Available online at www.sciencedirect.com
ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 43 (2007) 298–303
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

Efavirenz related compounds preparation by hydrolysis procedure: Setting reference standards for chromatographic purity analysis

José Antônio de Aquino Ribeiro *, Ligia Maria Moreira de Campos, Ricardo José Alves, Gustavo Portela Lages, Gerson Antônio Pianetti

Departamento de Produtos Farmacêuticos, Faculdade de Farmácia, Universidade Federal de Minas Gerais, Avenida Presidente Antônio Carlos 6627, 31270-910, Belo Horizonte, MG, Brazil

Received 24 March 2006; received in revised form 18 May 2006; accepted 2 June 2006
Available online 18 July 2006

Abstract

A simple procedure for obtaining and purifying two degradation products of efavirenz (amino alcohol and quinoline derivatives) from drug substance hydrolysis is described. These impurities are known to exhibit very different UV absorbance properties from those of the parent compound, making determination using a quantitation factor (QF) inaccurate. The obtained hydrolysis products were characterized by physicochemical methods to assure identity, purity and strength. Quinoline derivative was of high purity degree (100%) and amino alcohol was 98.74% pure. Both were set as reference standards in chromatographic related compounds test for efavirenz drug substance and tablets analyses.

Keywords: Efavirenz; Degradation; Related compounds; Chromatographic purity

1. Introduction

Efavirenz is a non-nucleoside reverse transcriptase inhibitor (NNRTI) specifically used in the treatment of human immunodeficiency virus type-1 (HIV-1) [1]. Brazilian government distributes efavirenz 600 mg tablets (Stocrin®, Merck Sharp & Dohme) free of charge for seropositive patients as part of the National AIDS Program. This policy of universal treatment access has attracted the recognition of the international community [2]. However, the self-sustainability of the AIDS Program has been threatened by increasing expenses with imported drugs in the last years [3]. Recently, Brazilian Ministry of Health has announced the possibility of efavirenz patent compulsory licensing in Brazil. Since then, perspectives of production of the generic drug product by governmental laboratories could be possible, with significant economy for the public safes [4].

Efavirenz pharmacopoeial monographs are not yet available in national or foreign official compendia. Compendial committees define what is an acceptable article and give test procedures that demonstrate that the article is in compliance [5]. Therefore, there is a need for technical specifications establishment to assure the user of the generic drug product of their identity, strength, quality and purity.

Impurity profiling is now receiving important critical attention from regulatory authorities. Different compendia, such as the British, the United States and the Brazilian pharmacopoeias have been specifying limits to allowable levels of impurities present in drug substances or products. Besides, ICH and FDA have published guidelines on residual solvents and impurities in new drug substances and products [6–9].

The control of drug chemical impurities is currently a critical issue to the pharmaceutical industry. The presence of unwanted chemicals, even in small amounts, may influence the efficacy and safety of the pharmaceutical products [10].

In Brazil, nowadays, the purchase of reference standards of pharmaceutical substances impurities is restrictive, because only few pharmacopoeias, such the American or the European offer these substances, at high prices and in small amount. Standards for efavirenz and its impurities are not yet available from these compendia or other known sources.

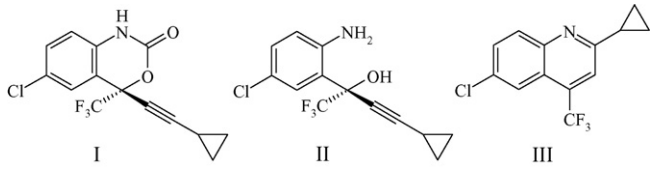
Two types of standards are recognized by FDA. A reference standard (i.e., primary standard) may be obtained from USP or other official sources and does not require further characterization. A working standard (i.e., in-house or secondary standard) is

* Corresponding author. Tel.: +55 31 34996982; fax: +55 31 34996976.
E-mail address: josedeaquino@farmacia.ufmg.br (J.A. de Aquino Ribeiro).

0731-7085/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2006.06.010

a standard that is qualified against to and used instead of the reference standard. In case there is no official source, a reference standard of the highest purity can be obtained by reasonable effort; however, it should be thoroughly characterized to ensure its identity, purity and strength [11].

