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Short communication

# Development and validation of a LC–MS method with electrospray ionization for the determination of the imidazole $H_3$ antagonist ROS203 in rat plasma

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**Abstract**

A rapid, simple and sensitive liquid chromatography–mass spectrometry (LC–MS) method was developed and validated for the determination of the imidazole $H_3$ antagonist ROS203 in rat plasma, using the superior homologue ROS287 as internal standard. Analyses were performed on an Agilent 1100 Series HPLC system employing a Supelco Ascentis $C_{18}$ column and isocratic elution with acetonitrile-10 mM ammonium acetate buffer pH 4.0 (30:70, v/v) at a flow rate of 0.25 mL/min. An Applied Biosystems/MDS Sciex 150-EX single quadrupole mass spectrometer, equipped with an electrospray ionization interface was employed, operating in the positive ion mode. Plasma samples were deproteinized with acetonitrile (1:2), evaporated under nitrogen stream, reconstituted in the mobile phase and 5 μL were injected into the system. The retention times of ROS203 and IS were 2.20 and 2.90 min, respectively. Calibration curves in spiked plasma were linear over the concentration range of 2610–2.61 ng/mL with determination coefficients >0.99. The lower limit of quantification (LLOQ) was 2.61 ng/mL. The accuracy of the method was within 15%. Intra- and inter-day relative standard deviations were less or equal to 9.50% or 7.19%, respectively. The applicability of the LC–MS method was tested employing plasma samples obtained after *i.p.* administration of ROS203 to female Wistar rats to support a behavioral *in vivo* study. The specificity of the method was confirmed by the absence of interferences from endogenous substances. The reported method can provide the necessary sensitivity, linearity, precision, accuracy and specificity to allow the determination of ROS203 in rat plasma samples to support further pharmacokinetic assays.


*Keywords:* $H_3$ antagonists; Mass spectrometry; Liquid chromatography; Plasma analysis; LC–MS

## 1. Introduction

The histamine $H_3$ receptor, discovered in the early 1980s [1], cloned and characterized in 1999 [2] is a G protein-coupled auto- and heteroreceptor (GPCR) regulating the synthesis and release of histamine [3] and of other important neurotransmitters [4]. Rat and human $H_3$ receptors display significant pharmacological differences for the affinity of a number of $H_3$ receptor antagonists [5]. $H_3$ receptors are localized primarily in the central nervous system, in those brain regions where histamine plays a central role for cognition processes and for sleep and homeostatic regulation [6,7]. Histamine $H_3$ antagonists have therefore promising therapeutic potential towards CNS-associated disorders, such as attention-deficit hyperactivity disorder, narcolepsy, epilepsy, neurodegenerative diseases such as Alzheimer's or Parkinson's and obesity [8]. Many prototypic $H_3$ antagonists, such as thioperamide [9] or ciproxifan [10], are imidazole-containing molecules. More recently, industrial and academic research has shifted towards potent non-imidazole $H_3$ antagonists, which have largely been described in the literature. However, despite the high number of potent and selective imidazole and non-imidazole $H_3$ antagonists synthesized, some of which have entered early phases of clinical trials, no clinical data for an $H_3$ receptor antagonist are yet available [11]. Our research

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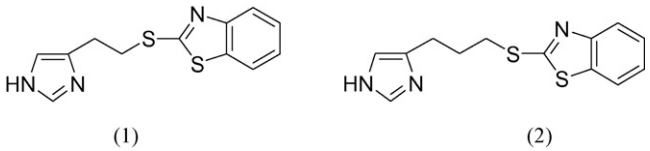

(1) (2)

Fig. 1. Chemical structures of (1) ROS 203 and (2) its analytical internal standard, the superior homologue ROS 287.

group has been involved for years in the design, synthesis, pharmacological and physico-chemical characterization of new compounds endowed with $H_3$ antagonist activity [12–16]. The present work was focused on the development and validation of a liquid chromatography/mass spectrometry (LC–MS) method for the determination of the imidazole $H_3$ antagonist 2-[2-(1H-imidazol-4-yl)ethylthio]benzothiazole ROS203 (see Fig. 1) in rat plasma [12]. ROS203 presented an *in vitro* rat $H_3$ receptor affinity similar to that of the reference $H_3$ antagonist thioperamide ($pK_i = 8.53$ versus 8.59) [14,15], and a higher affinity at human $H_3$ ($pK_i = 8.17$ [17] versus 7.28 [18]). When administered *i.p.*, ROS203 displayed higher *ex vivo* potency in displacing [$^3$H]-(R)-α-methylhistamine ([$^3$H]RAMHA) from rat brain, with an $ED_{50}$, 1 h after administration, of 1.35 mg kg$^{-1}$ [14] versus 2.04 mg kg$^{-1}$ for thioperamide [15].

ROS203, *i.p.* administered at the dose of 5 mg kg$^{-1}$, has shown to significantly ameliorate scopolamine-induced learning deficit in a rat model of passive-avoidance task [19], with a potency two fold higher than thioperamide. The anti-amnesic effect was rapid in onset and long-lasting, still significative 4 h after its *i.p.* administration (unpublished results) [17].

In this work, a liquid chromatography–mass spectrometry (LC–MS) method was developed and validated for the determination of ROS203 in rat plasma using the superior homologue ROS287 as analytical internal standard (Fig. 1).

In the literature, few papers reported bioanalytical methods for the quantitative determination of imidazole $H_3$ antagonist in biological fluids, either presenting a pre-run derivatisation step [20] or high quantitation limits [21].

The LC–MS bioanalytical methodology proposed here was validated for specificity, linearity, precision, accuracy and lower limit of quantification. The applicability of the LC–MS method was assayed by monitoring rat plasma levels of ROS203 after peripheral *i.p.* administration in association with the *in vivo* behavioral experiment for $H_3$ receptor antagonist-mediated passive-avoidance task.

## 2. Experimental

### 2.1. Chemicals and reagents

ROS203 was synthesized in our labs as previously described [12] and the purity was >99%. The superior homologue (ROS287), used as analytical internal standard (IS), was also synthesized in our labs and checked for purity. Drug-free plasma was obtained from healthy Wistar rats (250–300 g weight) provided by Charles-Liver laboratories (Charles-Liver srl, Milan, Italy). Acetonitrile (HPLC grade) was purchased from Sigma Aldrich srl (Sigma–Aldrich srl, Milan, Italy). Water was freshly bidistilled before use. All other chemicals were of analytical grade.

### 2.2. Preparation of calibration standards (CS) and quality control (QC) samples

Stock solutions for CS and QC were prepared separately in methanol. CS samples were prepared by spiking into heparinised rat plasma stock solutions of ROS203 to yield final concentrations of 2610, 1305, 522, 261, 130.5, 52.2, 26.1, 13.05, 2.61 ng/mL, corresponding to the 10 μM–10 nM concentration range. Quality control samples were prepared at concentrations equal to 10.4, 104, 1040 ng/mL. Standard stock solution of ROS287 (IS) was prepared in acetonitrile and successively diluted with acetonitrile to result in a final concentration of 137.5 ng/mL.

### 2.3. Sample preparation

A 100 μL aliquot of each plasma sample was deproteinized by addition of 200 μL of acetonitrile (containing 137.5 ng/mL of IS), vortexed for 30 s and centrifuged at 9000 × *g* for 10 min at 4 °C. A fixed aliquot of the supernatant was evaporated to dryness under a nitrogen stream, reconstituted in mobile phase and a 5 μL volume of the samples was injected into LC–MS. All plasma samples, including CS, QC and samples from behavioral experiments were processed following the same procedure.

### 2.4. Liquid chromatography/mass spectrometry

The HPLC system was an Agilent 1100 series equipped with a G1312A binary pump and a G1379A degasser (Agilent Technologies, Waldbronn, Germany). The HPLC column was a Supelco Ascentis HPLC column (100 mm × 2.1 mm i.d.) packed with 5 μm ODS stationary phase. The HPLC phase consisted of acetonitrile/10 mM ammonium acetate buffer pH 4.0 (30/70, v/v). The flow rate was set at 0.25 mL/min and analysis was performed in isocratic mode. Total run time was less than 5 min for each injection. A divert valve was used to discard the LC effluent during the first 1.5 min of each chromatographic run. The mass spectrometer was an API-150 EX single quadrupole with an electrospray ionization (ESI) interface (Applied Biosystems/MDS Sciex, Toronto, Canada). Data were acquired and processed by Analyst software (Version 1.4.1). Detection was performed by monitoring the positive ions with single ion monitoring mode (SIM). The theoretical *m/z* values of the $[M+H]^+$ ions were set at 262.0 for ROS203 and at 276.1 for analytical IS, ROS287. The following parameters were retained for optimal ROS203 detection: nebulizer gas: 15 psi; turbo ion spray gas: 6 psi; curtain gas: 10 psi; cone voltage: 20.30 V; skimmer voltage: 160.30 V; entrance potential: 3.20 V; ion source temperature: 450 °C. The dwell time used for acquiring data for each SIM analysis was 1.0 s.

### 2.5. Validation of the analytical method

The bioanalytical method was validated to determine specificity, accuracy, precision, calibration curve range, and reproducibility according to the FDA guidance for bioanalytical method validation [22]. A nine-point calibration curve was constructed by plotting the peak area ratio ($y$) of analyte to IS versus analyte concentration ($x$). Analysis of CS samples at each concentration was performed each day in duplicate. Results for blank samples were not used as part of the calibration curve. Slope, intercept and coefficient of determination ($r^2$) were calculated as regression parameters by weighted ($1/x$) linear regression. Precision and accuracy were evaluated by determining the concentration in five replicates of each QC sample at three different concentrations on three separate days. Each run consisted of CS samples, blank plasma sample with and without the IS in duplicate and QC samples in five replicates. The specificity of the method was investigated by analyzing six individual rat blank plasma samples. Matrix effect and absolute recovery were investigated by analyzing five individual rat plasma samples at three ROS203 concentrations: 10.4, 104, 1040 ng/mL. The stability of ROS203 in plasma after 8 h at room temperature (short-term) and after three freeze-and-thaw cycles was also investigated.

### 2.6. Rat plasma dosing

The method was applied to the determination of rat plasma concentrations from six female Wistar rats following *i.p.* administration of 5 mg kg$^{-1}$ ROS203 to support a behavioral study. Pre-training administration of ROS203 (5 mg kg$^{-1}$ *i.p.*) had shown to ameliorate scopolamine-induced learning deficit, in a rat model of passive-avoidance task, with a two fold higher potency than thioperamide [17]. Pyrilamine pre-treatment reverted the cognitive improvement confirming the involvement of endogenous histamine, via $H_1$ receptors. Two hundred microliters blood samples were collected from each rat at 5, 15, 30, 60, 120, 240 min post-dosing and centrifuged (2000 × $g$, 20 min) to obtain the plasma fraction. The plasma samples were deproteinized by organic solvent (acetonitrile) addition (1:2), centrifuged, dried, reconstituted in mobile phase and analysed immediately after withdrawal.

## 3. Results and discussions

### 3.1. Sample preparation and LC–MS conditions

Several elution conditions were assayed employing acidic aqueous phases (0.1% formic acid; 0.1% acetic acid; 0.05% TFA), changing the organic cosolvent and various gradient conditions. Finally, since ROS203 is a diprotic weak base (p$K_a$ values: 2.19; 6.88 [15]), the pH of the elution buffer was set at 4.0 for 10 mM ammonium acetate. LC separation was performed in isocratic mode to maximize sample throughput during the day.

Due to the presence of the basic imidazole nitrogen in its molecule (see also Fig. 1) ROS203 exhibited favorable sensitivity in positive ion mode because of the efficiency of ionization of the analyte. Optimization of LC–MS conditions was performed by flow injection analysis (FIA). The optimized parameters are reported in Section 2.4.

### 3.2. Selectivity

Assay selectivity was assessed by analysis of drug-free plasma from six individual rats and evaluation of the peaks that interfered with ROS203 and the IS.

Representative chromatograms obtained from blank rat plasma and plasma spiked with ROS203 and the IS are shown in Fig. 2. No interfering peak was observed in the samples at the retention times of either the analyte or IS. The retention times of ROS203 and the IS were 2.20 and 2.90 min, respectively.

