.0043	0.0097	205	19.8366	99.1832
30	0.6141	0.0043	0.0065	205	29.9010	99.6700
40	0.8158	0.0060	0.0068	204	39.8861	99.7153
50	1.0215	0.0054	0.0048	204	50.0693	100.1386
					Mean, n = 5	100,0384
					SD, µg/ml	0.8767
					%RSD	0.0078
					Accuracy, %	100.0384±0.8767
					Recovery, %	100.0384±0.0078

[a]Found concentration: (Absorbance – intercept)/slope;
[b]Recovery: found concentration /labeled concentration*100.

The accuracy of the method was proven by calculating the recovery at five different concentrations in table 1 (100.04 ± 0.88%). The mean percentage of recoveries was found to be 100.04 ± 0.01%. For example, the standard deviation of the proposed method was higher (0.88 µg/ml), compared to 0.14 µg/ml reported by RP HPLC[9], but RSD data was much lower: 0.0078% against reported 0.68%.

According to the Beer's law, regression coefficient, obtained similar specific absorbance (204-205), the calibration curve of Octopirox with good linearity was found in the concentration range 10.0-50.0 µg/ml (Table 1, Figure 2).

Despite RP HPLC results of 0.5-3.0 µg/ml, this method could be used in common laboratory without expensive apparatuses,[10] as well as presented calibration curve - for simple and fast evaluation of unknown Octopirox solution percentage.

Slope, intercept and correlation coefficient were determined from the regression analysis calculations (Table 2).

Table 2. Linearity, accuracy and precision of Octopirox in ethanol-water (1:3, v:v) solution.

Parameters	Results
Slope	0.0202
Intercept	0.0101
Linearity (µg/mL)	10.0-50.0
Regression equation	y = 0.0202x+0.0101
r^2	0.999
SE of intercept	0.0032
SD of intercept	0.0072
LOD (µg/mL)	1.1831
LOQ (µg/mL)	3.5852

The limit of detection (LOD) was found to be 1.18 µg/ml, while the limit of quantification (LOQ) was 3.58 µg/ml.

The ruggedness of the method was determined by performing the same assay by different researches and performing the assay for 0.0035% Octoripox ethanol-water (1:3) solution during week to check its reproducibility. The results were found to be highly reproducible during the day – RSD was 0.0032%, and during the week increased to 0.076%, still with high precision (Table 3). It's worth to mention, that maximum absorbance wavelength shifted to 306 nm in 3 h, due to protonation of Octoripox , and in the next three weeks it was found at 302-301 nm.

Table 3. The intra-day and inter-day precision of 0.0035% Octoripox solution in ethanol-water (1:3, v:v).

#	Intra-day	Inter-day
1	0.7226	0.7162
2	0.7215	0.7245
3	0.7217	0.7301
4	0.7202	0.7321
5	0.7178	0.7275
6	0.7162	0.7203
Mean	0.7200	0.7251
SD	0.0025	0.0060
%RSD	0.0032	0.0076

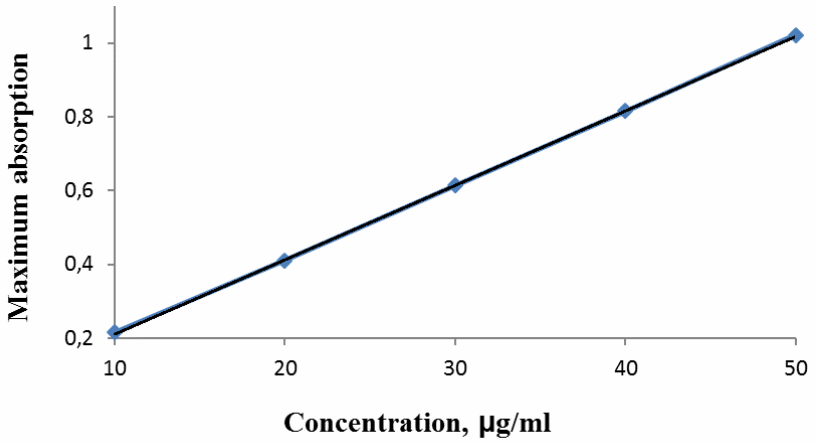

Figure 2. Calibration curve of Octopirox.

To determine the robustness of the method, experimental conditions like room temperature, stirring time, different ethanol series and distilled water were checked. It is very important to dilute Octoripox firstly in the 96% ethanol, because in case of usage ready-to-use ethanol - water (1:3, v:v) solution, substance was worse soluble, even in the concentration 0.05% and the next day opacity appeared. Furthermore, stirring was essential to obtain accurate results. Additionally, the second dilution in the higher amount of solvent like 50 ml also shifted the maximum absorbance results to the

lower values. In addition, usage of different distilled water series also caused its 307±1 nm due to different pH. The temperature very slightly effected the results, still the 20-22°C was the most appropriate one. Hence, to obtain absorption in the range 0.6-0.9 and to detect the amount of Octopirox in pure substance, the next method is proposed.

Quantitatively 0.040-0.0550 of Octopirox was placed in the 100.0 ml flask, dissolved with 25.0 ml of 95% ethanol, stir for 5 min, add distilled water to obtain 0.045-0.055% solution and stir for 5 min. Then quantitatively transfer 2.00 ml of the obtained solution in 25.00 ml flask, add ethanol-water (1:3) solution to obtain final 0.0036-0.0044% solution, stir for 5 min. Determine the concentration of Octopirox by measuring maximum UV absorption at wavelength of 307±1 nm in comparison to ethanol-water (1:3, v:v) solution in 3 mL cuvette with 1 cm layer.