Few methods reporting efavirenz impurities analysis have been described in the literature [12–14]. The development and validation of a reverse-phase HPLC method for analysis of efavirenz (I) and its related compounds in the drug substance and in capsules have previously been described by Montgomery et al. [12]. The method allows for quantitation of several efavirenz related compounds without their authentic standards, using efavirenz diluted solution as reference standard and applying quantitation factors (QF). However, in an interlaboratory qualification study, the authors verified poor reproducibility for the QF of two specific related compounds, amino alcohol (II) and quinoline (III) derivatives, which are known to be the primary degradation products of efavirenz. These impurities exhibit very different UV absorbance properties from those of efavirenz. According to the authors, to accurately measure these potential degradation products on a routine basis, the quantitation factor should be measured on the same instrument or authentic substances used.



This paper describes the preparation and purification of two efavirenz degradation products, an amino alcohol and a quinoline derivative from efavirenz drug substance hydrolysis. Their characterization by physicochemical methods and use as potential reference standards for related compounds quantitative analysis in efavirenz drug substance and tablets are also discussed.

2. Experimental

2.1. Chemicals

Efavirenz working standard (Labogen S.A., Indaiatuba, Brazil) was fully characterized by physicochemical methods and assayed by in-house non-aqueous titration (99.66% pure). Efavirenz drug substance (batches PL03101501 and PL03101701, Labogen S.A.) and efavirenz 600 mg tablets (Stocrin®, batch 144NA, Merck Sharp & Dohme, Campinas, Brazil) were used. All reagents were chromatographic or analytical grade.

2.2. Preparation and purification of degradation products

Efavirenz batch PL03101501 (200 mg) was charged into a 100 mL round-bottom flask equipped with a magnetic stirrer and dissolved with 5 mL of methanol. Sodium hydroxide (0.1 mol/L, 25 mL) was added dropwise with magnetic stirring. A condenser was coupled to the flask and the mixture was stirred and heated in a water bath at 60 °C for 24 h or until total consumption of efavirenz (as judged by TLC). Aluminum plate coated with silica gel F_{254} (Merck KGaA, Darmstadt, Germany) was used as support; elution was carried out with a mixture of ethyl acetate and *n*-hexane (50:50, v/v); 5 μL of efavirenz working standard solution (2 mg/mL in methanol) and 5 μL of reaction mixture were separately and jointly applied in the support. Spots were observed under ultraviolet light (254 nm). After completion of the reaction, the mixture was cooled to 8 °C in a refrigerator.

Quinoline was collected from the neck of the round-bottom flask and from condenser as white odorous crystals. Quinoline was also collected by filtration of the cooled mixture, washed with 3-fold 2 mL portions of water and dried under vacuum to give white odorous crystals. Amino alcohol was obtained by acidification of the filtrate with glacial acetic acid. The acidified filtrate was set aside for 30 min and filtered. The residue was washed with 5-fold 2 mL portions of water and dried under vacuum to give a pale yellow solid. The solid was dissolved in methyl *tert*-butyl ether (0.5 mL) and toluene (1.5 mL). The solution was concentrated to 1 mL at room temperature and heptane (1.5 mL) was added dropwise. The resulting slurry was set aside at room temperature for 1 h. The solid produced was collected by filtration, washed with 3-fold 1 mL portions of heptane and dried under vacuum to give a white odorless solid (amino alcohol).

2.3. Characterization of degradation products

Analytical HPLC were run on a HP1100 system (Agilent Technologies, Palo Alto, USA) equipped with a quaternary pump, an automatic injector, a diode array detector and a CH-500 column oven (Eppendorf AG, Hamburg, Germany). Chromatographic conditions (Table 1) were those previously described by Montgomery et al. [12]. Solutions of quinoline (200 μg/mL), amino alcohol (200 μg/mL) and efavirenz working standard (250 μg/mL) were prepared in acetonitrile–water (50:50, v/v). The relative retention times of any obtained peak (regarding efavirenz peak) along with the percentage area (relative to area sum of all peaks) were registered for each substance.

Melting points of quinoline and amino alcohol were determined by capillary method on a FP62 optoelectronic sensor (Mettler-Toledo GmbH, Greifensee, Switzerland).

