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Article

The Determination of Miconazole and its Related Production Impurities Together with Basic Solution Stability Studies Using a Sub 2 μm Chromatographic Column

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A selective and sensitive method for the analysis of Miconazole and its associated impurities is developed. The separation is carried out using a Thermo Scientific Hypersil Gold C18 Column (50 mm x 4.6 mm i.d., 1.9 μm particle size) with a mobile phase of acetonitrile–methanol–ammonium acetate (1.5 w/v) (30:32:38 v/v) at a flow rate of 2.5 mL/min and UV detection at 235 nm. The method is validated according to ICH guidelines with respect to precision, accuracy, linearity, specificity, robustness, and limits of detection and quantification. All parameters examined are found to be well within the stated guidelines. Naturally aged samples are also tested to determine sample stability. A profile of sample and impurity breakdown was presented. The analysis time was more than halved from just under 20 min (the current European Pharmacopeia Method) to under 8 min (developed method) and the method is applicable for assay and related substance determination.

Introduction

Miconazole, 1-[(2RS)-2-[(2,4-dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl]-1H-imidazole (Figure 1) is an imidazole antifungal agent. It is commonly applied topically to fungal infections as it has strong activity against dermatophytes and candida alibicans. It works by inhibiting the synthesis of ergosterol, a critical component of fungal cell membranes and creating a sterol, which is toxic to the yeast species cell membrane (1). It has a number of known impurities, which are close in structure to the main compound. These are listed and their structures shown (Figure 1). The high-performance liquid chromatography (HPLC) method for the determination of the seven main related substances is described in the European Pharmacopeia (Ph.Eur), a set of texts concerning the qualitative and quantitative composition of medicines, the tests to be carried out on medicines, on the raw materials used in the production of medicines, and on the intermediates of synthesis (2).

There are also a number of methods described in the literature for the analysis of miconazole by HPLC in different formulations, such as bulk and cream (3), pharmaceutical dosage forms (4, 5, 6), and in biological samples such as plasma (7, 8) and saliva (9). In addition, there are papers discussing the use of other analytical techniques such as derivative spectrophotometry (10) and other spectroscopic methods (11). These are often complicated and time-consuming methods or cannot be used for the simultaneous determination of the active pharmaceutical ingredient (API) and all of its impurities.

The introduction of a number of commercially available ultra high performance liquid (UHPLC) chromatographs and sub-2 μm columns has permitted the reduction of analysis time and solvent consumption in liquid chromatography. With the sub-2 μm particles, the mobile phase linear velocity is increased giving greater resolution and speed (12). Evidence suggests that the new systems combined with the smaller particles can allow up to nine fold reductions in analysis time compared to conventional high performance liquid chromatography. Many reports have shown the advantages of using these shorter columns, including the reduction in analysis times while maintaining or increasing resolution, and the sensitivity and selectivity have been increased (12–15). This improvement in speed and sensitivity is useful in all areas of the pharmaceutical process.

As a result of this improvement, the pharmaceutical industry has begun embracing the new technology. The significance of the savings in solvent consumption and analysis mean that the company can work more economically and reduce their waste solvent. The aim of this research is to increase the speed of separation without any loss of resolution or sensitivity thereby increasing sample throughput while reducing solvent and analysis costs.

Experimental

Materials and reagents

Samples of Miconazole and the seven impurities were received from Janssen Pharmaceutical Ltd., (Cork, Ireland). The chemical structure of Miconazole and its production impurities are shown in Figure 1. HPLC-grade acetonitrile and methanol and HPLC grade ammonium acetate was purchased from VWR (Radnor, PA). Ultra-pure water was obtained using a TKA water purification system (Niederelbert, Germany).

