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Quantitative determination of armodafinil in human plasma by liquid chromatography-electrospray mass spectrometry: Application to a clinical study

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Abstract

Armodafinil is a wake-promoting agent approved in 2007 by the US Food and Drug Administration for the treatment of excessive sleepiness. A rapid, sensitive and selective liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the determination of armodafinil in human plasma was developed and validated. Armodafinil and internal standard (armodafinil d-10) were extracted from human plasma using protein precipitation combined with liquid-liquid extraction. This developed method only requires 50 μ L of plasma for the analysis. The chromatographic separation was performed with a Waters symmetry, C18, 4.6 $\times$ 150 mm, 5 μ m column using formic acid, water, and acetonitrile as solvent delivered at a 0.7 mL/min flow rate. The total run time of the method was 3 min. The method was validated according to regulatory guidance in terms of specificity, selectivity, linearity, matrix effect, recovery and stability. Optimized Q1/Q3 mass transitions for armodafinil and armodafinil d-10 were 274.1/167.2 (m/z) and 284.4/177.4 (m/z) respectively. The method showed linearity within the tested concentration range of 10–10,000 ng/mL. The method was successfully applied to quantify armodafinil concentrations after single oral administration of a 250 mg tablet in a clinical study conducted in healthy volunteers. Significant advantages of this method are minimal sample volume, short run time and a lower LLOQ.

Keywords

Armodafinil; Pharmacokinetics; LC-MS/MS; Clinical Study; Modafinil

1. INTRODUCTION

Armodafinil (*R*-modafinil) is the *R*-isomer of the racemate modafinil. Chemically, armodafinil is a 2-[(*R*) (diphenyl methyl) sulfinyl] acetamide with a molecular weight of 273.55 g/mol. It promotes wakefulness in patients with excessive sleepiness associated with

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Conflict of interest

The authors declare no conflict of interest.

narcolepsy, shift work disorder, and obstructive sleep apnea. (Czeisler, Walsh, Wesnes, Arora and Roth 2009, Russo, Stetz, Jenkins and Folen 2009). While the exact mechanism of action is unknown, armodafinil binds *in vitro* to dopamine transporters, inhibiting dopamine reuptake (Garnock-Jones, Dhillon and Scott 2009). A recent study suggests that armodafinil may also promote wakefulness by inhibition of GABA-releasing parafacial zone (PZ^{Vgat}) neurons (Anaclet, Griffith and Fuller 2017). The recommended dosage of armodafinil in patients with narcolepsy or obstructive sleep apnea is 150mg to 250 mg orally once a day taken in the morning. For patients with shift work disorder, the recommended dosage is 150 mg taken one hour prior to the start of shift work (Garnock-Jones, Dhillon and Scott 2009).

Armodafinil exhibits linear pharmacokinetics over a dose range of 50 to 400 mg. Time-independent pharmacokinetics were observed during 12 weeks of dosing. The apparent steady-state is reached within 7 days of dosing. Armodafinil is rapidly absorbed after oral administration, and the absolute bioavailability is unknown. In a fasted state, peak plasma concentrations are reached within 2 hours, and in a fed state, peak concentrations occur within 2–4 hours. The drug is primarily metabolized to R-modafinil acid and modafinil sulfone, both are inactive, circulatory metabolites. The half-life of armodafinil is approximately 15 hrs, as compared to the S-enantiomer, which has a half-life of 4 hrs (Conrado 2012, Darwish, Kirby, D'Andrea, Yang, Hellriegel and Robertson 2010, Darwish, Kirby, Hellriegel and Robertson 2009, Darwish, Kirby, Hellriegel, Yang and Robertson 2009, Garnock-Jones, Dhillon and Scott 2009).

