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Article

UPLC and LC–MS Studies on Degradation Behavior of Irinotecan Hydrochloride and Development of a Validated Stability-Indicating Ultra-Performance Liquid Chromatographic Method for Determination of Irinotecan Hydrochloride and its Impurities in Pharmaceutical Dosage Forms

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The objective of the current investigation was to study the degradation behavior of irinotecan hydrochloride under different International Conference on Harmonization (ICH) recommended stress conditions using ultra-performance liquid chromatography and liquid chromatography–mass spectrometry and to establish a validated stability-indicating reverse-phase ultra-performance liquid chromatographic method for the quantitative determination of irinotecan hydrochloride and its seven impurities and degradation products in pharmaceutical dosage forms. Irinotecan hydrochloride was subjected to the stress conditions of oxidative, acid, base, hydrolytic, thermal and photolytic degradation. Irinotecan hydrochloride was found to degrade significantly in oxidative and base hydrolysis and photolytic degradation conditions. The degradation products were well resolved from the main peak and its impurities, thus proving the stability-indicating power of the method. Chromatographic separation was achieved on a Waters Acquity BEH C8 (100 × 2.1 mm) 1.7-μm column with a mobile phase containing a gradient mixture of solvent A (0.02M KH_2PO_4 buffer, pH 3.4) and solvent B (a mixture of acetonitrile and methanol in the ratio of 62:38 v/v). The mobile phase was delivered at a flow rate of 0.3 mL/min with ultraviolet detection at 220 nm. The run time was 8 min, within which irinotecan and its seven impurities and degradation products were satisfactorily separated. The developed method was validated as per ICH guidelines with respect to specificity, linearity, limit of detection, limit of quantification, accuracy, precision and robustness. This method was also suitable for the assay determination of irinotecan hydrochloride in pharmaceutical dosage forms.

Introduction

The aim of the present study to establish the inherent stability of irinotecan hydrochloride through stress studies under a variety of International Conference on Harmonization (ICH) recommended stress conditions. Irinotecan hydrochloride (HCl) [(S)-4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo1Hpyrano[3′,4′:6,7]-indolizino[1,2-b]quinolin-9-yl-[1,4′bipiperidine]-1′-carboxylate, monohydrochloride, trihydrate] (Figure 1), a semisynthetic water-soluble derivative of camptothecin, an alkaloid isolated from *Camptotheca acuminata* (1), has unique antitumor activity, preventing DNA synthesis by inhibiting topoisomerase I (2).

In the literature, few liquid chromatography (LC) methods have been reported for the determination of irinotecan HCl in pharmaceutical preparation. Few LC–tandem mass spectrometry (MS-MS), high-performance liquid chromatography–ultraviolet (HPLC–UV) or fluorescence detection methods have been reported for the estimation of irinotecan in biological fluids (3–7). One reported assay method (8, 9) describes the quantification of irinotecan HCl only, but it was out of scope because it did not separate or determine the impurities. Only one HPLC method has been reported for the determination of related compounds (metabolites) of irinotecan HCl (10), but this reported method deals with only two metabolites and has a run time of 20 min. Currently, no literature is available on forced degradation studies of irinotecan HCl as per ICH guidelines, and no stability-indicating ultra-performance liquid chromatography (UPLC) method has been reported for the estimation of all seven impurities of irinotecan HCl in pharmaceutical formulation. We have developed a stability-indicating reverse-phase (RP) LC method that can separate and determine irinotecan HCl and its seven impurities; namely, imp-A, imp-B, imp-C, imp-D, imp-E, imp-F and imp-G (Figure 1).

In LC, the analysis time can be reduced by using small columns packed with sub-2 μm particles. In addition, with sub-2 μm particles, due to the higher efficiency and smaller retention volume, sensitivity is also improved over conventional HPLC. A dedicated low dispersion system for ultra-high pressure separation with the particle size of the stationary phase reduced to 1.7 μm, small dwell and extra column volume, is able to work up to 1,000 bar (15,000 psi). In this way, the analysis time can be reduced ninefold over the conventional HPLC system using 5-μm particle size analytical columns without compromise on overall separation (11–13).

Hence, a rapid, simple, reproducible gradient stability-indicating RP-UPLC method was developed and validated for the quantitative determination of irinotecan HCl, its seven impurities, and its degradation products in pharmaceutical dosage forms.

