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Measurement and stability of FTY720 in human whole blood by high-performance liquid chromatography–atmospheric pressure chemical ionization–tandem mass spectrometry

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Abstract

We report here a validated method for the quantification of a new immunosuppressant drug FTY720, using HPLC–tandem mass spectrometry. Whole blood samples (500 μl) were subjected to liquid–liquid extraction, in the presence of an internal standard (Y-32919). Mass spectrometric detection was by selected reaction monitoring with an atmospheric pressure chemical ionization source in positive ionization mode (FTY720: m/z 308.3 → 255.3). The assay was linear from 0.2 to 25 μg/l ($r^2 > 0.997$, $n = 5$). The inter- and intra-day analytical recovery and imprecision for quality control samples (0.5, 7 and 15 μg/l) were 95.8–103.2 and <5.5%, respectively. At the lower limit of quantification (0.2 μg/l) the inter- and intra-day analytical recovery was 99.0–102.8% with imprecision of <7.6% ($n = 5$). The assay had a mean relative recovery of 100.5 ± 5.8% ($n = 15$). Extracted samples were stable for 16 h. FTY720 quality control samples were stable at room temperature for 16 h, at 4 °C for at least 8 days and when taken through at least three freeze–thaw cycles. In conclusion, the method described displays analytical performance characteristics that are suitable for pharmacokinetic studies in humans.

Keywords: FTY720; HPLC; Mass spectrometry; APCI; Immunosuppressant drug

1. Introduction

FTY720 is a novel immunomodulator (Fig. 1) currently under investigation as a combination therapy with other immunosuppressant drugs for the prophylaxis of acute rejection after solid organ transplantation [1,2] and for the treatment of multiple sclerosis [3]. Upon administration, FTY720 is rapidly phosphorylated *in vivo* to the active moiety FTY720-phosphate, which binds to the sphingosine-1-phosphate receptor [4]. The mode of action is to reduce the recirculation of lymphocytes from lymph nodes to blood and peripheral tissue, including the graft site [5,6]. In a phase 2 study of *de novo* kidney graft recipients receiving FTY720 (co-administered with cyclosporin and corticosteroids) compared with current clinical practice of mycophenolate mofetil, FTY720 at 2.5 mg was found to be as effective in the prevention of acute rejection episodes [2]. Currently, FTY720 at doses of 2.5 and 5 mg/day are being assessed in global renal transplant trials [4].

The pharmacokinetics of FTY720 in stable and *de novo* renal transplant recipients are characterized by a long half-life (>100 h) and a dose proportional pharmacokinetic profile [1,2]. Pre-clinical and phase 2 studies have shown that FTY720 will be administered in low mg/day dosing regimens and thus the circulating drug is expected to be in the low μg/l concentration range [2,7,8]. Whole blood is the matrix of choice for measurement of FTY720 as this drug is highly distributed into the cellular blood components [9].

The aim of this study was to develop and validate a HPLC–tandem mass spectrometry method (HPLC–MS), using atmospheric pressure chemical ionization, to measure clinically relevant concentrations of FTY720 in human blood. The validation was designed to fulfil the requirements described by Shah et al. [10] and to incorporate current regulatory opinion [11].

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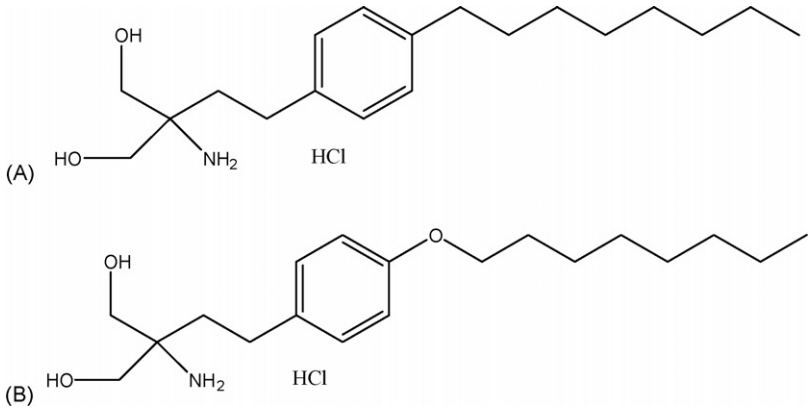

Fig. 1. The chemical structures of: (A) FTY720 and (B) Y-32919 (internal standard). The molecular weights of free bases FTY720 and Y-32919 are 307.5 and 323.5 Da, respectively.

