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Bio analytical method development and validation of solriamfetol in rat plasma by LCMS/MS

Dr. M Sreelakshmi[1], A Naga Chandra sekhar[2]
[1] Professor, Department of Pharmaceutical Analysis, MAM College of pharmacy, Acharya Nagarjuna University, Andhra Pradesh, India
[2] Department of Pharmaceutical Analysis, MAM College of pharmacy, Acharya Nagarjuna University, Andhra Pradesh, India

Abstract
A simple, rapid, sensitive, and reproducible LC–MS/MS bio analytical method was developed and validated for the quantitative determination of Solriamfetol in rat plasma using D5-Solriamfetol as the internal standard. Plasma samples were prepared by protein precipitation with acetonitrile, followed by chromatographic separation under optimized LC–MS/MS conditions. The method was validated according to US FDA bio analytical method validation guidelines by evaluating selectivity, sensitivity, linearity, precision, accuracy, recovery, matrix effect, and stability. The assay exhibited excellent linearity over the concentration range of 2–40ng/ml with a correlation coefficient (R^2 = 0.9994). The limit of detection (LOD) and lower limit of quantification (LLOQ) were 0.27ng/ml and 2.7ng/ml, respectively. No endogenous interference was observed at the retention times of Solriamfetol and the internal standard. The method demonstrated acceptable precision and accuracy within the prescribed regulatory limits, with an overall mean extraction recovery of 98.99% and negligible matrix effects. Stability studies, including bench-top, auto-sampler, wet extract, and freeze–thaw stability, confirmed the stability of Solriamfetol under all tested conditions. The validated LC–MS/MS method proved to be selective, accurate, precise, and robust, making it suitable for the routine quantitative estimation of Solriamfetol in rat plasma and its application in pharmacokinetic and other bioanalytical studies.

Keywords: Solriamfetol, bioanalytical method validation, rat plasma, pharmacokinetics, bioequivalance

Introduction

Solriamfetol, a selective dopamine and norepinephrine reuptake inhibitor, is widely prescribed for the management of excessive daytime sleepiness associated with narcolepsy and obstructive sleep apnea. Accurate and highly sensitive quantification of Solriamfetol in biological matrices is essential for pharmacokinetic, bioavailability, bioequivalence, and toxicokinetic investigations. The present study aimed to develop and validate a robust, rapid, and highly sensitive LC–MS/MS bioanalytical method for the determination of Solriamfetol in rat plasma in accordance with the United States Food and Drug Administration (US FDA).

