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RESEARCH ARTICLE

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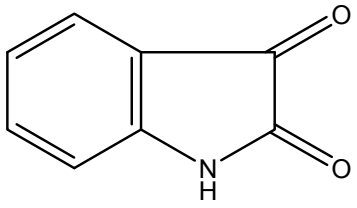
ESTIMATION OF ISATIN IN SPIKED PLASMA SAMPLES USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

An isocratic analytical method based on liquid chromatographic determination with UV detection is described for the determination of isatin in spiked plasma and in its synthetic mixture. The separation of the isatin was achieved on a betasil C-8 column using a mobile phase consisting of a binary mixture of acetonitrile and buffer (Na_2CO_3 + $NaHCO_3$). The linearity, accuracy and precision were found to be acceptable over the concentration range (5-150 mg/L). LOD and LOQ were found to be 1.09 and 3.3 mg/L, respectively. The developed LC method with UV-visible detection offers simplicity, selectivity, precision and accuracy. It produces a symmetric peak shape and reasonable retention time. No interference was observed with the excipients found in the drug formulation. Forced degradation studies were also conducted on the isatin samples and the results shows that the newly developed method is able to determine the content of isatin in presence of its degradation products. The proposed method when applied to the determination of isatin in spiked plasma samples produced a recovery ranging from 96.0-98.2%.

Keywords: HPLC, isatin, plasma samples, method validation and development.

Isatin, an orange red crystalline compound $C_8H_5NO_2$, is a heterocyclic molecule with an indole nucleus. Chemically, this compound is known as 1*H*--indole-2,3-dione and it was first obtained by Erdman and Laurent [1] in 1841. In their experiment, isatin was formed as a product from the oxidation of indigo dye by nitric acid and chromic acid. In the literature, an important method for the synthesis of isatin is reported, performed by cyclicizing the condensation product of chloral hydrate, aniline and hydroxylamine in H_2SO_4 [2,3]. This reaction is named "Sandmeyer isonitrosoacetanilide isatin synthesis" and it was discovered by Traugott Sandmeyer in 1919. Isatin forms a blue dye when mixed with sulfuric acid and crude benzene.

Isatin's derivatives have remarkable anti-bacterial and anti-fungal properties [4] and great significance in medicinal chemistry. Isatin is reported to be present in the mammalian tissues as one of the major components of tribulin [5]. In the mammalian tissue, isatin is formed probably from the heme-protein bound trypthophan in an iron catalyzed oxidation reaction. In addition to its presence in mammalian tissue, isatin is also found in various plants [6]. The structure of isatin is shown in Scheme 1.



Scheme 1. Structure of isatin.

Several biological activities are associated with isatin, including CNS effects and analgesic, anticonvulsant, anti-depressant, anti-inflammatory and antimicrobial activities. It is also reported to be able of crossing the blood-brain barrier [7]. Several other pharmacological importances highlighted in the literature include induction of arousal, reduction in duration of slow-wave sleep and increase of the seizure thres-

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hold in rats. Isatin was primarily discovered to aid as an inhibitor of monoamine oxidase (MAO) and subsequently recognized as selective inhibitor of MAO B [8-9]. Further studies on isatin have shown that it acts as an antagonist of both arterial natriuretic peptide-stimulated [10] and nitric oxide-stimulated [11] guanylatecyclase activity. Isatin is also considered as an endogenous marker of stress and anxiety [12]. As reported [13], stress can increase isatin levels in tissues (brain and heart) and serum, males having greater increase in isatin level as compared to females.

Isatin has various technological applications too. It is known to be a coloring reagent for the amino acid proline, forming a blue derivative. This property has been exploited for the determination of the levels of this amino acid in pollens and other vegetal materials using paper chromatography. Another application of isatin is its use in colorimetric screening test for human serum hyperprolinemia, in a colorimetric assay of HIV-1 proteinase. The property of isatin to produce fluorogenic derivative upon reaction with tryptophan is applied for the detection of tryptophan by thin layer chromatography [6].

In view of such important pharmacological role of isatin, this molecule needs to have a simple and sensitive method for its quality control. There are several analytical methods reported for the quantitative analysis of isatin, which include HPLC [14,15], electroanalytical methods [16,17], liquid chromatography-mass spectrometry [18], GC-MS [19], colorimetry [20] and fluorimetry [21]. The present work offers a simple HPLC method using a betasil C-8 column and binary mixture of acetonitrile and buffer (Na_2CO_3 + $NaHCO_3$).

EXPERIMENTAL

Instrumentation

Chromatography was performed on a Thermo surveyor HPLC system containing an autosampler, quaternary pump and a Thermo UV/Vis detector and computer aided recorder. Integration of the chromatograms was made by ChromQuest software. The column used in the present study was betasil C-8 manufactured by Thermo Scientific. The centrifugation processes for the preparation of plasma samples were performed on a Hermle Labortechnik centrifuge (GmbH).

