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Determination of rimonabant in human plasma and hair by liquid chromatography–mass spectrometry

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ABSTRACT

Rimonabant is the first therapeutically relevant cannabinoid antagonist, licensed in Europe for treatment of obesity when a risk factor is associated. The objective of this study was to develop and validate a method for measurement of rimonabant in human plasma and hair using liquid chromatography coupled to mass spectrometry (LC–MS/MS). Rimonabant and AM-251 (internal standard) were extracted from 50 μL of plasma or 10 mg of hair using diethylether. Chromatography was performed on a 150 mm × 2.1 mm C18 column using a mobile phase constituted of formate buffer/acetonitrile. Rimonabant was ionized by electrospray in positive mode, followed by detection with mass spectrometry. Data were collected either in full-scan MS or in full-scan MS/MS mode, selecting the ion *m/z* 463.1 for rimonabant and *m/z* 555.1 for IS. The most intense product ion of rimonabant (*m/z* 380.9) and IS (*m/z* 472.8) were used for quantification. Calibration curves covered a range from 2.5 (lower limit of quantification) to 1000.0 ng/mL (upper limit of quantification) in plasma and from 2.5 to 1000.0 pg/mg in hair. Validation results demonstrated that rimonabant could be accurately and precisely quantified in both matrixes: accuracy and precision were within 85–115% and within 15% of standard deviation, respectively. Stability studies in plasma showed that rimonabant was stable during the assay procedure, but a 30% decrease was observed for one concentration after 3 weeks at −20 °C. This simple and robust LC–MS/MS method can be used for measuring rimonabant concentrations in human plasma and hair either in clinical or in forensic toxicology.

1. Introduction

Rimonabant (Acomplia®) is the first therapeutically relevant cannabinoid antagonist [1]. It is a selective antagonist of the cannabinoid CB_1 receptor [2,3], licensed in Europe for treatment of obesity when another risk factor is associated (dyslipidemia, diabetes, etc.). Rimonabant is a new drug for an important public health concern, knowing that the World Health Organization declared that more than 1 billion people worldwide are overweight [4]. In four double-blind trials with more than 6600 overweight or obese patients, it reduced body weight and waist circumference, and also improved the profile of several metabolic risk factors (mainly increase of HDL cholesterol and reduction of triglycerides) [5–8]. The most frequent adverse events leading to drug discontinuation in these trials were mood-related disorders (depression, suicidal tendencies), which were the reason why the Food and Drug Administration did not approve rimonabant in the United States yet [9,10]. Many other effects have been described, such as attenuation of effects of smoked cannabis [11,12], hepatoprotective functions [13,14], modulation of cardiometabolic risk factors [15,16], anti-inflammatory and anti-hyperalgesic properties [17], bronchospasm [18] and actions on neurotransmission [19,20]. Rimonabant may also play a role in treatment of cocaine addiction [21].

Following multiple once-daily doses of 20 mg to healthy subjects, maximum plasma concentrations of rimonabant are achieved in approximately 2 h with steady state plasma concentrations achieved within 13 days (C_{max} = 196 ± 28.1 ng/mL; C_{min} = 91.6 ± 14.1 ng/mL; AUC_{0-24} = 2960 ± 268 ng h/mL) [22]. The *in vitro* human plasma protein binding of rimonabant is more than 99.9%. Rimonabant is metabolized by both CYP3A and amidohydrolase pathways but circulating metabolites do not contribute to its pharmacologic activity. Rimonabant is mainly eliminated by metabolism and subsequent biliary excretion of metabolites. Approximately 3% of the dose is eliminated in the urine, while about 86% is excreted in the faeces as unchanged drug and metabolites. In obese patients, the elimination half-life is longer (about 16 days) than in non-obese patients (about 9 days) due to a larger volume of distribution [22].

To our knowledge, quantification of rimonabant in human matrix has been reported in plasma only. Nirogi et al. [23]