Table 1
Chromatographic conditions[a] for efavirenz related compounds analysis

Column	Zorbax® SB-CN, 15 cm × 4.6 mm i.d. (5 μm)
Sample solvents	Acetonitrile–water (50:50, v/v)
Mobile phases	Solvent A: water–methanol–trifluoracetic acid (90:10:0.05, v/v/v) Solvent B: water–methanol–trifluoracetic acid (10:90:0.05, v/v/v)
Gradient program	Linear gradient from 60:40 (A:B) to 50:50 over 16 min, then to 35:65 over 7 min, then to 30:70 over 5 min, then to 20:80 over 1 min. Hold for 2 min and return to 60:40 over 1 min. Re-equilibrate for 8 min before next injection
Flow rate	1.5 mL/min
Injection volume	35 μL
Column temperature	40 °C
Detector	UV, 250 nm

[a] As in Montgomery et al. [12].

Infrared absorption spectra of potassium bromide solid dispersions of quinoline and amino alcohol (1:120, w/w) were recorded on a Spectrum 1000 spectrophotometer (Perkin-Elmer Inc., Wellesley, USA) in the 4000–400 cm^{-1} range.

The ^{1}H NMR (200 MHz) and ^{13}C NMR (50 MHz) spectra were recorded on a DPX 200 NMR spectrometer (Bruker Biospin GmbH, Rheinstetten, Germany). Aliquots of quinoline and amino alcohol were dissolved in deuterated chloroform ($CDCl_3$). Chemical shifts are given in δ units with respect to TMS and coupling constants (J) are in Hz.

Ultraviolet absorption spectra of methanol solutions of quinoline (0.001%, w/v) and amino alcohol (0.002%, w/v) were achieved on a UV-160A spectrophotometer (Shimadzu Corporation, Kyoto, Japan) in the 400–200 nm range.

The mass spectra of quinoline and amino alcohol were achieved on a Micromass Quattro LC mass spectrometer (Waters Corporation, Milford, USA) equipped with an electrospray (ES) source operated in the positive ionization mode (ES+). Quinoline and amino alcohol solutions (500 ng/mL) were prepared in acetonitrile-formic acid 0.1% (50:50, v/v) and directly introduced into the ion spray source at a constant flow rate of 10 μL/min. The cone voltage/collision energy was, respectively, 60 V/30 eV for quinoline and 20V/10 eV for amino alcohol. *MS scan* and *daughter scan* were achieved for each substance. The mass/charge ratio (*m/z*) was registered for precursor and fragment ions.

2.4. Amino alcohol assay

Solutions of amino alcohol (200 μg/mL) and quinoline (2 μg/mL) were prepared in acetonitrile-water (50:50, v/v). The solutions were analyzed by HPLC as described in Table 1.

2.5. Quantitation of related compounds in efavirenz drug substance and tablets

For quantitation of amino alcohol and quinoline in the samples, analytical curves were constructed preparing seven standard concentrations of each impurity ranging from 0.125 to 3.125 μg/mL (0.05–1.25% of target) in acetonitrile–water (50:50, v/v). Solutions of efavirenz working standard (1.25 μg/mL, 0.5% of target), efavirenz drug substance (250 μg/mL) and efavirenz tablets (250 μg/mL) were prepared in the same solvent. All solutions were immediately injected in the chromatographic system as described in Table 1, except that column temperature was set at 30 °C instead of 40 °C. Efavirenz sample solutions were also reinjected 24 h after preparation to check solution stability.

3. Results and discussion

3.1. Preparation and purification of degradation products

The alkaline hydrolysis conditions employed were based in efavirenz degradation data previously reported [12,14]. Kinetics and mechanism of efavirenz hydrolysis have been described by Maurin et al. [14]. In such work, the amino alcohol and quinoline were reported as the main hydrolysis products. However, the degradation products have not been isolated by the authors.

TLC results indicated total consumption of efavirenz and production of two degradation products after 24 h of reaction. The retention factors (R_f) under described conditions were 0.49, 0.54 and 0.68 for efavirenz, amino alcohol and quinoline, respectively. The proposed scheme for alkaline hydrolysis of efavirenz is shown in Fig. 1.

In the employed conditions, the efavirenz benzoxazinone ring is cleaved to give amino alcohol and carbon dioxide. Amino alcohol dehydrates and cyclizes to give quinoline or loses a proton to give a sodium alkoxide. Quinoline crystallizes in condenser by vapor drag or precipitates in the bulk, being separated by filtration. The amino alcohol precipitates in the mixture after acidification with glacial acetic acid and is separated by filtration. Recristallization conditions for amino alcohol were performed according to described in Pierce et al. [15].

Circa of 60 mg of quinoline and 77 mg of amino alcohol were obtained, corresponding to 35 and 42% yield, respectively.