Reagents and chemicals

Acetonitrile (ACN), HPLC grade was obtained from BDH prolabo, EC. Sodium carbonate (Na_2CO_3) and sodium bicarbonate both AR grade manufactured by BDH Chemicals Ltd., Poole England. Milli-Q water was used throughout the process. Acid, base and the oxidant used in the forced degradation studies were of analytical reagent grade. The isatin was obtained from Merck Germany and used without further purification.

Mobile phase

The buffer solution for the HPLC method was prepared by dissolving 0.5 g of sodium carbonate and 0.5 g sodium bicarbonate in 1000 mL of Milli-Q grade water (pH 9.71). The content of the mixture was sonicated prior to the analysis. The mobile phase consisted of buffer and acetonitrile in equal ratio.

Chromatographic conditions

The mobile phase was pumped at a flow rate of 0.5 mL min^{-1}. Chromatography was carried out at room temperature (30 °C).

Stationary phase. Betasil C-8 Column 150 mm× ×4.6 mm, particle size 3 μm, packed with high purity base deactivated silica with carbon load 12% manufactured by Thermo Scientific.

Mobile phase. Buffer:acetonitrile, 50:50; flow rate, 0.5 mL min^{-1}; injection volume, 10 μL; column temperature, 30 °C; UV-detector, 5 cm optical path length and cell volume of 10 μL; detection wavelength, 290 nm; run time, 10 min.

Standard solution preparation

Standard stock solution of isatin was prepared by dissolving 10 mg of isatin in 100 mL Milli-Q water. The drug was dissolved by continuously shaking the content and adding water to it, thus preparing a 100 mg/L solution. The injection volume was 10 μL to produce adequate UV responses to detect the drug.

Data analysis

Isatin was determined by injecting equal volumes of the standard and the assay preparation into the chromatograph. The chromatograms were obtained and the responses were measured for the major peak.

Procedure for the determination of isatin in synthetic mixtures

Synthetic mixtures of isatin were prepared by taking various excipients commonly used in tablet dosage forms with 100 mg/L of isatin in standard volumetric flask and tested to study the interferences of these excipients. The mixture for solid dosage forms was prepared by taking isatin (100 mg), lactose (280 mg), silicon dioxide (40 mg), corn starch (360 mg), glucose (100 mg) and magnesium stearate (25 mg). In the synthetic mixture, the mass ratio of isatin

to lactose, silicon dioxide, glucose and magnesium stearate was 1:2.8:0.4:3.6:1:0.25, respectively. It was observed that there was no interference from glucose, lactose, talc and magnesium stearate and these excipients could be well tolerated. In the literature, synthetic mixture has been used in the determination process of various instrumental methods [22,23].

Preparation of plasma

For the preparation of plasma samples, human blood samples were collected into tubes containing sodium citrate. Each tube was centrifuged for 15 min at 8500 rpm and the supernatant was collected in another tube. To the supernatant 1 mL of acetonitrile was added and kept for 10 min for the plasma proteins to precipitate and then the supernatant was collected for further use.

Procedure for the determination of isatin in plasma samples

The proposed method was applied for determination the isatin in plasma samples. Blood samples were collected with the help of King Khalid University Hospital, Riyadh. The centrifugation was done using a Hermle Labortechnik (GmbH), Germany. In a falcon tube, isatin was mixed with plasma to get a concentration of 1 mg mL^{-1}. The plasma sample was then mixed with water and acetonitrile (1:2) to prepare a solution of 0.5 and 0.75 mg/L, followed by incubation for 30 min at 4 °C and centrifugation for 15 min at 5000 rpm, after which the supernatant was collected and analyzed by the proposed method [24]. The quality controlled plasma samples were prepared similarly, according to the "preparation of the plasma" for checking the recovery of the analyte. Many analysts in the past have applied spiked plasma samples to quantify the pharmaceutical compound by their developed method [25].

Methodology of validation

Validation of a method is the process used to confirm that the analytical procedure employed for a specific test is suitable for the purpose that it was developed. Results obtained from various parameters of the method validation can be used to evaluate the quality, reliability and constancy of analytical results. Some parameters tested during the validation process are system suitability, selectivity, linearity, limit of detection (LOD), limit of quantitation (LOQ), accuracy and precision, and stability of analytical solution.

System suitability. The success of the system suitability test runs to guarantee that the complete system meets the analyst's expectations under the particular test conditions. It is meant for the system performance consistency and chromatographic suitability. In the system suitability test, six standard solutions of selected concentration are injected into the HPLC system. The relative standard deviation (*RSD*) of the six replicates should not be more than 2.0%.

Selectivity. In this parameter, the test mixture is prepared to contain the analyte and other potential sample components. The results are compared with the response of the analyte. One of the important steps in selectivity test is generation of degradation products, which can be accelerated by putting the sample under stress conditions, such as elevated temperature, humidity or light.

Linearity. ICH recommends at least five concentration levels to judge the linearity of the analytical procedures. Following the ICH guidelines, in the current analytical procedure eight concentration levels ranging from 5–150 mg/L were selected to study the linearity, and each concentration level three samples were injected and the mean area was plotted against concentration to evaluate the linearity.

