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Determination of rifampicin in human plasma and blood spots by high performance liquid chromatography with UV detection: A potential method for therapeutic drug monitoring

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Abstract

A high performance liquid chromatography method has been developed that allows quantification of concentrations of rifampicin in human plasma and blood spots. Rifampicin and papaverine hydrochloride (internal standard) were extracted from plasma using a Strata-X-CW extraction cartridge. These analytes were also extracted into acetonitrile from blood spots dried onto a specimen collection card. The recovery of rifampicin from plasma and blood spots was 84.5% and 65.0%, respectively. Separation was achieved by HPLC on a Kromasil C_{18} column with a mobile phase composed of ammonium acetate (20 mM, pH 4.0) and acetonitrile, delivered on a gradient programme. Optimum detection was at 334 nm. The assay was linear over the concentration range of 0.5–20 μg/ml. The limit of quantification was 0.5 μg/ml in plasma; 1.5 μg/ml in blood spots. Both intraday and interday precision data showed reproducibility (R.S.D. ≤ 8.0, $n = 9$). Stability studies showed rifampicin was stable in plasma for up to 9 h after thawing; the samples were also stable for up to 9 h after preparation. Five patient samples were analysed using the methods described. A correlation was found between the concentrations of RIF in plasma and blood spots ($r^2 = 0.92$). This method is proposed as a means of therapeutic drug monitoring of rifampicin in patients with tuberculosis.

Keywords: Rifampicin; HPLC; Dried blood spots; Therapeutic drug monitoring; Tuberculosis

1. Introduction

Tuberculosis (TB), an infection caused by *Mycobacterium tuberculosis* currently infects approximately one third of the world's population. There are approximately eight million new cases each year worldwide, almost all of them preventable or treatable [1]. Incomplete treatment of TB is common and is usually a consequence of non-compliance with the therapeutic regime or an interrupted supply of drugs. Therapeutic drug monitoring (TDM) may provide a means of determining compliance, particularly in remote areas of developing countries. However, outpatient treatment of TB patients raises logistical obstacles for TDM. The convenience of non-hospital based blood sampling could be of immense benefit to the quality of treatment for these patients. Sampling for TDM can be undertaken by collecting blood on specimen collection cards similar to the Guthrie cards used to test for phenylketonuria in neonates. These cards do not require specific storage or transportation conditions and blood dried on the cards is considered the lowest risk by the International Air Transportation Association [2].

Rifampicin (RIF, Fig. 1) is an important first line drug prescribed throughout TB therapy [3], often as part of fixed dose combination (FDC) tablets, which may also contain isoniazid and pyrazinamide. Although FDCs simplify the prescribing process and encourage compliance [4], the absorption of RIF from these formulations may vary, especially in tablets containing isoniazid [5], and contribute to treatment failure. Treatment failure and the development of drug resistance may be attributed to non-compliance with the treatment regime, poor bioavailability of RIF in some preparations, including some FDCs as described; counterfeit preparations, or malabsorption of RIF. While directly observed therapy (DOT) may address the first issue, it cannot

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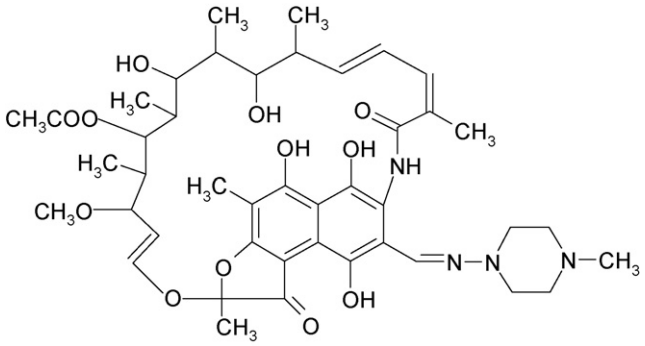

Fig. 1. Chemical structure of rifampicin.

address treatment failure due to the latter two causes. Currently, plasma levels of RIF are not monitored routinely in TB patients but it is clear that this would be advantageous if a simple and effective quantitative test were available. A number of methods for the determination of RIF in plasma have been reported [6–13]. These methods are characterised by lengthy sample preparation procedures [10,12], non-ideal chromatographic retention parameters (low analyte capacity factor) [6,7,12] and poor selectivity in the presence of the major metabolite (desacetlyrifampicin (DRIF)) [7,8,10] and the degradant (RIF-quinone) [6–10,12,13].

