

DETERMINATION OF AZOLE ANTIFUNGAL MEDICINES USING
ZERO-ORDER AND DERIVATIVE UV SPECTROPHOTOMETRY

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Abstract: This paper presents a new methodology of quantitative determination of seven azole antifungal medicines widely used in therapy. Analyses were performed directly by using zero-order (fluconazole), first derivative (bifonazole, clotrimazole, econazole, itraconazole, miconazole) and second derivative (ketoconazole) UV spectrophotometry. Validation of all methods confirms their proper precision (%RSD = 0.47 – 2.86), recovery (98.7 – 101.4) and linearity (r coefficient over 0.999) in concentrations under investigation. The parameters received enable the developed procedure to be used in quantitative and as auxiliary in qualitative pharmaceutical analysis.

Keywords: azole antifungal medicines, zero-order and derivative UV spectrophotometry, pharmaceutical analysis

Among antifungal substances used in therapy, azole compounds being imidazole and triazole derivatives are the most important synthetic ones. Due to high efficacy and acceptable safety profile these compounds gained a strong position in therapy (1-3). In this paper bifonazole, clotrimazole, econazole, ketoconazole and miconazole as imidazole derivatives and fluconazole and itraconazole as representatives of triazole derivatives were analyzed.

So far, the compounds mentioned above were determined using various techniques, exemplified by chromatographic (4), biological (5) and electro-analytical ones (6). However, there is a limited amount of published papers related to UV spectrophotometry and derivative spectrophotometry.

Bifonazole was determined directly (7, 8) and by forming complex compound with indicator bromophenol blue (9). For clotrimazole analyses were performed after prior acidic hydrolysis (10), complexation reaction (11, 12) or directly (8). Econazole was analyzed directly (8, 13) and after complexation (12). Fluconazole was also determined (14, 15), whereas for itraconazole no analysis was reported till now. Ketoconazole determination included direct analyses (16-18) and complexes formation (12, 19-22). In case of miconazole analyses were done indirectly with use of internal

standard (23), after complexation reaction (12) and directly (8, 13, 24).

The above listing of publications illustrates continuous demand for new research studies related to commonly available spectrophotometrical technique within the group of antifungal azole medicines. At first place, there is a need for direct analyses. Because of possibility of derivatization of zero-order absorption spectra by computer programmes, enabling valuable analytical operations, there is a need to use more accurate and sensitive derivative spectrophotometry.

The aim of this paper was to establish the conditions for quantitative determination of analyzed compounds for routine pharmaceutical analysis and define essential parameters helpful in identification. Moreover, analysis of several compounds from the same azole group under uniform conditions enables a comparison of variability of analytical parameters depending on differences in their chemical structure. This publication is a continuation of authors' research dealing with analytical assessment of azole antifungal drugs. Hitherto, analyses were performed using such techniques as TLC (25) and GC (26) and justified their usefulness in identification, separation and quantitative determination of the investigated compounds.

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EXPERIMENTAL

Apparatus

Spectrophotometer UV-Vis Cary 100 Conc (Varian Analytical Instruments, Palo Alto, USA) was used for analysis. Scan speed was 3000 nm/min and slit was 4 nm. Handling of results was enabled by Cary WIN UV Conc software. Deuterium lamp was a radiation source. Quartz cells of 10 mm width were used.

Reference standards, reagents and investigated preparations

An analysis involved organic solutions with concentration $4.8 \mu\text{g mL}^{-1}$ in case of bifonazole, econazole i miconazole, $8.0 \mu\text{g mL}^{-1}$ for clotrimazole and $48.0 \mu\text{g mL}^{-1}$ in case of fluconazole, itraconazole and ketoconazole (standard solutions). Itraconazole was dissolved in chloroform (1 : 4, v/v) and filled up with methanol (3 : 4, v/v), whereas other compounds were dissolved only in methanol. All reference standards were supplied by Sigma (St. Louis, USA) and met the pharmacopoeia requirements. Two of the investigated substances: econazole and miconazole, occurred in nitrate salt form (pharmacopoeial form). Solutions were kept at low temperature (4°C) and protected from light. Solvents of analytical grade came from POCh (Gliwice, Poland).

The analysis conditions established on reference standards were used for quantitative analysis of selected analytes in medicinal products. The studies were carried out on the preparation Fluconazole, 50 mg tablets (Polfarmex) and drug Ketokonazol, 200 mg tablets (Anpharm). The samples were prepared

by mixing a selected amount of average sample of powdered tablets with methanol, shaking for 15 min at frequency approx. 3 cycles/s and filtering through filter paper No. 2. The prepared solutions had the concentration of $48.0 \mu\text{g} \times \text{mL}^{-1}$ (sample solutions).