Limit of detection and limit of quantitation. To determine the *LOD* and *LOQ*, six replicates of test sample of isatin at 4 mg/L concentration were injected and the height of each peak that corresponds to the component concerned was obtained. Prior to this, six times the blank was injected into the HPLC system to get the noise and subsequently *S*/*N* ratio was calculated. Thus, the *LOD* and *LOQ* of the developed method were experimentally verified and were found to be 1.09 and 3.3, respectively.

Accuracy and precision. Accuracy and precision in the current method were assessed in terms of the recovery studies. The studies were performed at three concentration levels of 50, 100 and 125 mg/l. Analysis at the mentioned concentration levels were performed on one day (intraday) and for three days.

Solution stability

Isatin solution of 100 mg/L was prepared; the prepared solution was assayed for its content and then stored at two different storage conditions, *i.e.*, room temperature and refrigerator. The sample was analyzed at intervals of six hours up to 24 h to obtain the content of isatin. The stability of solution can be judged with % change in the content of isatin with respect to assay at time zero.

RESULT AND DISCUSSION

The structure of the analyte plays an important role in its retention characteristics. In general, a larger hydrophobic surface area (C-H, C-C) of the analyte will result in longer retention, as it increases the mole-

cule's non-polar surface area, which is non-interacting with the water structure; meanwhile polar groups such as -OH, COO^-, NH_2 reduce retention as they are well integrated into water. This concept was taken into consideration while developing the current reversed phase HPLC method. With the amide group and the carbonyl group in its moiety, isatin was easy eluted on the C-8 column. After some trials, carbonate buffer was finally selected. Various solvents such as acetonitrile, methanol and tetrahydrofuran were tried, but a good peak shape was obtained only with acetonitrile. Thus binary mixture of buffer (Na_2CO_3-$NaHCO_3$) and acetonitrile was selected as initial mobile phase for the estimation of isatin. Then, the proportion of acetonitrile and the buffer was determined by varying the acetonitrile and the buffer from 20:80, 25:75, 40:60, 50:50 and 75:25. Finally, 50:50 ratio of ACN:buffer was selected for detection of isatin, since this ratio produced good peak shape and reasonable retention time. The retention time of the replicates of isatin were found to be 3.679±0.025 min. Since the peak appeared at about 4 min, the sample was made to run up to 10 min.

Method validation and determination of Isatin in pure form

A newly developed analytical method cannot be considered accurate and authentic unless the validation of the proposed method is done. The fundamental parameters for a bioanalytical method validation are system suitability accuracy and precision, selectivity, linearity, *LOD* and *LOQ*, reproducibility, and stability. Measurements for analyte in the biological matrix should be validated. A typical chromatogram obtained from sample preparation is illustrated in Figure 1. In order to demonstrate that the current method has a performance that can guarantee reliable results, this method was validated with respect to its linearity, precision, accuracy, specificity, solution stability and robustness under the selected operating conditions. The method validation was done accordingly the ICH guidelines.

System suitability

System suitability tests ensure that the complete testing system (which includes the instrument, reagents, columns and the analysts) is suitable for the proposed application. Accordingly, the United States Pharmacopoeia system suitability tests are a vital part of the high performance liquid chromatographic method; they are used to authenticate that the reproducibility and the resolution (in case of more than one analyte) of the liquid chromatographic system are adequate for the study to be done. The system suitability test was performed based on USP 30 [2]. The observed *RSD* value for the isatin was found to be within the usually accepted value (≤ 2%). The capacity factor (k), theoretical plate per meter and tailing factor (T_f) were also determined.

Acceptance criteria for system suitability

- The *RSD* for the retention times of the principal peak from six replicates analyses of each standard solution should be not more than 2.0 %.
- The number of theoretical plates (N) for the isatin peaks should be not less than 2000.
- The tailing factor (T) for the isatin peaks should be not more than 2.0.