In the present study, an optimised sample preparation technique for quantitative and reproducible recovery of RIF from human plasma is described. A validated HPLC method for subsequent quantification is reported and its suitability in the monitoring of RIF in dried blood on specimen collection cards is demonstrated. These improved techniques should aid the TDM of RIF in TB patients.

2. Experimental

2.1. Chemicals and materials

RIF (95%, w/w), papaverine hydrochloride (≥99.0%, w/w) and sulindac (99.0%, w/w) were all obtained from Sigma–Aldrich (Dorset, UK). Rifabutin (96.3%, w/w) was obtained from the European directorate for the quality of medicines (EDQM, France). Desacetylrifampicin (DRIF) was kindly provided by Professor Mitchison (St. George's Hospital, London, UK). All solvents were of HPLC grade and were obtained from VWR Ltd. (Lutterworth, UK). Ammonium acetate was obtained from BDH (Lutterworth, UK) and glacial acetic acid was obtained from Sigma–Aldrich (Poole, UK). Strata-X, C_{18} and Strata-X-CW SPE cartridges (30 mg/1 ml) were obtained from Phenomenex (Macclesfield, UK). Whatman BFC-180 blood specimen collection cards were obtained from Camlab (Over, UK).

2.2. Collection of biological material

Ethical approval was obtained from North Glasgow University Hospitals NHS Trust Research Ethics Committee and the University of Strathclyde Research Ethics Committee.

Whole blood was obtained from the Glasgow Royal Infirmary (GRI), Glasgow, UK. An aliquot of the whole blood was kept at 4 °C until use. The remainder was centrifuged at 3500 × *g* for 7 min, the plasma removed and stored at −20 °C until use. Samples of venous blood and capillary blood, obtained by a pricking a single finger and spotting droplets of blood onto specimen collection cards (blood spots), were obtained from patients attending the TB clinic at GRI. The venous sample was centrifuged using the conditions described for whole blood and stored at −20 °C until use. The blood spots were allowed to dry and were stored at ambient temperature away from sources of heat and light until use.

2.3. Preparation of standard solutions

A stock solution of RIF (200 μg/ml) was prepared in methanol containing 0.5 mg/ml ascorbic acid to prevent oxidation of RIF. From this, standards containing 5, 25 and 50 μg/ml RIF were prepared. An aqueous stock solution of papaverine hydrochloride (1 mg/ml) was also prepared. This was diluted to 50 μg/ml with water for the blood spot samples. Standard solutions containing 1.08, 3.6 and 7.2 μg/ml RIF were also prepared and used to determine the recovery of RIF from blood spots.

2.4. Preparation of spiked plasma and blood spot samples

RIF spiked plasma samples were prepared by mixing 0.45 ml blank plasma with appropriate volumes of the standard RIF solutions (ranging in concentration from 5 to 200 μg/ml) and an aliquot (40 μl) of the stock papaverine solution to produce final RIF concentrations of 0.5, 1, 2.5, 5, 10 and 20 μg/ml. A blank was also prepared containing 0.45 ml blank plasma and papaverine hydrochloride. RIF spiked blood samples with final RIF concentrations of 1.5, 2.5, 5, 10 and 20 μg/ml were produced by mixing volumes of the standard RIF solutions (ranging in concentration from 5 to 200 μg/ml) with blank blood along with a blank containing only blood and papverine hydrochloride. The resulting blood sample was spotted onto the blood specimen collection card, using a glass Pasteur pipette, to produce four spots of approximately 10 mm in diameter. The spots were allowed to dry at ambient temperature.

