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Quantification of risperidone and 9-hydroxyrisperidone in plasma and saliva from adult and pediatric patients by liquid chromatography–mass spectrometry

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Abstract

A robust and validated LC–MS–MS quantitative method, using column switching and mutiple reaction monitoring was developed for the analysis of risperidone (RIS) and 9-hydroxyrisperidone in human plasma and saliva. The analytical range was 1–100 ng/ml. The method used 25 μl of sample precipitated with 75 μl of acetonitrile containing internal standard (R068808). Analyses were conducted on a PE Sciex API-III + triple quadrupole mass spectrometer fitted with a Turbo IonSpray source. The method was validated for human plasma using EDTA as the anticoagulant and cross-validated to heparinized human plasma and saliva. The recoveries of risperidone and 9-hydroxyrisperidone were 90–93 and 89–93%, respectively. The validated method was applied to clinical samples to study risperidone and 9-hydroxyrisperidone concentrations in plasma and saliva. Risperidone and 9-hydroxyrisperidone appear in the saliva of patients treated with risperidone. Their detection/quantification in saliva provides evidence for recent adherence with therapy.
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Keywords: Column switching; Validation; Risperidone; Hydroxyrisperidone

1. Introduction

Risperidone (RIS), an atypical neuroleptic drug, reported to have fewer adverse effects than traditional agents, is effective in psychoses, such as schizophrenia, and other psychiatric illnesses in adults and children, including pervasive developmental disorders (PDD), autism, and attention-deficit disorder (ADD) [1–3]. It acts primarily by selective antagonism of dopamine Type 2 (D_2) and serotonin Type 2 ($5HT_2$) receptors in the brain [4–6]. Apart from minor N-dealkylation, the major pathway of biotransformation of RIS is hydroxylation at the 9′ position on the pyrido-pyrimidone ring to 9-hyroxyrisperidone (9-OH-RIS), mediated by the liver enzyme, CYP2D6 [7]. The activity of CYP2D6, a genetically determined trait, is high in about 90% of the population (extensive metabolizers, EMs) and much lower in 10% (poor metabolizers, PMs) [8,9]. Many studies, including in vitro comparisons of affinity for dopamine receptors, indicate that the metabolite, 9-OH-RIS, is equally potent as the parent drug, RIS. In the majority of patients, EMs, 9-OH-RIS has a longer half-life (21–30 h) than RIS (3 and 20 h in EMs and PMs, respectively) [10,11]. Thus, both compounds are routinely assayed in patients. Recommended doses of RIS for adults range from 4 to 8 mg per day leading to plasma concentrations of 5–100 nM of RIS and 9-OH-RIS [1,12]. The plasma concentrations of RIS and 9-OH-RIS depend on the dose and individual pharmacokinetic characteristics of the patient. Typically, 1–100 ng/ml of RIS and 9-OH-RIS in plasma is an adequate range for most assays to cover concentrations encountered in patients [13,14].

RIS and 9-OH-RIS have most often been measured by HPLC using ultraviolet (UV) or electrochemical detection (ED) with quantification limits of about 2 and 0.1 ng/ml, respectively [14,15]. To date, few mass spectrometric methods have been published [13,16,17]. Two of these methods use

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initial plasma volumes of 0.5 ml and both report a limit of quantification (LOQ) of 0.1 ng/ml.

Most measurements of RIS and 9-OH-RIS have been in plasma [13–17]. To our knowledge, no assays have examined concentrations in saliva or undertaken parallel analyses in saliva and plasma. Quantitative and/or qualitative measurement of drugs in saliva has become a useful and suitable alternative to plasma in therapeutic drug monitoring (TDM) because collections are easier, non-invasive, painless and more acceptable to patients, particularly children. Previous studies of concurrent concentrations of drugs in plasma and saliva in TDM have been reported [18,19]. Some investigations of concurrent salivary/plasma (S/P) levels of drugs, including carbamazepine, fluconazole, lamotrigine, phenytoin, primidone, valproic acid and others, have shown a good correlation between salivary and plasma concentrations [20] while others have revealed marked inter- and intra-individual differences of S/P ratios for different drugs [21–24].

The purpose of this study was to: (1) develop and validate a robust, rapid, highly sensitive and specific LC–MS–MS method for the simultaneous measurement of RIS and 9-OH-RIS in small sample volumes (25 μl) of human saliva and plasma, using either heparin or EDTA as the anticoagulant, (2) determine recovery, accuracy, precision, linearity of standard curves, and limit of quantification and (3) demonstrate stability of analytes under standard conditions of time and temperature in the different matrices. This work describes a fast and specific method suitable for TDM in pediatric and other patients where blood draws may be difficult, inappropriate or only small volumes of sample are available for analysis. Furthermore, it has demonstrated that RIS and 9-OH-RIS are present in saliva of patients, and although the S/P concentration ratio is variable, their detection in saliva is evidence that the patient has received medication recently.