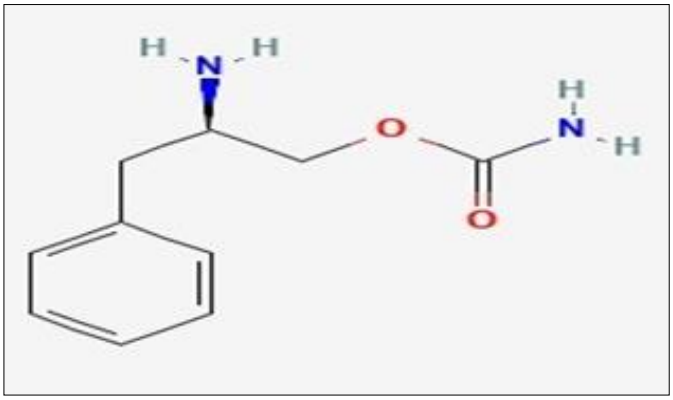

Fig 1: Solriamfetol structure

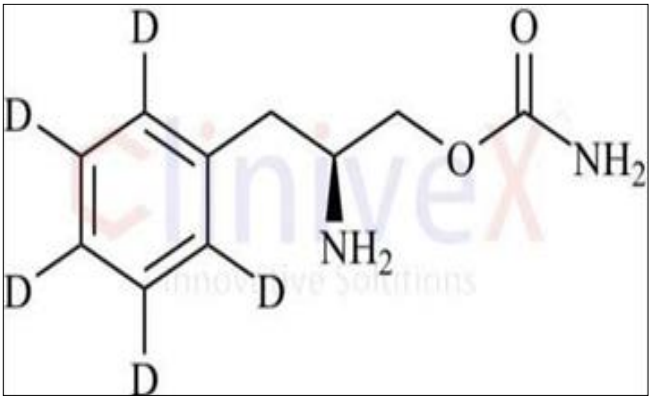

Fig 2: Internal standard D6 Solriamfetol

Materials and Methods

Chemicals and Reagents

Solriamfetol reference standard and D5-Solriamfetol (internal standard) were obtained from Gland Pharma Pvt. Ltd., Hyderabad, India. LC–MS grade acetonitrile and methanol were procured from Rankem and Merck, Hyderabad, while ammonium formate was purchased from Merck. Purified water was generated using an in-house Milli-Q water purification system. All solvents and reagents used were of HPLC or analytical reagent grade.

Instrumentation

Chromatographic analysis was carried out using a Waters Alliance e2695 LC system equipped with a quaternary gradient pump, degasser, and autosampler. Detection was performed on a SCIEX QTRAP 5500 triple quadrupole mass spectrometer fitted with an electrospray ionization (ESI) interface. Data acquisition and processing were performed using Analyst software. Auxiliary instruments included a Eutech pH meter, Sartorius analytical balance,

Unichrome ultrasonicator, Remi centrifuge, Remi cyclomixer, and Borosil volumetric glassware.

Preparation of Mobile Phase

The mobile phase consisted of 10 mM ammonium formate buffer and acetonitrile in the ratio of 30:70 (v/v). The chromatographic conditions were optimized by varying the composition of the mobile phase to obtain satisfactory peak shape, retention time, resolution, and system suitability.

Preparation of Standard Solutions

Stock solutions of Solriamfetol and D5-Solriamfetol were prepared in acetonitrile. Calibration standards were prepared by appropriate dilution of the stock solution to obtain plasma concentrations ranging from 2 to 40ng/ml.

Plasma Sample Preparation

Rat plasma samples (200 µL) were transferred into centrifuge tubes, followed by the addition of 300µL acetonitrile for protein precipitation. Appropriate volumes of Solriamfetol working solution and D5-Solriamfetol internal standard were added, and the mixture was diluted with the specified diluent. Samples were vortex-mixed for 5 min and centrifuged at 5000 rpm for 20 min. The clear supernatant was collected, and an aliquot was injected into the LC–MS/MS system for analysis.

LC–MS/MS Method Development

Method development involved optimization of chromatographic and mass spectrometric parameters, including mobile phase composition, stationary phase selection, flow rate, ionization conditions, and internal standard selection. Different extraction solvents and chromatographic conditions were evaluated to achieve optimum selectivity, sensitivity, peak symmetry,
and reproducibility. D5-Solriamfetol was selected as the internal standard because of its structural similarity and comparable chromatographic behavior.

Method Validation

The developed LC–MS/MS method was validated according to the US FDA bio analytical method validation guidelines. Validation parameters included system suitability, selectivity, sensitivity, matrix effect, linearity, precision, accuracy, recovery, carryover, and stability. Calibration curves were established over the concentration range of 2–40 ng/ml using linear regression analysis.

Stability Studies

The stability of Solriamfetol in rat plasma was evaluated under different storage and processing conditions, including bench-top stability, stock solution stability, auto-sampler stability, wet extract stability, freeze–thaw stability, and long-term storage. Stability was assessed by comparing the measured concentrations of stability samples with freshly prepared quality control samples, and acceptance criteria were based on US FDA recommendations.

Results and Discussion

Method Development and Optimization

The LC–MS/MS method was successfully developed for the quantitative determination of Solriamfetol in rat plasma using D5-Solriamfetol as the internal standard. Chromatographic conditions, including the mobile phase composition, stationary phase, extraction procedure, and mass spectrometric parameters, were systematically optimized to obtain maximum sensitivity, selectivity, and reproducibility. Protein precipitation with acetonitrile provided efficient sample cleanup with high analyte recovery and minimal matrix interference. Under the optimized conditions, Solriamfetol and the internal standard produced well-resolved, symmetrical chromatographic peaks with retention times of approximately 5.02 min.

