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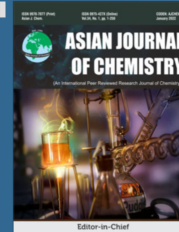

Assessment of Novel Stability-Indicating Technique-based HPLC Approach for One Genotoxic Impurity and Three Related Substance Impurities of Lurasidone Hydrochloride: Degradation Studies on Lurasidone Hydrochloride

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The present work communicates the selective quantification of a genotoxic impurity (P-LUH) and lurasidone hydrochloride (LUH) related compounds (I1-LUH, I2-LUH and I3-LUH) as potential impurities in the LUH active therapeutic ingredient using novel stability-indicating technique-based HPLC approach. The P-LUH, I1-LUH, I2-LUH and I3-LUH were separated on column Inerstil ODS 3V having length size of 250 mm, identification value of 4.6 mm and particle dimension of 5 µm. After that, a high sensitivity PDA detector at 231 nm was used to detect the signal of impurities. The method was thoroughly validated and established to be accurate and also precise with a rectilinear concentration range of 0.01% to 0.18% for P-LUH, 0.03% to 0.18% for I1-LUH, 0.033% to 0.18% for I2-LUH, 0.031% to 0.18% for I3-LUH and 0.03% to 0.12% for LUH with regard to a 10 µL injection. The LUH sample was exposed to UV-visible light open/closed conditions, heat for 10 days, relative humidity for 10 days, 4 N HCl for 3 days, 4 N NaOH for 1 h, 6% peroxide for 7 h, Milli Q water for 88 h and to 0.05 M Cu^{2+} for 88 h. The LUH was satisfactorily determined in the existence of degradation products including impurities, the LUH was satisfactorily determined. The formation of P-LUH, I1-LUH, I2-LUH and I3-LUH during degradation studies on LUH was also studied. The P-LUH, I1-LUH, I2-LUH and I3-LUH were effectively determined in LUH batches and the findings indicated the use of a novel stability-indicating technique-based HPLC approach for the selective assessment of trace impurities in LUH drug substances.

Keywords: Lurasidone hydrochloride, Genotoxic impurity, Related substances, Stability indicating, HPLC approach.

INTRODUCTION

Schizophrenia is a kind of psychosis characterized by emotions, distorted thinking, perception, language, behaviour and sense of self [1,2]. Schizophrenia impacts 20 million individuals globally, yet it is less frequent than many other mental illnesses. Schizophrenia causes significant impairment and can have an effect on academic and occupational functioning. Lurasidone hydrochloride (LUH) is an FDA-approved medication for the treatment of schizophrenia's clinical symptoms [3,4]. The effectiveness of LUH in the treatment of schizophrenia is assumed to be mediated primarily *via* antagonisms of central D2 and 5-HT2a receptors.

3-(1-Piperazinyl)-1,2-benzisothiazole (P-LUH impurity) is a precursor in LUH synthesis and also a genotoxic impurity. The subsequent molecules mentioned are the intermediates *viz.* (3aR,7aR)-4′-(benzo[*d*]isothiazol-3-yl)octahydrospiro-[isoindole-2,1′-piperazin]-1′-ium methanesulfonate (I1-LUH impurity), (3aR,4R,7S,7aS)-2-(((1R,2R)-2-((4-(benzo[*d*]-isothiazol-3-yl)piperazin-1-yl)methyl) cyclohexyl)methyl)-hexahydro-1*H*-4,7-methanoisoindole-1,3(2*H*)-dione (I2-LUH impurity), and (3aR,4S,7R,7aS)-2-(((R,S)-(1,2)-2-((4-(benzo-[*d*]isothiazol-3-yl)piperazin-1-yl)methyl)cyclohexyl)methyl)-hexahydro-1*H*-4,7-methanoisoindole-1,3(2*H*)-dione (I3-LUH impurity) generated in the course of lurasidone hydrochloride (LUH) synthesis:

According to several regulatory organizations, impurities are limited to 0.15% in pure drug material [5,6]. In order to execute batch release testing and perform stability testing process for lurasidone hydrochloride drug material, a stability-

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indicating quantitative method is deemed necessary to distinguish the LUH peak from all the peaks of potential lurasidone hydrochloride degradation products as well as process linked LUH related impurities (P-LUH, I1-LUH, I2-LUH and I3-LUH) and to separate these molecules from one another. Impurities in LUH must also be evaluated at the time of product release and during the product's shelf life.

As per the different regulatory regulations and worldwide management standards, developing analytical techniques for determining impurities, particularly active drug ingredient correlated impurities, is currently a key priority. At all phases of the study, from sample gathering and preparation to separation and ultimate evaluation, chromatographic methods have the ability to match worldwide trends toward sustainable chemistry [7]. In pharmaceutical sectors, these chromatographic approaches are a well-accepted as well as multi-purpose strategy for drug quantification as well as impurity analysis applications [8,9].

Due to the multiple, structurally identical components (Fig. 1) that must be separated and monitored throughout the shelf-life of the drug, developing a stability-indicating technique for lurasidone hydrochloride containing medicinal substances is difficult. According to a literature exploration, there is no recognized analytical technique that can detect and evaluate both genotoxic impurity (P-LUH) and all LUH related substances (I1-LUH, I2-LUH and I3-LUH) in LUH drug material by one stability-indicating technique-based method. Accordingly, the goal of this research process was to create a simple and novel stability-indicating technique-based approach for the simultaneous quantitative measurement of genotoxic impurity (P-LUH) and all lurasidone hydrochloride (LUH) related compounds (I1-LUH, I2-LUH and I3-LUH) in the drug material. Further, this approach may also be used to assess the purity of lurasidone hydrochloride (LUH) batch samples.

