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HPLC BASED STABILITY INDICATING QUANTITATIVE APPROACH FOR CONTENT AND STABILITY EVALUATION OF OLANZAPINE AND SAMIDORPHAN IN TABLET PRODUCTS

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Abstract:

Olanzapine (OZPE) and samidorphan (SMPN) was officially authorised recently by the FDA for the management of bipolar illness including schizophrenia. Analytical techniques for measuring OZPE and SMPN in tablet products have still not been disclosed. As a result, the authors wish to develop and validate a "stability indicating HPLC" procedure for determining OZPE and SMPN concentrations in tablet products.

OZPE and SMPN analysis employed “Sunsil C18 column” as stationary phase and mixture of 0.1 M Na_2SO_4 (pH 4.2): acetonitrile (70:30, v/v) was used as the mobile phase with 1.0 ml/min flow stream. At 242 nm, OZPE and SMPN were detected using a PDA. Stress conditions were established on OZPE and SMPN in full compliance with the ICH approach.

The validation met the ICH Q2 criteria of specificity, linearity (10–30 µg/ml for OZPE and 5–15 µg/ml for SMPN), precision (RSD- 0.281% for OZPE and 0.208% for SMPN) and accuracy (Assay percent-98.88% for OZPE and 98.76% for SMPN), robustness (RSD- 0.1% to 1.8% for OZPE and 0.1% to 1.6% for SMPN), quantification limit (0.041 µg/ml for OZPE and 0.045 µg/ml for SMPN), and detection (0.138 µg/ml for OZPE and 0.149 µg/ml for SMPN). The peaks for OZPE and SMPN were significantly alienated from the peaks for the degradation components. HPLC was used to develop an analytical stability indicating approach that allowed the rapid and precise quantification of OZPE and SMPN, and their stability in the existence of their degradation components.

Keywords: Olanzapine, Schizophrenia, Samidorphan, Stability indicating, HPLC.

INTRODUCTION

Profile of the drugs

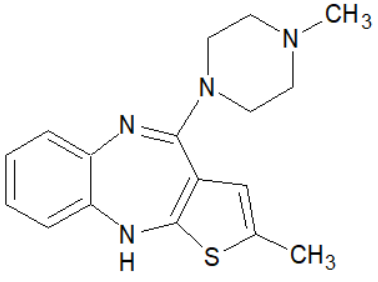
Schizophrenia is a complicated, chronic psychological health illness marked by a wide array of symptoms: hallucinations, delusions, disordered speech or behaviour, and reduced cognitive abilities [1,2]. The disease's early start, including its chronic nature, makes it a devastating ailment for many sufferers including their families. In 2017, one in every seven Indians suffered from a psychological illness of different severity. Since 1990, the proportionate contribution of psychological diseases to the overall disease burden in India have nearly doubled [3].

People sick with mental illnesses, particularly schizophrenia, have been disproportionately impacted by the COVID 19 pandemic. People having psychological illnesses may disregard their clinical status and fail to exhibit COVID-appropriate behaviour [4]. Lybalvi tablets were officially authorised recently by the FDA for the management of bipolar illness including schizophrenia [5]. The Lybalvi tablet includes 2 medications: olanzapine (OZPE) and samidorphan (SMPN).

OZPE (Fig. 1), a derivative of thienobenzodiazepine, is a second generation antipsychotic medication that has been reported to be successful in treating both positive as well as negative symptoms in patients having schizophrenia [6-9]. OZPE, when compared to traditional antipsychotics representative drugs, has a better selectivity for receptors of serotonin 5-HT2A than receptors of dopamine D2.

SMPN (Fig. 1), a new opioid-system regulator that was recently invented, works exclusively as a receptor ‘μ’ antagonist in nervous system [10]. When taken orally, SAM has a five-fold stronger affinity for the ‘μ’ receptor and a substantially higher bioavailability. SMPN binds to receptors ‘μ’, ‘κ’, and ‘δ’ in nervous system with a significant affinity in vitro, acting as an antagonist at receptor ‘μ’and a mild agonist at receptors ‘κ’, and ‘δ’ [11]. The effectiveness of SMPN to reduce weight gain related with OZPE has also been investigated [12]. Analytical methods are designed to ascertain the purity, identification, physical properties, and potency of medications. Methods are being developed to aid in drug assessment against specifications throughout production and quality release activities, and long-term stability investigations [13]. Methods may also be used to assist safety and characterisation investigations, as well as drug effectiveness evaluations.