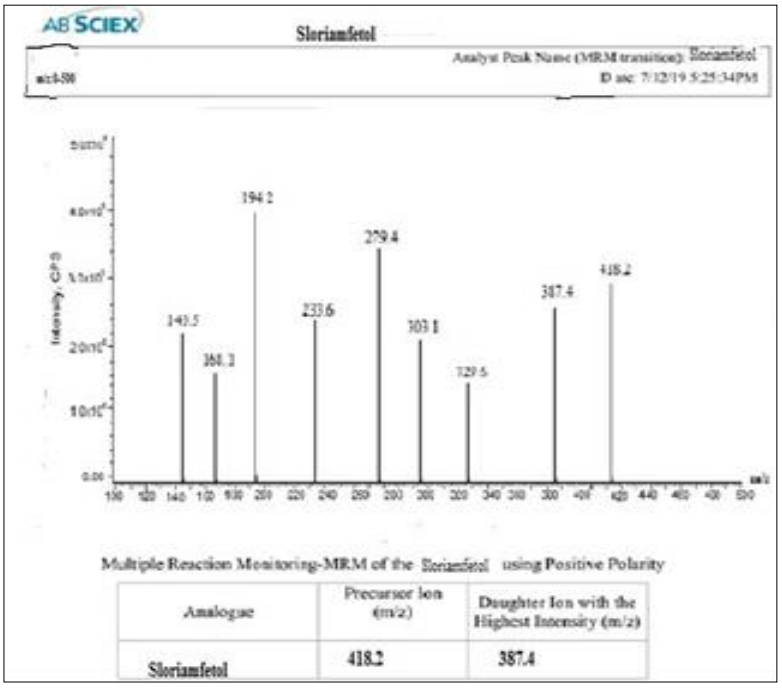

Fig 3: MS Spectra of Solriamfetol

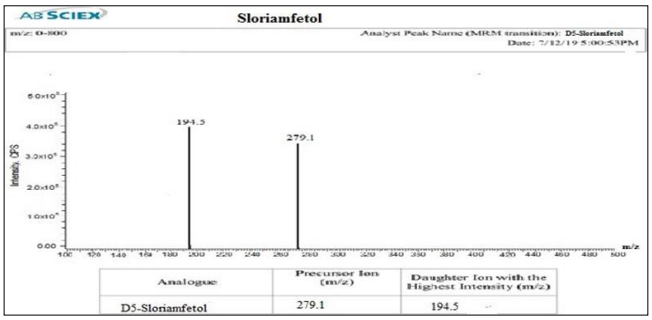

Fig 4: MS Spectra of D5-Solriamfetol

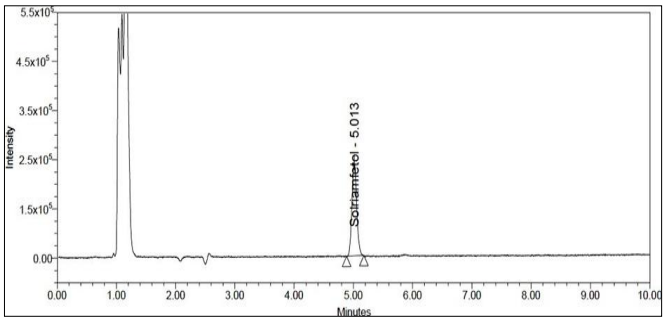

Fig 5: Typical Chromatogram of Sloriamfetol

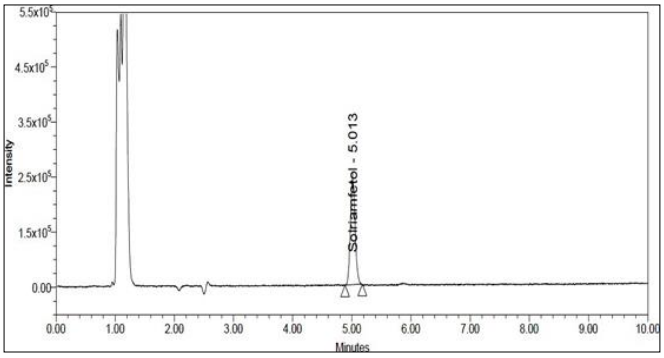

Fig 6: Analyte spiked by blank plasma at LLOQ

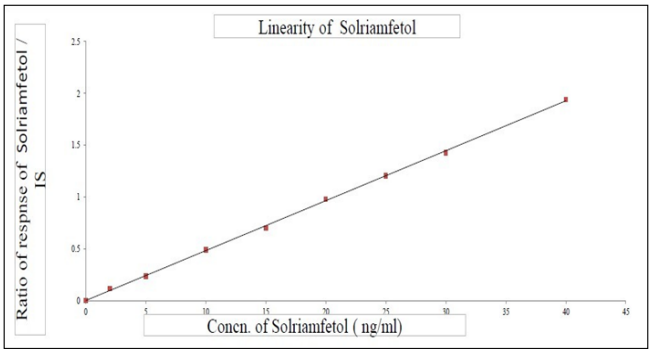

Fig 7: Calibration plot for concentration v/s Area ratio of Solriamfetol

Method Validation

Selectivity and Specificity

The developed method demonstrated excellent selectivity, as no endogenous plasma components interfered with the detection of Solriamfetol or the internal standard at their respective retention times. Blank plasma chromatograms obtained from six different rat plasma lots showed no significant interfering peaks, confirming the specificity of the assay.

System Suitability

System suitability evaluation indicated consistent chromatographic performance. Six replicate injections produced highly reproducible retention times and peak area ratios with %RSD values below the acceptance limits.

The %RSD for analyte retention time and area ratio were 0.61% and 0.46%, respectively, demonstrating satisfactory instrument performance throughout the analytical run.

System suitability: For Solriamfetol and Ist d are a ratio, the %RSD was0.61 and 0.46%, respectively. It thus passed the system suitability test.

Table 1: System suitability Results of Solriamfetol