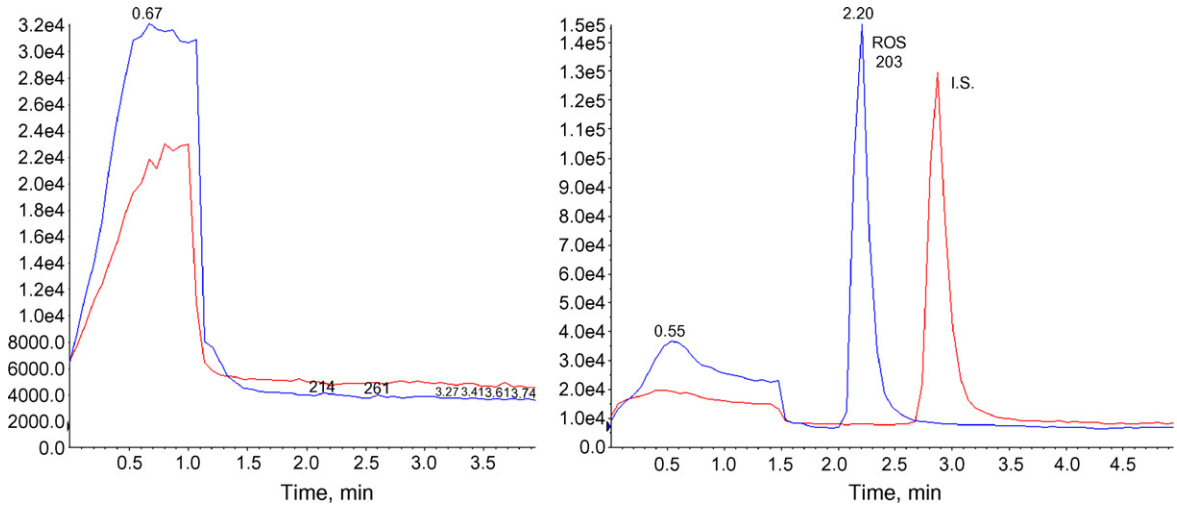


Fig. 2. Blank plasma and ROS203 (blue line) and IS (red line) chromatogram in SIM mode. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Table 1
Matrix effect and absolute recovery for ROS203 ($n = 5$; mean ± S.D.)

| | ROS203 concentration (ng/mL)[a] | | |
|---|---|---|---|
| | 10.3 | 103 | 1030 |
| Matrix effects (%) | 103.57 ± 11.81 | 101.89 ± 10.09 | 99.78 ± 4.58 |
| Absolute recovery (%) | 72.98 ± 7.11 | 69.48 ± 7.78 | 67.87 ± 3.76 |

[a] Reported are mean values ± S.D. ($n = 5$).

### 3.3. Matrix effects and absolute recovery

Matrix effects generally take the form of either ion suppression or ion enhancement and their magnitude may vary between sources of plasma. Matrix effects were calculated comparing the analyte-to-IS ratios in QC prepared spiking processed plasma with ROS203 and IS just before LC–MS injection and QC prepared in the mobile phase directly. The results are shown in Table 1. Matrix effects were in the range 99.78–103.57% for ROS203. The absolute recovery was calculated comparing the analyte-to-IS ratios in QC prepared in rat plasma and reconstituted in the mobile phase and QC prepared in the mobile phase directly (see Table 1). The overall mean recovery ranged from 67.87% to 72.98%. SPE $C_{18}$ extraction was also assayed, but did not improve the reported recoveries; therefore, protein precipitation was chosen as isolation procedure.

### 3.4. Linearity of calibration curves and lower limits of quantification (LLOQ)

Weighted ($1/x$) least-squares linear regression of ratio of the area of the analyte to that of IS versus concentration was used for calibration. Good linearity was obtained in the range 2610–2.61 ng/mL. Sixty-seven percent of each back-calculated standard concentrations within 15% deviation from nominal value (20% for the LLOQ) was the acceptance criterion for the regression curve. For calibration standards, the inter-run precision and accuracy results from the three analytical batches are listed in Table 2. The inter-run accuracy ranged from a 93.9% to 106.7%. The inter-run precision (%R.S.D.) ranged from 2.58% to 9.16%.

### 3.5. Precision and accuracy

Intra- and inter-day precision and accuracy were assessed by extracting and analyzing five replicates of each of the three QC concentrations (Table 3). The intra-day assay precision (expressed as %R.S.D.) ranged from 0.53% to 9.50%, while the intra-day assay accuracy (expressed as percent of nominal values) ranged from 98.4% to 113.3%. The inter-assay precision and accuracy were determined by analyzing five replicates of each QC concentration in each of the three assay runs (total: $n = 15$; Table 4). Method reproducibility exhibited inter-assay precision ranging from 3.34% to 7.19%. Inter-assay accuracy ranged from 103.1% to 107.1% (Table 4).

