Lat. Am. J. Pharm. **22** (4): 339-42 (2003)
Recibido el 21 de febrero de 2003
Aceptado el 7 de junio de 2003

Non-aqueous Titration of Gatifloxacin in Pharmaceutical Formulations using Perchloric Acid

Hérida R. N. MARONA [1]*; Cristiani C. G. O. LOPES [1] & Simone G. CARDOSO [2]

[1] *Programa de Pós-Graduação em Ciências Farmacêuticas, FCF, UNESP, Araraquara, SP, Brazil.*
[2] *Centro de Ciências da Saúde, UFSM, Santa Maria, RS, Brazil*

SUMMARY. An inexpensive, simple, precise and rapid method for the determination of fluoroquinolone gatifloxacin in tablets is described. The procedure is based on the use of volumetric dosage in a non-aqueous medium in glacial acetic acid with 0.1 M perchloric acid. The method validation yielded good results and included the precision, recovery and accuracy. It was also found that the excipients in the commercial tablet preparation did not interfere with the assay.

RESUMEN. Se describe un método rápido, simple, económico y preciso para la determinación de la fluoroquinona gatifloxacina en comprimidos. El procedimiento está basado en el uso de dosaje volumétrico en un medio no acuoso de ácido acético glacial con ácido perclórico 0,1 M. La validación del método produjo buenos resultados que incluyen precisión, recuperación y seguridad. Se encontró además que los excipientes del comprimido no interfieren en el ensayo.

INTRODUCTION

Gatifloxacin, [(±)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(cis-3,5-dimethyl-1-piperazinyl)-4-oxo-3-quinoline carboxylic acid), Fig. 1], an oral fluoroquinolone antibacterial agent, is active against most aerobic Gram-positive and Gram-negative organisms [1] and demonstrates moderate activity against anaerobes and *Mycobacteria*, which the quinolones in general have low activity [2,3]. Gatifloxacin presents as pale yellow prisms from methanol as hemihydrate which melting point is 162 °C [4].

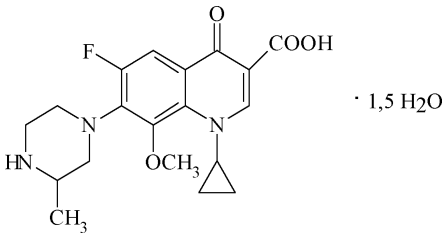

Figure 1. Chemical structure of gatifloxacin - $C_{19}H_{22}FN_3O_4$ (M.W. 375).

Although gatifloxacin (GATX) has been studied in terms of therapeutic activity [3,5-8] and commercialized, there is no official pharmacopoeial monograph on its quantification in raw material and tablets. Few reports about analytical methods in biological fluids are available in the literature such as UV-spectrophotometry, HPLC [1,9]. Marona *et al.* [10] have presented a UV-spectrophotometric method for gatifloxacin in raw material and tablets. High performance liquid chromatography of another fluoroquinolone, sparfloxacin, both as a raw material and in tablets was developed by Marona & Schapoval [11]. Recent papers have reported analytical methods for determination of sparfloxacin as UV-spectrophotometry [12], visible-spectrophotometry [13], microbiological assay [14], and non-aqueous titration [15], which we had validated by statistical analysis.

This paper reports a procedure for the quantitative determination of the drug in pharmaceu-

KEYWORDS: Fluoroquinolone, Gatifloxacin, Non-aqueous titration, Pharmaceutical analysis.
PALABRAS CLAVE: Análisis farmacéutico, Fluoroquinolona, Gatifloxacina, Titulación en medio no acuoso.

* Corresponding author. E-mail: maronah@fcfar.unesp.br

tical forms by non-aqueous titration, providing precise and accurate results, which could be verified by statistical methods.

MATERIALS

Gatifloxacin and gatifloxacin tablets were kindly supplied by Bristol-Myers Squibb do Brasil S.A. (Brazil). The GATX tablets (Tequin ™) were claimed to contain 400 mg of active drug. All other chemicals were analytical grade. Glacial acetic acid (Merck); perchloric acid 0.1 *M;* crystal violet 0.1% (in acetic acid) as indicator.

METHODS

Tablets of GATX

In order to determine the average weight, 20 tablets were accurately weighted and they were crushed and dried at 105 °C by 2 h. Each amount was accurately weighted, transferred into a 250 mL conical flask and 40 mL of glacial acetic acid and 5 drops of indicator were added. In order to determine the percentage, these solutions were titrated with perchloric acid 0.1 *M* until colour change, which indicated the final point was reached.

