UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

Faculdade de Farmácia

Disciplina de Trabalho de Conclusão de Curso de Farmácia

Validation of a simple HPLC method for the determination of Chlorhexidine Digluconate in solution

Mariana Colombo

Porto Alegre, novembro de 2013.

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

Faculdade de Farmácia

Disciplina de Trabalho de Conclusão de Curso de Farmácia

Validation of a simple HPLC method for the determination of Chlorhexidine Digluconate in solution

Mariana Colombo

Trabalho final da Disciplina de Trabalho de Conclusão de Curso em Farmácia

Orientador: Prof[a]. Dr. Letícia Scherer Koester

Coorientadora: Dr. Letícia Lenz Sfair

Porto Alegre, novembro de 2013.

“Happiness only real when shared.”

Christopher McCandless

AGRADECIMENTOS

Aos meus pais pelo apoio, amor e exemplo de todos os dias.

À Dr. Letícia S. Koester pela orientação deste trabalho e durante a iniciação científica.

À Dr. Letícia L. Sfair pela coorientação, dedicação e amizade durante a realização deste trabalho.

A Vidora Farmacêutica pela oportunidade e em especial ao pessoal do Controle de Qualidade pela amizade e companheirismo durante meu estágio.

Ao Fabrício por me mostrar que a vida pode ser e é bem mais fácil e simples.

À minha família, principalmente aos meus avós, Xande, Lu e Ana Maira pelo apoio e ensinamentos em todos os momentos.

APRESENTAÇÃO

Este trabalho está apresentado sob a forma de artigo científico, dividido nos seguintes tópicos: Introdução, Materiais e Métodos, Resultados e Discussão, Conclusão e Referências e será submetido à Latin American Journal of Pharmacy. Os experimentos foram realizados na Indústria Vidora Farmacêutica, Porto Alegre, Rio Grande do Sul.

SUMÁRIO

VALIDATION OF A SIMPLE HPLC METHOD FOR THE DETERMINATION OF CHLORHEXIDINE DIGLUCONATE IN SOLUTION

Mariana Colombo, Letícia L.Sfair, Letícia S. Koester*

Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul (UFRGS), Av. Ipiranga, 2752, 90610-000, Porto Alegre, RS, Brazil.

KEY WORDS. Chlorhexidine Digluconate. High performance liquid chromatography. Stability studies.

*Author to whom correspondence should be addressed. Email: leticia.koester@ufrgs.br

SUMMARY. Chlorhexidine Digluconate (CDG) is an antiseptic of biguadines class widely used as skin disinfectant. A high performance liquid chromatography method has been developed and validated for a rapid determination of CDG in commercial product (Kuratop®, 1%) and raw material. The method proved to be fast, specific, linear, precise, exact and robust for quantifying CDG in commercial product and raw material. Furthermore, the method was applied to quantification of CDG in the product filled in two different packings and submitted to accelerated (up to 6 months) and long-term (up to 1 year) stability studies. Throughout the period evaluated CDG content remained between 9.04 and 9.86 % and RSDs of the analysis were lower than 2,46% demonstrating the development of a precise analytical methodology.

INTRODUÇÃO

Chlorhexidine Digluconate (CDG) (Figure 1) [1], [N,N" -bis (4-chlorophenyl)-3, 12-diimino-2, 4, 11, 13-tetraazatetradecanediimidamide di-*D*-gluconate] is the most popular antiseptic of biguadines class. It has been described a high antibacterial activity for this compound, mainly against most gram-positive and some gram-negative bacteria, and is often used as a skin disinfectant [2]. The antimicrobial properties have been attributed to its di-cationic structure [3]. Moreover, CDG induces rupture of cytoplasmic membrane (lipopolysaccharides) by changing the membrane potential [4].

