https://doi.org/10.31925/farmacia.2023.5.19 **ORIGINAL ARTICLE**

DEVELOPMENT AND VALIDATION OF A NEW VISIBLE SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF FLUCONAZOLE IN THE PRESENCE OF SOME ESSENTIAL OILS

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Abstract

This study aimed to develop and validate a visible spectrophotometry analysis method for fluconazole quantification in the presence of some essential oils (cinnamon, oregano and clove essential oil), by forming a coloured complex with Cu^{2+} ion. For the spectrophotometric method validation the spectra of the fluconazole-Cu(II) complex in the presence of volatile oils were analysed and the wavelengths corresponding to the maximum absorbance values of the complex were determined. Evaluating the precision, linearity, accuracy and robustness of the elaborated visible spectrophotometric method, the obtained values of the specific parameters fell within the limits provided by the standards in force. Linearity was very good for the following fluconazole concentration ranges: 0.268 - 2.68 mg/mL for oregano essential oil, 0.2073 - 2.073 mg/mL for cinnamon essential oil and 0.2317 - 2.317 mg/mL in the case of clove essential oil. The proposed visible spectrophotometric method has been successfully applied for the assay of fluconazole in clove, cinnamon and oregano essential oils.

Rezumat

Acest studiu a avut ca obiectiv elaborarea și validarea unei metode de analiză prin spectrofotometrie în vizibil pentru cuantificarea fluconazolului în prezența unor uleiuri esențiale (de scorțișoară, de oregano și de cuișoare), prin formarea unui complex colorat al acestuia cu ionul Cu^{2+}. Pentru validarea metodei spectrofotometrice, au fost analizate spectrele complexului fluconazol-Cu(II) în prezența uleiurilor volatile și au fost stabilite lungimile de undă corespunzătoare valorilor maxime de absorbanță ale complexului. Valorile parametrilor specifici obținute la evaluarea preciziei, liniarității, acurateței și robusteței metodei spectrofotometrice elaborate s-au încadrat în limitele prevăzute de standardele în vigoare. Liniaritatea a fost foarte bună pentru următoarele domenii de concentrație ale fluconazolului: 0,268 - 2,68 mg/mL în cazul uleiului esențial de oregano, 0,2073 - 2,073 mg/mL în cazul uleiului esențial de scorțișoară și 0,2317 - 2,317 mg/mL în cazul uleiului esențial de cuișoare. Metoda spectrofotometrică în vizibil propusă a fost aplicată cu succes pentru dozarea fluconazolului în ulei esențial de cuișoare, de scorțișoară și de oregano.

Keywords: visible spectrophotometry, fluconazole, fluconazole-Cu(II) complex, oregano essential oil

Introduction

Fluconazole, an antifungal triazol derivative, is chemically named 2-(2,4-Difluorophenyl)-1,3-bis(1*H*-1,2,4-triazol-1-yl)propan-2-ol (M_r 306.3), according to European Pharmacopoeia [4].

Fluconazole is still frequently orally administered to treat various systemic and superficial fungal infections, despite its well-known gastro-intestinal adverse effects

and potential interactions with numerous drugs [7]. To overcome these drawbacks in case of superficial fungal infections and dermatophytoses, in the last decade several studies on various topical dosage forms as alternatives to oral or intravenous ones were performed [2, 11, 13, 19], considering the high fluconazole affinity for the stratum corneum [10]. In recent years, one of the approaches of formulating safe and effective topical fluconazole formulations was its combination with essential oils, which act both as antifungal agents in synergy with fluconazole [3, 9, 21, 22] and as skin penetration enhancers of drug [8, 18]. On this line, our group formulated and characterized some new biocompatible chitosan/hydroxypropylmethylcellulose-based hydrogels containing fluconazole in combination with essential oils possessing intrinsic antifungal activity, namely clove and cinnamon essential oil [14]. In the formulation stage of these hydrogels, the selection of essential oil was based on its solubilization potential for fluconazole, being necessary to measure drug solubility in three essential oils: clove oil, cinnamon oil and oregano oil. For this purpose, a spectrophotometric method was chosen considering the following issues: a) the advantages of spectrophotometric methods, including accessibility and rapidity; b) the spectral characteristics of fluconazole and of essential oils selected for screening; c) the ability of fluconazole to form coloured complexes with ions of transition metals (*i.e.*, Cu^{2+}), described in literature [1, 6, 15]. However, it turned out to be problematic to perform an adequate quantification of fluconazole in respective essential oils by an UV spectrophotometric technique previously set up for other solvents (media), because of the interferences between the UV absorption spectra of the fluconazole and essential oils in the organic solvents commonly used for drug solubility determination (ethanol, methanol and acetonitrile). More precisely, in these UV spectra the maximum absorption value of fluconazole interferes with the maximum absorption value of essential oils. In addition, the spectrum of volatile oils on the wavelength range of 190 nm to 280 nm present very high absorption values. This can be attributed to the complex composition of the essential oils used in the study. Thus, up to 50 chemical compounds, mainly linalool, methyl-eugenol and limonene, have been identified in the cinnamon essential oil composition [20]; approximately 35 compounds have been identified in the oregano essential oil, most of them carvacrol, thymol and γ-terpinen [5]; in clove essential oil, eugenol was identified in proportion of about 70% of the 18 identified compounds [12].

