Stability indicating reverse phase (RP)-high-performance liquid chromatography method development and validation for the simultaneous estimation of olanzapine and samidorphan in bulk and tablets

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Background
Patients with schizophrenia are given a combination tablet combining olanzapine (OLA), an atypical antipsychotic and samidorphan (SAM), an opioid receptor antagonist.
Objectives
In bulk and tablet dosage forms, a stability-indicating reverse phase (RP)-high-performance liquid chromatography technique for the simultaneous determination of OLA and SAM has been developed and validated.
Materials and methods
The chromatographic analysis was performed using an isocratic mobile phase of 0.1% formic acid in water : methanol : acetonitrile (10 : 40 : 50, v/v) at a flow rate of 0.8 ml/min, and the eluents were monitored at an isosbestic point of 285 nm.
Results and conclusion
The suggested method's specificity, precision, accuracy, linearity, and robustness were all validated according as per International Conference on Harmonization guidelines. The method's stability was validated by forced degradation experiments. Retention times for OLA and SAM were 2.85 and 4.79 min, respectively. The method was found to be precise and accurate. SAM linearity was found to be between 14.0 and 45.0 μg/ml, whereas OLA linearity was found to be between 22.5 and 67.5 μg/ml. The limit of detection (LOD) and limit of quantification (LOQ) of OLA were 2.65 and 8.85 μg/ml, respectively, whereas the LOD and LOQ of SAM were 8.12 and 27.06 μg/ml, respectively. As a result, the suggested high-performance liquid chromatography method for the quantification of OLA and SAM was reliable, repeatable, accurate, and sensitive.

Keywords:
high-performance liquid chromatography method, olanzapine, samidorphan, stability indicating and validation

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Introduction

Schizophrenia is a complicated and chronic disease that requires a long-term antipsychotic drug therapy with the primary objectives of symptom reduction, improved functioning and quality of life, and relapse prevention [1,2]. Persistent cognitive, behavioral, and emotional problems are characteristic features of this condition [3]. To control symptoms, enhance social functioning and quality of life, and avoid relapse, current clinical practice recommendations suggest a long-term therapy using pharmacological (antipsychotic medicines) and psychosocial treatments [4,5].

Olanzapine (OLA) is one of the most effective antipsychotics, having well-established effectiveness and benefits such as a lower rate of extra-pyramidal symptoms [6]. However, OLA has certain safety and tolerability issues, such as widespread weight gain and metabolic irregularities, which are linked to substantial health concerns and may impact patient adherence and retention on OLA treatment [7,8]. A previous study in people with schizophrenia and schizoaffective disorder indicates that using an opioid antagonist while taking an antipsychotic medication may help reduce weight gain [9]. Samidorphan (SAM) is a new opioid system modulator that has been demonstrated to work as a μ-opioid antagonist in vivo [10,11]. SAM binds to human μ-opioid, κ-opioid, and δ-opioid receptors with high affinity in vitro and functions as an antagonist at μ-opioid receptors, with minimal intrinsic activity at and –opioid receptors [12]. Lybalvi is a once-daily oral bilayer tablet with one layer containing a flexible dose of OLA (5, 10, 15, and

20 mg) and the other layer containing a fixed dose of 10 mg SAM, designed to provide OLA's established antipsychotic efficacy with a favorable weight and metabolic profile. Coadministering SAM with OLA has proved to reduce OLA-induced weight gain in phase I and phase II clinical trials [13,14]. OLA is mostly metabolized by hepatic metabolism, with just 7% of the OLA dosage given being excreted unaltered in the urine. Direct glucuronidation through uridine diphosphate glucuronosyltransferase 1A4 and cytochrome P-450-mediated oxidation, mostly by cytochrome P-450 1A2, are the two major metabolic routes for OLA. Hepatic metabolism and renal excretion of SAM are the primary routes of elimination. There was no pharmacokinetic drug–drug interaction between OLA and SAM in a prior clinical trial, which is consistent with the two drugs' different metabolic routes [9,15].

