Kantipudi and Pavuluri
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RESEARCH

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Bioanalytical method development and validation for the simultaneous estimation of Olanzapine and Samidorphan in rabbit plasma by using HPLC–MS/MS and application to pharmacokinetic study

Rambabu Kantipudi[1*] and Sugandha Kumar Pavuluri[1]

Abstract

Background Samidorphan is an opioid antagonist while Olanzapine is an effective medication for schizophrenia and bipolar disorder. A unique and accurate MS/HPLC approach due to simultaneous measurement of Olanzapine and Samidorphan is, therefore, more urgently required. Simultaneous quantification of Olanzapine and Samidorphan in rabbit plasma using HPLC-MS. Using a buffer composed of 1 mL of formic acid in 1 L of water and a mixture of two components, buffer and acetonitrile in a ratio of 50:50 and a flow rate of 1 mL/min at room temperature, we separated compounds on an Inertsil ODS column (250 × 4.6 mm, 5 m).

Results Analysis was performed within 8 min over a satisfactory linear concentration range of 2–40 ng/mL for Olanzapine ($r^2 = 0.99901$ 0.024) and 2–40 ng/mL for Samidorphan ($r^2 = 0.99927$ 0.012). The matrix effect recoveries of Olanzapine and Samidorphan at various QC concentration levels were 104.5, 100.51% and 110.36, 99.25%, respectively. The precision and recovery study outcomes fall within the acceptable range. An electrospray ionization source was used to analysis of Olanzapine and Samidorphan at m/z 313.40 → 192.54, m/z 371.45 → 220.61 for Olanzapine and Samidorphan, m/z 316.40 → 237.58, m/z 374.41 → 223.61 for D_3 Olanzapine and D_3 Samidorphan that were ion pairs of mass analysis.

Conclusions Liquid–liquid extraction was used to remove Olanzapine (0.17 mg/kg) and its reference standard (D_3-Olanzapine) from rabbit plasma. Both the active compound Samidorphan (0.17 mg/kg) and its reference, D_3-samidorphan, were isolated from rabbit plasma. We conducted stability studies to ensure that the medications would remain stable in accordance with USFDA regulations.

Keywords Development, HPLC–MS/MS, Olanzapine, Samidorphan, Validation, Rabbit plasma

Background

The atypical antipsychotic Olanzapine [1, 2] sold under the brand name Zyprexa is effective in treating schizophrenia [3, 4] and bipolar disorder [5, 6]. Both short-term treatment and long-term upkeep of schizophrenia are possible with this method. It can be injected into the muscle or taken orally [7, 8]. Common side effects

*Correspondence:
Rambabu Kantipudi
kantipudirambabu@gmail.com
[1] Department of Chemistry, R.V.R&J.C. College of Engineering, Chowdavaram, Guntur, AP 522019, India

include: weight gain [9, 10], movement abnormalities [11], dizziness [12, 13], feeling tired all the time, constipation [14], and dry mouth [15]. Among the other potential side effects include dizziness when standing (low blood pressure) [16, 17], allergic reactions [18], neuroleptic malignant syndrome [19, 20], high blood sugar [21], seizures, gynecomastia [22], erectile dysfunction [23], and tardive dyskinesia [24, 25]. It raises the chance of death in the elderly with dementia who take it. Using during the third trimester of pregnancy increases the risk that the baby will have mobility issues. Although its method of action is unknown, it blocks dopamine and serotonin receptors. As olanzapine/samidorphan (trade name Lybalvi), samidorphan (INN, USAN) (developmental code names ALKS-33, RDC-0313) is a treatment for mental disorders like schizophrenia and bipolar. The effects of olanzapine-induced weight gain are mitigated by samidorphan. Oral use of samidorphan is standard. Samidorphan's side effects include drowsiness [26] and stomach upset [27]. The research and development of samidorphan as a stand-alone drug for use in a variety of conditions has been halted.

The recovery rate is high, the run time is reduced, the accuracy is increased, the cost is decreased, the calibration curves are linear, the MRM transitions are optimised, and our method has been validated in accordance with USFDA criteria [28, 29]. Olanzapine (Fig. 1) and Samidorphan (Fig. 2) detection by HPLC–MS/MS simultaneously in rabbit plasma and its application to pharmacokinetic study is a topic that has not been covered in any published studies as of yet, and only few articles were reported on normal method development, validation and simultaneous determination in human plasma by using RP-HPLC [30–32].The pharmacokinetic research of olanzapine and samidorphan made excellent use of the bioanalytical assay. However, there are no current methods available for the determination of Olanzapine and Samidorphan at the present time. The current study aimed to (a) determine the pharmacokinetics of Olanzapine and Samidorphan following intravenous administration of test extracts to rabbits, and (b) create and validate a precise and sensitive MS/HPLC assay for measuring olanzapine and samidorphan in rabbit plasma.

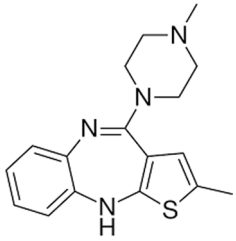

Fig. 1 Chemical structure of Olanzapine

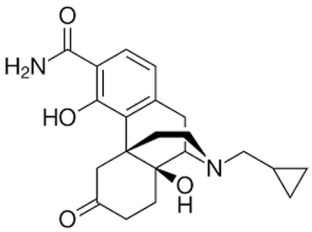

Fig. 2 Chemical structure of Samidorphan

Methods

Chemicals and materials

Olanzapine and Samidorphan ($C_{17}H_{20}N_4S$ and $C_{21}H_{26}N_2O_4$) and D_3-Olanzapine and D_3 –Samidorphan (Internal Standards, ($C_{17}H_{17}D_3N_4S$ and $C_{21}H_{23}D_3N_4S$) with purity levels 99% were obtained from Zydus Cadila, Ahmadabad. Acetonitrile (HPLC–MS Grade, 99.99 purity), Water (Milli Q), and Formic acid (HPLC grade, 99.0% purity) were all supplied by Merck (India) Ltd., Worli and Mumbai, India. All other materials and reagents were of commercial quality AR availability.

Instruments and conditions

The HPLC system (Waters Alliance model) and the mass spectrometer QTRAP 5500 triple quadrupole instrument (SCIEX) were used to construct the bioanalytical assay. Chromatographic separation was performed at room temperature using an isocratic model and an Inertsil ODS (250×4.6 mm×5 m) column. The mobile phase consisted of acetonitrile and formic acid at a ratio of 50:50 (by volume) at a flow rate of 1.0 mL/min. Ten litres of liquid were injected, and the entire cycle lasted eight minutes. For this study, we employed a QTRAP 5500 triple quadrupole mass spectrometer equipped with a positive ion electrospray ionisation interface. Mass ion pair monitoring using MRM mode: m/z 313.40→192.54, m/z 371.45→220.61 for Olanzapine and Samidorphan, m/z 316.40→237.58, m/z 374.41→223.61 for D_3 Olanzapine and D_3 Samidorphan (Internal standards of Olanzapine and Samidorphan). Ion spray voltage 5500 V; source temperature 550 °C; drying gas temperature 120–250 °C; collision gas nitrogen; pressure 55psi; drying gas flow rate 5 mL/min; declustering potential 40 V; entrance potential 45 V; exit potential 15 V; capillary voltage 5500 V; dwell time 1Sec. Table 1 clears necessary information on Instrumentation.