In terms of chemical reactivity, fluconazole is considered a semi-flexible or semirigid organic ligand, due to the presence of the saturated flexible C-C chain, as well as the two symmetrical rigid 1,2,4-triazole heterocycles in its structure. These heterocycles may bind to each other and may generate Me(II)-fluconazole complexes by coordination at the N atom in position 4; so fluconazole can be a good chelator for a large number of metals, especially for Cu(II) ions [6]. In an exhaustive study, Nagaj J. and coworkers demonstrated the formation of the complex between fluconazole and Cu(II) ions by modern physical methods such as atomic absorption spectroscopy, electron paramagnetic resonance, nuclear magnetic resonance and X-ray crystallography. Also, it has been shown that this complex is formed when the fluconazole ligand and cupric ion are present in a molar ratio of 1:1 and 1:2 [15]. According to these findings, it was considered possible to quantify fluconazole in the presence of essential oils as its coloured coordinative complex with Cu(II), whose absorption maximum falls in visible domain (380 - 740 nm) and therefore would not coincide with that of fluconazole recorded at wavelengths 200 - 300 nm (falling in UV domain). One can assume that small differences in absorption maximum of fluconazole-Cu(II) complex could occur depending on its concentration and colour intensity.

So, the present study aimed to develop and validate a sensitive and rapid spectrophotometric method in visible domain for quantification of fluconazole dissolved in three essential oils, namely clove oil, cinnamon oil and oregano oil. This method is intended for determination of fluconazole solubility in these volatile oils.

Materials and Methods

Materials

Chemicals and essential oils. Fluconazole has been generously donated by SC Vim Spectrum SRL (Romania). Copper (II) chloride ($CuCl_2 \cdot H_2O$) was purchased from Sigma Aldrich (Germany). Pure essential oils of cloves, cinnamon and oregano were obtained from Elemental (Romania) and acetonitrile (HPLC grade) from Acros Organics (Belgium). Distilled water was used to prepare aqueous solutions. All chemicals and essential oils used were of pharmaceutical or analytical grade.

Instrumental. Spectral and absorbance measurements were performed using a single split beam spectrophotometer T70 + UV-VIS spectrophotometer (PG Instruments, United Kingdom), supplied with a pair of 1 cm quartz cuvettes and UV-Win5 software for data acquisition and analysis. The scan system operated over the wavelength range from 600 to 900 nm. Analytical balance (Kern, Germany, ABJ 220-4NM model) was used also.

Methods

Preparation of standard solutions. To determine the optimal working conditions, the following solutions were prepared: the solution used for the fluconazole complexation (coded ACN-Cu(II)-H_2O), was prepared by mixing aqueous solution of cupric chloride 53

mg/mL with acetonitrile in ratio of 1:9; the solutions used to obtain fluconazole calibration curves in the tested essential oils (coded FZ-EO), were prepared by dissolving fluconazole at a concentration of 110 mg/mL, 95 mg/mL and 85 mg/mL in oregano essential oil, cloves essential oil and cinnamon essential oil respectively; the solutions containing the Cu(II)-fluconazole complex (coded FZ-Cu(II)-EO), were prepared by adding 0.1 mL solution of known concentration of fluconazole dissolved in each essential oil (cloves, cinnamon or oregano) to 4 mL of ACN-Cu(II)-H_2O solution; the standard solutions used in all spectrophotometric measurements (coded Cu(II)-EO), were prepared by dissolving 0.1 mL of essential oil (cloves, cinnamon or oregano) in 4 mL of ACN-Cu(II)-H_2O solution.

