AKADÉMIAI KIADÓ

Acta Chromatographica

35 (2023) 2, 187–192

DOI:
10.1556/1326.2022.01041

ORIGINAL RESEARCH PAPER

Determination of modafinil in rat plasma by UPLC-MS/MS and a study of its pharmacokinetics and bioavailability

YAN HE[1†], YIZHE MA[2†], LVQI LUO[2], JUNYING CHEN[2], CONGCONG WEN[2*] and MEILING ZHANG[3**]

[1] The Molecular Neuropharmacology Laboratory and the Eye-Brain Research Center, The State Key Laboratory of Ophthalmology, Optometry and Vision Science, Wenzhou Medical University, Wenzhou, China

[2] Laboratory Animal Center, Wenzhou Medical University, Wenzhou, China

[3] Analytical and Testing Center, School of Pharmaceutical Sciences, Wenzhou Medical University, Wenzhou, China

Received: March 17, 2022 • Accepted: April 19, 2022
Published online: May 13, 2022

ABSTRACT

Modafinil has a strong and long-lasting awakening effect. Short-term use can improve cognitive and work efficiency. Therefore, it has been known to be abused by students and parents as a "smart drug." It is in the first category of psychotropic drugs and strictly controlled. To detect modafinil in rat plasma and study the differences in the pharmacokinetics of modafinil between oral and sublingual administration in rats, an ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method was developed. Rats were injected with modafinil by oral gavage and sublingual vein, respectively, blood was collected within a certain period, and the plasma was obtained by centrifugation. Midazolam was used as the internal standard, and the concentration of modafinil in the plasma was determined by UPLC-MS/MS, where a drug-time curve was created to calculate the pharmacokinetic parameters. The standard curve for modafinil ranged from 1 to 2000 ng mL^{-1} with good linearity. The intra-day accuracy of modafinil was between 86% and 104%, and the intra-day accuracy was between 90% and 103%. Intra-day precision (RSD%) was less than 15%, inter-day precision (RSD%) was less than 15%. The matrix effect was between 93% and 102%, and the recovery was greater than 91%. The UPLC-MS/MS method established in this work has good selectivity and high sensitivity, and the UPLC-MS/MS method was successfully applied to the pharmacokinetics of modafinil by oral gavage and sublingual injection in rats. The bioavailability of modafinil was calculated to be 55.8%.

KEYWORDS

modafinil, pharmacokinetics, UPLC-MS/MS, bioavailability

INTRODUCTION

Modafinil was launched in France in 1990 and first approved by the European Medicines Agency (EMA) and the US Food and Drug Administration (FDA) for the treatment of narcolepsy [1–3]. It has since been approved by the FDA for use in obstructive sleep apnea and excessive sleepiness caused by shift work sleep disorders [4, 5]. While providing an effective treatment for drowsy patients, modafinil has also produced positive effects as a pro-cognitive agent of choice in the management of disorders associated with cognitive deficits. Modafinil can also be used to treat fatigue associated with depression, cancer, and stroke [6, 7]. It can also relieve drowsiness and fatigue, enhance memory, and improve cognitive ability in healthy special populations, such as military personnel and chess players. Modafinil has a strong and long-lasting awakening effect, and short-term use can improve learning and work

†These authors contributed equally to this work.

*Corresponding author.
E-mail: bluce494949@wmu.edu.cn

**Corresponding author.
E-mail: meiling308@163.com

efficiency, so has been sought after by students and parents as a "smart drug" and subsequently abused [5, 8]. It has been listed in the first category of psychotropic drugs is strictly controlled.

At present, the analytical and detection methods of modafinil primarily include spectrofluorimetry [9], high performance liquid chromatography (HPLC) [10–15], gas chromatography-mass spectrometry (GC-MS) [16], and high performance liquid chromatography-mass spectrometry (HPLC-MS/MS) [17]. Among them, liquid chromatography-tandem mass spectrometry has high separation ability, strong selectivity, high sensitivity, and can provide relative molecular weight and structural information, especially for complex samples [18–20]. Therefore, it is currently the main method used for drug detection in biological samples. McKinney developed a LC-MS/MS method for the detection of modafinil and its major metabolite, modafinil acid, in equine urine by solid-phase extraction and positive ion electrospray ionization; this method needed 14 min runtimes for each sample and method validation was not performed [17]. In this work, a method based on ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) was established for the determination of modafinil in the plasma of rats, and its pharmacokinetics were studied under different administration routes (gavage and sublingual intravenous injection).