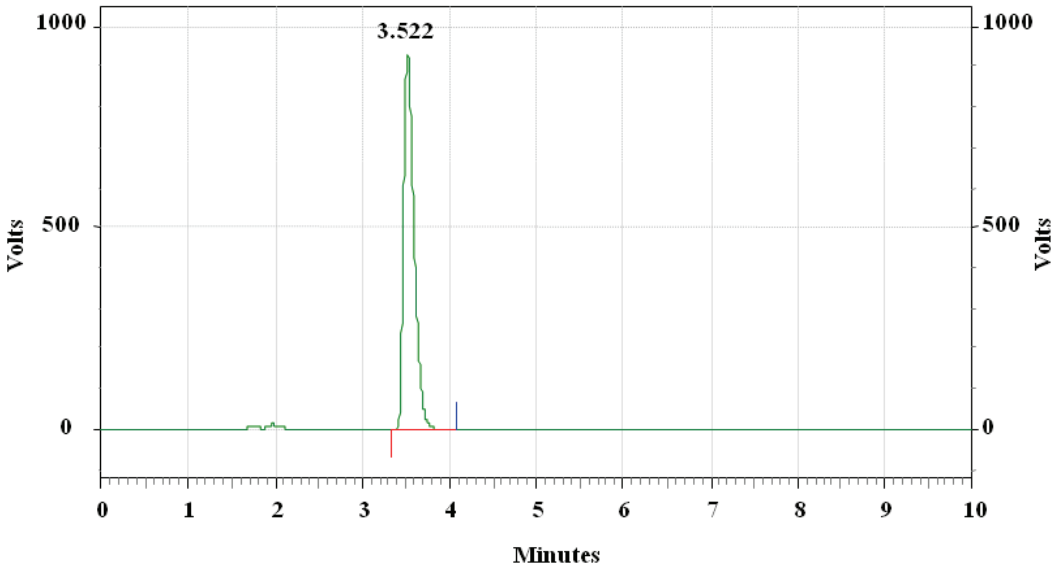

Figure 1. Sample chromatogram of isatin obtained by the current method.

The results are summarized in Table 1, from which we can conclude that the instrument, reagents and the column are suitable for the analysis of isatin.

Table 1. System performance parameters for isatin (n = 5); t_R, retention time, N, theoretical plate, k, capacity factor, A_f, asymmetry factor

Compound	t_R / min	N	k	A_f
Isatin	3.522	5036	8.6	1.64

Selectivity

Selectivity of an analytical method refers to its ability to differentiate and quantify the analytes in the presence of other components of the sample. Forced degradation study or stress study is assumed to demonstrate selectivity while developing a stability-indication analytical method. According to the regulatory authorities, forced degradation studies are carried out for the reasons discussed below.

- While developing and validating stability indicating methods.
- Determination of degradation route for drug substances and drug products.
- Discernment of degradation products in pharmaceutical preparations that is associated with drug substances against those that are related to non-drug materials viz. excipients.
- Structure interpretation of degradation product(s).
- Determination of the inherent stability of a pharmaceutical molecule.

Forced degradation studies have several defining characteristics mentioned below:

- They can be done in solution and / also in solid state .
- Forced degradation study involve conditions more severe than accelerated testing (*e.g.*, 40 °C; 75% relative humidity condition; the pharmaceutical samples are subjected to acidic, basic, oxidation and thermal stress).

As per the regulations, isatin samples were stressed by thermal, acidic, basic and oxidative environment over a fixed period of time.

Base degradation. 10 mg of isatin was transferred to 100 mL beaker followed by addition of 10 mL 0.5 N NaOH and it was heated in the water bath at 60 °C for 1 h. Then the sample was cooled and neutralized with 0.5 N HCl and diluted up to 100 mL with Milli-Q water. This solution was filtered and injected into the HPLC System. The chromatogram is shown in Figure 2.

Thermal degradation. Accurately weighed quantity of isatin equivalent to 10 mg was transferred to 100 mL beaker and was kept as such in oven at 40 °C for three days. After cooling, the sample was dissolved in 100 mL Milli-Q water. This solution was injected after filtration into the HPLC system. The chromatogram is shown in Figure 3.

Peroxide degradation. Isatin equivalent to 10 mg was transferred to a 100 mL beaker. 10 mL of 3% H_2O_2 was added and kept in water bath at 60 °C for 1 h. The content was cooled and sample was dissolved in 100 mL Milli-Q water. This solution was injected into the HPLC system. The chromatogram is shown in Figure 4.

Acid degradation. 10 mg of isatin was transferred to a 100 mL beaker followed by addition of 10 mL 0.5 N HCl and heating in a water bath at 60 °C for 1 h. The sample was then cooled, neutralized with 0.5 N NaOH and diluted up to 100 mL with Milli-Q water. This solution was filtered and injected into the HPLC

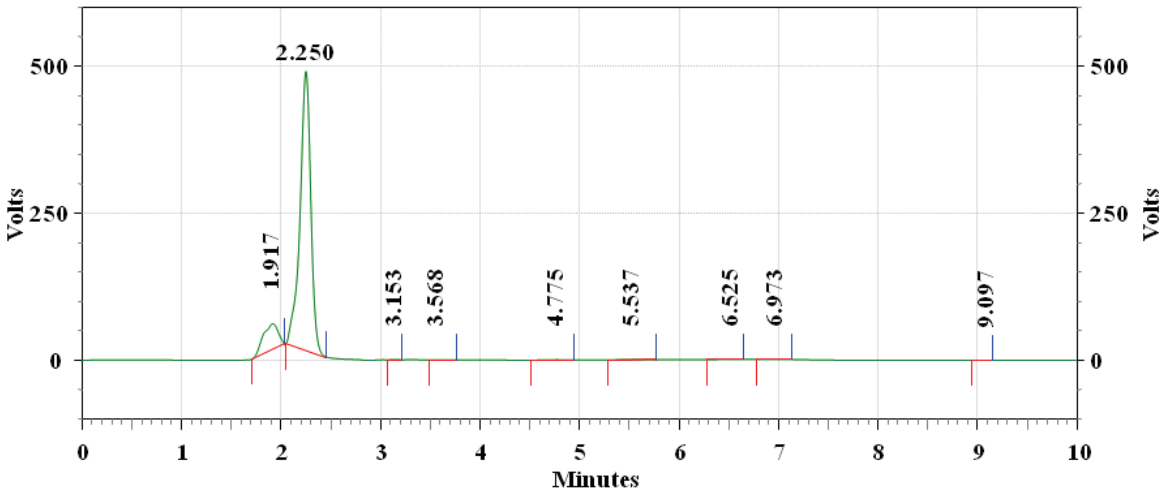

Figure 2. Chromatogram of the base degradation of isatin.