Recommended procedure for spectrophotometric determination of fluconazole in the essential oil (selection of maximum analytical wavelength λ_{max}). To select the measuring maximum wavelength (λ_{max}), the working solutions coded FZ-Cu(II)-EO, containing the Cu(II)-fluconazole complex in the presence of essential oil, were analysed spectrophotometrically. After 15 minutes from the solutions preparation, in case of each essential oil, absorption spectrum was recorded in the range of 600 - 900 nm, against a standard solution (coded Cu(II)-EO) prepared under the same conditions.

Spectrophotometric method validation. The proposed analytical method has been validated according to recommendations pointed out in the ICH guidelines. The relevant attributes of a conventional validation procedure including precision, linearity, recovery and robustness were determined.

Precision. To evaluate the precision of the proposed spectrophotometric method, six measurements were performed for six different concentrations of fluconazole solutions in each essential oil, namely: (i) 0.268 mg/mL, 0.537 mg/mL, 1.072 mg/mL, 1.608 mg/mL, 2.144 mg/mL and 2.680 mg/mL for fluconazole solutions in oregano essential oil; (ii) 0.2073 mg/mL, 0.4146 mg/mL, 0.8293 mg/mL, 1.2439 mg/mL, 1.6585 mg/mL and 2.073 mg/mL for fluconazole solutions in cinnamon essential oil; (iii) 0.2317 mg/mL, 0.4634 mg/mL, 0.9268 mg/mL, 1.39 mg/mL, 1.854 mg/mL and 2.317 mg/mL for solutions of fluconazole dissolved in clove essential oil. These concentration values refer to the 4.1 mL mixture obtained by adding 0.1 mL of fluconazole solution in volatile oil to 4 mL of ACN-Cu (II)-H_2O solution. The six samples of each concentration were analysed in five replicates within the same day (intra-day precision) and in three consecutive days (inter-day precision). The obtained data were analysed by calculating the standard deviation (SD) and the percentage relative standard deviation (%RSD).

Accuracy. To test the accuracy of the proposed spectrophotometric method, recovery experiments were conducted using standard addition technique. To 0.05 mL standard solution of a known concentration of fluconazole in essential oil (87.9 mg/mL in oregano essential oil, 68.00 mg/mL in cinnamon essential oil and 75.98 mg/mL in clove essential oil) was added other 0.05 mL fluconazole solution in essential oil with the following concentrations: (i) 0 mg/mL, 29.3 mg/mL, 58.6 mg/mL, 87.9 mg/mL and 117.2 mg/mL in oregano essential oil; (ii) 0 mg/mL, 22.67 mg/mL, 45.34 mg/mL, 68.00 mg/mL and 90.67 mg/mL in cinnamon essential oil; (iii) 0 mg/mL, 25.32 mg/mL, 50.64 mg/mL, 75.96 mg/mL and 101.28 mg/mL in clove essential oil. The resulting solution of 0.1 mL was mixed with 4 mL of ACN-Cu(II)-H_2O solution, then the absorbance of the final solution was recorded. Each level was repeated 3 times. The absorbance values of the samples (the final solutions containing the FZ-Cu(II) complex in the essential oil) were plotted *versus* concentration, yielding the calibration lines. The nominal concentration of the samples (mg/mL) was given by the ratio between the x-axis intercept and the slope of the regression line.

Linearity. For testing the linearity of proposed method, the solutions of FZ-Cu(II)-EO, containing FZ in six concentration levels (see *Precision* paragraph) were analysed spectrophotometrically at selected maximum wavelength. The absorbances for each concentration level were recorded in five replicates and their mean values were plotted against the concentration of fluconazole in the assessed samples. The regression equations of the calibration curves obtained for each series of solutions were statistically evaluated and the linearity was determined based on the determination coefficient.