Method parameters

For solutions of reference standards and preparations with fluconazole and ketoconazole the UV absorption spectra in range 200-400 nm were obtained. The usefulness of zero-order spectrophotometry was verified with regard to quantitative analysis. The results fulfilling accuracy requirement were received for fluconazole (Figure 1), whereas for ketoconazole interferences with matrix constituents for zero-order and first derivative (D1) spectra had negative influence on determination, thus the second derivative (D2) was used (Figure 2). For other compounds, for which there were no determination in medicinal products performed, method validation was done for first derivative D1 (Figures 3 – 7). In case of drugs analyses “baseline-to-peak” technique was used for determination. Analytical wavelengths for all compounds are shown in Tables 1 – 3.

RESULTS

Qualitative characteristics

Because of different shape of zero-order spectra and specific λ_{max} values, method enabled differentiation of investigated medicines. The wavelength, for which the maximum absorption occurred equaled: bifonazole – 254 nm, clotrimazole – 205 nm, econazole nitrate – 204 nm with fold at 219 nm,

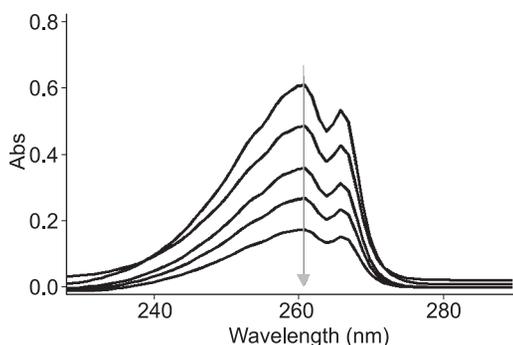


Figure 1. Zero-order spectra for fluconazole with signed analytical wavelength $\lambda = 261 \text{ nm}$.

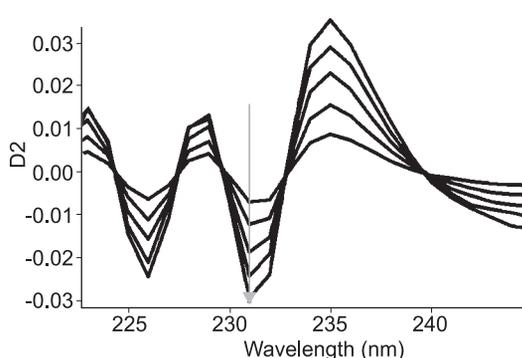


Figure 2. Second order derivative spectra for ketoconazole with signed analytical wavelength $\lambda = 231 \text{ nm}$.

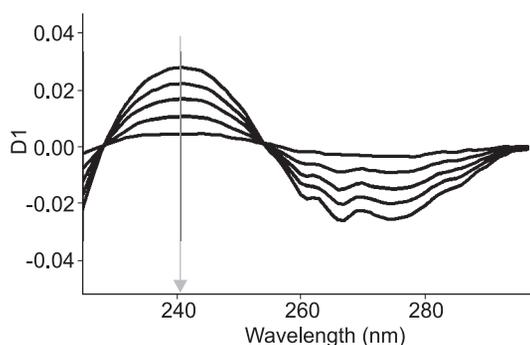


Figure 3. First order derivative spectra for bifonazole with signed analytical wavelength $\lambda = 241$ nm.

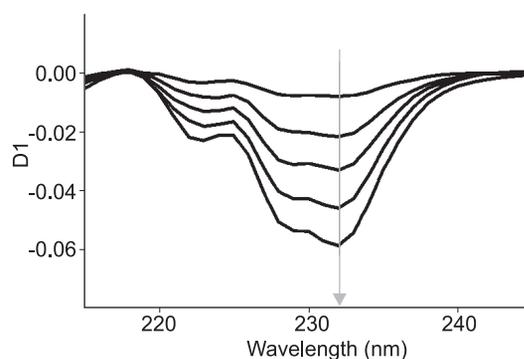


Figure 5. First order derivative spectra for econazole nitrate with signed analytical wavelength $\lambda = 232$ nm.

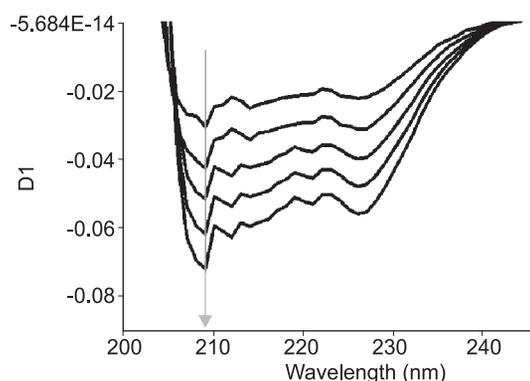


Figure 4. First order derivative spectra for clotrimazole with signed analytical wavelength $\lambda = 209$ nm.

