IJPSR (2020), Volume 11, Issue 4 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 07 June 2019; received in revised form, 07 November 2019; accepted, 06 February 2020; published 01 April 2020

METHOD DEVELOPMENT AND VALIDATION OF LC-ESI-MS/MS TECHNIQUE FOR THE ESTIMATION OF MODAFINIL IN HUMAN PLASMA; APPLICATION TO PHARMACOKINETICS IN HEALTHY RABBITS

Adluri Phanindra * and Y. Shravan Kumar

Mewar University, Chittorgarh - 312901, Rajasthan, India.

Keywords:

Modafinil, Narcolepsy, LC-ESI-MS/MS, FDA guidelines, Precision and Accuracy

Correspondence to Author:
Adluri Phanindra

Research Scholar,
Mewar University, Chittorgarh - 312901, Rajasthan, India.

E-mail: adluriphanindra@gmail.com

ABSTRACT: A new simple and sensitive liquid chromatography-electrospray ionization-tandem-mass spectrometric (LC-ESI-MS/MS) technique was developed and validated for the estimation of modafinil in human plasma and the method was applied to study pharmacokinetics in healthy rabbits. Chromatography was attained on Phenomenex- C_{18} (50mm × 4mm) 5µ column and acetonitrile, methanol and 0.1% formic acid (25:60:15 v/v) mixture as the movable phase at 0.7 ml/min flowrate. Modafinil and modafinil-D5 internal standards were detected at m/z 274.2/229.0, m/z 279.1/234.0 respectively. Modafinil and modafinil-D5 (internal standard) were separated with liquid-liquid extraction. The technique was linear over the 2.0-600.0 ng/ml concentration range. This technique established with intrabatch and inter batch precision within 1.54-7.18% and 1.82-6.25%. This technique established with intrabatch and inter batch accuracy within 98.56-102.80% and 97.62-102.76 %.The drug was shown mean T_{max} of 3.833; average $C_{max,}$ AUC0_t and AUC0_α, for test formulation is 677.667; 6306 and 6471 respectively in the pharmacokinetic study on healthy rabbits.

INTRODUCTION: Modafinil acts as a eugeroic for the treatment of narcolepsy (sleepiness), sleep disorder due to different shift work and more day-time sleepiness which was associated with OPA (obstructive sleep apnea) [1-4]. It was administered by the oral route. It acts by inhibiting selectively and weakly the dopamine reuptake process and indirectly promotes the releasing of histamines and orexin neurological peptides from the tuberomammillary nucleus and lateral hypothalamus, respectively and leads to contribution to heightened-arousal.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(4).1837-44

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(4).1837-44

Chemically Modafinil designated as 2-[(diphenylmethyl) sulfinul] acetamide **Fig. 1** having a molecular mass of 273.35 g/mol [5, 6].

Modafinil activates the cytochrome-P450 enzymes CYP-1A2, CYP-3A4, and CYP-2B6, as well as inhibition of CYP-2C9 and CYP-2C19 *in-vitro*. Modafinil also produces P-glycoprotein (Pgp) material which affects drug transportation by this glycoprotein (as digoxin). The bioavailability of modafinil is greater than 80% of the administered dose. An *in-vitro* study of the medicament indicates that 60% of the drug is bound only to plasma proteins in the clinical concentration level. The percentage sometimes changes with change in the concentration. C_{max} occurs nearly at 2-3 h after drug administration. In the presence of food, the drug will show slow absorption, but it will not affect total AUC. The half-life of the drug was approximately between 10–12 h range, which was

differed by CYP-genotypes, functioning of liver and kidney. The drug is metabolized in the liver and resulting inactive metabolite is excreted in the urine [7-9].

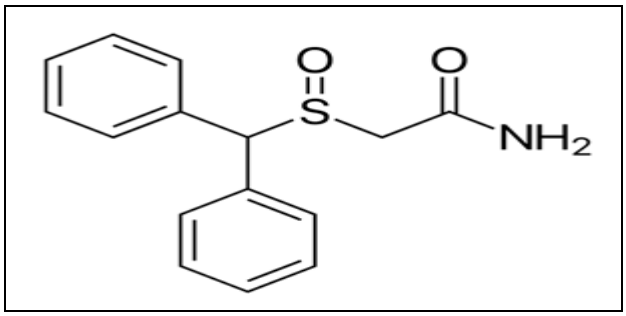

FIG. 1: STRUCTURE OF MODAFINIL

Literature review of modafinil reveals that few analytical quantitative methods were available for the quantification of modafinil in pharmaceuticals which includes UV-spectroscopic method [10-12], high-performance liquid chromatographic [13-17], HPTLC [18] and LC-MS/MS [19, 20] methods. Aim of the present work is to develop simple and sensitive LC-ESI-MS/MS technique for the quantification of modafinil and application of the developed method to study pharmacokinetics in healthy rabbits.

