International Scholars Journals

International Journal of Pharmacy and Pharmacology ISSN 2326-7267 Vol. 14 (1), pp. 001-007, January, 2025. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Utilizing Fluorine-19 NMR for Accurate Measurement of Efavirenz in Serum and Pharmaceuticals

Mojtaba Shamsipur[1]*, Masoumeh Sarkouhi[2], Jalal Hassan[3] and Soheila Haghgoo[4]

[1]Department of Chemistry, Razi University, Kermanshah, Iran.
[2]Department of Chemistry, Tarbiat Modares University, Tehran, Iran.
[3]Department of Toxicology, School of Veterinary Medicine, University of Tehran, Tehran, Iran.
[4]Food and Drug Quality Control Laboratory, Ministry of Health and Medical Education, Tehran, Iran.

Accepted 16 September, 2024

^{19}F NMR provided a highly specific tool for identification of efavirenz in human serum and pharmaceutical preparations as well as a suitable analytical technique for their absolute quantification. The method is based on the integration of appropriate signals of efavirenz and potassium fluoride, as an internal standard. The proposed method is simple and reliable, but when it is used without any sample pretreatment, there is a manipulation of large sample volumes and lengthy analysis time. The calibration curve for efavirenz in human serum was linear over the range of 0.8 to 1000 mg L^{-1}, with detection limit of 0.3 mg L^{-1}. The mean error on human serum samples ranged from about - 4 to -6%, with relative standard deviations of <8%. The method was also applied successfully for the determination of efavirenz in real pharmaceutical samples, and compared with the results obtained by a reference high-performance liquid chromatography-ultraviolet (HPLC-UV) method.

Key words: Efavirenz, ^{19}F NMR analyses, HIV, serum, pharmaceuticals.

INTRODUCTION

Efavirenz is chemically described as (S)-6-chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one. Its empirical formula is $C_{14}H_9ClF_3NO_2$ with the structural formula shown in Figure 1. It is a white to slightly pink crystalline powder that is practically insoluble in water (< 10 mgL^{-1}). Efavirenz, as a non-nucleoside reverse transcriptase inhibitor (NNRTI), is being increasingly used since 1998 in association with other antiretroviral agents in the treatment of HIV infection (Csajka et al., 2003). Its long half-life allows once-daily dosing and, therefore, presents an advantage for the compliance and efficacy of treatment. Efavirenz is the preferential choice to treat HIV-tuberculosis (TB) co-infected patients (Katijah, 2010). Management of both of these diseases is complicated due to pharmacological drug-drug interactions between efavirenz and rifampicin, which is a first-line anti-TB drug and a potent inducer of the cytochrome P-450 system (Ramachandran et al., 2006). Treatment failure and central nervous system side effects have been found to be associated with low and high efavirenz plasma levels. Thus, monitoring of efavirenz levels in plasma could be useful in the clinical management of HIV disease, especially in HIV-TB co-infected patients, who are being treated with efavirenz and rifampicin concomitantly (Novakova and Vlckova, 2009).

Several analytical methods for the analysis of efavirenz either alone or in combination with other drugs in plasma/serum have been published using reversed-phase HPLC- UV (Aymard et al., 2000; Choi et al., 2007; Kappelhoff et al., 2003; Lakshmi et al, 2007; Langmann et al., 2001; Notari et al., 2006; Rao et al.,.2009; Rezk et al., 2004; Sarasa- Nacenta et al., 2001; Simon et al., 2001; Turner et al., 2003; Usami et al., 2003; Weller et al., 2007) liquid chromatography coupled to mass

*Corresponding author. E-mail: mshamsipur@yahoo.com. Tel: +98 21 66908032.

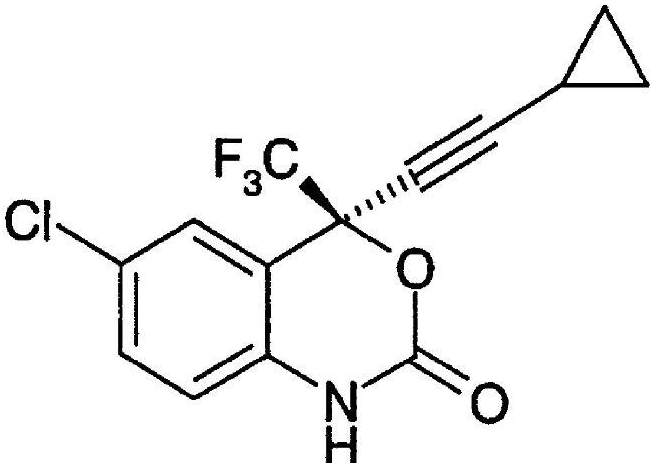

Figure 1. Structural formula of efavirenz.