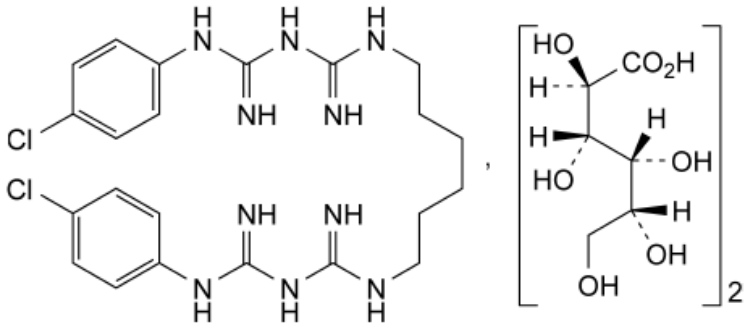

Figure 1.

Several analytical methods have been reported to chlorhexidine analysis by high performance liquid chromatography (HPLC) in complex biological matrices as, for instance, saliva [5,6,7,8], urine [9,10] and human serum [11,9]. With respect to pharmaceutical products CDG has been evaluated by HPLC in topical ointment [12], ophthalmic solution [13], suspension [14], pastille [15], spray and gargle dosage forms [16]. The official HPLC method for CDG determination has been described in USP 34 using a gradient of acetonitrile and buffer solution [17].

In order to ensure maintenance of quality in pharmaceutical product, stability testing must be performed at several stages. The efficacy and safety of a new formulation depends of a robust assay before commercialization. Therefore, the product under development must be subjected to accelerated and long-term stability testing. The accelerated stability testing provides an early

indication of product shelf life since the product is submitted to relatively high temperatures and/or humidity, that is, an accelarate degradation condition. In parallel, long-term stability testing under less rigorous conditions has been recommended to determine a product shelf life [18].

Considering that isocratic methods are fast and favourable to routine analysis and that the official method is a long standing gradient analysis assay, the purpose of this study was to develop and validate a fast, simple and efficient isocratic high-performance liquid chromatography method to determinate CDG for both the commercial product and its raw material. Furthermore, the stability of the commercial product was assessed and the method was applied to CDG quantification.

MATERIALS AND METHODS

Chemicals

Chlorhexidine Acetate USP reference standard (batch I0J311) and p-Chloroaniline USP reference standard (batch G0K0206) were obtained from US Pharmacopeia. The Chlorhexidine Digluconate 20% (raw material) and 1% aqueous solution (Kuratop®, commercial product) was obtained from Smaart Pharmac (India) and Vidora Farmacêutica (Porto Alegre, Brazil), respectively. Methanol and Acetonitrile HPLC grade were purchased from Panreac (Barcelona, Spain) and Merck (Darmstadt, Germany), respectively. Trifluoroacetic acid (TFA) and triethylamine (TEA) were purchased from Vetec (Rio de Janeiro, Brazil). Purified water was obtained by Line Master System Gehaka (São Paulo, Brazil).

Equipment and Chromatographic Conditions

The HPLC system consisted of a Merck Hitachi LaChrom equipped with a model L-2130 pump, L-2200 auto sampler, L-2300 column oven, L-2400 detector and EZChrom Elite software was used for data processing.

The column used was a SGE Analytical Science 250x4.6mm P $C_{18}H_{125}$ 5µm particle size, coupled to a C_{18} guard column (Australia). The mobile phase was composed of methanol, aqueous solution containing 0.5% TEA adjusted to pH 3.0 with TFA and acetonitrile (40:42:18, v/v/v). The mobile phase was filtered through 0.45 µm filter prior to use.

The HPLC system was operated in isocratic mode at a flow rate of 1.4 $mL.min^{-1}$, with detection at 240 nm, temperature of 35 °C and injection volume set at 20 µL.

Method validation

The method was validated according to the International Conference on Harmonization–ICH guideline (Q2(R1), 2005) [19].

Specificity was determined by analyzing a solution containing the excipients employed for the preparation of the commercial product, and a solution containing the degradation product (*p*-Chloroaniline) [14,17,20,21,22]. All solutions were injected in triplicate.

The linearity of the method was evaluated by injecting five standard solutions ranging from 60.0 to 100.0 $\mu g.mL^{-1}$, in three different days. Linearity was evaluated by calculation of a regression line using the least square method. The linearity was statistically evaluated by analysis of variance (ANOVA) and by the value of the correlation coefficient of the standard curve.