2. Experimental

2.1. Materials

FTY720 and Y-32919 (internal standard) were a kind gift from Novartis Pharma AG (Basel, Switzerland) (Fig. 1). Stock solutions of FTY720 and internal standard were prepared in methanol and stored at −80 °C. Drugs used for the selectivity study were obtained from their respective suppliers. HPLC grade acetonitrile and methanol were purchased from EMD Chemicals (Gibbstown, NJ, USA). Diethyl ether (Pronalys AR grade) and dichloromethane (HPLC grade) were sourced from BioLab (Clayton, Vic., Australia) and Mallinckrodt Chemicals (Phillipsburg, NJ, USA), respectively. All other chemicals used were analytical reagent grade. Deionized water was obtained from a Milli-Q water purification system (Millipore, Milford, MA, USA).

Stock solutions of FTY720 and internal standard were prepared in methanol and stored at −80 °C. Independent stock solutions were used for FTY720 standard (10 μg/ml and 100 ng/ml) and control (10 μg/ml and 100 ng/ml) preparation. A methanol stock solution of internal standard (1 mg/ml) was used in the preparation of the working stock (100 μg/l) that was made in methanol:deionized water (50:50, v/v).

Calibration and quality control material were prepared in-house using whole blood containing ethylenediaminetetraacetic acid as anticoagulant. Prior to use, this blood was screened for interference to the assay. Calibration samples were prepared from methanol stock solutions that were volumetrically added to blank blood to achieve concentrations of 0.2, 0.4, 1.0, 5.0, 10 and 25 μg/l. Similarly, quality control samples were prepared in the same manner to achieve concentrations of 0.2, 0.5, 7.0, 15 and 25 μg/l.

2.2. HPLC–mass spectrometry apparatus and conditions

The HPLC system was an integrated 1100 series (Agilent Technologies, Waldbronn, Germany). To remove sample carry-over effects, a programmed injector wash function utilising a needle wash in the flush port (methanol:deionized water, 70:30, v/v) and an injection valve clean using three consecutive valve switches after analyte elution (4 min) were used. The HPLC analytical column was an Allure^{TM} pentafluorophenylpropyl reverse phase column (50 mm × 2.1 mm i.d., 5 μm, Restek, Belloefonte, PA, USA) maintained at 50 °C in the column oven. An isocratic flow was delivered via a binary pump, in a composition of 30% 40 mM ammonium acetate in water (pH 5.1) and 70% methanol (v/v), at flow rate of 1 ml/min.

Mass spectrometric detection was performed on a linear ion trap quadrupole tandem mass spectrometer (4000 Q Trap^{TM}, Applied Biosystems/MDS Sciex Instruments, Concord, Ontario, Canada) using selected reaction monitoring. Ions were generated in positive ionization mode using an atmospheric pressure chemical ionization (APCI) interface. The heated nebulizer was set at 500 °C with a nebulizer current of 3 μA and nebulizer gas pressure of 45 PSI. Under these conditions, the predominant analyte precursor ion was the protonated species $[M+H]^+$. For collision-activated dissociation, nitrogen was used in combination with a collision energy of 24 V. Peak area ratios obtained from selected reaction monitoring of the mass transitions for FTY720 (m/z 308.3 → 255.3) and the internal standard (m/z 324.3 → 159.3) were used for quantification (Fig. 2). Standard curves (0.2, 0.4, 1, 5, 10 and 25 μg/l) were constructed using weighted ($1/\chi^2$) linear least squares regression. Data were collected and analysed using Analyst^{TM} software Version 1.4.1 (Applera Corporation, Norwalk, CT, USA).

2.3. Sample preparation

Standard and quality control whole blood samples (500 μl), containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant, were treated with 0.1 M sodium hydroxide solution (500 μl) and 50 μl of internal standard solution, in 15 ml glass culture tubes. Samples were mixed and 5 ml of diethyl ether/dichloromethane (75:25, v/v) was added. The contents were mixed for 60 min in a Multi Reax mixer (Heidolph Instruments, Schwaback, Germany) and centrifuged (5 min, 850 × g). The aqueous layer was frozen and the organic layer was decanted into respective clean glass tubes and evaporated to dryness at 60 °C under a stream of air. Samples were re-dissolved in mobile phase (200 μl) and submitted to the mass spectrometer (50 μl).