EXPERIMENTAL

Reference impurities and LUH materials: The reference materials of lurasidone hydrochloride (LUH) (batch no. API-LUH-01, purity 99.7%), P-LUH (batch no. API-P-LUH-01, purity 95.8%), I1-LUH (batch no. API-I1-LUH-02, purity 95.5%), I2-LUH (batch no. API-I2-LUH-ENDO-03, purity 98.8%) and I3-LUH (batch no. API-I3-LUH-04, purity 96.2%) were obtained as gift materials from Inogent Laboratories, Hyderabad, India. Lurasidone hydrochloride (LUH) tablets, Lurastar (labelled content 40 mg, batch No: D2106048) was purchase from Linux Laboratories Pvt. Ltd, Chennai, India.

Purchased potassium hydroxide (batch no. STBH4233), triethyl amine (batch no. 0010000280) from Sigma-Aldrich

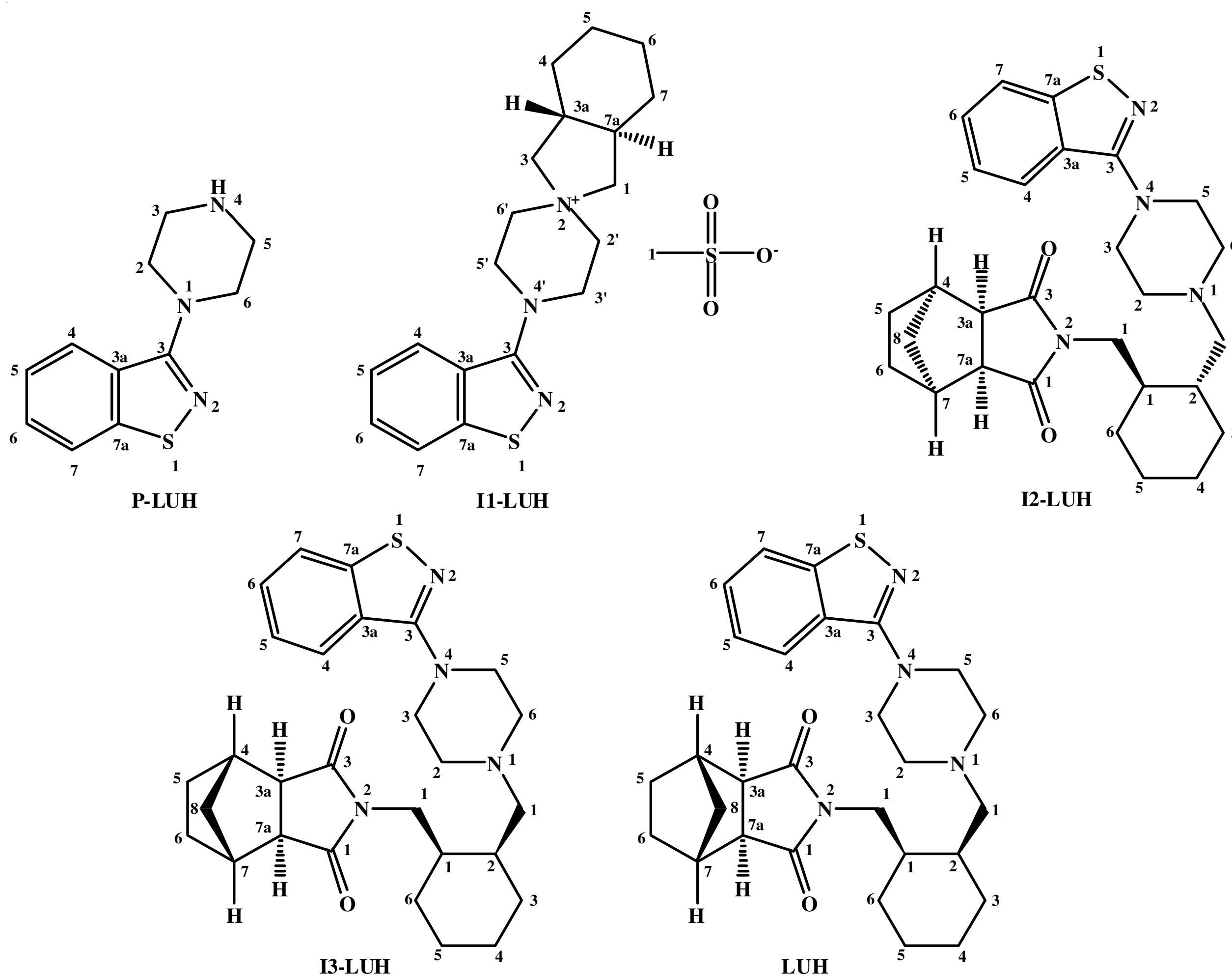

Fig. 1. Lurasidone hydrochloride (LUH) and its genotoxic and related impurities

and phosphoric acid (batch no. R269G20) from Rankem™ Chemicals, India. Acetonitrile (batch no. 0000265177) and methanol (batch no. 321351024FT) were purchased from Standard Reagents Pvt. Ltd., India. The Milli Q water was purchased from Merck Ltd., India.

Instruments: Aligent make HPLC system (identification no. ARDHPLC-002 & AMVHPLC-005) and column Inerstil ODS 3V (identification no. LC-01-20/001 and LC-11-21/001) having length size of 250 mm, identification value of 4.6 mm and particle dimension of 5 µm were employed for the simultaneous quantitative measurement of genotoxic impurity (P-LUH) and all LUH related compounds (I1-LUH, I2-LUH and I3-LUH) in LUH drug material and formulation of LUH.

Optimized conditions for assay, method validation and stability testing: The mobile phase buffer was a potassium dihydrogen phosphate solution (pH 3.2; 2.0 M). Solvent system I (SS-I) consisted of 95% volume of mobile phase buffer and 5% volume of acetonitrile, whereas solvent system II (SS-II) consisted of 20% volume of mobile phase buffer and 80% volume of acetonitrile. SS-I and SS-II were employed in linear gradient manner elution with a column flow speed of 0.8 mL/min. Elution was made in linear gradient manner for 70 min in the succeeding sequence: 0 min (SS-I 70% volume: SS-II 30% volume), 5 min (SS-I 70% volume: SS-II 30% volume), 30 min (SS-I 40% volume: SS-II 60% volume), 42 min (SS-I 0.0% volume: SS-II 100% volume), 55 min (SS-I 0.0% volume: SS-II 100% volume), 55.1 min (SS-I 70% volume: SS-II 30% volume) and 70 min (SS-I 70% volume: SS-II 30% volume). Diluent used was a mixture of 70% volume of SS-I and 30 % volume of SS-II. Needle wash solution consisted of 70% volume of acetonitrile and 30 % volume of water. The quantification of the genotoxic impurity (P-LUH) and all LUH related compounds (I1-LUH, I2-LUH and I3-LUH) was based on computing their peak response areas at 231 nm. The injection volume, column set temperature and sampler temperature were 10 µL, 35 ºC and room temperature, respectively.

Impurities solution: In volumetric flask of 10 mL size, a mixed standard solution of P-LUH, I1-LUH, I2-LUH and I3-LUH was formulated with diluent to yield an absolute concentration of 0.15% for each impurity.

System suitability solution: In a volumetric flask of 50 mL size, weighed accurately 7 mg of I3-LUH and 7 mg of LUH standard and sonicated to dissolve using 15 mL of SS-II and bring up to volume (50 mL) with SS-I. To volumetric flask of 10 mL size containing 3 mL of SS-II, spiked 50 µL of above solution and bring up to volume (10 mL) with SS-I.

Test LUH sample: LUH sample was made in volumetric flask of 10 mL size thrrough weighing 7 mg of LUH sample, sonicated to disperse uniformly using 3 mL of SS-II and make up to volume (10 mL) with SS-I.

Test LUH tablet, lurastar, sample: The powdered and weighed ten lurastar tablets sample was made in volumetric flask of 10 mL size through weighing powered tablet sample equal to 7 mg of LUH sample, sonicated to disperse uniformly using 3 mL of SS-II and make up to volume (10 mL) with SS-I.

Determination of known and unknown impurities in LUH drug substances and tablet, Lurastar: Injected (10 µL) diluent blank (2 injections), impurities solution (1 injection), system suitability solution (6 injections) and test LUH drug material sample/LUH Lurastar sample (1 injection) into column Inerstil ODS 3V and analyzed for known & unknown impurities using proposed HPLC conditions. The chromatograms were ascertained at 231 nm and the peaks owing to blanks were removed. Then lock the peaks due to HCl, DMF and toluene emanating at RRT around 0.12, 0.14 and 1.5, respectively. Reported the results by external calculation using the below given formulae. Integrated the known impurities at the specified wavelength (231 nm) only. Calculated each of the single maximum unknown impurities at 231 nm wavelength by external calculation and reported the results at higher area percentage.

$$\text{Known impurity (\%)} = \frac{\text{AKI} \times \text{SP} \times \text{Ws} \times \text{DF} \times \text{SD} \times 100 \times \text{CF}}{\text{Avg. area of LUH (SST solution)} \times \text{Wt} \times 100}$$

$$\text{Unknown impurity (\%)} = \frac{\text{AUKI} \times \text{SP} \times \text{Ws} \times \text{DF} \times \text{SD} \times 100 \times \text{CF}}{\text{Avg. area of LUH (SST solution)} \times \text{Wt} \times 100}$$

where, AKI = area of known impurity; AUKI = area of unknown impurity; SP = standard potency of impurity; Ws = weight of impurity standard; CF = correction factor; DF = dilution factor; SST = system suitability; and Wt = weight of test LUH drug material sample/LUH lurastar sample.

Degradation studies on test LUH sample: According to International Council for Harmonization (ICH) requirements [10], the degradation studies on test LUH sample were evaluated using suggested HPLC approach.

Thermal degradation: Test LUH sample was subjected to heat (60 ºC) for 10 days. The sample was processed as discussed in Test LUH sample section, then analyzed by deploying the proposed HPLC conditions for LUH-related substances and the percentile degradation of LUH was recorded.

Humidity: Test LUH sample was subjected to relative humidity (90%) for 10 days. The sample was processed as discussed in the Test LUH sample section, then analyzed deploying proposed HPLC conditions for LUH-related substances and the percentile degradation of LUH was recorded.

Photodegradation: The test LUH sample was subjected to visible and UV lights in a photostability enclosure chamber for at least 1.2 million lux hour & 200 Watt h/m^2 in photo open state. The test LUH sample packed with aluminium cover was subjected to visible and UV lights in a photostability enclosure chamber for at least 1.2 million lux hour & 200 Watt h/m^2 in photo closed state.

The above test LUH samples were processed as discussed in the subsections "Test LUH sample" and Test LUH tablet, Lurastar, sample", then analyzed deploying proposed HPLC conditions for LUH-related substances and the percentile degradation of LUH was recorded in photo open state, photo closed state and photo closed actual packing conditions.

Acid hydrolysis: Weighed and placed 250.32 mg of LUH sample into volumetric flask of size 100 mL, then added 1:1 acetonitrile:water solution (50 mL), brought up to volume (100 mL) adding 4 N HCl solution and well mixed. For three days, this prepared sample was held in a water bath (70 ºC). Following 3 days, transferred 2.8 mL of acid-degraded LUH sample to

the volumetric flask of size 10 mL and diluted with diluent to volume (10 mL). Analyzed this sample deploying proposed HPLC conditions for LUH-related substances and the percentile degradation of LUH was recorded.

Base hydrolysis: The LUH sample (250.43 mg) was precisely weighed and placed into the volumetric flask of size 100 mL. Added methanol (95 mL), brought up to final volume (100 mL) by adding 4 N NaOH solution and completely mixed. For 1 h, this prepared sample was held at room temperature. Following 1 h, transferred 2.8 mL of NaOH degraded LUH sample to volumetric flask of size 10 mL and diluted with diluent to volume (10 mL). Analyzed this sample deploying proposed HPLC conditions for LUH-related substances and the percentile degradation of LUH was recorded.

