ijlpr 2021; doi 10.22376/ijpbs/lpr.2021.11.3.L129-138

International Journal of Life science and Pharma Research

Research Article

Chemistry for New drug discovery

A Novel Analytical Method for the Estimation of Solriamfetol in Plasma Samples by Lc-Ms/Ms

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Abstract: Solriamfetol is a selective dopamine and norepinephrine reuptake inhibitor with wake-promoting effects. The aim of the present study is to develop a rapid, sensitive and reliable method for the estimation of Solriamfetol in plasma samples using LC-MS. In the present investigation, a rapid, specific, selective and novel method has been optimized for evaluation of solriamfetol in in plasma using modafinil as an internal standard and identification of degradants by LC-MS/MS. The solriamfetol and internal standard were extracted from plasma in a single step using acetonitrile. The principle analytes were eluted with the conditions of mobile phase having the 5mM ammonium format in methanol: 50% Methanol in acetonitrile (90:10%, v/v). The Chromatographic column used is Xterra MS C_{18}, 3.5µ.m, 1mmX150mm analytical column with the 0.5 ml/min flow rate. The detector is CEM array detector. The retention times of solriamfetol and modafinil were 1.50min-1.51min with a total run time of 3 min. The curve indicates correlation coefficient (r^2) for modafinil was superior by having the value 1.000 with linear range of 5ng/ml to 500ng/ml. The correlation coefficient (r^2) for solriamfetol was found to be 0.999. The LOQ and LOD for the solriamfetol was 33.70pg/ml and 11.12pg/ml respectively The developed method was validated by evaluating system suitability, selectivity, sensitivity, linearity, precision, accuracy, ruggedness and stability in conformity with the guidelines of the United States Food and Drug Administration (US-FDA). The results of validation parameters were found to be within the acceptance limits. Hence, the developed and validated method can be utilized for the routine determination of solriamfetol in plasma samples.

Keywords: Solriamfetol, Modafinil, Plasma, Validation, US-FDA, LC-MS/MS.

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Received On 3 June, 2021
Revised On 24 August, 2021
Accepted On 25 August, 2021
Published On 15 September, 2021

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

Citation Perina.Ratnakumari, N.Kannappan and P.Premkumar , A Novel Analytical Method for the Estimation of Solriamfetol in Plasma Samples by Lc-Ms/Ms.(2021).Int. J. Life Sci. Pharma Res.11(5), 129-138 http://dx.doi.org/10.22376/ijpbs/lpr.2021.11.3.L129-138

1. INTRODUCTION

Solriamfetol ([*R*] -2 -amino -3- phenylpropylcarbamate hydrochloride) is a selective dopamine and norepinephrine reuptake inhibitor. Solriamfetol is a medication used for the treatment of excessive sleepiness associated with narcolepsy and sleep apnea. At micro molar concentrations, solriamfetol selectively binds to and inhibits reuptake at dopamine and norepinephrine transporters without promoting monoamine release. Solriamfetol is a phenylalanine derivative with the systematic name (R)-2-amino-3-phenylpropylcarbamate hydrochloride. The molecular formula is $C_{10}H_{14}N_2O_2$, and the molecular weight is 230.69.[1-8]

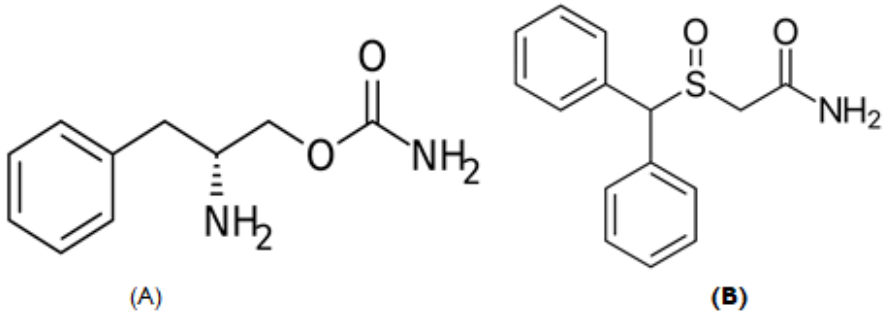


Fig 1: (A). Solriamfetol (B). Modafinil

Analytical method development is to be considered in the discovery, development and manufacturing of active pharmaceutical ingredients and dosage forms. The main objective of an analytical method is to get realistic, consistent, and accurate information. Validation is important and plays major role to choose the standard, reliability, and consistency of analytical results, that is associated with integral part of any sensible analytical practice.[9] Validation of analytical methods is additionally needed by most regulatory authorities and quality standards that impact laboratories. Dosage forms developed with more than one drug, generally said as combination products, are meant to satisfy patients, and would like analytical method development and validation by combining the therapeutic effects of two or more drugs (API) in one product.[10]The official test methods from these processes are carried out by quality control laboratories to make sure the identity, potency, purity, and performance of active pharmaceutical products. Identification of impurities and their quantification is a major task in pharmaceutical method development for quality and efficacy.[11]Various International regulatory Agencies have set the quality and fixed the protocol to match the reference for granting approval, authentication and registration. It is essential to use well-characterized and absolutely valid analytical strategies to yield reliable results in the laboratories whereas analysing the registration batch and accelerated stability testing samples. It is additionally necessary to emphasise that every analytical technique has its own characteristics, which will vary from analyte to analyte. In these instances, specific validation criteria might have to be developed for every analyte.[12]LC-MS/MS technique is considered to be more suitable for analysis of drugs since it has advantages over the other techniques like minimal sample manipulation, rapid analysis and simultaneous quantification of multicomponent samples with excellent specificity, accuracy and precision. There are only two reports on the determination of solriamfetol in plasma and bulk samples using HPLC and LC-MS/MS.[12] One LC-MS/MS method has been presented for the assay of solriamfetol in human plasma.[13] As per the knowledge of the authors, till now no LC-MS/MS method was reported for the determination of the solriamfetol in human plasma with sensitivity range 5 to 500ng/ml using modafinil as internal standard. Modafinil is an analeptic drug sold under the brand names Provigil® or Modvigil® and approved by the US FDA for the treatment of narcolepsy, shift work sleep disorder, and excessive daytime sleepiness. It is a novel internal standard suitable for numerous GC/MS and LC/MS applications from clinical toxicology testing and forensic analysis to urine drug testing and isotope dilution methods.[14] Thus the aim of present study is to develop a rapid, sensitive and reliable method for the estimation of Solriamfetol in plasma samples using LC-MS.. The developed method has been validated as per the guidelines of FDA.[15]

2. MATERIALS AND METHODS

Solriamfetol and Modafinil were received as gift samples from Ray Analytical Labs., methanol, acetonitrile, ammonium format, Water of HPLC grade were procured from Merck Pvt. Ltd, Worli, Mumbai.

2.1 Preparation of Buffer (5 mM ammonium format in methanol)

Precisely weighed 315g of ammonium format and taken into 1000 ml beaker. 500 ml methanol was added and stirred with the glass rod up to complete dissolving the salts then make up to 1000 ml with methanol[12].

2.2 Preparation of mobile phase

About 900 ml of buffer was transferred to 1000 ml reagent bottle. 100 ml of 50% Methanol in acetonitrile was added to the buffer and degassed to prepare 1000 ml of mobile phase[12].

2.3 Spiked plasma calibration curve standards and quality control samples

Solriamfetol and Modafinil standard stock solutions were prepared by taking 10mg in a 10 ml volumetric flask. To the above solution 5 ml acetonitrile was added and sonicated for 3 minutes. Then make up to 10 ml with acetonitrile. All standard solutions were spiked with 50 ng/ml of modafinil to perform the validation parameters. Solriamfetol (5ng/ml to 500 ng/ml) and modafinil(50 ng/ml) spiking solutions were prepared from standard stock solutions of drugs[16]. Calibration curve standards were prepared by spiking