Robustness. The method robustness was evaluated by assessing the impact of an operational parameter namely the concentration of Cu(II) ion in the ACN-Cu(II)-H_2O solution used for the fluconazole complexation. The values of this operational parameter varied as follows: 53 mg/mL, 52 mg/mL, 51 mg/mL, 50 mg/mL and 49 mg/mL. Following the proposed procedure, 4 mL of each of these ACN-Cu(II)-H_2O solutions were added to 0.1 mL fluconazole solution of known concentration in each essential oil (44 mg/mL in oregano essential oil, 34 mg/mL in cinnamon essential oil and 38 mg/mL in clove essential oil). Afterwards, the obtained solutions were analysed spectrophotometrically at the settled maximum wavelength and the measured absorbance values were entered into the regression equations of the calibration curves corresponding to each essential oil in order to obtain the calculated FZ concentration in the samples, which is required to calculate the recovery percentage for each sample. Each measurement was performed in triplicate.

Statistical analysis of the results

Statistical analysis of the method validation results was carried out using Microsoft Excel, 2016. Means

± SD and percentage relative standard deviation (%RSD) were used to express the experimental results. Regression analysis of the experimental data obtained in assessing the method linearity and accuracy was also performed.

Results and Discussion

Recommended procedure for spectrophotometric determination of fluconazole in the essential oil (selection of maximum analytical wavelength λ_{max})

In the absorption spectrum of FZ in the water-acetonitrile mixture (1:9) (Figure 1), three maximum absorbance values in the UV range were measured at wavelengths 208 nm, 261 nm and 266 nm. Also, the fluconazole maximum absorbance in acetonitrile is found at the same wavelength values (Figure 2).

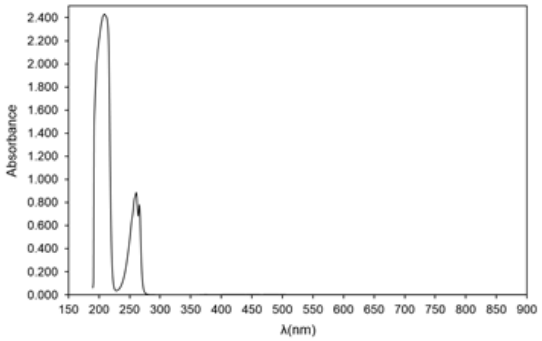

Figure 1.
The absorption spectrum of fluconazole in water-acetonitrile mixture (1: 9)

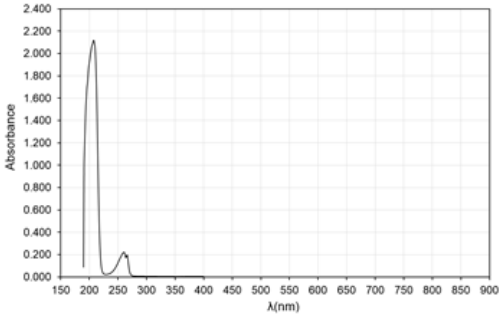

Figure 2.
The absorption spectrum of fluconazole in acetonitrile

Figure 3 shows the absorption spectra of the studied essential oils (oregano essential oil, cinnamon essential oil and clove essential oil) in acetonitrile, recorded in the UV domain, between 190 - 400 nm.

If comparing the fluconazole and essential oils spectra in acetonitrile (Figures 2 and Figure 3), it can be observed that the wavelengths at which FZ and the tested oils have maximum absorbance values coincide. Furthermore, it is found that at wavelengths of 211 nm and 266 nm (corresponding to the maximum absorption of FZ), the absorbance values of essentials oils are very low compared to those of FZ, but they were measured at a dilution of 1:1000000 essential oil in acetonitrile. It can be therefore considered that at a much lower dilution, identical to that of the FZ solution in acetonitrile, the essential oils will produce significantly higher absorbance values at the respective wavelengths.