Peroxide hydrolysis: Weighed and placed 249.48 mg of LUH sample into a flask sized 100 mL volume, then added 30 mL of 1:1 acetonitrile:water solution, brought up to volume (100 mL) adding 6% peroxide solution and well blended. For 7 h, this prepared sample was held at dark (protected from light) in room temperature. Following 7 h, transferred 2.8 mL of peroxide-degraded LUH sample to volumetric flask of size 10 mL and diluted with diluent to volume (10 mL). Analyzed this sample deploying proposed HPLC conditions for LUH-related substances and the percentile degradation of LUH was recorded.

Water hydrolysis: Accurately weighed and deposited LUH sample (249.59 mg) into volumetric flask sized 100 mL volume, then appended 1:1 acetonitrile:water solution (50 mL), brought up to volume (100 mL) thru adding Milli Q water and blended thoroughly for 2 days in a waterbath (70 ºC). Following 2 days, transferred 2.8 mL of milli Q water-degraded LUH sample to volumetric flask sixed 25 mL volume and diluted with diluent to final volume (25 mL). Analyzed this sample deploying proposed HPLC conditions for LUH-related substances and the percentile degradation of LUH was recorded.

Metallic ion hydrolysis: Weighed and placed 250.83 mg of LUH sample into volumetric flask of size 100 mL, then added 1:1 acetonitrile:water solution (50 mL), brought up to volume (100 mL) adding 0.05 M Cu^{2+} solution and well mixed. For 4 days, this prepared sample was held at room temperature. Following 4 days, transferred 2.8 mL of 0.05 M Cu^{2+}-degraded LUH sample to volumetric flask of size 25 mL and diluted with diluent to volume (25 mL). Analyzed this sample deploying proposed HPLC conditions for LUH-related substances and the percentile degradation of LUH was recorded.

RESULTS AND DISCUSSION

Optimization methodology: The recommended HPLC method was intended to use in routine concurrent quantitative measurements of genotoxic impurity (P-LUH) and all LUH related compounds (I1-LUH, I2-LUH and I3-LUH) in LUH drug material and lurastar LUH tablets without negatively impacting the environment by using no harmful chemicals and producing minimal waste. Some solvents, including toluene, benzene and chloroform were omitted from the mobile phase preparation due to their toxicity and detrimental environmental impact. To regulate the analysis time and acquire the optimal parameters, a gradient manner elution investigation was triggered using varied time-solvent programmes. Optimal separation of the genotoxic impurity (P-LUH) and all LUH related compounds (I1-LUH, I2-LUH and I3-LUH) was obtained using solvent system I (SS-I) consisted of 95% volume of mobile phase KH_2PO_4 buffer (pH 3.2; 2.0 M) and 5% volume of acetonitrile, whereas solvent system II (SS-II) consisted of 20% volume of mobile phase KH_2PO_4 buffer (pH 3.2; 2.0 M) buffer and 80% volume of acetonitrile. The gradient mode program followed was: 0 min (SS-I 70% volume: SS-II 30% volume), 5 min (SS-I 70% volume: SS-II 30% volume), 30 min (SS-I 40% volume: SS-II 60% volume), 42 min (SS-I 0.0% volume: SS-II 100% volume), 55 min (SS-I 0.0% volume: SS-II 100% volume), 55.1 min (SS-I 70% volume: SS-II 30% volume) and 70 min (SS-I 70% volume: SS-II 30% volume) drove at a stream rate of 0.8 mL/min. Furthermore, scanning at 231 nm revealed high sensitivity for the genotoxic impurity (P-LUH) and all LUH related compounds (I1-LUH, I2-LUH and I3-LUH). Fig. 2 shows a chromatogram with adequate resolution and an appropriate analysis time that shows all separated peaks.

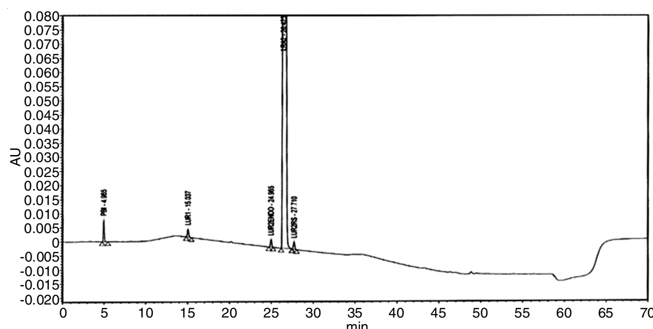

Fig. 2. Chromatogram of genotoxic impurity (P-LUH) and all LUH related compounds (I1-LUH, I2-LUH and I3-LUH)

Method validation: According with International Council for Harmonization (ICH) requirements [11], the suggested HPLC approach was evaluated.

System suitability: The system suitability (LUH – 0.15% and I3-LUH – 0.15%) solution and standard LUH (0.15%) solution were analyzed. Resolution between LUH and I3-LUH from system suitability (LUH – 0.15% and I3-LUH – 0.15%) solution injection was 3.4. Percentile RSD for the area of LUH and I3-LUH from six system suitability solution injections at 231 nm were 0.77% and 1.1%, respectively. Tailing factor for LUH from standard LUH (0.15%) solution injection was 1.8. The computed system suitability parameter's values were consistent with USP reference values [12], indicating that suggested HPLC technique performed well.