2.5. Extraction of RIF from plasma

RIF was extracted from the plasma using Strata-X, C_{18} and Strata-X-CW solid phase extraction cartridges. All cartridges were washed with 1 ml methanol (MeOH) and 1 ml water sequentially before loading the plasma. For Strata-X-CW the plasma was diluted 1:1 with 25 mM ammonium acetate (adjusted to pH 6.0 with glacial acetic acid) before to loading onto the cartridge. Interferents were washed off the Strata-X and C_{18} cartridges with water (2× 1 ml) and RIF was eluted with MeOH (2× 1 ml). The interferent washing solvent for the Strata-X-CW SPE cartridge was 25 mM ammonium acetate pH 6.0 and RIF was eluted with 3% (v/v) methanolic ammonia (2× 1 ml). All samples were then reduced to dryness under a stream of nitrogen

at ambient temperature and reconstituted in 200 μl ACN:H_2O (1:4, v/v).

The efficiency of the recovery of RIF from plasma was evaluated at concentrations of 1, 5 and 10 μg/ml. This was compared with RIF prepared from the standard solutions.

2.6. Extraction of RIF from blood spots

The blood spots were extracted using the method described by Oliveira et al., 2002 [14]. Four discs, 7 mm in diameter and each containing approximately 18 μl blood, were punched out of the blood specimen collection card and transferred to a 3.5 ml sample vial. Ammonium acetate buffer (0.4 ml, 20 mM, pH 4.0) was added and the samples vortexed for 2 min. Papaverine hydrochloride stock solution (20 μl) and ACN (3 ml) were added and the samples centrifuged at 3500 × *g* for 7 min. The supernatant was reduced to dryness under a stream of nitrogen at ambient temperature and the residue was reconstituted in 100 μl ACN:H_2O (1:4, v/v). The recovery of RIF from dried blood spots was evaluated at concentrations of 1.5, 5 and 10 μg/ml. This was compared with RIF prepared from the standard solutions containing 1.08, 3.6 and 7.2 μg/ml, which are the equivalent concentrations of RIF in 72 μl (the average volume of blood in four blood spots).

2.7. HPLC analyses

RIF was analysed at ambient temperature on a Kromasil C_{18} column (Phenomenex, UK, 100 mm × 3.2 mm i.d., 3 μm particle size) with an ODS Securigard® guard column 4 mm × 3 mm (Phenomenex, Macclesfield, UK). The mobile phase (20 mM ammonium acetate buffer adjusted to pH 4.0 with glacial acetic acid and acetonitrile (ACN)) was delivered at 0.7 ml/min on a gradient programme (20–90% ACN over 18 min) by a spectra system P2000 pump (Thermoseparation Products, Salford, UK). Detection was by UV absorbance at 334 nm using a spectra system UV1000 detector (thermoseparation products). The injection volume for each sample was 20.0 μl.

HPLC–MS analysis of RIF and the degradant rifampicin quinone (RIF-Q) was carried out on a Finnegan Mat LC-Q system (Salford, UK) with ionisation in positive ion electrospray ionisation mode. The capillary temperature and voltage were 200 °C and 70 eV, respectively. The sheath gas flow was 63 ml/min and the auxiliary gas flow was 20 ml/min. The column and mobile phase described above were used. Continuous mass spectra were collected in the range 100–1000 Da (Daltons) using Xcaliber Version 1.2 software.

2.8. Stability

RIF plasma samples and blood spot samples were assessed at high (20 μg/ml) and low (1 μg/ml (plasma) and 1.5 μg/ml (blood spots)) concentrations for short term and post preparation stability at 0, 3, 6 and 9 h. Plasma samples at the same concentrations were also assessed for stability over three freeze–thaw cycles by comparing the peak area ratios of these samples with freshly prepared RIF plasma samples at the same concentrations and conducting ANOVA analysis on the data. RIF stock solutions were also assessed for stability at the same time points. One-way ANOVA (unstacked) analysis was performed on the results.

2.9. Patient samples

Blood spot and plasma samples collected from five patients were analysed using the methods described, to evaluate the correlation between data from the methods. The time from sample collection to analysis was between 2 weeks and 22 months. The correlation between the two methods was assessed using linear regression analysis.