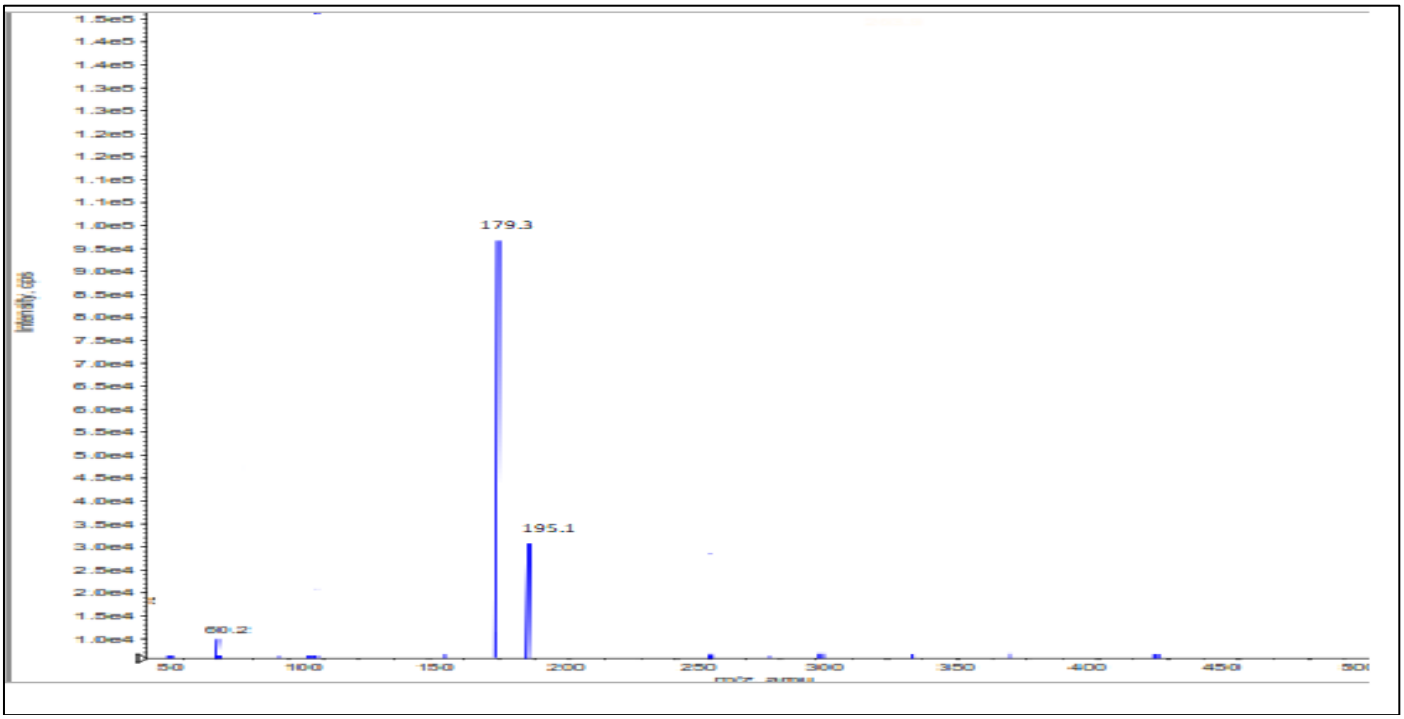
appropriate volume of working standard solution in drug free plasma (0.5ml) to obtain 5.0, 10.0, 20.0, 40.0, 80.0 and 100, 200, 400 and 500ng/ml of solriamfetol and 50 ng/ml of modafinil. Four quality control samples with concentrations 5ng/ml (LLOQ), 15ng/ml (LQC), 250 ng/ml (MQC) and 450 ng/ml (HQC) were prepared by spiking drug free plasma with appropriate volume of solriamfetol working solution. In all the quality control samples, modafinil is spiked at a concentration of 50 ng/ml. All samples were stored at -20 °C.

2.4 Processing of Plasma sample

Protein precipitation extraction technique was applied to extract solriamfetol and modafinil from plasma samples. The frozen calibration curve standards and quality control samples were thawed at room temperature and then homogenized using a vortex mixer. To 200 µl of spiked plasma samples, 2 ml of acetonitrile was added. The sample was mixed for 15 seconds in a vortex mixer. The mixture was vortexed for 2 min. Finally, the mixture was centrifuged at 3500 rpmfor 10 min. The organic layer obtained after centrifugation was collected and transferred into autosampler vials for injection (10 µl) into the LC-MS/MS system[15, 16].

2.5 Optimization of Chromatographic conditions and mass spectrometry

After series of trials, the chromatographic conditions were accomplished with the 5mM ammonium format in methanol: 50% Methanol in acetonitrile (90:10%, v/v) by utilizing the stationary phase Xterra MS C_{18}, 3.5 µ.m, 1mm X 150mm to obtain the best peak shape with the column temperature 40°C and sample compartment temperature 10° C with the flow rate of 0.5 ml/min with the sample volume 10µl. Solriamfetol and Modafinil (Internal standard) analysis was performed using MRM positive ion mode with mass transitions of m/z (amu) 195.1→179.3 and 254.1→177.2. The mass spectra of parent and product ions were depicted in Fig.2.



