ECRONICON OPEN ACCESS

EC MICROBIOLOGY
Research Article

Rifaximin in Tablets: Is it Possible to Evaluate the Quality by UV, IR, CE, HPLC and Turbidimetric Methods?

Ana Carolina Kogawa and Hérida Regina Nunes Salgado*

São Paulo State University (UNESP), School of Pharmaceutical Sciences, Campus Araraquara, São Paulo, Brazil

***Corresponding Author**: Hérida Regina Nunes Salgado, São Paulo State University (UNESP), School of Pharmaceutical Sciences, Campus Araraquara, São Paulo, Brazil.

Received: November 23, 2017; **Published:** January 03, 2018

Abstract

An adequate analytical method can be the first step in the rational use of pharmaceuticals. Currently, however, the effectiveness of the method of analysis is not enough. It should also be environmentally friendly, dynamic and low cost. A product can present more than one analytical method for its evaluation, and then, before adopting any of them, it is necessary to know if they are equivalent. Rifaximin, an antimicrobial, presents analytical methods by spectrophotometry in the ultraviolet region (UV), spectrophotometry in the infrared region (IR), capillary electrophoresis (CE), high performance liquid chromatography (HPLC) and turbidimetric bioassay for evaluation of its tablets. This work shows the comparison of these methods in the evaluation of the final product quality of rifaximin, in addition to comparing the equivalence of physical (UV, IR, CE, HPLC) and turbidimetric microbiological methods. The analysis of rifaximin tablets by physico-chemical and microbiological methods were statistically equivalent and can be interchangeable.

Keywords: *Rifaximin; Tablets; UV; IR; CE; HPLC; Turbidimetry Bioassay*

Introduction

Analytical methods are tools which aim to evaluate the quality of a product.

A practical and accurate method of analysis can be the first step in the rational use of medicines, since the quality of a pharmaceutical product is directly related to the health of patients [1,2].

Thus, effective analytical methods are extremely important and aim to provide reliable information on the true status of the pharmaceutical product available for the population.

However, currently, in addition to the requirement of the analytical methods are adequate, they should also be environmentally friendly. The use of methods considered green makes unnecessary the remediation of environmental impacts frequently observed today. Currently, the adoption of ecologically correct and effective methods is a growing and required reality in the chemical-pharmaceutical world [3-5].

Other items to be considered in the choice or development of an analytical method, aiming the current analytical needs, are detection and separation of all compounds of interest, rapid analysis to optimize equipment and analysts, reduction of the number of steps, reduction of sample pretreatment, low cost of analysis and prices of reagents, procedures, accessories and machines [6,7].

In the presence of more than one analytical method for the evaluation of a pharmaceutical product or if the existing analytical method is not accessible, it is necessary to evaluate if they are equivalent before adopting one.

Comparison of methodologies is necessary to determine if the variability of the methods differs significantly. USP 39 [8] recommends evaluating the parameters of precision and accuracy to prove the equivalence of the methodologies.

Rifaximin is an antimicrobial used for the treatment of hepatic encephalopathy, travelers' diarrhea, irritable bowel syndrome, infections by *Clostridium difficile*, ulcerative colitis and acute diarrhea, marketed in the form of tablets [9-17]. Analytical methods by spectrophotometry in the ultraviolet region (UV) [18,19], spectrophotometry in the infrared region (IR) [20], capillary electrophoresis (CE) [21], high performance liquid chromatography (HPLC) [22] and turbidimetry [23,24] are available for evaluation of its tablets.

This work shows the comparison of these methods in the evaluation of the quality of the final product of rifaximin, besides comparing the equivalence of the physical methods and the microbiological method. It also raises a discussion about the association of analytical methods and advantages of one with respect to another.

Experimental Part

Material

The standard used was rifaximin, 99.0%, obtained from NutraTech Development Limited (China).

The pharmaceutical form used was rifaximin 200 mg tablets (labeled content), from Gonher Farmaceutica Ltda. The average weight of 20 tablets was used for the sample preparations.

Method

The methods used in the quantification of rifaximin tablets for evaluation and subsequent comparison of content between them were UV [19], IR [20], CE [21], HPLC [22,23] and turbidimetry [23,24].

All methods were previously validated according to International Conference on Harmonisation (ICH) [25].

UV

Rifaximin solutions at concentration of 22 µg mL^{-1} were prepared in purified water (Millipore™) with 20% ethyl alcohol (Synth™) and subsequently filtered. Spectrophotometer UV (Shimadzu™) and quartz cuvettes with 1 cm optical path at 290 nm were used for the readings at absorbance [19].