The sample concentration is calculated in accordance with the next equation:

$$\text{C, µg/ml of final solution} = \frac{\text{A}i - 0.0101}{0.0202},$$

where A*i* – maximum absorption of the investigated sample in ethanol-water (1:3, v:v) at 307 ± 1 nm in 3 ml cuvette with 1 cm layer;

Or, in comparison to the measured absorbance of standard solution:

$$\text{C, \% of final solution} = \frac{\text{A}i * 0.004}{0.8181},$$

where A*i* – maximum absorption of the investigated sample in ethanol-water (1:3, v:v) at 307 ± 1 nm in 3 ml cuvette with 1 cm layer; 0.004 - concentration (%) of the standard solution with absorbance of 0.8181 at 307 ± 1 nm. or concentration in the initial sample:

$$\text{C, \% (w/V) in bulk} = \frac{\text{A}i \cdot \text{C}_0 \cdot 100.0 \cdot 25.00}{\text{A}_0 \cdot Vp \cdot l \cdot a},$$

where Ai – maximum absorption of the final experimental sample solution;

C_0 - concentration of the Octopirox standard solution is 0.004 %;

100.0, 25.00 - flasks dilutions volume, ml;

A_0 - maximum absorption of 0.004% standard solution at 307±1 nm is 0.8181,

V*p* - sample volume taken by pipette is 2 ml;

l -cuvette layer is 1 cm;

a - sample weight, g.

CONCLUSIONS

It was found, that Octopirox in ethanol-water (1:3, v:v) solution could be simple, fast and accurate as qualitatively and quantitatively determined by UV spectroscopy by the maximum absorbance at 307 ± 1 nm. The exact method of determination is proposed. Validation showed, that calibration curve had good linearity ($r^2 = 0.99$) in the concentration range 10-50 µg/ml. The LOD was calculated to be 1.18 µg/ml and LQD – 3.59 µg/ml. Such criteria like accuracy, precision, robustness and ruggedness also showed high validity and reproducibility, noticing the high importance of substance first step dilution in 96% ethanol.

REFERENCES

1. Pirocton-Olamin. http://www.wikiwand.com/de/Pirocton-Olamin.
2. Octopirox. Antidandruff active ingredient. Hoechst AG. EHOE. 4815E-0391/134. http://www.fda.gov/ohrms/dockets/dailys/04/oct04/101904/04n-0050-rpt0001-E-03-Hoechst-vol7.pdf.
3. Unilever Plc, Unilever N.V. *Antimicrobial Personal Care Compositions*. Patent WO 1998023258 A1. Issued date June 04, 1998. https://www.google.com/patents/WO1998023258 A1?cl=en&hl=ru.
4. Dubini, F., Bellotti, M.G., Frangi, A., Monti, D., Saccomani, L. 2005. *In vitro* antimycotic activity and nail permeation models of a piroctone olamine (octopirox) containing transungual water soluble technology. *Arzneimittel-Forschung*. **55**, 478–83. doi:10.1055/s-0031-1296892.
5. Safety data sheet. Octopirox. Clariant Corporation. USA. http://www.essentialingredients.com/msds/Octopirox.pdf.
6. Octopirox - 105273. Revision: 3. Product Specification. Höchst/Germany. Date of Issue: 03/18/2014. http://www.essentialingredients. com/spec/Octopirox.pdf

7. Cosmetics. EN16342:2013. Analysis of cosmetic products. Quantitative determination of zinc pyrithione, piroctone olamine and climbazole in surfactant containing cosmetic anti-dandruff products. BSI British Standards. doi:10.3403/30252298u.
8. Stability test of Octopirox (piroctone olamine) substance for 3 years at room temperature, Dr. Futterer, Hoechst AG, 30.06.1981.http://www.fda.gov/ohrms/dockets/dailys/04/oct04/101904/04n-0050-rpt0001-E-11-Futterer-vol7.pdf.
9. Determination of Octopirox in Shampoos by HPLC, Clariant GmbH, 02.03.2001. http://www.fda.gov/ohrms/dockets/dailys/04/oct04/101904/04n-0050-rpt0001-E-20-Clariant-GmbH-2001-vol7.pdf.
10. Chao, L. 2001. Simultaneous determination of four anti-dandruff agents including octopirox in shampoo products by reversed-phase liquid chromatography. *Int. J. Cosmet. Sci.* **23**, 183-188. doi:10.1046/j.1467-2494.2001.00090.x.
11. Gagliardi, L., Multari, G., Cavazzutti, G., De Orsi, D., Tonelli, D. 1998. HPLC determination of ciclopirox, octopirox and pyrithiones in pharmaceuticals and antidandruff preparations. *J. Liq. Chrom. Relat. Tech.* **21**, 2365-2373. doi:10.1080/ 10826079808000544.
12. Etheridge, D. 2007. Microsoft Office Excel 2007 Data Analysis: Your visual blueprint for creating and analyzing data, Charts, and PivotTables. 3rd ed. Wiley Publishing, Indianapolis, Indiana.
13. Validation of analytical procedures: Methodology. 1996. Geneva Q2B. Incorporated in Q2(R1), 2005. http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf.