DEVELOPMENT OF A RAPID AND SENSITIVE METHOD FOR ESTIMATION OF ARMODAFINIL IN HUMAN PLASMA BY LCMS/MS

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ABSTRACT: Modafinil (Provigil), a new wake-promoting drug, is currently used for the management of excessive sleepiness. Since there is a chance of potent drug abuse of this compound, stringent drug screening methods will be necessary to avoid this. Armodafinil and dulotexine (IS) were extracted from the plasma after liquid – liquid extraction after adding 0.2 ml of 5% tri potassium phosphate to each RIA vial, vortexed for 30 sec followed by addition of 2.5ml of extraction solvent mixture (Diethyl ether: Ethyl acetate 70:30, v/v).. The drug was separated on a Chromolith Performance RP -18e, 100 mm x 4.6 mm column using the binary pump [pump A - 0.1% formic acid: pump B – Acetonitrile 65:35] at a flow rate of 1.2 ml/min in Shimadzu UFLC attached to API 3000 Mass spectrometer with an ESI interface. The column oven temperature and the run time were 35^0C and 3.5 min, respectively. The analytes were detected in the positive electrospray ionization mode (split ratio 1:4) with multiple reactions monitoring (MRM). The MRM transitions monitored were m/z for parent ion 274.2 & daughter ion 167.1 (armodafinil), m/z for parent ion 298.4 & daughter ion 154.1 (dulotexine). A linear calibration plot of armodafinil and I.S. was achieved in the concentration ranges of 0.0779 to 10.3871 μg/ml (correlation coefficient >0.9971). Recoveries were consistently more than 66.33%. The assay was specific, accurate (101.11%), precise and reproducible (intra- and inter-day precisions R.S.D. 6.95% and 8.115 %, respectively). Sensitivity, simplicity, reproducibility and rapidity are the main advantages of this method.

Key words: Armodafinil, Liquid chromatography mass spectrometry, Human plasma,

Method development & validation

INTRODUCTION

Modafinil (Provigil), a new wake-promoting drug, is currently used for the management of excessive sleepiness. Armodafinil, [2-[(R)-(diphenylmethyl) sulfinyl] acetamide], molecular formula $C_{15}H_{15}NO_2S$ and molecular weight 273.35, is the R-enantiomer of modafinil and has a longer half live than the S - isomer. The chemical structure is shown below figure-1:
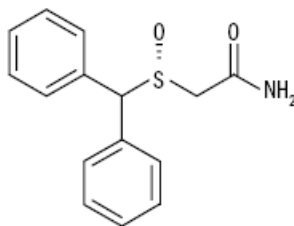
Figure-1: Chemical structure of Armodafinal

Armodafinil is crystalline white powder soluble in methanol. The action of this drug is similar to sympathomimetic agents like amphetamine but with fewer side effects (Hirshkowitz2007, Fiocchi 2009, Russo 2009). Since there is a chance of potent drug abuse of this compound, stringent drug screening methods will probably necessary to avoid this. Although a number of methods are available for assaying the armodafinil, most of them are complicated and not cost effective (Schwertner 2005, Rao 2008, Rao 2009). In this paper, we are reporting a rapid, sensitive and a relatively simpler method for estimation of this drug in human plasma using LCMS/MS.

Experimental

Chemicals and Reagents

Acetonitrile and methanol (HPLC grade) were purchased from Spectrochem whereas ethyl acetate (HPLC grade) was from Merck. Other reagents used were of analytical grade. Millipore (USA) deionized water was used throughout the procedure.

Armodafinil and duloxetine were from Vardha Scientific, Ahmedabad. Stock solution (1mg/ml) of duloxetine was prepared in methanol. Final concentration is made using the potency and actual amount weighed. Serial dilutions were made using methanol: water (1:1, v/v) to obtain a final concentration 1.5 $\Box$g /ml.

Stock solution (2mg/ml) of armodafinil was prepared in methanol. Similar to dulotexine, final concentration was corrected based on its potency and actual amount weighed. Stock solution was serially diluted with methanol: water (1:1, v/v) to obtain a concentration range of 3.84μg /ml to 500$\Box\Box$μg /ml.

Quality control (QC) samples for analyte were prepared in the range of 3.876 – 375.0 $\Box$μg /ml using methanol: water (1:1, v/v) as the diluent.