3. Results

3.1. Specificity of analysis

Fig. 2a and b shows the chromatograms obtained for the analysis of RIF (t_R = 10.9 min) at the LOQs in plasma and blood spots, respectively. To determine the resolution of RIF from DRIF, three different batches of blank plasma and whole blood were spiked with both RIF and DRIF and extracted as described. The resulting solutions, along with aqueous solutions containing RIF and DRIF were analysed by HPLC. In all studies, RIF was well resolved from its major metabolite, DRIF, with resolution factor (Rs) >2.0 (Fig. 3). In addition, no interfering peaks were observed at the retention times of RIF or DRIF in three different batches of blank plasma and blood spots.

A secondary peak was observed in all chromatograms generated for solutions containing RIF. Its identity was confirmed by HPLC–MS analysis as RIF-Q (Fig. 5, m/z = 821), the product of oxidation of two phenol groups on RIF (m/z = 823). The proposed method is specific and selective for RIF in the presence of DRIF and RIF-Q (t_R = 10.1 and 11.3 min, respectively, Fig. 3).

3.2. SPE method development for plasma samples

Recovery of RIF at two different concentrations in plasma from each of the three SPE cartridges was obtained. The average percentage recoveries for Strata-X was low (Table 1) and the relative standard deviation (R.S.D.) was high (approximately 20.7%). The C_{18} cartridge afforded higher percentage recoveries (average 83.1%) but the R.S.D. was still high (approximately 14.2%). With the Strata-X cartridge the average recovery was in excess of 80.0% and the R.S.D. was no more than 6.8%.

Table 1
Comparison of recovery of RIF from plasma using three different SPE cartridges

SPE cartridge	Concentration of RIF (μg/ml)	Average recovery (%) ($x \pm$ S.D., n = 3)
Strata-X	5	55.84 ± 8.12
	10	72.95 ± 12.59
C_{18}	5	88.30 ± 14.55
	10	77.95 ± 7.70
Strata-X-CW	5	83.48 ± 4.33
	10	84.02 ± 6.75