The detection (LOD) and quantification (LOQ) limits were calculated based on the standard deviation of the response (σ) and the slope (S) of the calibration curve, using the equations: LOD = 3.3σ/S and LOQ =10σ/S.

The accuracy of the proposed method was determined by the recovery of known amounts of chemical reference samples added to the samples solution (60 μg.mL^{-1}). The added levels were 20, 40 and 60%, that is, 12, 24 and 36 μg.mL^{-1}. Three samples were used for each recovery level.

The intra-day precision (repeatability) was evaluated by assaying six samples at CDG concentration of 80 μg.mL^{-1}, during the same day and under the same experimental conditions. Inter-day precision was studied by comparing the response of same solutions in three different days. The relative standard deviation (RSD) was calculated.

The robustness of the method was evaluated by testing the susceptibility of the measurements to deliberate variations of the analytical conditions, as pH values of the aqueous phase of the mobile phase, column temperature and wavelength.

Preparation of standard and sample solutions

The stock solution of Chlorhexidine Acetate USP (200 μg.mL-1) was prepared in water and subsequent dilutions were carried out to obtain five standard solutions (60.0, 70.0, 80.0, 90.0 and 100.0 μg.mL^{-1}) in mobile phase. The solution of p-Chloroaniline USP (100 μg.mL^{-1}) was prepared using mobile phase as diluting solution.

To prepare working sample solution, separately, 1 mL of the Chlorhexidine Digluconate 20% and 1% aqueous solution were diluted in mobile phase until the concentration of 80.0 μg.mL^{-1}. The solutions were filtered through a 0.45 μm membrane filter.

To calculate the concentration of the samples, a correction factor was used, considering the molecular weight of Chlorhexidine Acetate (625.55) and Chlorhexidine Digluconate (897.76).

Stability studies

The stability of CDG in the 1% aqueous solution was assessed using the validated method. Different batches of commercial products filled in two different types of packing, amber polyethylene terephthalate (batches I, II and III) and polyethylene (batches IV, V, and VI) were monitored for 3 and 6 months stored in original packing in stability chamber (40 °C and 75% relative humidity) for accelerated stability testing and for 3, 6, 9 and 12 months in stability chamber (30°C and 75% relative humidity) for long term stability testing [23]. We also evaluated organoleptic characteristics, density, pH and microbial contamination.

RESULTS AND DISCUSSION

Method validation

The chromatographic conditions were adjusted based on system suitability parameters. During the optimization of the analytical method, different organic solvents and aqueous phase with different pH values were tested. The best condition with a retention time of 6.8 min was obtained using a SGE Analytical Science 250x4.6mm $PC_{18}H_{125}$ (5µm particle size) column and mobile phase composed of methanol, aqueous solution containing 0.5% TEA adjusted to pH 3.0 with TFA and acetonitrile (40:42:18, v/v/v). The addition of TEA improved peak symmetry. Upon performing the

system suitability test, the column efficiency (plates) was >5000, the USP Tailing Factor < 1.5 and the Capacity Factor (k') was 1 <k´< 5.

The chromatograms shown in Figure 2 demonstrate that the method is specific and has no interference from the excipients or degradation product (*p*-Chloroaniline).