3.2. Characterization of degradation products

Relative retention times (t_{RR}) as well as relative percentage areas of peaks registered for quinoline and amino alcohol are presented in Table 2. The t_{RR} of the peak registered for quinoline

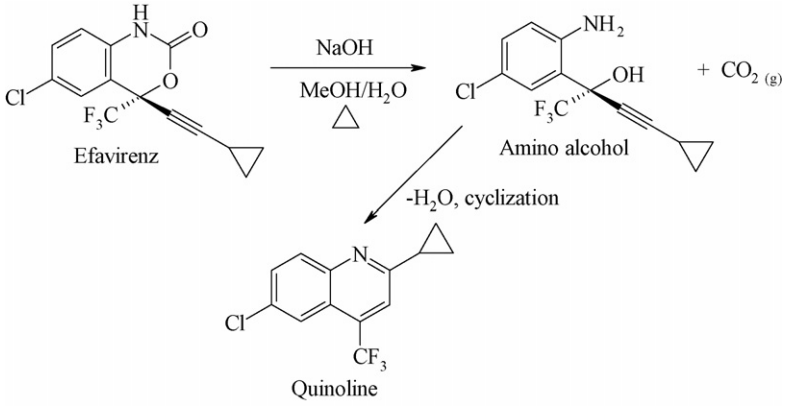


Fig. 1. Reaction scheme proposed for preparation of amino alcohol and quinoline from efavirenz alkaline hydrolysis.

Table 2
Results of relative retention times (t_{RR}) and relative percentage areas (%Area) of peaks registered for quinoline and amino alcohol

t_{RR}[a]	Quinoline		Amino alcohol	
	Area (mAU s)	Area[b] (%)	Area (mAU s)	Area[b] (%)
0.5	–	–	5653.2	96.05
1.4	22159.9	100.00	232.68	3.95
Total	22159.9	100.00	5885.9	100.00

[a] t_{RR}, relative retention time of any registered peak regarding efavirenz peak.
[b] %Area = 100(A_i/A_t), in which A_i is the individual area of any registered peak, and A_t is the sum of the areas of all registered peaks.

was consistent with that previously reported for this compound [12]. No chromatographic impurities were detected for quinoline. The t_{RR} of the main peak registered for amino alcohol was consistent with that previously reported for this compound; nevertheless a small fraction of quinoline was found as a chromatographic impurity.

The melting point obtained for quinoline (61.8–62.0 °C) was very close to the reference range (62.1–63.9 °C) previously reported for this compound [16]. The melting point of amino alcohol was 132.8–133.7 °C while the reference range was found 141–143 °C [15]. The difference between values can be attributed to the presence of quinoline as impurity in the product.

For quinoline, IR absorption bands, as well as ^{1}H and ^{13}C NMR chemical shifts were: IR (KBr, cm^{-1}) 3022, 1618, 1495, 1409, 1317, 1130, 1080; ^{1}H (200 MHz, $CDCl_3$) δ 8.00–7.53 (m, 4H), 2.25 (br-s, 1H), 1.22 (br-s, 4H); ^{13}C (50 MHz, $CDCl_3$) δ 163.6, 147.5, 132.9, 131.3, 131.2, 123.1, 122.1, 118.4, 18.3, 11.4. Spectral data were consistent with those of quinoline chemical structure, previously reported for this compound [16].

For amino alcohol, IR absorption bands, ^{1}H and ^{13}C NMR chemical shifts were: IR (KBr, cm^{-1}) 3421, 3332, 3071, 2237, 1618, 1490, 1410, 1264, 1164, 1092; ^{1}H (200 MHz, $CDCl_3$) δ 7.49 (s, 1H), 7.09 (d, 1H, J = 8.45), 6.58 (d, 1H, J = 8.45), 4.53 (br-s, 3H), 1.38 (br-s, 1H), 0.91–0.82 (m, 4H); ^{13}C (50 MHz, $CDCl_3$) δ 143.5, 130.6, 130.2, 124.0, 121.2, 120.9, 93.7, 75.3, 70.7, 8.7, −0.4. Spectral data were consistent with those of amino alcohol chemical structure previously reported for this compound [15].

The UV spectra obtained for amino alcohol showed absorption maxima at 207, 250 and 309 nm, and minima at 222 and 276 nm. For quinoline, absorption maxima were observed at 212, 243, 326 and 339 nm, and minima at 225, 299 and 333 nm. No UV spectral data were found for amino alcohol and quinoline in the literature searched to the best of our knowledge. These data presented can be set as future reference.