The World Anti-Doping Agency (WADA) banned modafinil in sports in 2004 as many athletes were allegedly using it as a doping agent. Clinical and forensic labs were mainly using gas chromatography-mass spectrometry (GC-MS) to detect modafinil in biological fluids (Dubey, Ahi, Reddy, Kaur, Beotra and Jain 2009). Since armodafinil is an isomer of modafinil, these methods might be applicable for the quantification of armodafinil as well. Several bioanalytical methods such as high-performance liquid chromatography (HPLC) coupled with photodiode array detector (PDA) or ultraviolet detector (UV) detection, GC-MS and LC-MS/MS have been reported in the literature for the determination of modafinil and modafinil isomers (Burnat, Robles and Do 1998, Cass and Ferreira Galatti 2008, Dubey, Ahi, Reddy, Kaur, Beotra and Jain 2009, Harvanova and Gondova 2017, McKinney, Suann and Stenhouse 2005, Moachon and Matinier 1994, Schwertner and Kong 2005, Tseng, Uralets, Lin and Kuo 2005). However, all of these published methods require more than 100 µL of plasma volume makes it difficult sometimes in a patient population where multiple samples needed for therapeutic drug monitoring. Other disadvantages associated with these methods are labor intensive extraction process, expensive, time-consuming. Due to the favorable pharmacokinetic properties of armodafinil as compared to modafinil, most recent studies were performed with armodafinil (Darwish, Kirby, Hellriegel and Robertson 2009, Ramachandra 2016).

To date, there are only a few bioanalytical methods reported in the literature that explicitly describe the quantification of armodafinil in human plasma. Devadiga et al. developed a LC-MS/MS method (lowest level of quantification (LLOQ) 77 ng/mL) using 200 µL of plasma and duloxetine as the internal standard (M. Devadiga 2011). Ramesh et al. reported a method with an LLOQ of 50 ng/mL, carbamazepine as the internal standard and a required plasma

volume of 250 μ L (D. Ramesh 2012). Both methods described method development and validation but did not show any application of the developed method and did not use the stable isotope of armodafinil as the internal standard. To the best of our knowledge, estimation of armodafinil in human plasma using stable labeled internal standard has not been published yet.

The present work aimed to develop and validate a simple, sensitive, selective and precise bioanalytical method for the quantification of armodafinil in human plasma with LC-MS/MS. The developed method requires only 50 μ L of plasma and uses the stable labeled armodafinil-d10 isotope as an internal standard. The LLOQ of our method is 10 ng/mL which is sensitive enough to capture elimination phase. The method was applied in a clinical pharmacokinetics/pharmacodynamics study of armodafinil where it was used to quantify armodafinil concentrations in healthy volunteers.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

Armodafinil (purity 98%) was purchased from Sigma-Aldrich (St. Louis, MO). Armodafinil-d10 was purchased from Clearsynth (Mumbai, India). Structures of both compounds are given in Figure 1. Blank human plasma was obtained from the local blood bank. Double distilled water was obtained in-house (Department of Pharmaceutics, University of Florida). All other reagents such as acetonitrile (purity 99.5%), dimethyl sulfoxide (DMSO, purity 99.7%), and formic acid (purity 99.5%) were purchased from Fisher Scientific (PA, USA).

2.2. Stock solutions, standard solutions, and quality control sample preparation

Stock solutions (1 mg/mL) of armodafinil and armodafinil-d10 were prepared in DMSO. The calibration standards (10–10,000 ng/mL) were prepared via stepwise dilution in acetonitrile. Quality control (QC) samples were prepared with a different armodafinil stock solution (1 mg/mL) at five different concentration levels as follows: lowest level of quantification (LLOQ-QC-10 ng/mL), lower level of quality control (LQC-30 ng/mL), middle1 level of quality control (M1QC-450 ng/mL), middle2 level of quality control (M2QC-4500 ng/mL), higher level of quality control (HQC-7500 ng/mL) using acetonitrile as a diluent. Armodafinil-d10 (internal standard (IS)) was diluted with acetonitrile to a final concentration of 1000 ng/mL before spiking into the plasma samples. All the stock solutions including the primary stocks and the intermediate stocks were stored at 2–8°C. Stability studies proved stock stability for the entire validation period. The CS and QC samples were prepared by spiking 2.5 μ L of stock solutions with 47.5 μ L of blank human plasma to establish the final calibration range of 10–10,000 ng/mL.