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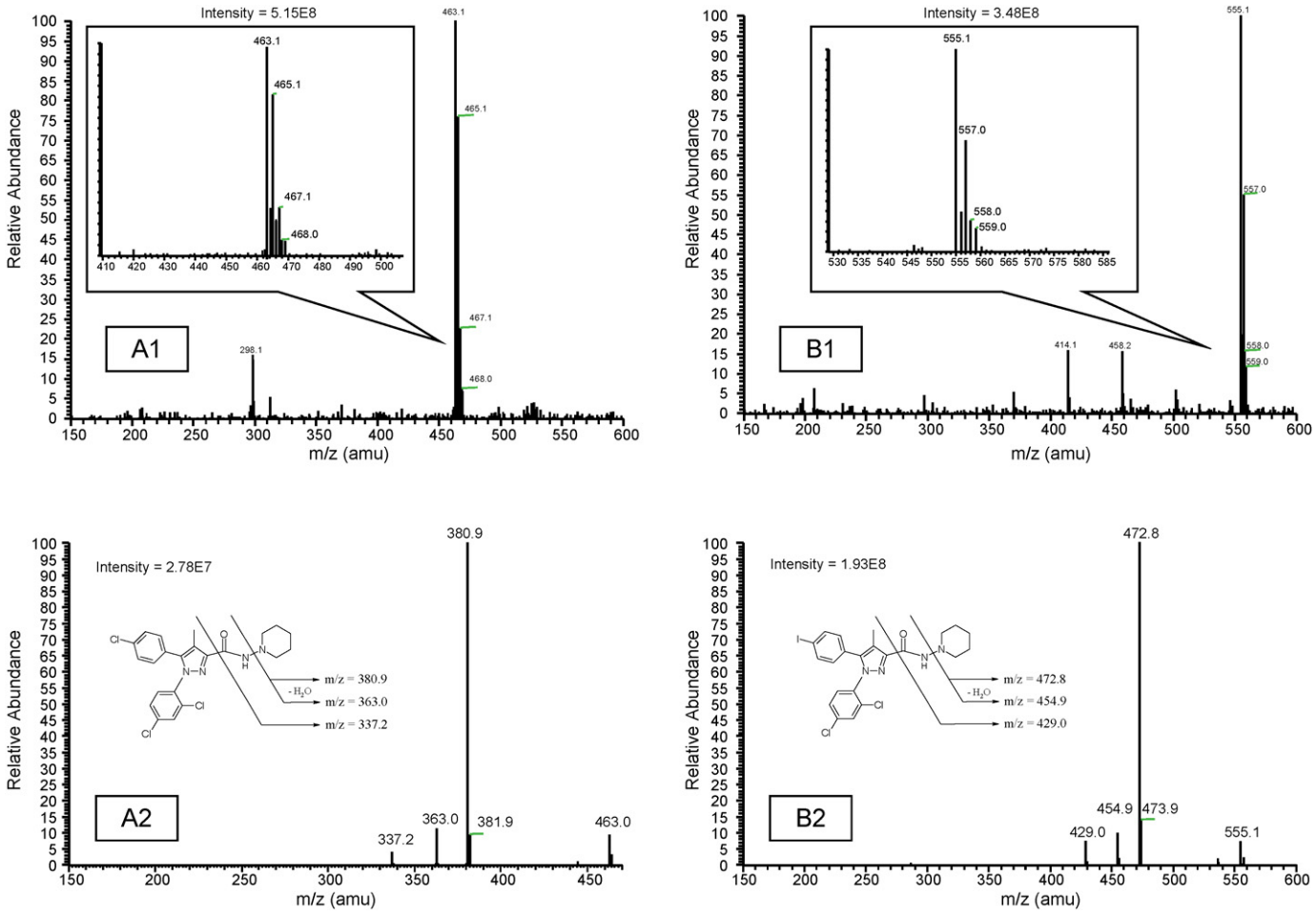

Fig. 1. Full positive ion scan (upper panel) and full scan MS/MS spectra (lower panel) of extracted plasma spiked with 500.0 ng/mL of rimonabant (A) and extracted plasma spiked with 400.0 ng/mL IS AM-251 (B). Regions of full ion scans are zoomed and presented in boxes. Chemical structures of rimonabant and AM-251 are also presented in A2 and B2, respectively.

reported an LC–MS/MS method for quantification of rimonabant in a short range of concentration (0.1–100 ng/mL) from 200 μL of plasma, while therapeutic concentrations range from about 100 to 200 ng/mL. McCulloch et al. [24] also reported quantification of rimonabant in plasma, but after a single protein precipitation from 200 μL of plasma. Another study reported quantification of rimonabant in mouse plasma using fused-core silica column [25], without any validation of the method. The purpose of our study was to develop and validate a rapid and sensitive LC–MS/MS method for determining rimonabant concentrations in human plasma and hair. This latest matrix may be interesting for determining the cumulative month-to-month exposure to rimonabant with patients chronically treated, or in forensic toxicology when blood samples are not available, and because of the weak excretion of rimonabant in urine. This method had to require small sample amounts and should be easily applied to clinical pharmacokinetics. The developed assay was applied to hair samples of two volunteers receiving rimonabant, which was also measured in plasma for one of them.

2. Experimental

2.1. Chemicals and reagents

Rimonabant [*N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide] (Fig. 1A2, $C_{22}H_{21}Cl_3N_4O$; monoisotopic mass: 462.08) was a kind gift from Sanofi-Synthelabo and the internal standard (IS) AM-251 (Fig. 1B2, $C_{22}H_{21}Cl_2IN_4O$; monoisotopic mass: 554.01) was supplied by Tocris Cookson Ltd. (Bristol, United Kingdom). Acetonitrile, methanol, dichloromethane and diethylether (all HPLC-grade) were purchased from Sigma Aldrich (Paris, France), Prolabo (Paris, France) and Merck (Darmstadt, Germany), respectively. Formic acid was purchased from Sigma–Aldrich, sodium carbonate and sodium hydrogenocarbonate from Prolabo. Ultra-pure water was obtained by ultrafiltration using a Direct-Q UV3 apparatus (Millipore Corp., Molsheim, France). All other chemicals were of analytical grade.