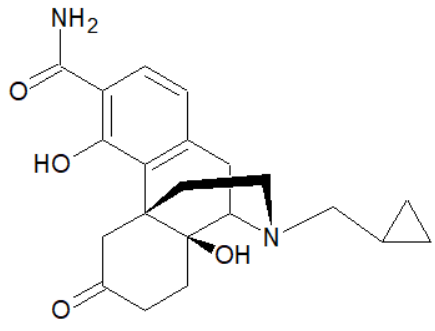
Quantification of OZPE and SMPN in tablet products is unavoidably required for quality management during manufacturing as well as storage. However, there are no published data on analytical procedures for quantifying OZPE and SMPN in tablet products. Hence, the authors want to design and validate a "stability indicating HPLC" approach for assessing OZPE and SMPN in tablet products.



Olanzapine

IUPAC name: 2-Methyl-4-(4-methyl-1-piperazinyl)-10H-thieno[2,3-b][1,5]benzodiazepine

(A)



Samidorphan

IUPAC name: 17-(Cyclopropylmethyl)-4, 14-dihydroxy-6-oxomorphinan-3-carboxamide

(B)

Fig. 1: OZPE and SMPN structures and their IUPAC names

MATERIALS AND METHODS

Instrumentation

HPLC based OZPE and SMPN analysis was achieved using an HPLC system (model 2995, Waters Company, USA) with PDA detector (model 2998, Waters Company, USA). The system was run by the empower edition 2.0 software.

Chemicals

OZPE and SMPN were contributed from "Rainbow Pharma Training Labs" Telangana. Lybalvi tablets (Alkermes, Inc.) were utilised, which included 20 mg OZPE and 10 mg SMPN. peroxide, HCl, Na_2SO_4, and NaOH were obtained from "SD Fine Chemicals Ltd", Tamilnadu. "Merck India Ltd" in Tamilnadu provided the acetonitrile.

Standard solutions

OZPE and SMPN stock samples were made in mobile phase at concentrations of 200 µg/ml and 100 µg/ml, respectively. OZPE and SMPN working samples were also made in mobile phase with OZPE and SMPN stock samples at concentrations of 20 µg/ml and 10 µg/ml, respectively.

Conditions

HPLC based OZPE and SMPN analysis method employs "Sunsil C18 column" as the stationary phase with length 250 mm, identification 45 mm, particle size 3.5, and the mixture of 0.1 M Na_2SO_4 (pH 4.2): acetonitrile (70:30, vol:vol) was used as the mobile phase with 1.0 ml/min flow stream. PDA detection-based analysis of OZPE and SMPN was carried out at 242 nm. Test OZPE and SMPN, and standard OZPE and SMPN samples of 10 µL were injected for their analysis.

Calibration curve

Calibration curves for OZPE and SMPN standards were produced using standard concentrations varying from 10–30 µg/ml (OZPE) and 5–15 µg/ml (SMPN). Each of the standard OZPE and SMPN solution (10, 15, 20, 25, and 30 µg/ml of OZPE and 5.0, 7.5, 10.0, 12.5 and 15 µg/ml of SMPN) was applied on the "Sunsil C18 column" separately. The OZPE and SMPN samples are assessed by chromatography conditions stated. The calibration curves been created by graphing peak area *vs* concentration (µg/ml). The linearity (R^2) of the OZPE and SMPN calibration graphs were evaluated from regression assessment.

Preparation of OZPE and SMPN sample solution

A fine powder of OZPE and SMPN tablets was made by initially weighing and afterwards grinding 20 Lybalvi tablets. A precisely weighed tablet fine powder portion having 20 mg of OZPE and 10 mg of SMPN was transferred into separate volumetric flask that already holds 30 ml of HPLC mobile phase. The solution mix was subsequently sonicated over 30 min, cooled, and brought to a total volume of 100 ml with HPLC mobile phase. The obtained solution was sieved and diluted subsequently utilizing the HPLC mobile phase to achieve a standard OZPE and SMPN solution having concentration of 20 µg/ml (OZPE) and 10 µg/ml (SMPN). The detailed process given under "Conditions" and "Calibration curve" sections was preceded. The regression formula was engaged to calculate the OZPE and SMPN concentrations, as well as their recovery percent.