MATERIALS AND METHODS:

Chemical and Reagent Materials: Modafinil and Modafinil-D5 of pure drugs were obtained from MSN Labs, Hyderabad as gift samples. Phenomenex-C_{18} (50mm × 4mm) 5µ columns were bought from Thermo-Fischer Pvt., Ltd., HPLC-grade Acetonitrile, methanol, and analytical grade formic acid were bought from S.D-Fine Chemicals. The water utilized in the research work from Milli-Q purification system belongs to Millipore. The animal studies on healthy rabbits were authenticated by Institutional Ethical Committee no- 1292/ac/09/CPCSEA/17-39/A.

Instrument: The LC-MS/MS system consists of a Shimadzu-LC10 chromatographic system from Shimadzu united with an MS/MS-API-3000 from Applied Bio-systems Sciex, Canada, equipped with a Turbo-Ion-Spray source for ion induction. Chromatographic data were monitored and integrated by Software belongs to Analyst of Applied Bio-systems, version 1.4.1.

Liquid Chromatography: Modafinil and modafinil-D5 were separated by injecting the sample solution into Phenomenex-C_{18} (50 mm × 4mm) 5µ column utilizing a mobile phase mixture of acetonitrile, methanol and 0.1% formic acid in the proportion of 25:60:15 v/v. Degasification of the mobile phase was done by filtration through 0.5µ filter paper and followed by sonication. Drug and IS where eluted with isocratic elution by injecting the mobile phase through the stationary column at 0.7ml/min. The autosampler temperature was adjusted to 5 °C.

Mass Scanning Optimization: Modafinil stock solution was processed by dissolving the drug in HPLC-grade methanol. Further dilutions were made with the solvent composition of water and methanol in the proportion of 20:80 v/v. The components were determined by multiple reaction monitoring (MRM) of the transition pairs of transitions m/z 274.2/229.0, m/z 279.1/234.0 for Modafinil and modafinil-D5 respectively.

Mass Spectrometry: The curtain gas (N_2) was tuned to a constant figure of 11 units and the temperature of source (at the setpoint) was 500 °C. The electro-spray interface heater (IHE) was switch to on mode and Ion spray (IS) Voltage was fixed at 5000 V for ionization. The mass instrument parameters were adjusted to attain high sensitivity at unit resolution. The MRM mode for modafinil and modafinil-D5 were detected at m/z 274.2/229.0, m/z 279.1/234.01 respectively.

Preparation of Quality Control and Calibration Standards: Modafinil stock solution was prepared in 80% methanol to get a concentration of 1mg/ml. Calibration standards were 2.0, 4.0, 20.0, 40.0, 80.0, 160.0, 300.0 and 600.0 ng/ml. These solutions were processed from the stock solution by serial dilution method with 80% methanol. High, medium and low concentration quality control (QC) standards for modafinil were (480.0, 240.0 and 4.0 ng/ml) were processed in the same way. The stock solution of IS (1 mg/ml) was also processed in 80% methanol and further it was diluted to 5 ng/ml concentration. All the processed solutions were reserved in a freezer at 2-8 °C until the samples to be analysed.

Sample Preparation: To 400µl of plasma, 100µl of IS were vortexed. To the sample 400 µl methanol was added and subjected for centrifugation at 3000 rpm for 15-20 min at 5 °C. The organic portion was dried in lyophiliser. To the

residue, 250 µl of the mobile phase was added and transfer a suitable volume of samples into labeled Auto-sampler vials and infused into the LC-ESI-MS/MS system.

Pharmacokinetic Study: Six healthy rabbits (Male) of about 2.5 to 3 kg have opted for the pharmacokinetics of modafinil. Before 12 h and after 24 h of the drug administration, food was avoided for rabbits. Water has given for rabbits for the entire study. The drug dose of 4 mg/kg was given to the rabbits and collected 0.6 ml of blood samples from marginal ear-vein of rabbits before dosing (zero-time) and at the time interval of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 8.0, 12.0, 16.0, 24.0, 36.0 and 48.0 h. The resulting solution was subjected to centrifugation at 4500 rpm for 15 min and separated plasma was transferred into labeled polypropylene tubes at -20 °C.

Validation of Analytical Method: Validation of the Method was processed according to the Food and Drug Administration (FDA) guidelines on Bioanalytical Method Validation [21-25].