Instrumentation

The system used was an Agilent 1200 Rapid Resolution Liquid Chromatograph with a 1200 Series binary pump SL and vacuum degasser, a 1200 Series high-performance autosampler, a 1200 Series thermostatted column compartment SL, and a 1200 Series DAD SL for up to 80 Hz operation, and it was controlled by ChemStation B.02.01.SR1 data acquisition and evaluation software (Agilent, Santa Clara, CA). The column used was a Thermo Scientific Hypersil Gold C18 Column (50 mm x 4.6 mm i.d., 1.9 μm particle size) (Waltham, MA).

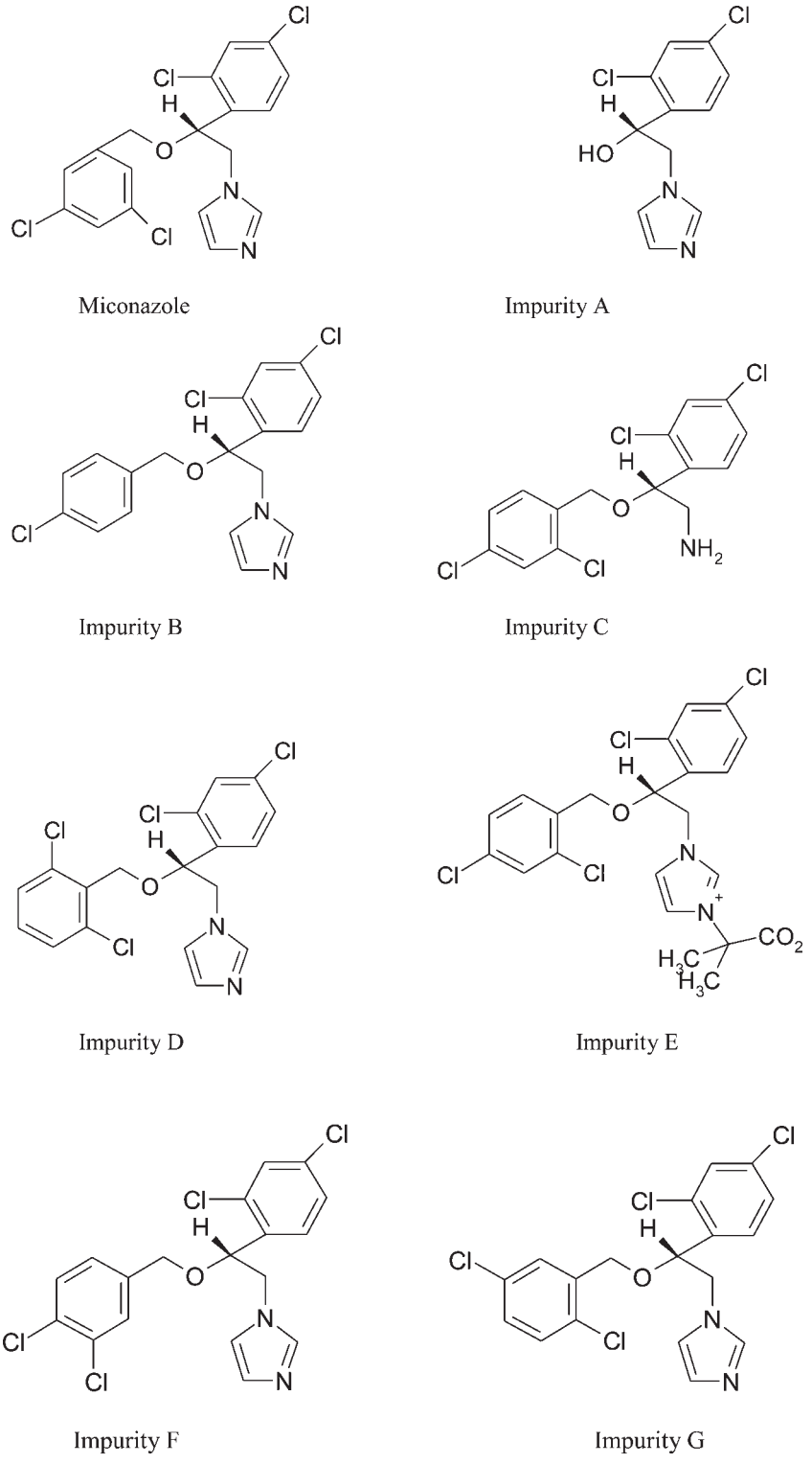

Figure 1. Structural Formulae for Miconazole and Impurities.