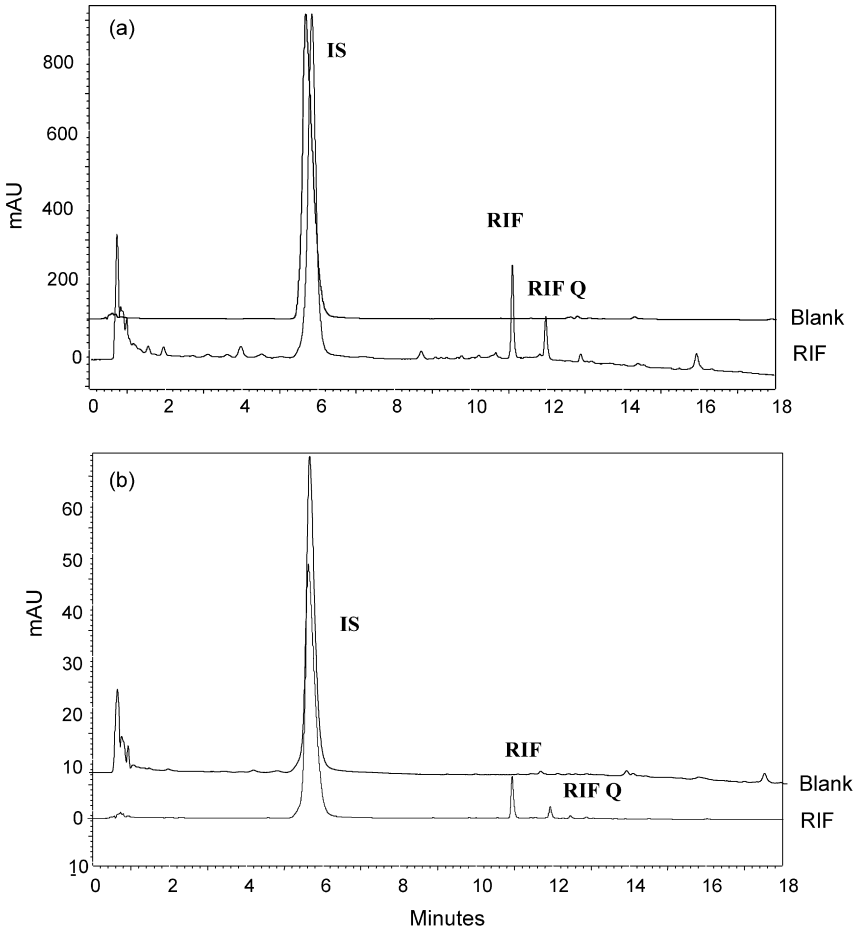

Fig. 2. (a) Chromatogram obtained after analysis of plasma spiked with 0.5 μg/ml RIF. IS = internal standard (papaverine hydrochloride), RIF = rifampicin, RifQ = rifampicin quinone; (b) chromatogram obtained after analysis of blood spots spiked with 1.5 μg/ml RIF. IS = internal standard (papaverine hydrochloride), RIF = rifampicin, RifQ = rifampicin quinone.

3.3. Linearity

A rectilinear relationship was established between the concentration of RIF and the peak area ratio (peak area of RIF/peak area of internal standard). The concentrations of RIF used were 0.5 (plasma only), 1.0 (plasma only), 1.5 (blood spots only), 2.5, 5.0, 10.0 and 20.0 μg/ml. The correlation coefficients and intercepts were as follows:

$$\text{Plasma}: \quad y = (0.0458 \pm 0.004)x - (0.0012 \pm 0.006), \quad r^2 = 0.9986 \pm 0.009 \quad (n = 3)$$

$$\text{Blood spots}: \quad y = (0.0537 \pm 0.0018)x - (0.0023 \pm 0.014), \quad r^2 = 0.9976 \pm 0.0019 \quad (n = 3)$$

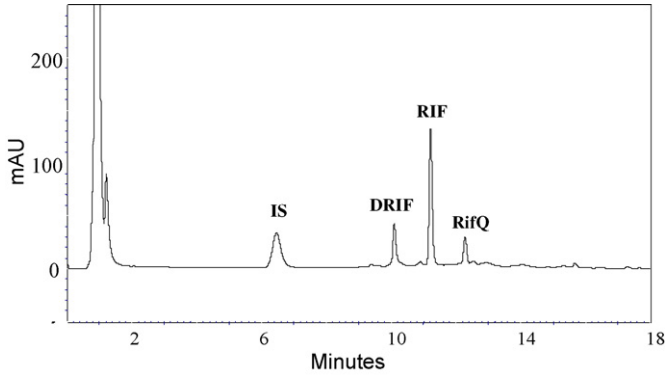

Fig. 3. Chromatogram obtained from patient blood spots. IS = internal standard (papaverine hydrochloride), DRIF = desacetylrifampicin, RIF = rifampicin, RifQ = rifampicin quinone.

3.4. Accuracy and precision

The results from the accuracy and precision experiments are tabulated in Tables 2a and 2b. Accuracy was determined by calculating the concentrations of RIF from the peak area ratios, and expressing this as a percentage of the actual RIF concentration. This was repeated once on each of three consecutive days at three concentrations (1.0, 5.0 and 10.0 μg/ml for plasma and 1.5, 5.0 and 10.0 μg/ml for blood spots). All the samples for both plasma and blood spots were calculated as being between 85.0% and 115.0% of the actual RIF concentration. Intraday precision was determined by calculating the R.S.D. of the percentages of RIF found in the samples analysed on the same day (Tables 2a and 2b). The R.S.D. did not exceed 7.2% ($n = 9$) for

Table 2a
Accuracy and precision of the plasma method

RIF concentration (μg/ml)	Intraday		Interday	
	Mean (% of actual RIF concentration) ±S.D. ($n=3$)	R.S.D. (%) ($n=3$)	Mean of means (%) ±S.D. ($n=9$)	R.S.D. (%) ($n=9$)
10	103.87 ± 2.25	2.16	98.36 ± 4.99	5.07
5	99.93 ± 7.18	7.19	101.43 ± 1.59	1.57
0.5	93.52 ± 6.62	7.07	100.71 ± 7.51	7.46

Table 2b
Accuracy and precision of blood spot method

RIF concentration (μg/ml)	Intraday		Interday	
	Mean (% of actual RIF concentration) ±S.D. ($n=3$)	R.S.D. (%) ($n=3$)	Mean of means (%) ±S.D. ($n=9$)	R.S.D. (%) ($n=9$)
10	102.09 ± 8.01	7.85	104.58 ± 5.0	4.78
5	91.13 ± 6.82	7.02	98.07 ± 4.19	4.27
1.5	88.36 ± 1.22	1.38	90.97 ± 3.06	3.37

plasma and 7.9% ($n=9$) for blood spots. The interday precision was also calculated for both plasma and blood spots over three days ($n=27$ determinations). The R.S.D. did not exceed 7.5% for plasma and 4.8% for blood spots, at any concentrations, on any of the three days.