2. Experimental

2.1. Chemicals and materials

RIS, 9-OH-RIS and the internal standard R068808 (IS) were purchased from Janssen Research Foundation (Beerse, Belgium). Ammonium acetate was obtained from Sigma (St. Louis, MO, USA). Deionized water was generated by a Milli-Q-Plus water system from Millipore (Waltham, MA, USA). HPLC-grade acetonitrile was purchased from Fisher Scientific (Pittsburgh, PA, USA). Samples were centrifuged in 1.5 ml siliconized microcentrifuge tubes with the Marathon 21000 (R) refrigerated multipurpose centrifuge. Both were purchased from Fisher Scientific.

2.2. Column switching configuration

A Valco six-port switch-valve (Valco Instruments, Houston, TX, USA) was used for online clean-up of extracts, to prolong column life and separate/remove compounds that might interfere with the analysis. The column switching system is depicted in Fig. 1 and employs a two-column, two-pump configuration. The first pump, with the weaker mobile phase delivers the sample onto a loading column. After 1 min, the valve is switched so the loading column is in-line with the analytical column. This allows the second pump, having the stronger mobile phase, to back-flush the analytes from the loading column onto the analytical column for further separation. Back-flushing the analytes gave a more compressed peak and better signal to noise ratio compared to direct forward elution onto the analytical column.

2.3. HPLC conditions

The loading and analytical columns were a Zorbax SB C_{18} 12.5 × 4.6 mm, 5 μm (Wilmington, DE, USA) and a

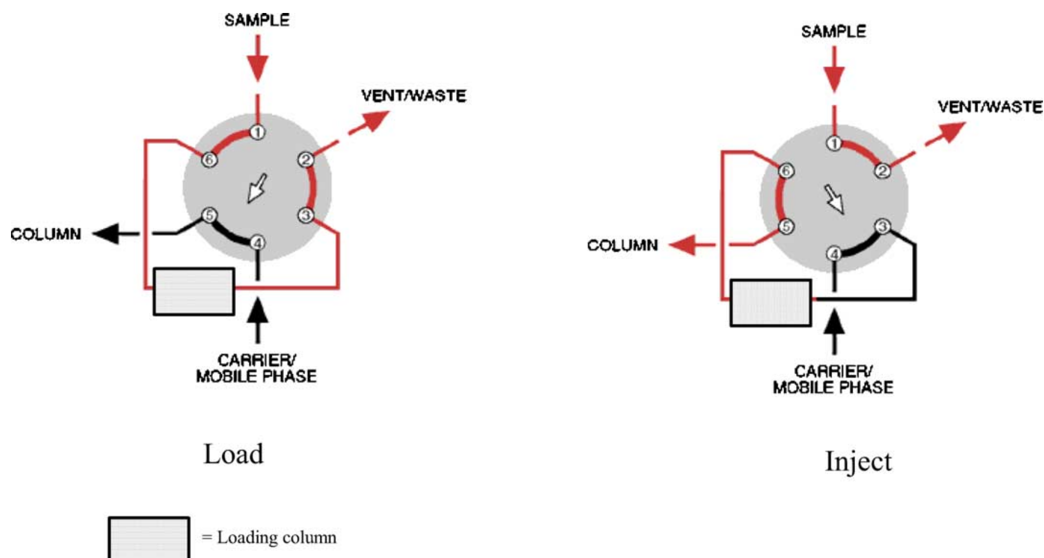

Fig. 1. Column switching setup in the back-flush mode.

Zorbax SB C_{18}, 30 × 2.1 mm, 3.5 μm (Wilmington, DE, USA). The loading mobile phase consisted of 10 mM ammonium acetate–acetonitrile (85:15, v/v), at a flow rate of 0.7 ml/min. The analytical mobile phase consisted of 10 mM ammonium acetate–acetonitrile (20:80, v/v) at a flow rate of 0.35 ml/min kept at ambient temperature. The eluting compounds were introduced into a PE-Sciex API-III + triple quadrupole tandem mass spectrometer (Thornhill, Canada).

2.4. Standard solutions

Approximately 10 mg each of RIS and 9-OH-RIS were dissolved separately in final volumes of 10 ml of methanol, yielding concentrations of ca. 1 mg/ml. A 100 μl aliquot of each solution, added to a 10 ml volumetric flask, was dissolved in acetonitrile to produce a stock solution containing RIS and 9-OH-RIS (10 μg/ml of each). The internal standard was treated similarly with methanol and acetonitrile to yield a final internal standard working solution (ISWS) of 10 μg/ml in acetonitrile. A standard (REF LOQ), containing RIS and 9-OH-RIS at a concentration of 250 pg/ml in acetonitrile–10 mM ammonium acetate (3:1, v/v), served both as a quality control (QC) of the lower limit of quantification and extraction efficiency. Similarly, a reference solution (REF) of 10 ng/ml of RIS, 9-OH-RIS and IS was used to ensure suitability of the system for measurement of samples.