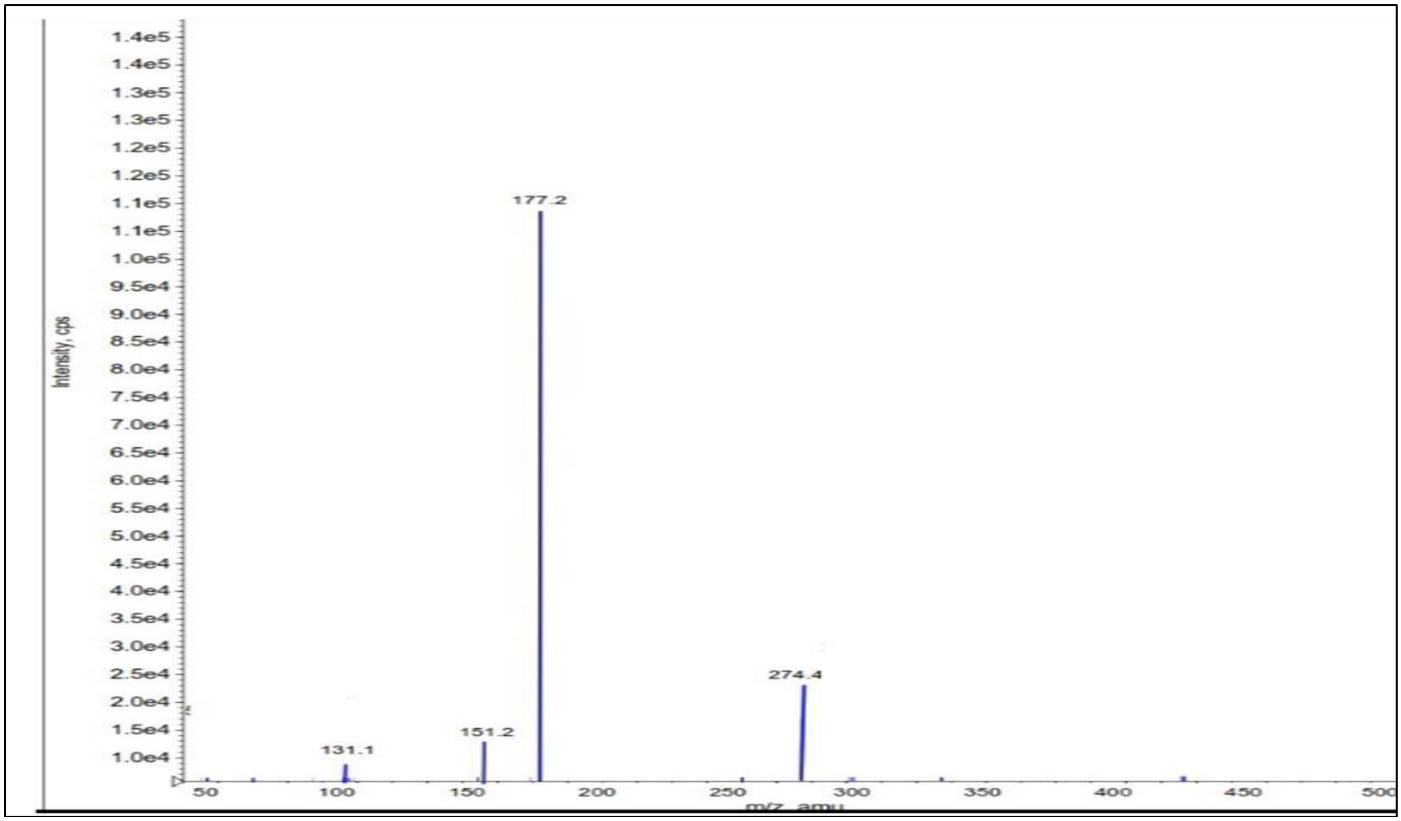


Fig 2: Mass chromatograms of (A). Solriamfetol and (B).Modafinil

2.6 Validation of analytical method

Validation was performed as per FDA guidelines for the developed method with the stringent limit to prove the efficiency of this method [10].

2.7 System suitability

To assess system suitability, middle quality control (MQC) samples along with internal standards were injected into the HPLC with six replicates. The percent relative standard deviation (%RSD) for the peak area response and retention time of solriamfetol and internal standard was calculated. The percent relative deviation value for peak area ratio was also determined[15].

2.8 Specificity

Specificity was performed to demonstrate the absence of chromatographic interference from blank plasma components. Specificity was assessed using six blank plasma and LLOQ level (5 ng/ml) samples. The samples were checked for any interference of blank and sample response. The peak area of any interference peak should be ≤20% of the solriamfetol peak area and ≤5% of the internal standard peak area.