Chromatographic conditions

An isocratic separation was carried out using a mobile phase consisting of acetonitrile–methanol–ammonium acetate (1.5 w/v) (30:32:38 v/v) and was used at a flow rate of 2.5 mL/min with UV detection at 235 nm. The column was maintained at ambient temperatures, and an injection volume of 3 μL was used. The mobile phase was filtered through 0.45 μm nylon filters and degassed in an ultrasonic bath prior to use.

Sample preparation

A standard solution (50 ppm) of Miconazole and impurities was prepared using acetonitrile–methanol–ammonium acetate (1.5 w/v) (30:32:38 v/v) as the diluent. The specificity solution (10 mg/mL) was prepared using a particular batch with the same diluents as detailed earlier.

For linearity standards, a stock solution of 50 ppm was prepared by weighing 5 mg each of the API and impurities A–G and adding them to a 100-mL volumetric flask. This was diluted to make standards ranging from 10–22 ppm in intervals of 2 ppm.

Results and Discussion

Method development and optimization

Initially, as detailed in the Ph.Eur, a Thermo Scientific ODS C18 column (100 mm x 4.6 mm i.d., 3-µm particle size) maintained at ambient temperatures was used for the determination of Miconazole and its impurities. The flow rate of 2 mL/min eluted the active ingredient in 19.175 min.

After a review of the columns available and some experimental work, a Thermo Scientific Hypersil Gold C18 Column was chosen to develop a new method. It has the same United States Pharmacopeia (USP) column classification and showed very promising initial results with respect to the reduction of run time.

In the root method, the resolution between the F and G impurities was always poor. Baseline resolution was not achievable, though 2 peaks were visible (Figure 2).

Initial method development on the new column (Thermo Scientific Hypersil Gold C18) showed a cumulative peak for impurities F and G. They were inseparable with the new column dimensions without extending the run time beyond its original.

A number of mobile phase compositions were tested, combined with a reduction in flow rates. However, the best resolution possible was at a run time greater than the original, thus negating any positive effect the smaller dimensions had on analysis time.