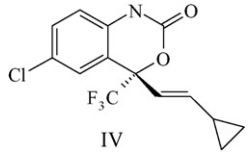
Full scan mass spectrum of amino alcohol showed protoned molecular ion [M + H$^+$] at m/z 290 consistent with amino alcohol precursor ion (elemental composition of $C_{13}H_{11}NOClF_3$). The most abundant ion in daughter scan spectrum was at m/z 272 corresponding to the loss of 18 Da (H_2O). The fragment mass is consistent with elemental composition of $C_{13}H_9NClF_3$ which corresponds to protoned quinoline molecule, a known dehydration-cyclization product of amino alcohol. Full scan mass spectrum of quinoline showed protoned molecular ion [M + H$^+$] at m/z 272 consistent with quinoline precursor ion (elemental composition of $C_{13}H_9NOClF_3$). Daughter scan spectrum showed fragment ion at m/z 244 and 189 corresponding to the loss of 28 and 83 Da, respectively. No assignments to these fragments were attempted.

3.3. Amino alcohol assay

Amino alcohol was obtained along with the quinoline impurity. To determine the amino alcohol content of the obtained product, it was assayed for the presence of quinoline using the quinoline obtained from efavirenz hydrolysis as reference standard; the amino alcohol content was determined by difference (%amino alcohol = 100 − %quinoline). The strength of amino alcohol obtained from efavirenz hydrolysis was 98.74%, as determined from the area values registered for amino alcohol (200 μg/mL) and quinoline (2 μg/mL) solutions.

3.4. Quantitation of related compounds in efavirenz drug substance and tablets

The column temperature of the chromatographic method used was changed to 30 °C instead 40 °C to improve the separation of efavirenz and a critical impurity as previously described by a study of the same research group from Montgomery et al. [13]. This impurity (IV) differs from efavirenz in that it contains a *trans*-alkene bond while efavirenz contains a triple bond.



IV

A summary of linear regression data of analytical curves constructed for amino alcohol and quinoline is given in Table 3. The correlation coefficients were greater than 0.9999 and the y-intercepts were statistically not different from zero ($p > 0.05$). In addition, the residuals were randomly scattered around zero, which shows a good fit with the linear model.

The mean area values registered for the impurities detected in the drug substance and tablet samples are presented in Table 4. All relative standard deviations were adequate for related com-

Table 3
Linear regression data for amino alcohol and quinoline analytical curves ranging from 0.125 to 3.125 μg/mL (0.05–1.25% of target)

Compound	Slope [mAU s/(μg/mL)]	Slope S.E.[a]	y-Intercept (mAU s)	y-Intercept S.E.[a]	p-Value for intercept	Correlation coefficient
Amino alcohol	15.7741	0.0392	0.0485	0.0688	0.4942	0.99996
Quinoline	126.369	0.3733	−0.436	0.6557	0.5190	0.99995

[a] S.E., standard error.

Table 4
Results of impurities percentage in efavirenz drug substance (batches PL03101501 and PL03101701) and tablets (Stocrin® 600 mg batch 144NA) samples

Sample	t_{RR}	Impurity[a]	QF[b]	Area (mAU s)	R.S.D. (%) ($n = 3$)	Impurity (%)
PL03101501	0.5	SD573	–	1.6627	2.39	0.04
	0.9	SR695	1.1	12.188	1.19	0.08
	1.1	SP234	1.0	6.5975	2.29	0.04
	1.3	SM097	–	20.557	1.33	0.07
	1.7	SE563	1.4	3.5579	4.70	0.03
Total						0.26
PL03101701	0.5	SD573	–	1.3028	4.18	0.03
	0.9	SR695	1.1	26.123	0.83	0.18
	1.1	SP234	1.0	6.5668	1.60	0.04
	1.3	SM097	–	16.9449	1.28	0.06
	1.7	SE563	1.4	6.9335	0.35	0.06
Total						0.37
Tablets	1.1	SP234	1.0	6.646	2.04	0.04
Total						0.04

[a] Impurities codes as previously reported for each compound [12], wherein SD573 and SM097 correspond to amino alcohol and quinoline, respectively.
[b] QF = 1/RF, in which RF is the response factor as calculated from [related substance peak area at 1.25 μg/mL]/[efavirenz peak area at 1.25 μg/mL] ratio. Values presented are those previously reported for each compound [12].

pounds assay (<5%). The identification codes and QF used are those previously reported for each compound [12]. The percentage of amino alcohol and quinoline (SD573 and SM097 codes, respectively) in the samples were calculated from the analytical curve equations. The concentrations of other related compounds [SR695 (*trans*-alkene impurity), SP234 and SE563] were calculated from the peak response of 1.25 μg/mL efavirenz standard solution applying the appropriate QF. The chemical structures of the impurities SP234 and SE563 were not found in the literature searched.

