International Journal of Pharma Sciences and Scientific Research

Volume 7 Issue 4,
April 2021

Citation

Cristina Duarte Vianna-Soares et al. (2021), GC method for quantification of DMF and DMSO. Int J Pharm Sci & Scient Res. 7:4, 28-39

ISSN 2471-6782

Published by
Biocore Group|
www.biocoreopen.org/ijpsr/archive.php

Research Article

GC method for quantification of DMF and DMSO

Daniel Marques de Sá e Silva [1, 2], Gabriela Hermont Duarte [2], Cristina Duarte Viana-Soares[1*]

[1]*Universidade Federal de Minas Gerais, Av. Dep. Anuar Menhem, 271 - Belo Horizonte - MG, 31560-200, Brazil*
[2]*Belfar Ltda., Belo Horizonte, MG, Brazil*

Corresponding author: Cristina Duarte Vianna-Soares
Universidade Federal de Minas Gerais, Av. Dep. Anuar Menhem, 271 - Belo Horizonte - MG, 31560-200 Brazil. **Tel No:** +55 31 3409 6959, **E-mail:** cviannas@ufmg.br

Article History: **Received:** March 7, 2021;
Accepted: March 14, 2021;
Published: April 15, 2021.

Abstract

Residual solvents are volatile organic substances, derived from synthesis or manufacturing processes, and can be present in active pharmaceutical ingredients (API). Due to their toxic potential, they must be eliminated, or at least have their content controlled according to official specifications. We aimed to optimize and validate an analytical method by gas chromatography with liquid injection to determine the residual solvents dimethylformamide (DMF) and dimethyl sulfoxide (DMSO) in the API ketoconazole. A divinylbenzene column coupled to a flame ionization detector (FID) was used and helium was chosen as the carrier gas. In selectivity there was a good separation of both peaks besides other impurities present in the sample, compared to the reference chemical substances. The method presented linearity, showing correlation coefficients of 0.99984 and 0.99976 and determination coefficients of 0.99951 and 0.999984 for DMF and DMSO, respectively. The residuals evaluation showed homoscedastic distribution and the slope met the requirements for the F test. In precision, the tested solutions revealed a relative standard deviation (RSD) value of 1.88% for DMF and 1.56% for DMSO, below the limit of 10.0%. In verifying accuracy using the recovery test, the results from 101.4 to 106.9% of the established limit, were satisfactory. Finally, robustness was assessed by means of premeditated variations of critical parameters for the method, such as sim-ultaneous changes in the column lot (+1.34% for DMF and –0.16% for DMSO) or gas flow (–1.85% for DMF and –1.52% for DMSO). Thus, the method developed is satisfactory for the purpose for which it's intended.

Key Words: Gas chromatography, method development, method validation, ketoconazole, residual solvents, dimethylformamide, dimethyl sulfoxide.

Declaration of Conflicting Interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Introduction

Nowadays, it is estimated that there are over 200.000 fungi species known by humankind, but only a few hundred are harmful to human beings. Such infections are especially relevant to immunodeficient patients (Giacomazzi et al., 2015). One of the drugs used to combat such infections is the imidazole antifungal agent ketoconazole, available in the Brazilian market in dosage forms as tablets, capsules, creams, oral suspension, spray, shampoos, alone or in association with other drugs (Goodman & Gilman, 2017, Brasil, 2019, The United, 2020a).

In the manufacture of drugs in which various chemical compounds are utilized it is critical to test for residual solvents, as a step for quality control. In general, residual solvents include organic volatile compounds used or produced in the manufacture of pharmaceutical products. Such substances have no therapeutic value and must be removed, as much as possible from the final product, and should have their content evaluated (The International, 2019).

In order to better classify the residual solvents The International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) rank those substances in three groups, according to the risk that they represent for the human health and the environment. The solvents grouped in Class 1 represent the higher risk for the human health and must be avoided at all costs, for its potential carcinogenic activity. Class 2 solvents are those non-genotoxic carcinogens and can cause irreversible damage to the body system. Finally, Class 3 solvents are those which offer less risk if consumed, for they have lower toxic potential (The International, 2019).

The organic and amphoteric solvent dimethylformamide (DMF) (73.09 g/mol, boiling point 153 ºC) is used in ketoconazole synthesis as a reagent for the formation of one of the reaction intermediates (Zhejiang, 2019). It is a Class 2 solvent, with potential toxicity affecting reproduction, in addition to damage in the eyes, liver and gastrointestinal tract. The ICH guideline establishes a limit at 880 ppm for DMF, which is also adopted by The United States Pharmacopeia – USP (The United, 2017; The International, 2019).