spectrometry (LC-MS) (Rouzes et al., 2004), HPLC with fluorescence detection (Matthews et al., 2002) and a capillary electrophoresis method (Pereira et al., 2005). Since the sample preparation and pre-concentration are usually a critical part of high put quantitative bioanalysis, various methods such as liquid–liquid extraction (Ramachandran et al., 2006; Lakshmi et al., 2007; Langmann et al., 2001), solid phase extraction (Aymard et al., 2000; Rezk et al., 2004; Sarasa-Nacenta et al., 2001; Simon et al.; 2001) and a protein precipitation method followed by a dilution step with distilled water (Kappelhoff et al., 2003) have been used to extract efavirenz from plasma.

Nuclear magnetic resonance (NMR) has gained widespread acceptance over recent years as a most versatile tool for industrial drug research (Aman et al., 2005; Gil Garcya et al., 2006; Ellis et al., 2000; Lenz et al., 2002; Shamsipur et al., 2002, a, b; Talebpour et al., 2003a, b, 2004a, b; Wenyi et al., 2006). Since fluorine atoms are not naturally present in biological fluids, the ^{19}F NMR spectra of these matrices containing desirable F-substituted analytes and their metabolites are generally highly simplified, in comparison with the corresponding ^{1}H NMR spectra. In addition, in the case of ^{1}H NMR spectra, a water suppression method is also necessary for the analysis of pharmaceuticals in biological fluid matrices, to eliminate the intense water signal observed in these matrices.

As a basis for quantitative determination of fluorinated species, ^{19}F NMR offers several potential advantages compared to chromatography; these include nuclear spin of 1/2 , relatively narrow lines, 100% natural abundance, high sensitivity for fluorine-19 nucleus (83% that of proton) and correct sensitivity, large chemical shift range (300 ppm), which minimizes signal overlap used as a powerful, universal and fast-screening technique to evaluate the quality and quantity of drugs (Hull et al., 1988; Lenz et al., 2002; Shamsipur et al., 2007a, b). An important advantage, specific to ^{19}F NMR, is that the resonances of fluorine nuclei can be observed without problems of interfering background signals, since the level of endogenous fluorine-containing compounds is very low (Hull et al., 1988).

A number of previously published results revealed that ^{19}F NMR spectroscopy could be employed as a powerful selective tool to analyze different fluorinated drugs (Lenz et al., 2002; Malet- Martino et al., 2006; Martino et al., 2005; Shamsipur et al., 2003, 2007a, b; Talebpour et al., 2003a). In this work, the presence of three fluorine atoms in the efavirenz structure enabled us to use ^{19}F NMR for the selective and sensitive analysis of drug contents of human serum samples and pharmaceutical formulations. The experimental parameters for ^{19}F NMR analysis of efavirenz were selected to optimize the NMR method with respect to accuracy, precision and analysis time. In addition, the salt assisted solvent extraction was also used for extraction of efavirenz from plasma followed by its HPLC-UV determination, for confirmation of the ^{19}F NMR results.

MATERIALS AND METHOD

Chemicals and reagents

Pure reference standard of efavirenz with chemical purity of >99.3% was obtained from Food and Drug Quality Control Laboratory, Ministry of Health and Medical Education (Tehran, Iran) and was used without any further purification. Potassium fluoride, as internal standard, HPLC grade acetonitrile, ammonium acetate and methanol were supplied by Merck (Darmstadt, Germany). Deuterium oxide and perdeuterated methanol, CD_3OD, (>99.8%) were purchased from Fluka (Buchs, Switzerland). The efavirenz tablet was obtained from Bakhtar Biochemical Pharmaceutical Company (Kermanshah, Iran) and drug-free human plasma and serum were obtained from the Pathobiology Laboratory Center (Tehran, Iran).

Apparatus

All ^{19}F NMR spectra were recorded on a BRUKER DRX 500 AVANCE (11.7 T) spectrometer operating at 470.59 MHz ^{19}F observation frequency, equipped with a dedicated 5-mm QNP probe head and running XWIN-NMR 2.6 software, using 500 µl of samples. In all experiments, a known amount of CD_3OD or D_2O was added as an internal field frequency lock. The spectra were acquired using 90° pulses with 16 to 512 scans collected into 128 k data points over a spectral width. The 90° pulse width was measured to be 12.10 µs for human serum. The acquisition time was 0.332 s followed by a relaxation delay of 10 s, to ensure full T_1 relaxation The spectra were recorded at 300 K and ^{19}F NMR chemical shifts were reported relative to trichlorofluoromethane ($CFCl_3$) at $_F$ = 0.0 ppm. The NMR processing for final solutions of all samples included phase correction (performed manually for each replicate) and baseline correction over the entire spectral range. In all instances, the baseline was additionally corrected over the integrated regions. Areas of the peaks were determined by electronic integration of the expanded regions around diagnostic resonances, using an integral limit of ± 20 Hz around the corresponding signals. The percentage of error (reported in %RSD) was found to be less than 1%.