Stress degradation studies of OZPE and SMPN

During studies on OZPE and SMPN degradation, the mentioned stress conditions were established in full compliance with the ICH approach [14].

Study with acid

Stock tablet OZPE and SMPN solution (10 ml) was treated using HCl solution (0.1 N, 10 ml) at 27 °C with sonication for over 30 min time, cooled, and brought to a total volume of 100 ml with HPLC mobile phase and analysed with detailed process given under "Conditions" sections

Study with alkali

Stock tablet OZPE and SMPN solution (10 ml) was processed with NaOH solution (0.1 N, 10 ml) at 27 °C with ultrasonic treatment for over 30 min time, cooled, and brought to a total volume of 100 ml with HPLC mobile phase, and then analysed using the comprehensive steps outlined in the "Conditions" sections.

Study with peroxide

Stock tablet OZPE and SMPN solution (10 ml) was processed with peroxide solution (30%, 10 ml) at 27 °C with ultrasonic mixing for over 30 min time, cooled, and brought to a total volume of 100 ml with HPLC mobile phase, and then analysed using the ample steps outlined in the "Conditions" sections.

Study with dry heat

Placed stock tablet OZPE and SMPN solution (10 ml) at 105 °C in oven for over 30 min time. The sample was cooled and brought to a total volume of 100 ml with HPLC mobile phase. Analysed using the ample steps outlined in the "Conditions" sections.

Study with sun light

Exposed stock tablet OZPE and SMPN solution (10 ml) to direct sun for over six-hour time. The sample was cooled and brought to a total volume of 100 ml with HPLC mobile phase. Analysed utilising the detailed procedures specified in the "Conditions" sections.

RESULTS

Development of HPLC based OZPE and SMPN analysis process

A sequence of trials of the proposed approach were made based on the physical and chemical characteristics of the OZPE and SMPN, as well as scientific data gathered from the literature. The sequence of trials include: different constituents of mobile phase (phosphoric acid/methanol; KH_2PO_4/acetonitrile; Na_2SO_4/acetonitrile), detection wavelength, and column (ACE C18, 250 mm, identification 45 mm, particle size 3.5; Sunsil C18, 250 mm, identification 45 mm, particle size 3.5), with distinct pH values. An excellent separation with better symmetric peak profile for OZPE and SMPN was accomplished with “Sunsil C18 column” as the stationary phase with length 250 mm, identification 45 mm, particle size 3.5, and the mixture of 0.1 M Na_2SO_4 (pH 4.2): acetonitrile (70:30, vol:vol) as the mobile phase with 1.0 ml/min flow stream. A wavelength light of 242 nm was opted for measurement in order to maximise sensitivity for OZPE and SMPN as well as applicability for regular work. As illustrated in Fig. 2, the retention times for OZPE and SMPN were 3.547 and 4.906 min, respectively, underneath the given HPLC based OZPE and SMPN analysis conditions.