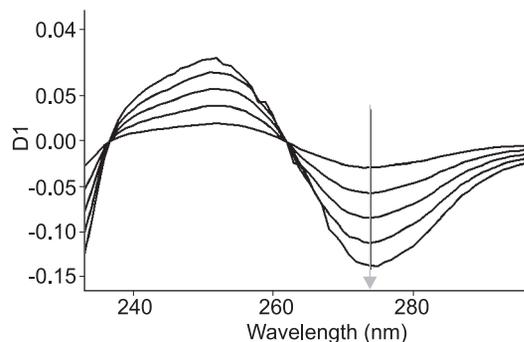


Figure 6. First order derivative spectra for itraconazole with signed analytical wavelength $\lambda = 274$ nm.

fluconazole – 261 and 266 nm, itraconazole- 230 and 261 nm, ketoconazole 243 and 296 nm, and 204 nm in case of miconazole nitrate.

For each compound the value of molar absorptivity factor ϵ was established and the value of specific absorptivity factor $A_{1\text{cm}}^{1\%}$ was defined. These values, determined at wavelength λ_{max} , which are of importance for qualitative analyses, are shown in Tables 1 – 3.

Method validation and quantitative analysis

After parameters of the method were established, as described above, the method was validated. The validation (27) allowed precision, accuracy and linearity ($n=5$) within the specified concentration range to be verified. Precision was measured in the terms of repeatability of the series of obtained results ($n = 6$) and was additionally checked inter-day. Accuracy was determined as percentage recovery ($n=3$) at three concentration levels (80, 100 and 120% addition of the reference standard). The limit

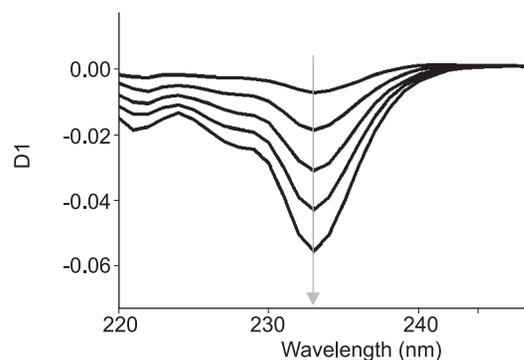


Figure 7. First order derivative spectra for miconazole nitrate with signed analytical wavelength $\lambda = 233$ nm.

of detection (LOD) and limit of quantification (LOQ) was found from ratio of the signal (S) to the noise of the baseline (N) at the required ratio S/N for LOD equal to 1:3, and 1:10 for LOQ.

Table 1. Method parameters for fluconazole and ketoconazole.

	Fluconazole [50 mg/ tablet]	Ketoconazole [200 mg/ tablet]
Method	zero-order	second derivative
Analytical wavelength [nm]	261	231
Mean [mg/ tablet]	50.2	198.6
Precision [%RSD]	1.53	1.89
Recovery [%]	99.2 ÷ 101.4	98.7 ÷ 100.6
Linearity range [$\mu\text{g mL}^{-1}$]	32.0 – 96.0	16.0 – 80.0
Correlation coefficient (r)	0.9994	- 0.9993
LOD [$\mu\text{g mL}^{-1}$]	5.0	0.6
LOQ [$\mu\text{g mL}^{-1}$]	24.0	2.1
ϵ [$\text{dm}^3 \text{mol}^{-1} \text{cm}^{-1}$]	1713.5	15758.6
$A_{1\text{cm}}^{1\%}$ [$100 \text{mL cm}^{-1} \text{g}^{-1}$]	55.9	296.5

Table 2. Method parameters for bifonazole and clotrimazole.

	Bifonazole	Clotrimazole
Analytical wavelength [nm]	241	209
Mean [D1 value]	0.0166	- 0.0522
Precision [%RSD]	1.17	1.92
Linearity range [$\mu\text{g mL}^{-1}$]	1.6 – 8.0	4.8 – 11.2
Correlation coefficient (r)	0.9996	- 0.9992
LOD [$\mu\text{g mL}^{-1}$]	0.3	2.0
LOQ [$\mu\text{g mL}^{-1}$]	1.1	4.4
ϵ [$\text{dm}^3 \text{mol}^{-1} \text{cm}^{-1}$]	29077.4	50258.6
$A_{1\text{cm}}^{1\%}$ [$100 \text{mL cm}^{-1} \text{g}^{-1}$]	939.0	1457.5

Table 3. Method parameters for econazole nitrate, itraconazole and miconazole nitrate.