2.3. Apparatus and software

A Shimadzu UFLC-Nexera X2 system (Kyoto, Japan) consisting of a LC-30AD pump, SIL-30AC autosampler, CTO-30A column oven and DGU-20ASR degasser was used for reverse phase chromatography. The ionization and detection of the analytes was carried out on the ABSCIEX API 5500 Q-Trap mass spectrometer (AB Sciex, Framingham, MA, USA)

coupled with an electrospray ionization (ESI) source operating in positive mode. All data analysis was performed with the Analyst 1.6 software (AB Sciex, Framingham, MA, USA).

2.4. Chromatographic conditions

Chromatographic separation was achieved with a Waters symmetry, C18 (150×4.6 mm i.d., 5 µm) analytical column maintained at room temperature. Mobile phase A consisted of a mixture of water with 0.1% formic acid. Mobile phase B was a mixture of acetonitrile: water with 0.1% formic acid (95:5% v/v). The isocratic elution was carried out at a 90:10% v/v ratio mobile phase A: mobile Phase B delivered at a flow rate of 0.7 mL/min. The total run time of a single injection was 3 min. The sample injection volume was 5 µL.

2.5. Mass spectrometric conditions

Bioanalysis of armodafinil was performed using an ABSCIEX API 5500 Q-Trap mass spectrometer (AB Sciex, Framingham, MA, USA) coupled with a Turbo V Ion Source operated in the positive electrospray ionization (ESI) mode. The mass spectrometric detection of armodafinil and IS was performed in the multiple reaction monitoring (MRM) mode. To determine various compound dependent and source dependent parameters of the analyte and the IS, tuning was performed by continuously infusing both analyte and IS into the mass spectrometer at 10 µL/min.

2.6. Sample preparation

All samples were purified with protein precipitation (PPT) combined with liquid-liquid extraction (LLE) in order to improve recovery of the analytes and to minimize unwanted matrix effects. All samples were processed as follows: 100 µL of IS solution was added to 50 µL of sample volume, which was then vortexed for 2 mins to precipitate the proteins. The ratio of plasma to precipitating solvent was 1:2. Then 1 mL of ethyl acetate was added, and the mixture was vortexed again for 5 min to ensure adequate partitioning of armodafinil and IS into the extraction solvent. The ratio of plasma to extracting solvent was 1:20 and overall plasma to total solvent ratio for the extraction was 1:22 including precipitating solvent. Subsequently, the samples were centrifuged at 10,000 rpm for 10 min. After centrifugation, 750 µL of the clear supernatant was transferred into microfuge tubes and dried under a nitrogen stream at 40°C. The dried samples were reconstituted with 100 µL of acetonitrile. Finally, 5 µL of the processed sample were injected into the LC-MS/MS system.

2.7. Assay validation procedures

The developed electrospray ionization tandem mass spectrometry (ESI-MS/MS) method was fully validated according to the FDA Bioanalytical Method Validation Guidelines with respect to accuracy, precision, selectivity, sensitivity, reproducibility, and stability (US Food and Drug Administration, 2018).

The linearity of each of the calibration curves was determined by plotting the peak area ratio of the analyte and IS against the nominal concentration of the calibration curve. The sensitivity of the method was determined from the signal to noise ratio (S/N ratio) of the lowest concentration in calibration curve with acceptable precision and accuracy.

2.7.1. Accuracy and precision of the quality control samples—The accuracy and precision of the within-run analysis was calculated by analyzing the set of QC samples (n=5) at five concentration levels (LLOQC, LQC, M1QC, M2QC, HQC) on a single day. In a similar way, between batch accuracy and precision was calculated by analyzing five QC levels (n=5) on three different days. According to the guideline, the accuracy of the QCs (within and between batches) should be within 85–115% of the nominal concentration for the LQC, M1QC, M2QC and HQC samples and within 80–120% for the LLOQ samples. Similarly, the precision of the QCs (within and between batches) needs to be within $\pm 15\%$ coefficient of variation (CV), except for the LLOQ, which needs to be within $\pm 20\%$ CV of the nominal value.