The protic and polar solvent dimethylsulfoxide DMSO (78.13 g/mol, boiling point 189 ºC) is used at the condensation step of ketoconazole synthesis (Zhejiang, 2019). It is a Class 3 residual solvent, thus, less toxic than other organic volatile compounds in Classes 1 and 2. Nevertheless, it is known to cause gastrointestinal disturbs, headache and fatigue. Its limit, as any other of Class 3 residual solvents, is established at 5000 ppm (The United, 2018; The International, 2019).

Besides DMF and DMSO, four other solvents like toluene, methanol (Class 2), ethyl acetate and ethanol (Class 3) are known to be used in the manufacture of ketoconazole. The analyses of these solvents require, however, gas chromatographic technique attached to a headspace sampler (Restek, 2008) hence, they were not determined in this work.

Brazilian and international official guidelines determine the use of gas chromatography as the chosen method for residual solvents analysis. The fundamentals of this technique are based on the difference of affinity of the analyte between the mobile phase – an inert gas – and the stationary phase. The volatility of the residual solvents is what makes this technique an ideal choice, since only gaseous compounds are carried inside the column by the mobile phase (The United, 2020b).

In this study we present the optimization and validation of a gas chromatographic method using a coupled liquid injection and a flame ionization detector (FID) to determine two residual solvents, DMF and DMSO in ketoconazole active drug.

Material and Methods

Ketoconazole was acquired from Zhejiang East-Asia (Pubagang, China). Methanol (J.T. Baker, Phillipsburg, NJ, USA) of a chromatographic purity was used as diluent. Analytical standards DMF (batch R04410, purity 99.99%, density 0.944 g/mL), DMSO (batch R056E0, purity 100%, density 1.100 g/mL) and ethyl acetate (batch R093550, purity 99.9%, density 0.902 g/mL) were all purchased from the USP (Rockville, MD, USA). Other analytical standards as ethanol (batch LRAC2434, purity 91.5%, density 0.789 g/mL) and toluene (batch LRAC2115, purity 99.9%, density 0.867 g/mL) were also acquired from USP.

Preparation of solutions

In the preparation of the sample solution, 400.0 mg of ketoconazole were transferred to a 10 mL volumetric flask, homogenized and completed with methanol. The final concentration was 40.0 mg/mL (40000 µ/mL).

The residual solvents analytical standards stock solution was jointly prepared by transferring 46.6 µL of DMF and 227.0 µL of DMSO standards to a 50 mL volumetric flask. Standard solutions were prepared by dilution of the stock solution to a 25 mL volumetric flask. Final concentrations of the standard solutions were 35.0 µg/mL for DMF and 200.0 µg/mL for DMSO. The volume was completed with the diluent methanol in all flasks. Such amounts of final concentrations match the specifications for each residual solvent (880 ppm for DMF, 5000 ppm for DMSO in 40 mg of ketoconazole) according to

the ICH guidelines, USP and Brazilian Pharmacopeia (The International, 2019; The United, 2019; Brasil, 2019). Other solvents tested also were prepared at the concentration limits (ethanol: 5000 ppm; ethyl acetate: 5000 ppm; toluene: 890 ppm; methanol: 3000 ppm) in methanol as diluent.

GC Parameters setting and method validation

The analysis was performed in an Agilent 8860 gas chromatograph, with internal OpenLab software. The column used was a DB-624 (dimensions 30.0 m × 0.53 mm × 3.0 µm) coated with divinylbenzene (Agilent Technologies, Santa Clara, CA, USA). The samples were analyzed using a liquid injector at temperature 250ºC. The injection volume was 2.0 µL and the split ratio was 5:1. A flame ionizing detector (FID) set at temperature 250ºC and a data acquisition frequency of 20 Hz/0.01 min was used. The carrier gas, helium, was used at a constant flow of 3.3 mL/min. The oven was initially set at 90ºC for 4.0 min, and then heated at a rate of 50ºC/min until 190ºC, for 5.0 min.