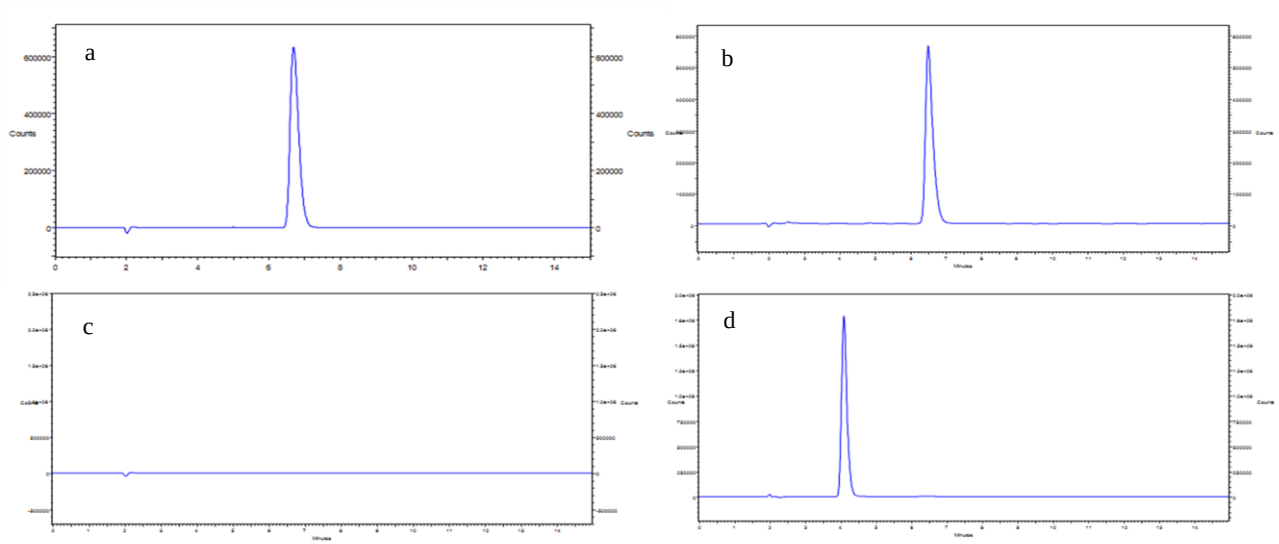

Figure 2.

The method demonstrated good linearity in the range of 60 – 100 µg.mL^{-1} and the standard equation y = 125238.407x + 1033434.369 showed excellent correlation coefficient (r> 0.999). According to ANOVA there is linear regression and there is no deviation from linearity (p=0.05). The detection and quantification limits were 1.7 and 5.17 µg.mL^{-1}, respectively.

The low RSD values for intra-day and inter-day analysis indicated the acceptable precision of the method. The results were presented in Table 1.

		Theoretical amount ($\mu g.mL^{-1}$)	Experimental amount ($\mu g.mL^{-1}$±SD)	RSD (%)
Raw material	**Intra-day**			
	Day 1 (n=6)	80.0	80.71 ± 0,76	0.94
	Day 2(n=6)	80.0	81.77 ± 0.48	0.58
	Day 3(n=6)	80.0	81.38 ± 0.61	0.75
	Inter-day (n=18)	80.0	81.29 ± 0.54	0.66
Commercial product	**Intra-day**			
	Day 1 (n=6)	80.0	74.55 ±1.09	1.46
	Day 2(n=6)	80.0	75.90 ±1.26	1.67
	Day 3(n=6)	80.0	76.59±1.18	1.54
	Inter-day (n=18)	80.0	75.68±1.03	1.37

Table 1.

The table 2 shows the accuracy results. The recovery values ranged from 98.99% to 103.51% (raw material) and from 97.76% to 99.49% (commercial product) demonstrating the accuracy of the method.

	Added ($\mu g.mL^{-1}$)	Found ($\mu g.mL^{-1}$ ±SD)	Recovery (%)
Raw material	12.07	12.04 ± 0.46	100.35
	24.14	23.73 ± 0.64	98.99
	36.20	37.26 ± 0.41	103.51
Commercial product	11.72	11.94± 0.05	99.49
	23.44	23.46 ± 0.07	97.76
	35.16	35.44 ± 0.60	98.44

Table 2.

The robustness results show that there were no relevant changes in the concentration, retention time, tailing factor and theoretical plate, after slight modifications of the analytical conditions, showing the robustness of the proposed method (Table 3a and 3b).

Modifications	Concentration ($\mu g.mL^{-1}$)	Retention Time (min)	Tailing Factor (TF)	Theoretical Plate (N)
pH Mobile Phase				
2.8	80.30	6.6 min	1.46	5502
3.2	80.72	6.4 min	1.50	5532
Temperature (°C)				
33	80.82	7.0 min	1.43	6002
37	79.87	6.5 min	1.45	5754
Wavelength (nm)				
238	79.35	6.8 min	1.43	5631
242	80.70	6.8 min	1.46	5565
None	80.55	6.8 min	1.43	5547

a.