Specificity: Impurities solution (P-LUH – 0.15%, I1-LUH – 0.15%, I2-LUH – 0.15% and I3-LUH – 0.15%) and test LUH sample spiked with genotoxic impurity (P-LUH – 0.15%) and all LUH related compounds (I1-LUH – 0.15%, I2-LUH – 0.15% and I3-LUH – 0.15%) were analyzed. The retention times and peak purities of P-LUH, I1-LUH, I2-LUH and I3-LUH were determined from impurities solution injection and spiked test LUH sample injection (Table-1). Purity angles of P-LUH, I1-LUH, I2-LUH and I3-LUH was smaller than its

TABLE-1
RETENTION TIMES AND PEAK PURITIES OF P-LUH, I1-LUH, I2-LUH AND I3-LUH

Impurity	Spiked test LUH sample			Impurities solution		
	Retention time (min)	Purity1 angle	Purity1 threshold	Retention time (min)	Purity1 angle	Purity1 threshold
P-LUH	5.856	6.302	20.244	5.895	2.101	9.407
I1-LUH	17.986	13.742	54.430	18.033	6.037	23.003
I2-LUH	28.943	10.431	43.309	28.937	11.936	44.876
I3-LUH	32.073	11.544	54.717	32.028	6.886	23.359

purity threshold measures and purity testing revealed no flags on the peaks of P-LUH, I1-LUH, I2-LUH and I3-LUH. The results confirmed that P-LUH, I1-LUH, I2-LUH and I3-LUH are quite well separated and specified and the peak purities of P-LUH, I1-LUH, I2-LUH and I3-LUH is within acceptable standards.

Limit of detection and limit of quantification: In contrast to blank diluent, the lowest concentrations of P-LUH, I1-LUH, I2-LUH and I3-LUH was regarded as detection limit and quan-titation limit with a "signal-to-noise" ratio of ≥ 3 and ≥ 10, respectively (Table-2). The low values conquered demonstrate the recommended method's excellent sensitivity, which enables for trace analysis and identification of extremely small levels of P-LUH, I1-LUH, I2-LUH and I3-LUH below their specified concentration limits.

TABLE-2
DETECTION AND QUANTITATION LIMITS OF P-LUH, I1-LUH, I2-LUH AND I3-LUH

Impurity	Detection limits		Quantitation limits	
	Conc. (%)	Signal: noise proportion	Conc. (%)	Signal: noise proportion
P-LUH	0.003374	5.404673	0.010223	47.99
I1-LUH	0.009915	8.623896	0.030046	46.12
I2-LUH	0.010810	8.165650	0.032759	41.46
I3-LUH	0.010249	7.470848	0.031058	40.55
LUH	0.009756	8.060441	0.029565	40.86

Linearity: The linearity of genotoxic impurity (P-LUH - from 0.01% to 0.18%), all LUH related compounds (I1-LUH - from 0.03% to 0.18%; I2-LUH - from 0.033% to 0.18%; I3-LUH - from 0.031 to 0.18%) and LUH (from 0.03% to 0.12%) was analyzed, which corresponds to quantitation limit to 120% of specified concentration. The correlation coefficient, Y-intercept measures at 100% of test concentration, slope, residual sum of squares and intercept were evaluated for LUH, P-LUH, I1-LUH, I2-LUH and I3-LUH (Table-3). The values conquered demonstrate the recommended method's excellent linearity for assessment of P-LUH, I1-LUH, I2-LUH and I3-LUH in LUH drug substances.

Precision: Test LUH sample spiked with the genotoxic impurity (P-LUH) and all LUH related compounds (I1-LUH – 0.15%, I2-LUH – 0.15% and I3-LUH – 0.15%) were analyzed for precision. On the same day, the precision was assessed by redoing the assessment of three different chosen concentrations (80%, 100% and 120% of specified test levels) of each impurity six times. The averages, standard deviations and relative deviations for P-LUH, I1-LUH, I2-LUH and I3-LUH were calculated (Table-4). Satisfactory values ensured the precision.

TABLE-3
DETECTION AND QUANTITATION LIMITS OF P-LUH, I1-LUH, I2-LUH AND I3-LUH

	P-LUH	I1-LUH	I2-LUH	I3-LUH	LUH
Range (% concentration)	0.01 to 0.18	0.03 to 0.18	0.033 to 0.18	0.031 to 0.18	0.03 to 0.12
Correlation coefficient	0.999	0.999	0.999	0.999	0.998
Y-Intercept value near 100% test concentration	-0.850585	-1.40152	0.755715	-1.79842	2.227
Residual sum of squares	5193659	1805370	1114487	824177	540090
Slope	60833878	32388279	26831909	27782840	24896152
Intercept	-515.156	-445.751	210.987	-499.309	399.2906

TABLE-4
PRECISION MEASURES OF P-LUH, I1-LUH, I2-LUH AND I3-LUH

	P-LUH	I1-LUH	I2-LUH	I3-LUH
80% specification level				
Average* (% concentration)	0.128641	0.115786	0.156374	0.150720
Stdev	0.00237323	0.00224146	0.00290756	0.00275994
%RSD	1.8%	1.9%	1.9%	1.8%
100% specification level				
Average* (% concentration)	0.139085	0.117427	0.164027	0.158345
Stdev	0.00181169	0.00245971	0.00187692	0.00239984
%RSD	1.3%	2.1%	1.1%	1.5%
120% specification level				
Average* (% concentration)	0.193412	0.180844	0.224541	0.224541
Stdev	0.00266244	0.00461951	0.00444263	0.00403650
%RSD	1.4%	2.5%	1.9%	1.8%

*Average of six % concentration values determined

Accuracy: The accuracy research was performed in the 80%, 100% and 120% of test concentration ranges. Prepared the impurities spiked test LUH solutions three times and analyzed each solution deploying proposed HPLC conditions for genotoxic impurity (P-LUH) and LUH-related substances (I1-LUH, I2-LUH and I3-LUH). Calculated the percentile impurity for the impurities (P-LUH, I1-LUH, I2-LUH and I3-LUH) content from three preparations. At each test concentration range, the percentile recovery of P-LUH, I1-LUH, I2-LUH and I3-LUH were calculated. Satisfactory values (Table-5) ensured the accuracy.