Table 2
Back-calculated ROS203 concentrations in rat plasma calibration standards and calibration curve parameters[a]

| Concentration (ng/mL) | STD 1 2610 ng/mL | STD 2 1305 ng/mL | STD 3 522 ng/mL | STD 4 261 ng/mL | STD 5 130.5 ng/mL | STD 6 52.2 ng/mL | STD 7 26.1 ng/mL | STD 8 13.05 ng/mL | STD 9 2.61 ng/mL | Slope | Intercept | Coefficient of determination ($r^2$) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Batch 1 | | | | | | | | | | | | |
| Run 1 | 2570 | 1380 | 554 | 273 | 134 | 50.2 | 25.6 | 13.4 | 2.73 | 0.00458 | 0.0323 | 0.9990 |
| Run 2 | 2380 | 1290 | 581 | 278 | 135 | 52.3 | 26.5 | 12.2 | 2.53 | | | |
| Batch 2 | | | | | | | | | | | | |
| Run 1 | 2370 | 1340 | 520 | 268 | 146 | 51.8 | 24.9 | 12.0 | 2.46 | 0.00454 | 0.0412 | 0.9984 |
| Run 2 | 2500 | 1370 | 565 | 293 | 110 | 53.6 | 26.5 | 11.7 | 2.74 | | | |
| Batch 3 | | | | | | | | | | | | |
| Run 1 | 2510 | 1310 | 580 | 293 | 138 | 47.2 | 23.5 | 13.0 | 2.57 | 0.00416 | 0.0375 | 0.9982 |
| Run 2 | 2490 | 1330 | 541 | 258 | 130 | 48.2 | 26.1 | 11.5 | 2.72 | | | |
| $n$ | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | | | |
| Overall mean | 2470 | 1337 | 557 | 277 | 132 | 50.6 | 25.5 | 12.3 | 2.61 | | | |
| S.D. | 78.7 | 34.4 | 23.7 | 13.9 | 12.1 | 2.48 | 1.16 | 0.748 | 0.111 | | | |
| %R.S.D. | 3.19 | 2.58 | 4.25 | 5.03 | 9.16 | 4.91 | 4.55 | 6.08 | 4.26 | | | |
| %Accuracy | 94.6 | 102.3 | 106.7 | 106.2 | 100.9 | 96.8 | 97.8 | 93.9 | 99.9 | | | |

[a] $y = mx + q$, weigthed 1/concentration where $y$ is the peak area ratio of ROS203 to IS, $x$ the concentration of ROS203 and $m$, $q$ are slope and intercept.

Table 3
Intra-day assay precision and accuracy for ROS203 in rat plasma ($n = 5$)

| Nominal concentration (ng/mL) | Observed concentration (ng/mL, mean ± S.D.) | Precision[a] (%) | Accuracy[b] (%) |
|---|---|---|---|
| 10.4 | | | |
| (Day 1) | 10.24 ± 0.652 | 6.37 | 98.4 |
| (Day 2) | 11.38 ± 0.604 | 5.31 | 109.5 |
| (Day 3) | 11.80 ± 1.050 | 8.91 | 113.3 |
| 104 | | | |
| (Day 1) | 107.4 ± 2.63 | 2.45 | 103.3 |
| (Day 2) | 103.2 ± 9.81 | 9.50 | 99.3 |
| (Day 3) | 111.2 ± 2.95 | 2.65 | 106.9 |
| 1040 | | | |
| (Day 1) | 1031 ± 90.0 | 8.73 | 99.1 |
| (Day 2) | 1094 ± 33.6 | 3.07 | 105.2 |
| (Day 3) | 1092 ± 5.7 | 0.53 | 105.0 |

[a] Expressed as %R.S.D.: (S.D./mean) × 100.
[b] Calculated as (mean determined concentration/nominal concentration) × 100.

Table 4
Inter-day assay precision and accuracy for ROS203 in rat plasma ($n = 15$)

| Nominal concentration (ng/mL) | Observed concentration[a] (ng/mL, mean ± S.D.) | Precision[b] (%) | Accuracy[c] (%) |
|---|---|---|---|
| 10.40 | 11.13 ± 0.801 | 7.19 | 107.1 |
| 104.0 | 107.3 ± 3.98 | 3.71 | 103.3 |
| 1040 | 1072 ± 35.8 | 3.34 | 103.1 |

[a] $n = 3$ days with five replicates per day.
[b] Expressed as %R.S.D.: (S.D./mean) × 100.
[c] Calculated as (mean determined concentration/nominal concentration) × 100.