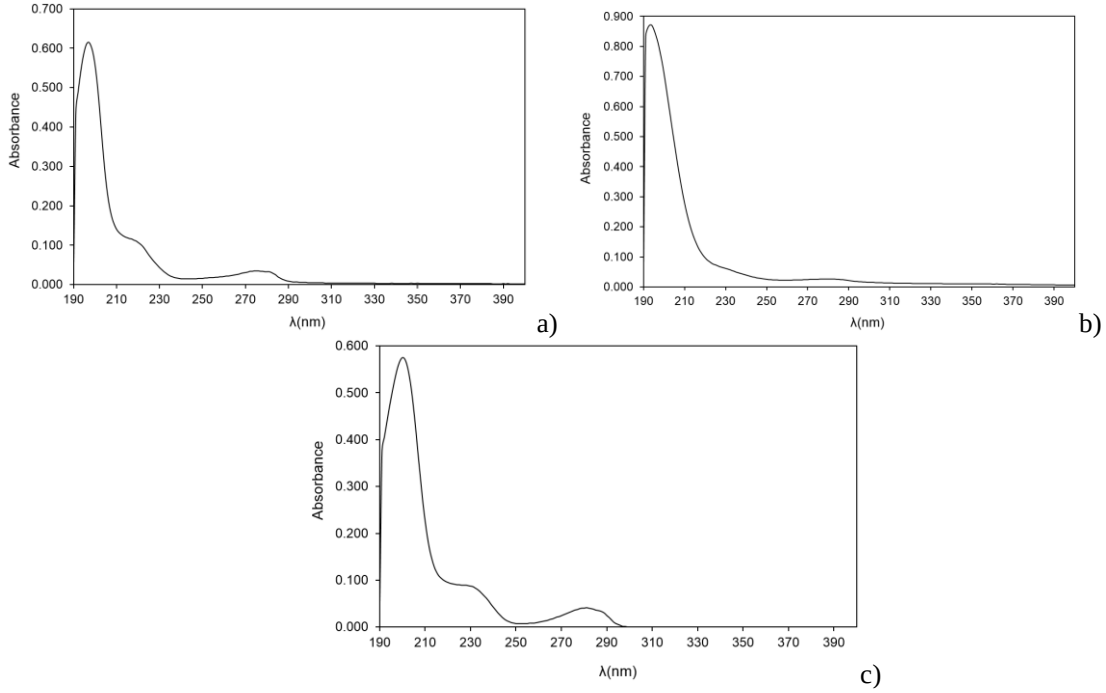

Figure 3.
The absorption spectra of studied essential oils in acetonitrile (dilution 1:1000000):
a) oregano essential oil; b) cinnamon essential oil; c) clove essential oil

Under these conditions, the spectrophotometric quantification of fluconazole in the presence of essential oils is not possible because their absorbance values cannot be compensated by the standard so as to obtain an absorbance value attributable only to the presence of fluconazole.

By the complexation of fluconazole with Cu(II), its absorption peak wavelength shifts towards higher wavelengths in the visible domain. Due to this modification, fluconazole can be quantified in the presence of essential oils, because it avoids the superposing of spectra over the wavelength range which have been observed to absorb the compounds of essential oil as well.

For obtaining this complex, it was taken into account the presence and immiscibility with water of volatile oils, in which fluconazole is soluble or slightly soluble [16, 17], as well as the very low solubility of fluconazole in water. As a result, 0.1 mL of volatile oil was maintained dissolved in a mixture of cupric chloride aqueous solution-acetonitrile in a 1:9 volume ratio. The mixture of aqueous solution cupric chloride-acetonitrile (1:9) was clear, light-green coloured (Figure 4).

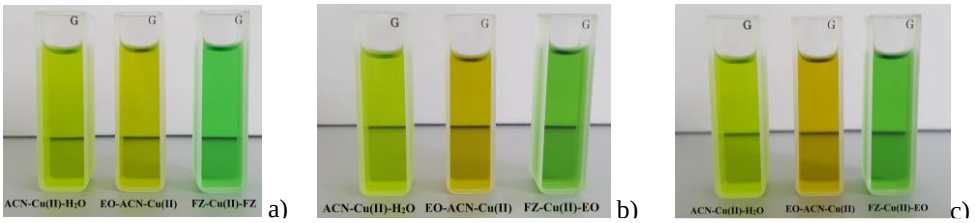

Figure 4.

Formation of the FZ-Cu(II) complex in the presence of oregano essential oil (a), cinnamon essential oil (b) and clove essential oil (c)