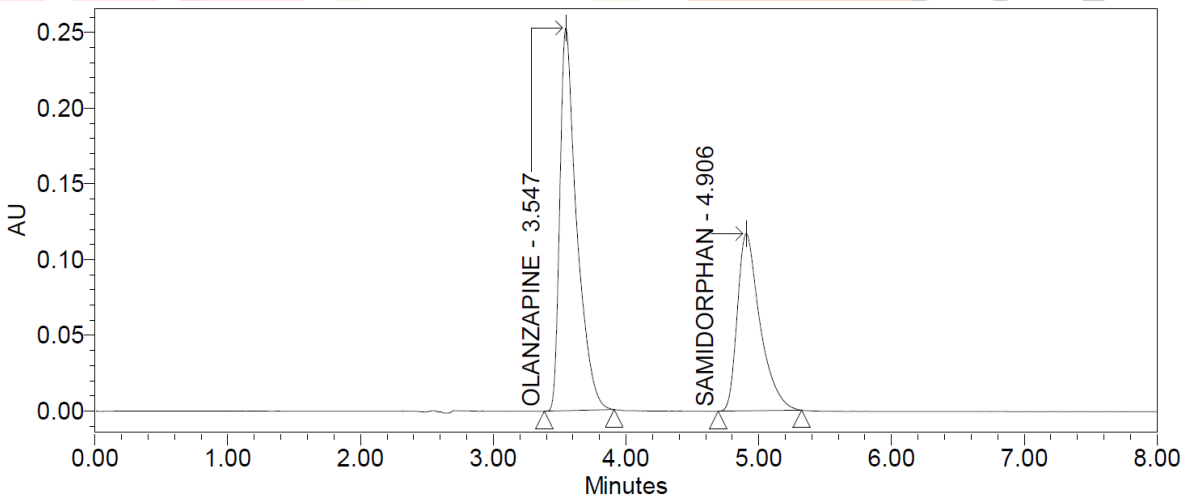
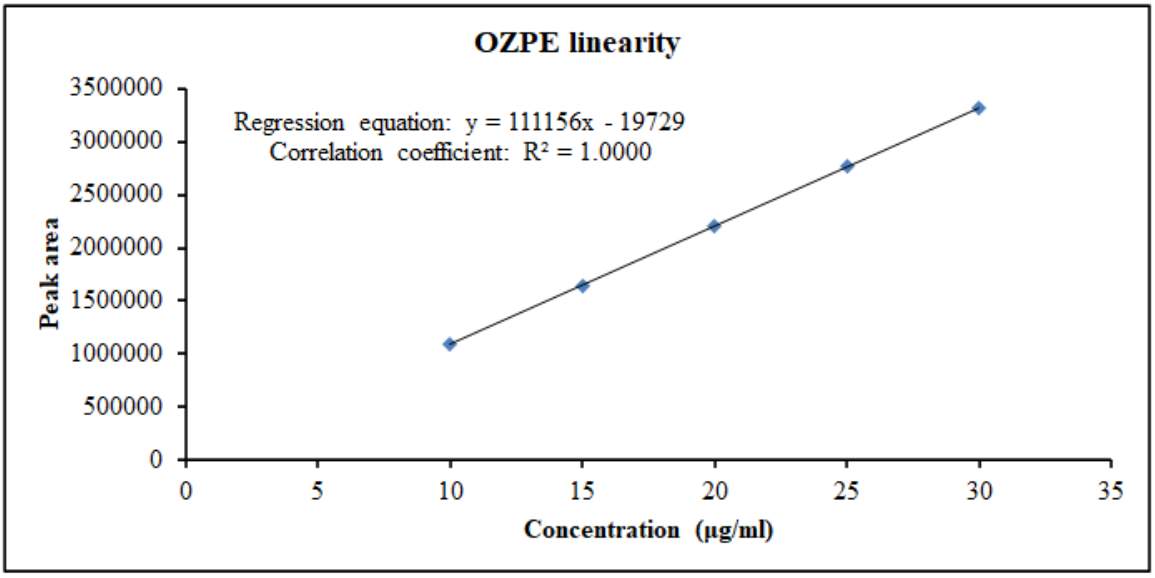


Fig. 2: OZPE and SMPN chromatogram with optimized analysis conditions

Validation

Linearity

The OZPE and SMPN calibration curves ranged from 10 - 30 µg/ml (OZPE, Fig. 3) and 5 - 15 µg/ml (SMPN, Fig. 3). The OZPE and SMPN linearities were established between 10 and 30 µg/ml, 5 and 15 µg/ml with a strong correlation coefficient (R^2) of higher than 0.9990.