IR

Rifaximin pellets with 2 mg of drug were prepared in potassium bromide (Synth™), previously dried for 24 hours in an oven at 105°C, and the readings were carried out on spectrophotometer IR (Shimadzu™) using the carbonyl band at 1767 - 1701 cm^{-1} [20].

CE

Rifaximin solutions at concentration of 300 µg mL^{-1} were prepared in purified water (Millipore™) with 20% ethyl alcohol (J.T. Baker™) and subsequently filtered. Capillary Electrophoresis System (Beckman Coulter™), uncoated fused silica capillary, borate buffer 25 mM pH 9.5, +20 kV at 290 nm were used for the readings [21].

HPLC

Rifaximin solutions at concentration of 30 µg mL^{-1} were prepared in the mobile phase (water (Millipore™) + 0.1% glacial acetic acid (J.T. Baker™) and ethyl alcohol (PanReac AppliChem™), 52:48 v/v). Chromatographic System (Waters™), C18 column (Eclipse Plus™), flow rate 0.9 mL min^{-1}, injection volume 20 µL, 25°C at 290 nm [22,23].

Turbidimetry

Rifaximin solutions at concentration of 50, 70 and 98 µg mL^{-1} were prepared in purified water (Millipore™) with 20% ethyl alcohol (Synth™). Spectrophotometer (Quimis™) at 530 nm, shaker (Marconi™), *Escherichia coli* ATCC 10536 at 8% and broth Brain Heart Infusion (BHI) (Acumedia™) were used. Positive and negative controls were done at each analysis [23,24].

The content of rifaximin in the tablets was calculated by comparing the results from standard and sample, both prepared under the same conditions and simultaneously aiming to avoid differences and errors not inherent to the product.

Results and Discussion

Table 1 presents the different parameters determined for the quantitative methods of UV, IR, CE, HPLC and turbidimetry.

Parameter	Methods				
	UV	IR	HPLC	CE	T
Reading range	290 nm	1767 - 1701 cm^{-1}	290 nm	290 nm	530 nm
Concentration range	10 - 30 µg mL^{-1}	1.0 - 3.5 mg	5 - 50 µg mL^{-1}	50 - 500 µg mL^{-1}	50 - 98 µg mL^{-1}
n	6	5	6	6	3
Equation: y = ax + b					
a	0.0270	0.2243	38.006	144	-0.8143
b	0.0131	0.0120	38.501	-1861	4.0717
r	0.9999	0.9997	0.9997	0.9993	0.9976
Intraday precision (RSD %)	0.57	2.51	1.77	1.30	7.93
Interday precision (RSD %)	1.08	4.00	1.19	1.56	7.05
Accuracy (%)	100.17	100.13	100.41	100.24	100.70

Table 1: *Applicability, precision and accuracy of validated methods of spectrophotometry in the ultraviolet region, spectrophotometry in the infrared region, capillary electrophoresis, high performance liquid chromatography and turbidimetry.*

The methods can be considered satisfactory, since RSD values for precision are less than 5% for physico-chemical methods and less than 15% for the microbiological method and the accuracy is close to 100% [26,27].

Another way to prove the equivalence of the methods is to compare the dosing data, shown in table 2, using ANOVA, Fisher's Test-F and Student's Test-t for parametric data.

Parameter	Methods				
	UV	IR	HPLC	CE	T
Content (%)	106.95	110.49	107.94	106.12	107.79
	107.16	110.19	106.03	104.10	107.14
	113.71	101.45	113.27	112.02	105.42
Average content (%)	109.28	107.38	109.08	107.42	106.78
RSD (%)	3.52	4.79	3.44	3.83	1.15

Table 2: *Values obtained in the determination of rifaximin content in tablets using spectrophotometry in the ultraviolet region, spectrophotometry in the infrared region, capillary electrophoresis, high performance liquid chromatography and turbidimetry.*

The results of the statistical treatment are presented in tables 3 and 4.

Variation sources	SS	DF	QM	$F_{calculated}$	P_{value}	$F_{critical}$
Between groups	9.58	3	3.19	0.18	0.91	**4.07**
Within groups	144.31	8	18.04			
Total	153.89	11				

Table 3: *Analysis of variance of the contents obtained in physicochemical methods.*
significant for p < 5%
SS: Sum of Squares; DF: Degrees of Freedom; QM: Quadratic Mean

Test	P_{value}	Calculated	Critical
F	0.10	9.33	19.40*
t	0.51	0.67	2.16*

Table 4: *Test-F and Test-t for the contents obtained using physico-chemical and microbiological methods.*
**significant for p < 5%*

The results of rifaximin dosing in tablets obtained by physico-chemical methods of UV, IR, CE and HPLC were analyzed for variance. They were statistically the same ($F_{cal} < F_{crit}$) and they can be interchangeable for the physico-chemical quantitative determination of rifaximin tablets.