Experimental

Stock preparedness, calibration and quality control specimens

Olanzapine, D_3-Olanzapine (IS), and Samidorphan, D_3-Samidorphan (IS), were dissolved in Formic acid 0.1% in water-ACN (80:20, v/v) to create stock solutions

Table 1 Optimised liquid chromatography and mass spectroscopic conditions

LC parameters		MS parameters	
HPLC	**Waters Alliance**	**MS**	**Sciex QTRAP 5500**
Isocratic step mobile	ACN: Formic acid 0.1% in water 50:50 v/v	Ionisation source	Drying gas: N_2 gas@Drying flow rate: 5 ml/min@Pressure: 55 psi
	Flow level: 1 ml/min		Source temperature: 550 °C
	Injection volume: 10 µl		Capillary voltage: 5500 V
Inertsil ODS	250 mm length	Collision cell gas	Nitrogen with high purity
	4.6 mm ID	Mode	MRM[b]
	5 µm PS		
Analyte	Olanzapine	Olanzapine MRM transitions	m/z-313.40 → m/z-192.54@ CE[a]—14 V
	Samidorphan	Samidorphan MRM transitions	m/z-371.45 → m/z220.61@ CE[a]—15 V
Internal standard	D_3-Olanzapine	D_3-Olanzapine MRM transitions	m/z-316.40 → m/z-237.58@ CE[a]—14 V
	D_3-Samidorphan	D_3-Samidorphan MRM transitions	m/z-374.41 → m/z-223.61@ CE[a]—15 V

[a] *CE* Collision energy

[b] *MRM* Multi reaction monitoring transitions

with concentrations ranging from 2.0 to 40 ng/mL. Calibration and quality control samples were made by mixing the aforementioned working solutions with plasma and then distributing the resulting mixture. Eight calibration specimens were used, with values of 2, 5, 10, 15, 20, 25, 30, and 40 ng/mL. The quality control (QC) samples were made in the same way, but their final concentrations ranged from 2 ng/mL (LLOQ) to 30 ng/mL (HQC). Before being brought back to room temperature for examination, all samples were frozen to a temperature of − 20 °C.

Preparation a solution for plasma samples

In order to prepare the samples, 500 µL of working standard stock solution and 500 µL of internal standard (IS) were injected into 200 µL aliquots of rabbit plasma samples. After 15 min of vortexing 300 µL of acetonitrile and 500 µL of diluent, centrifuging the samples at 5000 rpm for 15 min, then dividing, collecting, filtering, and injecting the supernatant-managed solution into the HPLC machine, we have our final product.

Animal parameters

In order to conduct this research, female rabbits were procured from Bioneeds India pvt. Ltd. in Bangalore. Six rabbits were used in this study (Table 2 represents average body weights of rabbits). Animal ethics committee (Reg. No. 1074/PO/Re.S/05/CPCSEA) at the institute approved the experiment protocol. The circumstances resemble those of a laboratory, and the animals have access to fresh endive, carrots, and maize. Feed for animals should be kept between 21 and 24 degrees Celsius, with humidity between 50 and 55 per cent. All animals fasted for an entire day and drank water at will before being used in an experiment. Olanzapine and Samidorphan solid dispersion tablets' pharmacokinetics were studied. Olanzapine and Samidorphan were given orally to all rabbits at a dose of 0.2 mg/kg. Blood samples were obtained from rabbits at 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, and 35 h, and the concentrations of Olanzapine (1.62 g/mL) and Samidorphan (1.64 ng/mL) ranged from 1.67 ng/mL to 18.4 ng/mL. The plasma was centrifuged for 30 min at 5000 rpm. Until analysis could be performed, plasma samples were kept at 2–8 degrees Celsius, and the supernatant solution was injected into a chromatographic column.

Table 2 Mean average body weights of rabbits

Group name	Average weight of rabbits
Rabbit-1	2538.32 ± 2.65
Rabbit-2	2521.48 ± 3.84
Rabbit-3	2556.47 ± 2.52

Selectivity

The retention times of Olanzapine, Samidorphan, and IS were measured, and interference from untested samples was tested by analysing rabbit plasma samples from six distinct rabbits to assess selectivity.

Matrix effect

By comparing the peak zone fraction in the post-extract plasma sample of six separate plasma samples devoid of medicine and slick recovery samples, we were able to assess the Effect matrix for Olanzapine and Samidorphan. Six different lots of plasma were tested at MQC levels in duplicate, with satisfactory accuracy (% CV 15%).

Recovery

The recovery was calculated by comparing the peak areas of standards that had not been extracted with those of the extracted Olanzapine and Samidorphan (6 replicates per QC concentration).

Dilution integrity

A matrix with an analyte concentration over the ULOQC must be injected, and the test must be diluted using a blank matrix to prove that the dilution was done correctly.

Carry over

The retention of an analyte in the chromatographic system after the injection of a sample is referred to as carry over and can be identified in subsequent blank or unknown samples.

Precision and accuracy

Quality control replication study was performed on a total of six samples to determine the results at four different quality control levels: low, medium, and high. Except for the LLOQ, which should be less than 20%, the CV level should be less than 15%.

Stability

Comparing the area response of the analyte in the stability samples with the region response of the sample obtained from the fresh stock solution allowed us to draw conclusions about the stock solution's stability. The effects of LQC and HQC concentrations on plasma stability were tested using six dose replicates. The US Food and Drug Administration (USFDA) define stability as a coefficient of variation (CV) of less than 15% for an analyte. Injected rabbit plasma samples were tested for 24 h of shelf life (bench top stability) after being kept at room temperature. The autosampler stability of increased rabbit plasma was measured over a period of 24 h at 2–8 °C. Extract plasma samples were injected immediately or stored in the autosampler at 2–8 °C for 24 h to assess the stability of the autosampler. Freeze–thaw stability was evaluated by contrasting newly infused quality control samples with those that had been frozen at − 30 °C and thawed three times. Six aliquots were utilised to test the freeze–thaw stability of both the low- and high-quality control concentrations. To evaluate the long-term stability, the 24-h concentration was compared to the starting concentration.

Results

Bioanalytical method development

With this method, atmospheric pressure chemical ionisation (APCI) mode is avoided in favour of ESI's more powerful reaction. Quantifying the ionisation of Olanzapine and Samidorphan with the MRM mode. Ion pair scan of Olanzapine and Samidorphan formed major ions of $[M+H]^+$ at m/z 313.4, m/z 362.5, m/z 234.55, m/z 192.54 and m/z 371.45, m/z 296.82, m/z 220.61, m/z 164.57. D_3-Olanzapine and D_3-Samidorphan, both internal standards, formed high intensity daughter ions of $[M+H]^+$ at m/z 237.58 and m/z 223.61. When compared to ion-negative mode, Olanzapine and Samidorphan exhibit a positive ion response mode. Figures 3, 4, 5 and 6 show the details of the mass spectrums.