According to a study of the literature, only a few analytical methods using different techniques have been reported for determining OLA alone and in combination with other analytes. Instrumental approaches such as LC/MS [16,17], LC-MS/MS [18–21], high-performance liquid chromatography (HPLC) [22–28], and voltammetry [27] and spectrophotometry [22,29–31] are among these methods. For the determination of SAM alone or in combination with OLA, no analytical technique has been described. Given the rising global demand for the aforementioned drugs, it is necessary to develop a new economical, accurate, and rapid HPLC analytical technique for the simultaneous estimation of both drugs in the pharmaceutical formulation, as well as to conduct forced degradation studies in five different conditions that could be used to evaluate the quality, efficacy, and storage conditions of each drug.

Materials and methods

Chemicals and reagents

Both drug standards were gifted from Alembic Pharmaceuticals, Vadodara, India. Methanol, water, and acetonitrile (LC grade) were purchased from Sigma-Aldrich, Ronkonkoma, New York, USA. Analytical grade sodium hydroxide (NaOH), hydrogen peroxide (H_2O_2), hydrochloric acid (HCl), and a 0.22 mm membrane filter were purchased from Sigma-Aldrich. Lybalvi tablets contain OLA and SAM with a label claim of 15 and 10 mg, respectively, and are purchased from the native pharmaceutical market. All chemicals were analytical or LC grade.

High-performance liquid chromatography instrumental condition

An Acquity HPLC system (Waters, Milford, Massachusetts, USA) with a model 2996 PDA detector and Empower software was used to create the method. The two analytes were separated at room temperature using 0.1% formic acid in a mixture of water, methanol, and acetonitrile (10 : 40 : 50, v/v) in isocratic mode at a flow rate of 0.8 ml/min. At 285 nm, the PDA detector was utilized to monitor the two medications. The solvents were filtered through a 0.22 mm membrane filter and degassed in an ultrasonic bath before being utilized. As a diluent, the mobile phase was utilized.

Standard solution preparation

A standard stock solution of OLA (45 µg/ml) and SAM (30 µg/ml) was made by carefully weighing 10 mg of each and dissolving in a 10 ml volumetric flask containing 5 ml of methanol, then sonicating the flask to dissolve the contents, and topping up with the same. These samples were aliquoted into a 10 ml volumetric flask with 5 ml diluent (mobile phase), sonicated for 5 min, and the residual volume brought up to the mark with a diluent to get final concentrations of 45 µg/ml for OLA and 30 µg/ml for SAM, respectively.

Formulation analysis

Twenty tablets were carefully weighed and crushed into a fine powder in a mortar. In a volumetric flask, 10 mg of SAM was transferred, 5 ml of diluent was added, and the mixture was sonicated to guarantee solubility. Finally, to get the tablet's principal stock solution, the volume was raised to 10 ml. A 0.30 ml aliquot was collected and put to a volumetric flask with a capacity of 10 ml. For OLA and SAM, the mobile phase was employed to produce volumes of 45 and 30 µg/ml, respectively. The resulting solution was filtered, if necessary, with 0.45 m Millipore nylon filter paper. The peak areas for OLA and SAM were measured after 10 µl was introduced into the HPLC equipment. The percent assay of the preparation was determined.

Validation of the chromatographic method

The developed method was validated according to International Conference on Harmonization (ICH) criteria (ICH Guidelines, Q2 (R1), 2005) [32–34].

Suitability of the high-performance liquid chromatography system

To validate system performance, system suitability characteristics were assessed. Six injections of

standard preparations were used to assess the system's accuracy. The size of the peak, resolution of the peaks, and the theoretical plate number were all taken into consideration.

Accuracy (recovery)

Accuracy is represented (ICH Guidelines, Q2 (R1), 2005) and determined by recovery experiments. In this process, it was tested at three different levels namely 50, 100, and 150% and analyzed chromatogram.

Specificity

To assess the specificity, a working placebo solution (blank) in the absence of the OLA and SAM and standard solution having a concentration of 5 and 30 µg/ml for OLA and SAM respectively, as well as formulations were injected into the HPLC system and analyzed chromatograms.

Precision

Six replicate injections of optimum concentrations of OLA and SAM were used to demonstrate the analytical technique's precision (intraday and interday). Chromatograms were used to calculate the average and percent RSD of peak area and assay.