Selectivity and Specificity: Potential interference between analyte and matrix constituents was studied by the analysis of 6 blank plasma lots from 6 different sources. A double blank and an LLOQ sample were prepared, infused and analyzed from each lot [21]. To estimate potential nosiness between IS and analyte, blank samples spiked with analytes separately (at the upper limit of quantification) and IS were infused and estimated. Peak areas of components co-eluting with analytes should be < 20% of the LLOQ sample peak area. Peak areas of co-eluents of IS should be < 5% of the average IS peak area. The measured concentrations of the LLOQ standard samples should be < 20% from the nominal concentrations.

Calibration Curve: For the determination of linearity, calibration graphs of 8non-zero standards were used. Eight non-zero points of 2.0, 4.0, 20.0, 40.0, 80.0, 160.0, 300.0 and 600.0 ng/ml were analysed.

The data from 3 accuracy and precision batches were considered to analyze the goodness of fit using $1/x$ and $1/x^2$ weighing factor. Deviation from nominal concentration should be within ±20% for LLOQ and within ±15% for remaining concentrations. The linear coefficient of correlation (r^2) should be ≥ 0.98.

Precision and Accuracy: These parameters were determined by analyzing 5 similar QC- samples of modafinil at the concentration level of LLOQ, LQC, MQC and HQC standards in 3 analytical runs. Inter-assay accuracy was analyzed as the relative difference between the average measured concentration after 3 runs and the nominal concentration [21-23]. Accuracy should be ±20% for the LLOQ and ±15% for the remaining concentrations. Inter and Intra-assay precisions were represented by the coefficient of variation (%CV), which should be <20% for the LLOQ and <15% for the remaining standards.

Matrix Factor: To determine matrix effect plasma was processed at the concentrations of HQC and LQC levels after extracting the six different blank matrix batches. Simultaneously six duplicates of equivalent neat quality control samples were processed and analyzed. It was determined by the application of formula:

Matrix Factor = Peak area in the presence of matrix components / Average peak area in aqueous samples

Recovery: The recovery of the method was estimated by comparing the average peak response of 6 extracted LQC, MQC and HQC samples (4.0, 240.0 and 480.0 ng/ml) to the average peak area of 6 spiked samples with the same quantities of high, medium and low-quality control samples.

Auto-Sampler Stability: This parameter was estimated by processing 6 sets QC-standards (LQC and HQC) and kept in an autosampler. These standard samples were injected after 24 h and were assessed against freshly spiked calibration standards. The resulting values, when compared with nominal concentrations, should be within ±15%.

Dilution Integrity: The drug concentration of above the upper limit of quantification (ULOQ) was processed and precision and accuracy data were determined. The percentage of nominal concentration should be ± 15%.

Stability: LQC and HQC stored samples (n=6) were collected from the freezer after 3 freeze and thaw cycles. Samples were stored at -30 °C in 3

cycles of 24.0, 48.0, 72.0 h. For the long term stability of the drug in QC samples were also assessed by analysis after 4 months of storage at -25 °C &-70 °C. Benchtop stability was evaluated for 7 h period with standard concentrations [23-25]. Stability solutions were processed and extracted along with freshly spiked calibration standards. The accuracy and precision of the stability solutions should be ±15% of their nominal concentrations [24].

RESULTS AND DISCUSSION:

Method Development and Validation: A procedure for sample extraction was employed for the extraction of drugs and IS from the plasma samples. Chromatographic parameters were improved through different trials to get better resolution and to increase S/N (signal to noise) ratio of analyte and IS. The Mass parameters were monitored by injecting the solution directly into the electrospray ionization source of the mass system.

Acetonitrile portion was increased to get a specific and accurate method after the MRM transitions were fixed. A better resolution and separation were obtained utilizing an isocratic mobile phase of acetonitrile, methanol and 0.1% formic acid (25:60:15 v/v) at 0.7 ml/min flow rate.

Selectivity: Six different lots of blank human plasma samples have opted and interference of interfering constituents at retaining time of analyte and internal standard were evaluated. Interference of matrix constituents was not detected at the retention time and m/z of modafinil and IS in all the lots selected and **Fig. 2** and **3**, clarifies the chromatograms for blank, blank with IS and LLOQ injections.

Linearity: Linearity graph was constructed over the concentration level of 2.0-600.0 ng/ml in plasma with a correlation coefficient [r^2] of 0.998. Three calibration graphs were linear in working concentration range with 8-point calibration utilized for the quantitation by linear-regression.

The linear curve regression equation was found to be y =0.9976x + 1.6425. The precision (%CV) detected for the linear curve was found to be ≤ 4.93 for modafinil, and the results were shown in **Table 1**.