Different buffers were tested with acetonitrile as the mobile phase in isocratic and gradient modes to determine the best conditions for chromatography. With each run, the mobile phase composition was tweaked to achieve higher resolution and shorter retention durations. For the best results with the specified drugs, an isocratic mobile phase of 0.1% formic acid and ACN (50:50 v/v) was chosen. In our optimisation procedure, we made use of a number of stationary phases, such as C18, C8, and CN-propyl. We get reliable peak shapes for Olanzapine and Samidorphan using an Inertsil ODS column with dimensions of 250 mm × 4.6 mm × 5 coupled to a PDA detector. Mobile phase flow rates were at 1 mL/min. Using these parameters, we find that Olanzapine and Samidorphan have retention durations of 2,241 and 5,098 min, respectively. Six replicate injections of Olanzapine and Samidorphan show coefficients of variation (CV) of 0.31 and 0.29 per cent, respectively, demonstrating the high precision of the suggested approach. The current procedure has been proven reliable in accordance with USFDA standards. Figures 7, 8 and 9 show the details of the chromatograms.

Validation of bioanalytical process

Matrix effect

At the Low Quality Control (LQC) and High Quality Control (HQC) levels, Olanzapine and Samidorphan's matrix effect results were 104.5 and 100.51 per cent, respectively (Table 3). The CV% for both compounds was calculated to be 0.77 at the LQC and 0.69 at the HQC. According to