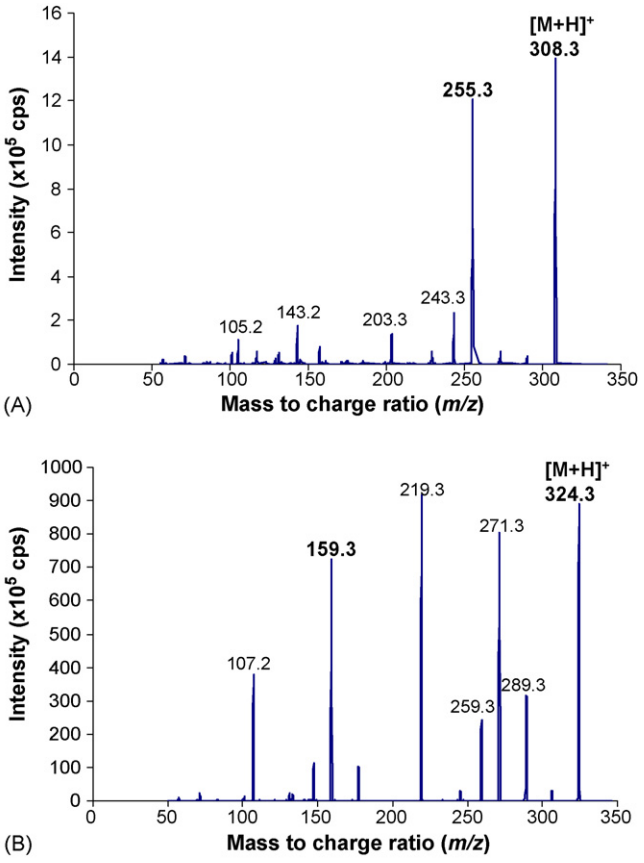

Fig. 2. The collision assisted dissociation mass spectra of: (A) FTY720 and (B) Y-32919 (internal standard). The mass transitions m/z 308.3 → 255.3 (FTY720) and m/z 324.3 → 159.3 (IS) were used for detection by selected reaction monitoring.

2.4. Assay validation studies

The selectivity of the method was evaluated for potential endogenous interferences by analysing whole blood samples from 20 transplant patients not receiving FTY720 therapy. A peak or response at the respective retention times for either FTY720 or the internal standard (signal to noise < 5:1) was considered to be insignificant. The patients studied consisted of renal (75%) and liver (25%) transplant recipients receiving multiple drug immunosuppressant therapy of tacrolimus, prednisone and mycophenolate mofetil. Potential xenobiotic interferences were assessed with a range of commonly used immunosuppressant drugs, tacrolimus (50 μg/l), sirolimus (100 μg/l), everolimus (100 μg/l), cyclosporin A (2000 μg/l), mycophenolic acid (50 mg/l) and mycophenolic acid glucuronide (500 mg/l). Potential carry over effects were studied by injecting an extract containing FTY720 (25 μg/l) and internal standard followed by four blank extracts and observing any potential residual peaks at the retention time for each analyte. This process was repeated four times.

Linearity was evaluated by analysis of whole blood standard samples at concentrations of 0.2, 0.4, 1, 5, 10 and 25 μg/l ($n = 5$). A weighted linear regression model ($1/\chi^2$) was used throughout the study for construction of calibration curves. The inter-day analytical recovery and imprecision (co-efficient of variation) of standard samples were determined from the back-calculated results of the linearity study. The analytical performance of the method was further assessed based on the analytical recovery and imprecision of quality control samples at the lower limit of quantification (0.2 μg/l), within the linear range (0.5, 7 and 15 μg/l) and at the upper limit of quantification (25 μg/l). Control samples were analysed in replicates of five on 1 day (intra-day) and once on 5 days (inter-day). Analytical recovery was expressed as the mean assayed result for the quality control samples ($n = 5$) as a percentage of the weighed-in concentration.

The mean absolute recoveries of the analytes were determined by comparing the peak areas obtained from FTY720 (0.5, 7.0 and 15 μg/l) and internal standard added to and extracted from whole blood samples of five different subjects for each concentration, compared to the peak areas obtained from the analytes added post-extraction to their respective subject blank extracts. The relative recovery of FTY720 was calculated from the ratio of FTY720 and internal standard absolute recoveries, expressed as a percentage. Dilution suitability for samples within (7.0 and 15 μg/l) and above (100 μg/l) the linear range was assessed based on 250 μl:250 μl (1 in 2) and 100 μl:400 μl (1 in 5) dilutions with FTY720 free EDTA blood. Further, a 1 in 2 dilution of a low concentration within the linear range (0.5 μg/l) was tested. Intra-day analytical recovery and imprecision of diluted samples were determined by assaying quality control samples in replicates of five within 1 day.

Matrix effects were investigated by the post-column infusion method [12]. The experiment was undertaken using the HPLC and mass spectrometric conditions described and FTY720 was infused post-column at 20 μl/min. A blank blood extract was injected and the response for the mass transition recorded. Inter-subject variability was investigated by analyzing samples supplemented with FTY720 at the three quality control concentrations using five blood samples from different subjects at each concentration.