Linearity

Pure analytical standard preparation was prepared and analyzed at five different concentrations to ensure linearity. For OLA and SAM, the proposed method has excellent linearities across a range of 22.5, 33.75, 45.00, 56.25, and 67.50 µg/ml and 15.00, 22.50, 30.00, 37.50, and 45.00 µg/ml, respectively.

Detection and quantification limit

The signal-to-noise technique was used to calculate the limit of detection (LOD) and limit of quantification (LOQ) of OLA and SAM, as stated by the ICH standard (ICH Guidelines, Q2 (R1), 2005). The signal-to-noise ratio was determined at each concentration by injecting increasingly dilute solutions of each medication and contaminant into the chromatograph.

Robustness

The impact of modest changes in flow rate (±5%), change in column temperature (±5°C), and change in detection wave length (±2 nm) on resilience as a measure of method capacity to stay unaffected by small, but purposeful changes in chromatographic conditions was investigated.

Forced degradation studies

Stability testing of novel drug substances and products is mandated by the ICH (ICH Guidelines, Q1A(R2), 2003) [32,33] guidelines, which involves stress testing to define the active substance's intrinsic stability properties. The objective of this research was to use the proposed technique to conduct stress degradation experiments on the OLA and SAM.

Hydrolysis in acidic and alkaline conditions

Transfer 0.3 ml of the primary stock solution to two 10 ml volumetric flasks. For acidic conditions, 1 ml of 0.1 N HCl was added to the aforementioned solution in a pair of 10 ml standard flasks. In another set of 10 ml standard flasks, 1 ml of 0.1 N NaOH was added for alkaline degradation. The standard flask was then maintained in a water bath for 3 h at 85°C and 5 h at 70°C for acid and alkaline samples, respectively. Both solutions were neutralized and diluted to 10 ml with a diluent, yielding 45 µg/ml for OLA and 30 µg/ml for SAM, respectively. Allow the solution to cool to room temperature. Filter the solution with a 0.22 mm syringe before injecting it into the vials of the HPLC system.

Degradation by oxidation

Transfer 0.3 ml of main stock solution to a 10 ml standard flask . The volume was brought up to the mark using diluents after adding 2 ml of 3% (w/v) H_2O_2, resulting in 45 µg/ml for OLA and 30 µg/ml for SAM.. The standard flask was heated for 1 h at 80°C. After filtering using a 0.22 mm syringe filter, the resultant solution was cooled and injected into the vials of the HPLC system.

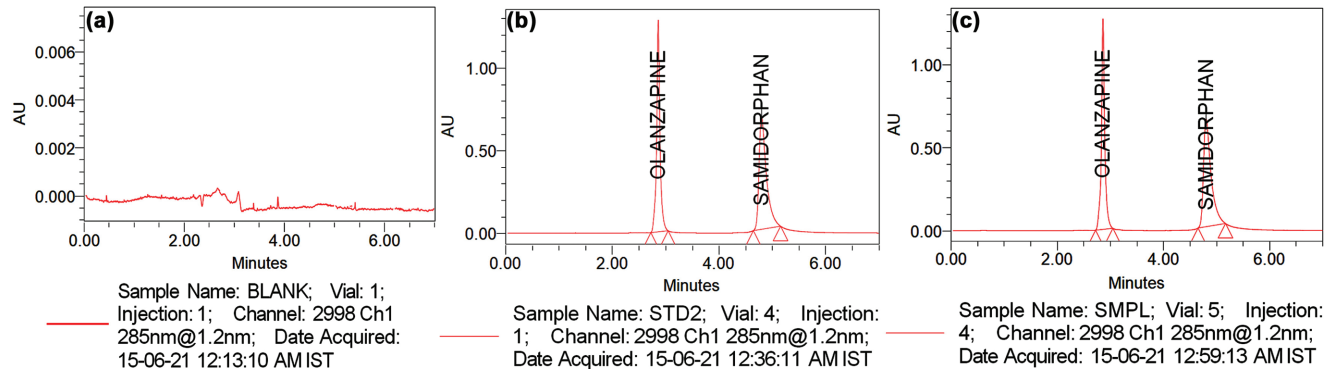
Degradation caused by heat

Transfer 0.3 ml of main stock solution to a 10 ml standard flask and dilute to the desired amount with a diluent to obtain 45 µg/ml for OLA and 30 µg/ml for SAM. The resultant solution was then refluxed for 10 h at 85°C. The solution was then brought to room temperature. After filtering with a 0.22 mm syringe filter, place the vials in the HPLC system.