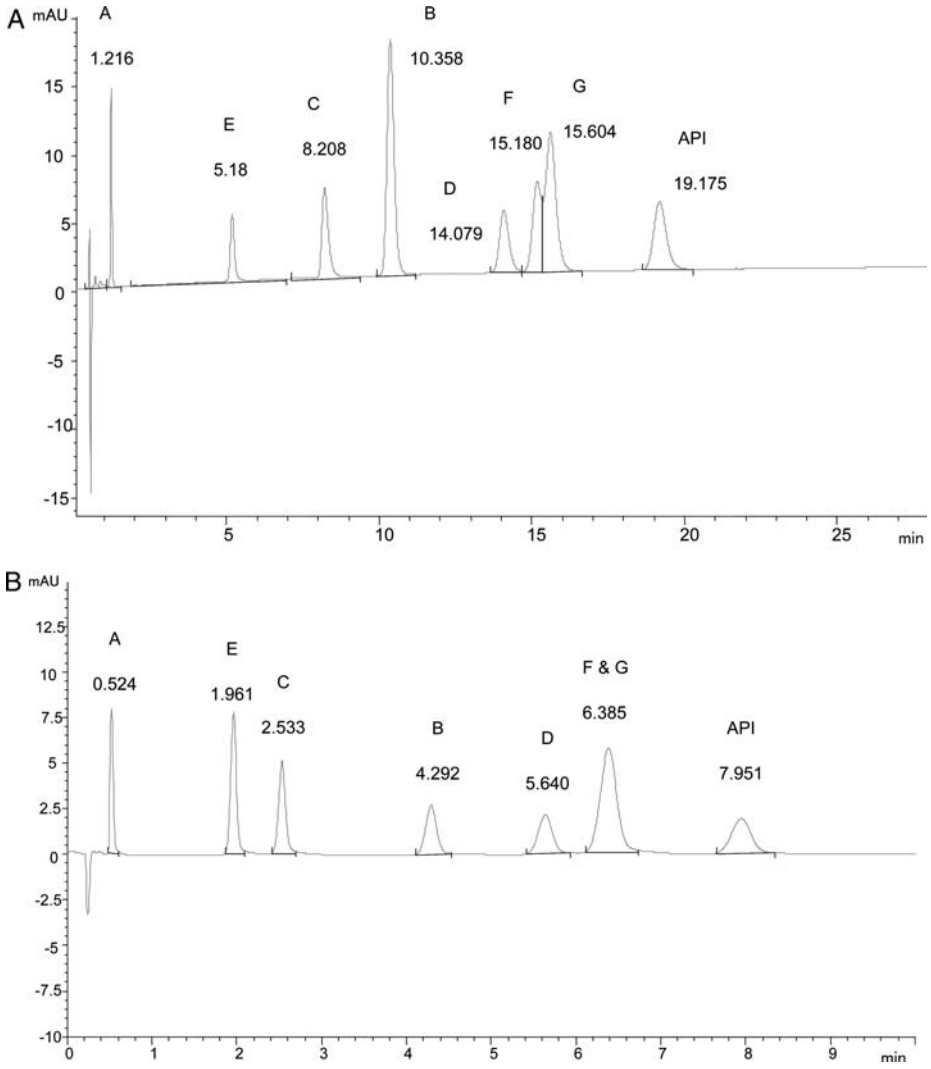

Figure 2. Comparison of chromatograms of API and the main impurities obtained with original method [Thermo Scientific ODS C18 column (100 mm x 4.6 mm i.d., 3 µm particle size), Acetonitrile/Methanol/Ammonium Acetate (1.5 w/v) (30/32/38 v/v) at a flow rate of 2 mL/min] (A) and new method [Thermo Scientific Hypersil Gold C18 Column (50 mm x 4.6 mm i.d., 1.9 µm particle size), Acetonitrile/Methanol/Ammonium Acetate (1.5 w/v) (30/32/38 v/v) with a flow rate of 2.5 mL/min] (B).

The reason for the co-eluting impurities may be linked to their similar structures. Impurities D, F, and G have a very similar structure to Miconazole, differing only in the positions of the chlorine groups on the lower aromatic ring. However, impurities F and G are the only structures that have a chlorine group in a Meta position relative to the rest of the molecule, which may have some bearing on the resolution. Further study would have to be done to determine all the bonding interactions involved in the retention of the molecule on a reversed-phase column.

Taking into account that the F and G impurities could not be separated, and due to the fact that they were reported together in the original method, the new method was developed to provide the best separation of all the other peaks in the fastest possible timeframe. As the theory suggests, the smaller particles allowed the use of higher flow rates without loss of efficiency.

For method optimization, a systematic examination of flow rate was conducted. The flow rate was increased in increments taking pressure and resolution into account. An isocratic method using acetonitrile–methanol–ammonium acetate (1.5 w/v) (30:32:38 v/v) with a flow rate of 2.5 mL/min at an ambient temperature was found to give a retention time of between 7.5–8.5 min with satisfactory resolution between all peaks while maintaining a reasonable pressure. The detection wavelength was kept constant, as was the buffer and solvent composition. Figure 2 shows the comparison of the run times and chromatography between the original and new methods.

The order of elution does not vary between the two methods: Impurity A is first, followed by E, C, B, D, with F and G co-eluting, and finally the Miconazole.

Validation of the method

The method was validated with respect to parameters laid out by the International Conference on Harmonization (ICH 2002), including linearity, precision, accuracy, specificity, LOD, and LOQ.