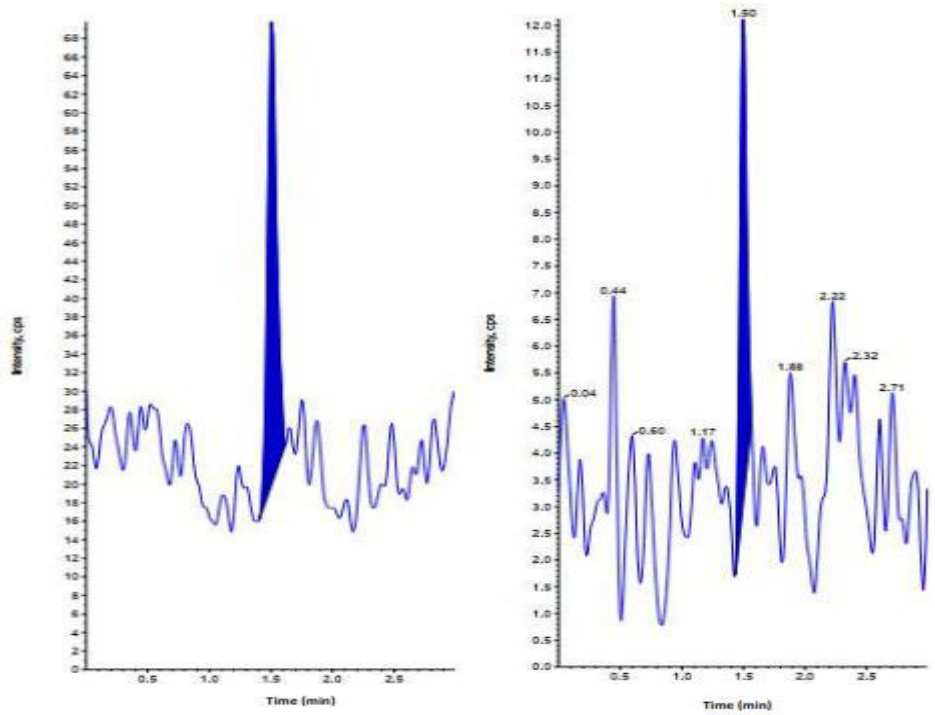


Fig 3: Blank chromatograms of (A). Solriamfetol and (B).Modafinil

2.9 *Sensitivity*

To detect the lowest limit of detection of the method, sensitivity test was performed at LLOQ concentration (5ng/ml) level. For this purpose, LLOQ samples were injected into the HPLC system six times and percent relative standard deviation was calculated.