The analytical chromatographic system used consisted of an Agilent 1200 series vacuum degasser, an automatic sample injector, a quaternary pump, a variable wavelength detector (VWD) (all from Agilent Technologies, Palo Alto, USA), and a Symmetric C18 column, 5 µm particle diameter, 100 Å pore diameter, 4.6 mm i.d., 250 mm length, with guard column (Waters Corporation, Massachusetts, U.S.A.) and controlled by a computer running Chem Station software (Agilent Technologies). Mobile phases were filtered through a Millipore 0.22-µm membrane filter before use. The column was stabilized at 25 ± 2°C (room temperature) for 1 h before chromatography. A flow-rate of 1.0 ml min^{-1} was applied at laboratory temperature. The mobile phase was a 50:50, v/v mixture of acetonirile-0.5% ammonium acetate in water (pH = 7.50 ± 0.05) and the detection wavelength was 252 nm.

Dissolution of efavirenz tablets

A pharmaceutical formulation containing 600 mg of efavirenz was available as film- coated tablets for oral administration, and its active ingredients were analyzed using the proposed method. For sample preparation, 5 tablets were weighed, powdered and thoroughly mixed. An accurately weighed portion of the well-mixed powder was then dissolved in an appropriate volume methanol. The content of the flask was sonicated for about 10 min and then the solution was finally filtered.

Determination of efavirenz by ^{19}F NMR

Stock solutions of efavirenz and the internal standard (potassium fluoride) were prepared separately at concentrations of 1000 and 3000 mg L^{-1} in methanol, respectively.

In the case of tablet analysis, the calibration samples were prepared by appropriate diluting of the stock solutions in methanol to final concentrations of 0.6 to 1000 mg L^{-1} of efavirenz and 30 mg L^{-1} potassium fluoride as internal standard in methanol (400 µl final volume). In all NMR measurements, 100 µl (20% v/v of final volume) of CD_3OD was added to lock the field frequency of the instrument. The appropriate volumes of solutions obtained from dissolution of tablet with internal standard and D_2O were then transferred into analytical NMR tubes (500- µl) and the spectra were recorded. In all cases, three determinations were performed.

In the case of serum analysis, 300 µl portions of drug-free serum samples from healthy donors were used for calibration and validation studies. The calibration of serum samples was carried out by spiking appropriate amounts of the stock solutions of efavirenz and internal standard, to yield spiked solutions of 0.8 to 1000 mg L^{-1} efavirenz containing 30 mg L^{-1} internal standard of in deuterium oxide, with a volumes of 400 µl final volume, for all samples. Then 100 µl D_2O was added to the thus prepared human serum samples to lock the field frequency of the instrument. For the construction of each calibration curve, the spiked serum samples were analyzed in duplicate. Serum samples spiked with different concentrations of the analyte (that is, 25, 50 and 100 mg L^{-1}) were prepared to test the accuracy and precision of the method.

Determination of efavirenz by HPLC-UV

The efavirenz in spiked plasma samples was extracted with salting out assisted-solvent extraction method (Zhang et al., 2010) and determined by HPLC-UV, as follows. The plasma sample (500 µl) was transferred into a 2.0 ml polypropylene micro-centrifuge tube. The sample was deproteinized by the addition of 600 µl acetonitrile, vortexed for 30 s and then added 500 µl saturated potassium carbonate and mixed. The supernatant fluid (450 µl) was transferred into another 2.0 ml polypropylene microcentrifuge tube and 20 µl of the solution was injected onto the HPLC column.

The assay of tablet was also performed by a standard USP method, based on determination with HPLC (U.S. Department of Health and Human Services, 2001).

Statistical analysis and method validation

The quantification of the efavirenz was performed using the internal standard method with the integration of fluorine-19 signal of the drug related to that of an internal standard (KF) in each calibration solution. The ^{19}F peak area ratios were plotted against the corresponding efavirenz concentration, and a linear regression variance analysis was performed to determine linearity and correlation coefficients. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated by using signal-to-noise (S/N) ratios of 3 and 10, respectively. The precision of proposed method, expressed as relative standard deviation (RSD), for the spiked serum samples at 50 and 110 µg mL^{-1} concentration levels (n=3 for each case) was studied. The accuracy was checked by utilizing the results obtained for a pharmaceutical sample (n=3) as compared with those obtained by the USP reference method (U.S. Department of Health and Human Services, 2001) and also the claimed amount, and the validity was checked by the *t*-test method.