2.2. LC–MS/MS system and conditions

Chromatography was performed on Surveyor liquid chromatography system (Thermofisher, Les Ulis, France), using a 5-μm HyPurity C18 ThermoHypersil column (150 mm × 2.1 mm i.d.) maintained at 30 °C. The device was completed with a precolumn (HyPurity C18 ThermoHypersil, 5 μm, 4 mm × 2.1 mm i.d.). Elution was achieved isocratically with a mobile phase of 2 mmol/L NH_4COOH pH 3.8 buffer/acetonitrile (20:80, *v/v*) at a flow rate of 300 μL/min and run time was 5 min. Compounds were detected by a LCQ Deca XP mass spectrometer (Thermofisher) equipped with an electrospray ionization (ESI) source.

Nitrogen (Nitrox UHPLCMS 18, nitrogen generator, Domnick Hunter, Villefranche sur Saône, France) was employed as sheath and auxiliary gas at a pressure of 50 and 10 arbitrary units, respec-

tively. The ESI source was set in positive ionization mode, and an ion-spray voltage of +4.0 kV was applied. Capillary temperature was set to 250 °C under a voltage of +3.2 V. The system was tuned using a continuous 12 μL/min infusion of rimonabant (1 mg/L in mobile phase). Acquisition was performed either in full-scan mode or in full-scan MS/MS mode (*m*/*z* 150.0–600.0). Protonated molecular ions $[M+H]^+$ of rimonabant (*m*/*z* 463.1) and IS (*m*/*z* 555.1) were trapped with a mass resolution of 1.0 atomic mass unit (amu) and fragmented with a collision energy of 34 and 33%, respectively. The most intense product ions resulting from these fragmentations were *m*/*z* 380.9 (used as quantification ion), *m*/*z* 363.0, and *m*/*z* 337.2 (used as confirmation ions) for rimonabant; *m*/*z* 472.8 (quantification ion), *m*/*z* 454.9 and *m*/*z* 429.0 (confirmation ions) for IS. Chromatographic data acquisition and quantification were performed using the Xcalibur software (v1.3, Thermofisher).

2.3. Working solutions, calibration standard, and quality controls

Stock solution of rimonabant and IS (1 g/L) were prepared in methanol. Working solutions of rimonabant for calibration standards (CS) were prepared at three concentrations in each matrix (0.025, 0.25 and 2.5 mg/L for plasma; 0.005, 0.05 and 0.5 mg/L for hair) by dilution of the stock solution in methanol. Working solutions of rimonabant for quality control (QC) preparation were also prepared at three concentrations (1, 10 and 100 mg/L for plasma; 0.00375, 0.0375 and 0.375 mg/L for hair) by dilution in methanol of another 1 g/L stock solution prepared separately. Working solutions of IS (0.25 mg/L for plasma and 0.025 mg/L for hair) were obtained by dilution of stock solution in methanol.

Calibration curves were prepared by spiking with appropriate volumes of the previously mentioned working solutions either blank plasma to produce the CS equivalent to 2.5, 5.0, 10.0, 25.0, 50.0, 100.0, 250.0, 500.0 and 1000.0 ng/mL; or blank hair to obtain CS equivalent to 2.5, 5.0, 10.0, 25.0, 50.0, 100.0, 250.0, 500.0 and 1000.0 pg/mg. QC samples were also prepared in bulk in blank plasma at concentrations of 15.0, 150.0 and 750.0 ng/mL or in blank hair at concentrations of 7.5, 75.0 and 750.0 pg/mg. Blank human hair samples were obtained from healthy volunteers and processed as described in the hair sample preparation section. CS solutions and plasma QCs were stored at −20 °C while capillary QCs were prepared extemporaneously.

2.4. Sample preparation

2.4.1. Plasma samples

Samples were processed using a liquid-liquid extraction. 20 μL of the IS working solution (AM-251, 0.25 mg/L) and 500 μL of diethylether were added to each tube containing 50 μL of plasma. The samples were then shaken for 15 min and centrifuged at 3500 rpm for 10 min. The upper organic layer was decanted into another tube and evaporated to dryness under a nitrogen stream. Samples were reconstituted with 150 μL of mobile phase, vortex mixed for 10 s, and transferred into injection vials for analysis (injection volume 10 μL).