	Econazole nitrate	Itraconazole	Miconazole nitrate
Analytical wavelength [nm]	232	274	233
Mean [D1 value]	- 0.0331	- 0.0841	- 0.0308
Precision [%RSD]	2.81	0.47	2.86
Linearity range [$\mu\text{g mL}^{-1}$]	1.6 – 8.0	16.0 – 80.0	1.6 – 8.0
Correlation coefficient (r)	- 0.9998	- 0.9998	- 0.9999
LOD [$\mu\text{g mL}^{-1}$]	0.2	0.3	0.2
LOQ [$\mu\text{g mL}^{-1}$]	0.8	1.1	0.8
ϵ [$\text{dm}^3 \text{mol}^{-1} \text{cm}^{-1}$]	53412.7	31970.6	65565.2
$A_{1\text{cm}}^{1\%}$ [$100 \text{mL cm}^{-1} \text{g}^{-1}$]	1402.1	452.9	1570.8

Under the established conditions the quantitative determination of analyzed compounds was carried out. For fluconazole and ketoconazole, determinations were made in medicinal products in the form of tablets of unmodified release (Table 1). For these compounds the method selectivity was evaluated by comparing the spectra shapes and λ_{max} val-

ues for the analyzed product with those of the reference standard. No interferences at the analytical wavelength with the preparation auxiliary substances were found. Fluconazole product included lactose, maize starch, starch glyconate sodium salt, polyvidon and magnesium stearate, whereas ketoconazol preparation contained lactose, potato

starch, microcrystalline cellulose, polyvidon, talc and magnesium stearate as auxiliary substances. Recovery for 80, 100 and 120% addition of the reference standard equal 99.7%, 99.2%, 101.4% for fluconazole and 98.7%, 99.9%, 100.6% for ketoconazole, respectively.

Results of validation for bifonazole and clotrimazole are collected in Table 2, whereas parameters for econazole nitrate, itraconazole and miconazole nitrate are shown in Table 3.

The presented data on validation of determination method indicate proper parameters of the newly developed procedure for all investigated compounds, which enable making routine analysis in medicinal products.

DISCUSSION

In order to perform quantitative determination of investigated compounds, referential pharmacopoeial monographs (European Pharmacopoeia 6, Polish Pharmacopoeia VI, International Pharmacopoeia 4), for compounds under examination require testing of reference standards *per se* by titration method with potentiometric determination of the end-point. However, this method is unsuitable for analysis of these compounds in medicinal products or biological material, where the presence of number of additional substances can affect the results of determination. For analysis of such complex samples as drugs a selective method is required, e.g. the spectrophotometric one. It should be noted that monograph Ketoconazoli tabulettae from Polish Pharmacopoeia VI (28) recommends testing the tablets with ketoconazole using spectrophotometric method, but only with regard to prove the active substance identity.

Quantitative analysis in medicinal products performed basing on zero-order spectra often do not fulfilled the accuracy requirement because of interferences with auxiliary substances from sample matrix. By derivatization of zero-order spectra, derivative spectra are received for which determination results are usually characterized by a smaller error. Derivative spectrophotometry technique is more selective and sensitive enabling more accurate analyses (29).

Hitherto, research in azole antifungal medicines group using UV spectrophotometric technique, do not covered itraconazole determination. In this paper conditions and results of its analysis is shown for the first time. Compounds were dissolved in methanol (itraconazole in chloroform and methanol), what should be considered as a method

attitude – methanol is the most common and well available solvent.

Unfortunately, UV spectrophotometric technique cannot be used for unequivocal qualitative analysis. Qualitative determination was elaborated by authors using chromatographic methods; TLC (25) and GC (26). Moreover, chromatographic techniques enabled separation of compounds under investigation, what was not possible using only UV spectroscopy. TLC-densitometric procedure was the best for purity examination, whereas GC method enabled determination of the smallest quantities of medicines.

CONCLUSION

A new analytical procedure for direct determination of seven common azole medicines using UV spectrophotometry was evaluated. Essential parameters which are of importance for qualitative analyses were established. The method of quantitative analysis of bifonazole, clotrimazole, econazole, fluconazole, itraconazole, ketoconazole and miconazole is suitable for routine determination of these compounds. Validation parameters are proper and proves method's usefulness for intended purpose of pharmaceutical analysis. Probably, this methodology can be also applied to analyze the above mentioned compounds in biological and environmental materials after appropriate preliminary preparation.

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