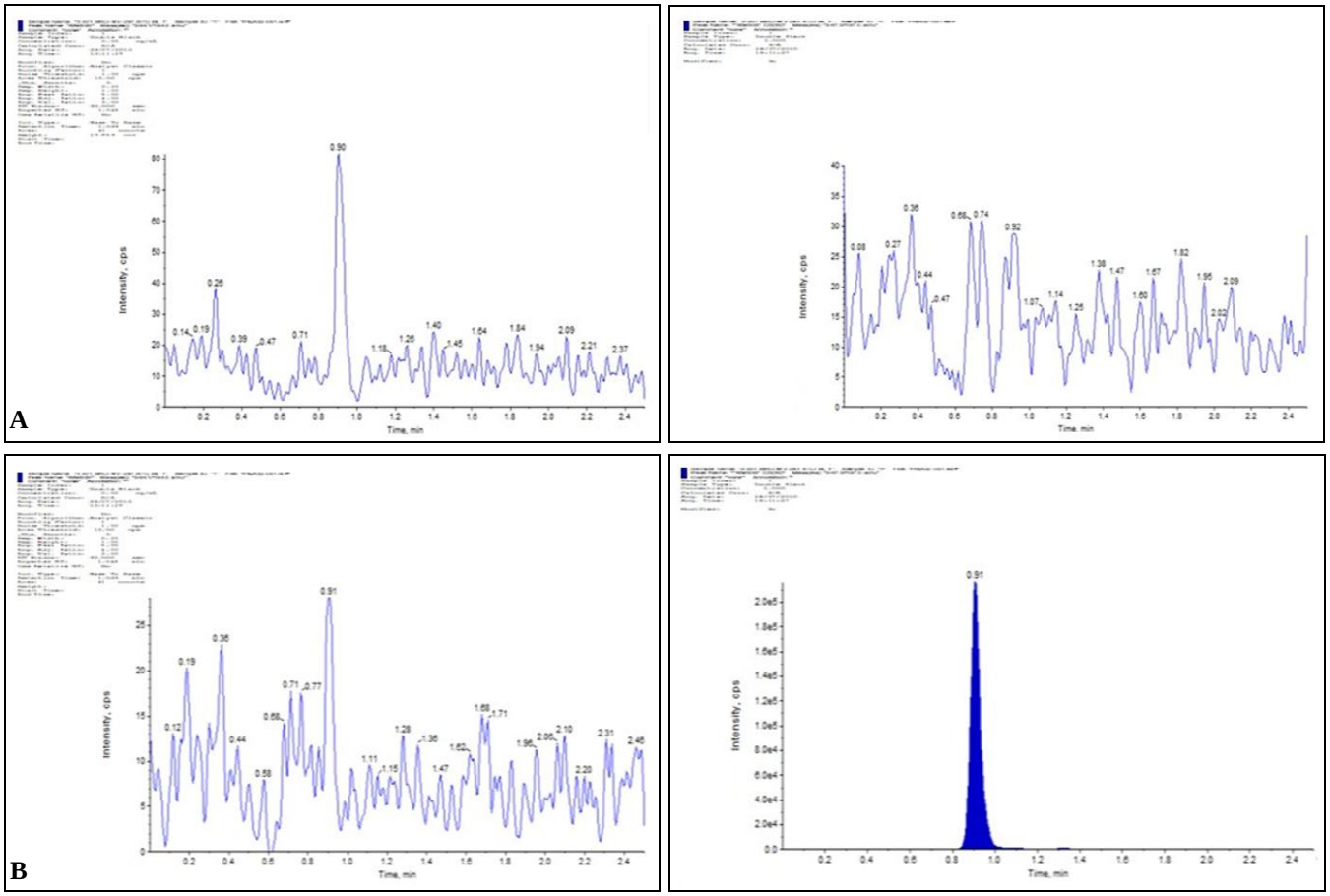

FIG. 2: TYPICAL CHROMATOGRAMS A) BLANK PLASMA, B) BLANK PLASMA SPIKED WITH IS

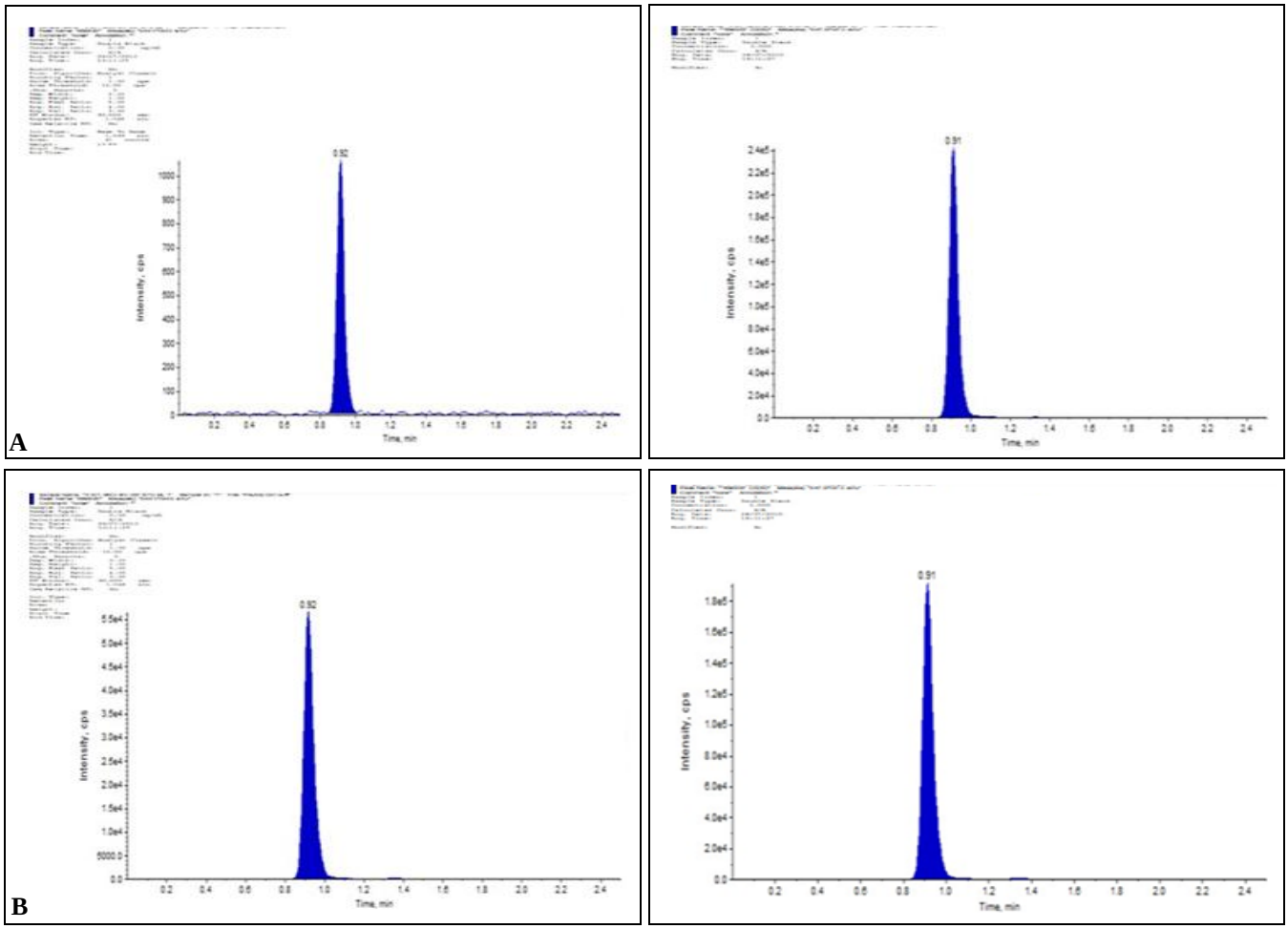

FIG. 3: MODAFINIL CHROMATOGRAMS A) LLOQ, B) RABBIT PLASMA SAMPLE

Linearity: Linearity graph was constructed over the concentration level of 2.0-600.0 ng/ml in plasma with a correlation coefficient [r^2] of 0.998. Three calibration graphs were linear in working concentration range with 8-point calibration utilized for the quantitation by linear-regression. The linear curve regression equation was found to be y =0.9976x+1.6425. The precision (%CV) detected for the linear curve was found to be ≤ 4.93 for modafinil, and the results were shown in **Table 1**.

TABLE 1: SPIKED PLASMA CONCENTRATION AND RSD (%) FOR MODAFINIL

Plasma Concentration (ng/ml)	Concentration estimated mean (ng/ml) ± SD (n)	% RSD	Accuracy in %
2.0	1.92 ± 0.0.78	3.71	96.52
4.0	3.88 ± 0.201	2.77	97.26
20.0	20.7 ± 0.284	4.80	103.45
40.0	40.884 ± 0.213	2.37	102.21
80.0	86.9 ± 6.234	2.23	108.71
160.0	162.1 ± 2.90	1.53	101.31
300.0	309.52 ± 9.208	0.74	103.21
600.0	616.75 ± 15.562	1.52	102.81

n=6 replicates; SD = Standard deviation; RSD=Relative standard deviation

Precision and Accuracy: Method precision and accuracy were assessed by determining intraday and interday batch deviancies of 3 quality control standard samples in six replicate: 4.0, 240.0 and 480.0 ng/ml as represented in **Table 2**. Intraday precision and accuracy were within 1.54-7.18% and 98.56-102.80% respectively. Interday precision and accuracy were within 1.82-6.25% and 97.62-102.76% respectively. These results direct that the technique was reliable, specific, accurate and reproducible.