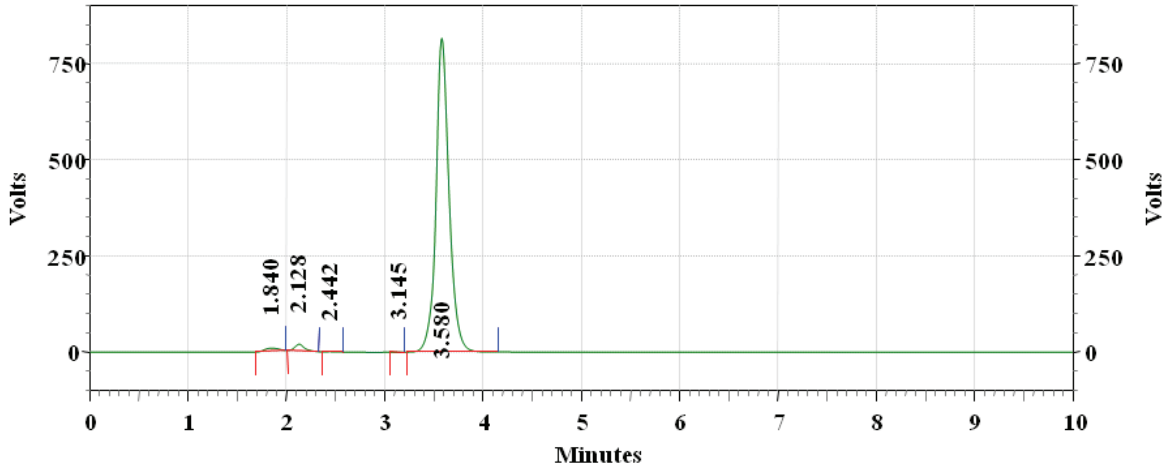

Figure 3. Chromatogram of the thermal degradation of isatin.

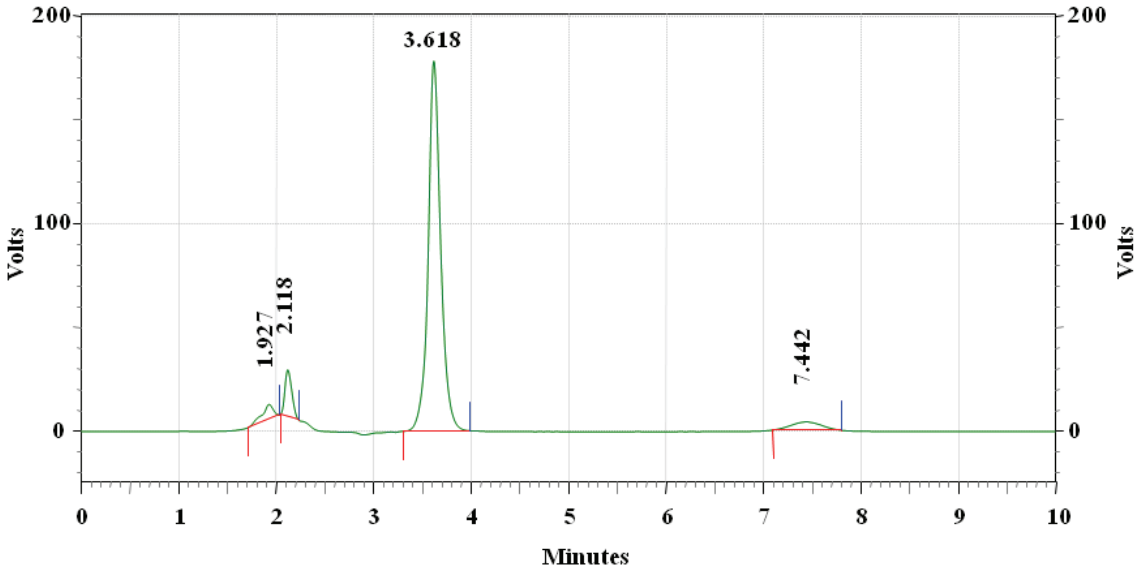

Figure 4. Chromatogram of the forced degradation of isatin by oxidant.

System. The chromatogram is shown in Figure 5.

The isatin samples were analyzed upon exposure to stress conditions. It was observed that the stressed samples degraded to a level, where the content of isatin samples was lower than that of the original level. The isatin samples exposed to thermal stress showed less degradation, while in basic condition isatin was found to degrade preferably. In terms of the impurities formed after the stress testing, basic stress exposure caused the formation of the most number of impurities, while the acidic degradation led to formation of fewer impurities. No interfering peaks at the retention time of isatin were observed in any of the forced degraded samples. Besides this, the placebo formulations were also determined to check the interference from the excipients. A clean chromatogram was obtained showing that there was no interference from the excipients. The above experiments prove the ability of the current method to separate the drugs from their degradation products and without interference from their excipients indicating good selectivity of the developed method.