Linearity

Each of the impurities and the API gave a linear response over the concentration range tested. The mean values of the slope, intercept, and correlation co-efficient are given in Table I.

Accuracy

The percent recovery of the linearity samples was calculated and is shown in Table II. Good recoveries were obtained ranging from 98.7% to 101.99% for the API. The percent relative error was also calculated for each concentration giving relative standard deviation (RSD) values of 0.44% and 1.15% for the API.

Table I
Linearity Data

Compound Name	Slope	y-Intercept	Correlation Coefficient
Impurity A	1.0141	−0.18175	0.999
Impurity B	1.1686	−0.86278	0.999
Impurity C	1.4967	−1.82971	0.999
Impurity D	1.2103	−1.09721	0.998
Impurity E	1.8777	−1.92952	0.999
Impurity F & G	3.8461	−4.10818	0.999
Miconazole	1.4922	−0.99506	0.998

LOQ and LOD

The LOQ and LOD for all impurities and the API were determined based on signal-to-noise ratios, where the analytical responses of approximately 10 and 3, respectively, were used. The concentrations found are seen in Table III. A chromatograph for the LOQ for the API is seen in Figure 3.

Precision

Precision was assessed with respect to analysis repeatability, injection repeatability, and intermediate precision in accordance with Janssens' specific requirements.

Analysis repeatability

Studies were performed by injecting six replicates of a solution of Miconazole and impurities at two different concentrations (16 ppm and 22 ppm) where %RSD values were found to range between 0.33% and 1.33% for the 16 ppm sample and 0.17% and 0.99% for the 22 ppm sample (Table IV).

Injection repeatability

Injection repeatability was examined by injecting a solution of Miconazole and impurities at 2 different concentrations (16 ppm and 22 ppm) six times. The %RSD values were found to range between 0.18% and 1.35% for the 16 ppm sample and 0.12% and 0.93% for the 22 ppm sample (Table IV). The results for analysis repeatability and injection repeatability were all within specified limits as all had % RSD values less than 1.5%.

Selectivity

The specificity solution, which contains an overload of the API but small impurity peaks at around the maximum expected

Table II
Recovery Data

Concentration (ppm)	% Recovery	% RSD
10	99.0581	0.7842
12	98.71119	0.6723
14	98.7029	1.389
16	98.74368	1.1565
18	101.99323	0.5748
20	101.4375	0.4429
22	101.3534	0.6223

Table III
Limits of Detection and Quantification

	LOD	LOQ
Impurity A	0.02 μg/mL	0.06 μg/mL
Impurity E	0.045 μg/mL	0.1 μg/mL
Impurity C	0.05 μg/mL	0.1 μg/mL
Impurity B	0.1 μg/mL	0.35 μg/mL
Impurity D	0.1 μg/mL	0.3 μg/mL
Impurity F	0.07 μg/mL	0.18 μg/mL
Impurity G	0.09 μg/mL	0.22 μg/mL
API	0.09 μg/mL	0.3 μg/mL

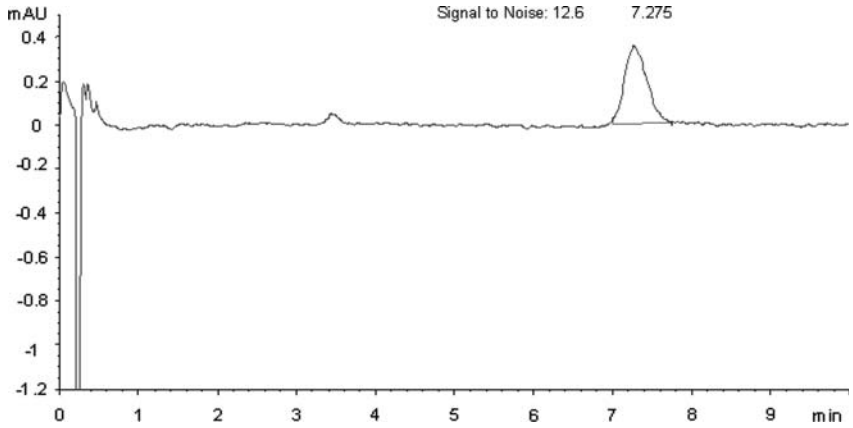

Figure 3. Limit of quantification of API [0.3 μg/mL, chromatographic conditions same as Figure 2B].