The suitability of the chromatographic system was evaluated by the injection of five standard solutions, prepared as described. The acceptance criteria for the suitability test was set as between 0.8 and 2.0 for the analyte's peak asymmetry; RSD ≤10.0% between the replicates; and a resolution between interest peaks of at least 1.5. Official guidelines for method validation (Resolution RDC 166) according to the National Health Surveillance Agency (Anvisa) were followed (Brasil, 2017). The selectivity was evaluated by the comparison of the chromatograms of a ketoconazole sample solution fortified with the identification analytical standards (DMF and DMSO) solutions. Other residual solvents were assessed, but not quantified. They were tested just to check possible interferences in the method. The assessment followed the same procedure stated for DMF and DMSO. The chromatograms were compared with chromatograms of a fortified sample and identification solutions, all prepared at the concentration limit stated as in the literature (The International, 2019).

According to RDC 166 and to ICH guidelines the limit of quantification (LQ) is the smallest amount of compound that can be determined with precision and accuracy, with a minimum signal-to-noise (S/N) ratio 10:1. Therefore, three standard solutions at 100% of the specification limit (concentrations 14.1 µg/mL for DMF and 79.9 µg/mL for DMSO) were evaluated for their S/N ratio and thereby, the concentration with S/N ratio 10:1 was estimated and tested. Finally, the precision and accuracy of the LQ was determined following the same procedure described in RDC 166 (Brasil, 2017, The International, 2019).

Once the LQ has been established, the precision and accuracy of the method were tested. To evaluate precision a standard solution, a sample solution and six independent fortified samples were injected. In the repeatability test the standard deviation of samples prepared in the same day were evaluated, meanwhile in the intermediate precision, sample solutions prepared by different analysts in different days were assessed. The acceptance criteria was set at relative standard deviation (RSD) <10% (Brasil, 2017).

The linearity of the method was assessed in triplicate, using five different concentration levels (14, 21, 28, 35 and 42 µg/mL for DMF and 80, 120, 160, 200 and 240 µg/mL for DMSO). With these results, three separate plots of concentration versus peak area were obtained, each one originated from a different stock solution. The data were analyzed in order to check if the results comply with the current legislation (Pearson correlation coefficient, r >0.990; determination coefficient r^2 ≥0.98 and angular coefficient significantly different from zero) (Brasil, 2017).

Accuracy was demonstrated by the recovery of the analyte from the fortified samples, when compared with the sample with no addition of the residual solvents. For this purpose, three concentration levels were evaluated: at the LQ, 100% and 120% of the linearity range. Each concentration was analyzed in triplicate, and the recovery acceptance range was set between 90.0 and 110.0% (Brasil, 2017).

Finally, the robustness of the method was evaluated, following Youden's method. For this, eight experiments were prepared, altering one or more parameters of the method conditions. The precision of the replicates, the chromatogram profile, and the amount of analyte were evaluated to determine whether or not the conditions were critical for the results. Table 1 presents the parameters at nominal and altered conditions, and how those parameters were attributed in the experiment (Youden, 1975).

Parameter	(Max/min value)	Nominal conditions	Altered conditions	Experiment							
				1	2	3	4	5	6	7	8
Carrrier gas flow	mL/min (A/a)	A - 3.3	a – 4.0	A	A	A	A	a	a	a	A
Column temperature	°C (B/b)	B - 90	b – 95	B	B	b	b	B	B	b	B
Detector temperature	°C (C/c)	C - 280	c – 285	C	c	C	c	C	c	C	C
Column	(D/d)	D - batch 1	d - batch 2	D	D	d	d	d	d	D	D

a: nominal conditions

Table 1 Parameters verified for robustness test of the nominala and altered conditions in order to configure optimal conditions through Youden´s method, by GC-FID.

Results

The peaks in the chromatograms 1, 2, and 3 in Figure 1 represent the compounds in the standard solutions (DMF and DMSO), the diluent (methanol) and ketoconazole sample, respectively The chromatogram of diluent methanol shows impurities 1 and 2, and ketoconazole sample showed an unknown impurity, all with different retention times when compared to the analytes (DMF, 6.16 min; DMSO, 6.84 min). The retention times of the compounds of interest in the fortified sample solution matched the identification solutions. Therefore, the method is considered to be selective for DMF and DMSO. Table 2 presents the chromatographic parameters (retention time, asymmetry, resolution and peak area) obtained for the ketoconazole sample fortified with DMF and DMSO, used in the selectivity test.