EXPERIMENTAL

Chemicals and reagents

Modafinil (>98% purity, Fig. 1A and B) with European Pharmacopoeia Reference Standards was obtained from Beijing Northern Weiye Metrology Technology Research Institute (Beijing, China). Midazolam (internal standard, ≥98% purity, Fig. 1B) was purchased from Merck (Darmstadt, Germany). Chromatographically pure methanol and acetonitrile were purchased from Merck (Darmstadt, Germany); experimental ultrapure water was prepared by a Millipore Milli-Q purification system (Bedford, MA, USA). Sprague-Dawley (SD) rats (male, body weight 200–250 g) were obtained from the Animal Experiment Center of Wenzhou Medical University.

UPLC-MS/MS conditions

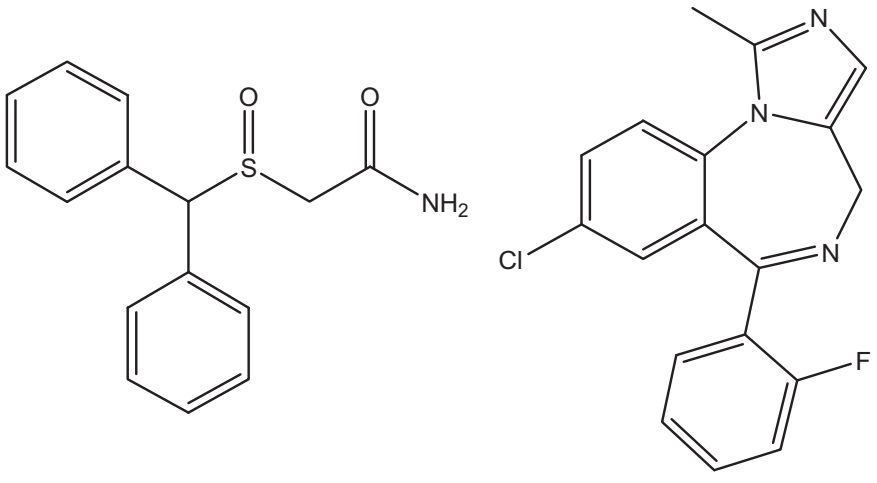
A Waters XEVO TQ-S micro triple quadrupole tandem mass spectrometer was used to analyze the compounds. Data acquisition and instrument control were performed using Masslynx 4.1 software (Waters Corp.).

Chromatographic conditions: UPLC BEH C18 column (50 mm × 2.1 mm, 1.7 µm), flow rate of 0.4 mL min^{-1}, column temperature of 40°C, the mobile phase was acetonitrile-0.1% formic acid water, gradient elution, and the gradient program was as follows: 0–0.2 min, 10% acetonitrile; 0.2–1.0 min, 10–70% acetonitrile; 1.0–2.5 min, 70–90% acetonitrile; 2.5–2.8 min, 90–10% acetonitrile; 2.8–4.0 min, 10% acetonitrile.

Mass spectrometry conditions: Nitrogen was used as a desolvation gas (900 Lh^{-1}) and cone gas (50 Lh^{-1}). The ion monitoring conditions were defined as a capillary voltage of 3.1 kV, a source temperature of 150°C, and a desolvation temperature of 500°C. The technique was quantified in multiple reaction monitoring (MRM) mode with electrospray ionization positive mode, *m/z* 274→167 for modafinil and *m/z* 326→291 for internal standard, Fig. 2.

Preparation of reference solution

Stock solutions of modafinil (100 µg mL^{-1}) and midazolam (100 µg mL^{-1}) were prepared in methanol-water (50:50). Working solutions of modafinil were diluted by methanol, and working solutions of midazolam were diluted by acetonitrile. All solutions were stored at 4°C and brought to room temperature before use.