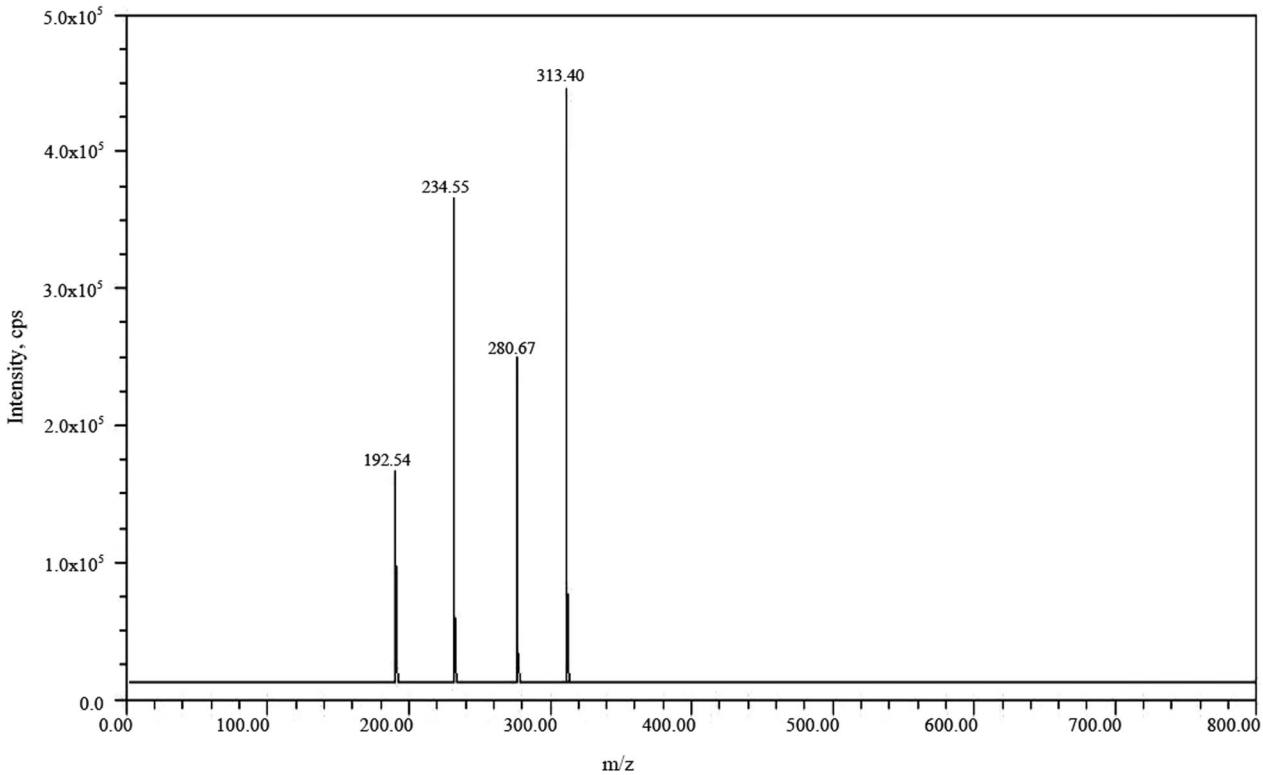

Fig. 3 Mass spectrum of Olanzapine

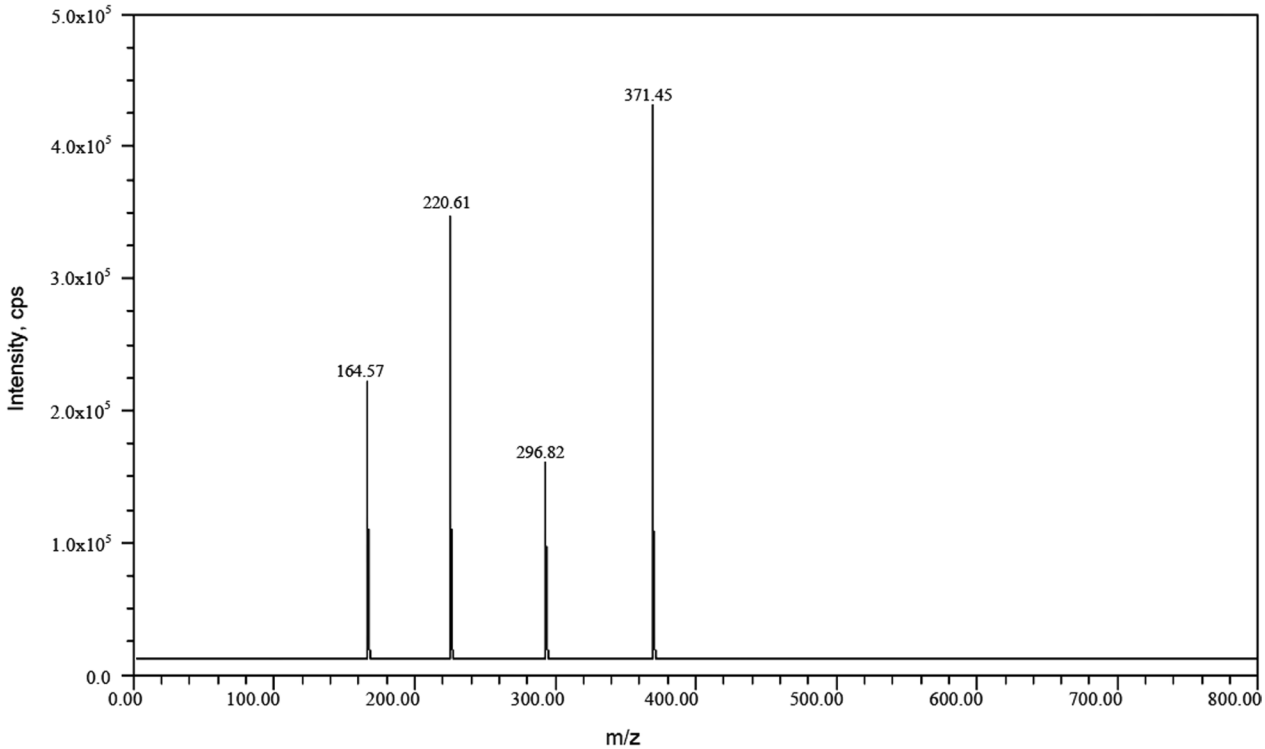

Fig. 4 Mass spectrum of Samidorphan

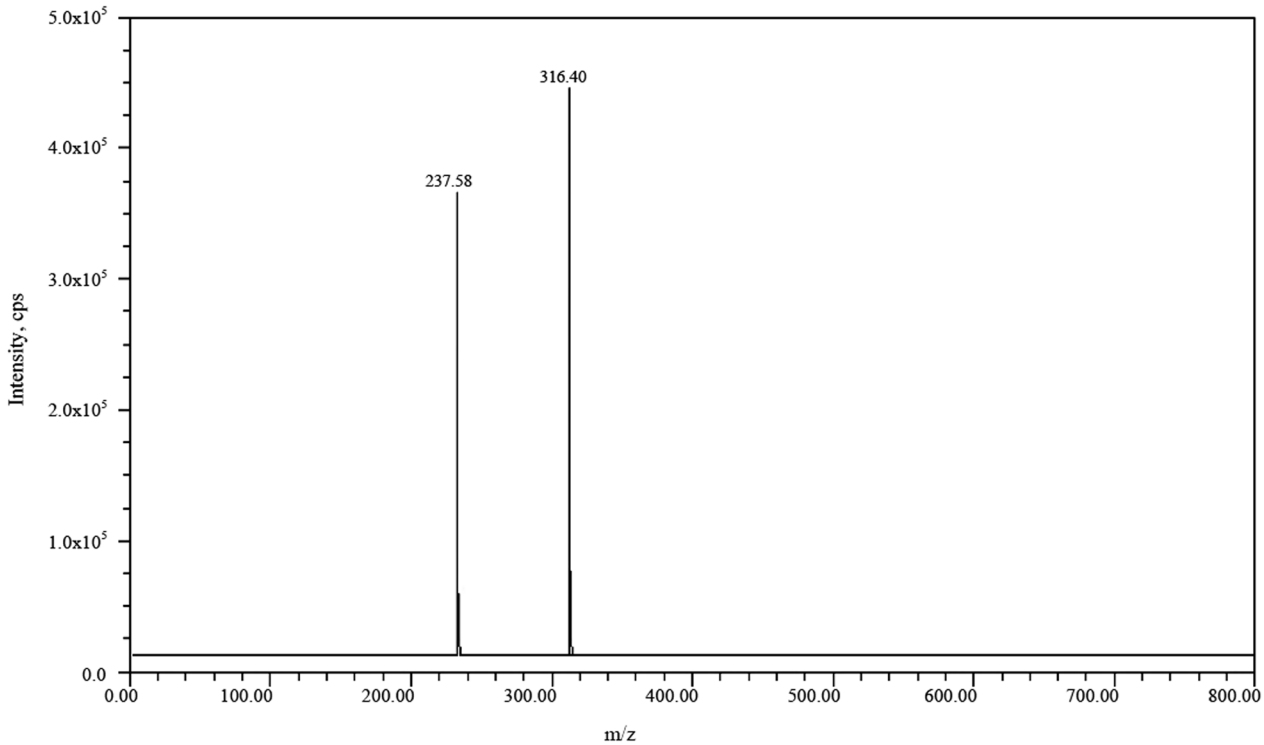

Fig. 5 Mass spectrum of D_3-Olanzapine

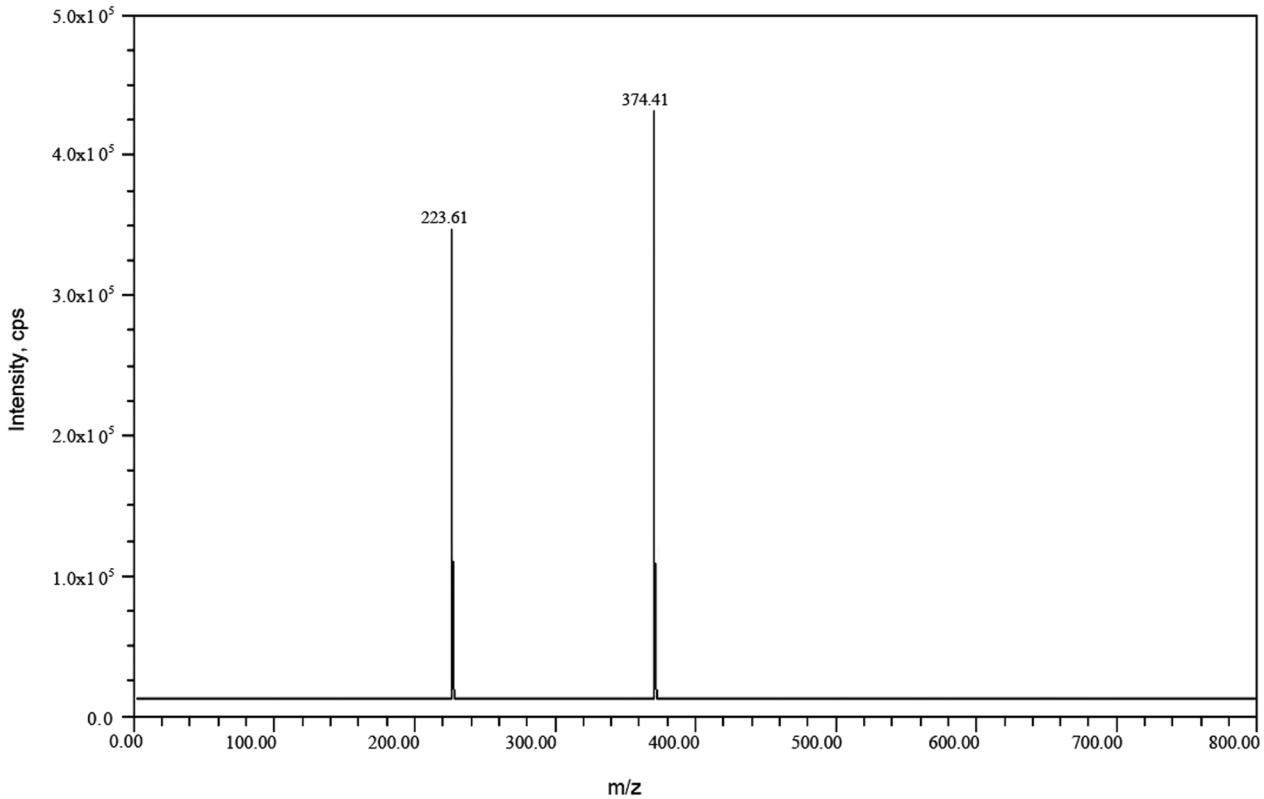

Fig. 6 Mass spectrum of D_3-Samidorphan

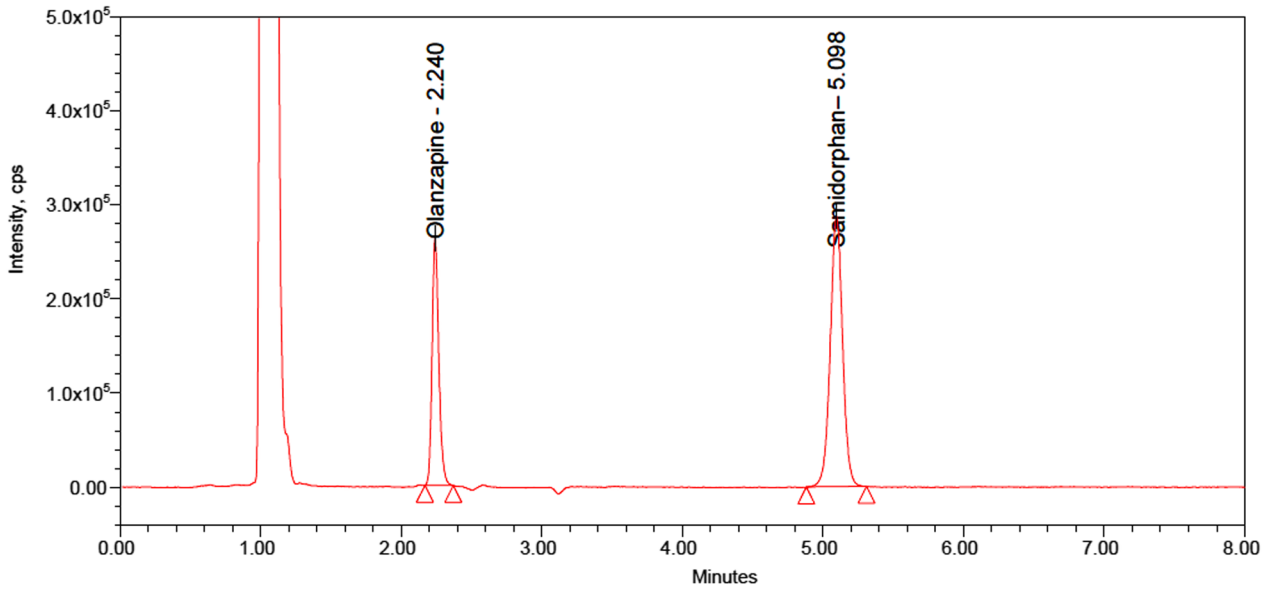

Fig. 7 Chromatogram of standard

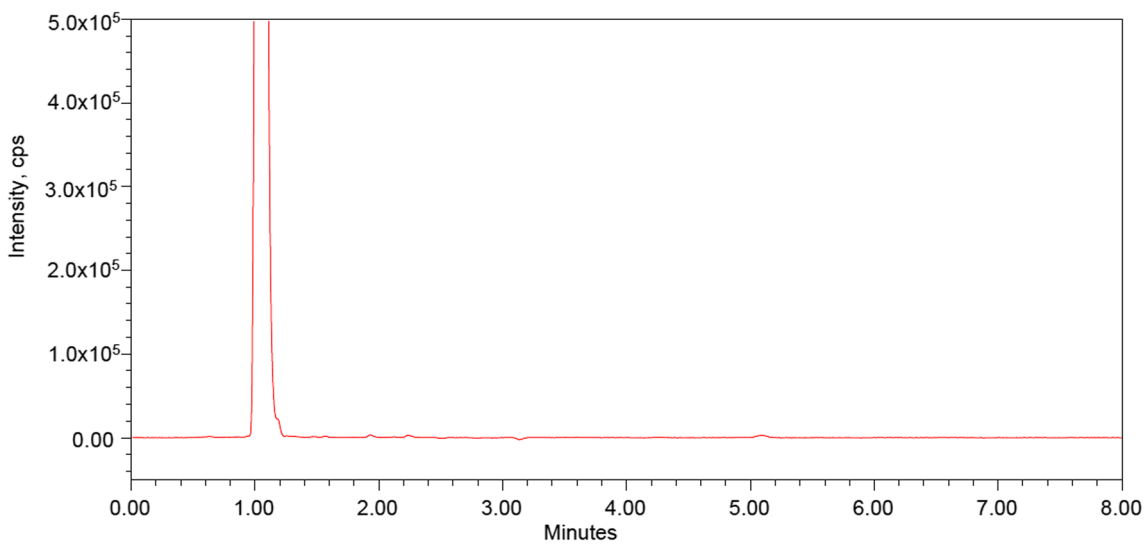

Fig. 8 Chromatogram of Blank Plasma

the findings, the matrix's effect on analyte ionisation and internal conditions was within acceptable limits.

Recovery

Recovery rates for olanzapine and samidorphan in rabbit plasma at 10, 20, and 30 ng/mL are 101.33, 100.59, and 100.31%, respectively, at low, medium, and high focal concentrations. Olanzapine and Samidorphan are clearly effective in their extraction (Table 3).