Table IV
% RSD data for precision studies

Compound	16 ppm		22 ppm	
	Analysis repeatability % RSD	Inj. Repeatability % RSD	Analysis repeatability % RSD	Inj. Repeatability % RSD
Imp A	0.33312	0.17537	0.176826	0.119866
Imp E	0.469591	0.319418	0.525312	0.298029
Imp C	1.144045	0.598781	0.722355	0.374892
Imp B	0.563425	0.575533	0.770611	0.877159
Imp D	1.280599	0.99129	0.821618	0.93146
Imp F	1.325748	0.881045	1.224151	0.41227
API	0.444873	0.538874	1.354311	0.670715

areas in a normal batch, was injected six times. All peaks were sufficiently separated and no interference was noted (Figure 4).

Sample stability

The stability of the samples stored in clear and amber glassware in ambient temperatures and in clear glassware in the fridge was tested at five intervals: immediately, after 24 h, after 48 h, after 2 weeks, and after 4 weeks.

There was no notable degradation of any peaks for the first four testing intervals. However, after a month, the later peaks in the chromatograms were significantly reduced. The earlier peaks had increased considerably which also would suggest that the later peaks degraded into products that elute closely to these impurities or are in fact the impurities themselves.

Though all samples showed similar degradation, the clear ambient sample was the most degraded. The Miconazole peak was very small with only a peak area of 3.2 compared to 122.4 on day 1. The dark ambient sample showed the least degradation, indicating that light more than temperature is responsible for the degradation of the clear samples. Figure 5 shows the degradation of the clear ambient samples from day 1 to day 28.

Method robustness

Flow rate, detection wavelength and mobile phase composition were varied to determine the robustness of the method experimental conditions.

At the flow rate of 2.25 mL/min, the retention time of the final peak was increased to over 8.5 min as opposed to 7.7 min at the method flow rate of 2.5 mL/min. At the faster flow rate of 2.75 mL/min, the retention time of the final peak was 6.967 min; however, the pressure was much higher. The resolution was satisfactory at all flow rates.

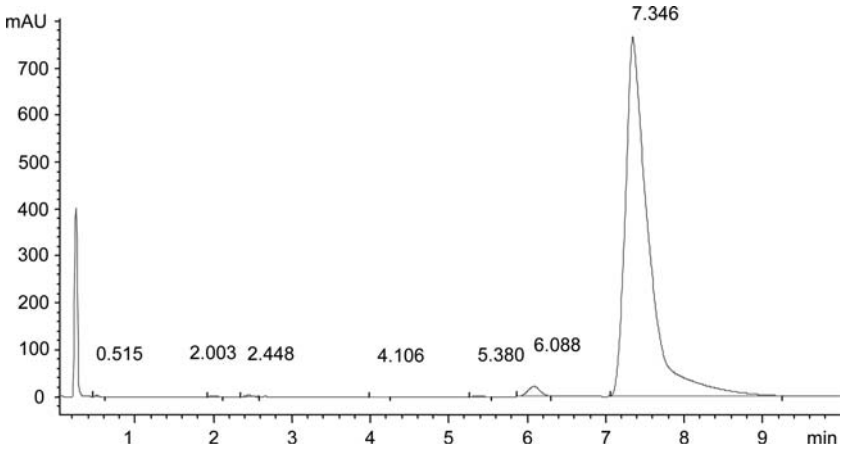

Figure 4. Selectivity Chromatogram [10 mg/mL, chromatographic conditions same as Figure 2B].