3.5. Recovery

Recovery was determined by comparing absolute peak areas for RIF in plasma and blood spots with the standard RIF solutions described above. The results for the recovery of RIF from both plasma and blood spots are shown in Table 3. The average recovery for the plasma method (84.8%) was higher than for the blood spots (65.0%). The R.S.D. of replicate recovery studies ($n=9$) was found to be less than 9.5% for all results.

3.6. Sensitivity

The limit of detection for the procedure was defined as the concentration of RIF that produces a peak whose height is three times the height of the noise from blank sample. This value was found to be 0.2 μg/ml in plasma and 0.5 μg/ml for blood spots. The limit of quantification for the procedure was defined, according to the International Conference on Harmonisation (ICH) guidelines, as the concentration of RIF that produces a peak whose height is 10 times the height of the noise from blank sample. This value was found to be 0.5 μg/ml in plasma and 1.5 μg/ml for blood spots. Although the LOQ value for plasma is higher than previously reported values which range from 0.05 to 0.1 μg/ml [9,15,16], the present method has the advantage of demonstrating specificity/selectivity for RIF in the presence of both the major metabolite (DRIF) and the major degradation product (RifQ).

3.7. Stability

In short term stability studies in plasma, no degradation was observed in thawed samples prepared at $t=0$, 3, 6 and 9 h. The response for RIF (measured as peak area ratio, RIF/IS) in the samples prepared and analysed after 9 h were 93.7% and 96.1% of the response at $t=0$ h at RIF concentrations of 0.5 and 20 μg/ml, respectively, in plasma. Similarly, the response for RIF in the post preparative samples analysed after 9 h were 93.1% and 101.0% that of samples analysed at $t=0$ h at RIF concentrations of 0.5 and 20 μg/ml, respectively, in plasma. Degradation was observed at the lower concentration (0.5 μg/ml) in the freeze–thaw samples: the RIF response of freeze–thaw samples was 59.3% that of fresh samples at the same concentration. However, at the higher concentration (20 μg/ml), this degradation was less evident: the RIF response of the freeze–thaw samples was 91.7% that of fresh samples at the same concentration.

Table 3
Recovery of rifampicin from plasma and blood spots

Matrix	RIF concentration (μg/ml)	Average recovery (%) ($n=3$)	R.S.D. of recoveries ($n=3$)
Plasma	1.0	84.72 ± 7.05	8.35
	5.0	84.18 ± 4.82	5.72
	10.0	85.34 ± 3.74	4.39
Blood spots	1.5	71.35 ± 1.45	2.03
	5.0	60.38 ± 5.53	9.16
	10.0	63.12 ± 4.77	7.55

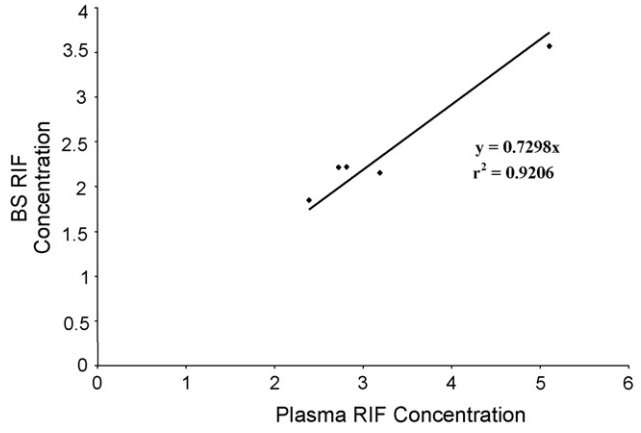

Fig. 4. Graph showing correlation between RIF concentration in plasma and blood spots of patients with TB.