2.5. Preparation of standard curves

Aliquots of the stock solution (10 μg/ml of RIS/9-OH-RIS in acetonitrile) were further diluted in acetonitrile to yield solutions with concentrations of 0.1, 0.2, 0.5, 1.0, 5.0, 9.0 and 10 μg/ml of RIS and 9-OH-RIS (spiking solutions). A 10 μl aliquot of each spiking solution was added to 1 ml of human plasma to provide calibration concentrations of 1, 2, 5, 10, 50, 90, 100 ng/ml. Similarly, quality control samples of 3.0, 25 and 75 ng/ml were prepared. QC and sample concentrations were calculated from the calibration curve using a linear regression ($1/X$) algorithm, where X represents concentration. The calibration curves were constructed from the ratios of the peak areas of the analytes, RIS and 9-OH-RIS, versus the IS. The software was McQuan Version 1.4 (MDS SCIEX, Thornhill, Canada).

2.6. Sample preparation

Volumes (25 μl) of plasma or saliva were added to siliconized microcentrifuge tubes (1.5 ml) accompanied by gentle tapping to avoid adherence of sample to the side of the vial. The sample was precipitated by addition of 75 μl of ISWS, vortexed for 5 s, and centrifuged at 14 000 rpm for 5 min at 4 °C. The supernatant (75 μl) was transferred to an HPLC vial and 10 μl injected into the LC–MS–MS system.

3. Results and discussion

3.1. Specificity

Method validation principles have been described previously [25–27]. Accordingly, six "blank" samples of plasma and saliva from different donors were analyzed separately to ensure that no endogenous compounds or other impurities interfered with the assay. These, analyses, as well as independent analyses of the Standard A (IS only), ruled out interferences with the measurement of RIS and 9-OH-RIS in the matrix or the IS. In addition, REF LOQ samples were analyzed at the beginning, middle and end of each run to ensure that the system performed with adequate sensitivity.

Fig. 2 shows the product ion mass spectra of RIS, 9-OH-RIS and the IS. The major MS–MS transitions utilized for LC–MS analysis were m/z 411.4 → 191.2 for RIS, m/z 427.3 → 207.2 for 9-OH-RIS and m/z 421.4 → 201.2 for the IS. Tuning the system to maximize intensity of product ions was conducted by infusing a stock solution of analyte (10 μg/ml), at a flow rate of 10 μl/min through a tee into a stream of mobile phase and adjusting the flow rates of both the nebulizer and auxiliary gases as well as the temperature of the Turbo IonSpray probe. This procedure allowed maximization of the analyte intensity at the actual flow rate used for analysis of the samples. Fig. 3 shows a representative extracted ion chromatogram of a reference solution, standard A and human plasma blank. Standard A and the blank have no interferences at the expected retention time of the analytes and internal standard, indicating good specificity of the method.

3.2. System suitability of the method

The performance of the system was shown to be optimal by analyses of REF in triplicate at the beginning, middle and end of each sample sequence. The criterion was 10% relative standard deviation (R.S.D.) of analyte/IS ratio for all five REF samples. Noncompliance with this criterion, resulted in rejection of the analysis. Furthermore, if the %R.S.D.s [(S.D./mean) 100] exceeded this criterion, then the sampling variation of the system was considered suspect. Our samples for checking the suitability of the system were well within the acceptable %R.S.D.s. Thus, any variability attributable to faulty operation of the system was minimized and evaluation of system suitability permitted immediate identification of minor problems before they evolved into major complications, requiring rejection of the entire "run".

3.3. Calibration curves

Calibration curves were linear for RIS and 9-OH-RIS over the concentration range 1–100 ng/ml with correlation coefficients >0.99 and percent accuracy of ±15%.