Linearity, consistency and precision

At its height, emphasis was placed on the region's relative importance in determining adjustment standards. Figures 10 and 11 show that the method had a linearity range of 2.0–40 ng/mL for Olanzapine and 2–40 ng/mL for Samidorphan. Olanzapine and Samidorphan's correlation coefficients at different QC levels were greater than 0.9993, and their presented calibration curves covered the linear concentration range. The linearity and

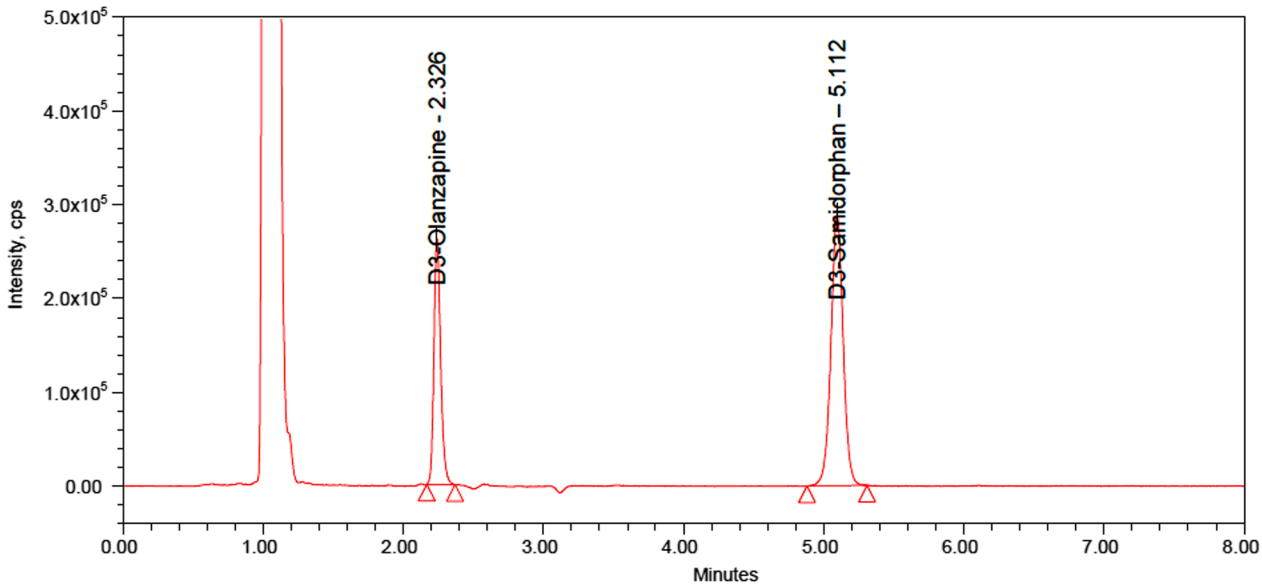

Fig. 9 Blank plasma spiked with internal standard

Table 3 Results of matrix variability and Recovery (%) of Olanzapine and Samidorphan in rabbit plasma