The short term and post preparative blood spot samples showed no degradation over 9 h, as with the plasma samples. RIF responses for the 9 h short term stability samples were 95.0% and 96.1% that of samples prepared and analysed immediately after the spots had dried ($t = 0$) at RIF concentrations of 0.5 and 20 μg/ml, respectively, in blood spots. The responses due to RIF for the 9 h post preparative samples were 82.6% and 79.4% that of samples analysed immediately after preparation at RIF concentrations of 0.5 and 20 μg/ml, respectively, in blood spots.

3.8. Patient samples

Fig. 3 shows the chromatogram obtained from analysis of a blood spot sample taken from one patient. There was a rectilinear relationship between the concentrations of RIF in plasma and that in the blood spots with a correlation coefficient of 0.92 (Fig. 4).

4. Discussion

TDM of RIF is not routinely carried out in TB patients. However, due to the problems with malabsorption and erratic compliance, such testing would be advantageous. There are many examples in the literature of the extraction and analysis of RIF in plasma [6–13]. SPE using silica based reverse phase (RP) cartridges has been used to extract RIF from plasma [7,8,15] with reasonable success: recoveries ranged from 88 to 98% and linearity has been demonstrated in the ranges 0.16–20 μg/ml [7] and 0.05–35 μg/ml [15]. In our study, the C_{18} SPE cartridges produced variable recoveries as did the polymeric Strata-X cartridges. There was also a large drop in recovery of RIF at 5 μg/ml compared to 10 μg/ml with the Strata-X cartridges The cation exchange (Strata-X-CW) cartridges provided an average recovery of 83.3%, which was reproduced over replicates with three concentrations of RIF (1, 5 and 10 μg/ml) (Table 1).

A number of substances were evaluated for use as an internal standard for RIF. Rifabutin and rifapentine were rejected on the grounds of asymmetrical peaks (using the proposed method, symmetry factor As = 3) and lack of availability, respectively. Sulindac and papaverine hydrochloride, although structurally unrelated to RIF, have been reported as internal standards for RIF [7,8,15]. Using the method described sulindac co-eluted with RIF and so was rejected. Papaverine hydrochloride was baseline resolved from RIF and produced reproducible results with the proposed method and so was chosen as the internal standard for RIF.

RIF oxidises in solution to form rifampicin quinone (RIF-Q) [17]. Ascorbic acid is often added to solutions of RIF (in plasma or aqueous) to slow down this oxidation [7–9,12,15]. A quinone peak can be observed by HPLC approximately 2 h after sample preparation so it is important to analyse samples rapidly and to prepare fresh calibration standards regularly. In addition to this degradant, RIF is metabolised *in vivo* to DRIF via a desacetylation pathway [12]. It is essential that analytical methods show both specificity and selectivity for RIF in the presence of both RIF-Q and DRIF. Few published methods [6,9,12] report the resolution of RIF from these related substances. The United States Pharmacopoeia (USP) [18] method for analysis of anti-TB drugs in an FDC tablet [12,15] separates RIF, DRIF and RIF-Q with baseline resolution between all compounds but the analysis is lengthy (>23 min) and the analytes were extracted from simple matrices such as a tablet. The proposed method is specific and selective for RIF in the presence of both RIF-Q and DRIF. A DRIF standard was used to identify the presence of RIF in patient samples. It is baseline resolved from RIF, with an average ($n = 3$) Rs of 9.0 (Fig. 2a and b). LC–MS analysis of RIF and its major degradant (Fig. 5) confirmed the identity of the degradant as RIF-Q. The average Rs between the two analytes was calculated as 6.0 ($n = 3$).

Validation of both methods was carried out according to the International Conference of Harmonisation (ICH) guidelines on validation. The stability studies were carried out according to the food and drug administration guidelines as the advice was more specific to biological samples than that given in the ICH guidelines.

Calibration curves for average peak area ratios (RIF/IS) were found to be linear in the range 0.5–20 μg/ml for plasma and 1.5–20 μg/ml for blood spots. This should be adequate for most patients taking doses of 450 or 600 mg of RIF per day (C_{max} range is approximately 1.9–20 μg/ml) [19].