In the comparison between averages for quantitative variables containing two samples, Fisher's variance Test for independent samples showed equal variances for rifaximin dosing data by physico-chemical (UV, IR, CE, HPLC) and microbiological techniques (Turbidimetry), in which $F_{cal} < F_{crit}$. Thus, the Test-t used was homoscedastic.

The results obtained through the Student's Test-t showed no significant difference between the proposed physico-chemical and microbiological methods, at the significance level of 5%. Thus, the validated methods are equivalent and therefore interchangeable for the physico-chemical and microbiological quantitative determination of rifaximin in tablets.

Although the methods show interchangeability, each one has its own characteristics. It is worth highlighting that the main difference between these methods is the advantages and limitations of one in relation to the other.

Price of equipment, price and quantity of reagents, price of accessories, cost of maintenance and training, time of analysis, number of stages and processes, sample pretreatment, type of reagent, waste treatment are items to be evaluated in the choice of method to be adopted in the evaluation of pharmaceutical products [4,7,28].

This is the current view of chemical-pharmaceutical analyzes, in which the analysis is seen as a whole. It contemplates the time concern that invigorates processes, financial resources, environmental contamination and the health of the operator.

In the case of antimicrobials, the association of physico-chemical and microbiological methods is extremely important. Not always the part of the molecule detected in the physico-chemical method is the same responsible for the activity. So, the association of these two types of methods is mandatory [29-36].

The analysis of rifaximin tablets by physico-chemical (UV, IR, CE, HPLC) and Turbidimetric microbiological methods were statistically equivalent, and any methods can be used for its evaluation. However, it should be remembered that in the case of antimicrobials, such as rifaximin, the simultaneous analysis of a physico-chemical method and a microbiological method is fundamental for the release of reliable results.

Conclusion

The evaluation of the content of rifaximin tablets can be performed by physicochemical methods, UV, IR, CE and HPLC, as well as by turbidimetric microbiological method. However, their association is crucial.

Acknowledgments

The authors acknowledge CNPq (Brasília, Brazil), FAPESP (São Paulo, Brazil) and CAPES (São Paulo, Brazil).

Declaration of Interest

The authors report no declarations of interest.

Bibliography

1. Nakashima K., *et al*. "Analytical studies on the development of high-performance liquid chromatographic methods with fluorescence or chemiluminescence detections and their practical applications". *Analytical Sciences* 25 (2009): 21-31.

2. Menezes MN., *et al*. "Should we be Concerned about Non Sterile Pharmaceutical Products?" *EC Microbiology* 10.6 (2017) 221-226.

3. Kogawa AC., *et al*. "Development and validation of infrared spectroscopy method for the determination of darunavir in tablets". *Physical Chemistry* 3.1 (2013): 1-6.

4. Kogawa AC., *et al*. "Validation of a stability indicating capillary electrophoresis method for the determination of darunavir in tablets and comparison with the of infrared absorption spectroscopic method". *World Journal Pharmacy and Pharmaceutical Sciences* 3.6 (2014): 283-297.

5. Marco BA., *et al*. "Development and validation of an innovative method for the determination of cefadroxil monohydrate in capsules". *Physical Chemistry* 6.3 (2016): 67-74.

6. Watzig H. "Validation of analytical methods using capillary electrophoresis". *Separation Science and Technology* 9 (2008): 225-244.

7. Kogawa AC., *et al*. "Comparative study over methods developed for quantification of darunavir in tablets by environmental friendly infrared and capillary electrophoretic techniques". *Journal of International Research in Medical and Pharmaceutical Sciences* 2.3 (2015): 99-105.

8. USP, The United States Pharmacopeia. 39th Edition, Rockville, United States Convention (2016).

9. Castro R., *et al*. "Rifaximin treatment for acute recurrent diarrhea in children with genitourinary disorders". *Current Therapeutic Research* 59.10 (1998): 746-752.

10. Ruiz J. "*In vitro* antimicrobial activity of rifaximin against enteropathogens causing traveler's diarrhea". *Diagnostic Microbiology and Infectious Disease* 59.4 (2007): 473-475.

11. Zhang X., *et al*. "Sensitive quantification of rifaximin in human plasma by liquid chromatography–tandem mass spectrometry". *Journal of Chromatography B* 850 (2007): 348-355.

12. Jiang Z., *et al*. "Rifaximin - induced alteration of virulence of diarrhoea producing Escherichia coli and Shigella sonnei". *International Journal of Antimicrobial Agents* 35.3 (2010): 278-281.