2.4.2. Hair samples

Hair was decontaminated successively 2 min with dichloromethane, 2 min with hot water, and 2 min with dichloromethane. It was then allowed to dry at room temperature, cut into small pieces of less than 3 mm, and pulverized in a ball mill (MM200, Fisher Scientific, Illkirch, France). 10 mg of powered hair was incubated overnight in 500 μL of 1 mol/L carbonate buffer (pH 9.7) in presence of 20 μL of IS working solution (AM-251, 0.025 mg/L) and the appropriate volume of CS or QC working solution when necessary. The aqueous homogenate was extracted with 1000 μL of diethylether by the same procedure than for plasma, and dry extracts were reconstituted with 75 μL of mobile phase, before injection of 10 μL in the system.

2.5. Method validation procedure

2.5.1. Linearity

Calibration curves included a blank sample, a zero sample, and nine CS over the following concentration range: 2.5 ng/mL (lower limit of quantification (LLOQ)) to 1000.0 ng/mL (upper limit of quantification (ULOQ)) for plasma; and 2.5 pg/mg (LLOQ) to 1000.0 pg/mg for hair. Eleven (plasma) and six (hair) calibration curves obtained over a period of 1 month were taken into account for the determination of the best fit. The best fit among linear and quadratic equations was determined using various weighting factors of the inverse concentration (e.g. $1/x$ and $1/x^2$). The equation showing the lowest and most constant percentage total bias from nominal CS values was considered as the best-fit model. The IS method was used for quantification: rimonabant/IS ratios were plotted against the spiked concentrations. Back-calculated concentrations of the CS had to be within 85–115% of the nominal concentrations.

2.5.2. Specificity, carry over

To investigate whether endogenous matrix constituents interfered with the assay, drug-free matrix blank samples, zero samples and samples spiked at the LLOQ were analyzed according to the described procedure. Assay specificity was defined by evidence of non-interference at retention times and ion channels identical to that of rimonabant and IS in the blank samples. A blank sample was also analyzed immediately following the highest CS in each run to monitor the carry over of rimonabant and IS.

2.5.3. Lower limit of quantification

The LLOQ was defined as the lowest concentration for which an accuracy between 80 and 120% and a precision with a coefficient of variation of ±20% or less that was obtained over six measurements.

2.5.4. Accuracy and precision

Accuracy (measured value/nominal value) and precision (coefficient of variation) were determined for the three QC levels in each matrix. For the intraday assay, six replicates of each QC level were processed the same day. For the interday assay, each QC level was processed six times three different days over a period of 1 month. The values obtained were analyzed using analysis of variance (ANOVA), which separated the intra and interday standard deviation and consequently the corresponding coefficients of variation (CV). An accuracy within the range 85–115% of the nominal values and a precision with a CV of ±15% were required, except for the LLOQ for which a range of 80–120% and a CV of ±20% were accepted for accuracy and precision, respectively.

2.5.5. Recovery and matrix effect

For plasma assay, three procedures (A–C) were performed on five different matrix sources at three concentrations (15.0, 150.0 and 750.0 ng/mL) in order to evaluate extraction yield, matrix effect and overall method recovery: (A) rimonabant and the IS were spiked in the mobile phase and directly injected; (B) rimonabant and the IS were spiked afterwards in extracted blank matrix samples and injected; (C) rimonabant and IS were spiked in plasma samples, the complete extraction procedure was carried through, and the samples were injected into the system. The mean chromatographic peaks obtained using the three procedures were compared. The ratios C/B, B/A, and C/A determined the yield of extraction, the matrix effect, and the overall method recovery,

respectively, and were calculated for both rimonabant ($n = 5$ for each QC concentration) and IS ($n = 15$). Overall method recovery of the IS had to be ±15% of rimonabant recovery.

For hair assay, the same A and B procedures were employed to determine cumulative effects of hydrolysis and matrix on six different matrix sources, determined by the B/A ratio.

2.5.6. Analyte plasma stability

To test the short- and long-term stabilities of the extracted rimonabant, three plasma QC samples (15.0, 150.0 and 750.0 ng/mL) were analyzed in triplicate after three freeze and thaw cycles by complete thawing at room temperature and freezing at −20 °C for 12–24 h. The short-term stabilities of extracted samples were also determined during storage for 24 h at +20 °C, at +4 °C and at −20 °C. Long-term storage stability at −20 °C was determined after 21 days. Subsequently, the rimonabant concentrations were measured and compared with freshly prepared samples. Analytes were considered stable in plasma at each concentration when the differences were found to be not exceeding 15%. Stability of processed samples was assessed by re-injection of three plasma QC levels ($n = 6$) after conservation 24 h in the autosampler (set at 20 °C) or at +4 °C and comparison with the previously obtained values.

2.5.7. Robustness

Consequences of small variations of the analytical conditions were determined by quantification of triplicates of the three plasma QC levels under normal conditions (see Section 2.2), and when changing the flow rate of mobile phase (250 μL/min instead of 300 μL/min), the composition of the mobile phase (75% of acetonitrile instead of 80%) and the column temperature (27 °C instead of 30 °C).