RESULTS AND DISCUSSION

Evaluation of longitudinal relaxation time of efavirenz

The inversion recovery pulse sequence method was employed to measure the longitudinal relaxation time (T_1) of efavirenz, using the T_1 cal BRUKER program, via fitting the data to the exponential equation $I = I_0 + P \exp(-/T_1)$ (Shamsipur et al., 2007a), where I is the intensity of efavirenz resonance at inversion delay times (= 0, 10, 0.25, 0.75, 1.00, 2.50, 5.00, 7.50, 10.00, 15.00 and 20.00 s) and I_0 is that at the equilibrium state and P is a constant.. The T_1 value thus obtained for efavirenz was 1.69 ± 0.01 s.

Optimization of ^{19}F NMR parameters for the assay of efavirenz

The ^{19}F NMR spectrum of efavirenz, possessing a sharp singlet ^{19}F signal at - 82.6 ± 0.1 ppm, which is well separated from that of potassium fluoride, used as an internal standard, located at -150.8 ± 0.1 ppm in methanol and at 129.3 ± 0.1 ppm in human serum samples (Talebpour et al., 2003a). The high sensitivity of ^{19}F nucleus in conjunction with the wide range of fluorine chemical shifts (Aman et al., 2005; Lenz et al., 2002; Shamsipur et al., 2002, Wenyi et al., 2006) provided a suitable and simple method for the identification and determination of efavirenz.

To provide quantitative information needed for the efavirenz assay, different NMR data collection para-meters have been optimized. The magnitude and duration

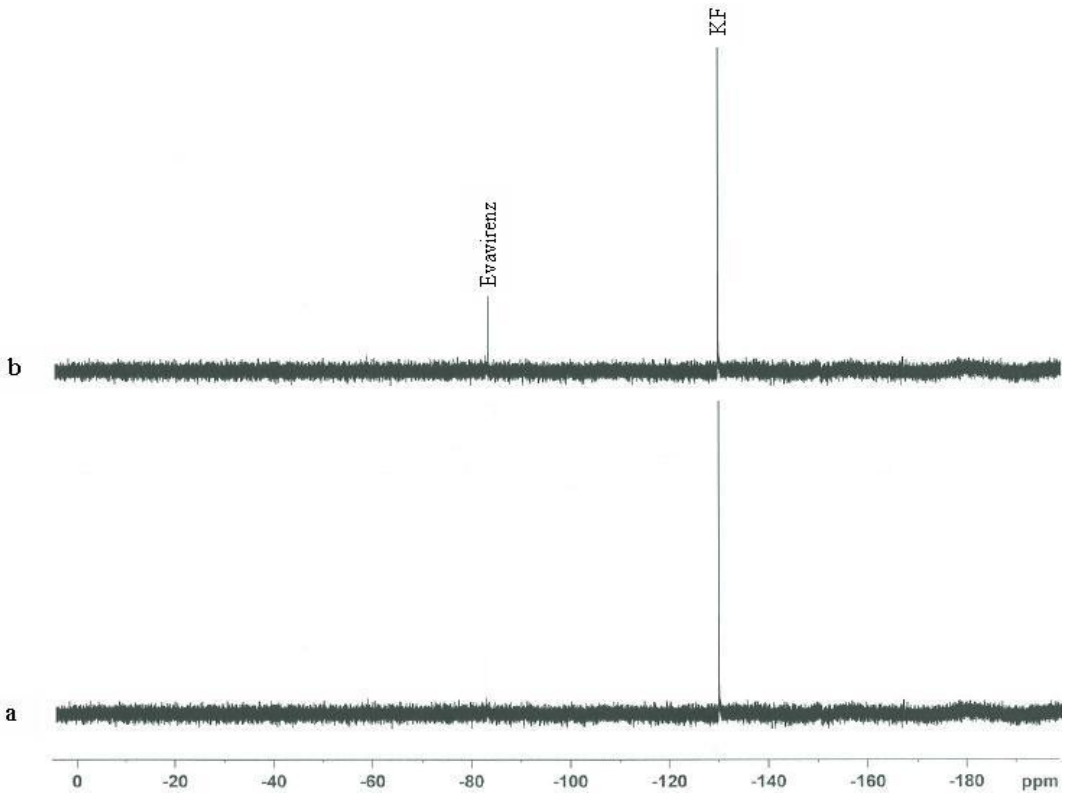

Figure 2. The ^{19}F NMR spectra of (a) blank human serum, and serum spiked with (b) 50 mg L^{-1}.

of the applied RF pulse are important parameters, which affect both the signal-to-noise ratio and the quantitative accuracy of the signal integrals (Shamsipur et al., 2007b). The other parameter for optimizing sensitivity is the recycling delay time (D_1), necessary to return all magnetizations to equilibrium between pulses (Wenyi et al., 2006) . This is obviously related to the spin-lattice relaxation time (T_1) of the nuclei.