13. Mantry PS., *et al*. "Rifaximin for the treatment of hepatic encephalopathy". *Transplantation Proceedings* 42.10 (2010): 4543-4547.

14. Rivkin A., *et al*. "Rifaximin: new therapeutic indication and future directions". *Clinical Therapeutics* 33.7 (2011): 812-827.

15. Valentin T., *et al*. "Rifaximin intake leads to emergence of rifampin-resistant staphylococci". *Journal of Infection* 62.1 (2010): 34-38.

16. Mullen KD., *et al*. "Rifaximin is safe and well tolerated for long-term maintenance of remission from overt hepatic encephalopathy". *Clinical Gastroenterology and Hepatology* 12.8 (2014): 1390-1397.

17. Pistiki A., *et al*. "*In vitro* activity of rifaximin against isolates from patients with small intestinal bacterial overgrowth". *International Journal of Antimicrobial Agents* 43.3 (2014): 236-241.

18. Sudha T., *et al*. "Spectrophotometric estimation methods for rifaximin in tablet dosage form". *International Journal of Pharmacy and Pharmaceutical Sciences* 2 (2010): 43-46.

19. Kogawa AC., *et al*. "Quantification of rifaximin in tablets by spectrophotometric method ecofriendly in ultraviolet region". *Scientifica* (2016).

20. Kogawa AC., *et al*. "Spectrophotometry in infrared region: a new, low cost and green way to analyze tablets of rifaximin". *Current Pharmaceutical Analysis* 13 (2017).

21. Kogawa AC., *et al*. "Capillary zone electrophoresis: a potential and conscious technique for evaluation of rifaximin in tablets". *Current Pharmaceutical Analysis* (2017).

22. Kogawa AC., *et al*. "Method indicative of stability for determination of rifaximin and its degradation products by thin chromatographic". *Current Pharmaceutical Analysis* 13.6 (2017): 520-524.

23. Kogawa AC., *et al*. "Optimization of microbiological method by turbidimetry for quantification of rifaximin tablets: validation, application and evaluation of degraded compounds". *Pharmaceutica Analytica Acta* 7 (2016): 518-524.

24. Kogawa AC., *et al*. "Impact of rifaximin potency after treatment with different solvents". *Journal of Pharmaceutical and Biological Sciences* 5.3 (2017): 77-80.

25. International Conference on Harmonization "Validation of analytical procedures: text and methodology Q2(R1)" (2005).

26. Horwitz W., *et al*. "Quality assurance in the analysis of foods and trace constituents". *Association of Official Analytical Chemists* 63.6 (1980): 1344-1354.

27. National Agency for Health Surveillance RE No. 899, of May 29, 2003. "Guide for the validation of analytical and bioanalytical methods". Official Journal of the Union, Brasília (2003).

28. Kogawa AC., *et al*. "Golden Age of Green Chemistry". *EC Microbiology* 12.2 (2017): 52-54.

29. Kogawa AC., *et al*. "Development and validation of a stability-indicative turbidimetric assay to determine the potency of doxycycline hyclate in tablets". *International Journal of Microbiology Research* 4.8 (2012): 316-321.

30. Cazedey ECL., *et al*. "A novel and rapid microbiological assay for ciprofloxacin hydrochloride". *Journal of Pharmaceutical Analysis* 3.5 (2013): 382-386.

31. Tótoli EG., *et al*. "Development and validation of a rapid turbidimetric assay to determine the potency of ampicillin sodium in powder for solution for injection". *Analytical Methods* 5.21 (2013): 5923-5928.

32. Pedroso TM., *et al*. "Development and validation of a microbiological assay by turbidimetry to determine the potency of cefazolin sodium in lyophilized powder". *Analytical Methods* 6.5 (2014): 1391-1396.

33. Vieira D., *et al*. "Development and validation of a rapid turbidimetric assay to determine the potency of cefuroxime sodium in powder for dissolution for injection". *Pathogens* 3.3 (2014): 656-666.

34. Silva LM., *et al*. "Rapid turbidimetric assay to potency evaluation of tigecycline in lyophilized powder". *Journal of Microbiological Methods* 110 (2015): 49-53.

35. Tótoli EG., *et al*. "Rapid turbidimetric assay to determine the potency of daptomycin in lyophilized powder". *Pharmaceutics* 7.3 (2015): 106-121.

36. Curbete MM., *et al*. "Rapid turbidimetric assay for quantification of fusidic acid in a dermatological cream". *Talanta* 153 (2016): 51-56.

Volume 14 Issue 1 January 2018