TABLE 2: MODAFINIL INTRA-DAY AND INTER-DAY ACCURACY AND PRECISION

Nominal (ng/ml)	Intra-day[a]			Inter-day[a]		
	Mean detected (ng/ml)	% Precision	% Mean accuracy	Mean detected (ng/ml)	% Precision	% Mean accuracy
4.0	3.91	1.54	99.50	3.88	7.14	97.62
240.0	235.86	7.18	98.56	235.96	6.32	98.76
480.0	483.02	2.65	102.80	482.19	1.25	102.76

a = 6 replicates

TABLE 3: MODAFINIL RESULTS FOR MATRIX EFFECT

Modafinil		
QC sample	LQC	HQC
Original concentration (ng/ml)	4	480
1	3.89	479.1
2	3.95	478.21
3	3.91	478.23
4	3.94	481.02
5	3.81	478.9
6	3.85	479.89
Mean	3.89	479.225
± SD	0.049	0.98
% CV	1.46	0.28
% Accuracy	97.78	98.06

SD = Standard deviation; CV=Coefficient of variation

Matrix Factor: The matrix factor was assessed in % coefficient of variance and the values for HQC and LQC standards were 0.28% and 1.46% respectively. The measured values were within the acceptable limit. The findings were represented in **Table 3**.

Dilution Integrity: Modafinil was diluted up to 20 fold by blank plasma and were analyzed with spiked samples above the upper limit of the calibration standard and samples with the highest concentration. The % nominal was within ±15 and the observed precision was within ≤15%. This demonstrates that the sample can be diluted up to 20 times, and yet the results are predictable and reproducible.

Stability Studies: The stability data of modafinil, which includes auto-sampler, freeze-thaw, long-term and bench-top, were within the acceptance limit. Results were revealed in **Table 4**.

TABLE 4: STABILITY DATA FOR MODAFINIL

Conc. (ng/ml)	Freeze-thaw stability		Auto-sampler stability		Bench-top stability		Long-term stability	
	Mean ± SD (ng/ml)	%CV	Mean ± SD (ng/ml)	% CV	Mean ± SD (ng/ml)	% CV	Mean ± SD (ng/ml)	% CV
4.0	4.0 ± 0.19	7.6	4.0 ± 0.14	4.8	4.0 ± 0.21	4.85	4.0 ± 0.14	7.1
480.0	480.0 ± 6.12	8.1	480.0 ± 5.86	7.25	480.0 ± 6.41	4.94	480.0 ± 6.32	4.9

SD = Standard deviation; CV= Coefficient of variation

Recovery: The percentage recovery was assessed by estimating the absolute peak response of drugs and IS from a human plasma sample processed according to the method. The extent of retrieval of drug analyte and of the internal reference standard should be consistent, precise and reproducible. The mean overall recovery of analyte and IS was found to be 97.26% and 98.01% respectively.

Pharmacokinetics: The Pharmacokinetic parameter of modafinil was calculated from the graph obtained by taking plasma concentration on Y-axis and time on X-axis using Pk-solver software. In this study trapezoidal rule was considered for the calculation of area under the curve from 0 to 48 hours (AUC_{0-48}). Modafinil was shown mean T_{max} of 3.833; mean $C_{max,}$ AUC0_t, and AUC0_α for test formulation is 677.667; 6306 and 6471 respectively. The results were shown in **Table 5, 6** and **Fig. 4**.