3. Results and discussion

3.1. LC–MS/MS analysis

In LC–MS/MS analysis, the choice of an appropriate internal standard is a crucial point. The better candidates are isotopically labelled analytes because they usually behave like the studied drugs and produce similar extraction recovery and matrix effect. In our case, as there was no isotopically labelled rimonabant available, AM-251 was chosen because of its chemical structure similar to rimonabant (Fig. 1A2 and B2), as in the method of McCulloch et al. [24]. In the previously described method of Niragi et al. [23], sitagliptin was used, but provided an extraction recovery 17.8% lower than the one of rimonabant. Fig. 1 presents the full-scan positive ion mass spectra of rimonabant (Fig. 1A1) and IS (Fig. 1B1). Full-scan MS spectra show a major peak at m/z 463.1 for rimonabant and m/z 555.1 for IS, corresponding to the protonated $[M+H]^+$ ions. Two other peaks at m/z 465.1 and m/z 467.1 for rimonabant and at m/z 557.0 and m/z 559.1 for IS were observed, which correspond to isotopes of these compounds. MS/MS spectrum of rimonabant (Fig. 1A2) shows that the most intense product ion is observed at m/z 380.9, which was used for quantification. Ions at m/z 363.0 and 337.2 were used as confirmation ions. Fig. 2 shows typical chromatograms of the hair sample analysis: an extracted blank sample injected just after the highest CS (Fig. 2A), a sample spiked only with IS (Fig. 2B), and calibration standards at LLOQ (Fig. 2C) and ULOQ (Fig. 2D). Under optimized conditions, rimonabant and IS were separated with retention times of 3.3 and 3.8 min, respectively. No interferences from constituents of drug-free human plasma and hair at the retention times and the ion channels of rimonabant and IS were observed (Fig. 2A). When a blank sample was analyzed immediately after the highest calibration standard, mean carry over was lower than 0.3%.

3.2. Method validation

3.2.1. Linearity

A quadratic equation weighted by the inverse concentration ($1/x$) resulted in the best fit. The mean bias of 99 measurements from 11 calibration curves was 7.2 ± 6.1% for plasma. Mean fitted equation obtained from these same 11 curves for nine points was: $y = 0.003675\ (\pm 0.003957) + 0.006215\ (\pm 0.000554)x - 7.414\text{E}{-07}\ (\pm 6.037\text{E}{-07})x^2$ ($r^2 = 0.9972\ (\pm 0.0015)$). For hair, the mean bias of 63 measurements from 6 calibration curves was 8.7 ± 5.9% and the mean fitted equation was: $y = -0.01251\ (\pm 0.01451) + 0.01162\ (\pm 0.00147)x - 1.473\text{E}{-06}\ (\pm 2.2604\text{E}{-06})x^2$ ($r^2 = 0.9961\ (\pm 0.0021)$). Interday CV ranged from 1.5% to 12.4% and bias ranged from 91.9% to 108.3% for the back-calculated concentrations of the nine calibration standards in plasma. In hair, these values ranged from 0.7% to 14.6% and 86.0% to 111.2%, respectively (data not shown). The LLOQ was 2.5 ng/mL for plasma and 2.5 pg/mg for hair, demonstrating a CV of less than 20%, and accuracy greater than 80%, with a signal-to-noise ratio greater than 10. The ULOQ was 1000.0 ng/mL for plasma and 1000.0 pg/mg for hair. This wide range of concentration should allow quantification of rimonabant in patient's plasma without diluting samples and should also allow easy assay of rimonabant in human hair.

3.2.2. Precision and accuracy

Intra and interday precision and accuracy of QC samples are summarized in Table 1 for plasma and Table 2 for hair. In plasma, intraday precision ranged from 5.0% to 7.3% with an accuracy ranging from 97.5% to 106.5%, and interday precision ranged from 4.0% to 6.0% with an accuracy from 96.7% to 102.9%. In hair, intraday and interday precisions were included between 2.3–11.0% and 6.9–13.9%, respectively. Intra and interday accuracies were also acceptable, between 92.5 and 102.6%. These data confirm that the present method has a satisfactory accuracy and precision for quantification of rimonabant throughout a wide dynamic range.

3.2.3. Recovery and matrix effect

In plasma, extraction yield, matrix effect, and overall recovery of the method were independent of rimonabant concentration (Table 3). The mean extraction yield of rimonabant was 74.4 ± 10.0%. The mean matrix effect was 117.4 ± 19.5%, suggesting that plasma extraction residues may moderately enhance rimonabant detection. The mean overall recovery of rimonabant was 95.1 ± 18.1%. Mean extraction yield, matrix effect and overall recovery of the IS were in the same range each time, with less than 5% of difference compared to rimonabant, showing that this molecule behaves like rimonabant. In hair, evaluation of hydrolysis and matrix effect provided results showing a mean effect of 70.7 ± 15.3% for rimonabant, and 70.9 ± 16.7% for IS (Table 4), highlighting that hair process induces a slight loss of signal, identical for rimonabant and IS.