Modifications	Concentration ($\mu g.mL^{-1}$)	Retention Time (min)	TailingFactor (TF)	Theoretical Plate (N)
pH mobile phase				
2.8	74.63	6.6 min	1.47	5487
3.2	76.96	6.4 min	1.51	5457
Temperature (°C)				
33	75.90	7.0 min	1.43	6084
37	74.36	6.5 min	1.46	5685
Wavelength (nm)				
238	74.76	6.8 min	1.43	5770
242	76.75	6.8 min	1.47	5572
None	75.10	6.8 min	1.43	5502

b.

Table 3.

CDG assay during stability studies

Finally, the method was applied to CDG assay in the commercial product under stability studies. No relevant change was observed during the period examined in both the accelerated stability and the long-term stability regarding organoleptic characteristics, pH, density and microbiological contamination (data not shown). Table 4 shows the results obtained for the assay of CDG in different periods. The drug content remained within the specified range (not less than 9.0 $mg.mL^{-1}$ and not more than 11.0 $mg.mL^{-1}$) between 9.04 and 9.86 % and the RSD of the analysis were lower than 2,46%.

		Concentration ($mg.mL^{-1}$) ± SD					
		Accelerated stability testing		Long term stability testing			
Batch	Initial	3 months	6 months	3 months	6 months	9 months	12 months
I	9.80± 0.08	9.63±0.01	9.58±0.47	9.71±0.01	9.67±0.03	9.20±0.10	9.26±0.01
II	9.82±0.00	9.55±0.06	9.53±0.09	9.67±0.01	9.58±0.02	9.29±0.12	9.23±0.07
III	9.77±0.06	9.68±0.01	9.64±0.06	9.70±0.01	9.65±0.14	9.53±0.02	9.23±0.09
IV	9.54±0.00	9.52±0.02	9.47±0.00	9.52±0.01	9.56±0.07	9.06±0.01	9.34±0.28
V	9.56±0.03	9.45±0.00	9.50±0.05	9.50±0.01	9.45±0.00	9.28±0.02	9.18±0.00
VI	9.67±0.10	9.30±0.01	9.86±0.11	9.41±0.00	9.37±0.02	9.04±0.08	9.37±0.08

Table 4.

CONCLUSION

The proposed HPLC method can be used for Chlorhexidine Digluconate assay in raw material and commercial product. The method is faster and uses simple reagents, compared to the pharmacopeial method and was successfully applied to drug monitoring during stability studies.

REFERENCES

1. European Pharmacopoeia (2005). Council of Europe: Strasbourg.
2. Sweetman, S. C. (2009). "Martindale: The Complet Drug Reference 36th ed. London, UK. pp. 1635-38.
3. Moshrefi, A. J. (2002) West Soc. Periodontol. Periodontal Abstr. **50**: 5-9.
4. Kuyyakanond, T; Quesnel, L.B. (1992) FEMS Microbiol. Lett. **79**: 211-15.
5. Lam, Y.W.F., Chan, D.C.N., Rodriguez, S.Y., Lintakoon, J.H., Lam, T.H. (**1993)** J. Chromatogr. Biomed. Appl. **612**: 166-71.
6. Pesonen, T., Holmalahti J., Pohjola, J. (1995) J. Chromatogr. B. **665**: 222-25.
7. Medlicott, N.J., Ferry, D.G., Tucker, I.G., Rathbone, M.J., Holborow, D.W., Jones, D.S. (1994) J. Liq. Chromatogr. **17**: 1605-20.
8. Tsuchiya, H., Miyazaki, T., Ohmoto, S. (1999) Caries Research. **33**: 156-63.
9. Brougham, L.R., Cheng, H., Pittman, K.A. (1986) J. Chromatogr. Biomed. Appl. **383**: 365-37.
10. Wainwright, P., Cooke, M. (1986) The Analyst. **111**: 1343-44.
11. Kudo, K., Ikeda, N., Kiyoshima, Hino, A., Y., Nishida, N., N. Inoue. (2002) J. Anal. Toxicol. **26**: 119-22.
12. Havl´ıkov´a, L., Matysov´a, L., Nov´akov´a, L., H´ajkov´a, R., Solich, P. (2007) Journal of Pharmaceutical and Biomedical Analysis. **43**: 1169-73.