2.5. Stability studies

Post-preparative extract stability and whole blood short-term, medium-term and freeze–thaw stability of FTY720 was evaluated at three quality control sample concentrations (0.5, 7 and 15 μg/l), using a calibration curve based on freshly prepared blood samples. Post-preparative stability was assessed based on comparing results from sample extracts (in mobile phase) at each of the quality control concentrations ($n = 5$) with the expected concentration after storage in an autosampler at room temperature for a 16-h period. Stability of FTY720 in blood in the short-term (at room temperature and exposed to light for 16 h) and medium-term (at 4 °C for 8 days) were evaluated based on comparing concentrations from quality control samples with expected weighed-in concentrations ($n = 3$). Freeze–thaw stability was studied based on the comparison of the results obtained from quality control whole blood samples after three freeze–thaw cycles, performed over a 3-day period, with expected weighed-in concentrations ($n = 3$).

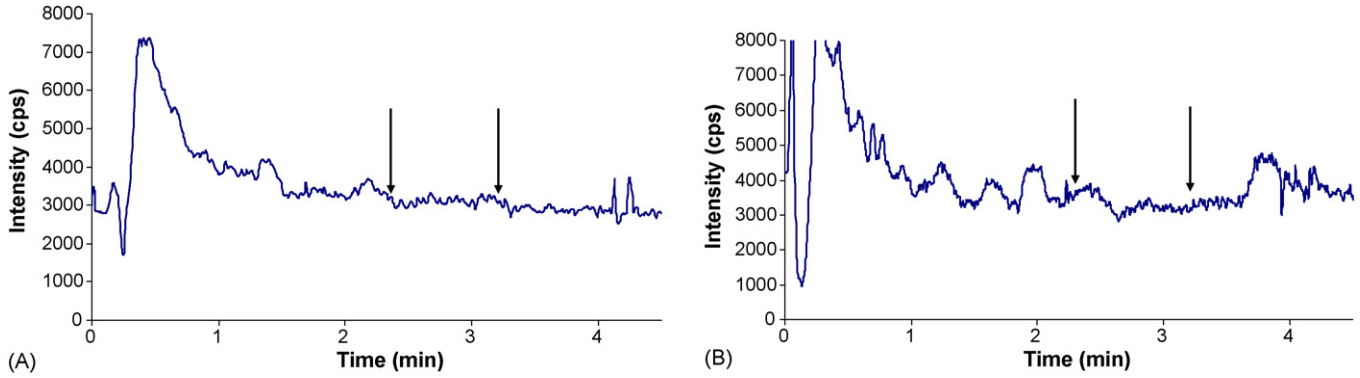

Fig. 3. The monitoring of the FTY720 mass transition, during a post-column infusion experiment as described by King et al. [12] under (A) APCI and (B) electrospray conditions, in which an extract of patient sample was injected. Details of this experiment are described in Section 2.4. The arrows represent the retention times of the analytes.

3. Results and discussion

3.1. Sample preparation

Initial attempts to develop a sample preparation method using solid phase extraction were unsuccessful. The use of organic solvent protein precipitation followed by C_{18} solid phase extraction have been used for the extraction of other immunosuppressant drugs such as cyclosporin and tacrolimus from whole blood [13]. Using this approach, the recovery of FTY720 was <20%. Various mixtures of organic solvents at various organic strengths and pH were investigated as elution solvents without success. One possible explanation for the poor recovery is that FTY720 may be highly bound to proteins and when a blood sample is treated with organic solvent, to cause protein precipitation, the majority of the drug is not released into the precipitation solvent but remains in the protein pellet.

A liquid–liquid extraction process was investigated as a possible alternative. To ensure reproducible extraction efficiency the pH of the sample was increased by the addition of sodium hydroxide. Under these basic conditions, the primary amine functional group is not charged and the analyte would be expected to be extracted into the organic phase. Several solvents including butanol, diethyl ether, chloroform, dichloromethane and methanol in various combinations were assessed for extraction efficiency. All had poor extraction efficiency except for the diethyl ether/dichloromethane mixture (75:25, v/v) and chloroform which both had similar recoveries (>80%). The diethyl ether/dichloromethane mixture was the preferred extraction solvent as this solution formed the top layer in the aqueous–organic mixture. As compared with chloroform which formed the bottom layer. This enabled the aqueous layer, after centrifugation of the aqueous–organic mixture, to be frozen and the organic layer to be decanted. The transfer process was thus simplified. Investigations into the optimal mixing time of the aqueous–organic mixture revealed that the maximum recovery of the drug was obtained after 60 min. The required length of mixing to obtain maximum recovery may reflect the highly bound nature of FTY720.