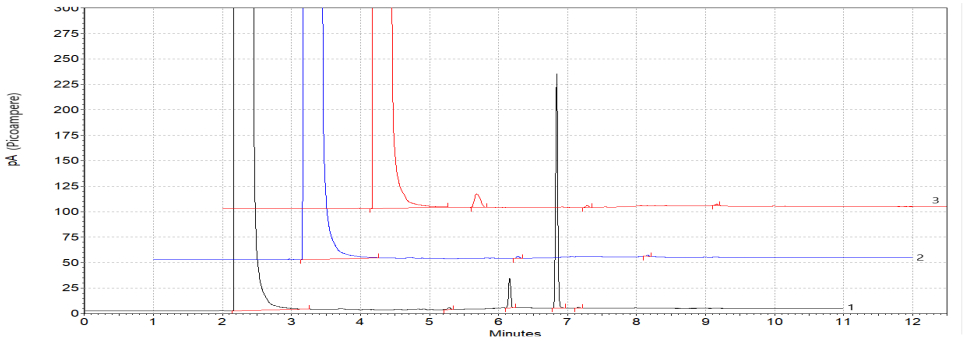

Fig1 Overlaid selectivity chromatograms indicating the separation between the peaks of interest, DMF (tr= 6.16 min) and DMSO (tr= 6.84 min) in the solutions, by GC-FID. 1) DMF and DMSO identification standard solutions; 2) diluent methanol; 3) sample solution. The retention times for all peaks are shown in Table 2.

Substance	Retention time (min)	Retention factor	Asymmetry	Resolution	Area
DMF	6.16	2.08	0.97	12.54	504,364
DMSO	6.84	2.42	1.04	12.00	3,799,163
Methanol	2.18	0.09	21.24	nda	8,496,590,158
Unknown impurity	3.68	0.84	1.30	7.36	600,958
Diluent impurity 1	5.28	1.64	0.98	13.22	48,071
Diluent impurity 2	7.16	2.58	0.83	5.53	22,880
Ethanol	2.792	0.40	nda	1.3	6,822,900
Ethyl acetate	4.036	1.02	1.03	8.2	2,523,166
Toluene	5.723	1.86	1.04	3.4	1,104,993

a: not determined.

Table 2 Chromatographic parameters obtained for the ketoconazole sample fortified with DMF and DMSO and for identification solutions used in the method validation, by GC-FID.

As shown in Table 2, all the relevant compounds match the specifications for asymmetry (between 0.8 and 2.0) and resolution (>1.5). For the measurement of these parameters, the calculation recommended in the USP was used (The United, 2020b).

Although not quantified by the present method, the manufacturer of ketoconazole based in China informs that the residual solvents ethanol, ethyl acetate and toluene have been used in the ketoconazole synthesis (Zhejiang, 2019). As previously mentioned, these residual solvents require a quantitation method by gas chromatography including an attached headspace sampler. Thus, Figure 2 illustrates the elution in chromatogram 1 for ethanol, ethyl acetate and toluene (from left to right) and in chromatogram 2, for methanol. As shown at table 2, their retention times do not coincide with those from DMF and DMSO.

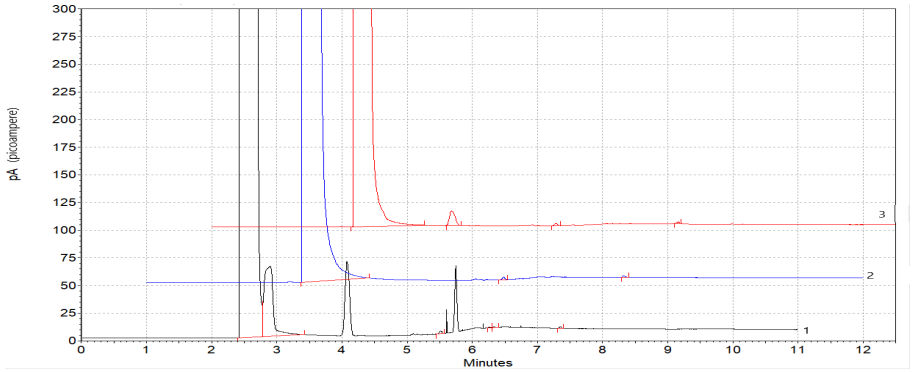

Fig2 - Overlaid selectivity chromatograms indicating the retention times of residual solvents by GC-FID. 1) identification standard solution (containing ethanol, ethyl acetate and toluene, left to right, respectively); 2) methanol (highest peak in all); 3) ketoconazole sample solution (impurity on right). The retention times of all peaks are shown in Table 2.