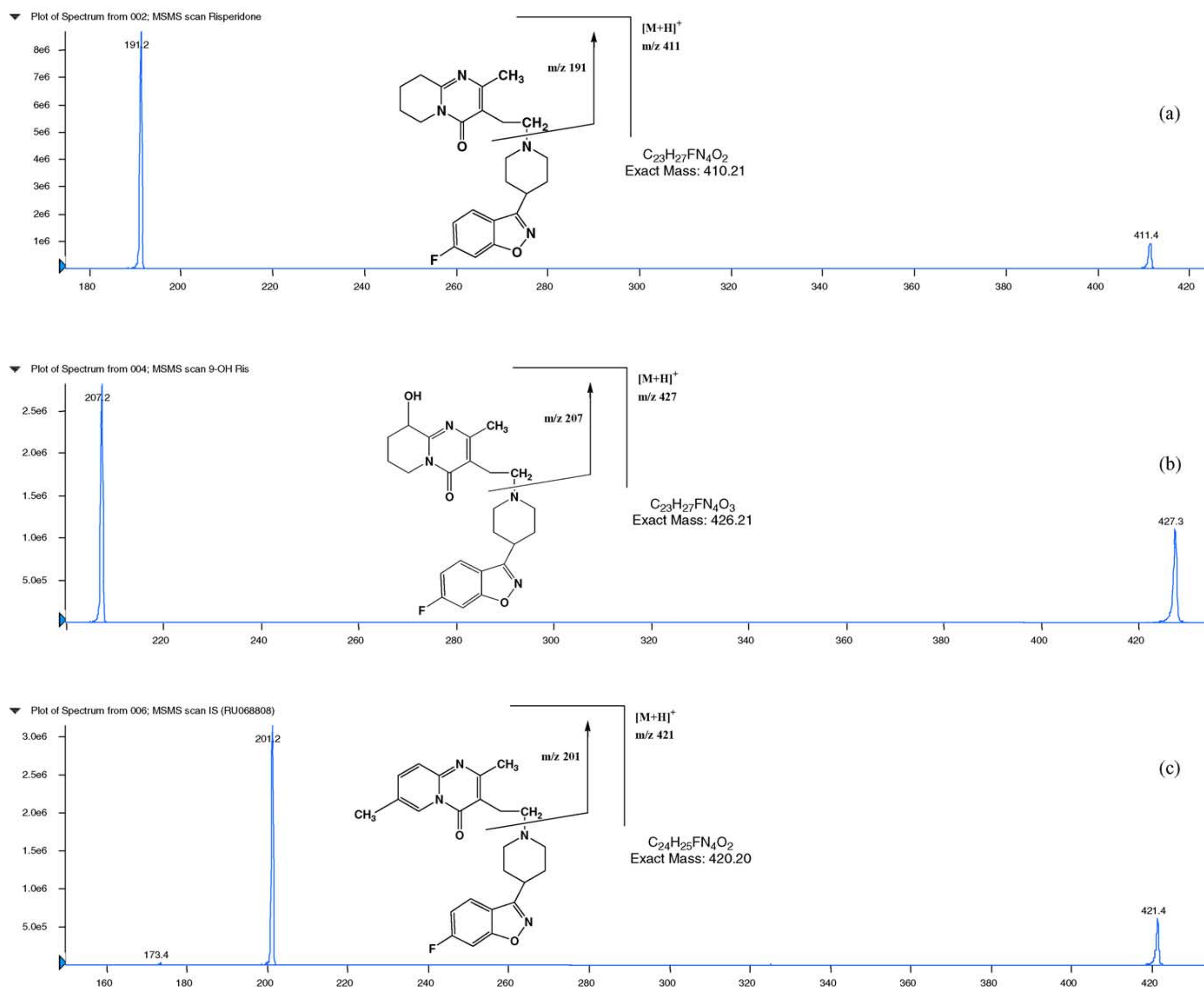

Fig. 2. Product ion mass spectra of (a) RIS, (b) 9-OH-RIS and (c) R08808 and respective structures.

3.4. Precision and accuracy

Four batches of quality control samples, each at three concentrations (3, 25, 75 ng/ml in human plasma) measured six times, on four different days with calibration curves (1–100 ng/ml) established the precision, accuracy and inter-day variability of the assay. Results are summarized in Tables 1 and 2, including mean and S.D. of each day. The R.S.D.s ranged from 1.01 to 10.4% (RIS) and 1.68 to 6.63% (9-OH-RIS). Similarly, the accuracy [(mean/nominal) mean 100] ranged from 94.3 to 108 and 90.2 to 108% for RIS and 9-OH-RIS, respectively.

3.5. Recovery of RIS and 9-OH-RIS

Samples of plasma (25 μl) at low (10 ng/ml) and high (75 ng/ml) concentrations of analytes and 1S (20 ng/ml), selected to fit the linear range of the assay (0–100 ng/ml), were extracted and assayed as described. The recovery was evaluated by evaporation to dryness of blank samples of plasma (25 μl) in vials, and addition of analytes (RIS and 9-OH-RIS) in 75 μl of acetonitrile to the residue to provide final concentrations of 18.75 ng/ml and 2.5 ng/ml. These two levels represent high and low concentrations, respectively. The percent recovery was calculated as follows:

$$\frac{\text{mean of analyte area of extracted samples}}{\text{mean analyte area of 100\% recovery samples}} 100$$

The recoveries for RIS were 90.0 and 93.4% while those of 9-OH-RIS were 92.8 and 89.6%.

3.6. Cross-validation

Cross-validation experiments in plasma (using EDTA and heparin as anticoagulant) and saliva showed that the assay was comparable in each matrix. Ten quality control samples