Degradation of light

Pipette 0.3 ml from the stock solution into a 10 ml standard flask, then dilute to the desired amount with a diluent to obtain 45 µg/ml for OLA and 30 µg/ml for SAM, respectively. The samples were then transferred to a Petri plate and placed in a photostability chamber for 30 h at 200 Wh/m^2 ultraviolet (UV) light and 1.2 million lux hours UV light. Allow the finished product to cool to room temperature. Filter the solution with a 0.22 mm syringe before injecting it into the vials of the HPLC system.

Figure 1



Representative chromatograms of (a) blank, (b) standard, and (c) sample (tablets).

Table 1 System suitability parameters

S. No	Parameter[a]	OLA	SAM
1	Theoretical plate count	3630	7753
2	Average peak area	5 570 645.167	6 481 226.83
3	RT (min)	2.85	4.79
4	Tailing	1.72	1.18
5	Resolution	–	22.57
6	S/N	1669.82	464.25

S/N, signal-to-noise. [a]Average of six replicates.

Table 2 Recovery study

			Olanzapine			
S. No	Accuracy level (%)	Weight. of sample (mg)	Peak area[a]	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery
1	50	224.99	2 760 635	22.47	22.30	99.24
2	100	449.98	5 544 767.333	44.94	44.79	99.66
3	150	674.97	8 332 433	67.41	67.31	99.84
Samidorphan						
4	50	224.99	3 245 336	15.00	15.02	100.13
5	100	449.98	6 465 543	30.00	29.93	99.75
6	150	674.97	9 797 883	45.00	45.35	100.77

[a]Mean of three determinations at each level.

Results and discussion

When compared with the OLA and SAM standard, OLA and SAM samples were confirmed as a function of retention time. In addition, OLA and SAM were identified by adding the standard to the sample before analysis, resulting in a proportional increase in the sample's peak area. The mean OLA and SAM retention durations were 2.85 and 4.79 min, respectively, at a flow rate of 0.8 ml/min. Other degradation products had little effect on the system. Figure 1a–c shows the OLA and SAM blank, standard, and sample chromatograms.

Validation of the high-performance liquid chromatography method

System suitability study

System suitability was attained by checking the various parameters and found within the ICH limit. The results are presented in Table 1.

Accuracy (recovery)

Accuracy was determined at three distinct levels: 50, 100, and 150%. Table 2 displays the results. At 50, 100, and 150%, mean percent recoveries for OLA were determined to be 99.24, 99.66, and 99.84%, respectively. SAM 100.13, 99.75, and 100.77%, respectively, at 50, 100, and 150%.

Precision

Precision of the analytical method was established for both intraday and interday using a concentration of 45 µg/ml and 30 µg/ml by OLA and SAM six replicate injections. The results are shown in Table 3.

Specificity

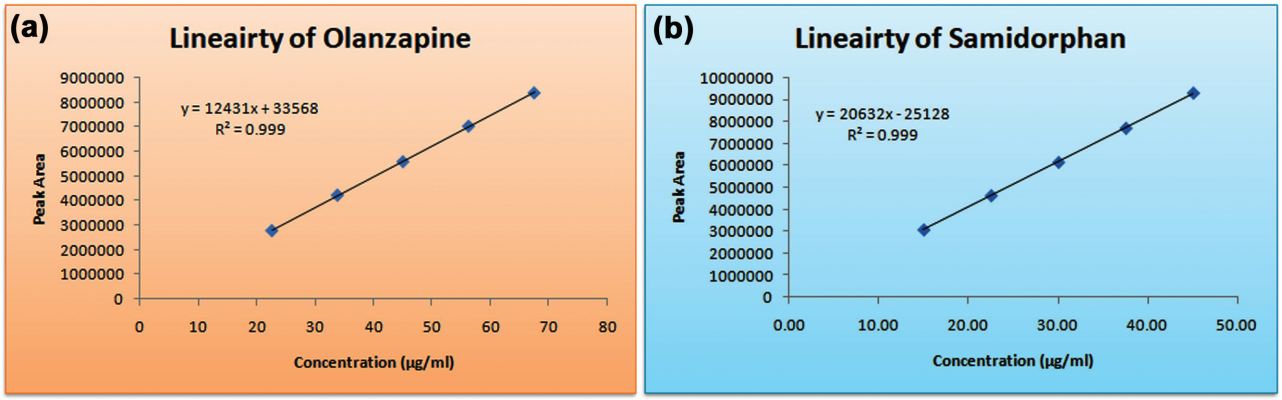
The method's specificity was demonstrated by the fact that the chromatogram of the functioning placebo solution showed no interference at the OLA and