To carry out quantitative determinations, we first measured the T_1 value of efavirenz by the inversion recovery pulse sequence method, and the resulting value found to be 1.69 ± 0.01 s. It has been suggested that a good approach for the quantitative analysis of complex mixtures containing nuclei with a short range of T_1 values is to use large pulse angles (70 to 90°) and a repetition time higher than the maximum T_1 by three- to five-fold. After performing optimization studies on the efavirenz, a standard pulse angle of 90° and a relaxation delay of 10 s associated with an acquisition time of 0.332 s were selected, which allowed the accurate quantification of efavirenz in real samples.

Application of the proposed method to the assay of efavirenz in human serum

Figure 2a shows the ^{19}F NMR spectrum of a blank serum sample, which contains no ^{19}F NMR signal in chemical shift windows employed for the quantitative studies of the efavirenz. The quantitative behavior of the proposed method was evaluated by spiking known concentrations of the analyte into control serum samples (Talebpour et al., 2003a). Figure 2b shows the ^{19}F NMR spectra of a serum sample spiked with 50 mg L^{-1} of efavirenz.

The calibration graph was constructed using a least-squares linear regression of the efavirenz-to-potassium fluoride integral ratios versus the corresponding concentration of the drug. The resulting regression equation, in the efavirenz concentration range of 0.8-1000 mg L^{-1}, found to be I_{EF}/I_{IS} = 0.1525C + 0.0873 (n = 7, r = 0.9981), where I_{EF}/I_{IS} is the efavirenz-to-potassium fluoride integral ratio and *C* is the efavirenz concentration (Table 1). The limit of detection (LOD), calculated from the calibration graph signal-to-noise (S/N) ratios of 3, was0.3 mg L^{-1}. This LOD would be obtained without any pre-concentration step.

Since the S/N ratio for a peak in the NMR spectrum is known to increase with scan number as the $(S/N)^{1/2}$ (Shamsipur et al., 2007b; Talebpour et al, 2003a), minimum quantitable limit of analyte may be attained with ^{19}F NMR by increasing the number of scans. The precision of proposed method, expressed as relative standard deviation (RSD), for the spiked serum samples at 50 and 110 µg ml^{-1} concentration levels was studied.

Table 1. Figures of merit for ^{19}F NMR and HPLC-UV methods.

Method	Equation	DLR (mg L^{-1})	LOD (mg L^{-1})	LOQ (mg L^{-1})	RSD
^{19}F NMR in methanol	I = 0.1525C + 0.0873 (r = 0.9998)	0.6-1000	0.2	0.6	1.1-7.8
^{19}F NMR in serum	I = 0.1235C + 0.0712 (r = 0.9981)	0.8-1000	0.3	0.8	2.1-4.5
HPLC-UV	A = 26.882C - 1.1982 (r = 0.9990)	0.1-250	0.03	0.1	0.5-3.0

DLR, Dynamic linear range; LOD, limit of detection; LOQ, limit of quantification; RSD, relative standard deviation.

Table 2. Analysis of efavirenz in human serum samples.

Method	Concentration added (mg L^{-1})	Mean concentration found ± %RSD (mg L^{-1})	Error (%)
HPLC-UV	2	1.9 ± 0.3	-5
	5	4.8 ± 0.6	-4
^{19}F NMR	50	48±8	-4
	110	103±6	-6