(a) (b)

Fig. 1. Chemical structure of modafinil (a) and internal standard (b)

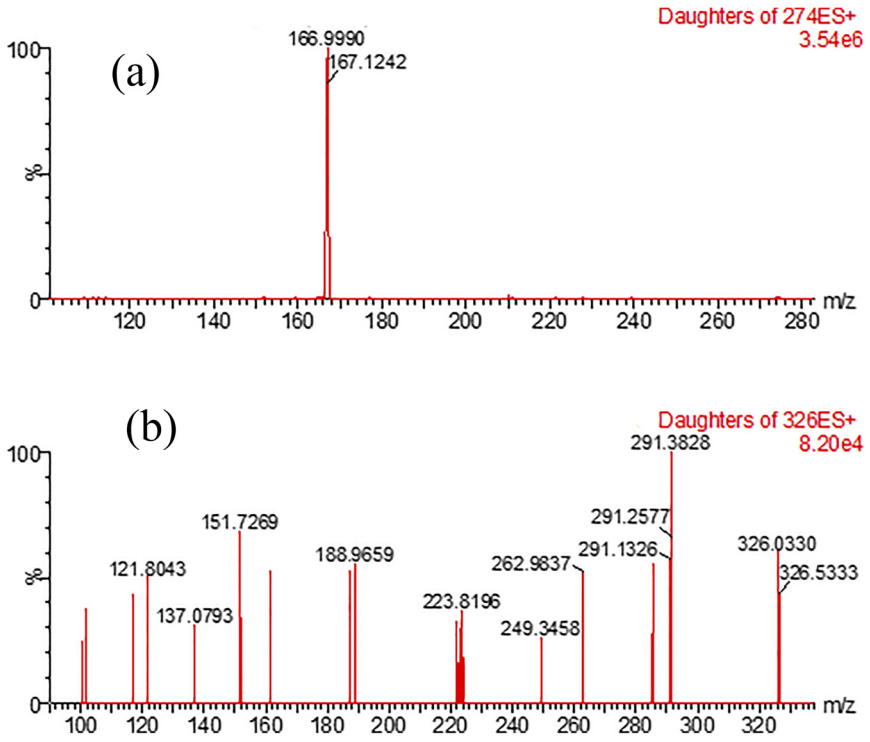


Fig. 2. Mass spectrum of modafinil (a) and internal standard (b)

Standard curve preparation

Modafinil standards were prepared by adding an appropriate amount of working solution to blank rat plasma (1, 5, 10, 20, 50, 100, 200, 500, 1,000, and 2,000 ng mL^{-1}), followed by vortex mixing, where the standard curve range was 1–2,000 ng mL^{-1}. Quality control (QC) samples were prepared at three different plasma drug concentrations (3, 160, and 1,600 ng mL^{-1}) in the same manner as standard samples. Calibration standards and QC samples were protein precipitated with acetonitrile prior to UPLC-MS/MS analysis.

Sample processing

A plasma sample (50 µL) was thawed in 1.5 mL tube, 100 µL of acetonitrile (containing 20 ng mL^{-1} internal standard) was added, vortexed for 0.5 min, and then centrifuge (13,000 rpm, 4°C, 10 min). Supernatant (2 µL) was injected into the UPLC-MS/MS for analysis.

Pharmacokinetic studies

Six rats were injected sublingually with a drug concentration of 1 mg kg^{-1}, and six rats were administered a 10 mg kg^{-1} drug concentration via oral gavage. All experimental procedures and protocols were approved by the Animal Care Committee of Wenzhou Medical University (Wydw 2019-0982). After administration, 500 µL of blood was collected from the tail vein of the rats at 0.08333, 0.25, 1, 2, 4, 6, 8, 12, and 24 h after administration, centrifuged (8,000 r/min, 8 min), and 100 µl of the supernatant was collected and stored frozen at −80°C. Masslynx 4.1 software (Waters Corp.) was used for data acquisition, and the main pharmacokinetic parameters were analyzed by DAS 2.0 statistical software.