Ruggedness: The ruggedness of the procedure was proved by analyzing the same sample deploying proposed HPLC conditions on multiple systems (system 1 identification no. ARDHPLC-002, ARDHPLC-008, APQC-041 and system 2 identification no. AMVHPLC-005), by different analysts (Analyst 1 and 2), in different columns (column 1 identification no. LC-01-20/001 and column 2 identification no. LC-11-21/001) and in different labs (Lab 1: ADL-LAB and Lab 2: AMV-LAB) on the same day. Ruggedness was demonstrated by injecting 100% impurities spiked test LUH solutions at six different preparations and calculated percentile relative deviations for the impurities results. Calculated the impurities content and percentile relative deviations for six 100% impurities spiked test results (Table-6). Satisfactory values ensured the ruggedness.

Mobile phase and test LUH solution stability: Established the stability of 100% impurities spiked LUH sample solution and mobile phase, which were exploited in the estimation of percentile of impurities (P-LUH, I1-LUH, I2-LUH and I3-LUH), over a three-day period. Prepared the sample LUH solution spiked with P-LUH, I1-LUH, I2-LUH and I3-LUH at 100% of specification. Prepared the mobile phase according to the test technique and kept it tightly closed. Prepared and analyzed the sample LUH solution by spiking with impurities at 100% specification level freshly. Calculated the percentile of impurities (P-LUH, I1-LUH, I2-LUH and I3-LUH) for the spiked LUH sample solution. Stored the mobile phase and impurities spiked LUH sample solution on refrigerated. On day 1 and day 3, used the stored mobile phase and injected freshly prepared impurities spiked LUH sample solution followed by stored impurities spiked LUH sample solution. Calculated the percentile of P-LUH, I1-LUH, I2-LUH and I3-LUH for stored and freshly prepared solutions following the planned HPLC conditions for the estimation of stabilities of sample LUH solution (Table-7) and mobile phase (Table-8). Satisfactory values ensured the stabilities of sample LUH solution and mobile phase over a three-day period.

Robustness: During robustness testing, impurities spiked LUH test solution at 100% of specification level was analyzed with alterated conditions like mobile phase buffer in SS-II (± 10% change), flow rate (± 0.2 mL per min change), acetonitrile in SS-I (± 10% change), temperature (± 5°C change) and mobile phase pH (± 0.2 units change). Calculated percentile recoveries for all impurities (P-LUH, I1-LUH, I2-LUH and

TABLE-5
ACCURACY MEASURES OF P-LUH, I1-LUH, I2-LUH AND I3-LUH

Impurity	Specification level					
	80%		100%		120%	
	Theoretical conc. (%)	Mean* % recovery	Theoretical conc. (%)	Mean* % recovery	Theoretical conc. (%)	Mean* % recovery
P-LUH	0.119547	92.197	0.146985	90.010	0.181281	92.486
I1-LUH	0.116417	100.512	0.145522	92.438	0.179477	93.877
I2-LUH	0.125547	109.893	0.152665	105.450	0.185809	109.304
I3-LUH	0.123411	94.275	0.150068	95.348	0.182648	97.490

*Mean of three recovery values

TABLE-6
RUGGEDNESS MEASURES OF P-LUH, I1-LUH, I2-LUH AND I3-LUH

Condition	% Concentration determined			
	P-LUH	I1-LUH	I2-LUH	I3-LUH
Analyst 1; System 1 identification no – ARDHPLC-002; Column 1 identification no. LC-01-20/001; Lab 1 – ADL-LAB	0.14122	0.12019	0.16434	0.16042
	0.13954	0.11791	0.16391	0.15635
	0.13872	0.11698	0.16577	0.15857
	0.14004	0.11851	0.16581	0.16106
	0.13916	0.11808	0.16364	0.15890
	0.13583	0.11289	0.16069	0.15477
Analyst 2; System 2 identification no – AMVHPLC-005; Column 2 identification no. LC-11-21/001; Lab 2 – AMV-LAB	0.14163	0.12679	0.15290	0.16268
	0.14180	0.12700	0.15518	0.16382
	0.13739	0.12312	0.15037	0.15764
	0.14154	0.12742	0.15571	0.16397
	0.14364	0.12759	0.15598	0.16539
	0.15610	0.13852	0.16819	0.18021
Average*	0.14139	0.12292	0.16021	0.16198
Stdev	0.00510	0.00693	0.00590	0.00660
%RSD	3.60653	5.63919	3.68067	4.07310

*Average of 12% concentration values determined

TABLE-7
RESULTS OF SAMPLE LURASIDONE HYDROCHLORIDE (LUH) SOLUTION STABILITY

Impurity	Impurity (%)		Variation	Acceptance criteria
	Initial	1st day bench top		
P-LUH	0.138848	0.127094	8.4654	± 20%
I1-LUH	0.135016	0.119319	11.6260	± 20%
I2-LUH	0.167965	0.150508	10.3932	± 20%
I3-LUH	0.160545	0.143092	10.8711	± 20%
Unspecified impurity	0.022830	0.020778	0.002052	± 0.03%
Total impurities	0.772364	0.690023	10.6609	± 15%
	Initial	1st day 2-8 °C	Variation	Acceptance criteria
P-LUH	0.138848	0.119474		
I1-LUH	0.135016	0.114939	14.8701	± 20%
I2-LUH	0.167965	0.143683	14.4566	± 20%
I3-LUH	0.160545	0.134554	16.1892	± 20%
Unspecified impurity	0.022830	0.020226	0.002604	± 0.03%
Total impurities	0.772364	0.654156	15.3047	± 15%
	Initial	3rd day	Variation	Acceptance criteria
P-LUH	0.138848	0.145572		
I1-LUH	0.135016	0.134721	0.218	± 20%
I2-LUH	0.167965	0.170744	-1.654	± 20%
I3-LUH	0.160545	0.162142	-0.995	± 20%
Unspecified impurity	0.022830	Not detected	0.022830	± 0.03%
Total impurities	0.772364	0.752063	2.628	± 15%

TABLE-8
RESULTS OF MOBILE PHASE STABILITY

Impurity	Impurity (%)		Variation	Impurity (%)	Variation	Acceptance criteria
	Initial	1st day		3rd day		
P-LUH	0.138848	0.127094	8.4654	0.149655	-7.783	± 20%
I1-LUH	0.135016	0.119319	11.6260	0.142648	-5.653	± 20%
I2-LUH	0.167965	0.150508	10.3932	0.174469	-3.872	± 20%
I3-LUH	0.160545	0.143092	10.8711	0.166776	-3.881	± 20%
Unspecified impurity	0.022830	0.020778	0.002052	Not detected	0.022830	± 0.03%
Total impurities	0.772364	0.690023	10.6609	0.778288	-0.767%	± 15%

I3-LUH) results with altered conditions. The results (Table-9) revealed method's rugged nature at 100% of specification level.