It has been reported that efavirenz and related compounds solutions were stable for 7 or more days in a previous work [12]. In the present study, sample solutions were injected immediately and 24 h after preparation to confirm stability. The results are depicted by the representative chromatograms obtained for efavirenz drug substance batch PL03101501, shown in Fig. 2. It was observed that, although the peak areas of efavirenz and SR695, SP234 and SE563 impurities did not change, the amino alcohol and quinoline peak areas increased significantly over 24 h. The latter two impurities are known to be the primary degradation products of efavirenz. Therefore, the solutions prepared for efavirenz related compounds assay should be analyzed immediately after preparation.

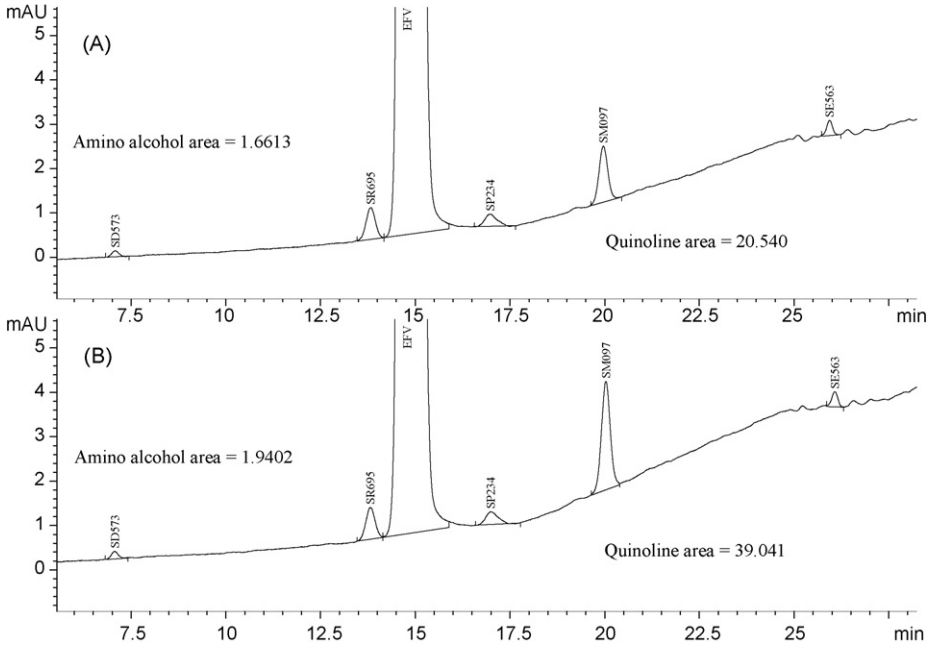


Fig. 2. Representative chromatograms registered for efavirenz sample solution (batch PL03101501) immediately (A) and 24 h (B) after preparation.

Table 5
Amino alcohol and quinoline quantitation factors (QF) calculated from experimental data and values reported in literature

Compound	Reported QF[a]	Calculated QF
Amino alcohol	3.8	4.1
Quinoline	0.51	0.51

[a] As in literature [12].

3.5. Quantitation factors for amino alcohol and quinoline

Amino alcohol and quinoline quantitation factors calculated from analytical curve slopes and from the peak response of 1.25 μg/mL efavirenz standard solution are presented in Table 5. The QF calculated for quinoline was identical to that previously reported for this compound. However, the QF calculated for amino alcohol was higher than that reported [12]. Therefore, if the reported QF was applied, the percentage of amino alcohol in the analyzed samples would have been underestimated. This reinforces the requirement of determining the amino alcohol quantitation factor in the same instrument on a routine basis.