3.2. HPLC–mass spectrometry

Chromatography was performed on a pentafluorophenylpropyl bonded silica column. This type of stationary phase has shown excellent retention of both basic and acid drugs at high mobile phase organic content when compared to C_{18} phase columns [14,15]. The use of relatively high organic content in the mobile phase (70% methanol) for retention of FTY720 leads to good ionization efficiency and thus response.

The response for FTY720 under these chromatographic conditions was similar for electrospray and APCI. FTY720 is readily protonated by either ion source, with no other adduct formation occurring (*i.e.* $[M+NH_4]^+$). Post-column infusion assessment of matrix effects for both ion sources were undertaken as described by King et al. [12]. There was minimal signal suppression at the retention times of FTY720 or the internal standard using APCI but some suppression was observed using electrospray between 2 and 3.5 min (Fig. 3). APCI is less prone to suffer from matrix effects than electrospray [16] and thus was selected for this study. Fig. 2 shows the precursor ions ($[M+H]^+$) and product ions of FTY720 and the internal standard under APCI conditions.

3.3. Validation

The chromatographic conditions used in this method achieve retention times of 3.2 min for FTY720 and 2.4 min for the internal standard, with a total chromatographic run time of 4.5 min. Fig. 4 shows representative chromatograms of a blood sample obtained from (a) a subject not receiving FTY720 therapy (blank), (b) a FTY720 quality control sample at the lower limit of quantification (0.2 μg/l) and (c) a patient sample (11.0 μg/l). No significant peaks or signals (signal to noise < 5:1) were detected at their respective retention times for either FTY720 or the internal standard in the screening of potential interference from endogenous compounds in samples from transplant recipients not receiving FTY720 therapy ($n = 20$) or in the assessment of carry over effects between sample extracts from autosampler injections ($n = 16$). Similarly, no interferences were detected for either FTY720 or the internal standard when