Table 3 Precision study

Precision	Mean peak area[a]		% RSD[a]		Mean assay[a]		% RSD[a]	
	OLA	SAM	OLA	SAM	OLA	SAM	OLA	SAM
Intraday	5 562 261.67	6 498 702.67	0.38	0.88	99.85	100.27	0.38	0.88
Interday	5 563 605.50	6 481 944.33	0.35	0.78	99.87	100.01	0.35	0.78

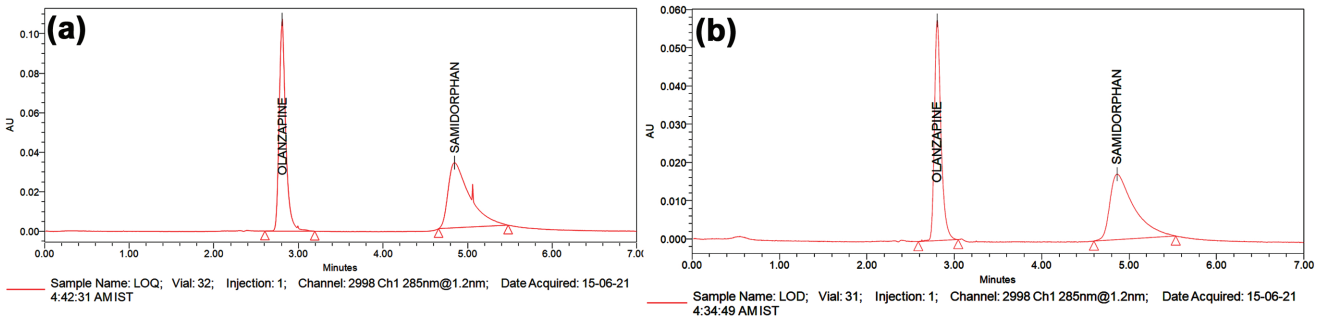
[a]Mean of six determinations.

Figure 2



Linearity curves of (a) olanzapine and (b) samidorphan.

Figure 3



Chromatograms of (a) LOD and (b) LOQ. LOD, limit of detection; LOQ, limit of quantification.

SAM retention times. As a result, it can be stated that the major excipients used in the formulations had no effect on the analytical technique used to determine OLA and SAM. Figure 1a–c shows chromatograms of blanks, standards, and formulations.

Linearity

For OLA and SAM, the proposed technique has excellent linearity across a range of 22.5, 33.75, 45.00, 56.25, and 67.50 µg/ml and 15.00, 22.50, 30.00, 37.50, and 45.00 µg/ml for both medications. The coefficient correlation was more than 0.999. Figure 2a and b depicts the linearity curves.

Detection and quantification limits

The method's limit of detection for OLA and SAM was found to be 2.65 and 8.85 µg/ml, respectively, while its limit of quantification was found to be 8.12 and 27.06 µg/ml, suggesting that it was highly fast and sensitive. The chromatograms of LOD and LOQ are shown in Fig. 3a and b.

Study of robustness

According to the findings, there was no significant difference in the robust chromatograms when compared with the optimized one. Table 4 displays the results.

Assay

The assay % of a marketed product was determined. Table 2 summarizes the results achieved within the ICH limit.