4. Conclusion

The proposed efavirenz alkaline hydrolysis procedure allowed for the preparation of efavirenz primary degradation products, the amino alcohol and quinoline related compounds. Quinoline was obtained with a high purity degree (100%) and amino alcohol was 98.74% pure. Both impurity substances were successfully used as references for quantitative analysis of amino alcohol and quinoline in two batches of efavirenz drug substance. In addition, it was verified that sample solutions for efavirenz related compounds assay should be analyzed immediately after preparation, since amounts of amino alcohol and quinoline can significantly increase over 24 h.

Acknowledgements

The authors would like to thank Mrs. M.A.F. Prado, Ph.D., for NMR spectra, Mrs. S.M. Lucas da Silva, M.Sc., for mass spectra and Mrs. C.D. Vianna-Soares, Ph.D., for manuscript review. Labogen S.A., for supplying efavirenz reference standard and drug substance samples and the Brazilian Ministry of Health, for supplying efavirenz tablets samples are also thanked. This research was supported by the Brazilian Pharmacopoeia.

References

[1] S.D. Young, S.F. Britcher, L.O. Tran, L.S. Payne, W.C. Lumma, T.A. Lyle, J.R. Huff, P.S. Anderson, D.B. Olsen, S.S. Carroll, D.J. Pettibone, J.A. O'Brien, R.G. Ball, S.K. Balani, J.H. Lin, I.W. Chen, W.A. Schleif, V.V. Sardana, W.J. Long, V.W. Byrnes, E.A. Emini, Antimicrob. Agents Chemother. 39 (1995) 2602–2605.
[2] J. Galvão, Lancet 360 (2002) 1862–1865.
[3] P. Chequer, Third International AIDS Society Conference on HIV Pathogenesis and Treatment Oral presentation, Rio de Janeiro, 2005, http://www.ias-2005.org/planner/Presentations/ppt/3333.ppt.
[4] The sustainability of universal access to antiretroviral medicines in Brazil, Brazilian Ministry of Health, National STD/AIDS Program, National Health Council, Brasília, 2005. Available at http://www.aids.gov.br/data/documents/storedDocuments/%7BB8EF5DAF-23AE-4891-AD36-1903553A3174%7D/%7B6AB55CB8-AD0A-42C7-BE9C-1E86CFE66F40%7D/Cons._nacional_sustainability.doc.
[5] General Notices, USP 28-NF 23, United States Pharmacopeia, Rockville, 2005, p. 3–12.
[6] ICH, Q3A(R1): Impurities in New Drug Substances (Revised Guideline), Geneva, 2002.
[7] ICH, Q3B(R1): Impurities in New Drug Products (Revised Guideline), Geneva, 2003.
[8] ICH, Q3C(R3): Impurities: Guideline For Residual Solvents, Geneva, 1997.
[9] Food and Drug Administration, Center for Drug Evaluation and Research. Guidance for Industry (Draft Guidance), ANDAs: Impurities in Drug Substances, Rockville, 2004.
[10] J. Roy, AAPS PharmSciTech. 3 (2002) article 6. Available at http://www.aapspharmscitech.org/.
[11] Food and Drug Administration, Center for Drug Evaluation and Research. Guidance for Industry (Draft Guidance), ANDAs: Analytical Procedures and Methods Validation, Rockville, 2000.
[12] E.R. Montgomery, A.L. Edmanson, S.C. Cook, P.K. Hovsepian, J. Pharm. Biomed. Anal. 25 (2001) 267–284.
[13] R.P. Weissburg, E.R. Montgomery, L.A. Junnier, J. Segretario, S. Cook, P.K. Hovsepian, J. Pharm. Biomed. Anal. 28 (2002) 45–46.
[14] M.B. Maurin, S.M. Rowe, K. Blom, M.E. Pierce, Pharm. Res. 19 (2002) 517–521.
[15] M.E. Pierce, R.L. Parsons Jr., L.A. Radesca, Y.S. Lo, S. Silverman, J.R. Moore, Q. Islam, A. Choudhury, J.M.D. Fortunak, D. Nguyen, C. Luo, S.J. Morgan, W.P. Davis, P.N. Confalone, C. Chen, R.D. Tillyer, L. Frey, L. Tan, F. Xu, D. Zhao, A.S. Thompson, E.G. Corley, E.J.J. Grabowski, R. Reamer, P.J. Reider, J. Org. Chem. 63 (1998) 8536–8543.
[16] B. Jiang, Y.G. Si, J. Org. Chem. 67 (2002) 9449–9451.