2.7.2. Matrix effect—The matrix effect was determined by comparison of the peak area of the analytes that were spiked into the blank extracted human plasma samples with the peak area of the analytes spiked into the neat solution at the same concentration (QCs) in each of the six replicates. Human plasma from six different lots was used to determine the matrix effect on the analytes.

2.7.3. Recovery—The % mean extraction recovery was calculated by comparing the peak area response of armodafinil in the extracted plasma samples at LLOQC, LQC, M1QC, M2QC and HQC with those of unextracted quality control samples at LLOQC, LQC, M1QC, M2QC and HQC. Recovery of IS was determined with the same methodology.

2.7.4. Stability studies—The analyte stability at the LQC and HQC was evaluated by a series of different stability experiments in human plasma. These experiments included auto-sampler stability ($6 \pm 2^\circ\text{C}$ for 24 h), bench-top stability (6 hours at ambient temperature), long-term stability (at $-70 \pm 10^\circ\text{C}$ and $-20 \pm 5^\circ\text{C}$ for 30 days), dry extract stability (extracted samples kept at $-70 \pm 10^\circ\text{C}$ for 24 hours) and freeze-thaw stability (at $-70 \pm 10^\circ\text{C}$ for up to three cycles). Each of the stability studies was performed in six replicates. The samples were compared with freshly prepared calibration curve and QC samples and considered stable if the deviation was within the range of 85–115% of the nominal concentration.

2.8. Application to a clinical study

Blood samples were collected from three subjects enrolled in the trial “*Effect of Armodafinil on Simulated Driving, Electroencephalogram and Cognitive Performance in Sleep Deprived Healthy Subjects*” (NCT02468856), conducted in the Clinical Research Center of the Clinical and Translational Science Institute at the University of Florida. The study was reviewed and approved by the Institutional Review Board of the University of Florida and was in accordance with the Declaration of Helsinki. Each participant gave written consent before participation. In this randomized placebo-controlled, double-blinded crossover study, participants underwent a sleep deprivation period of 38 hours and received either placebo or armodafinil 250 mg in each of the two study sessions. Blood samples were taken before administration of the study drug and at the following time points: 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 10 hours post-dose through an intravenous catheter placed in the arm vein of the subjects. For each blood sample, approximately 3mL plasma was collected into EDTA Vacutainer

tubes. After blood collection, the tubes were centrifuged in a refrigerated centrifuge for 15 minutes at 3000 rpm and stored in a -80°C freezer until analysis.

3. RESULTS AND DISCUSSION

3.1. LC-MS/MS optimization

To obtain adequate sensitivity and selectivity for armodafinil and IS, numerous chromatographic and mass spectrometric conditions were optimized. The selection of proper ionization mode is essential for the proper signal intensity of analyte. The sensitivity of armodafinil was found higher in ESI mode compared to atmospheric pressure chemical ionization (APCI) source. Further ionization was optimized by comparing signal intensity in positive as well as in a negative ESI mode. The response obtained in positive ionization mode was found much higher and stable. The optimized Q1/Q3 mass transitions for armodafinil and IS were 274.1/167.2 mass-to-charge ratio (m/z) and 284.4/177.4 (m/z) respectively (Figure 2). Other compound dependent and source dependent parameters were optimized gradually to improve sensitivity and stability of analyte response. The optimized parameters are provided in Table 1.