According to RDC (Brasil, 2017), the S/N ratio for the LQ of each solvent should be of at least 10:1. The S/N ratios found for DMF and DMSO were 12.68:1 and 85.57:1, respectively. Thus, calculated LQ was found to be 14.1 µg/mL for DMF and 79.9 µg/mL for DMSO, respectively, according to the Equation 1 (Brasil, 2017).

$$LQ = \frac{Concentration\ at\ 100\% \times S\text{–}R}{S\text{–}R_{100}}$$

Equation 1 - Calculation of the LQ. S-R_{LQ}: Signal-to-noise ratio at LQ; S-R_{100}: Signal-to-noise ratio at the concentration of 100%.

The accuracy and precision at the LQ were also evaluated. The results for both analytes yielded RSD <10% (1.18 for DMF and 1.72 for DMSO) and a recovery percentage (104.0 to 106.9% for DMF; 101.4 to 106.3% for DMSO) within the acceptance range (100 ± 10%) was found for all the replicates.

The calibration curves data were evaluated to assess linearity parameters for DMF and DMSO. The calcu-lated curve equation for DMF was Y = 13,779,019.0 X + 9,495.73, while the equation for DMSO was Y = 17,830,347.5 X + 10,924.93. Both linearity curves were tested for Cochram test and the results (0.603 for DMF and 0.619 for DMSO) were lower than the critical value of 0.684. The residual plots evaluated for verification of homoscedasticity of data from the DMF and DMSO calibration curves were considered in the positive and negative confidence limits (α= 0.10) showing homocedasticity for DMF and DMSO determination.

The analytical curve equation was determined by the ordinary least squares method. The angular coefficient was determined by analysis of variance (ANOVA) test, and all values found were satisfactory. Fisher (F) value for DMF was 6.68 and 4.80 for DMSO. Both values were higher than the theoretical limit of 4.67. Pearson (r) correlation coefficients were 0.99984 for DMF and 0.99976 for DMSO, and the coefficient of determination, r2 values were 0.99951 and 0.99984, respectively.

The repeatability results for both DMF and DMSO were inside the established limits (<10%). For DMF the RSD was found to be 1.88%, while for DMSO, 1.56%. Intermediate precision was evaluated for solutions prepared by two different analysts in subsequent days. The RSD between the two different preparations was of 1.57% for DMF and 1.29% for DMSO.

The accuracy of the method was determined by the standard addition method to calculate the percentage recovery, by means of sample solutions fortified with standards solutions compared to non-

fortified sample solutions. The results of the accuracy test in the concentration levels at the limit of quantitation (LQ), 100% and 120% of the established limits were satisfactory and the RSD values were equal to or smaller than 1.23% for DMF and 2.64% for DMSO, as well.

Solution		Peak area (pA × min)	Found	Theoretical	%Recovery	%RSD
			Conc.(µg/mL)	Conc. (µg/mL]		
			DMF			
Control	0		0	-	-	-
	1	201,604	15.0	14.1	106.9	
LQ[a]	2	200,491	15.0	14.1	106.3	0.97
	3	197,825	14.8	14.1	104.9	
	1	493,944	36.8	35.2	104.8	
	2	492,199	36.7	35.2	104.4	1.23
100%	3	503,524	37.6	35.2	106.8	
	1	597,738	44.6	42.2	105.7	
	2	601,575	44.9	42.2	106.4	0.44
120%	3	602,857	45.0	42.2	106.6	
DMSO						
Control		0	0	-	-	-
LQ[a]	1	1,495,158	84.9	79.9	106.3	
	2	1,492,105	84.8	79.9	106.1	2.64
100%	3	1,426,477	81.0	79.9	101.4	
	1	3,632,445	206.4	199.8	103.3	
	2	3,675,704	208.8	199.8	104.5	0.69
	3	3,676,569	208.9	199.8	104.6	
120%	1	4,398,744	249.9	239.7	104.3	
	2	4,448,871	252.8	239.7	105.4	0.58
	3	4,414,264	250.8	239.7	104.6	

a: LQ = 40% of established limits (880 ppm for DMF; 5000 ppm for DMSO); b: CV, coefficient of variation.

Table 3 Results obtained for evaluation of the accuracy of the method for DMF and DMSO, at the levels of concentration zero (control), limit of quantitation (LQ), 100% and 120% of established limits, by GC-FID.