13. Cheung, A.P., Mavar, R., Carlson, C., Chiang, W.K. (1991) J. Pharm. Biomed. Anal. **9**: 41-45.
14. Xu, Y.M., Wong, G.Y. (1999) J. Liq. Chromatogr. Relat. Technol. **22**: 2071-91.
15. Bauer, M., Degude, C., Mailhe, L. (1984) J. Chromatogr. **315**: 457-64.
16. Dogan, A., Nursabah, E. B. (2011) Current Pharmaceutical Analysis. **7**: 167-75.
17. The United States Pharmacopeia USP34-NF29 (2011). The United States Pharmacopoeial Convention: Rockville.
18. Bajaj, S., Singla, D., Sakhuja, N. (2012) J. App. Pharm. Sci. **2**: 129-38
19. International Conference on Harmonisation of Technical Requirements for Registration Of Pharmaceuticals for Human Use (2005) Validation of Analytical procedure: Text and Methodology Q2(R1).
20. Basrani, B. R.; Manek, S.; Sodhi, R. N. S.; Fillery, E.; Manzur, A. (2007) Basic Res.Tech. **33**: 966-69.
21. Rasimick, B. J.; Nekich, M.; Hladek, M. M.; Musikant, B. L.; Deutsch, A. S. (2008) J. Endod. **34**: 1512-13.
22. British Pharmacopoeia (2012). British Pharmacopoeia Commission Office, London.
23. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (2003) Stability testing guidelines : Stability testing ofnew drug substances and products Q1A(R2).

Captions of Figures

Figure 1. Chemical structure of Chlorhexidine Digluconate.

Figure 2.Chromatograms of (a) Chlorhexidine Acetate (80.0 μg.mL^{-1}); (b) Chlorhexidine Digluconate (80.0 μg.mL^{-1}); (c) Placebo Solution; (d) p-Chloroaniline (100.0 μg.mL^{-1}).

Captions of Tables

Table 1. Intra-day and inter-day precision of the method.

Table 2. Recovery of standard solution added to samples.

Table 3. Robustness of the method (3a: Raw material; 3b: Commercial product).

Table 4. Results of stability studies.

ANEXO

Normas para publicação

Manuscripts submitted to *Latin American Journal of Pharmacy* are only accepted on the understanding that they are subject to editorial review and that they have not been, and will not be, published in whole or in part in any other journal. Papers must be written in English. If English is not authors' native language, the manuscript should be checked by someone proficient in the language before submission. Manuscripts in which English is difficult to understand may be returned to the author for revision before scientific review.

Types of Contribution

Original articles should contain material that has not been previously published elsewhere, except in a preliminary form. These papers should not exceed 5000 words including tables, references and legends of tables and figures. *Short Communications* are research papers constituting a concise but complete description of a limited investigation, which will not be included in a later paper. They should be as completely documented as a regular paper and should not occupy more than 2,500 words including tables, references and legends of tables and figures. *Reviews* and *mini-reviews* will be exceptionally accepted in areas of topical interest and will normally emphasize literature published over the previous five years. *Letters to the Editor* are published from time to time on subjects of topical interest.

Manuscript Preparation

Manuscripts must be neatly typed (size page A4), double-spaced throughout, including figures and tables, with at least 2 cm margins on all sides. The Editor reserves the right to adjust style to certain

standards of uniformity. Every page of the manuscript must be numbered at the right top, preceded by the name of the author to whom the correspondence should be sent. The usage of italics should be limited to scientific names of organisms. A cover letter is not required, but if included it should be placed at the beginning of the manuscript.