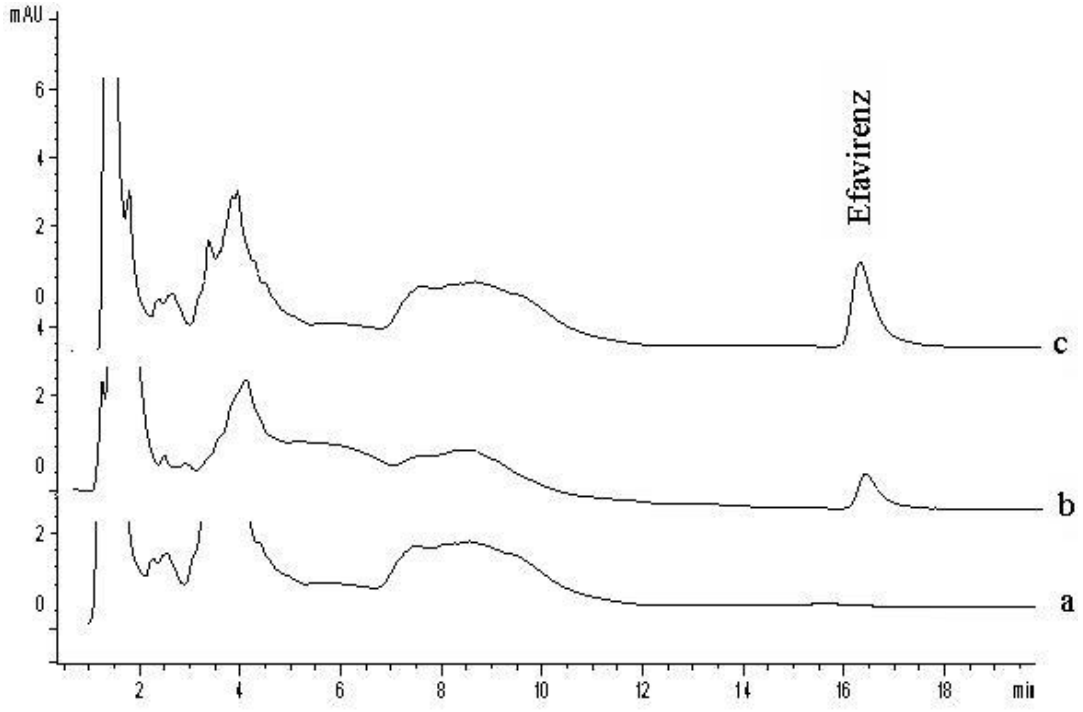

Figure 3. Typical chromatograms obtained from an extract of human blank plasma (a) and human blank serum spiked with 2.0 mg L^{-1} (b) and 5.0 mg L^{-1} efavirenz (c).

The obtained RSD values were at the most 6% and the recovery was found to be almost quantitative. A summary of results of the precision and accuracy experiments is given in Table 2.

For the confirmation of NMR results, we used HPLC-UV determination of the drug, following the salting out assisted liquid- liquid extraction of efavirenz from plasma (Zhang et al., 2010). Typical chromatograms obtained from an extract of human blank plasma and human blank serum spiked with 2.0 and 5.0 mg L^{-1} of efavirenz are shown in Figure 3. Calibration graphs were obtained using six points and coefficient of regression better than 0.999 was achieved. The figures of merit are also included in Table 1. Finally the proposed method was used for the determination of efavirenz in drug-free human serum and the results are summarized in Table 2.

Table 3. Analysis of efavirenz in pharmaceutical preparation.

Label claim (mg tablet^{-1})	Proposed NMR method (mg tablet^{-1})[a]	USP HPLC method (mg tablet^{-1})[a]
600	580 ± 15	590 ±10

[a] Mean ± %RSD (n = 3).

Assay of efavirenz in pharmaceutical formulations

The determination of efavirenz content of pharmaceutical samples was performed according to the procedure described previously, and the results are summarized in Table 3. The results showed that the contents of active ingredient of tablet and injection samples are consistent with their declared values. The relative standard deviations were less than 5%. Furthermore, the results obtained for three real samples are compared with those obtained by the USP reference method, which are also included in Table 3. The calculated t-test values did not exceed the theoretical values, indicating the absence of any significant difference in terms of precision and accuracy.

Conclusion

The proposed ^{19}F NMR spectroscopic method for efavirenz in pharmaceutical and human's serum samples, the determination is simple, fast, precise and selective. The minimum required sample volume is 300 μl, which makes the method applicable to clinical studies of the samples taken from infants and young children, or to the studies involving laboratory animals with small body blood volume. The total analysis time is less than 5 min, which is advantageous in daily routine monitoring of the drug. The assay covers the concentration range of interest and is suitable for pharmacokinetic studies in HIV infected patients.

REFERENCES

Aman R, Biehl J, Carle R (2005). Application of HPLC coupled with DAD, APcI-MS and NMR to the analysis of lutein and zeaxanthin stereoisomers in thermally processed vegetables. Food Chem., 92(4): 753-763.

Aymard G, Legrand M, Trichereau N, Diquet B (2000). Determination of twelve antiretroviral agents in human plasma sample using reversed-phase high-performance liquid chromatography. J. Chromatogr., B 744(2): 227–240.