In the assessment of robustness, as shown in Table 4, the parameters selected for deliberated variation were the carrier gas flow, the column initial temperature, the detector temperature and the batch of the column following Youden´s method (Youden, 1975). In order to calculate the magnitude of the change in the results, the average of the peak areas was used. The results are expressed in variation relative to the nominal conditions and were within the acceptance criteria of ±1.0, ±0.2, ±2.5, ±5.0 for retention time, asymmetry, resolution and %area, respectively, for DMF and DMSO.

Variation					
Condition	**t_R (min)**	**Asymmetry**	**Plates number**	**Resolution**	**%Area**
DMF					
Carrier gas flow	-0.38	0.00	-4.077	0.27	-1.85
Column temperature	-0.21	0.02	-24,749	-0.63	0.00
Detector temperature	0.00	0.01	-3,223	-0.28	0.54
Column batch	-0.02	0.15	21,670	2.24	1.34
DMSO					
Carrier gas flow	-0.43	0.02	2,481	0.30	-1.52
Column temperature	-0.19	-0.02	418	-0.09	-0.59
Detector temperature	0.00	-0.01	-33,047	-0.10	0.47
Column batch	-0.01	0.13	-14,518	0.65	-0.16
Column temperature	-0.19	-0.02	418	-0.09	-0.59
Detector temperature	0.00	-0.01	-33,047	-0.10	0.47
Column batch	-0.01	0.13	-14,518	0.65	-0.16

a: Acceptance criteria: ±1.0, ±0.2, ±2.5, ±5.0 for retention time, asymmetry, resolution and %area, respectively.

Table 4 Variation (in retention time tR, asymmetry, plates number, resolution and %area) obtained for robust-ness assay according to Youden's method in nominal conditions, for determination of DMF and DMSO, by GD-FID[a].

Lastly, the bench stability of the sample was tested by the injection (n=3) of samples at zero, 24 and 48h after preparation. The stability results obtained at controlled room temperature showed no significant changes between the content of the residual solvents at time zero (101.2% for DMF and 100.3% for DMSO) and that one at 48 h later (103.0% for DMF and 102.2% for DMSO). A supplementary material is available for consultation of data obtained.

Discussion

The starting point for this method optimization was the methodology stated by the ketoconazole manufacturer in order to analyze the residual solvent contents in the final ingredient (Zhejiang, 2019). Firstly, the optimization was focused on reducing the time of analysis. This was achieved by increasing the oven heating rate in order to reach a higher final temperature and a shorter chromatographic run, assuring that all compounds elut-ed off the column. Altogether, the analysis time was reduced from 16 to 11 min.

Secondly, the method was adapted in order to yield larger peak areas for the analytes. This was a necessary modification with the goal of reaching a higher S/N ratio, which would allow the establishment of lower LQ values, since the minimum ratio recommended for the calculation of LQ is 10:1. The proposed modification for solving this issue was to double the active ingredient amount in the sample.

By doubling the mass of ketoconazole, the amount of DMF and DMSO standards to be used in the fortified sample solutions would also increase twice as much, in order to maintain the residual solvents limits in ppm equal to the limits established by legislation. For instance, as the concentration of the ketoconazole solution changed from 20.0 mg/mL to 40.0 mg/mL, the concentration of DMF also rose from 17.5 µg/mL to 35.0 µg/mL, to keep the ratio 880 ppm of DMF in ketoconazole. In the same way, the DMSO concentration changed from 0.100 µg/mL to 0.200 µg/mL (equivalent to 5000 ppm for DMSO in ketoconazole). The amount of residual solvents used to prepare the standard solution was increased as well, in order to maintain the concentrations of the sample and standard solutions alike. With this approach, it was possible to raise the S/N ratio to a higher value.

The chromatograms shown in Figures 1 and 2 confirm the method selectivity. Since DMF and DMSO are substances with high boiling point values (153ºC and 189ºC, respectively) they were diluted in methanol (tallest peak), which is a low boiling point (64.7ºC) solvent (Pubchem, 2004).Figure 1 shows the main peaks of the solutions containing only DMF or DMSO (2.1 Preparation of solutions section) correspondent in area and retention time with the peaks found in the fortified sample. In order to assure no overlapping between other peaks, the diluent and ketoconazole sample chromatograms were also evaluated, as shown in Figure 2.