Intraday and interday precision were found by calculating the R.S.D.s for peak area ratios (RIF/IS) of spiked samples at three

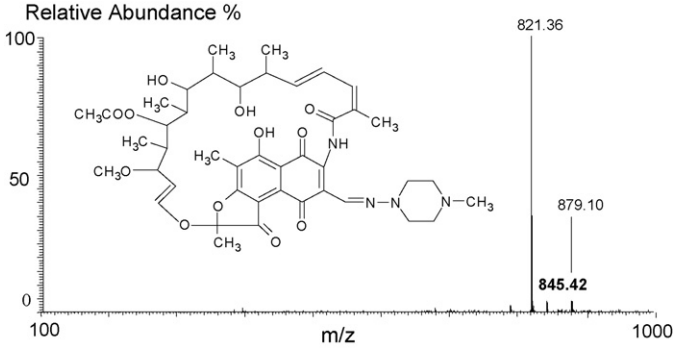

Fig. 5. Positive ion mass spectra of LC peak corresponding to the degradant RIF quinone ($m/z = 821$ amu). Inset: structure of RIF quinone.

different concentrations of RIF in both plasma and blood spots. This was repeated with three different samples (overall, $n = 9$). The intraday R.S.D. for plasma was found to be less than 7.2% ($n = 9$). In blood spots this was found to be less than 7.9% ($n = 9$). The interday R.S.D. was found to be less than 7.5% in plasma and less than 4.8% in blood spots ($n = 9$, nine determinations on each of 3 days). One-way ANOVA (unstacked) analysis showed no significant difference between the data on three separate days ($p > 0.05$) in either plasma or blood spots.

The calculated recovery of RIF in plasma is higher than for RIF in blood spots (Table 3). This can be attributed to the fact that only 80% of RIF is bound in plasma [20], the remainder binds to red blood cells. Therefore, approximately 20% of the RIF added to whole blood will be discarded with the red blood cells. If this is taken into account when calculating the recovery of RIF from blood spots, the recovery of the method is comparable with the plasma method.

The LOQ of the method was found to be 0.5 μg/ml in plasma and 1.5 μg/ml in blood spots. There are no accepted range of C_{max} values of RIF in plasma or blood as it varies significantly from patient to patient. However, Vekey et al. [19] reported a C_{max} range of 1.9–20 μg/ml. Any method that can quantify RIF at concentrations below 1.9 μg/ml should be suitable for the majority of patients with TB, provided samples are taken at a time when plasma concentrations of RIF are at a maximum (approximately 2 h after dosing).

In the stability studies, one-way ANOVA (unstacked) analysis showed no significant difference over time (up to 9 h) of the peak area ratios obtained with RIF samples in plasma and blood spots, $p > 0.05$. This is indicative of the stability of RIF in plasma, blood spots and water for up to 9 h. The post preparative samples were also stable for up to 9 h ($p > 0.05$, ANOVA). The freeze–thaw study showed that RIF is stable in plasma over three freeze–thaw cycles at a concentration of 20 μg/ml ($p > 0.05$, ANOVA). However, it was not found to be stable at the lower concentration of 0.5 μg/ml ($p < 0.05$, ANOVA). It is recommended that analyses are carried out on the day of preparation. Any plasma samples collected should be kept in the freezer (−20 °C) until required for analysis but should be analysed promptly once thawed. The patient sample analysis showed that samples are stable for up to almost 2 years, if stored in the conditions described.

5. Conclusions

The assay procedures described are suitable for the quantification of RIF in both plasma and blood spots. Linearity, accuracy, precision and resolution from major degradants and metabolites have been demonstrated and both methods are sensitive for the detection of therapeutic concentrations of RIF in both plasma and blood spots. Both methods afford high, consistent recovery of RIF. These methods could be used for therapeutic drug monitoring of rifampicin in TB patients to identify cases of RIF malabsorption or non-compliance. A good correlation between the plasma and blood spot methods has been shown using samples from five patients with TB. It has been demonstrated that the samples are stable for up to 9 h after preparation, and that patient samples are stable for long periods of time (up to 2 years) if properly stored. The blood spot method has particular potential, as it does not require the patient to attend a clinic; patients could take their own samples and send the cards to a laboratory. This would be especially useful in remote, developing areas.

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