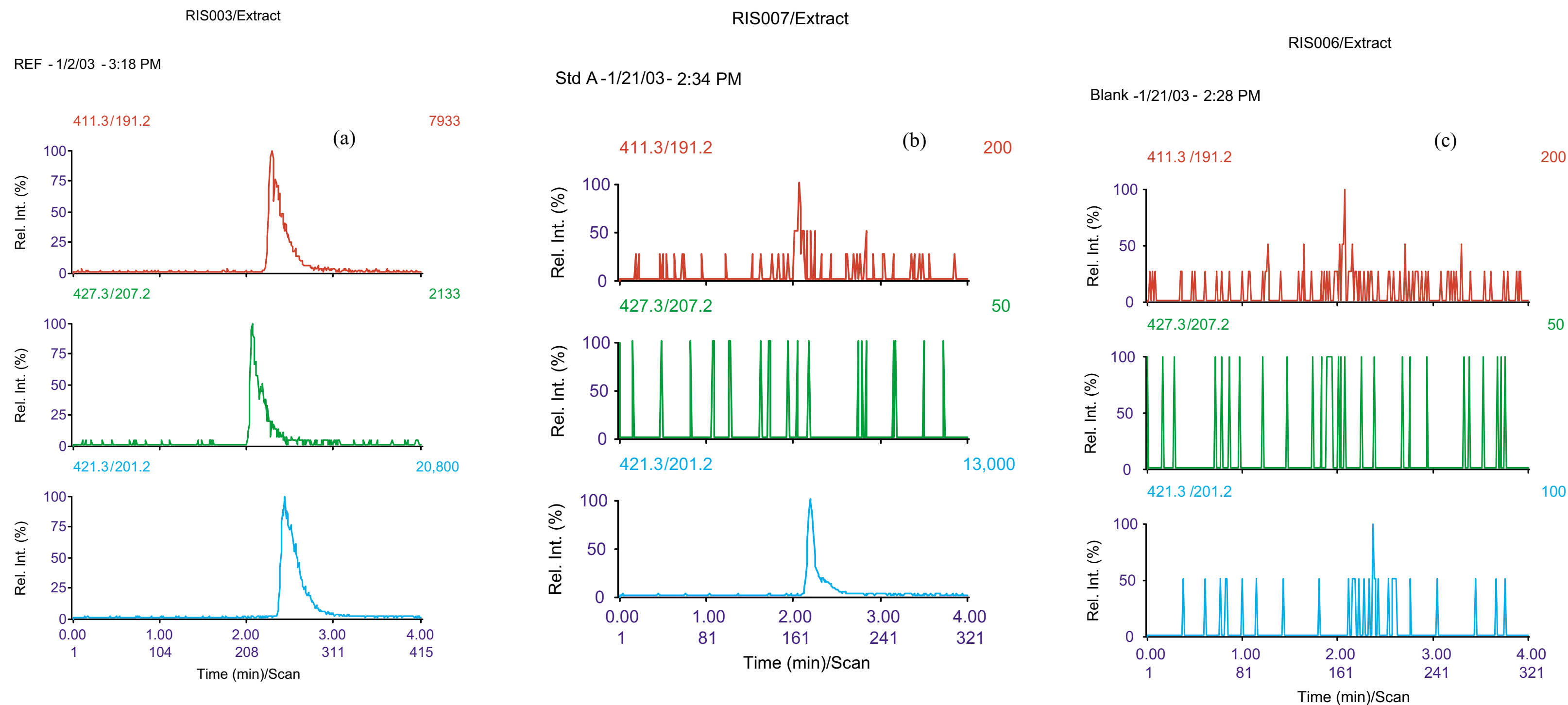

Fig. 3. Representative extracted ion chromatograms of RIS, 9-OH-RIS and IS: (a) REF, (b) standard A (IS only) and (c) human plasma blank.

Table 1
Intra-day variabilities for QCA (3 ng/ml), QCB (25 ng/ml) and QCC (75 ng/ml)

	Day 1			Day 2			Day 3			Day 4		
	QCA	QCB	QCC	QCA	QCB	QCC	QCA	QCB	QCC	QCA	QCB	QCC
RIS												
Mean	2.89	24.2	76.6	2.83	25.0	76.2	3.12	26.7	81.3	3.15	26.3	79.6
S.D.	0.299	0.458	0.942	0.219	0.577	0.772	0.297	2.21	1.29	0.108	0.547	1.23
R.S.D. (%)	10.3	1.89	1.23	7.72	2.31	1.01	9.56	8.29	1.59	3.44	2.08	1.54
Accuracy (%)	96.5	96.9	102	94.3	100	102	104	107	108	105	105	106
9-OH-RIS												
Mean	3.04	24.7	74.4	2.71	24.9	75.5	3.08	25.9	81.4	3.12	24.3	78.0
S.D.	0.093	0.694	1.54	0.060	0.741	2.38	0.201	6.63	1.68	0.124	0.619	1.91
R.S.D. (%)	3.06	2.81	2.07	2.21	2.98	3.16	6.53	6.63	1.68	3.99	2.55	2.45
Accuracy (%)	101	99.8	99.2	90.23	99.4	101	103	104	108	104	97.2	104

These are the quality control samples spiked in human plasma and run with a calibrations curve. Mean and standard deviation were calculated on the basis of $n = 6$ for each QC.

Table 2
Inter-day variability for QCA (3 ng/ml), QCB (25 ng/ml) and QCC (75 ng/ml)

	Inter-day variability (over 4 days)					
	RIS			9-OH-RIS		
	QCA	QCB	QCC	QCA	QCB	QCC
Mean	3.00	25.6	78.4	3.15	25.0	77.3
S.D.	0.268	1.50	2.38	0.492	1.17	3.22
R.S.D. (%)	8.94	5.88	3.04	15.6	4.58	4.17
Accuracy (%)	100	102	104	105	99.9	103

These are the quality control samples spiked in human plasma and run with a calibrations curve. Mean and standard deviation were calculated on the basis of $n = 24$ for each QC.

prepared in saliva and plasma (heparinized) were analyzed with a routine standard curve in plasma (EDTA). The R.S.D. ranged from 0.84 to 5.57% with a accuracy of 93–109% well within the acceptable range of the assay. Thus, the assay proved to be valid in all three matrices (Table 3).