Linearity

The linearity of an analytical procedure is the capability of that particular method to produce test results that are directly proportional to the concentration of analytes in samples within a given range by means of mathematical conversions. Linearity may be established directly on the test substance by preparation of multiple dilution of a standard stock solution and/or by taking separate weights of synthetic mixtures of the test product components, using the proposed procedure. Different concentrations of isatin were prepared for the linearity experiments. An eight-

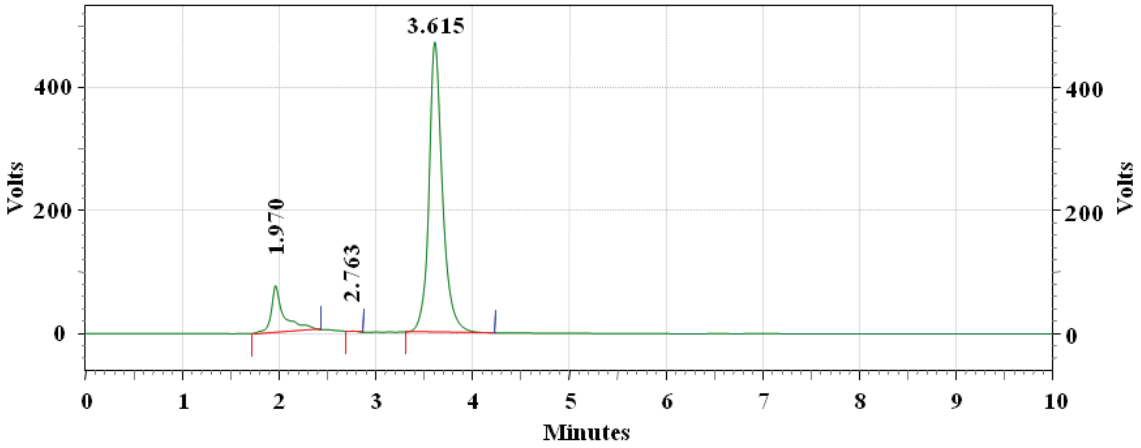

Figure 5. Chromatogram of the forced degradation of isatin by acid.

point calibration curve displays the linear response of the isatin samples. The peak area (A) and concentration of isatin was subjected to regression analysis to calculate the regression equation and the correlation coefficient. The regression analysis of calibration data yielded the linear equation $A = -1.20\times10^4 + 8.36\times10^4 c$ with correlation coefficient $r = 0.9975$. The range for the method has been set at 5–150 mg/L of the isatin concentration, since the method was shown to be precise, accurate and linear within this range. The results show that within the stated range there was an excellent correlation between the peak area and the concentration of each drug.

Limit of detection (*LOD*) and limit of quantification (*LOQ*)

The *LOD* is the smallest concentration of the analyte that gives a measurable response, while *LOQ* is the smallest concentration of the analyte, which gives a response that can be accurately quantified. The signal-to-noise method was used to calculate *LOD* and *LOQ*. For this, replicates of blank solution are injected and magnitude of the noise around is the retention time of analyte is measured by auto integrator of the HPLC system. Then, six replicates of the isatin test sample at lower concentration were injected and the height of the peak corresponding to the component was obtained; subsequently, the *S/N* ratio was calculated. Thus, the *LOD* and *LOQ* of the developed method were experimentally verified and were found to be 1.09 and 3.3, respectively.

Accuracy and precision

Accuracy is the difference between the observed value and the exact value. Accuracy is usually presented as a percent of nominal, although absolute bias is also acceptable according to the ICH guidelines. The term accuracy and precision are co-related; accuracy in the absence of precision has very little meaning. Accuracy claims should be made with acceptable precision. ICH guidelines suggest testing three replicates at a minimum of three concentrations. In our present experiment, the accuracy of the assay method was determined for both intra-day and inter-day variations using the triplicate analysis of the QC samples in terms of recovery studies. The precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability refers to the use of the analytical procedure within a laboratory over a short period of time that was evaluated by assaying the quality control (QC) samples during the same day. Intermediate precision was assessed by comparing the assays on 3 different days. An amount of the product synthetic mixture equivalent to 50, 100 and 150% of the isatin selected (100 ppm) was weighed accurately and assayed in five replicates for each of the three concentration levels. The results for the recovery studies and the precision are shown in Table 2. It can be seen that the relative standard deviation (*RSD*) values are well within the acceptable range. The results in the table reflect small standard deviation values ranging from 0.329–0.702.