Degradation studies on LUH sample: The LUH sample was exposed to UV-visible light open/closed conditions in photo chamber, to heat of 60 ºC for 10 days, to 90% relative humidity for 10 days, to 4 N HCl at 70 ºC for 3 days, to 4 N NaOH at ambient temperature for 1 h, to 6% peroxide at ambient temperature for 7 h, to Milli Q water at 70 ºC for 88 h and to 0.05 M Cu^{2+} at ambient temperature for 88 h. After specified exposure conditions, the degraded LUH sample was analyzed for formation of genotoxic impurity (P-LUH), LUH related impurities (I1-LUH, I2-LUH and I3-LUH), unknown impurities and total impurities. The results are summarized in Table-10. The genotoxic impurity, P-LUH, was formed only in 0.05 M Cu^{2+} stress condition. The LUH related substance impurity, I1-LUH, was not seen in any of the conditions of stress applied. I2-LUH impurity formation is seen only in 4 N HCl stress condition. The LUH related substance impurity, I3-LUH, was seen in 0.05 M Cu^{2+} stress and 4 N NaOH stress applied. The chromatograms of this experiments are shown in Fig. 3. The results demonstrated the non-interventions of impurities, degradation LUH products to establish the method is specific and stability indicating. Interferences of unknown impurities with analyte peak has been demonstrated by proven separation between analyte peak and all other impurities peaks.

Applicability of method: The stability indicating technique based HPLC approach was applied for content quantification of genotoxic impurity (P-LUH) and LUH related impurity compounds (I1-LUH, I2-LUH and I3-LUH) in LUH (batch no. API-LUH-01) drug molecule and LUH tablets, Lurastar (labelled content 40 mg, batch No: D2106048). The impurities P-LUH and I1-LUH were not identified in the LUH drug molecule or LUH tablets. The impurities I2-LUH (0.0126745% concentration) and I3-LUH (0.0038870% concentration) in in the LUH drug molecule. These values were much below the established safe threshold. These findings demonstrated that genotoxic and associated impurities in the LUH drug molecule as well as in LUH tablets were properly regulated. Thus, the stability-indicating technique-based HPLC approach's great efficiency was demonstrated by quantitative assessment of genotoxic and related impurities in LUH substance batches including tablets.

Conclusion

In this investigation, a simple and novel stability-indicating technique based HPLC approach for the simultaneous quanti-