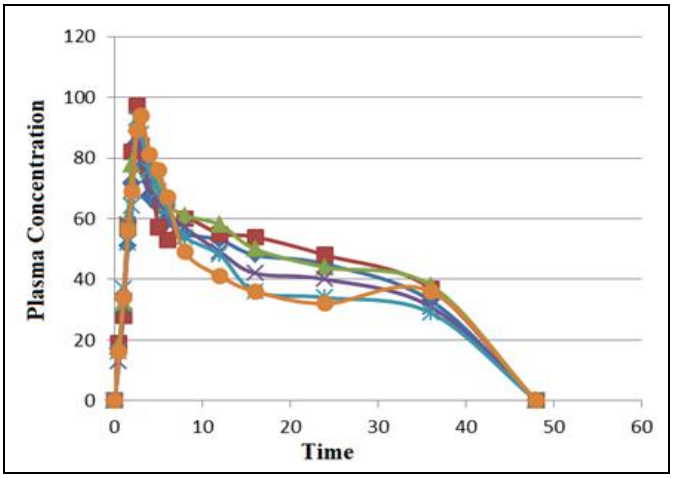

FIG. 4: PLASMA CONCENTRATION-TIME PROFILE CURVES OF 6-RABBITS

TABLE 5: PLASMA CONCENTRATIONS AT DIFFERENT TIME INTERVALS

Time in hrs	Measured concentrations (ng/ml)							
	Animal 1	Animal 2	Animal 3	Animal 4	Animal 5	Animal 6	Mean	SD
0	0	0	0	0	0	0	0	0
0.5	14	18	16	14	14	15	15.16667	1.46
1	33	32	32	31	36	33	32.83333	1.53
1.5	50	55	58	54	50	52	53.16667	2.84
2	78	81	76	75	73	70	75.5	3.5
2.5	84	86	92	90	89	85	87.66667	2.84
3	78	85	87	81	89	92	85.33333	4.75
4	65	75	79	75	74	80	74.66667	4.85
5	66	71	73	68	71	74	70.5	2.75
6	58	58	62	68	62	69	62.83333	4.33
8	60	59	58	51	51	50	54.83333	4.21
12	51	58	54	51	52	60	54.33333	3.49
16	41	51	49	41	34	35	41.83333	6.38
24	50	46	49	43	31	36	42.5	6.89
36	30	29	24	26	25	20	25.66667	3.29
48	0	0	0	0	0	0	0	0

SD- Standard deviation

TABLE 6: TEST ANIMALS (RABBITS) PK PARAMETERS MEAN VALUES

Parameters	Animal 1	Animal 2	Animal 3	Animal 4	Animal 5	Animal 6	Mean	SD
C_{max}	659	695	676	695	645	696	677.667	19.81
log C_{max}	2.8945	2.854	2.854	2.862	2.789	2.378	2.77192	0.18
T_{max}	3	4	4	5	3	4	3.83333	0.68
log T_{max}	0.4771	0.602	0.602	0.699	0.477	0.602	0.57652	0.07
$t_{1/2}$	9.1414	8.113	9.563	6.7059	8.331	6.028	7.98038	1.25
log $t_{1/2}$	0.971	0.909	0.981	0.8265	0.921	0.78	0.89808	0.07
Ke	0.0747	0.085	0.072	0.1033	0.083	0.115	0.08883	0.01
log Ke	-1.12	-1.068	-1.14	-0.986	-1.08	-0.94	-1.0557	0.07
AUC_{0-t}	6488.5	6455	5940	6218	5874	6306	6213.58	235.4
log AUC_{0-t}	3.8121	3.81	3.774	3.7937	3.769	3.8	3.79313	0.016
AUC-0-inf_obc	6791.8	6735	6229	6469.5	6150	6471	6474.38	235.9
log AUC-0-inf_obc	3.832	3.828	3.794	3.8109	3.789	3.811	3.81082	0.015

SD- Standard deviation

CONCLUSION: In this research article an LC-ESI-MS/MS technique for the quantification of modafinil in plasma was efficiently developed and validated. Chromatography was attained on Phenomenex C_{18} (50 mm × 4 mm) 5µ analytical column with acetonitrile, methanol and 0.1% formic acid (25:60:15 v/v) as mobile phase at 0.7 ml/min flow rate. All the validation parameters: selectivity, accuracy, precision, recovery, stability, matrix effect and dilution integrity were within the acceptance limit. This technique was successfully applied to study pharmacokinetic parameters in six male healthy rabbits and the drug was shown mean T_{max} of 3.833; mean $C_{max,}$ AUC0_t and AUC0_α, for the test formulation is 677.667; 6306 and 6471 respectively in the pharmacokinetic study on healthy rabbits.

AUTHORS CONTRIBUTION: All the authors contributed equally to this manuscript.

ACKNOWLEDGEMENT: Nil

CONFLICTS OF INTEREST: The authors claim that they have no conflict of interest.

REFERENCES:

1. Krishnan R and Chary KV: A rare case modafinil dependence. Journal of Pharmacology & Pharma-cotherapeutics 2015; 6(1): 49-50.
2. Stahl SM: Modafinil-prescriber's Guide: Stahl's Essential Psychopharmacology. Cambridge, United Kingdom, Cambridge University Press, Edition 2017: 491-95.
3. Kim W, Tateno A, Arakawa R, Sakayori T, Ikeda Y, Suzuki H and Okubo Y: *In-vivo* activity of modafinil on dopamine transporter measured with positron emission tomography and [^{18} F]FE-PE2I. The International Journal of Neuropsycho-Pharmacology 2014; 17(5): 697-03.
4. Mignot EJ: A practical guide to the therapy of narcolepsy and hypersomnia syndromes. Neurotherapeutics 2012; 9(4): 739-52.
5. Reith ME, Blough BE, Hong WC, Jones KT, Schmitt KC, Baumann MH, Partilla JS, Rothman RB and Katz JL: Behavioral, biological, and chemical perspectives on