Construction of Calibration Plot

The blank plasma was spiked with armodafinil to obtain the final concentration 0.077 – 10 μg /ml. Of course, the final concentration was again corrected based on potency and stock weight. 50μl of internal standard (dulotexine) was added to all these armodafinil spiked plasma (20μl). Armodafinil was extracted from the plasma by liquid –liquid extraction after adding 0.2 ml of 5% tri potassium phosphate to each RIA vial, vortexed for 30 sec followed by addition of 2.5ml of extraction solvent mixture (Diethyl ether: Ethyl acetate 70:30, v/v). The RIA vials were then shaken on the vibramax at 2500 rpm for 10 min. The organic layer was separated by centrifuging it at 4500 rpm for 10 min at 4^0C. Top organic layer (2ml) was transferred to a new RIA vial, evaporated to dryness with a stream of nitrogen gas at 50^0C for 15 min. It was then reconstituted with 1ml of Reconstitution solution [Acetonitrile: Formic acid (0.1%) 80:20, v/v]. 5$\Box$l was injected to LCMS/MS for analysis.

Quality control (QC) samples, marked as LLOQC, LQC, MQC and HQC respectively, containing 0.078 $\Box$μg /ml, 0.204 $\Box$μg /ml, 3.0 μg /ml and 7.5 $\Box$μg /ml of the drug were prepared in blank plasma.

Sample Analysis

Human blood containing K_2 EDTA as an anti-coagulant was centrifuged at 3500 rpm for 15 min at 4^0C to separate the plasma. Armodafinil was isolated from the plasma after liquid – liquid extraction followed by evaporation and reconstitution as mentioned above.

Chromatography

The drug was separated on a Chromolith Performance RP -18e, 100 mm x 4.6 mm column (Merck) using the binary pump [pump A - 0.1% formic acid: pump B – Acetonitrile 65:35] at a flow rate of 1.2 ml/min in Shimadzu UFLC Prominence attached to API 3000 Mass spectrometer (Applied Biosystems, USA) with an ESI interface. The column oven temperature was maintained at 35^0C and the run time was 3.5 min. The analytes were detected on mass spectrometer operating in the positive electrospray ionization mode (split ratio 1:4) with multiple reactions monitoring (MRM). The MRM transitions monitored were m/z for parent ion 274.2 & daughter ion 167.1 (armodafinil), m/z for parent ion 298.4 & daughter ion 154.1 (dulotexine). Data were acquired and processed with Analyst software 1.4.1.

## RESULT & DISCUSSION

Specificity

The retention times for armodafinil and the internal standard were 1.98 min and 2.02 min, respectively (Figure.2). No interfering peaks were observed in the blank at the retention times corresponding to drug and I.S. indicating that the procedure is specific to armodafinil. Similarly, no matrix effect was found while analyzing the human plasma samples, calibration standards and QC samples (Table 1).

Table 1. Matrix Effect for estimation of armodafinil

Aqueous LQC Analyte (Area count) (n=6)	Post spiked LQC analyte (Area count) (n=6)	Percentage matrix Effect (n=6)
25211	25878.50	102.65
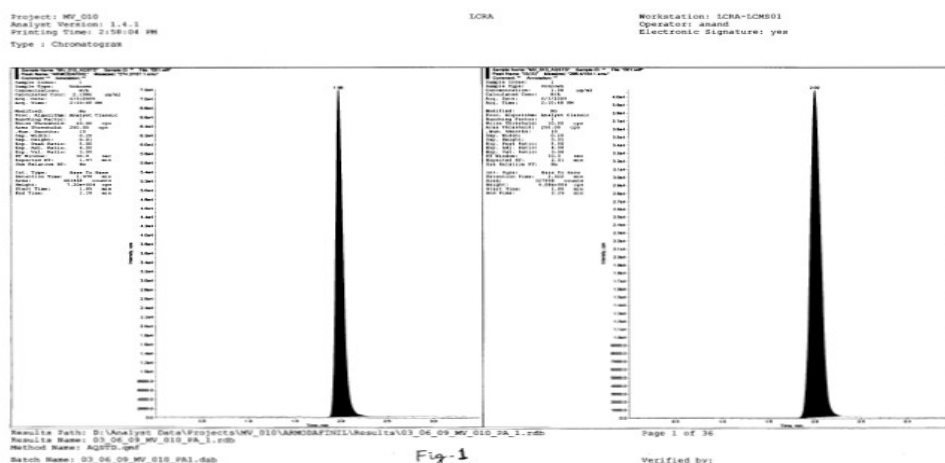
Figure-2: Chromatogram of Armodafinil and internal standard (Dulotexine)

Linearity of the Calibration Plot

A calibration plot of armodafinil and I.S. showed that the calibration is linear in the concentration ranges of 0.0779 to 10.3871 µg/ml and the correlation coefficient was > 0.9971. Inter day and Intra day precision and accuracy were determined by replicate analysis of LLOQC, LQC, MQC and HQC samples and the mean RSD for inter (n=30) and intra (n=12) day assay reproducibility were 8.115 and 6.95 respectively, which are within acceptable limit (Table 2).