Table 5
Stability of ROS203 in rat plasma ($n = 15$)[a]

| Analyte | Concentration (ng/mL) | 8 h stability (% of nominal) | Freeze-and-thaw stability (% of nominal) |
|---|---|---|---|
| ROS203 | 10.40 | 96.5 ± 5.58 | 97.4 ± 3.89 |
| | 104.0 | 96.1 ± 4.42 | 96.2 ± 9.90 |
| | 1040 | 97.9 ± 7.61 | 94.5 ± 9.66 |

[a] Calculated as (mean determined concentration/nominal concentration) × 100%.

### 3.6. Stability tests in processed samples

Stability tests of the analyte were performed on five replicates of three QC concentrations after 8 h at room temperature (short-term stability) and after three freeze-and-thaw cycles. The overall stability of ROS203 showed % nominal values ranging from 96.1% to 97.9% for room temperature short-term stability and from 94.5% to 97.4% for freeze-and-thaw stability (Table 5). However, all the pharmacological samples were processed and injected in the LC–MS system on the same day of withdrawal.

### 3.7. Application of the method

The analytical procedure described was applied to plasma samples obtained from six female Wistar rats which were *i.p.* administered a single dose of 5 mg kg$^{-1}$ ROS203.

A representative concentration-versus-time profile is shown in Fig. 3.

The concentration versus time profile of ROS203 after administration showed a $C_{max} = 1980$ ng mL$^{-1}$ at $t = 30$ min,

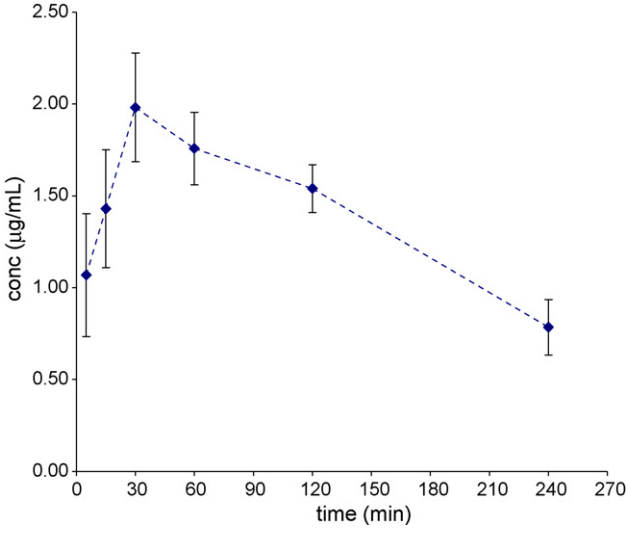


Fig. 3. Plasma concentrations vs. time profile after *i.p.* administration of 5 mg kg$^{-1}$ dose of ROS203 (mean ± S.D.; $n = 6$).

with high plasma concentrations of ROS203 up to 240 min ($C_{240min} = 780\ ng\ mL^{-1}$), in accordance with the behavioral effect, significative up to 4 h from ROS203 administration before training trail. The observed variability is estimated from individual response to the drug and not results of analysis, since the established analytical method exhibited high accuracy and precision without individual variability. The results at 6 h indicate that the LLOQ of the method is applicable to the behavioral study.

## 4. Conclusions

A rapid, selective and sensitive LC–MS method was developed and validated for the quantitative determination of the imidazole $H_3$ antagonist ROS203 in rat plasma. The analyte was extracted after protein precipitation, MS detection was performed with positive SIM mode and a quick isocratic LC separation was used. The estimated calibration range was 2610–2.61 ng/mL with practically no interference or matrix effects from endogenous plasma components. The method was successfully applied to quantify rat plasma concentrations of ROS203 in a behavioral model of $H_3$ antagonist activity and proved to be effective in determining plasma concentrations for further PK assessments.