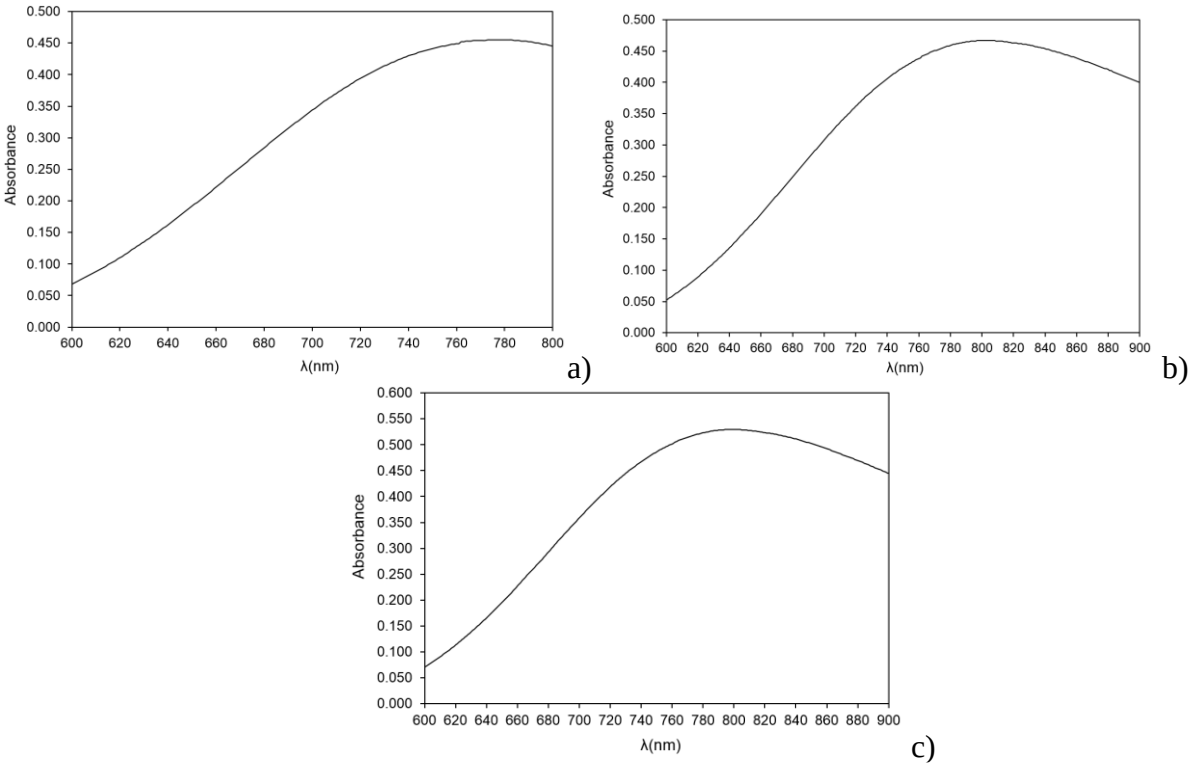

Figure 5.

The absorption spectra of FZ-Cu (II) complex in studied essential oils: a) oregano essential oil; b) cinnamon essential oil; c) clove essential oil

Mixing 0.1 mL of pure essential oil or 0.1 mL of fluconazole solution in essential oil with the ACN-Cu(II)-H_2O solution, the colour of the last solution changed into yellow-greenish and green respectively (Figure 5), indicating the formation of the Cu(II)-fluconazole complex. Since the obtained samples containing fluconazole were coloured, their absorption spectrum were recorded in the visible domain within the wavelength range 600 - 900 nm, using as a standard the mixture of ACN-Cu(II)-H_2O solution with pure essential oil (Figure 5).

In these spectra, the maximum absorbance values of the Cu(II)-fluconazole complex were measured at 762 nm wavelength for the oregano essential oil sample and at 800 nm wavelength for samples of cinnamon and clove essential oil (Figure 5).

Spectrophotometric method validation

Precision. The precision (intra-day and inter-day variability) of the method was determined by measuring the concentration of fluconazole in each of the three essential oils on six levels of concentration. The precision results are summarized in Tables I, II and III. It can be observed that the recovery (%) and the % standard relative deviation were within the following ranges of values: 96.22 - 109.25% and respectively 0.1801 - 1.5403% for oregano essential oil samples; 98.06 - 100.97% and respectively 0.1881 - 0.9398% for samples of cinnamon essential oil; 99.02 - 102.27% and respectively 0.0024 - 0.0087% for samples of clove essential oil.