Name of the sample	Area of Analyte	RT (min) of Analyte	Area of ISTD	RT(min) of ISTD	Area Ratio
MQC	$2.5729x10^5$	5.006	$2.5896x10^5$	5.002	0.9634
MQC	$2.5429x10^5$	5.011	$2.5462x10^5$	5.019	0.9672
MQC	$2.5989x10^5$	5.014	$2.5953x10^5$	5.017	0.9698
MQC	$2.5619x10^5$	5.017	$2.5642x10^5$	5.018	0.9677
MQC	$2.5669x10^5$	5.021	$2.5841x10^5$	5.021	0.9632
MQC	$2.5589x10^5$	5.013	$2.5952x10^5$	5.017	0.9573
Mean	$2.5671x10^5$	5.093	$2.5791x10^5$	5.016	0.9648
SD	0.0186	0.0053	0.0198	0.00686	0.00447
%RSD	0.61	0.10	0.62	0.14	0.46

Sensitivity

The developed method exhibited excellent analytical sensitivity. The lower limit of quantification (LLOQ) showed acceptable precision and accuracy with a %RSD of 0.30% and a mean accuracy of 98.89%, confirming its suitability for the determination of low plasma concentrations of Solriamfetol.

Table 2: Sensitivity Results of Solriamfetol

Replicate Number	LLOQ
	Nominal Concentration (ng/ml)
	1.1559
	Nominal Concentration Range (ng/ml)
	(1.1585-1.1596)

	Calculated Concentration (µg/ml)
1	1.1598
2	1.1507
3	1.1548
4	1.1556
5	1.1596
6	1.1548
n	6
Mean	1.1559
SD	0.00342
%RSD	0.30
%Mean Accuracy	98.89%

Matrix Effect

Matrix effect evaluation using six independent plasma lots demonstrated negligible ion suppression or enhancement. The %CV values obtained for low- and high-quality control samples were 1.49% and 0.36%, respectively, while mean accuracy remained close to 100%, indicating that endogenous plasma constituents did not significantly influence analyte ionization.