Chromatographic conditions mainly include mobile phase composition, column and flow rates were evaluated and optimized to achieve better symmetrical peak shape, acceptable resolution and shorter run time. Several varieties of reversed phase columns with different lengths (ranging from 50 mm to 150 mm in length) like Supelco Discovery, Thermo Synchronis, Phenomenex Luna, Phenomenex Ultracarb, and Waters Symmetry were assessed for the optimal sensitivity and better peak shape. In case of Supelco Discovery and Thermo Synchronis, poor peak shape was observed where retention time was increased in case of Phenomenex Luna and Ultracarb. Waters Symmetry column found most appropriate for the analytes with symmetric peak shape and in terms of better signal. Various combinations of aqueous buffers such as 0.05%–0.5% formic acid or acetic acid, ammonium formate or ammonium acetate (2–10 mM) with organic phase solvents such as methanol, acetonitrile, and the combination of both were tried at different compositions as well as different flow rates. Formation of $[M+H]^+$ ions and chromatographic separation significantly influenced by the addition of formic acid in the mobile phase. Changing aqueous composition from water to 0.1% formic acid in water showed marked improved in the peak shape and sensitivity of the armodafinil. Thus formic acid was also added to the organic phase as well. Thus, Waters Symmetry, C18 column (150 mm $\times$ 4.6 mm i.d. 5 μm) column combined with mobile phases of acetonitrile: water containing 0.1% formic acid (95:5% v/v) and 0.1 % formic acid in water (90:10, v/v) delivered isocratically at the flow rate of 0.7 mL/min were selected for the analysis. One of the best ways to reduce endogenous interferences is the use of a stable labeled internal standard. Thus, armodafinil-d10 was selected as an internal standard because it has a similar retention time, ionization efficiency and recovery as compared to armodafinil. The total run time of the method was 3 min, which allowed for high-throughput analysis.

3.2. Sample preparation

The following step after optimization LC-MS/MS condition optimization is to develop an efficient sample cleanup technique which could eliminate potential interference from the human plasma matrix. Among the sample preparation techniques for the human biological samples, PPT and LLE are widely used for its simplicity and economic advantages. When PPT was tried alone to extract out armodafinil from the human plasma, desired LLOQ was not achieved, and it also suppresses the signal drastically. PPT was tried with different plasma to solvent ratio such as 1:2, 1:3, 1:4 and 1:5. Solvents such as methanol, acetonitrile, and the combination of both tried, but acetonitrile gave reasonable extraction recovery and less ion suppression. In contrast to PPT when LLE method alone tried with different solvents such as ethyl acetate, hexane, tert-butyl methyl ether and chloroform, it has increased recovery significantly, but still, ion suppression was observed. Among different combinations of these solvents, ethyl acetate showed better recovery, and less ion suppression as armodafinil is slightly soluble in water it was able to extract out easily. Another technique solid-phase extraction (SPE) was also tried, but it showed inconsistency in the drug and IS recovery. Thus it was not selected. Finally, a combination of PPT followed by LLE provided the best results in regards to recovery and endogenous interferences and was therefore chosen as the sample preparation method. In the optimization steps, LLE provided a cleaner sample and less ion suppression as compared to PPT. In addition, it helps to maintain a low column pressure over long time periods when a large number of samples need to be analyzed within short time intervals. During the PPT, most of the proteins are denatured by acetonitrile and during the LLE armodafinil can easily be extracted from the plasma sample with ethyl acetate.

3.3. Method validation

3.3.1. System suitability—The CV of the peak area ratio of armodafinil to IS was found to be < 5% in each of the tested scenarios. System suitability was evaluated every time before the start of a sample analysis for either validation or clinical analysis.

3.3.2. Specificity and selectivity—The retention time of armodafinil and armodafinil-d10 (IS) were 1.63 and 1.62 min respectively. Endogenous matrix from human plasma did not interfere with the peak of interest. Thus the selected extraction methodology and mass transition provided selectivity and specificity for armodafinil and IS (Figure 3).

3.3.3. Calibration Curve—The calibration curve in human plasma ranged from 10 to 10,000 ng/mL. The curve was reproducible over the tested calibration range. The linearity of the method was determined using a $1/x^2$ weighted least square regression analysis of the peak area ratio (analyte peak area/IS peak area) versus standard nominal concentration. The average regression coefficient (r^2) was found to be >0.99. The LLOQ was 10 ng/mL with a signal/noise ratio that was higher than 10.

3.3.4. Accuracy and Precision—Within-batch and between batch accuracy and precision of this method was determined at five different QC (LLOQC, LQC, M1QC, M2QC, and HQC) concentration levels. The developed method was found to be precise and accurate, and the variability was within the acceptable limits (Table 2).