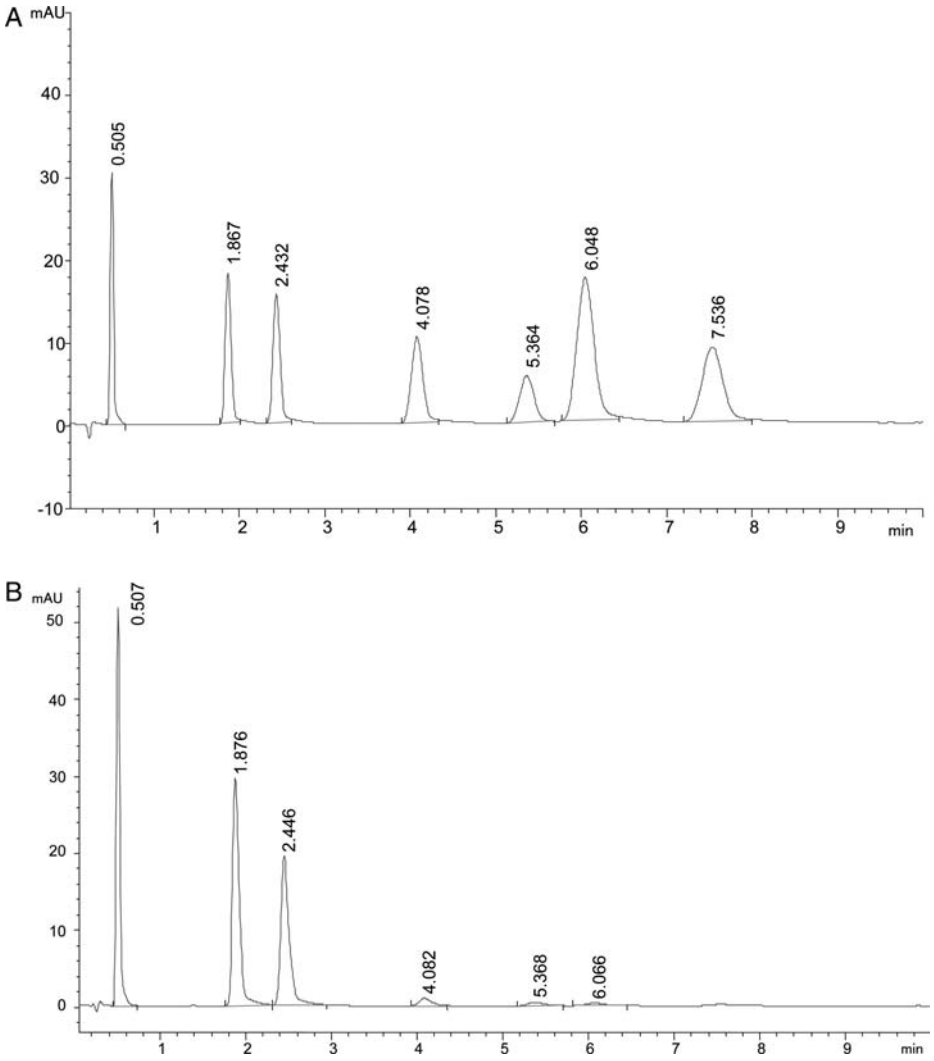

Figure 5. Shows the degradation from day 1 (A) to day 28 (B).

When the mobile phase was varied 36:33:31 (acetonitrile–methanol–ammonium acetate), the resolution between the impurity D and combined impurities F and G, was not sufficient. At 40:31:29 the resolution was satisfactory but the retention time was lengthened to over 10 min.

There was a noticeable difference between the chromatograms when the wavelength was varied by $\pm$ 2 nm. At 237 nm, the peak areas of all peaks were significantly decreased when compared with the method wavelength of 235 nm; whereas at 233 nm, the peak areas were increased considerably. However, the European Pharmacopeia guidelines don't allow a change to the detection wavelength of a method, so 235 nm was used.