The percent of GATX was determined by applying the equation:

$$A\ (\%) = \frac{v \times dmEq \times 100}{m}$$

where v = mL of 0.1 M perchloric acid; dmEq = decimiliequivalent of GATX; m = weight of GATX (g). A blank determination was performed at a temperature of 20 °C and any necessary corrections were made.

Recovery Test

The accuracy was determined by adding amounts of the reference substance to the samples at the beginning of this procedure according USP XXV [16] .

The recoveries were determined by adding known amounts of gatifloxacin reference substance GATX-RS (10.1, 20.3 and 39.7 mg) to the samples at the beginning of the procedure. The GATX-RS and tablets were dried at 105 °C for 2 h. The equivalent of 400 mg GATX in tablets was spiked with amounts of GATX -RS and was dissolved in 40 mL of glacial acetic acid. Finally, this sample was titrated with 0.1 M perchloric acid to quantify as described above. Three replicate determinations in 3 different days were carried out to test the precision of this method.

Precision

The accuracy and precision of this method were determined for intra- and inter-day on three different days. The precision was expressed as the percent coefficient of variation. The analysis of variance (ANOVA) is an important statistical tool to verify the internal validity of an analytical procedure.

RESULTS

The results obtained through the titrimetric analysis with GATX tablets are displayed in Table 1, which shows mean, s, CV% and RSD.

Recovery Test

The results of recovery test of GATX in tablets using the volumetric determination with perchloric acid 0.1 M are shown in Table 2.

	Day 1	Day 2	Day 3	Mean interday
I	100.48	99.72	101.37	
II	100.85	100.02	101.32	
III	99.65	99.72	100.79	
mean	100.28	99.82	101.16	100.42
s	0.666	0.173	0,321	0.681
CV%	0.66	0.17	0.32	0.68
RSD	0.384	0.10	0.186	0.393
n	3	3	3	3

Table 1. Titration of GATX tablets with perchloric acid 0.1 *M.* s: standard deviation; CV: coefficient of variation; RSD: relative standard deviation.

spiked amount of RS (mg)	recovery amount of RS (mg)	recovery %
10.1	9.91	98.1
20.3	20.42	100.6
39.7	39.56	99.6

Table 2. Recovery test of gatifloxacin tablets using the volumetric determination with perchloric acid 0.1 *M*.

DISCUSSION

Gatifloxacin demonstrated the character of the proton dissociation of carboxylic group in C_3 and methylpiperazinyl in C_7, respectively. The reaction between gatifloxacin and a non-aqueous medium and glacial acetic acid is an acid-base reaction where the strong acid can donate a proton to nitrogen from piperazinyl ring.

The proposed analytical method in a non-aqueous medium shows to be an important tool for precision and accuracy in the quantification of gatifloxacin raw material and tablets.

In the literature [4] the gatifloxacin is shown as having a decomposition at 255-257 °C or forming a light yellow crystalline solid which melt at 318-320 °C. Preliminary studies conducted with high temperature gatifloxacin appeared to present a thermal stability. The DSC curve to the gatifloxacin, reference material, already shows endothermic and exothermic peaks bellow 250 °C, which may be due to dehydration and some structure rearrangement (or phase transition). The probable melting can be observed through two consecutive endothermic peak between 300-350 °C followed by thermal decomposition. The DSC curve to the commercial product shows basically the same peaks except for an endothermic peak at 270 °C, which may indicate the partial thermal decomposition of the gatifloxacin. The decomposition at 255-257 °C did not be observed in either reference or commercial sample [17].

The standard deviation and the coefficient of variation were found to be less than 1%, indicating good repeatability of the non-aqueous titration. The relative standard deviation observed was approximately 0.39%.

The accuracy may be expressed as percent recovery by the assay of known, added amounts of analyte [16]. The mean absolute recovery test of non-aqueous titration was 99.4% and can indicate a good accuracy.

However non-aqueous titration as well as spectrophotometric analysis could quantify degradation products that have similar chemical structures, the non-aqueous titration is clearly the least expensive method and it does not require high cost equipment and specialized technician when it is compared with spectrophotometric analysis, HPLC or bioassay. Besides another characteristics of this method is the short time required for performance and ease of handling that could indicate this procedure as a laboratory routine method.

This volumetric method proposed is simple, rapid and inexpensive and can therefore be applied to the determination of gatifloxacin raw material and tablets. Method validation yielded good results and included precision and accuracy.