3.7. Ion suppression

In general, even after protein precipitation, the sample may contain ionizable contaminants that compete with the analyte(s) for the limited ion current, often distorting the signal(s), lowering precision, accuracy and sensitivity of the assay and shortening column life. Some of these difficulties may be circumvented by selecting different columns and/or mobile phases, thus changing partitioning (K') characteristics and retention times of solutes and promoting their separation from analytes of interest (RIS, 9-OH-RIS and IS). Alternatively, ion suppression may be reduced by extensive clean-up of samples as described recently [13,17]. Both approaches, however, would result in longer analysis times and lower sample throughput. Introduction of the column switching device overcame ion suppression effects, reduced extensive "clean-up", preserved column life time and still provided for high sample throughput. Matrix effects were tested by comparing spiked plasma and saliva blanks and extracting as described for the recovery determination. The

Table 3
Cross-validation results from human plasma–EDTA to human plasma heparin and human saliva

	RIS (ng/ml)						9-OH-RIS (ng/ml)					
	Saliva			Heparin			Saliva			Heparin		
	QCA	QCB	QCC	QCA	QCB	QCC	QCA	QCB	QCC	QCA	QCB	QCC
	2.77	25.93	80.10	3.02	24.94	79.19	2.89	25.88	83.87	3.18	24.36	77.09
	2.75	25.55	80.51	3.15	25.02	80.71	3.06	25.88	82.50	2.68	23.75	75.92
	2.77	25.35	79.58	3.06	24.91	78.19	2.92	26.61	83.07	2.84	23.79	74.83
	2.78	25.35	82.54	2.86	24.98	80.96	3.02	26.94	80.40	2.75	23.71	76.84
	2.81	25.43	79.64	3.01	24.60	82.10	2.95	27.38	80.30	2.77	23.98	76.16
	2.76	25.18	75.63	2.87	25.20	78.00	2.85	27.12	77.29	2.83	23.63	73.44
	2.75	25.93	77.80	3.08	24.90	80.25	3.00	27.47	83.45	2.68	23.76	74.82
	2.74	25.35	76.85	3.02	25.22	78.86	2.96	26.07	81.49	2.64	24.47	72.53
	2.78	25.38	79.36	3.09	25.25	83.68	3.01	26.02	83.27	2.70	23.72	74.91
	2.86	25.21	77.84	2.92	25.26	81.34	3.18	26.28	80.51	2.87	24.36	76.01
Mean	2.78	25.46	78.99	3.01	25.03	80.33	2.98	26.56	81.61	2.79	23.95	75.26
S.D.	0.03	0.26	1.99	0.10	0.21	1.81	0.09	0.62	2.03	0.16	0.32	1.45
R.S.D. (%)	1.23	1.04	2.52	3.20	0.84	2.25	3.18	2.34	2.49	5.57	1.33	1.93
Accuracy (%)	92.57	101.86	105.31	100.20	100.11	107.10	99.47	106.26	108.82	93.11	95.81	100.34

Table 4
Stability data in human plasma

	RIS (ng/ml)			9-OH-RIS (ng/ml)		
	QCA	QCB	QCC	QCA	QCB	QCC
Ambient temperature (24 h)						
Mean	3.15	26.33	79.57	3.12	24.31	77.96
S.D.	0.11	0.55	1.23	0.12	0.62	1.91
R.S.D. (%)	3.44	2.08	1.54	3.99	2.55	2.45
Accuracy (%)	105.01	105.32	106.10	103.92	97.22	103.95
Ambient temperature (−20 °C)						
Mean	3.08	26.58	79.42	3.08	23.66	77.47
S.D.	0.04	0.53	1.31	0.17	0.71	1.10
R.S.D. (%)	1.24	1.99	1.64	5.43	3.01	1.41
Accuracy (%)	102.74	106.32	105.90	102.78	94.62	103.30
Ambient temperature (−80 °C)						
Mean	3.11	26.28	78.98	2.80	24.29	76.09
S.D.	0.03	0.36	1.50	0.17	0.51	1.72
R.S.D. (%)	0.92	1.39	1.90	6.14	2.09	2.26
Accuracy (%)	103.69	105.11	105.31	93.18	97.17	101.46
Freeze/thaw (5 cycles)						
Mean	3.06	25.64	78.39	3.10	24.57	76.57
S.D.	0.17	0.53	4.10	0.29	1.29	3.43
R.S.D. (%)	5.42	2.08	5.23	9.29	5.25	4.47
Accuracy (%)	101.84	102.55	104.53	103.40	98.26	102.10

The six QCs were run at each level for each determination and back calculated from a freshly spiked calibration curve.

assay proved to be equivalent for measurements in both plasma and saliva.