3.3.5. Extraction recovery—The percent mean recovery of armodafinil and armodafinil d-10 are shown in Table 3. The recovery for armodafinil was measured at each of the QC concentration levels and the recovery for the IS armodafinil d-10 was determined at 1000 ng/mL. The data suggest that PPT plus LLE provides efficient and reproducible extraction of the analytes from human plasma with acceptable variability.

3.3.6. Matrix effect—No significant matrix effect was observed in all of the six-tested human plasma lots at the LLOQC, LQC, M1QC, M2QC and HQC concentration. The impact of the endogenous matrix was within the $\pm 15\%$ limit.

3.3.7. Stability—The result of bench-top, long-term auto-sampler, dry extract, and freeze-thaw stability revealed that the tested QC samples (LQC and HQC) were stable under and the variability was within the acceptable limit (Table 4).

3.4. Pharmacokinetic study

The proposed LC-MS/MS method was successfully applied to quantify armodafinil concentrations in healthy human subjects. Pharmacokinetic analysis of plasma armodafinil concentration versus time data was conducted by non-compartmental analysis (with linear trapezoidal method) using Phoenix WinNonlin 6.4 (Pharsight Corporation, Mountain View, CA). The concentration-time profile of armodafinil is given in Figure 4 and mean pharmacokinetic parameters are given in Table 5.

4. CONCLUSION

A rapid, sensitive, reproducible and robust bioanalytical method was developed for armodafinil in human plasma using liquid chromatography-tandem mass spectrometry. The present method is able to quantify armodafinil concentrations in the range of 10–10,000 ng/mL. This method utilizes PPT followed by LLE as an extraction method and requires only 50 μ L of human plasma. Moreover, the developed method for armodafinil is sensitive with an LLOQ of 10 ng/mL which is substantially lower than previously developed methods (D. Ramesh 2012, M. Devadiga 2011). The turnover rate of 3 min per sample enables high-throughput analysis of armodafinil. This method can be used in routinely performed clinical studies such as bioequivalence studies, drug-drug interaction studies, and therapeutic drug monitoring studies. To the best of our knowledge, this is the first developed and validated LC-MS/MS method of armodafinil using a stable labeled internal standard (armodafinil d-10) with shown application in a clinical pharmacokinetic/pharmacodynamics study.

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Figure 1.
Chemical structures of (a) armodafinil and (b) armodafinil d-10.

Figure 2.

Mass spectra of armodafinil and armodafinil d-10. Spectra were obtained by direct infusion of 500 ng/mL of each analyte in positive ion electrospray ionization mode. (a) MS/MS spectra of armodafinil (b) MS/MS spectra of armodafinil d-10.

Figure 3.

Representative MRM ion-chromatograms of (a) blank human plasma (armodafinil), (b) blank human plasma (IS), (c) blank human plasma with IS (armodafinil), (d) blank human plasma+ IS (armodafinil d-10) (e) Calibration curve sample (LLOQ) chromatogram armodafinil, (1.63 min) (f) chromatogram of IS (1.62 min.), (g) chromatogram of PK sample at 6hr of armodafinil and (h) chromatogram of PK sample at 6-hr of IS.

Figure 4.
Concentration-time profile of armodafinil in 3 different healthy individuals.

Table 1

Optimized source and compound dependent parameters for armodafinil and armodafinil-d10.

Source Parameters	Value	
Curtain Gas (psi)	10	
Collision Gas (psi)	Medium	
IonSpray Voltage (eV)	5500	
Source Temperature (°C)	450	
Ion Source Gas 1 (psi)	35	
Ion Source Gas 2 (psi)	40	

Compound Parameters	Armodafinil	Armodafinil-d10
Q1 Mass (Da)	274.1	284.4
Q3 Mass (Da)	167.2	177.4
DP (ev)	40	34
CE (ev)	12	11
EP (ev)	13	13
CXP (ev)	9	9
Dwell Time (msec)	200	200

DP-Declustering potential
CE-Collision energy
EP-Entrance potential
CXP-Collision exit potential