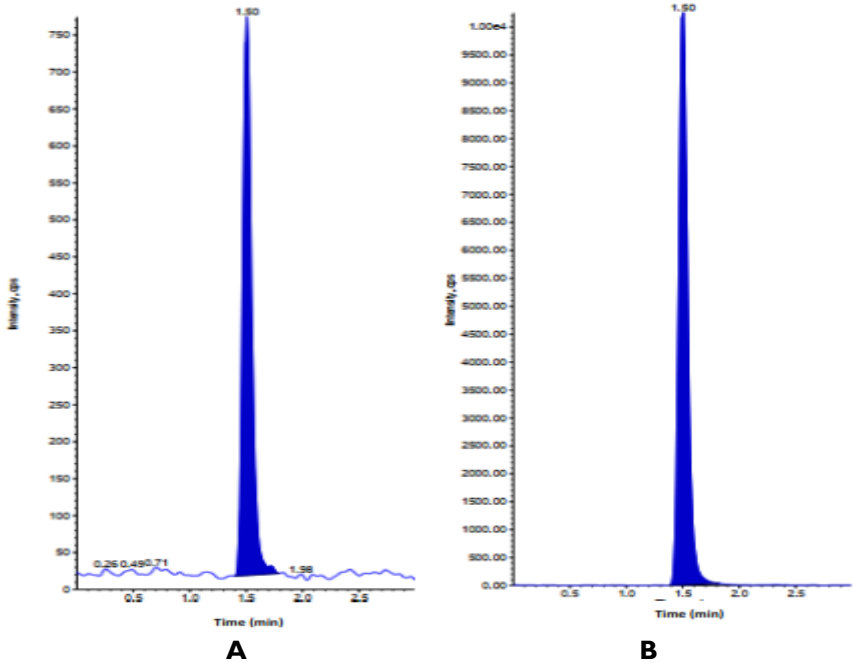


Fig 4: Chromatograms (LLOQ level) of (A). Solriamfetol and (B).Modafinil

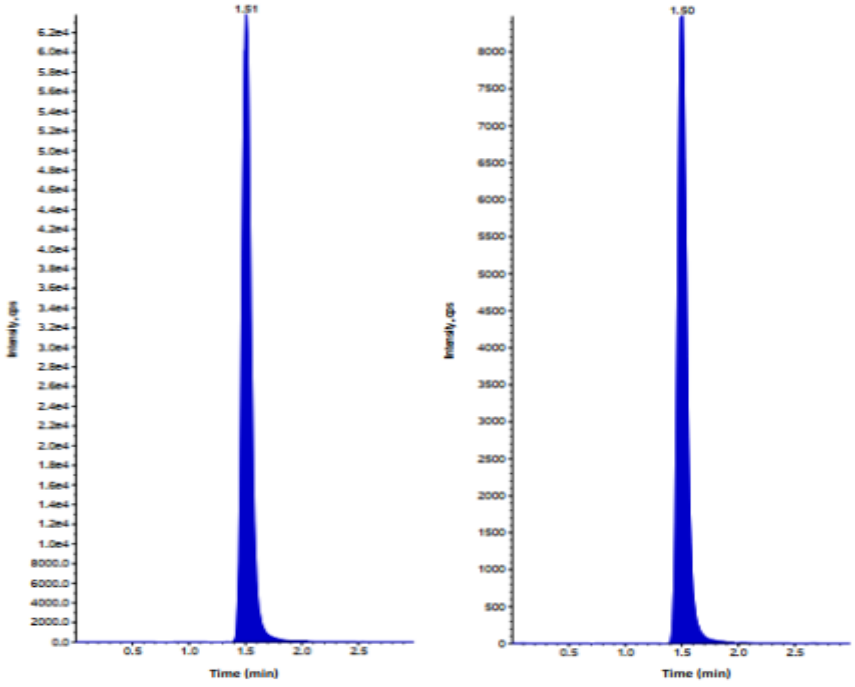


Fig 5: Chromatograms (ULOQ level) of (A). Solriamfetol and (B).Modafinil

2.10 Calibration graph

Ten µl of calibration curve standard solutions (5-500 ng/ml) were injected thrice onto the column. The corresponding chromatograms were recorded using the chromatography conditions described. Concentration of the unknown was computed from the calibration curve or regression equation derived using the peak area ratio and concentration data.

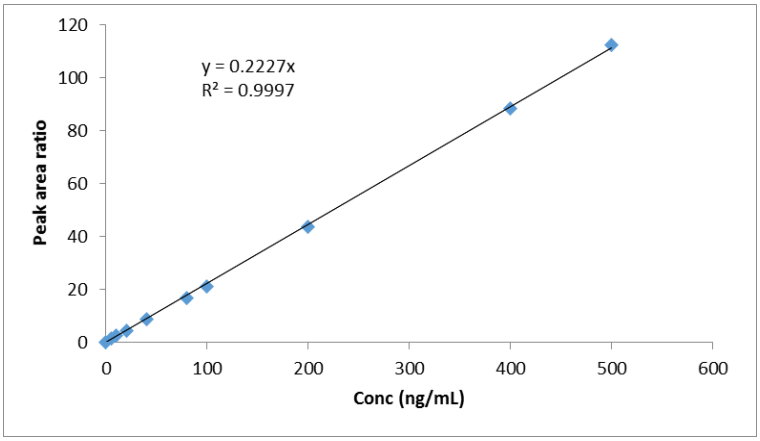


Fig.6: Linearity data

2.11 Precision and Accuracy

Accuracy and precision were established by analyzing quality control samples at four concentration levels (LLOQ, LQC, MQC and HQC) in six replicates on six validation runs. The percent recovery was used to assess accuracy and relative standard deviation was used to assess precision. The acceptable criterion of the inter-day and intra-day precision was ≤15 % for LQC, MQC and HQC, and ≤20% for LLOQ samples. The acceptable criterion for method accuracy was within ±15 % for LQC, MQC and HQC, and ± 20% for LLOQ samples. The inter- and intra-day precision and accuracy results of four quality control samples were depicted in Table 2.