Analyte	Matrix	Matrix factor bias (%)		% Recovery		
		LQC	HQC	LQC	MQC	HQC
Olanzapine	Plasma	98.51	99.51	98.33	99.59	99.31
Samidorphan	Plasma	99.36	99.25	98.82	99.04	99.62

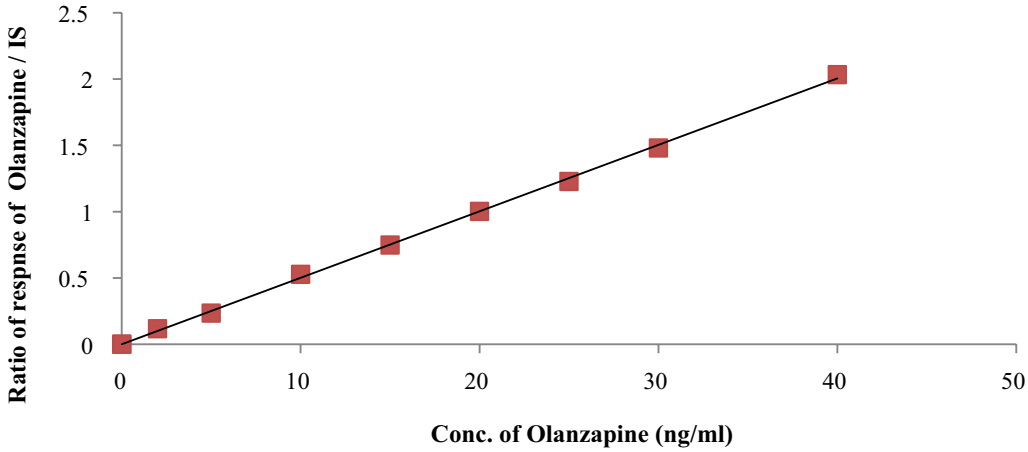

Fig. 10 Calibration plot of Olanzapine

correlation data for Olanzapine and Samidorphan are shown in Table 4, 5 and 6.

They ensured precision and exactness by combining the test results from numerous QC samples. The accuracy results of quality control samples for Olanzapine were 98.24–99.72% and for Samidorphan they were 98.03–99.72%. And %CV of Olanzapine and Samidorphan at various concentrations was 5% for all quality

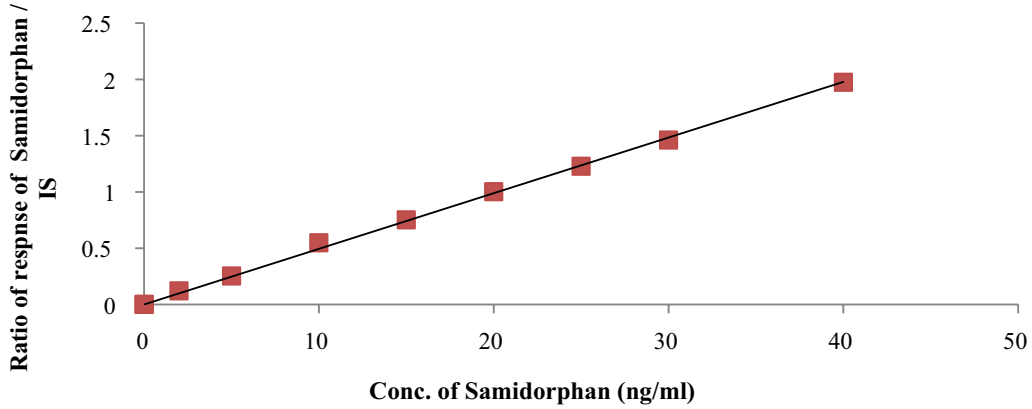

Fig. 11 Calibration plot of Samidorphan

Table 4 Linearity results of Olanzapine

Linearity	Olanzapine conc. (ng/ml)	Olanzapine area response ratio
1	2.00	0.118
2	5.00	0.235
3	10.00	0.527
4	15.00	0.749
5	20.00	1.002
6	25.00	1.228
7	30.00	1.481
8	40.00	2.034
Slope	0.0489	
Intercept	0.01688	
CC	0.99901	

Table 5 Linearity results of Samidorphan

Linearity	Samidorphan Conc. (ng/ml)	Samidorphan area response ratio
1	2.00	0.121
2	5.00	0.253
3	10.00	0.548
4	15.00	0.752
5	20.00	1.001
6	25.00	1.227
7	30.00	1.461
8	40.00	1.974
Slope	0.0490	
Intercept	0.02232	
CC	0.99927	

control samples. All exactness and precision results fell within the quantification range. Table 7 displays the results in detail.

Dilution integrity

Spiking the analyte matrix fixation over the ULOQC and diluting this specimen with blank matrix should be used to demonstrate dilution integrity. Olanzapine and samidorphan were both diluted to a concentration of 40 nanograms per millilitre (ng/mL) at a 2ULOQC. Olanzapine and Samidorphan were both tested with six replicate samples at 1:2 dilutions (20 ng/mL, 20 ng/mL) and 1:4 dilutions (10 ng/mL, 10 ng/mL). Two components' and% CV were found to be within a reasonable range. Table 8 displays the results.

Carry over

Carryover refers to any systematic error that might influence the sample's measured value. The following method was used to assess sample retention on an HPLC/MS system set up with Waters Alliance. The waters Z-spray triple quadruple mass detector underwent a flow injection system blank injection with a volume of 10L containing 0.1% Formic acid and Acetonitrile (50:50). We may conclude that the proposed strategy's accuracy and precision were not affected by the method used here. Olanzapine and Samidorphan sample carry over findings were LLQC (3.21%), ULQC (1.14%), and LLQC (6.78%), ULQC (0.93%), respectively, within the allowable limit. Table 9 displays the outcomes of the carryover.