Choi SO, Rezk NL, Kashub ADM (2007). High-performance liquid chromatography assay for the determination of the HIV-protease inhibitor tipranavir in human plasma in combination with nine other antiretroviral medications. J. Pharm. Biomed. Anal., 43(4): 1562–1567.

Csajka C, Marzolini C, Fattinger K, Decosterd LA, Fellay J, Telenti A, Biollaz J, Buclin T (2003). Population pharmacokinetics and effects of efavirenz in patients with human immunodeficiency virus infection, Clin. Pharmacol. Ther., 73(1): 20-30.

Ellis DA, Martin JW, Muir DC, Mabury A (2000). Development of an ^{19}F NMR method for the analysis of fluorinated acids in environmental water samples. Anal. Chem., 72(4): 726–731.

Gil Garcya MD, Martynez Galera M, Barranco Martynez D, Gallego G (2006). Determination of benzoylureas in ground water samples by fully automated on-line pre-concentration and liquid chromatography-fluorescence detection. J. Chromatogr., A1103(2): 271–277.

Hull WE, Port RE, Hermann R, Britsch B, Kunz W (1988). Metabolites of 5-fluorouracil in plasma and urine, as monitored by ^{19}F nuclear magnetic resonance spectroscopy, for patients receiving chemotherapy with or without methotrexate pretreatment. Cancer Res., 48(6): 1680–1688.

Kappelhoff BS, Rosing H, Huitema ADR, Beijnen JH (2003). Simple and rapid method for the simultaneous determination of the non-nucleoside reverse transcriptase inhibitors efavirenz and nevirapine in human plasma using liquid chromatography. J. Chromatogr., B 792(2): 353–362.

Katijah KS (2010) Is there a need for ototoxicity monitoring in patients HIV/AIDS? Afr. J. Pharm. Pharmacol., 4(9): 574-579.

Lakshmi SA, Kishore KK, Ravi KDVR, Mohan KC, Yugandhar NM, Srinubabu G (2007). Development and validation of a liquid chromatographic method for determination of efavirenz in human plasma. Chromatographia, 65(5-6): 359–361.

Langmann P, Schirmer D, Vath T, Zilly M, Klinker H (2001). High-performance liquid chromatographic method for the determination of HIV-1 non-nucleoside reverse transcriptase inhibitor efavirenz in plasma of patients during highly active antiretroviral therapy. J. Chromatogr., B 755(2): 151-156.

Lenz EM, Wilson ID, Wright B, Partridge EA, Rodgers CT, Haycock PR, Lindon JC, Nicholson JK (2002). A comparison of quantitative NMR and radiolabelling studies of the metabolism and excretion of Statil™ (3-(4-bromo-2-fluorobenzyl)-4-oxo-3H-phthalazin-1-ylacetic acid) in the rat. J. Pharm. Biomed. Anal., 28(1): 31-43.

Matthews CZ, Woolf EJ, Mazenko RS, Haddix-Wiener H, Chavez-Eng CM, Constanzer ML, Doss GA, Matuszewski BK (2002). Determination of efavirenz, a selective non-nucleoside reverse transcriptase inhibitor, in human plasma using HPLC with post-column photochemical derivatization and fluorescence detection. J. Pharm. Biomed. Anal., 28(5): 925–934.

Notari S, Bocedi A, Ippolito G, Narciso P, Pucillo L P, Tossini G, Donnorso RP, Gasparrini F, Ascenzi P (2006). Simultaneous determination of the HIV protease inhibitors indinavir, amprenavir, saquinavir, ritonavir, nelfinavir and the non-nucleoside reverse transcriptase inhibitor efavirenz by high-performance liquid chromatography after solid-phase extraction. J. Chromatogr., B 831(1-2): 258- 266.

Novakova L, Vlckova H (2009). A review of current trends and advances in modern bio-analytical methods: Chromatography and sample preparation, Anal. Chim. Acta, 656(1-2): 8-35.

Pereira EA, Micke GA, Tavares MFM (2005). Determination of antiretroviral agents in human serum by capillary electrophoresis. J. Chromatogr., A 1091(1-2): 169- 176.

Ramachandran G, Hemanth Kumar AK, Swaminathan S, Venkatesan P, Kumaraswami V, Greenblatt DJ (2006). Simple and rapid liquid chromatography method for determination of efavirenz in plasma. J. Chromatogr., B 835(1-2): 131-135.

Rao BU, Nikalje AP (2009). Stability- indicating HPLC method for the determination of efavirenz in bulk drug and in pharmaceutical dosage form. Afr. J. Pharm. Pharmacol., 3(12): 643-650.

Rezk NL, Tidwell RR, Kashuba ADM (2004). High-performance liquid chromatography assay for the quantification of HIV protease inhibitors and non-nucleoside reverse transcriptase inhibitors in human plasma. J. Chromatogr., B 805(2): 241-247.