Table-1. Precision

QC levels*	LLOQ (5ng/ml)	LQC (15ng/ml)	MQC (250ng/ml)	HQC (450 ng/ml)
Intra-day precision and accuracy				
Mean calculated (ng/ml)**	5.45	15.12	250.94	449.89
RSD (%)	0.06	0.20	0.41	0.16
Accuracy (%)	1.13	1.34	0.16	0.04
Inter-day precision and accuracy				

Mean (ng/ml)**	calculated	5.08	14.97	249.04	449.07
RSD (%)		0.04	0.25	0.79	0.70
Accuracy (%)		0.80	1.66	0.32	0.16

** QC – Quality control samples*
*** Mean of six determinations*

2.12 Robustness

To verify the method efficiency when the minor changes in the optimized method parameters like mobile phase flow and column temperature parameters were performed with the criteria, it should pass the system suitability criteria.

Table.3.0: Robustness data

Parameter	Flow Rate (+5%)	Flow Rate (-5%)	Column Temp High (+2°C)	Column Temp Low (-2°C)
Mean**	251.41	248.61	249.81	252.01
SD	0.95	2.21	0.63	0.95
% RSD	0.38	0.89	0.25	0.38
Mean %RSD		0.63	0.31	

***** Mean of six determinations***

2.13 Limit of Detection (LOD) and Limit of quantification (LOQ)

LOD and LOQ were determined using the signal-to-noise ratio (s/n) by comparing test results from samples with known concentrations of analytes with blank samples.

2.14 Analyte recovery

The analytic recovery of solriamfetol and modafinil was performed by spiking three concentration levels (LQC, MQC and HQC) into blank plasma samples. Analyte recovery was determined by comparing the solriamfetol and modafinil (internal standard) peak area obtained from extracted samples with un-extracted samples[17]. The acceptance criterion was that the relative standard deviation of recovery at each quality control concentration level and for internal standard should be ≤15 %.

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Table 3: Recovery data

Parameter	*Solriamfetol	*Modafinil
LQC		
Mean	5849	150664
SD(±)	31.80	2059.78
% RSD	0.54	1.37
% Recovery	92.91	98.56
MQC		
Mean*	525492	155159
SD(±)	27327.25	2059.78
% RSD	5.20	1.33
% Recovery	98.75	100.73
HQC		
Mean*	891956	152987
SD(±)	110.38	3646.01
% RSD	0.01	2.38
% Recovery	99.99	100.02
Mean %recovery	**97.21**	**99.77**
Mean %CV	**2.30**	**4.82**

****Average of six determinations***

2.15 Assessment of stability of standard stock solutions

The prepared stock standards of solriamfetol and modafinil (Internal standard) preparations were verified up to 48 hours for the stability at ambient and refrigerated conditions.

Table.4.0: Stability data of standard solutions				
Parameter	Solriamfetol		Modafinil	
	*Comparison samples	*ST Stability samples	*Comparison samples	*ST Stability samples
Mean	65152.00	64986.00	410660.00	407856.00
SD (±)	310.56	310.56	2622.90	2622.90
CV (%)	0.48	0.48	0.64	0.64
Mean %Accuracy	99.37		99.15	

** Mean of six determinations*

2.16 Stability of the sample

The stability of the solriamfetol in plasma was evaluated under different study conditions; i.e. standing at room temperature over 24 h and storing at −20 °C for one month (long-term stability)[17]. The results of the stability of solriamfetol in plasma at diverse storage conditions were expressed as percentage recoveries and relative standard deviation. The percent stability of solriamfetol stored -20°C were assessed by comparing stability samples with freshly spiked samples. The stability studies were performed with LQC (15ng/ml) and HQC (450ng/ml) samples. Percent accuracy should be within the range 85 - 115% and percent relative standard deviation should be ≤15%.

Table 5: Summary of stability of solriamfetol in plasma under different storage conditions		
Quality control sample	Concentration of solriamfetol (ng/ml)	
	LQC*	HQC*
Freeze and Thaw stability (-20°C)		
% CV	0.56	0.09
% Accuracy	100.47	99.94
Room temperature stability (24 hours)		
%CV	1.62	0.84
% Accuracy	101.13	99.79
Long term stability (1 month; -20 °C)		
%CV	0.95	0.45
%Accuracy	100.91	102.03

**Average of six determinations*

3. STATISTICAL ANALYSIS

All the values were expressed as Mean or average of six determinations.