Table 2

Accuracy and precision for within batch and between-batch QC samples

	LLOQ	QC	LQC	MIQC	M2QC	HQC
Nominal concentration (ng/mL)	10.00	30.00	450.00	4500.00	7500.00	
Within batch accuracy and precision						
Batch 01						
Mean	10.07	33.78	508.00	4955.00	8477.50	
SD	0.76	1.34	46.10	359.03	683.59	
% CV	7.54	3.97	9.07	7.25	8.06	
% Mean accuracy	100.70	112.58	112.89	110.11	113.03	
% RE	0.70	12.58	12.89	10.11	13.03	
Batch 02						
Mean	10.57	31.56	442.80	4978.00	8066.00	
SD	0.95	2.60	37.67	278.78	570.82	
% CV	8.98	8.23	8.51	5.60	7.08	
% Mean accuracy	105.74	105.20	98.40	110.62	107.55	
% RE	5.74	5.20	-1.60	10.62	7.55	
Batch 03						
Mean	10.67	32.44	466.40	5108.00	6828.00	
SD	0.67	1.19	27.33	176.83	382.91	
% CV	6.31	3.68	5.86	3.46	5.61	
% Mean accuracy	106.72	108.13	103.64	113.51	91.04	
% RE	6.72	8.13	3.64	13.51	-8.96	
Between-batch accuracy and precision						
Mean	10.44	32.51	472.40	5017.86	7741.43	
SD	0.79	1.94	44.77	261.22	883.93	
% CV	7.58	5.97	9.48	5.21	11.42	
% Mean accuracy	104.39	108.36	104.98	111.51	103.22	
% RE	4.39	8.36	4.98	11.51	3.22	

Table 3

Mean extraction recovery of armodafinil and armodafinil d-10 in human plasma.

Analyte	Nominal concentration (ng/mL)	Mean Extraction Recovery (%)
Armodafinil	10	75.91 ± 4.88
	30	73.13 ± 3.96
	450	74.20 ± 4.54
	4500	76.32 ± 2.43
	7500	75.03 ± 5.17
Armodafinil d-10 (IS)	1000	74.34 ± 6.75

Table 4

Stability data of armodafinil in human plasma.

Storage conditions	Nominal concentration (ng/mL)	Human Plasma		
		Measured mean concentration (ng/mL)	% CV	Accuracy (%)
Freeze-thaw stability ($-70 \pm 10^{\circ}\text{C}$)	30	30.70 ± 2.11	6.88	102.33
	7500	7803.33 ± 605.01	7.75	104.04
Long-term Stability ($-70 \pm 10^{\circ}\text{C}$, 30 days)	30	30.14 ± 2.73	9.07	100.47
	7500	8613.33 ± 156.95	1.92	108.84
Long-term Stability ($-20 \pm 5^{\circ}\text{C}$, 30 days)	30	29.20 ± 2.88	9.87	97.33
	7500	7631.50 ± 625.02	8.19	101.75
Auto-sampler stability (4°C , 24 h)	30	30.80 ± 2.02	6.56	102.67
	7500	402.00 ± 16.09	4.00	105.60
Dry Extract Stability	30	32.88 ± 1.49	4.52	109.60
	7500	6876.00 ± 241.83	3.52	91.68
Bench-top stability	30	30.80 ± 2.01	6.56	102.67
	7500	6816 ± 423.71	6.22	90.88

Table 5

Pharmacokinetic parameters of armodafinil in the healthy subjects following a single dose of 250 mg armodafinil.

Parameters	Estimates
C _{max} (ng/mL)	7053.33.33 ± 707.84
T _{max} (hr)	2.33 ± 0.31
AUC _{0-t} (hr*ng/mL)	54750.74 ± 7074.36
t _{1/2} (hr)	15.65 ± 1.89
Vd/F (L)	35.56 ± 3.84
Cl/F (L/hr)	1.58 ± 0.21

Abbreviation: C_{max}: Maximum concentration achieved in plasma, AUC: area under the curve from 0 to t hr, V_d: volume of distribution, Cl: clearance, t_{1/2}: terminal half-life. Each value represents mean ± SD (n=3).