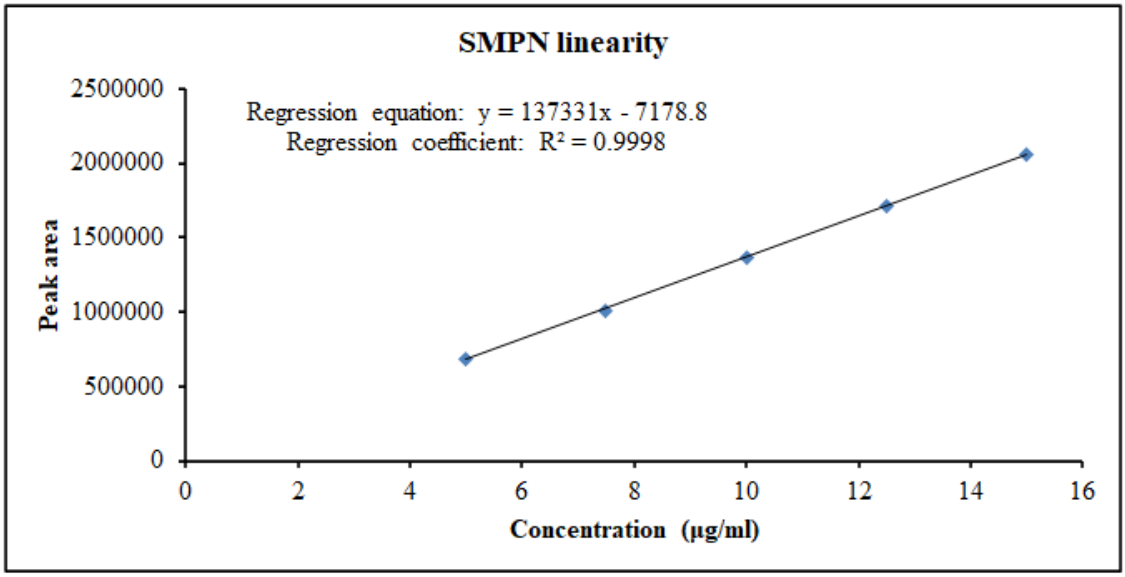


Fig. 3: OZPE and SMPN linearities and regression scores

Precision

Precision was conducted by assessing standard OZPE and SMPN solution samples (20 µg/ml of OZPE and 10 µg/ml of SMPN) six times on the similar day. The RSD and SD readings for the integration area were computed and used as a gauge of precision. Table 1 depicts the precision statistics of OZPE and SMPN in standard OZPE and SMPN solution specimens.

Accuracy

Accuracy was conducted by assessing standard OZPE and SMPN solution samples (20 µg/ml of OZPE and 10 µg/ml of SMPN) six times on the similar day. The assay percentage, RSD and SD readings from the integration area were computed and used as a gauge of accuracy. Table 1 depicts the accuracy statistics of OZPE and SMPN in standard OZPE and SMPN solution specimens.

Table 1: HPLC based method of OZPE and SMPN analysis precision/accuracy

Injection of OZPE and SMPN solution	OZPE Area	SMPN Area	% OZPE Assay	% SMPN Assay
	Precision		Accuracy	
1	2204798	1366733	98.72	98.80
2	2214072	1366719	99.14	98.80
3	2217262	1370855	99.28	99.10
4	2208891	1362761	98.91	98.51
5	2203623	1366693	98.67	98.80
6	2201635	1363739	98.58	98.58
Average gauged	2208380	1366250	98.88	98.76
SD gauged	6204.913	2840.203	0.278	0.205
RSD gauged	0.281	0.208	0.281	0.208

Selectivity

Selectivity was measured by adding specified quantities of OZPE and SMPN standard drugs (n = 3, at individual levels of 50, 100, and 150 percent) to tablet OZPE and SMPN samples (20 µg/ml of OZPE and 10 µg/ml of SMPN) in triplicate. During this study, the average recovery (Table 2) of the OZPE and SMPN concentrations was computed and utilised as a selectivity measure of method.

Table 2: HPLC based method of OZPE and SMPN analysis selectivity

µg/ml OZPE included	µg/ml OZPE found	% OZPE Recovered	% OZPE Mean recovered	SD gauged	RSD gauged
OZPE determined at 50% added range					
9.900	9.83	99.26			
9.900	9.86	99.65	99.23	0.4358	0.439
9.900	9.78	98.78			
OZPE determined at 100% added range					
19.800	19.73	99.64			
19.800	19.86	100.31	99.95	0.3378	0.338
19.800	19.78	99.90			
OZPE determined at 150% added range					
29.700	29.68	99.94			
29.700	29.65	99.83	99.97	0.1572	0.157
29.700	29.74	100.14			
µg/ml SMPN included	**µg/ml SMPN found**	**% SMPN Recovered**	**% SMPN Mean recovered**	**SD gauged**	**RSD gauged**
SMPN determined at 50% added range					
4.950	4.97	100.38	100.45	0.0651	0.065