Conclusion

A rapid method for the determination of Miconzole and its impurities was developed and optimized, taking into consideration the current European Pharmacopeia approved method. It was found that the analysis times could be reduced by more than half, while maintaining good efficiency. The method was proven to be linear, precise, and capable of satisfactorily separating all impurities in under 8 min. It can be used for the routine analysis of Miconazole in bulk API form. It is also both economically and environmentally friendly due to the decrease in solvent consumption. It was completely validated with acceptable data for all parameters tested.

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References

1. De Zan, M.; Camara, M.; Robles, J.; Kergaravat, S.; Goicoechea, H. Development and validation of a simple stability-indicating high performance liquid chromatographic method for the determination of miconazole nitrate in bulk and cream formulations. *Talanta* **2009** *79*(Issue 3), pp 762–767.

2. http://www.edqm.eu/site/European-Pharmacopoeia-1401.html.
3. Rao, D.; Satanarayana, N.; Malleswara Reddy, A.; Sait, S.; Chakole, D.; Mukkanti, K. *J. Pharmaceut. Biomed. Anal.* **2010** *51*(Issue 3), pp 736–742.
4. Akay, C.; Ozkan, S.; Senturk, Z.; Cevheroglu, S. Simultaneous determination of metronidazole and miconazole in pharmaceutical dosage forms. *Il Farmaco* **2002** *Vol. 57*(Issue 11), pp 953–957.
5. Cavrini, V.; Di Pietra, A.; Gatti, R. Analysis of miconazole and econazole in pharmaceutical formulations by derivative UV spectroscopy and liquid chromatography (HPLC). *J. Pharmac. Biomed. Anal.* **1989** *7*(12), 1535–1543.
6. Cavrini, V.; Di Pietra, A.; Raggi, M. High-pressure liquid chromatographic (HPLC) analysis of imidazole antifungals in commercial dosage forms. *Int. J. Pharmaceut.* **1982** *10*(2), 119–124.
7. Kobylinska, M.; Kobylinska, K.; Sobik, B. *J. Chromatography B* **1996** *685*(1), 191–195.
8. Sternson, L.; Patton, T.; King, T. *J. Chromatography B* **1982** *227*(1), 223–228.
9. Turner, A.; Warnock, D. Determination of miconazole in human saliva using high-performance liquid chromatography. *J. Chromatography B* **1982** *227*(1), 229–232.
10. Wróbel, K.; Wróbel, K.; de la Garza Rodríguez, I.; López-de-Alba, P.; López-Martínez, L. *J. Pharmaceut. Biomed. Anal.* **1999** *20*(1-2), 99–105.
11. Khashaba, P.; El-Shabouri, S.; Emara, K.; Mohamed, A. Analysis of come antifungal drugs by spectrophotometric and spectrofluorometric methods in different pharmaceutical dosage forms. *J. Pharmaceut. Biomed. Anal.* **2000** *22*(2), 363–376.
12. Dongre, V.; Karmuse, P.; Rao, P.; Kumar, A. Development and validation of UPLC method for determination of primaquine phosphate and its impurities. *J. Pharmaceut. Biomed. Anal.* **2008** *46*(2), 236–242.
13. De Villiers, A.; Lestremau, F.; Szucs, R.; Gélébart, S.; David, F.; Sandra, P. Evaluation of ultra performance liquid chromatography Part I. Possibilities and limitations. *J. Chromatography A* **2006** *1127*(1-2), 60–69.
14. Wren, S.; Tchelitcheff, P. Use of ultra-performance liquid chromatography in pharmaceutical development. *J. Chromatography A* **2006** *119*(1-2), 140–146.
15. Guillarme, D.; Nguyen, T.-T.; Rudaz, S.; Veuthey, J.-L. Recent developments in liquid chromatography—Impact on qualitative and quantitative performance. *J. Chromatography A* **2006** *1149*(1), 20–29.