RESULTS

Method development

Two columns, BEH C18 and HSS T3, were tested for optimal selectivity, retention time, and peak shape for both the analyte and internal standard. The resolution of modafinil and internal standard was set as a suitability requirement for column selection [21, 22]. BEH C18 and HSS T3 resulted in resolution = 1.6 and resolution = 1.2 respectively, therefore BEH was selected. Since acetonitrile has a stronger eluting power than methanol, acetonitrile was chosen as the eluent. When acetonitrile-water was used as the mobile phase system, it was found that the response of the analyte was relatively low, so an attempt was made to add acid to the mobile phase. These tests showed that 0.1% formic acid was better than 0.1% acetic acid to increase the response. By studying the relative proportions of acid in the sample, it was found that 0.1% formic acid showed the best response. The chromatographic run time was only 4 min, and the pretreatment method was protein precipitation.

Selectivity

The selectivity of the method was evaluated by chromatographic analysis of blank plasma samples, blank plasma spiked with modafinil, and the internal standard midazolam, and plasma samples collected from the tail vein of the administered rats. Figure 3 shows a typical chromatogram

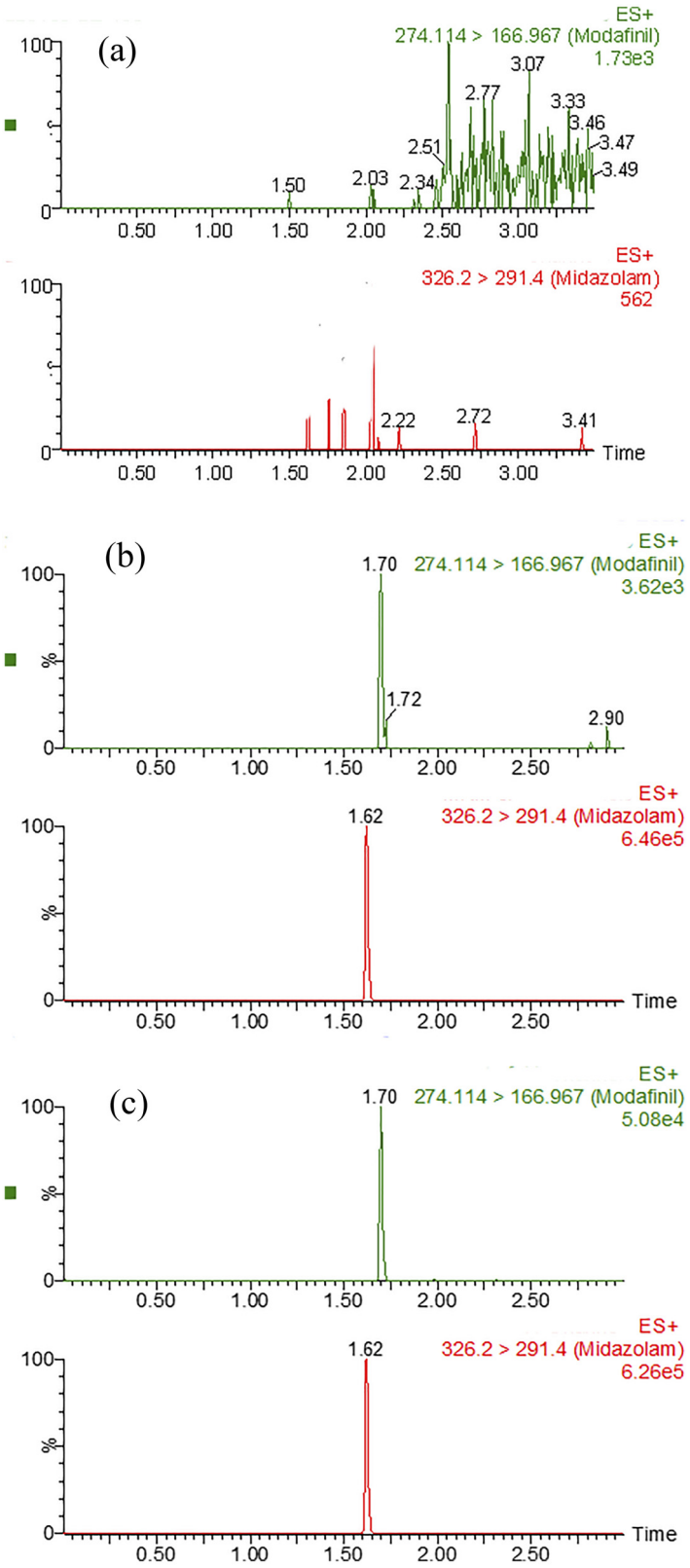


Fig. 3. UPLC-MS/MS of modafinil and internal standard in rat plasma; a) blank rat plasma, b) blank rat plasma spiked with modafinil and internal standard, c) a rat plasma after intravenous administration

of a plasma sample collected from the tail vein of a dosed rat. The retention time of modafinil and the internal standard midazolam were observed at 1.69 and 1.62 min, respectively. There was no obvious interference of impurities and endogenous substances on the assay, which shows that the method has good selectivity.

Table 1. Accuracy, precision, matrix effect and recovery of modafinil in rat plasma

Concentration (ng mL^{-1})	Measured Concentration (ng mL^{-1})	Accuracy (%)		Precision (RSD%)		Matrix effect (%)	Recovery (%)
		Intra-day	Inter-day	Intra-day	Inter-day		
1	0.9	86.0	90.1	14.2	14.9	93.9	94.6
3	2.8	92.7	97.2	12.5	5.0	94.1	94.4
160	162.9	101.8	94.2	9.5	4.2	101.5	91.6
1,600	1651.2	103.2	102.1	5.9	6.5	93.5	95.0

Table 2. Stability of modafinil in rat plasma