Table 3: Matrix effect Results of Solriamfetol

S.No.	Lot No. of Plasma	HQC	LQC
		Concentration in ng/ml (Nominal)	
		30.4733	10.5097
		Concentration Range in ng/ml (Nominal)	
		(30.298-30.645)	(10.206-10.734)
		Calculated Concentration (ng/ml)	
1.	Lot1	30.385	10.206
		30.348	10.315
		30.298	10.325
2.	Lot2	30.425	10.365
		30.598	10.524
		30.541	10.531
3.	Lot3	30.478	10.569
		30.471	10.489
		30.614	10.495
4.	Lot4	30.615	10.472
		30.629	10.641
		30.645	10.625
5.	Lot5	30.433	10.679
		30.461	10.398
		30.345	10.375
6.	Lot6	30.398	10.720
		30.384	10.712
		30.452	10.734
n		18	18
Mean		30.4733	10.5097
SD		0.10918	0.15656
%CV		0.36	1.49
%Mean of Accuracy		100.14%	99.84%
QC Failed		0	0

Linearity

Excellent linearity was achieved over the concentration range of 2–40 ng/mL. Linear regression analysis produced a correlation coefficient (R^2) of 0.9994, confirming a direct proportional relationship between analyte concentration and peak area ratio throughout the calibration range.

Table 4: Solriamfetol working stock solution preparation for standard curve

Linearity	Plasma (µl)	ACN (µl)	Std Stock (µl)	IS (µl)	M Padded (µl)	Solriamfetol Conc.(ng/ml)	Solr response	Area res ratio
Linearity-1	200	300	50	500	1450	2.00	0.364	0.116
Linearity-2	200	300	125	500	1375	5.00	0.742	0.235
Linearity-3	200	300	250	500	1250	10.00	1.533	0.491
Linearity-4	200	300	375	500	1125	15.00	2.186	0.699
Linearity-5	200	300	500	500	1000	20.00	3.064	0.979
Linearity-6	200	300	625	500	875	25.00	3.774	1.204
Linearity-7	200	300	750	500	750	30.00	4.456	1.425
Linearity-8	200	300	1000	500	500	40.00	6.057	1.940
SLOPE						0.0466		
INTERCEPT						0.02055		
r square						0.99942		

Precision and Accuracy

The developed assay exhibited excellent precision and accuracy at all quality control levels. Intraday precision ranged from 0.08% to 0.82%, while accuracy varied between 96.32% and 100.05%. These values complied with the US FDA acceptance criteria, confirming the reproducibility and reliability of the analytical method.

Table 5: Data for Precision and Accuracy of Solriamfetol

Quality control samples	Spiked concentration (ng/ml)	Mean(ng/ml)	SD	Accuracy (%)	RSD (%)
Intraday Precision					
LLOQ	1.1598	1.1585	0.0013	96.32	0.82
LQC	10.209	10.235	0.0244	98.28	0.16
MQC	20.113	20.1763	0.0987	100.05	0.33
HQC	30.385	30.3840	0.0375	99.89	0.08

Recovery

Extraction recovery was consistently high across all quality control concentrations. The overall mean recovery of Solriamfetol was 98.99% with an overall %RSD of 0.43%, demonstrating the efficiency and reproducibility of the protein precipitation extraction procedure.

Table 6: Recovery of analyte for Solriamfetol

Replicate Number	HQC		MQC		LQC	
	Extracted Response	Un Extracted Response	Extracted Response	Un Extracted Response	Extracted Response	Un Extracted Response
1	$3.362x10^5$	$3.969x10^5$	$2.415x10^5$	$2.858x10^5$	$1.215 x10^5$	$1.761x10^5$
2	$3.365x10^5$	$3.945x10^5$	$2.458x10^5$	$2.825x10^5$	$1.214x10^5$	$1.787x10^5$
3	$3.425x10^5$	$3.925x10^5$	$2.369x10^5$	$2.847x10^5$	$1.253x10^5$	$1.723x10^5$
4	3.345_x10^5	$3.945x10^5$	$2.487x10^5$	$2.869x10^5$	$1.263x10^5$	$1.741x10^5$
5	$3.432x10^5$	$3.969x10^5$	$2.341x10^5$	$2.873x10^5$	$1.259x10^5$	$1.721x10^5$
6	$3.397x10^5$	$3.981x10^5$	$2.462x10^5$	$2.883x10^5$	$1.254x10^5$	$1.743x10^5$
n	6	6	6	6	6	6
Mean	$3.3877x10^5$	$3.9223x10^5$	$2.4220x10^5$	$2.8258x10^5$	$1.2097x10^5$	$1.7127x10^5$
SD	0.0359	0.0394	0.0575	0.1108	0.0708	0.1173
%RSD	0.08	0.09	0.19	0.35	0.59	0.71
%Mean Recovery	98.12	99.89%	99.12%	100.18%	96.54%	99.63%
Overall% Mean Recovery	98.99%					
Overall SD	1.1604					
Overall %RSD	0.43					