Table I

Results of the method precision evaluation for oregano essential oil

Parameters	***Intra-day test***						***Inter-day test***		
							Day one	***Day two***	***Day three***
FZ taken (mg/mL)	0.2680	0.5370	1.0720	1.6080	2.1440	2.6800	2.6800	2.6800	2.6800
FZ found (mg/mL)	0.2928	0.5167	1.0661	1.5907	2.1687	2.6751	2.6751	2.6760	2.6901
SD*(mg/mL)	0.0045	0.0078	0.0090	0.0039	0.0039	0.0060	0.0060	0.0008	0.0028
Recovery (%)	109.25	96.22	99.45	98.92	101.15	99.82	99.82	99.85	100.37
RSD (%)	1.5403	1.5117	0.8460	0.2455	0.1801	0.2230	0.2230	0.0299	0.1027

*Mean for six independent determinations

Table II

Results of the method precision evaluation for cinnamon essential oil

Parameters	***Intra-day test***						***Inter-day test***		
							Day one	***Day two***	***Day three***
FZ taken (mg/mL)	0.2073	0.4146	0.8293	1.2439	1.6885	2.0730	2.0730	2.0730	2.0730
FZ found (mg/mL)	0.2086	0.4198	0.8145	1.2132	1.6291	2.0918	2.0918	2.0984	2.0840
SD*(mg/mL)	0.0060	0.0039	0.0023	0.0060	0.0039	0.0039	0.0039	0.0159	0.0118
Recovery (%)	100.97	101.25	98.22	98.06	100.03	100.91	100.91	101.22	100.53
RSD (%)	0.9398	0.9372	0.2789	0.4927	0.2371	0.1881	0.1881	0.7577	0.5664

*Mean for six independent determinations

Table III

Results of the method precision evaluation for cloves essential oil

Parameters	***Intra-day test***						***Inter-day test***		
							Day one	***Day two***	***Day three***
FZ taken (mg/mL)	0.2317	0.4634	0.9268	1.3900	1.8540	2.3170	2.3170	2.3170	2.3170
FZ found (mg/mL)	0.2266	0.4563	0.9479	1.4058	1.8358	2.3161	2.3161	2.3144	2.2853
SD*(mg/mL)	0.0024	0.0073	0.0042	0.0073	0.0087	0.0064	0.0064	0.0027	0.0052
Recovery (%)	97.80	98.46	102.27	101.14	99.02	99.96	99.96	99.89	99.72
RSD (%)	1.0705	1.5949	0.4433	0.5176	0.4764	0.2771	0.2771	0.1155	0.2258

*Mean for six independent determinations

The results of the intra-day and inter-day tests were compliant and denoted satisfactory recovery with low %RSD for all three essential oils, supporting the suitability of the proposed spectrophotometric method.

Linearity. The calibration curves were obtained by plotting the average absorbance values *versus* the corresponding FZ concentration. The Lambert-Beer's law was obeyed in the following concentration ranges: 0.268 - 2.68 mg/mL for oregano essential oil, 0.2073 - 2.073 mg/mL for cinnamon essential oil and 0.2317 - 2.317 mg/mL for clove essential oil. The regression analysis of the calibration data was performed by fitting them into the linear regression equation y = ax + b, where y is the absorbance at the maximum wavelength; x is the concentration in g/100 mL; a is the slope and b is the intercept of the calibration line. The values of the determination coefficient, R^2, of the three calibration lines were greater than 0.999 (0.9995 in case of oregano essential oil, 0.9992 in case of cinnamon essential oil and 0.9996 in case of cloves essential oil). The high values of determination coefficients calculated for all three volatile oils revealed an excellent linearity of the proposed method.

Accuracy. The standard addition technique is frequently used to evaluate the accuracy of an analytical method, being considered one of the most simple and direct technique for the removal of interferences effect on the results. This technique consists in adding of several different known amounts of analyte to several aliquots of test solution, thus increasing their concentration; after the absorbance measurement, the calibration line

is extrapolated to zero response. Using this technique, the accuracy is tested by performing recovery experiments. The analytical parameters and the results of both recovery test and statistical analysis of data are presented in Table IV.

Table IV

Results of method accuracy evaluation by standard addition technique

FZ concentration (mg/mL)			Parameters of linear regresion equation			Recovery (%)
Sample	***Addition fractions***	***Nominal concentration***	***Intercept***	***Slope (mL/mg)***	***R^2***	
Oregano essential oil						
87.90	0; 29.30; 58.60; 87.90; 117.20	88.23	0.349	0.00396	0.9998	100.37
Cinnamon essential oil						
68.00	0; 22.67; 45.34; 68.00; 90.67	67.41	0.204	0.00304	0.9998	99.13
Cloves essential oil						
75.96	0; 25.32; 50.64; 75.96; 101.28	76.11	0.403	0.00529	0.9998	100.17

These results revealed the good linearity of the absorbance *versus* fluconazole concentration obtained by the standard addition technique, indicating the accuracy of the proposed spectrophotometric method. Based on these results, it can be suggested that standard addition technique is adequate for testing the accuracy of the proposed analytical method also in the presence of other excipients besides the essential oils and analyte fluconazole.