Degradation studies

During the development of analytical methods, forced degradation studies are a helpful tool for anticipating

Table 4 Robustness data

Parameters	Condition	Olanzapine			Samidorphan		
		RT	Peak area	% assay	RT	Peak area	% assay
Flow	0.6 ml/min	3.523	5 476 665	98.31	5.902	6 464 947	99.75
	0.8 ml/min	2.850	5 570 645	100.00	4.790	6 481 227	100.00
	1.0 ml/min	2.387	5 526 688	99.21	4.037	6 390 579	98.60
Temp	25°C	2.845	5 498 542	98.71	4.795	6 447 497	99.48
	30°C	2.850	5 570 645	100.00	4.790	6 481 227	100.00
	35°C	2.842	5 499 293	98.72	4.756	6 497 781	100.26
Wave length	283 nm	2.814	5 597 131	100.48	4.220	6 452 436	99.56
	285 nm	2.850	5 570 645	100.00	4.790	6 481 227	100.00
	287 nm	2.681	5 556 765	99.75	5.263	6 443 379	99.42

Table 5 Forced degradation study

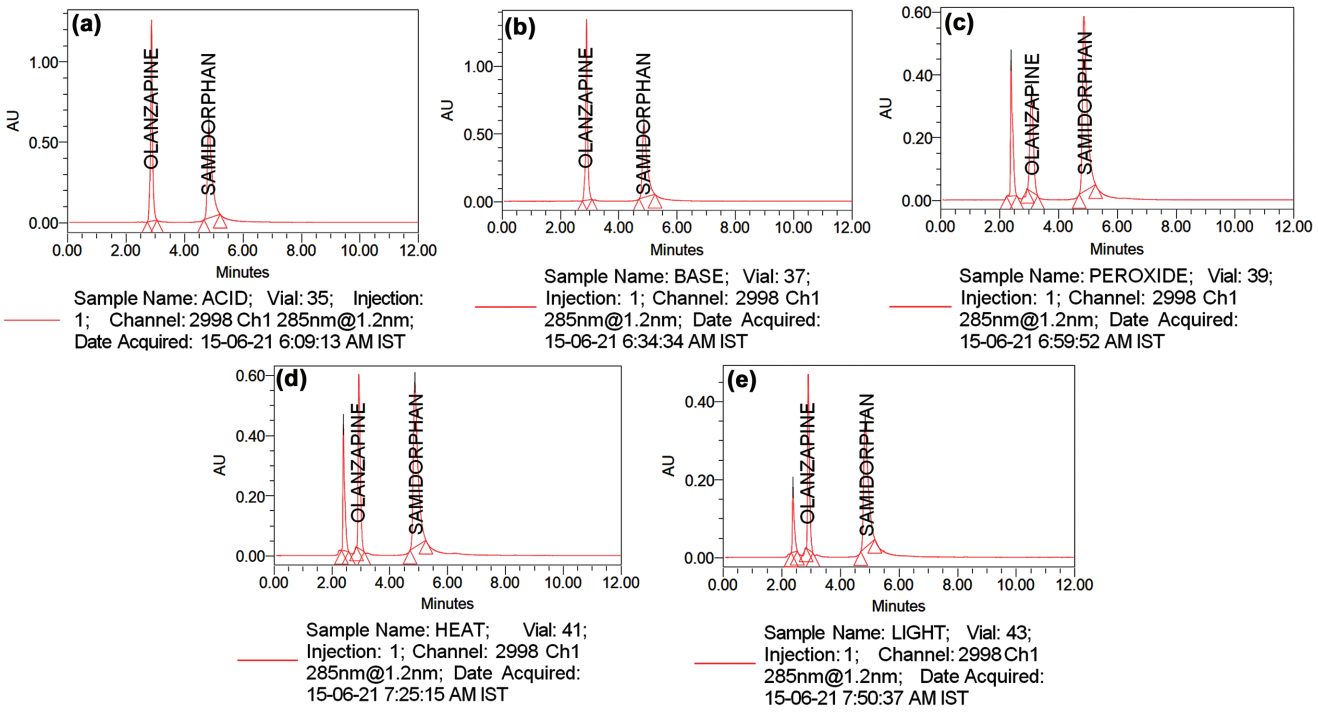
S. No	Condition	Olanzapine			Samidorphan		
		Peak area	% assay	% Degradation	Peak area	% assay	% degradation
1	Acid	5 085 098	91.28	8.72	5 889 514	90.87	9.13
2	Base	5 016 542	90.05	9.95	5 845 578	90.19	9.81
3	Peroxide	5 104 977	91.64	8.36	5 799 581	89.48	10.52
4	Thermal	5 020 713	90.13	9.87	5 864 601	90.49	9.51
5	UV	5 158 396	92.60	7.40	5 935 086	91.57	8.43