Stability Studies

The stability of Solriamfetol in rat plasma was successfully demonstrated under all investigated conditions. Bench-top, auto-sampler, wet extract, and freeze–thaw stability studies produced accuracy values within the prescribed acceptance limits, while precision remained below 2% in all cases. These findings confirm that Solriamfetol is stable during routine sample handling, storage, and analysis.

Table 7: Stability data on day zero of Solriamfetol

Replicate No.	HQC	LQC
	Nominal Concentration(ng/ml)	
	30.3380	10.4620
	Nominal Concentration Range (ng/ml)	
	(30.224-30.459)	(10.214-10.691)
	Back Calculated Concentration (ng/ml)	
1	30.342	10.214
2	30.224	10.295
3	30.257	10.456
4	30.365	10.489
5	30.381	10.627
6	30.459	10.691
n	6	6
Mean	30.3380	10.4620
SD	0.0858	0.1843
% RSD	0.19	1.19
%Mean Accuracy	98.42%	97.58%

Limit of Detection and Limit of Quantification

The method demonstrated high analytical sensitivity with a limit of detection (LOD) of 0.27 ng/mL and a lower limit of quantification (LLOQ) of 2.7 ng/mL. These results indicate the capability of the method to detect and accurately quantify Solriamfetol at very low concentrations.

Table 8: LOD and LOQ data for Solriamfetol

Name	LOD		LOQ	
	Concentration (ng/ml)	s/n	Concentration (ng/ml)	s/n
Solriamfetol	0.27	4	2.7	24

Discussion

The developed LC–MS/MS method fulfilled all bioanalytical validation requirements recommended by the US FDA. The method combined simple sample preparation with high chromatographic selectivity and excellent analytical sensitivity. The use of D5-Solriamfetol as the internal standard effectively compensated for analytical variability and contributed to the high precision and accuracy observed throughout validation.

The assay exhibited excellent linearity over the investigated concentration range, negligible matrix effects, high extraction recovery, and satisfactory stability under different storage and processing conditions. The low LOD and LLOQ values further demonstrate the capability of the method for quantifying trace levels of Solriamfetol in biological samples. Overall, the validated method proved to be robust, reproducible, and suitable for routine bioanalytical applications, including pharmacokinetic, bioavailability, bioequivalence, and toxicokinetic studies involving Solriamfetol.

Conclusion

A simple, rapid, sensitive, and robust LC–MS/MS bioanalytical method was successfully developed and validated for the quantitative determination of Solriamfetol in rat plasma using D5-Solriamfetol as the internal standard. The developed method demonstrated excellent selectivity, linearity over the concentration range of 2–40 ng/mL (R^2 = 0.9994), high sensitivity with an LOD of 0.27 ng/mL and an LLOQ of 2.7 ng/mL, and satisfactory precision and accuracy in accordance with US FDA bioanalytical method validation guidelines. The protein precipitation extraction procedure provided consistent and reproducible recovery with negligible matrix effects, while comprehensive stability studies confirmed the stability of Solriamfetol under all investigated storage and processing conditions.

The validated method fulfilled all regulatory acceptance criteria for bioanalytical method validation and demonstrated excellent reproducibility, reliability, and analytical performance. Owing to its simple sample preparation, high sensitivity, and robust analytical characteristics, the developed LC–MS/MS method is suitable for the routine quantitative estimation of Solriamfetol in rat plasma. Furthermore, it can be effectively applied in preclinical pharmacokinetic investigations, bioavailability and bioequivalence studies, toxicokinetic evaluations, and other bioanalytical applications requiring accurate and precise quantification of Solriamfetol.

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