3.2.4. Analyte plasma stability and robustness

Three freeze–thaw cycles did not result in significant changes of the rimonabant plasma concentrations in the QC samples. However, when QC samples were stored at −20 °C for 3 weeks, a decrease of 29.7% of rimonabant concentration has been observed for expected concentration at 750.0 ng/mL. When QC samples were stored at +20, +4 or −20 °C for 24 h, rimonabant concentrations remained within 85–115% of the initial values. These observations suggest that clinical plasma samples containing rimonabant should be measured in the following 24 h after blood collection if not frozen.

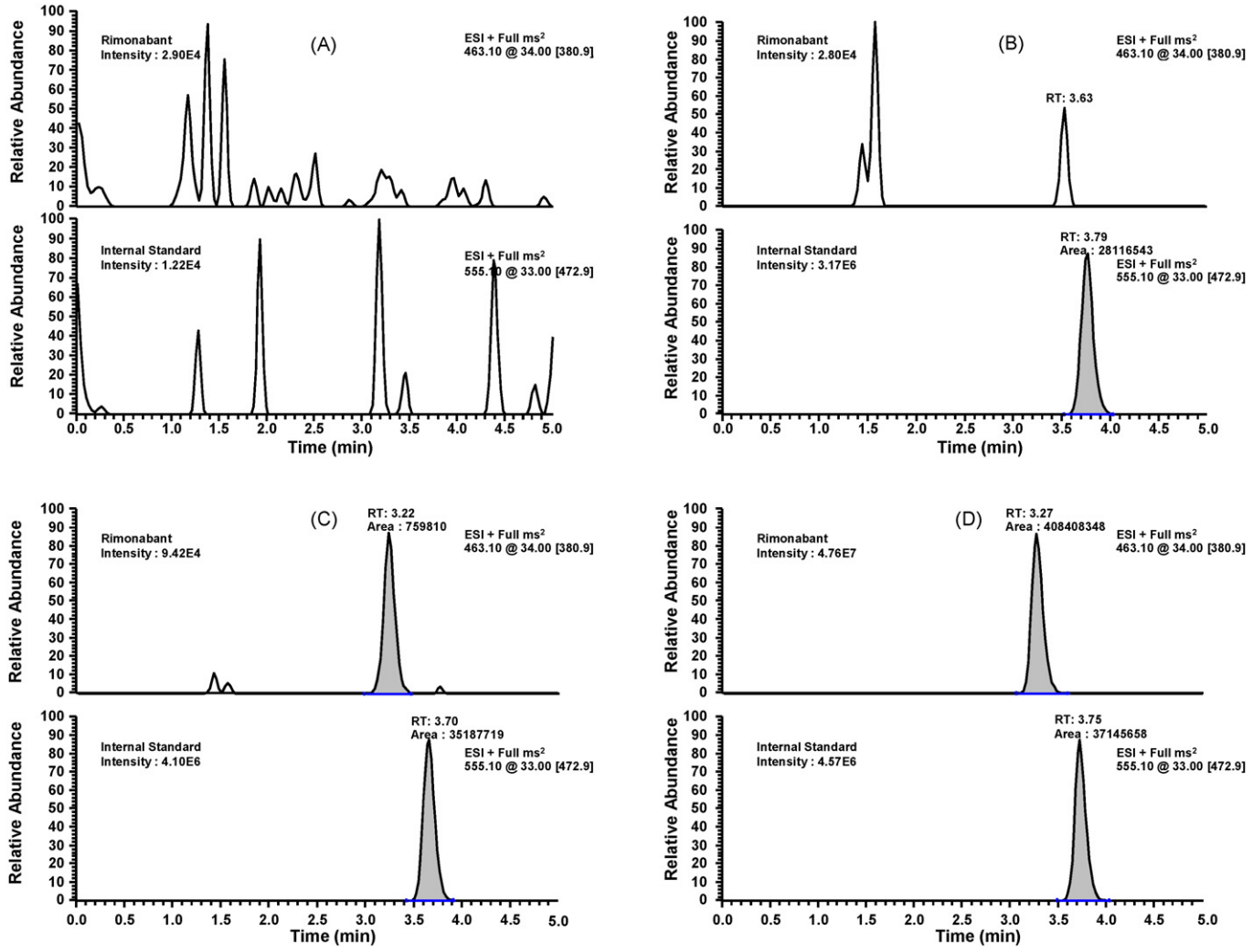

Fig. 2. LC–MS/MS chromatograms of rimonabant (upper graphs) and IS (lower graphs) of an extracted blank hair sample injected just after the highest calibration standard (1000.0 pg/mg) (A), hair spiked only with IS (B), and calibration standards at 2.5 pg/mg (LLOQ) (C) and 1000.0 pg/mg (ULOQ) (D).