UV, ultraviolet.

drug stability concerns and identifying the major degradation products. Degradation tests indicate the specificity of the proposed technique in the presence of degradation products delivered in bulk and therapeutic dose forms. The purity of drug peaks in a combination of two medications was determined using purity angles. The OLA and SAM formulations were subjected to all of the forced degradation conditions mentioned in the ICH guideline, including hydrolysis (acidic with 0.1 M HCl and basic with 0.1 M NaOH), oxidation (3% H_2O_2), photolysis, and heat stress conditions. The results of the OLA and SAM stability studies are summarized in Table 5. The degradation chromatograms of OLA and SAM are shown in Fig. 4a–e.

Various chromatographic trails have been carried out based on the physicochemical properties of the molecules. The stationary phase, mobile phase composition, flow rate, and column temperature were all taken into consideration throughout the tests [35]. To begin the trail, one variable was maintained constant while another value was altered. Methanol : water, 50 : 50 (v/v); acetonitrile : water, 30 : 70 (v/v); methanol : orthophosphoric acid buffer (pH 4.5–6.5), 50 : 50 (v/v); methanol : phosphate buffer (pH 3.0–6.5), 25 : 75 (v/v); and acetonitrile : phosphoric acid buffer (pH 3.0–6.5), 25 : 75 (v/v) (pH 3.2–4.5). The sensitivity of the test, suitability for stability studies, ease of preparation, and use of widely available solvents were all considerations in evaluating the acceptance of the mobile phase [36]. As a consequence, it was discovered that a mobile phase containing 0.1% formic acid in water, methanol, and acetonitrile (10 : 40 : 50, v/v) is the most efficient for isocratic simultaneous estimation of OLA and SAM. Scanning the usual OLA and SAM solution between 200 and 400 nm identified the detection wave length, and the isosbestic point of 285 nm was used to identify the analytes.After a set amount of time at 85°C for 3 h and 70°C for 5 h, 10% degradation was observed in both acid and alkaline degradation tests. There were no degradation products detected in these conditions (Fig. 4a and b). This might have occurred as a result of the creation of volatile degradants or degradants without chromophore groups in their structure, which the PDA detector would have missed [37]. Peroxide, thermal, and light degradation were all shown to be significant. Both analytes were found to be very unstable when exposed to peroxide. The OLA and SAM degraded to 8.36 and 10.52%, respectively, after a substantial deterioration product eluting at 2.56 min (3% H_2O_2 at 80°C for 1 h). On the chromatogram, the degradation peak could be detected (Fig. 4c). The analytes were sufficiently stable under milder oxidative conditions (3% H_2O_2 at 80°C for 10 min) (95.02%). The analytes suffered from heat degradation. Unaltered drug content was reduced to about 90.13 and 90.49% for OLA and SAM, respectively, after dry heat degradation (85°C for 10 h), with an unknown

Figure 4



Chromatograms of (a) acid, (b) base, (c) peroxide, (d) thermal, and (e) photolytic (UV light) degradation studies. UV, ultraviolet.

degradation product eluting after 2.489 min. In the chromatogram, the degradation product was visible (Fig. 4d). OLA and SAM were found to be very unstable when exposed to 1.2 million lux hours of near-UV radiation at 200 Wh/m^2 for 30 h. The chromatogram is shown in Fig. 4f. At 2.57 min, a substantial degradation product was eluted from the chromatogram. Long-term storage of analytes under photolytic circumstances produces deterioration, according to the results of the degradation tests, with a drop in the analyte content and a corresponding increase in degradation products.

Conclusion

This method was proved to be fast, precise, selective, robust, and easy, and it may be used to a recently FDA approved OLA and SAM drug combination. This type of analysis may be used to determine the drug's safety, effectiveness, and quality in a cost-effective way. The developed technique was validated in accordance with ICH guidelines, and stability studies showed that the approach was effective in monitoring drug stability. It may also be used for regular analysis in bioanalytical laboratories, hospital research institutes, quality control divisions of pharmaceutical companies, formulation dissolution studies, and accredited testing laboratories.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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