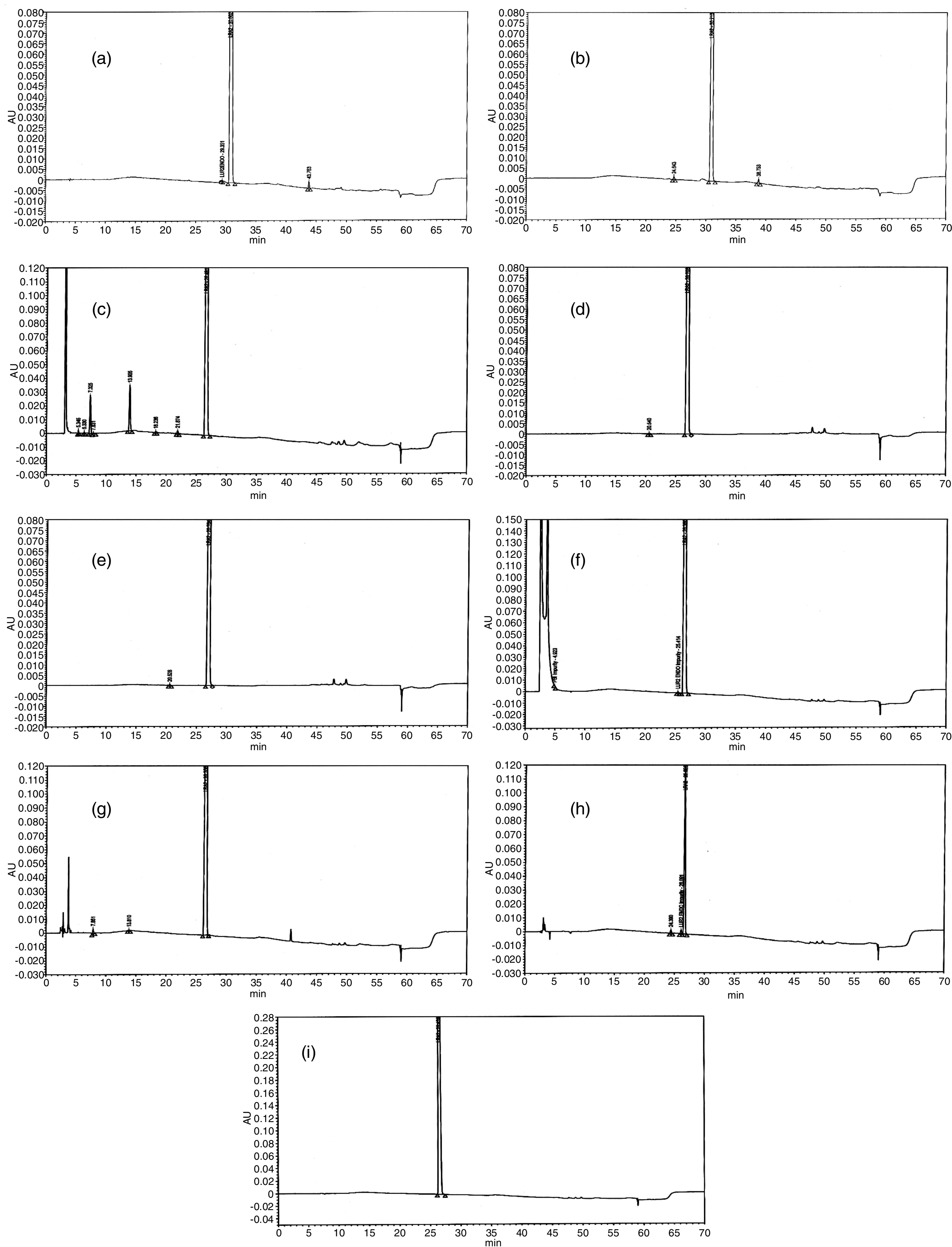

Fig. 3. Thermal treated (a), humidity treated (b), peroxide treated (c), photo open treated (d), photo closed treated (e), 0.05 M Cu^{2+} treated (f), 4 N HCl treated (g), 4 N NaOH treated (h) and Milli Q water treated (i) LUH sample chromatograms

TABLE-9
ROBUSTNESS MEASURES OF P-LUH, I1-LUH, I2-LUH AND I3-LUH

Impurity	Theoretical conc. (%)	Obtained conc. (%)	Recovery (%)	Theoretical conc. (%)	Obtained conc. (%)	Recovery (%)
	90% mobile phase buffer in SS-II			110% mobile phase buffer in SS-II		
P-LUH	0.153348	0.136911	89.281	0.153348	0.137239	89.495
I1-LUH	0.150228	0.131602	87.602	0.150228	0.133529	86.920
I2-LUH	0.153556	0.165561	102.578	0.153556	0.164494	102.479
I3-LUH	0.147825	0.155794	102.097	0.147825	0.142976	93.536
	95% acetonitrile in SS-I			105% acetonitrile in SS-I		
P-LUH	0.153348	0.138001	89.992	0.153348	0.133766	87.230
I1-LUH	0.150228	0.134031	89.218	0.150228	0.127766	85.048
I2-LUH	0.153556	0.154602	95.979	0.153556	0.155054	96.126
I3-LUH	0.147825	0.162036	103.558	0.147825	0.158159	101.506
	0.6 mL/min of flow rate			1.0 mL/min of flow rate		
P-LUH	0.153348	0.142698	93.055	0.153348	0.144817	94.437
I1-LUH	0.150228	0.128781	85.724	0.150228	0.129589	86.262
I2-LUH	0.153556	0.173861	113.223	0.153556	0.178246	109.503
I3-LUH	0.147825	0.156905	106.142	0.147825	0.155465	104.985
	Temperature 5 °C increased (40 °C)			Temperature 2 °C decreased (30 °C)		
P-LUH	0.153348	0.149464	96.830	0.153348	0.136752	89.178
I1-LUH	0.150228	0.138113	91.936	0.150228	0.128829	85.756
I2-LUH	0.153556	0.175117	109.103	0.153556	0.163362	101.088
I3-LUH	0.147825	0.162922	106.857	0.147825	0.157760	100.765
	Mobile phase pH variation (pH 3.4)			Mobile phase pH variation (pH 3.1)		
P-LUH	0.153348	0.135312	88.239	0.153348	0.140023	91.311
I1-LUH	0.150228	0.137703	89.111	0.150228	0.130166	86.646
I2-LUH	0.153556	0.164312	100.566	0.153556	0.162111	105.021
I3-LUH	0.147825	0.154831	100.049	0.147825	0.153971	98.665

*Mean of three recovery values

TABLE-10
DEGRADATION MEASURES OF LURASIDONE HYDROCHLORIDE (LUH) SAMPLE

Condition	Percentage content of					
	P-LUH	I1-LUH	I2-LUH	I3-LUH	Unknown impurity	Total impurities
Thermal	Not detected	Not detected	0.021407%	Not detected	0.157301	0.30
Humidity	Not detected	Not detected	Not detected	Not detected	0.138001	0.29
Photo open	Not detected	Not detected	Not detected	Not detected	0.0271216	0.04
Photo closed	Not detected	Not detected	Not detected	Not detected	0.0267157	0.03
Acid hydrolysis	Not detected	Not detected	Not detected	Not detected	Not detected	0.0277483
Base hydrolysis	Not detected	Not detected	Not detected	0.179843	Not detected	0.151212
Oxidation	Not detected	Not detected	Not detected	Not detected	Not detected	5.51
Water hydrolysis	Not detected	Not detected	Not detected	Not detected	Not detected	0.0609229
0.05 M Cu^{2+}	0.008268	Not detected	Not detected	0.0061711	Not detected	0.0309258

tative measurement of genotoxic impurity (P-LUH) and LUH related compounds (I1-LUH, I2-LUH and I3-LUH) in LUH drug material were established. The novel stability-indicating technique based HPLC approach that was designed is precise, linear, accurate, specific, robust and rugged, as evidenced by the aforementioned experimental findings on the different method validation characteristics. Also conducted degradation studies on LUH test sample. The impurities P-LUH and I2-LUH was formed in 0.05 M Cu^{2+} stress and 4 N HCl stress conditions. The impurity I3-LUH, was found in 0.05 M Cu^{2+} stress and 4 N NaOH stress applied. In none of stresses applied, the impurity I1-LUH was seen. This HPLC approach was found to be suitable for the intended purpose based on the findings of the aforementioned investigation. As a result, this approach is suitable for routine quality analysis of lurasidone hydrochloride (LUH).

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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