The British Pharmacopoeia [18] recommends a titrimetric assay for nalidixic acid, which is dissolved in dichloromethane and isopropanol, using ethanolic sodium hydroxide as the titrant. The detection of the end point is accomplished potentiometrically. Norfloxacin, a first generation quinolone, is titrated in glacial acetic acid with perchloric acid and detecting the end point potentiometrically.

The results obtained through the titrimetric analysis with gatifloxacin tablets using perchloric acid show 100.42% of gatifloxacin and coefficient of variation 0.68%.

Recovery Test

The accuracy of the method was measured by the recovery rate, which was obtained by comparing the experimental results to the calculated theoretical concentrations.

The results of recovery test of gatifloxacin in tablets using the volumetric determination with perchloric acid 0.1 *M* are in agreement with spiked amount of reference substance.

The non-aqueous titration can be applied routinely, because it does not require high cost equipments. There was no evidence of interference from excipients in the tablets analysed. The standard deviation and the coefficient of variation were found to be less than 1.0%, indicating good repeatability of the non-aqueous titration.

We had developed a non-aqueous titration method of gatifloxacin in raw material and tablets. We can compare this method with a UV-spectrophotometric analysis [10] on the basis of precision, accuracy, repeatability, cost an ease of handling.

Both methods proposed yielded coefficients of variation less than 1.0%. These results can indicate a good precision. Ease of handling, as measured by technician time required for performance of a single assay can be another determination factor to select a laboratory routine method. Our work leads to conclude that the validation of non-aqueous titration of gatifloxacin in pharmaceutical formulations using perchloric acid yielded good results.

CONCLUSION

The non-aqueous method proposed is simple, rapid and inexpensive and can therefore be applied to the determination of gatifloxacin raw material and tablets. Method validation yielded good results and included precision and accuracy. It was also found that the excipients in the commercial tablet preparation did not interfere with the assay.

Acknowledgements. The authors thank the Brystol-Myers Squibb Brasil S. A. for provinding gatifloxacin reference substance and gatifloxacin tablets. This work was supported by PACD-FCFAr-UNESP and CNPq, Brazil. C.L.C.G.O. was funded by CAPES-Brazil.

REFERENCES

1. Borner, K.; H. Hartwig & H. Lode (2000) Determination of gatifloxacin in human serum and urine by HPLC. *Chromatographia* **52**: S105-107.
2. Tomioka, H.; H. Saito & K. Sato (1993) Antimicrob. Agents Chemother. **37**: 1259-63.
3. Gatifloxacin, Tequin® (1999) *Drugs Fut.* **24**: 335-339.
4. The Merck Index (2001) 13 ed. Merck & Co. Inc., Whitehouse station.
5. Blondeau, J.M. (1999) *J. Antimicrob. Chemother.* **43**: S1-11.
6. Cynamon, M. & S. Chase (1999) *Activity of gatifloxacin in a murine tuberculosis model.* In: 20th Congress of European Society for Mycobacteriology, Lucerne, Switzerland.
7. Jones, R.N.; K.C. Kugler; M.E. Erwin; D.J. Biedenbach; M.L. Beach & M.A Pfaller (1999) *Diag. Microbiol. Infect. Dis.* **33**: 247-53.
8. Lober, S.; S. Ziege; M. Rau; G. Schreiber; A. Mignot; P. Koeppe & H. Lode (1999) *Antimicrob. Agents Chemother.* **43**:1067-71.
9. Liang, H.; M.B. Kays & K.M. Sowinski (2002) *J. Chrom.* **B 772**: 53-63.
10. Marona, H.R.N. & C.C.G.O. Lopes (2002) *Acta Pharm. Turcica.* **44**: 86
11. Marona, H.R.N. & E.E.S. Schapoval (1999) *J. Pharm. Biomed. Anal.* **20**: 413-7.
12. Marona, H.R.N. & E.E.S. Schapoval (1999) *J. Antimicrob. Chemother.* **44**: 136-7.
13. Marona, H.R.N. & E.E.S. Schapoval (2001) *J. Pharm. Biomed. Anal.* **26**: 501-4.
14. Marona, H.R.N. & E.E.S. Schapoval (1998) *Información Tecnológica* **9**: 251-4
15. Marona, H.R.N. & E.E.S. Schapoval (2001) *Eur. J. Pharm. Biopharm.* **52**: 227-9.
16. United States Pharmacopoeia (2002) 25th Ed. Rockville: United States Pharmacopeial Convention.
17. Marona, H.R.N.; J.D.R. Mingorance; C. Torres & C. A. Ribeiro (2002) *Acta Pharm. Turcica* **44** (Suppl.): 97.
18. British Pharmacopoeia (2001) London, Her Majesty's Stationery Office