Re-injection reproducibility

Reproducibility of re-injection was performed to validate the system after the hard product was disabled due to any

Table 6 Correlation results of Olanzapine and Samidorphan

Validation parameter	Olanzapine			Samidorphan		
Quality control levels	Low	Medium	High	Low	Medium	High
QC Conc. (ng /ml)	10	20	30	10	20	30
Linearity range	2.0–40 ng/ml			2–40 ng/ml		
Correlation (r^2)	0.9990 ± 0.010			0.9992 ± 0.017		

Table 7 Precision and accuracy results of Olanzapine and Samidorphan in rabbit plasma

Matrix	Sample	Olanzapine			Samidorphan		
		Accuracy bias (%)	Precision RSD (%)		Accuracy bias (%)	Precision RSD (%)	
			Intra-day	Inter-day		Intra-day	Inter-day
Plasma	LLOQC	−0.74	2.92	−0.88	−0.85	1.67	2.12
	LQC	1.11	0.46	1.23	0.89	0.21	0.17
	MQC	0.69	0.15	0.47	0.72	0.13	0.22
	HQC	0.59	0.27	0.06	0.66	0.49	0.58

Table 8 Results of dilution integrity

Analyte	ULOQC conc (ng/ml)	Calculated conc (ng/ml)	%CV
Olanzapine	40	40.29	0.23
Samidorphan	40	40.17	0.71

Table 9 Results of carry over

Concentration	% of carry over	
	Olanzapine	Samidorphan
Blank	0	0
LLOQC	4.06	3.70
ULOQC	0.37	0.58

instrument malfunction during analysis of real-subject samples. If an instrument fails during an investigation of a genuine subject specimen, the batch can be re-infused after 24 h if the results of the re-injection show a per cent change of less than 2.0 per cent at the LQC and HQC levels.

Stabilities

We prepared a stock solution of olanzapine and samidorphan and tested its stability by keeping it at room temperature for 18 h. A stock solution kept in an autosampler at room temperature for 24 h displays stable behaviour in terms of autosampler stability. Stock was maintained at (− 28 °C) for 24 h to evaluate freeze–thaw stability, 2–8 °C for 18 h to evaluate wet extract stability, and (− 20 ± 3)°C for 18 h to evaluate dry extract stability. Long-term stability was determined by holding the stock for 28 days at (− 20 ± 3)°C and injecting it into an HPLC–MS, whereas short-term stability was demonstrated by storing the medicines for 7 days at (5 ± 3)°C. Check the new stock solution's stability against the results of the stock solution prepared more than 24 h in advance. Olanzapine and Samidorphan showed negligible percentage changes of 1.15 and 0.68, respectively, during a 24-h period, demonstrating the stability of these solutions.

Plasma stability at room temperature was demonstrated for both olanzapine and samidorphan. Plasma samples spiked with Olanzapine and Samidorphan were tested and found to maintain their LQC, MQC, and HQC levels after being frozen and thawed multiple times. Olanzapine and Samidorphan were found to be stable at a freezing temperature of − 30 °C for 24 h, demonstrating their long-term stability. Tables 10 and 11 display the results for Olanzapine and Samidorphan's overall stability.

Discussion

A stock solution of olanzapine and samidorphan was made and left out at room temperature for 18 h to test their stability. When it comes to autosampler stability, a stock solution kept at room temperature for 24 h in the device exhibits stable performance. The stock solution was stored at 2–8 °C for 18 h to evaluate its damp extract stability, whereas the stock was held at (− 20 ± 3 °C) for

Table 10 Stability results of Olanzapine in plasma of rabbit under different storage conditions

Stability	Storage condition	Conc. level	Measured conc (ng/ml) (Mean ± SD, *n* = 6)	% RSD	% Recovery
Bench top stability	18 h at room temperature	10	10.215 ± 1.2	0.96	99.64
		20	20.324 ± 0.8	0.84	99.59
		30	29.042 ± 2.3	0.75	99.84
Autosampler stability	24 h in autosampler at room temperature	10	10.174 ± 0.7	1.92	99.47
		20	20.236 ± 2.4	0.53	99.53
		30	30.524 ± 0.6	1.14	99.54
Long-term stability	28 days at (− 20 ± 3)°C	10	9.231 ± 1.3	2.54	86.46
		20	20.529 ± 2.2	0.53	87.42
		30	30.112 ± 3.1	0.49	86.86
Freeze thaw stability	24 h at (− 28 ± 5)°C then exposed to three freeze and thawed cycles	10	10.432 ± 1.5	1.54	99.02
		20	19.362 ± 0.47	1.10	98.37
		30	30.047 ± 1.11	0.76	98.45
Wet extract stability	18 h at 2–8 °C	10	10.321 ± 0.6	1.55	98.48
		20	19.865 ± 2.3	0.43	99.24
		30	29.745 ± 1.7	0.28	98.61
Dry extract stability	18 h at (− 20 ± 3)°C	10	10.341 ± 3.5	0.95	98.85
		20	20.552 ± 0.7	1.43	98.74
		30	30.639 ± 4.1	0.52	99.53
Short-term stability	7 days at (5 ± 3)°C	10	10.063 ± 1.9	0.89	93.57
		20	20.597 ± 3.2	0.33	94.54
		30	30.552 ± 4.6	0.71	94.96

Table 11 Stability results of Samidorphan in rabbit plasma under different storage conditions

Stability	Storage condition	Conc. level	Quantified conc. (ng/ml) (Mean ± SD, *n* = 6)	% RSD	% Recovery
Bench top stability	18 h at room temperature	10	10.313 ± 5.1	1.54	99.54
		20	20.207 ± 1.6	0.37	98.96
		30	30.421 ± 2.0	0.59	99.42
Autosampler stability	24 h in autosampler at room temperature	10	10.078 ± 5.7	2.54	98.75
		20	20.112 ± 5.4	0.24	99.22
		30	30.328 ± 3.6	1.64	99.32
Long-term stability (Day 28)	28 days at (− 20 ± 3)°C	10	9.649 ± 4.8	2.22	85.76
		20	20.334 ± 6.1	0.58	86.26
		30	30.293 ± 20.0	0.94	87.54
Freeze thaw stability	24 h at (28 ± 5)°C then exposed to three freeze and thaw cycles	10	10.498 ± 5.3	2.47	98.85
		20	19.485 ± 7.4	1.36	99.45
		30	29.554 ± 6.7	0.94	98.51
Wet extract stability	18 h at 2–8 °C	10	10.368 ± 2.8	0.85	98.32
		20	20.491 ± 5.8	0.42	98.85
		30	30.589 ± 3.4	0.67	99.59
Dry extract stability	18 h at (− 20 ± 3)°C	10	10.647 ± 7.8	2.54	99.48
		20	19.328 ± 9.5	1.39	99.54
		30	30.514 ± 4.4	1.28	99.83
Short-term stability	7 days at (5 ± 3)°C	10	9.272 ± 7.6	0.42	94.65
		20	20.367 ± 2.8	2.51	95.74
		30	30.604 ± 12.6	3.84	94.59

24 h to evaluate its dry extract stability. Storage of medicines for 7 days at (5 ± 3 °C) demonstrates short-term stability, while storage of the stock for 28 days at (− 20 ± 3 °C) and injection into an HPLC–MS demonstrates long-term stability. Examine the differences between the stability results of stock solutions prepared immediately and those prepared more than 24 h in advance. The results showed that after 24 h, there were only a 1.15 per cent change in Olanzapine and a 0.68 per cent change in Samidorphan in the solution.

Both olanzapine and samidorphan were plasma-stable at room temperature under a range of circumstances. Repeated freezing and thawing had no effect on the levels of LQC, MQC, or HQC in plasma samples that had been artificially spiked with olanzapine and samidorphan. Long-term stability testing showed that both olanzapine and samidorphan could withstand temperatures as low as − 30 °C for 24 h. Tables 10 and 11 display the results for the overall stability of Olanzapine and Samidorphan, respectively.