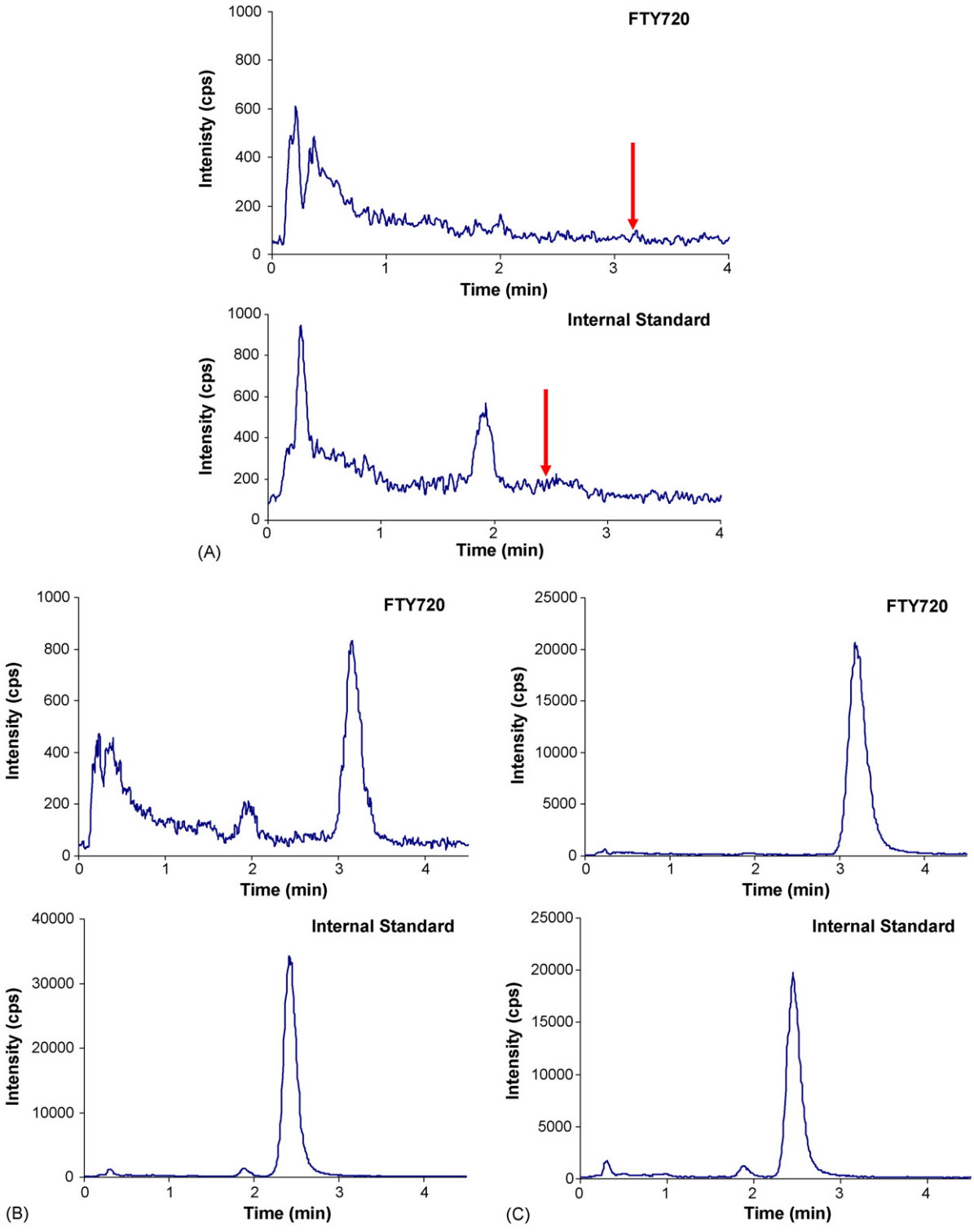

Fig. 4. Representative chromatograms of: (A) a blood sample obtained from a subject not receiving FTY720 therapy, (B) a FTY720 quality control sample at the lower limit of quantification (0.2 μg/l) and (C) a patient sample (11.0 μg/l). Retention times are 3.2 min for FTY720 and 2.4 min for the IS.

screening the immunosuppressant drugs; tacrolimus, sirolimus, everolimus, cyclosporin A and mycophenolic acid glucuronide. However, mycophenolic acid (50 mg/l) gave a response in the mass transition for FTY720 of approximately 0.1 μg/l response for FTY720. This interference may be due to a degree of in-source degradation of mycophenolic acid leading to production of the precursor ion *m/z* 308.3 and subsequent fragmentation to *m/z* 255.3. Since the expected mycophenolic acid patient trough concentrations would be 1.0–3.5 mg/l [17], this would represent approximately 3.5% of the FTY720 response at the LLOQ

Table 1
Analytical performance parameters of the whole blood FTY720 HPLC–tandem mass spectrometry method based on weighed-in quality control samples

	FTY720 weighed-in concentrations (μg/l) ($n = 5$)						IS[a] ($n = 15$)
	0.2	0.5	7	15	25	100	25 μg/l
Analytical recovery[b] (%)							
Inter-day	102.8	102.1	98.7	100.7	100.8	n/a	n/a
Intra-day	99.0	103.2	95.8	99.9	98.7	n/a	n/a
Dilution suitability[c] (1 in 2)	n/a	98.4	93.3	97.6	n/a	n/a	n/a
Dilution suitability (1 in 5)	n/a	n/a	93.1	97.3	n/a	101	n/a
Imprecision[d] (%)							
Inter-day	7.5	3.4	1.7	3.6	2.2	n/a	n/a
Intra-day	1.9	5.4	1.7	1.7	2.5	n/a	n/a
Dilution suitability (1 in 2)	n/a	5.3	1.6	0.6	n/a	n/a	n/a
Dilution suitability (1 in 5)	n/a	n/a	1.6	2.3	n/a	4.2	n/a
Absolute recovery[e] (%)	n/a	87.0	82.6	92.6	n/a	n/a	87.1
Relative recovery[f] ± S.D.(%)	n/a	105.2 ± 6.4	97.8 ± 4.7	98.4 ± 3.7	n/a	n/a	n/a
Inter-subject variability[g] (%)	n/a	2.9	4.0	3.4	n/a	n/a	n/a

n/a: not applicable.
[a] IS: internal standard, Y-32919.
[b] Analytical recovery was determined as the mean assayed concentration expressed as a percentage of the weighed-in concentration.
[c] Intra-day dilutions (100 μl:100 μl (1 in 2) and 100 μl:400 μl (1 in 5)) were performed with EDTA human whole blood screened as "blank" for FTY720 and internal standard.
[d] Imprecision was expressed in terms of coefficient of variation.
[e] Absolute recovery (extraction efficiency) of the analytes were determined by comparing the independent peak areas obtained from FTY720 and internal standard added before and after extraction.
[f] The relative recovery of FTY720 was calculated from the peak area ratios of weighed-in FTY720 and internal standard, before and after extraction and expressed as a percentage.
[g] Inter-subject variability was expressed in terms of coefficient of variation in the relative recoveries of different subjects.