Table 1
Accuracy and precision data for plasma QC samples of rimonabant

QC concentration (ng/mL)	Intraday ($n = 6$)			Interday ($n = 6$)		
	Concentration found (ng/mL)	CV (%)	Accuracy (%)	Concentration found (ng/mL)	CV (%)	Accuracy (%)
15	16.0 ± 0.8	5.2	106.5	15.4 ± 0.9	6.0	102.9
150	155.8 ± 11.3	7.3	103.9	148.8 ± 5.9	4.0	99.2
750	731.5 ± 36.7	5.0	97.5	725.6 ± 37.0	5.1	96.7

Similarly, when extracted samples were kept at +20 or +4 °C for 24 h, no significant difference has been observed compared to the initial value. Our robustness study confirmed that small variations in flow rate, mobile phase composition and column temperature did not affect the rimonabant resolution by LC analysis (data not shown).

3.2.5. Application

Measurement of rimonabant concentration has been done with this method for two volunteers in compliance with the Declaration of Helsinki. The first one was a 45-year-old man, weighing 95 kg for 1.72 m with a body mass index of 32. He was treated for 6 weeks with 20 mg daily rimonabant for his overweight, and

Table 2
Accuracy and precision data for hair QC samples of rimonabant

QC concentration (pg/mg)	Intraday ($n = 6$)			Interday ($n = 6$)		
	Concentration found (pg/mg)	CV (%)	Accuracy (%)	Concentration found (pg/mg)	CV (%)	Accuracy (%)
7.5	7.7 ± 0.9	11.1	102.6	7.4 ± 0.9	13.9	100.2
75	69.4 ± 4.4	6.3	92.5	71.7 ± 8.2	6.9	95.6
750	739.2 ± 16.8	2.3	98.6	747.7 ± 55.3	8.9	99.7

Table 3
Extraction yield, matrix effect, and overall method recovery of rimonabant and internal standard in human plasma

	Extraction yield	Matrix effect	Method recovery
Rimonabant			
15 ng/mL (n = 5)			
Mean (%)	71.2	120.5	102.7
CV (%)	12.0	25.8	19.1
150 ng/mL (n = 5)			
Mean (%)	77.1	115.0	89.7
CV (%)	10.3	11.9	14.1
750 ng/mL (n = 5)			
Mean (%)	74.8	116.7	92.8
CV (%)	18.7	10.8	23.8
AM-251 (IS)			
100 ng/mL (n = 155)			
Mean (%)	75.8	114.8	100.0
CV (%)	10.3	15.6	17.8

Table 4
Effect of hydrolysis and matrix on rimonabant and internal standard in hair

	Hydrolysis and matrix effect
Rimonabant	
7.5 pg/mg (n = 6)	
Mean (%)	74.8
CV (%)	15.7
75 pg/mg (n = 6)	
Mean (%)	67.9
CV (%)	8.5
750 pg/mg (n = 6)	
Mean (%)	69.4
CV (%)	21.6
AM-251 (IS)	
50 pg/mg (n = 18)	
Mean (%)	70.9
CV (%)	16.7

plasma quantification was done because he was suffering putative side effects, namely diarrhoea, nausea irritability and trouble with sleeping. Two blood samples were taken in the 6th week of treatment, one just before administration of the drug, and another one 2 h later. Results obtained were 114 ng/mL for C_{min} and 265 ng/mL for C_{max} (2 h after administration) which are in accordance with previously described concentrations [22]. Two samples of hair (salt-and-pepper) of this patient were obtained. Taking into account a mean hair growth speed of 1 cm per month, the first sample was representative of 3 weeks of exposure, and the second one of 4 weeks. In these samples, rimonabant was measured at 67.2 and 93.3 pg/mg.

The second volunteer was a 60-year-old woman, weighing 90 kg for 1.68 m with a body mass index of 32, and also receiving 20 mg daily rimonabant for hers overweight. She had brown hair, and a 4-cm long hair sample was cut. The first 2 cm (root side) represented only a few days of treatment while the two distal centimetres represented approximately 8 weeks of treatment. Rimonabant concentrations were measured in both samples and were found at 7 and 101 pg/mg, respectively.

To our knowledge, this is the first report of rimonabant concentration in human hair after chronic treatment, in one man and one woman with two different hair colours.

4. Conclusion

A fast LC–MS/MS method was developed and validated for quantification of rimonabant in human plasma and hair. The main advantages of this method are: (1) small amount of sample required (50 μL of plasma and 10 mg of hair); (2) processing is simple and rapid; (3) excellent recovery; (4) absence of carry over and observable matrix ionization in plasma; (5) short analytical time; and (6) validation over a large dynamic range including the whole spectrum of clinically relevant rimonabant concentrations. Compared to the previously described method, our technique prevents extraction solvent and mobile phase wasting, since lower volumes of both are used for one analysis. The IS which was used in our method behaves closely to rimonabant. While there are only few human pharmacokinetics data published on rimonabant, this simple, accurate, precise and robust analytical method could be useful for such studies, and could also help to identify rimonabant in a matrix such as hair in forensic toxicology. This matrix may also be helpful to measure month-to-month exposure to this drug.