3.8. Stability of analytes

The stability of RIS, 9-OH-RIS and the IS was studied in saliva, plasma, acetonitrile and methanol under varying conditions of time, temperature, and cycles of freezing/thawing. A freeze/thaw cycle was defined as an initial 24 h freezing period, followed by thaw and then freeze for a minimum of 12 h before the next thaw cycle. Peak areas in each matrix were compared with those in freshly prepared stock solutions (Table 4). Conditions for these studies included ambient temperature for 1 week at a concentration of 10 μg/ml; saliva and plasma at ambient temperature, −20 and −80 °C for 3 weeks and over 6 freeze/thaw cycles, each lasting 12–24 h. The percent accuracy under these condition did not deviate by more than ±6.3%.

3.9. Analysis of clinical samples

Following approval of the institutional review board (IRB), the protocol was discussed with each patient and/or guardian and written consent to participate was received.

Table 5
Predose sample concentrations for all adult and pediatric patients

	RIS (ng/ml)			9-OH-RIS (ng/ml)		
	Plasma (P)	Saliva (S)	P/S ratio	Plasma (P)	Saliva (S)	P/S ratio
Patient 1	9.30	7.79	1.19	93.3	110	0.851
Patient 2	114	40.1	2.83	84.6	42.4	2.00
Patient 3	1.64	0.792	2.08	83.4	60.2	1.39
Patient 4	1.37	0.669	2.06	124	77.2	1.61
Patient 5	22.8	8.16	2.80	148	80.7	1.84
Patient 6	5.62	2.68	2.10	30.0	23.0	1.31
Patient 7[a]	45.5	2.84	16.0	206	111	1.86
Pediatric 1	12.6	5.01	2.51	12.1	9.65	1.25
Pediatric 2	0.411	N/a	N/a	29.9	5.98	4.99
Pediatric 3	3.01	0.97	3.11	8.56	7.14	1.20
Pediatric 4	N/a	N/a	N/a	10.9	2.21	4.93
Pediatric 5	N/a	N/a	N/a	3.17	3.77	0.84
Pediatric 6	N/a	0.05	N/a	12.9	9.01	1.43

N/a: not available. For all values above 100 ng/ml, the calibration was extended with 6 diluted QCs.
[a] Dose 7.5 mg p.o.

Briefly, the patient was admitted to a clinical unit and given the usual evening dose of RIS. The following morning (8–9 a.m.), approximately 12 h after the last dose, concurrent samples of blood by venipuncture and saliva by expectoration into a plastic cup were collected from seven adults (fasting). Thereafter, each adult received an orally administered (p.o.) dose of 6 mg of RIS and fasted for an additional 1 h, prior to a light breakfast. Additional samples of blood and saliva were collected at 1, 2 and 3 h. Single specimens of blood and saliva were also collected concurrently from children on RIS attending an outpatient clinic.

The assay (1) tested the hypothesis that RIS and 9-OH RIS were present in the saliva of pediatric and adult patients receiving RIS and (2) examined the plasma/salivary concentration ratios for RIS and 9-OH-RIS and the applicability of salivary concentrations as a non-invasive test of compliance with therapy. Table 5 shows the plasma and salivary concentrations in seven adults approximately 12 h postdose, close to equilibrium conditions of RIS and 9-OH-RIS (after completion of both absorption and distribution phases). Seven adult patients showed P/S concentration ratios that ranged from 1.19 to 16.0 and 0.85 to 2.00 for RIS and 9-OH-RIS, respectively. In children the ratios were 2.51–3.11 (RIS) and 0.84–4.99 (9-OH-RIS). In some children ratios were not calculated owing to absence of a sample (see Table 5).

The results indicate that RIS and 9-OH-RIS are present in saliva of both pediatric and adult patients. A relatively wide range in the concentration ratios (plasma/saliva) of both