(0.2 μg/l). Therefore, this source of interference can be considered insignificant within the quantification limits of this current assay.

The assay was linear from 0.2 to 25 μg/l with a satisfactory co-efficient of determination on each study day ($r^2 > 0.997$, $n = 5$). Calibration samples had an inter-day analytical recovery of 97.6–105.0% and imprecision of <6%. The analytical performance of quality control samples over the analytical range is shown in Table 1. The lower limit of quantification for this investigation was deemed to be 0.2 μg/l with an inter- and intra-day analytical recovery of 99.0–102.8% and imprecision of <7.6%. Based on the guidelines of Shah et al. [10], these performance indicators at the lower quantification limit are within the analytical recovery (80–120%) and imprecision (<20%) acceptance criteria. However, measuring lower FTY720 concentrations may result in an overestimation due to potential interference from mycophenolic acid in patients co-administered with mycophenolate mofetil.

Quality control samples at 0.5, 7 and 15 μg/l had inter- and intra-day analytical recovery of 95.8–103.2% and imprecision <5.5%. The upper limit of quantification was determined to be 25 μg/l, with inter- and intra-day analytical recovery of 98.7–100.8% and imprecision <2.6%. The method was not tested above this concentration and the true upper limit of quantification may not have been reached.

The method displayed adequate extraction efficiency, with mean absolute recoveries (±S.D.) of FTY720 and the internal standard of 87.4 ± 11.4 and 87.1 ± 11.3%, respectively, with a mean relative recovery of 100.5 ± 5.8% for FTY720 ($n = 15$, Table 1).

Dilution suitability of quality control samples at 0.5 μg/l (based on a 1 in 2 dilution) and 7.0, 15 and 100 μg/l (based on a 1 in 2 and 1 in 5 dilutions), had intra-day analytical recovery of 93.1–101% and imprecision <5.4% (Table 1). The dilution capabilities permit the extraction of samples which would otherwise not be possible when <500 μl of blood specimen was collected in the first instance. Furthermore, the ability to perform dilutions would be advantageous in circumstances of analytical failure or assay rejection, where a repeat extraction would be required, but <1 ml of blood sample was originally provided. Furthermore, the suitable dilution performance at 100 μg/l (using a 1 in 5 dilution), extends the analytical range beyond the highest calibrator of 25 μg/l.

Post-column infusion assessment of matrix effects was undertaken as described by King et al. [12]. There was minimal signal suppression at the retention times of FTY720 or the internal standard (Fig. 3). While this approach gives a qualitative measure of matrix effects Matuszewski et al. [18] have shown that during validation of a HPLC–MS method, a quantitative approach of inter-subject variability (the influence of various matrices on results) should be assessed. We have previously described an approach, to assessing inter-subject variability, of analyzing three concentrations (typically at the quality control concentrations) using five blood samples from different subjects at each concentration [19]. Using this method, we found that the inter-subject variability, expressed as coefficient of variation, was <4.1% (Table 1). These data compare favourably with the variability (<5.5%) observed for pooled blood used for quality control samples and thus suggest between-subject differences in matrix are having little to no influence on results. Overall it can

Table 2
Short-term, medium-term and freeze–thaw stability of FTY720 in extract and whole blood, as measured by the HPLC–tandem mass spectrometry, using weighed-in quality control samples

Matrix and conditions	FTY720 weighed-in concentrations (μg/l)					
	0.5		7		15	
	AR[a] (%)	CV[b] (%)	AR (%)	CV (%)	AR (%)	CV (%)
Extract at 25 °C for 16 h ($n=5$)	99.2	6.2	96.2	2.9	100.4	2.6
Blood at 25 °C for 16 h ($n=3$)	103.0	5.0	92.1	2.9	96.4	1.6
Blood at 4 °C for 8 days ($n=3$)	99.4	0.9	95.1	1.9	99.3	2.4
Blood 3 × freeze–thaw cycles ($n=3$)	101.0	2.9	99.9	3.8	100.4	1.0

[a] AR: analytical recovery was determined as the mean assayed concentration expressed as a percentage of the weighed-in concentration.
[b] CV: imprecision was expressed in terms of coefficient of variation.

be concluded that matrix effects have negligible if any influence on the performance of this method.

The ability to measure FTY720 over such a wide analytical range makes this method suitable for pharmacokinetic studies. Based on the guidelines of Shah et al. [10] and current regulatory opinion [11] the assay displayed suitable analytical recovery and imprecision for all weighed-in whole blood calibration and quality control samples.