4.950	4.98	100.51			
4.950	4.97	100.45			
SMPN determined at 100% added range					
9.900	9.85	99.46			
9.900	9.89	99.95	100.03	0.6139	0.614
9.900	9.97	100.68			
SMPN determined at 150% added range					
14.850	14.88	100.21			
14.850	14.94	100.57	100.28	0.2621	0.261
14.850	14.86	100.06			

Degradation studies

In varied stress situations, OZPE and SMPN degradation was detected, and the peaks for OZPE and SMPN were significantly alienated from the peaks for the degradation components. About 10.37% and 9.0% of OZPE and SMPN, respectively, was destroyed in HCl treatment with the degradant peaks aroused at 1.075, 2.712, 6.544, and 7.301 min (Fig. 4a). OZPE and SMPN has shown 91.97% and 92.39%, respectively, of stability during alkaline treatment with the degradant peaks aroused at 1.803, 2.554, and 6.250 min (Fig. 4b). During peroxide treatment, OZPE and SMPN stabilities were 94.63% and 93.73%, respectively with degradant peaks aroused at 1.436, 6.448 and 7.389 min (Fig. 4c). The stability of OZPE and SMPN was 88.93% and 89.97%, respectively, after treatment with dry heat. During dry eat study degradant peaks aroused at 1.398, 2.405, 6.901, and 7.233 min (Fig. 4d). The OZPE and SMPN demonstrated 90.59% and 93.87% stability under photo treatment, respectively, with degradant peaks occurring at 2.407, 6.691, 7.088, and 7.768 min (Fig. 4e).