Robustness. The method's robustness was verified by measuring fluconazole concentration in the FZ-Cu(II)-EO sample solutions under minor deliberate variations of experimental conditions. The selected variable in this test was the concentration of cupric chloride aqueous solution which was mixed with acetonitrile in ratio of 1:9 to obtain the solution for fluconazole complexation. This method variable was selected upon its potential impact on the FZ-Cu(II)-EO solution properties, as the metal salt is used in excess compared to FZ in order to ensure that drug (analyte) has been completely transformed into the self-assembly of complex [6]. The results of the robustness analysis, namely the mean recovery and %RSD were the following: 99.276% and, respectively, 0.478 for oregano essential oil, 97.952% and, respectively, 0.228 for cinnamon essential oil and 101.638% and, respectively, 0.508 for cloves essential oil (Tables V, VI and VII).

Table V

Results of robustness evaluation of proposed spectrophotometric method in presence of oregano essential oil

Parameters	***Experimental data***				
FZ concentration taken (mg/mL)	1.072	1.072	1.072	1.072	1.072
Concentration of $CuCl_2$ solution (mg/mL)	53.00	52.00	51.00	50.00	49.00
FZ concentration found (mg/mL)	1.0595	1.0634	1.0673	1.0712	1.0595
Recovery (%)	98.8394	99.2037	99.5681	99.9324	98.8394
Mean recovery (%)	99.2766				
RSD* (%)	0.478				

*Relative standard deviation calculated from mean recovery

Table VI

Results of robustness evaluation of proposed spectrophotometric method in the presence of cinnamon essential oil

Parameters	***Experimental data***				
FZ concentration taken (mg/mL)	1.2439	1.2439	1.2439	1.2439	1.2439
Concentration of $CuCl_2$ solution (mg/mL)	53.00	52.00	51.00	50.00	49.00
FZ concentration found (mg/mL)	1.2184	1.2184	1.2224	1.2145	1.2184
Recovery (%)	97.9522	97.9522	98.2685	97.6360	97.9522
Mean recovery (%)	97.9522				
RSD* (%)	0.228				

*Relative standard deviation calculated from mean recovery

Table VII

Results of robustness evaluation of proposed spectrophotometric method in presence of cloves essential oil

Parameters	***Experimental data***				
FZ concentration taken (mg/mL)	0.9268	0.9268	0.9268	0.9268	0.9268
Concentration of $CuCl_2$ solution (mg/mL)	53.00	52.00	51.00	50.00	49.00
FZ concentration found (mg/mL)	0.9478	0.9437	0.9352	0.9437	0.9395
Recovery (%)	102.272	101.819	100.912	101.819	101.365
Mean recovery (%)	101.638				
RSD*(%)	0.508				

*Relative standard deviation calculated from mean recovery

The mean values of the recovery percentage being close to 100% and the very small %RSD in the conditions of the variation of the cupric ion concentration, indicate the robustness and reliability of the proposed method for all three essential oils.

Conclusions

The visible spectrophotometric method developed and validated is intended for quantitative determination of antifungal drug fluconazole dissolved in some essential oils with intrinsic antifungal activity, namely oregano essential oil, cinnamon essential oil and cloves essential oil. The method's major advantage is that it allows adequate quantification of fluconazole as coloured Cu(II)-fluconazole complex in visible domain (wavelength of 762 nm for oregano essential oil and 800 nm for cinnamon and cloves essential oil) without interference from active compounds of essential oils. The spectrophotometric method validation was performed by testing the following analytical attributes: precision, linearity, accuracy and robustness. The method showed good precision (intra-day and inter-day) and accuracy (through standard addition method), linearity and high robustness. Additionally, the method is simple and less expensive (use commonly available reagents). These features support its use in determination of fluconazole solubility in essential oils and its potential application for fluconazole quantification in topical dosage forms containing essential oils among the excipients.

Conflict of interest

The authors declare no conflict of interest.

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