In the linearity of the calibration curves, the Cochram (C) test values (0.603 for DMF and 0.619 for DMSO) prove the homocedasticity of data, since they were below the critical value (0.684). This parameter is used to show if the variance between the results is constant. If so, all points will have the same relevance in the calculation of the curve equation. Therefore, the establishment of data homocedasticity allows the use of the ordinary least squares method for the calculation of the calibration curve equation (Brasil, 2017, The International, 2019). Pearson's correlation coefficient values were higher than 0.999 for both residual solvents evaluated, showing that linearity of the method was adequate. ANOVA Fisher test results prove that the linear coefficient of the given model was significantly different from zero and it is adequate to describe the present data. The determination coefficient (r^2) values were also greater than the recommended value, 0.980, and are an indicative of how adequate is the proposed model expressed by the curve equation. All values for the linearity test were in accordance to Anvisa and ICH guidelines (The International, 2019; Brasil, 2017).

The precision results met the requirements for both repeatability (intra-assay) and intermediate precision (inter assay) precision tests. RSD found were lower than the established limit (RSD <10%), hence in accordance to the limitations of the method and the current legislation (Brasil, 2017).

The accuracy results also prove the suitability of the method for its purpose, given that all results showed a recovery range from 101.4% to 106.9%.

The established LQ satisfactorily showed accurate and precise results. Altogether, the LQ accuracy and linearity results are good enough to set the working concentration range between 40% and 120% of the specification for each residual solvent.

Finally, the robustness results showed that the most critical parameters are the gas flow rate and the new columns batch, for which modifications caused variations of –1.85% and +1.34% for DMF peak area, respectively. For DMSO, such changes caused a peak area variation of –0.16% and –1.52% in the gas flow rate and columns batch, respectively. Nevertheless, if only such results were to be assessed, it is possible to say that the robustness of the method is adequate, once all change variations were smaller than 5.0%. Nevertheless, the chromatographic profile must also be evaluated. Figure 3 shows that when both flow rate and column batches are changed, a coelution happens between the peaks of DMF and a diluent's impurity. Therefore, those parameters should be observed with special attention when analyzing residual solvents in ketoconazole.

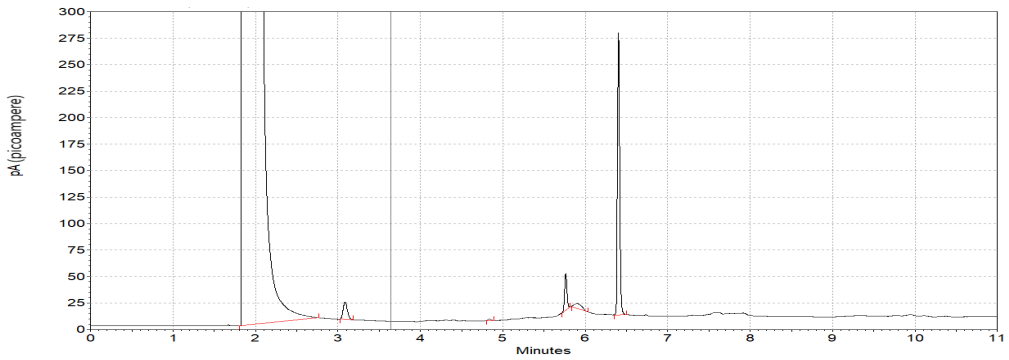

Fig3 - Representative chromatogram obtained in the robustness assay, showing a coelution of peaks of DMF and that of a diluent impurity in API ketoconazole methanolic solution, around 5.8 min, by GC-FID

The robustness test was also accomplished by evaluation of the bench stability of solutions. The stability re-sults obtained at room controlled temperature show that the solutions are stable for at least 48 h, since no great variations are reflected on %RSD values (0.94 to 1.02), nor in the chromatogram profile and the peak areas for both DMF and DMSO.

In summary, the changes made in this method led to a shorter time analysis than in the manufacturer's method, in a wide working concentration range, thus, a more suitable method for quality control routine was developed. The validation parameters assessed show that the method is selective, precise, accurate and linear, being adequate to the use of the quality control of ketoconazole.

Conclusions

The developed method led to a faster and equally effective procedure in regard to the original method. In addition, it met the requirements for the specifications adopted by the quality control of pharmaceuticals of this company, as well as, those by regulatory agencies. Its efficacy was proven by the results obtained in the method validation, which showed that the current method is capable of identifying and quantifying the residual solvents DMF and DMSO in the API ketoconazole.