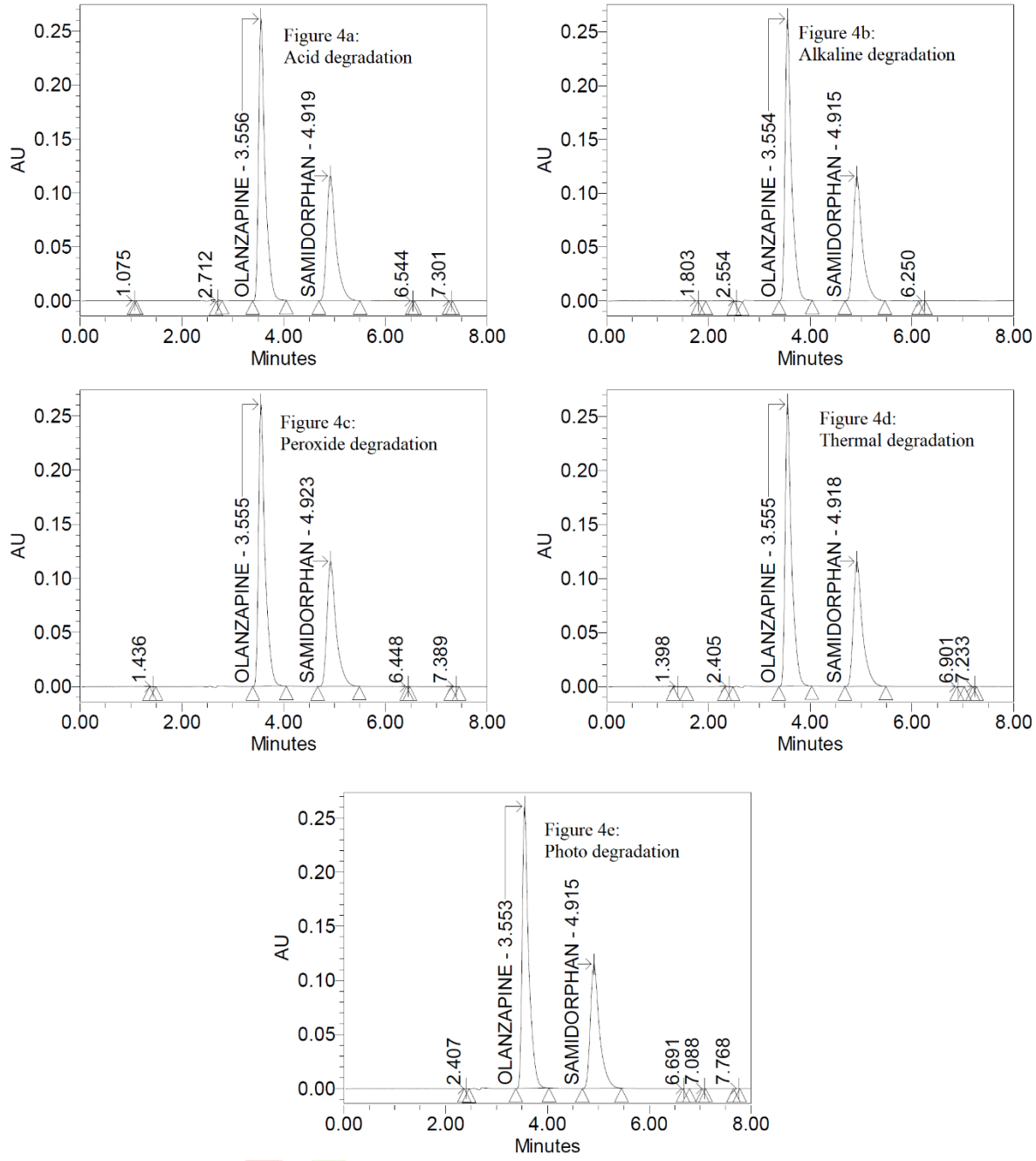


Fig. 4 a-e: OZPE and SMPN degradation chromatograms

Robustness

Small controlled changes in the flow rate (1.0±0.1 ml/min), column temperature (25± 2 °C), acetonitrile ratio (30±5%), detection nanometers (242±2 nm) and pH value (4.2±0.1 value) were applied to assess robustness. The RSD and SD readings for the integration area were computed and used as a gauge of robustness. Table 3 depicts the robustness statistics of OZPE and SMPN in minor variations of HPLC based OZPE and SMPN analysis method.

Table 3: HPLC based method of OZPE and SMPN analysis selectivity robustness

Robustness Conditions tested	OZPE				SMPN			
	Peak response	Mean gauged	SD gauged	RSD gauged	Peak response	Mean gauged	SD gauged	RSD gauged
Acetonitrile proportion								
Optimized 30% vol	2197968	2229168	31003.4	1.4	1352244	1377735	22163.3	1.6
Varied 25% vol.	2229567				1388517			
Varied 35% vol.	2259971				1392445			
Flow stream in column								
Optimized 1 ml/min	2197968	2235324	40543.3	1.8	1362244	1386201	22888.0	1.7
Varied 0.9 ml/min	2278439				1407844			
Varied 1.1 ml/min	2229567				1388517			
pH								
Optimized 4.2 value	2234798	2232812	2833.8	0.1	1386733	1387323	1033.9	0.1
Varied 4.1 value	2234072				1386719			
Varied 4.3 value	2229567				1388517			
Temperature inside analytical column								
Optimized 25 ºC	2229567	2235892	41281.1	1.8	1388517	1383377	22090.0	1.6
Varied 23ºC	2198139				1359171			
Varied 27ºC	2279971				1402445			
Wavelength								
Optimized 242 nm	2180163	2222587	39400.9	1.8	1408946	1386488	23538.2	1.7
Varied 240 nm	2258032				1362001			
Varied 244 nm	2229567				1388517			

Limit of detection (LOD) & quantitation (LOQ)

In this approach, the signal to noise relation was exploited to compute the Ld and Lq of OZPE and SMPN. The Ld is the content (µg/ml) of OZPE/SMPN with a signal to noise relation of 3:1, while their Lq is 10:1. OZPE's Ld and Lq were 0.041 µg/ml and 0.138 µg/ml, respectively. SMPN's Ld and Lq were 0.045 µg/ml and 0.149 µg/ml, respectively. Related chromatograms of OZPE and SMPN was given (Fig. 5a and 5b).