Concentration (ng mL^{-1})	Autosampler (4°C, 12 h)		Ambient (2 h)		–20°C (30 d)		Freeze-thaw	
	Accuracy	RSD	Accuracy	RSD	Accuracy	RSD	Accuracy	RSD
3	94.5	6.8	103.3	8.3	93.1	7.3	88.9	10.5
160	107.6	7.4	93.0	4.5	99.3	10.3	95.9	7.5
1,600	106.3	5.7	96.2	7.7	91.8	5.6	86.0	11.7

Calibration curve

The prepared standard series of different concentrations, the peak areas of each concentration, and internal standard were measured, and a standard curve was drawn to assess the linearity. Modafinil has a linear range of 1–2,000 ng mL^{-1}, and the equation for the standard curve was: $y = (0.00040 \pm 0.00003)\,x + (0.00005 \pm 0.00001)$, $r = 0.999$, where y represents the peak area ratio of modafinil to the internal standard, and x represents the concentration of modafinil. The lower limit of quantification of modafinil in rat plasma was 1 ng mL^{-1}.

Accuracy, precision, matrix effects, and recovery

QC samples with three different concentrations (3, 160 and 1,600 ng mL^{-1}) were operated in parallel for three consecutive days. The samples were prepared every day and measured according to the accompanying standard curve. The intra-day accuracy of modafinil was between 86 and 104%, and the inter-day accuracy was between 90 and 103%, where the intra-day precision (RSD%) was less than 15% and inter-day precision (RSD%) was less than 15%.

To evaluate the matrix effect, blank rat plasma was extracted and spiked with the analyte at 3, 160, and 1,600 ng mL^{-1} concentrations. The corresponding peak areas were then compared to those of neat standard solutions at equivalent concentrations. The recovery of modafinil was evaluated by comparing the peak area of extracted QC samples with those of reference QC solutions reconstituted in blank plasma extracts ($n = 6$) [23]. The matrix effect was between 93 and 102%, and the recovery was greater than 91%, Table 1. These results show that the established UPLC-MS/MS method is suitable for the pharmacokinetic study of modafinil in this paper.

Stability

The rat plasma was placed in the autosampler for 2 h, and the plasma samples were pretreated and placed at room temperature for 24 h. After three freeze-thaw cycles, a stability test was performed at −20°C for 30 d. The accuracy of modafinil was between 86 and 108%, and the RSD was within 12%, Table 2, indicating that the stability of modafinil was good.