Appendices

Figures and Tables

DMF Replicate	Peak area (pA × min)	S/N	Conc. (µg/mL)	Accuracy LQ	Precision LQ	
				%Recovery	RSD	Status
Diluent	0	-	-	-	-	-
1	201,604	12.14	15.0	106.9		
2	200,491	12.19	15.0	106.3		
3	197,825	12.38	14.8	104.9	1.18	Pass
4	200,273	12.79	14.9	106.2		
5	196,055	12.89	14.6	104.0		
6	196,343	13.68	14.6	104.1		
DMSO Replicate	**Area (pA × min)**	**S/N**	**Conc. (µg/mL)**	**Accuracy LQ**	**Precision LQ**	
				%Recovery	**RSD**	**Status**
Diluent	0	-	-	-	-	-
1	1,495,158	74.68	84.9	106.3		
2	1,492,105	88.86	84.8	106.1		
3	1,426,477	73.18	81.0	101.4	1.72	Pass
4	1,457,716	88.42	82.8	103.7		
5	1,463,975	88.09	83.2	104,1		
6	1,470,049	99.18	83.5	104.5		

Table 1 Accuracy and precision results and status obtained for DMF and DMSO according to the signal to noise ratio (S/N) in concentrations at their limit of quantitation (0.0799 mg/mL and 0.0141 mg/mL, respectively), by GC-FID.

DMF			DMSO		
Concentration (µg/mL)	**Peak area (pA × min)**	**Average (pA)**	**Concentration (µg/mL)**	**Peak area (pA × min)**	**Average (pA)**
	198,887			1,430,405	
14.00	203,711	200,262	80.00	1,435,973	1,429,967
	198,189			1,423,523	
	300,316			2,170,734	
21.00	303,298	300,564	120.00	2,154,044	2,153,269
	298,077			2,135,029	
	393,564			2,872,641	
28.00	396,147	394,602	160.00	2,856,236	2,858,277
	394,095			2,845,954	
	489,673			3,572,466	
35.00	498,426	496,607	200.00	3,621,717	3,609,437
	501,722			3,634,129	
	582,475			4,256,249	
42.00	587.671	584,506	240.00	4.323.808	4,267,952
	583.373			4.223.800	

Table 2 Results of the calibration curves obtained for determination of DMF and DMSO in methanol solution in the concentration range 80-240 g/mL, by GC-FID.

DMF			DMSO		
Concentration (µg/mL)	**Peak area (pA × min)**	**%Conc.**	**Conc. (µg/mL)**	**Peak area (pA × min))**	**%Conc.**
35.0	485,604	98.0	200.0	3,576,623	97.1
	501,119	101.1		3,694,698	100.3
	499,973	100.9		3,694,833	100.3
	507,775	102.5		3,703,865	100.5
	513,688	103.6		3,749,388	101.8
	500,757	101.0		3,681,718	99.9
RSD	1.88			1.56	
Analyst 1	**Analyst 2**	**%RSD**	**Analyst 1**	**Analyst 2**	**%RSD**
98.0	101.1	1.57	97.1	101.4	1.29
101.1	102.5		100.3	100.2	
100.9	103.9		100.3	99.0	
102.5	100.0		100.5	99.7	
103.6	101.6		101.8	101.2	
101.0	101.4		99.9	101.6	

Table 3 - Results obtained for the evaluation of repeatability (intra-assay) and intermediate precision (inter-assay) by two analysts for determination of DMF and DMSO in ketoconazole, by GC-FID.

Peak area (pA × min) / Time (h)	DMF				DMSO			
	0	24	48	%RSD	0	24	48	%RSD
Sample	482,898	546,381	513,616	-	3,516,936	3,491,426	3,744,658	-
Standard	479,522	540,357	501,007	-	3,500,628	3,466,599	3,658,082	-
%Content	101.2	101.6	103.0	0.94	100.3	100.6	102.2	1.02

Table 4 Robustness results for peak area obtained for the assessment of bench stability for determination of residual solvents DMF and DMSO in ketoconazole in different time intervals, by GC-FID.

Acknowledgment

The authors are grateful to Fernando Cesar da Costa and Lilian Silva Felix for their help in the review of the final manuscript and in the analysis, respectively. This research did not receive any specific grant from funding agencies in the public, commercial, or non-profit sectors.

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