Table 2. Results from determination of the accuracy and precision of analysis of armodafinil in the quality-control samples

Parameter	LOQC			LQC			MQC			HQC		
	Actual conc (ng/ml	Estimated Conc. (ng/ml)	%RSD	Actual conc (ng/ml	Estimated Conc. (ng/ml)	%RSD	Actual conc (ng/ml	Estimated Conc. (ng/ml)	%RSD	Actual conc (ng/ml	Estimated Conc. (ng/ml)	%RSD
Intraday	0.0779	0.0815	6.11	0.2030	0.2039	5.62	3.1223	3.1250	10.16	7.8058	8.0493	5.92
Interday	0.779	0.0779	9.33	0.2030	0.2002	5.57	3.1223	3.1719	8.61	7.8058	8.1428	8.94

Recovery

Absolute recovery percentage was determined by comparing the peak area of armodafinil obtained by injecting 6 extracted samples of LQC, MQC and HQC sample with the peak obtained by injection of standard solutions of the same concentration. Recoveries were consistently more than 66.33% (Table 3).

Table 3. Recovery of armodafinil from biological matrix

LQC			MQC			HQC		
Unextracted area (n=6)	Extracted area (n=6)	Mean Percentage Recovery	Unextracted area (n=6)	Extracted area (n=6)	Mean Percentage Recovery	Unextracted area (n=6)	Extracted area (n=6)	Mean Percentage Recovery
26632	17639	66.2313	432929	277018	63.9869	957809	673324	70.29835

Stability

Short – Term/bench - top stability

To check whether the sample is stable during analysis, six aliquots of LQC & HQC samples were thawed and kept at room temperature for 7 hours, which has been decided based on the time required for analysis. The samples were then processed and analyzed as mentioned above. No significant differences were noticed when these results were compared with those obtained from the freshly spiked samples indicating that the analyte was stable at room temperature (Table 4).

Auto sampler stability

The stability of the processed samples in the auto sampler during analysis was determined by using six aliquots of LQC, MQC & HQC samples. The stability of drug and IS were assessed for 24 hours, the expected run time for batches of validation samples. The results were then compared with that of freshly spiked samples. No significant difference in the results indicated that the armodafinil and IS are stable for at least 24 hour in the auto sampler (Table 4).

Table 4. Stability of armodafinil

Stability check Procedure	LQCActual conc. ng/ml)	LQC Avg. conc. Found± SD n=6) (ng/ml)	HQCActual conc.(ng/ml)	HQCAvg. conc. Found± SD n=6) (ng/ml)
Bench Top (7 hrs)	0.2030	0.1972 ± 0.0054	7.8058	8.2776 ± 0.4309
Freeze Thaw (after 3 Cycles at -70^{0}C)	0.2030	0.1877 ± 0.0087	7.8058	7.9741 ± 0.3761
Auto sampler at 10º C	0.2030	0.1967 ± 0.0062	7.8058	8.3746 ± 0.5721

Freeze – Thaw stability

Analyte stability was determined after three freeze – thaw cycles for six aliquots of each of the LQC and HQC. The samples were stored below – 70^{0}C for 24h and then allowed to thaw at room temperature unassisted. After complete thawing, the samples were stored at same temperature for 12h. The freeze – thaw cycle was repeated twice before analyzing the samples. Comparison of the results with the fresh QC samples indicated no differences (Table 4).

Conclusion

A rapid, sensitive and specific LCMS/MS method was developed for the determination of armodafinil in human plasma. The assay was specific, accurate (101.11%), precise and reproducible (intra- and inter-day precisions R.S.D. 6.95% and 8.115 %, respectively) for six replicate low, medium, and high quality control samples. Sensitivity, simplicity, reproducibility and rapidity are the main advantages of this method. The method is suitable for pharmacokinetic studies of armodafinil in human plasma as per the regulatory authorities.

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