Rouzes A, Berthoin K, Xuereb F, Djabarouti S, Pellegrin I, Pellegrin JL, Coupet AC, Augagneur S, Budzinski H, Saux MC, Breilh D (2004).

Simultaneous determination of the antiretroviral agents: amprenavir, lopinavir, ritonavir, saquinavir and efavirenz in human peripheral blood mononuclear cells by high-performance liquid chromatography–mass spectrometry J. Chromatogr., B 813(1-2): 209-216.

Sarasa-Nacenta M, López-Púa Y, López-Cortés LF, Mallolas J, Gatell JM, Carné X (2001). Determination of efavirenz in human plasma by high-performance liquid chromatography with ultraviolet detection. J. Chromatogr., B 763(1): 53-59.

Simon VA, Thiam MD, Lipford LC (2001). Determination of serum levels of thirteen human immunodeficiency virus-suppressing drugs by high-performance liquid chromatography. J. Chromatogr., A 913(1-2): 447-453.

Shamsipur M, Talebpour Z, Bijanzadeh HR, Tabatabaei S (2002). Monitoring of ampicillin and its related substances by NMR, J. Pharmaceut. Biomed. Anal., 30(4): 1075-1085.

Shamsipur M, Shafiee Dastjerdi L, Haghgoo S, Armspach D, Matt D, Aboul-Enein HY (2007a). Chiral selectors for enantioresolution and quantitation of the antidepressant drug fluoxetine in pharmaceutical formulations by ^{19}F-NMR spectroscopic method. Anal. Chim. Acta, 601(1): 130-138.

Shamsipur M, Shafiee-Dastgerdi L, Talebpour Z, Haghgoo S (2007b). ^{19}F NMR as a powerful technique for the assay of anti-psychotic drug haloperidol in human serum and pharmaceutical formulations. J. Pharm. Biomed. Anal., 43(3): 1116-1121.

Talebpour Z, Haghgoo S, Shamsipur M (2003). A selective ^{19}F nuclear magnetic resonance spectroscopic method for the assay of the neuroleptic drug cis(Z)-flupentixol in human serum, Anal. Biochem., 323: 205-210.

Talebpour Z, Maesum S, Jalali-Heravi M, Shamsipur M (2003). Simultaneous determination of theophylline and caffeine by proton magnetic resonance spectroscopy using partial least squares regression techniques, Anal. Sci., 19(6): 1079-1082.

Talebpour Z, Bijanzadeh HR, Haghgoo S, Shamsipur M (2004). Qualitative and quantitative determination of caffeine and theophylline in pharmaceutical preparations and human serum by using NMR spectroscopy, Chem. Anal., (Warsaw), 49(2): 369-381.

Talebpour Z, Haghgoo S, Shamsipur M (2004). ^{1}H nuclear magnetic resonance spectroscopy analysis for simultaneous determination of levodopa, carbidopa and methyldopa in human serum and pharmaceutical formulations. Anal. Chim. Acta, 506(2): 97-104.

Turner ML, Reed-Walker K, King JR, Acosta EP (2003). Simultaneous determination of nine antiretroviral compounds in human plasma using liquid chromatography. J. Chromatogr., B 784(2): 331-341.

U.S. Department of Health and Human Services. FDA. Guidance for Industry Bioanalytical Method Validaton (2001) http://www.fda.gov/CDER/ GUIDANCE/4252fnl.htm.

Usami Y, Oki T, Nakai M, Sagisaka M, Kaneda T (2003). A simple HPLC method for simultaneous determination of lopinavir, ritonavir and efavirenz. Chem. Pharm. Bull., 51(6): 715-718.

Weller DR, Brundage RC, Balfour J, Henry H, Vezina HE (2007). An isocratic liquid chromatography method for determining HIV non-nucleoside reverse transcriptase inhibitor and protease inhibitor concentrations in human plasma. J. Chromatogr. B., 848(2): 369-373.

Wenyi H, Fengpei D, Yan W, Yinghong W, Xin L, Hongyue L, Xiaodong Z (2006). Quantitative 19F NMR method validation and application to the quantitative analysis of a fluoro-polyphosphates mixture. Fluorine Chem., 127(6): 809-815.

Zhang J, Rodila R, Gage E, Hautman M, Fan L, King LL, Wu H, El-Shourbagy TA (2010). High-throughput salting-out assisted liquid/liquid extraction with acetonitrile for the simultaneous determination of simvastatin and simvastatin acid in human plasma with liquid chromatography. Anal. Chim. Acta, 661(2): 167-172.