## Acknowledgement

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## References

[1] J.-M. Arrang, M. Garbarg, J.-C. Schwartz, Nature 19 (1983) 832–837.
[2] T.W. Lovenberg, B.L. Roland, S.J. Wilson, X. Jiang, J. Pyati, A. Huvar, M.R. Jackson, M.G. Erlander, Mol. Pharmacol. 55 (1999) 1101–1107.
[3] J.-M. Arrang, M. Garbarg, J.-C. Schwartz, Neuroscience 15 (1985) 832–837.
[4] E. Schlicker, B. Malinowska, M. Kathmann, M. Göthert, Fundam. Clin. Pharmacol. 8 (1994) 128–137.
[5] R. Leurs, R.A. Bakker, H. Timmerman, Nat. Rev. Drug Discov. 4 (2005) 107–120.
[6] G. Drutel, N. Peltsaro, K. Karlstedt, K. Wieland, M.J. Smit, H. Timmerman, P. Panula, R. Leurs, Mol. Pharmacol. 55 (2001) 1101–1107.
[7] H. Haas, P. Panula, Nat. Rev. Neurosci. 4 (2003) 121–130.
[8] T.A. Esbenshade, G.B. Fox, M.D. Cowart, Mol. Interv. 6 (2006) 77–88.
[9] J.-M. Arrang, M. Garbarg, J.C. Lancelot, J.M. Lecomte, H. Pollard, M. Robba, W. Schunack, J.-C. Schwartz, Nature 327 (1987) 117–123.
[10] X. Ligneau, J.S. Lin, G. Vanni-Mercier, M. Jouvet, J.L. Muir, C.R. Ganellin, H. Stark, S. Elz, W. Schunack, J.C. Schwartz, J. Pharmacol. Exp. Ther. 287 (1998) 658–666.
[11] A.A. Hancock, Biochem. Pharmacol. 71 (2006) 1103–1113.
[12] P.V. Plazzi, F. Bordi, M. Mor, C. Silva, G. Morini, A. Caretta, E. Barocelli, T. Vitali, Eur. J. Med. Chem. 30 (1995) 881–889.
[13] M. Mor, F. Bordi, C. Silva, S. Rivara, P. Crivori, P.V. Plazzi, V. Ballabeni, A. Caretta, E. Barocelli, M. Impicciatore, P.A. Carrupt, B. Testa, J. Med. Chem. 40 (1997) 2571–2578.
[14] V. Ballabeni, M. Impicciatore, S. Bertoni, F. Magnanini, V. Zuliani, F. Vacondio, E. Barocelli, Inflamm. Res. 51 (2002) S55–S56.
[15] F. Vacondio, M. Mor, C. Silva, V. Zuliani, M. Rivara, S. Rivara, F. Bordi, P.V. Plazzi, F. Magnanini, S. Bertoni, V. Ballabeni, E. Barocelli, P.A. Carrupt, B. Testa, Eur. J. Pharm. Sci. 23 (2004) 89–98.
[16] G. Morini, M. Comini, M. Rivara, S. Rivara, S. Lorenzi, F. Bordi, M. Mor, L. Flammini, S. Bertoni, V. Ballabeni, E. Barocelli, P.V. Plazzi, Bioorg. Med. Chem. Lett. 16 (2006) 4063–4067.
[17] E. Barocelli, V. Ballabeni, L. Flammini, S. Bertoni, M. Tognolini, M. Chiavarini, V. Zuliani, M. Rivara, F. Vacondio, M. Impicciatore, 32° Congresso Nazionale della Società Italiana di Farmacologia, 2005.
[18] E. Barocelli, V. Ballabeni, V. Manenti, L. Flammini, S. Bertoni, G. Morini, M. Comini, M. Impicciatore, Pharmacol. Res. 53 (2006) 226–232.
[19] M. Orsetti, P. Ghi, G. Di Carlo, Behav. Brain Res. 124 (2001) 235–242.
[20] M.K. Handley, W.W. Hirth, J.G. Phillips, S.M. Ali, A. Khan, L. Fadnis, C.E. Tedford, J. Chromatogr. B. Biomed. Sci. Appl. 716 (1998) 239–249.
[21] E. Sakurai, E. Gunji, Y. Iizuka, N. Hikichi, K. Maeyama, T. Watanabe, J. Pharm. Pharmacol. 46 (1994) 209–212.
[22] Guidance for Industry: Bioanalytical Method Validation, US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER) and Center for Veterinary Medicine (CVM), 2001, http://www.fda.gov/cder/guidance/index.htm.