atypical agents targeting the dopamine transporter. Drug and Alcohol Dependence 2015; 147: 1-19.
6. Stahl SM: Modafinil Prescriber's Guide: Stahl's Essential Psychopharmacology (6th ed.). Cambridge, United Kingdom: Cambridge University Press 2017; 491-95.
7. Queensberry AJ and Baker LE: Dopaminergic mediation of the discriminative stimulus functions of modafinil in rats. Psychopharmacology 2015; 232(24): 4411-9.
8. Punzi M, Gili T, Petrosini L, Caltagirone C, Spalletta G and Sensi SL: Modafinil-induced changes in functional connectivity in the cortex and cerebellum of healthy elderly subjects. Frontiers in Aging Neuroscience 2017; 9: 1-9.
9. Kaser M, Deakin JB, Michael A, Zapata C, Bansal R, Ryan D, Cormack F, Rowe JB and Sahakian BJ: Modafinil Improves Episodic Memory and Working Memory Cognition in Patients With Remitted Depression: A Double-Blind, Randomized, Placebo-Controlled Study 2017; 2(2): 115-22.
10. Basniwal PK and Jain D: Determination of modafinil in tablet formulation using three new validated spectrophotometric methods. The Malaysian Journal of Analytical Sciences 2014; 18: 329-36.
11. Bijithra C, Ragan G and Shanmugasundaram P: Analytical method development and validation of modafinil in pure and tablet dosage form by UV spectroscopy. Research Journal of Pharmacy and Technology 2016; 9(8): 1303-08.
12. Jonnalagadda T and Katakam S: A simple visible spectrophotometric method for the determination of armodafinil in bulk and pharmaceutical dosage form. Int J Pharm Sci Res 2015; 6(6): 2579-81.
13. Shanmugasundaram P, Bijithra C, Ragan G, Sankar ASK and Sumithra M: Analyitcal method development and validation of a reversed-phase high-performance liquid chromatography for the determination of modafinil in bulk and pharmaceutical dosage forms. Asian J Pharm Clin Re 2016; l9: 177-81.
14. Reddy MNC, Sekhar KC and Babu PV: Method development and validation of related substances in asenapine tablets by reverse phase HPLC. World Journal of Pharmaceutical Research 2015; 5: 1653-63.
15. Mrinalini CD: Development and validation of HPLC method for determination of modafinil in human plasma. International Journal of Pharmacy & Technology 2016; 8: 11932-42.
16. Bhimanadhuni NR, Garikapati DR and Karamsetty S: Development and validation of RP-HPLC method for determination of Modafinil in bulk and dosage form. International Current Pharmaceutical J 2012; 1: 77-80.
17. Venkatesh V: Determination and validation of Modafinil in Pharmaceutical Formulation by Spectrophotometric and RP- HPLC Methods. Journal of Pharmacy Research 2011; 4(2): 509-11.
18. Pandya GP and Joshi HS: Stability indicating HPTLC method for estimation of Modafinil in the bulk and tablet formulation. IOSR Journal of Pharmacy and Biological Sciences 2013; 5: 22-28.
19. Bharatiya R: Liquid chromatography tandem mass spectrometry method for the estimation of modafinil in human plasma. World Journal of Pharmaceutical Sciences 2014; 2: 1191-97.
20. Ramachandra B: A critical review of properties of modafinil and analytical, bioanalytical methods for its determination. Critical Reviews in Analytical Chemistry 2016; 46(6): 482-89.
21. Rao RN: LC–ESI-MS determination and pharmacokinetics of adrafinil in rats. J Chromatogr. B 2008; 873: 119-23.
22. US FDA, Guidance for Industry Bioanalytical Method Validation, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Rockville, Maryland, USA 2001.
23. International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use, Validation of analytical procedures: Methodology, ICH-Q2B, Geneva 1996.
24. Validation of Analytical Procedures: Methodology. ICH Harmonised Tripartite Guidelines 1995.
25. Rezk MR, Basalious EB and Amin ME: Novel and sensitive UPLC- MS/MS method for quantification of sofobuvir in human plasma: application to a bioequivalence study. Biomed Chromatogr 2016; 30: 1354-62.

How to cite this article:
Phanindra A and Kumar YS: Method development and validation of LC-ESI-MS/MS technique for the estimation of modafinil in human plasma; application to pharmacokinetics in healthy rabbits. Int J Pharm Sci & Res 2020; 11(4): 1837-44. doi: 10.13040/IJPSR.0975-8232.11(4).1837-44.