Pharmacokinetic studies

Modafinil was administered to rats by sublingual intravenous injection and intragastric administration, respectively, and the plasma concentration-time curves were obtained as shown in Fig. 4. The main pharmacokinetic parameters are shown in Table 3. The formula for absolute bioavailability is: absolute bioavailability = AUC for oral administration/AUC for intravenous injection × 100%. The bioavailability of modafinil was 55.8%.

DISCUSSION

Modafinil, a new type of central wake-up stimulant, is mainly used to treat narcolepsy and spontaneous narcolepsy,

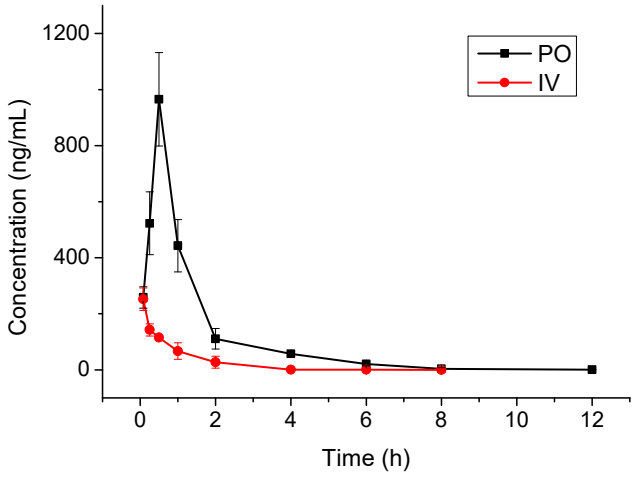


Fig. 4. The concentration-time curve of rats after intravenous (iv, 1 mg kg^{-1}) and oral (po, 10 mg kg^{-1}) administration of modafinil

Table 3. Main pharmacokinetic parameters after intravenous (iv) and oral (po) administration of modafinil in rats (mean ± SD)

Group	$AUC_{(0-t)}$ (ng mL^{-1}*h)	$AUC_{(0-\infty)}$ (ng mL^{-1} *h)	$t_{1/2z}$ (h)	$CL_{z/F}$ (L h^{-1} kg^{-1})	$V_{z/F}$ (L kg^{-1})	C_{max} (ng mL^{-1})
po, 10 mg kg^{-1}	1169.8 ± 180.9	1171.3 ± 180.2	1.1 ± 0.4	8.7 ± 1.1	14.3 ± 5.9	965.2 ± 166.9
IV, 1 mg kg^{-1}	209.6 ± 59.3	209.7 ± 59.3	0.8 ± 0.2	5.1 ± 1.4	5.9 ± 2.6	252.3 ± 39.9

$AUC_{(0-t)}$-the area under the curve from pre-dose to the last sampling time.
$AUC_{(0-\infty)}$-the area under the curve from pre-dose extrapolated to infinity.
T½ - Elimination half-life.
CL- Clearance.
$V_{z/F}$- Apparent volume of distribution.
C_{max}- Maximum plasma concentration.

as well as obstructive sleep apnea syndrome and sleep disturbances caused by shift work [24]. Compared with traditional central stimulants, it can effectively regulate sleep and promote wakefulness with less adverse reactions. It is a psychoactive drug, strictly controlled at home and abroad, and is a prescription drug in most countries [25, 26].

Due to the high detection sensitivity, the protein precipitation extract can be directly injected into the sample after dilution, avoiding the time-consuming concentration process [27, 28]. Both the pretreatment method and detection method are fast and convenient.

This method adopts rapid gradient elution for chromatographic separation, which increases the chromatographic retention of modafinil, improves the sensitivity, and avoids the accumulation of endogenous matrix. This established UPLC-MS/MS method was successfully applied to the pharmacokinetics of modafinil by oral gavage and sublingual injection in rats, and the bioavailability of modafinil was calculated to be 55.8%.

ACKNOWLEDGMENTS

This study was supported by grants from the Wenzhou Science and Technology Bureau (Y2020069).