3.4. Stability

The stability of FTY720 in extract and whole blood matrices were investigated for the purpose of determining the appropriate handling requirements for analysis, sample transport and storage. The correct handling of blood samples is important to ensure the integrity of the results obtained. As shown in Table 2, the response of sample extracts ($n=5$) at concentrations of 0.5, 7 and 15 μg/l, injected after storage in an autosampler at ambient temperature for 16 h, exhibited an analytical recovery of 96.2–100.4% and imprecision of <6.3%. Whole blood quality control samples ($n=3$) supplemented with FTY720 and exposed to light that were stored at room temperature for 16 h, or at 4 °C for 8 days or subjected to three freeze–thaw cycles had analytical recovery between 92.1 and 103.0% and imprecision of <5.1%. These performance measures compare favorably with the validation data of freshly prepared control material. This suggests that in the short-term (simulating work bench conditions) FTY720 is stable in its post-preparative form and in blood for 16 h at room temperature. Similarly in the medium-term, FTY720 is stable in blood at typical refrigeration temperatures for at least 8 days and is stable in blood despite three freeze–thaw cycles, permitting sample re-analysis after freezer storage.

4. Conclusion

This is the first reported HPLC–MS method for the measurement of FTY720 in human whole blood. The method provides accurate, precise and selective measurement of FTY720. Based on current and expected dosing strategies [4], this method would be suitable for pharmacokinetic investigations and if required, therapeutic drug monitoring.

Acknowledgements

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References

[1] K. Budde, R.L. Schmouder, R. Brunkhorst, B. Nashan, P.W. Lucker, T. Mayer, S. Choudhury, A. Skerjanec, G. Kraus, H.H. Neumayer, J. Am. Soc. Nephrol. 13 (2002) 1073.
[2] H. Tedesco-Silva, G. Mourad, B.D. Kahan, J.G. Boira, W. Weimar, S. Mulgaonkar, B. Nashan, S. Madsen, B. Charpentier, P. Pellet, Y. Vanrenterghem, Transplantation 79 (2005) 1553.
[3] R.E. Gonsette, J. Neurol. Sci. 223 (2004) 87.
[4] J.M. Kovarik, R.L. Schmouder, A.J. Slade, Ther. Drug Monit. 26 (2004) 585.
[5] K. Chiba, Y. Yanagawa, Y. Masubuchi, H. Kataoka, T. Kawaguchi, M. Ohtsuki, Y. Hoshino, J. Immunol. 160 (1998) 5037.
[6] S. Chen, K.B. Bacon, G. Garcia, R. Liao, Z.K. Pan, S.K. Sullivan, H. Nakano, A. Matsuzawa, V. Brinkmann, L. Feng, Transplant Proc. 33 (2001) 3057.
[7] P. Troncoso, B.D. Kahan, Clin. Biochem. 31 (1998) 369.
[8] M. Mourad, P. Wallemacq, J. Konig, E.H. de Frahan, D.C. Eddour, M. De Meyer, J. Malaise, J.P. Squifflet, Clin. Pharmacokinet 41 (2002) 319.
[9] K.L. Napoli, Ther. Drug Monit. 22 (2000) 47.
[10] V.P. Shah, K.K. Midha, J.W. Findlay, H.M. Hill, J.D. Hulse, I.J. McGilveray, G. McKay, K.J. Miller, R.N. Patnaik, M.L. Powell, A. Tonelli, C.T. Viswanathan, A. Yacobi, Pharm. Res. 17 (2000) 1551.
[11] Anon., www.fda.gov/cder/guidance/guidance.htm, 2001.
[12] R. King, R. Bonfiglio, C. Fernandez-Metzler, C. Miller-Stein, T. Olah, J. Am. Soc. Mass. Spectrom. 11 (2000) 942.
[13] P.J. Taylor, Ther. Drug Monit. 26 (2004) 215.
[14] S.R. Needham, P.M. Jeanville, P.R. Brown, E.S. Estape, J. Chromatogr. B Biomed. Sci. Appl. 748 (2000) 77.
[15] B. Atcheson, P.J. Taylor, D.W. Mudge, D.W. Johnson, P.I. Pillans, S.E. Tett, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 799 (2004) 157.
[16] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 70 (1998) 882.
[17] A.G. Johnson, R.J. Rigby, P.J. Taylor, C.E. Jones, J. Allen, K. Franzen, M.C. Falk, D. Nicol, Clin. Pharmacol. Ther. 66 (1999) 492.
[18] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 75 (2003) 3019.
[19] P.J. Taylor, Clin. Biochem. 38 (2005) 328.