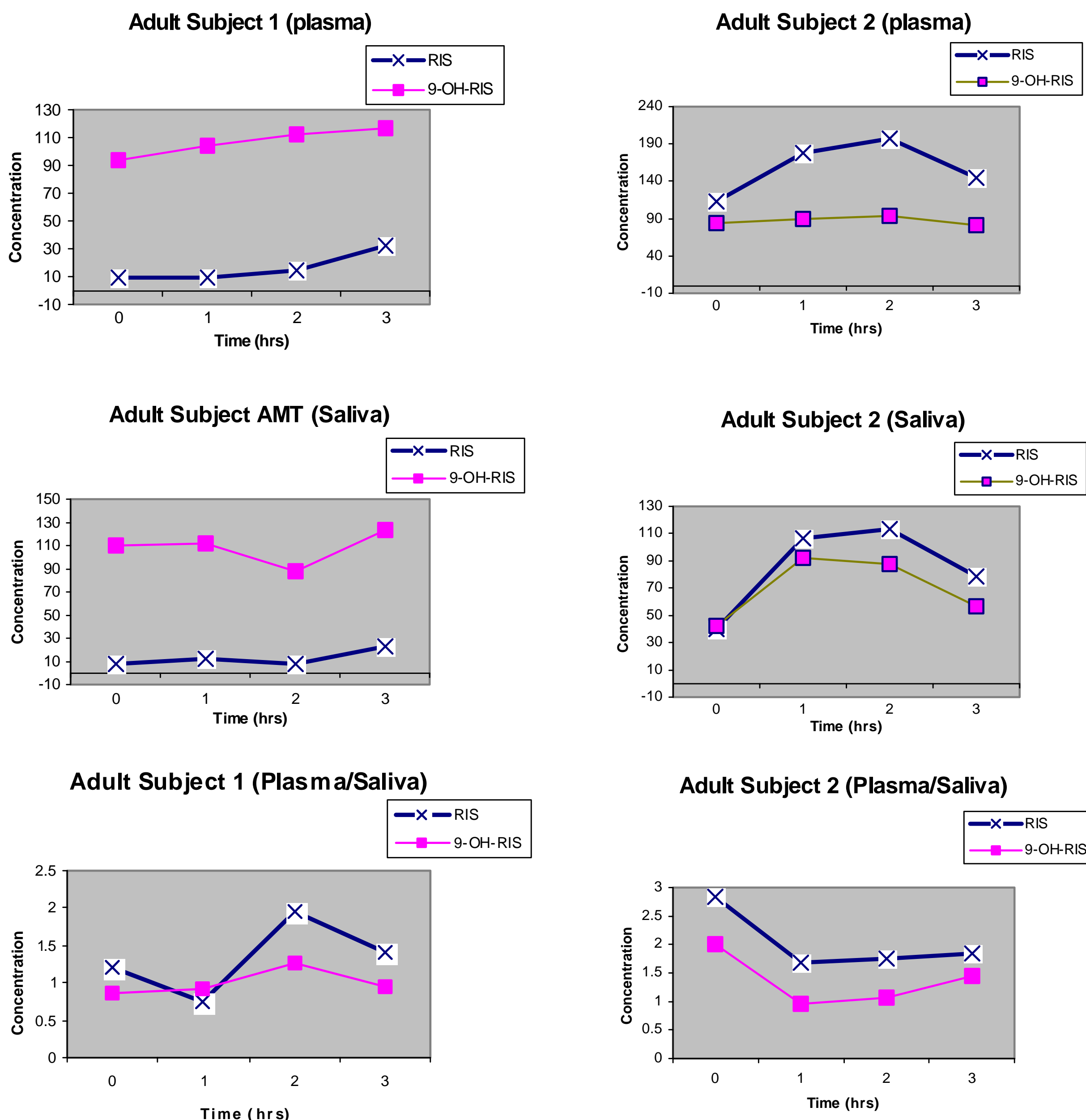

Fig. 4. Plasma/saliva ratios for adult and pediatric patient samples.

compounds makes it impossible to predict exact plasma concentrations from measurements in saliva. The long half-life of total drug in EMs, PMs and rarer allelic variants makes it difficult for the clinician to conclude precise estimates of daily adherence with a prescribed regimen, even from measurements in plasma. The assay of RIS and/or 9-OH-RIS in saliva is therefore a reasonable alternative to plasma for deducing evidence of recent compliance with therapy. The apparently wide inter-subject variation in therapeutic concentrations of total drug complicates any conclusions from a single measurement, whether from plasma or saliva. However, the absence of drug in saliva suggests a possible lack of compliance and should then be followed up by measurements in plasma and other techniques (pill counts, consultation with pharmacist regarding frequency of prescriptions and dates of refills).

Considerable variability may occur in the plasma/salivary concentration ratio (P/S) of RIS and 9-OH RIS (see Table 5). The wide P/S is influenced by numerous factors, including pH of saliva (6.5–7.3), pK_a, lipid solubility, protein binding, molecular mass and mechanism of excretion of drug into saliva (passive diffusion or active transport). Statistical analysis of the two patients depicted in Fig. 4 indicates that patient 1 belonged to the EM group in the population evidenced by higher activity of CYP2D6, elevated conversion rate of RIS to 9-OH-RIS and high concentrations of 9-OH-RIS compared to RIS. In contrast, patient 2 belonged to the group of poor metabolizers (or a minor allelic variant) showing reverse relationships with comparably higher concentrations of RIS, a lesser activity of CYP2D6 and lower conversion rate of RIS to 9-OH-RIS.

4. Conclusions

This work describes a robust, validated, sensitive LC–MS–MS assay for RIS and 9-OH-RIS requiring a sample volume of 25 μl. It is applicable to routine measurements in adult and pediatric patients. Moreover, we demonstrated that RIS and 9-OH-RIS, excreted into saliva of patients, are readily quantifiable. The plasma/salivary concentration ratios of both compounds vary and the concentration in one matrix cannot be used to predict the exact concentration in the other. From a clinical perspective, venipunctures are painful, often lead to bruising, and are expensive (US$ 50 or more). Therefore, a small volume (25 μl) of saliva is a painless, inexpensive alternative and regular detection of drug by LC–MS in samples of saliva at routine clinical visits may well be the preferred method in the future to ensure compliance.

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