4. RESULTS AND DISCUSSION

Analytical methods for the qualitative and quantitative determination of nor-epinephrine reuptake inhibitors (NRI) are chromatographic methods, electroanalytical, capillary electrophoresis (CE), or spectrometric methods[13]. Estimation of these drugs and their main metabolites in bulk form, pharmaceutical formulations, and biological fluids are generally done based on high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (HPLC/MS) and spectrometric methods.[18] Validation of the optimized LCMS method was done according to the current ICH guideline[19]. Linearity, sensitivity (LOD, LOQ), accuracy and precision for the determination - solriamfetol were investigated as the main important parameter in the validation. Method sensitivity was evaluated for the determination of Solriamfetol, by sequentially diluting sample solutions. From the results, figure 4 and 5 shows that there is a clear separation and good resolution and carryover was not observed and the system suitability acceptance criteria also found satisfactory. For the system precision parameter the %RSD obtained is 1.62%. The peak area response in all the six blank plasma samples is zero. The results demonstrated the non-interference from blank plasma components (Fig. 3). Hence the method is considered as selective and specific. The % RSD of solriamfetol at LLOQ level was 1.48%. The value was found to be within the acceptance limit (%RSD - ≤20%). Therefore, the proposal was sensitive. The data in table-2 indicates acceptable accuracy and precision (%RSD was NMT 2.0%) for both intra-day and inter-day samples at all the four concentration levels. The linearity parameter was quantified by peak area ratio vs concentration methodology. Different concentrations from 5 ng/ml to 500 ng/ml standard solutions were prepared and injected into the system. The calculated regression coefficient was 0.999 as shown in Figure 6. Liquid chromatographic separation methods are most widely used for the selective and sensitive analysis. Various detection techniques like UV, FL, and MS coupled with HPLC are used for the quantification of NRIs in biological fluids. In this study, the, chromatography conditions were subjected to minor variations (flow and column temperature) to evaluate the robustness of the method. The results were shown in Table 2. The results proved that the method was stable to produce consistent results with the minor variation of the method parameters. The LOQ and LOD were identified by injecting the lower concentrations with the S/N ratio criteria.[15] The LOD and LOQ for the solriamfetol was 11.12pg/ml and 33.70pg/ml. The recovery results are summarized in Table 3. The values are within the acceptance limits. The method provided good extraction efficiency for solriamfetol and internal standard in plasma samples. [20] As shown in Table 4 and 5, the stability results are within the acceptance limits.

The results indicated that solriamfetol was stable throughout the period of analysis. During method optimization initially organic solvents were used as a mobile phase with the water in different composition. But both compounds were not detected. Then started usage of buffer with organic solvent such as acetonitrile in different ratios and pH with the Xterra MS C_{18}, 3.5 µ.m, 1mm X 150mmcolumn.Finally the method was found optimized with the conditions of mobile phase (5mM ammonium format in methanol: 50% Methanol in acetonitrile (90:10%, v/v), flow rate of 0.5 ml/min, column temperature of 40°C, sample compartment temperature of 10°C, sample volume of 10µl. With this method both analytics (solriamfetol and modafinil) eluted at 1.50 min and 1.51 min with good resolution and symmetry. After the method optimization the method was validated as per FDA guidelines[21]. As per the results obtained in the method validation there was no interference of the blank and carryover problem even at the LLOQ level quantification. Both LOQ and LOD of this method were verified practically in the instrument with S/N ratio criteria[22-24]. The results inferred that method was accurate, precise and sensitive. Hence, this method can be used during product optimization at the time of pharmacokinetic studies.

5. CONCLUSION

The developed LC-MS/MS method is an appropriate technique for the determination of solriamfetol in plasma using modafinil as standard. The method is simple and utilizes acetonitrile to precipitate the proteins as the only sample preparation step prior to analysis. The rapid (runtime 3 min), single step plasma preparation coupled with the HPLC-Mass spectrometry isocratic chromatographic apparatus makes the method more rapid and suitable for analysis of a large number of samples. The developed method was proved to be sensitive, selective, rugged, precise and accurate in harmony with the FDA guidelines. The solriamfetol was stable in the plasma placed at room temperature for 1 day and for 30 days when stored at −20°C. As a result, the developed and validated HPLC coupled with Mass spectrometry method can be used for routine analysis of solriamfetol in plasma.

6. ACKNOWLEDGEMENTS

The authors are grateful to Ray analytical technologies, Hyderabad, India for providing support to carry out analysis work.

7. AUTHORS CONTRIBUTION STATEMENT

P. RatnaKumari did this research work under the guidance of Dr. N. Kannapan. Mr. Prem Kumar helped in drafting of the paper. All the authors read and approved the final version of the manuscript.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

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