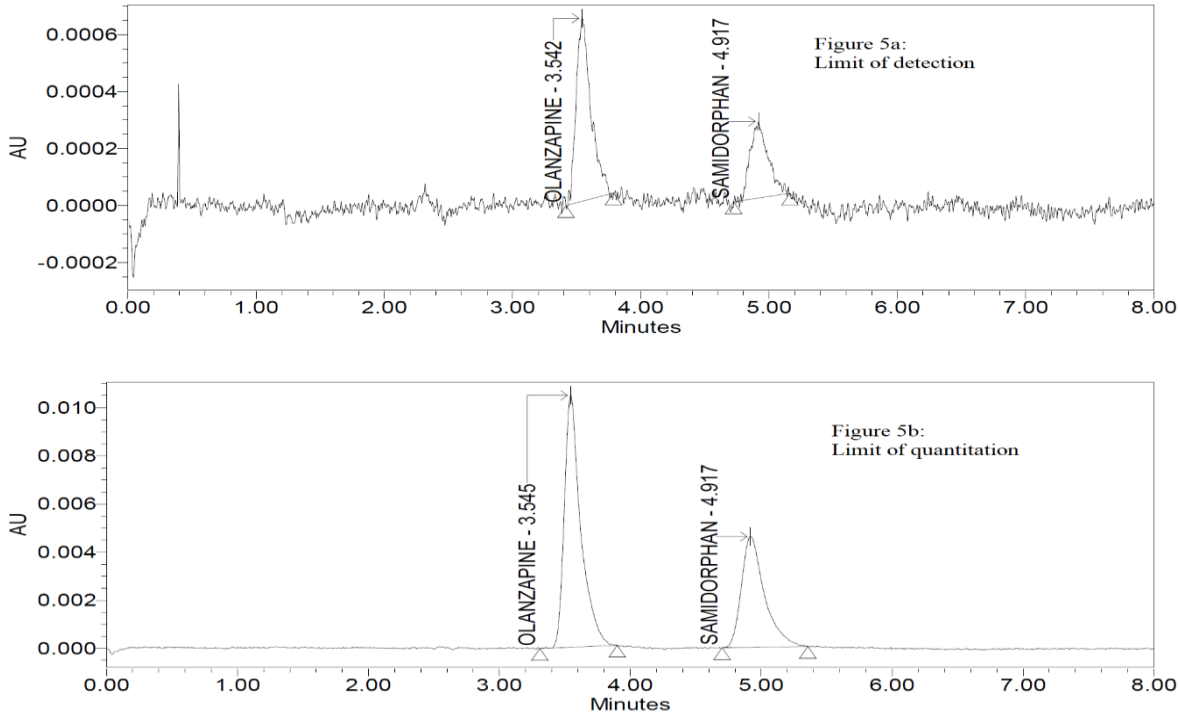


Fig. 5a, b: LOD and LOQ chromatograms for OZPE and SMPN

DISCUSSION

The pervasive use of antipsychotics (OZPE) and opioid antagonists (SMPN) for the therapy of mental illnesses, particularly schizophrenia, has piqued our attention in developing a quick and convenient "stability indicating HPLC" based evaluation method for the simultaneous estimation of OZPE and SMPN in raw bulk material and tablet products. The HPLC based OZPE and SMPN analysis method was validated following "ICH" standards for parameters [15]: sensitivity, linearity, selective, accuracy, specificity, precision and robustness.

The HPLC-based OZPE and SMPN analytical technique's linearity yielded correlation coefficients of 1.0000 (Fig. 3) and 0.9998 (Fig. 3) for OZPE and SMPN, respectively, criteria judged adequate [16]. The precision and accuracy findings (Table 1) were noticed to be well inside adequate limits, representing that the HPLC-based OZPE and SMPN analytical technique is precise and accurate [17]. The recovery percentage findings (Table 2) were noticed to be well inside adequate limits, representing that the HPLC-based OZPE and SMPN analytical technique is selective and demonstrates the absence of hindrance from excipients in OZPE and SMPN tablets [18,19]. The chromatograms of OZPE and SMPN after treatments with acid, peroxide, alkali, dry heat, and photo showed that the degradation products' peaks appeared alongside OZPE and SMPN peaks without interfering (Fig. 4a-e), showing that the approach is specific and also stability indicating [20-22]. Minor adjustments in flow rate, column temperature, acetonitrile content, wavelength and pH values (Table 3) were shown to have no effect on the assessment of the two drugs (OZPE and SMPN) investigated [23].

CONCLUSION

An analytical approach indicative of stability HPLC was devised. It enabled the quantification of OZPE and SMPN in raw bulk material and tablet products. The method turned out to be rapid, easy, and specific in the mere existence of OZPE and SMPN degradation products. The method also featured smaller times for retention, sample running, and smaller values measured for limit detection, and quantification. The validation characteristics stated in the ICH Q2 verification standards were fulfilled by the approach evaluated in this study.

V. ACKNOWLEDGMENT

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