REFERENCE

1. Minzenberg, M.J.; Carter, C.S. *Neuropsychopharmacology* **2008**, *33*, 1477–502.
2. Morein-Zamir, S.; Turner, D.C.; Sahakian, B.J. *Schizophr. Bull.* **2007**, *33*, 1298–306.
3. Gerrard, P.; Malcolm, R. *Neuropsychiatr Dis Treat* **2007**, *3*, 349–64.
4. Hashemian, S.M.; Farhadi, T. *J. Drug Assess* **2020**, *9*, 82–6.
5. Wilms, W.; Wozniak-Karczewska, M.; Corvini, P.F.; Chrzanowski, L. *Chemosphere* **2019**, *233*, 771–85.
6. Ramachandra, B. *Crit. Rev. Anal. Chem.* **2016**, *46*, 482–9.
7. Hawkes, N. *BMJ* **2015**, *351*, h4573.
8. Van Puyvelde, M.; Van Cutsem, J.; Lacroix, E.; Pattyn, N. *Mil. Med.* **2022**, *187*, 52–64.
9. Hosny, N.M.; Gadallah, M.I.; Gomila, R.M.; Qayed, W.S. *Talanta* **2022**, *236*, 122890.
10. Rao, R.N.; Shinde, D.D. *Biomed. Chromatogr.* **2009**, *23*, 811–6.
11. Nageswara Rao, R.; Shinde, D.D.; Kumar Talluri, M.V. *Talanta* **2007**, *73*, 407–14.
12. Schwertner, H.A.; Kong, S.B. *J. Pharm. Biomed. Anal.* **2005**, *37*, 475–9.
13. Gorman, S.H. *J. Chromatogr. B Analyt. Technol. Biomed. Life. Sci.* **2002**, *767*, 269–76.
14. Burnat, P.; Robles, F.; Do, B. *J. Chromatogr. B Biomed. Sci. Appl.* **1998**, *706*, 295–304.
15. Moachon, G.; Matinier, D. *J. Chromatogr. B Biomed. Appl.* **1994**, *654*, 91–6.
16. Tseng, Y.L.; Uralets, V.; Lin, C.T.; Kuo, F.H. *J. Pharm. Biomed. Anal.* **2005**, *39*, 1042–5.
17. McKinney, A.R.; Suann, C.J.; Stenhouse, A.M. *Rapid Commun. Mass Spectrom.* **2005**, *19*, 1217–20.
18. Sun, W.; Jiang, X.; Wang, X.; Bao, X. *Curr. Pharm. Anal.* **2021**, *17*, 547–53.
19. Chen, F.; Yu, Z.; Wang, X. *J. Chromatogr. B Analyt. Technol. Biomed. Life. Sci.* **2021**, 1180.
20. Bao, X.; Jiang, X.; Ma, J.; Wang, X.; Zhou, Q. *Biomed. Res. Int.* **2020**, *2020*.
21. Yao, Y.; Wang, H.; Ma, J. *Lat. Am. J. Pharm.* **2021**, *40*, 1727–31.
22. Ren, K.; Shi, H.; Hong, F.; Yu, Y.H.; Ma, W.C.; Ma, J.S. *Lat. Am. J. Pharm.* **2020**, *39*, 1875–9.
23. Qiu, E.; Yu, L.; Liang, Q.; Wen, C. *Int. J. Anal. Chem.* **2022**, *2022*, 1838645.
24. Gagnon, D.J.; Leclerc, A.M.; Riker, R.R.; Brown, C.S.; May, T.; Nocella, K.; Cote, J.; Eldridge, A.; Seder, D.B. *Neurocrit. Care* **2020**, *33*, 283–97.
25. Battleday, R.M.; Brem, A.K. *Eur. Neuropsychopharmacol* **2015**, *25*, 1865–81.
26. Hurst, D.L.; Lajara-Nanson, W.A.; Lance-Fish, M.E. *J. Child Neurol.* **2006**, *21*, 294–7.
27. Lin, Z.; Lu, X.; Yu, H.; Jiang, H.; Ma, J.; Bao, S. *Lat. Am. J. Pharm.* **2020**, *39*, 2221–5.
28. Zhang, D.; Hu, Y.; Zhou, C.; Feng, T.; Chen, L.; Ma, J. *Lat. Am. J. Pharm.* **2019**, *38*, 2401–5.