Analytical solution stability

The stability of the sample solution was determined after storage at room temperature and in refrigerator. The samples were analyzed over 24 h at intervals of 6 h. The data was evaluated as % content change with respect to time zero. It was found that at room temperature the % degradation after 6, 12, 18 and 24 h was 0.52, 0.91, 1.26 and 1.42%, respectively, whereas the % degradation of isatin solution at refrigerated conditions after 6, 12, 18 and 24 h was 0.20, 0.36, 0.48 and 0.57%, respectively. Thus, final degradation after 24 h at room temperature was

Table 2. Evaluation of accuracy and precision of the proposed method by inter-day and intra-day assay

Taken, ppm	Found±*SD*[a], ppm	*RSD*[a] / %	*SAE*[b]	*CL*[c]
	Intraday assay			
50	50.04±0.468	0.935	0.209	0.581
100	100.25±0.432	0.430	0.193	0.536
125	124.90±0.329	0.264	0.147	0.409
	Interday assay			
50	49.87±0.702	1.409	0.314	0.872
100	100.11±0.492	0.492	0.220	0.611
125	123.92±0.615	0.496	0.275	0.764

[a]Mean for five independent analyses; [b]standard analytical error; [c]confidence limit at 95% confidence level and four degrees of freedom (t = 2.776)

1.42% and at refrigerated conditions was 0.57% of 100 mg/L. These results show that there was no significant change over 24 h at either of the temperature conditions.

Comparison of the proposed method with existing methods

The proposed method was compared with existing methods in the literature. Table 3 shows clearly that the proposed method has advantages over the other methods in terms of the simplicity, although in the literature various other sensitive methods such as LC-MS [18] have been used for the estimation of isatin. The proposed method has a higher recovery of isatin. Other methods, such as fluorimetry [21], offer lower costs but require a lot of tedious pre-treatment and have less recovery than the proposed method. The proposed method is comparable to another reported HPLC method in terms of the correlation coefficient and coefficient of variance.

Validity of the proposed method

Oona McPolin in her book "An introduction to HPLC for pharmaceutical analysis" [27] mentioned various type of calibration methods namely internal standard, external standard and multi-level calibration. The authors of the present study adapted the multi-level calibration method. The authors have used the standard addition method in an attempt to make corrections for the uncontrollable random errors caused by other components in the system or the instrument itself. In the standard addition method, a known amount of pure isatin (25 mg/L) is added to its formulated mixture at three different concentration levels (25, 50 and 75) and the nominal value of isatin is calculated thereafter. The results of the standard addition experiments are summarized in Table 4. It can be seen from the table that the recoveries obtained are in the range of 99.7–100.1%, which is quite satisfactory, and the RSD is low as well.

Method applicability

The application of the proposed method was checked by determining the isatin in plasma samples. The plasma samples were analyzed at three concentration levels and the recovery was found to be 96.0--98.2%. A typical chromatogram for the blank plasma

Table 3. Comparison of proposed method with existing methods in the literature for determination of isatin

Method	Calibration range	Recovery, %	DL	Correlation coefficient	Precision	Ref.
Liquid chromatography/mass spectrometry	5 to 5000 ng/mL	80	50 ng/mL	Greater than 0.990	Inter-assay precision for the quality control; samples was less than 3% and inter-assay accuracy was within 5%	18
Fluorimetric determination of isatin	0.16-10.7 ng (1.1-72.7 pmol)	Greater than 94	0.11 ng (0.75 pmol) (s/n = 3)	0.998	The relative standard deviation of the standard IST was 1.9% at 0.62 ng (n = 8) and 1.2% at 4.0 ng (n = 8)	21
Flow-injection chemiluminescence (CL)	0.1-100.0 μM	97.6-102.3	10.0 nM (3σ)	0.999	The relative standard deviations (%) were in the range of 0.98-3.53%	26
High performance liquid chromatography with an ultraviolet detector (HPLC-UV)	2 to 20 nmol per mL	Around 96	Not mentioned	0.924	Intra-assay and inter-assay precesion was within 3%	15
HPLC-UV	5-150 mg/L	95.98-98.16	1.09 mg/L	0.9975	Relative standard deviation (*RSD*) 0.264-1.409	This method

Table 3. Standard addition method for the determination of isatin in synthetic mixture (formulation A); taken: 25 ppm

Added, ppm	Found±*SD*[a]	Recovery, %	*RSD* / %	*SAE*
25	49.84±0.452	99.68	0.907	0.202
50	75.06±0.174	100.08	0.232	0.078
75	99.73±0.153	99.73	0.153	0.068

[a]Mean of five independent analyses

and that spiked with isatin are presented in Figures 6 and 7, respectively.

The applicability of the proposed method for the assay of isatin was also tested on a synthetic mixture due to unavailability of commercially available any dosage forms of isatin in the local market. Results of both the plasma and the synthetic mixture shows that the proposed method is fast, accurate, and does not involve any pretreatment prior to analysis except for the preparation of the plasma samples. This method could be a better alternate for the researchers working on isatin in hospitals, research laboratories and pharmaceutical quality control laboratories.