References

[1] M.A. Carai, G. Colombo, G.L. Gessa, Life Sci. 77 (2005) 2339.
[2] M. Rinaldi-Carmona, F. Barth, M. Heaulme, R. Alonso, D. Shire, C. Congy, P. Soubrie, J.C. Breliere, G. Le Fur, Life Sci. 56 (1995) 1941.
[3] M. Rinaldi-Carmona, F. Barth, M. Heaulme, D. Shire, B. Calandra, C. Congy, S. Martinez, J. Maruani, G. Neliat, D. Caput, et al., FEBS Lett. 350 (1994) 240.
[4] M. Wadman, Nature 437 (2005) 618.
[5] F.X. Pi-Sunyer, L.J. Aronne, H.M. Heshmati, J. Devin, J. Rosenstock, JAMA 295 (2006) 761.
[6] A.J. Scheen, N. Finer, P. Hollander, M.D. Jensen, L.F. Van Gaal, Lancet 368 (2006) 1660.
[7] L.F. Van Gaal, A.M. Rissanen, A.J. Scheen, O. Ziegler, S. Rossner, Lancet 365 (2005) 1389.
[8] J.P. Despres, A. Golay, L. Sjostrom, N. Engl. J. Med. 353 (2005) 2121.
[9] R. Christensen, P.K. Kristensen, E.M. Bartels, H. Bliddal, A. Astrup, Lancet 370 (2007) 1706.
[10] P.B. Mitchell, M.J. Morris, Lancet 370 (2007) 1671.
[11] D.A. Gorelick, S.J. Heishman, K.L. Preston, R.A. Nelson, E.T. Moolchan, M.A. Huestis, Am. Heart J. 151 (2006) 754 e1.
[12] M.A. Huestis, S.J. Boyd, S.J. Heishman, K.L. Preston, D. Bonnet, G. Le Fur, D.A. Gorelick, Psychopharmacology (Berl.) 194 (2007) 505.
[13] F. Teixeira-Clerc, B. Julien, P. Grenard, J. Tran Van Nhieu, V. Deveaux, L. Li, V. Serriere-Lanneau, C. Ledent, A. Mallat, S. Lotersztajn, Nat. Med. 12 (2006) 671.
[14] M. Gary-Bobo, G. Elachouri, J.F. Gallas, P. Janiak, P. Marini, C. Ravinet-Trillou, M. Chabbert, N. Cruccioli, C. Pfersdorff, C. Roque, M. Arnone, T. Croci, P. Soubrie, F. Oury-Donat, J.P. Maffrand, B. Scatton, F. Lacheretz, G. Le Fur, J.M. Herbert, M. Bensaid, Hepatology 46 (2007) 122.
[15] L.J. Aronne, Am. J. Med. 120 (2007) S26.
[16] A.I. Kakafika, D.P. Mikhailidis, A. Karagiannis, V.G. Athyros, J. Clin. Pharmacol. 47 (2007) 642.
[17] B. Costa, Br. J. Pharmacol. 150 (2007) 535.
[18] A. Calignano, I. Katona, F. Desarnaud, A. Giuffrida, G. La Rana, K. Mackie, T.F. Freund, D. Piomelli, Nature 408 (2000) 96.
[19] E.T. Tzavara, R.J. Davis, K.W. Perry, X. Li, C. Salhoff, F.P. Bymaster, J.M. Witkin, G.G. Nomikos, Br. J. Pharmacol. 138 (2003) 544.
[20] B. Ferrer, M.A. Gorriti, A. Palomino, I. Gornemann, Y. de Diego, F.J. Bermudez-Silva, A. Bilbao, E. Fernandez-Espejo, R. Moratalla, M. Navarro, F. Rodriguez de Fonseca, Eur. J. Pharmacol. 559 (2007) 180.
[21] T.J. De Vries, Y. Shaham, J.R. Homberg, H. Crombag, K. Schuurman, J. Dieben, L.J. Vanderschuren, A.N. Schoffelmeer, Nat. Med. 7 (2001) 1151.
[22] European Agency for The Evaluation of Medicinal Products. Rimonabant: summary of product characteristics, 2006. http://www.emea.europa.eu/humandocs/PDFs/EPAR/acomplia/H-666-PI-en.pdf (18.07.2007).
[23] R. Nirogi, V. Kandikere, K. Mudigonda, D. Ajjala, Biomed. Chromatogr. 22 (2007) 469.
[24] M. McCulloch, X. Zhou, Y. Xu, S. Brunell, L. Spear, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 863 (2008) 258.
[25] Y. Hsieh, C.J. Duncan, J.M. Brisson, Anal. Chem. 79 (2007) 5668.