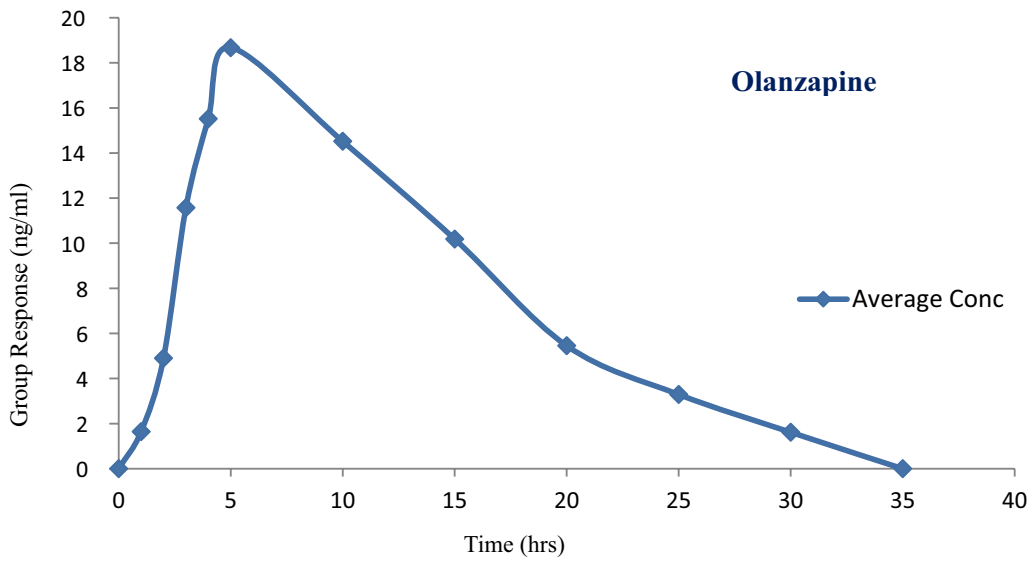

Fig. 12 Recovery plot of Olanzapine

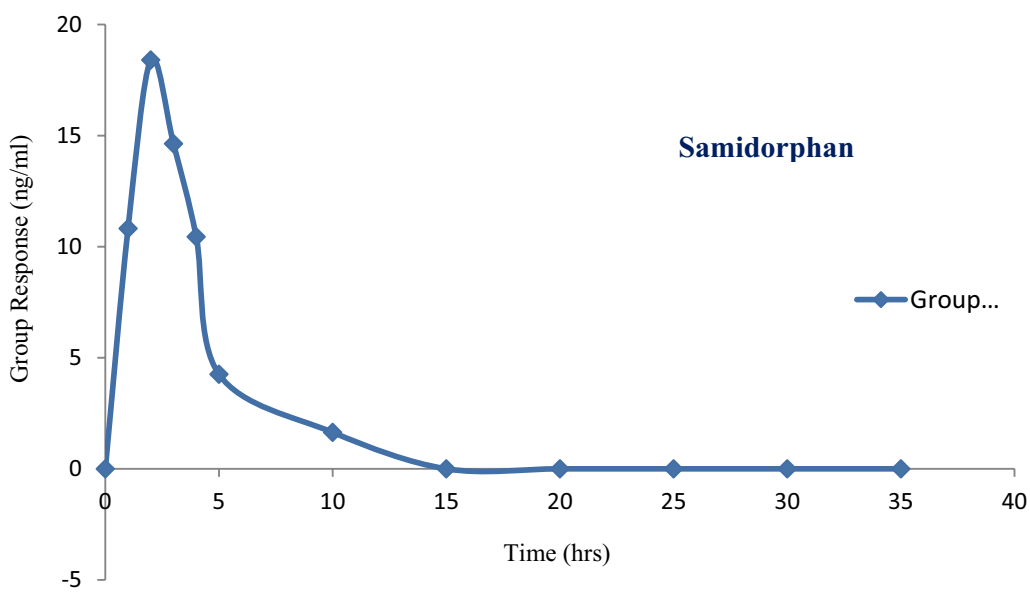

Fig. 13 Recovery plot of Samidorphan

Table 12 Pharmacokinetic studies of Olanzapine and Samidorphan

Pharmacokinetic parameters[a]	Olanzapine	Samidorphan
AUC_{0-t} (ng h/ml)	265 ± 2.841	75 ± 0.988
C_{max} (ng/ml)	18.7 ± 0.134	18.4 ± 0.111
$AUC_{0-\infty}$ (ng h/ml)	265 ± 1.427	75 ± 1.024
$T_{1/2}$ (h)	30 ± 0.41	10 ± 0.12
T_{max} (h)	5 ± 0.2	2 ± 0.15

[a] All parameters expressed as Mean ± SD, ($n = 6$) values except T_{max} and $T_{1/2}$ which are expressed in terms of median value

Pharmacokinetic study

After administering 0.17 mg/kg of Olanzapine and 0.17 mg/kg of Samidorphan intravenously to rabbits, respective mean plasma concentration–time profiles (Figs. 12, 13) were obtained for pharmacokinetic analysis. There are notable distinctions between Olanzapine and Samidorphan in intravenous pharmacokinetic studies. One, two, three, four, five, ten, fifteen, twenty-five, thirty, and three-and-a-half hours after the drugs were given, we collected samples from the rabbits' bodies at varying intervals. The values were recorded after the test sample was prepared and injected into the chromatographic apparatus. C_{max} and T_{max} after intravenous administration of Olanzapine and Samidorphan (18.6740.146 and 18.4010.657), Kel (obvious first request terminal rate constant calculated from semi-log plot of plasma concentration versus time bend, employing the least square relapse technique), and $t_{1/2}$ (terminal half-life as governed by 0.693/Kel ratio) were all determined to be accurate measures of bioavailability. C_{max}, AUC0-24, and AUC0- were all within the acceptable range of 18.70.134, 2652.841, and 2654.282, and 18.40.111, 750.988, and 751.024, respectively. In Table 12, we can see the Olanzapine and Samidorphan pharmacokinetic characteristics.

Conclusion

An innovative HPLC–MS/MS method for assessing Olanzapine and Samidorphan in 8-min rabbit plasma has been developed and validated for the first time. Both Olanzapine and Samidorphan were rapidly absorbed by the rabbit's body after being given intravenously, as would be expected based on their pharmacokinetic profiles. The procedure outlined here is efficient, reliable, and repeatable. It has a good linear concentration range and sufficient precision for application in pharmacokinetic investigations for checking analyte concentrations in bodily fluids. These investigations are essential for the future credibility of our findings as a benchmark.

Abbreviations

HPLC	High-performance liquid chromatography
ACN	Acetonitrile
USFDA	United States food and Drug Administration
MS	Mass spectrometry
ODS	Octadecyl-silica
MQC	Middle quality control
HQC	Higher quality control
LQC	Lower quality control
LLOQC	Lower limit of quantitation
MRM	Multiple reaction monitoring
IS	Internal standard
CV	Coefficient of variation
CC	Correlation coefficient
ULOQC	Upper limit of quantitation

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Author contributions

The author planned the study, developed and tested the methods, wrote the procedure, and wrote the first draught of the paper.

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Availability of data and materials

The data for verification are provided with a Supplementary file and the rest.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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