Manuscripts in general should be organized in the following order:

- *Title*: should be clear, concise, and unambiguously reflect the paper's contents.
- *Name(s) of author(s)*: first name, initial(s) of the middle name(s), and family name of each author. The corresponding author should be identified with an asterisk (*).
- *Affiliations*: include the name of department (if any), institution, city and state or country where the work was done, indicating which authors are associated with which affiliation.
- *E-mail address of the corresponding author*, as all correspondence, including proofs, should be sent only to him.
- *Summary*: not exceeding 150 words, reporting concisely on the major findings. Many abstracting services use abstracts without modification, so this section should be comprehensible in its own right.
- *Key Words*: at least three and not more than six in alphabetical order will be listed.
- *Introduction*: briefly review important prior publications and state the reasons for the investigation being reported.
- *Materials and methods*: description of methods, equipment and techniques (including statistical treatments used in the research).
- *Results*: efforts should be made to avoid jargon, to spell out all non-standard abbreviations the first time they are mentioned and to present the contents of the study as clearly and concisely as possible.
- *Discussion* (may be combined with the Results section).

- *Conclusions (at the author's discretion)*: must not reiterate any discussion or introductory comments, they must be genuine conclusions drawn from the results of the study.
- *Acknowledgements* and any additional information concerning research grants, etc.
- *References*: will be numerated correlatively as they are cited in the text and listed separately under the title "References" (please use a hanging indent: second and subsequent lines indented). The style used for citation of articles in journals (1), monographs (2), chapters in books (3), and internet references (4) which must be strictly observed, is given in the following examples:

(1) Medeiros R., G.F. Passos, C.E. Vitor J. Koepp, T.L. Mazzuco, L.F. Pianowski, M.M . Campos & J.B. Calixto (2007) *Brit. J. Pharmacol.* **151**: 618-27.

Journal names should be abbreviated according to ISI style (you are invited to consult the sites http://www.efm.leeds.ac.uk/~mark/ISIabbr/A_abrvjt.html or http://images.isiknowledge.com/WOK46/help/WOS/L_abrvjt.html)

(2) Vogel, W.H., B.A. Scholkens, J. Sandow & G. Muller (2002). *"Drug discovery and evaluation, Pharmacological assay"*, Second Edition, Spinger-Verlag, Berlin Heidelberg, New York, pp. 906-44.

(3) Aristide, V. & J.W. Martin (2005) *"Doxorubicin"*, in "Analytical profiles of drug substances" (F. Klaus, ed.), Academic Press, New York, pp. 245-74.

(4) Duke, J.A. *"Medical Botany. Module 8: Amazonian (Iberoamerican)"*.

Available at (http://www.ars-grin.gov/duke/syllabus/module8.htm).

For 2-6 authors all authors are to be listed, with "&" separating the last two authors; for more than six authors, use the first six authors followed by *et al*. For three or more authors use *et al.* in the text.

- *Tables and Figures*: will be numbered using Arabic numerals in the order they appear in the text. *Letters and symbols included into the figures should be made in a suitable size, since figures are usually reduced to half a column wide size (7.5 cm)*. Photographs, charts and diagrams are all to be referred to as "Figures". They should accompany the manuscript. All illustrations should be clearly marked with the figure number. All figures are to have a self-explanatory caption. Captions of Tables and Figures should be supplied on a separate sheet.

Page charges

- Page charges are required for publication in Latin American Journal of Pharmacy. These cover some of the costs of publication and allow us to more fairly share charges between researchers and libraries. Please be aware that the journal is published by the College of Pharmacists of Buenos Aires Province, Argentina, which does not receive direct funding from any external agencies. Support from page charges allows lower subscription prices and thus a greater circulation for the journal.
- Page charge forms will be sent automatically on acceptance of an article for publication in the journal. We thank you for your prompt submission of completed page charge forms so that we can publish your paper more quickly and efficiently. Please be advised that we cannot publish papers until we have received completed and signed page charge forms from the authors.

- Page charge during 2011 has been fixed in 20 dollars per print page. You will be advised of the total page charges when you receive the page proofs. As an example, Short Communications (no more than 2500 characters) usually are four pages long and the total page charge is at most 80 dollars. In the same way, total page charge for Original Articles (no more than 5000 characters) is at most 160 dollars.