CONCLUSION

The developed LC method with UV-visible detection offers simplicity, selectivity, precision and accuracy. It produces a symmetric peak shape and reasonable retention time. No interference has been observed with the excipients found in the drug formulation. Thus, it is concluded that the proposed method is suitable for determination of isatin and can be used for the routine quality control analyses of isatin.

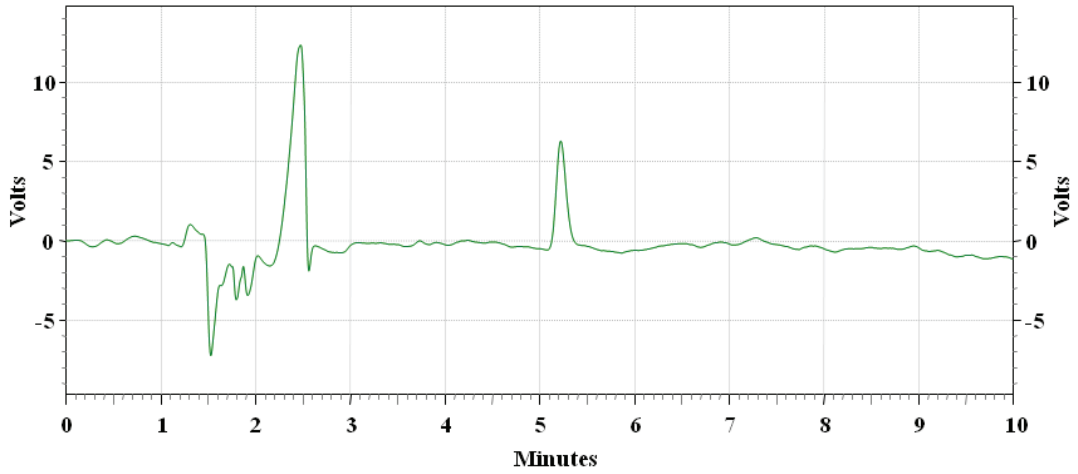

Figure 6. A typical chromatogram for the blank plasma.

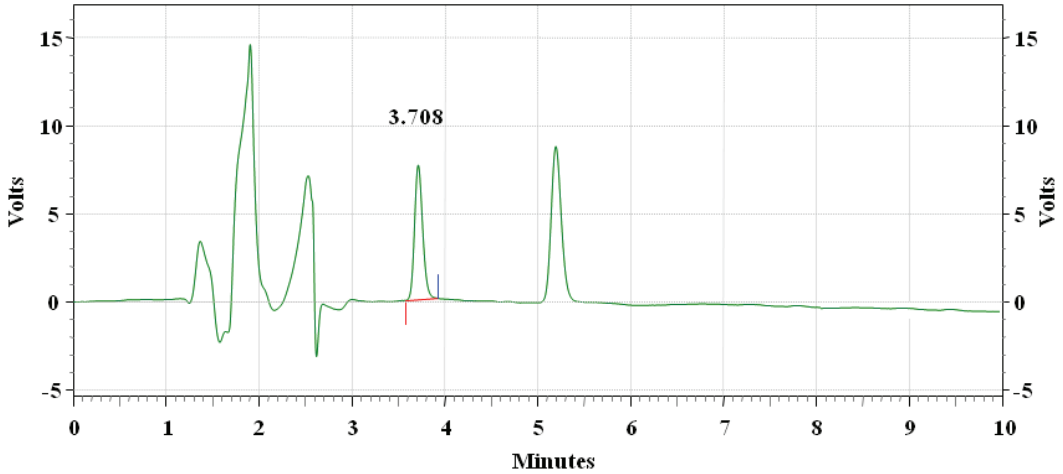

Figure 7. A typical chromatogram of the plasma spiked with isatin.

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STRUČNI RAD

HPLC ODREĐIVANJE IZATINA U OBOGAĆENIM UZORCIMA PLAZME

U radu je opisana izokratska metoda tečne hromatografije sa UV detekcijom za određivanje izatina u obogaćenoj plazmi i u njenim sintetičkim smešama. Za razdvajanje je korišćena betasil C-8 kolona, a kao mobilna faza je upotrebljena binarna smeša acetonitrila i pufera (Na_2CO_3 + $NaHCO_3$). Određena je lineranost, tačnost i preciznost u opsegu koncentracije od 5 do 150 mg/L. Određeni limit detekcije iznosi 1,09 mg/L, a limiti kvantifikacije 3,3 mg/L. Razvijena LC metoda sa UV-Vis detekcijom je jednostavna, selektivna, precizna i tačna. LC metodom se dobija dobro razdvajanje sa simetričnim pikovima i sa retencionim vremenima koje se dovoljno razlikuju. Ne postoji interferencija sa puniocima koji se koriste u formulaciji leka. Ubrzana degradacija uzoraka izatina je pokazala da je razvijena metoda sposobna za određivanje sadržaja izatina u prisustvu njegovih degradacionih produkta. Predložena metoda je primenjena za određivanje izatina u obogaćenim uzorcima plazme sa recovery vrednostima koje su u opsegu 96,0-98,2%.

Ključne reči: HPLC, izatin, obogaćena plazma, metoda validacije i razvoja.