Experimental

Chemicals and reagents

The certified irinotecan HCl working standard injection and its impurities; namely, imp-A, imp-B, imp-C, imp-D, imp-E, imp-F

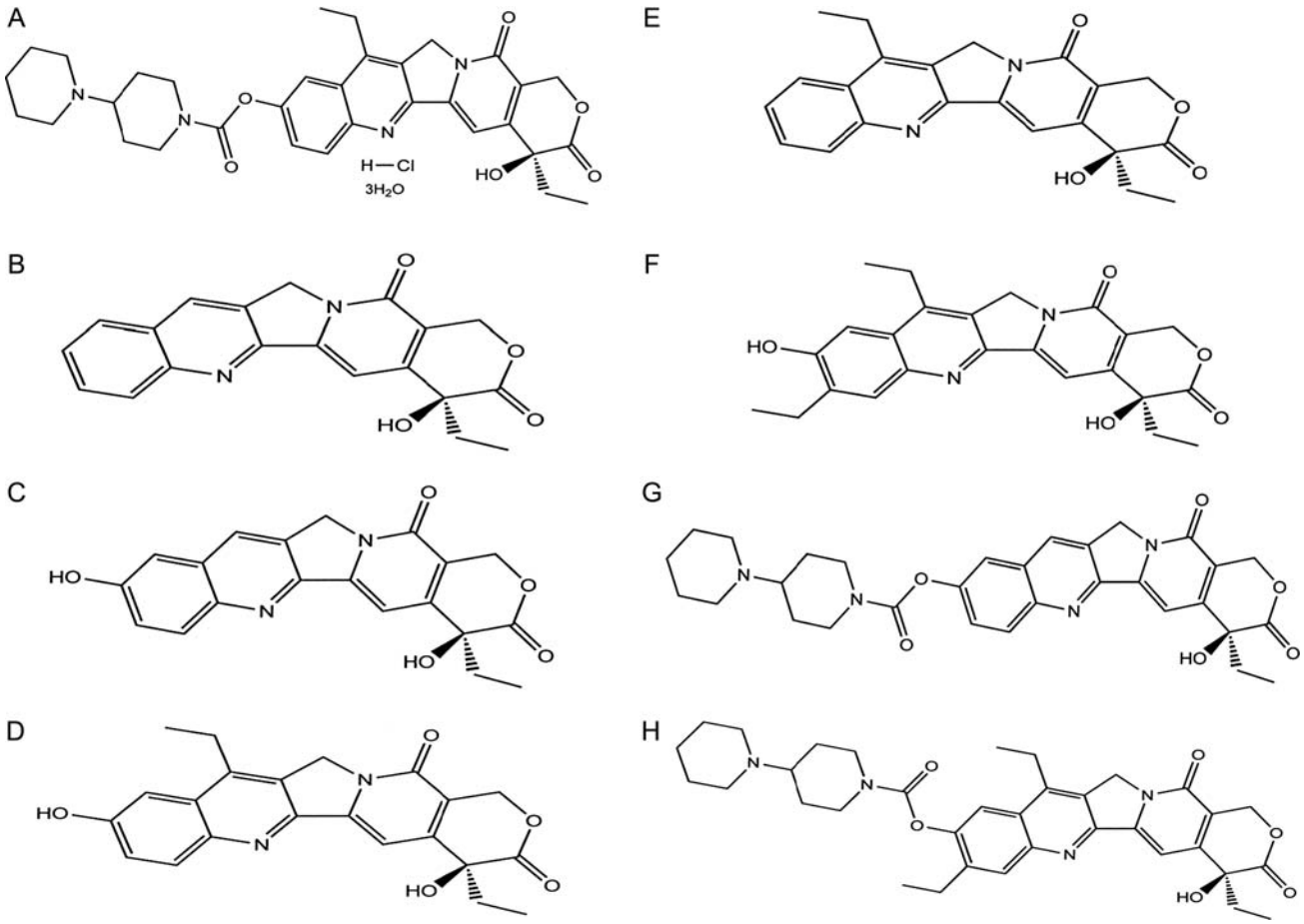

Figure 1. Structures of: irinotecan hydrochloride (A); impurity-A (B); impurity-B (C); impurity-C (D); impurity-D (E); impurity-E (F); impurity-F (G); impurity-G (H).

and imp-G, were supplied by Dr. Reddy's Laboratories (Hyderabad, India). The HPLC-grade acetonitrile and methanol, analytical-grade KH_2PO_4, ammonium formate, formic acid and ortho-phosphoric acid were purchased from Merck (Mumbai, India). High-purity water was prepared by using a Milli-Q Plus water purification system (Millipore; Milford, MA)

Instrumentation

The Acquity UPLC (Water, Milforde, USA) used consists of a binary solvent manager, a sample manager and a photodiode array (PDA) detector. The output signal was monitored and processed using Empower 2 software. A Cintex digital water bath was used for hydrolysis studies. Photo-stability studies were carried out in a photo-stability chamber (Sanyo; Leicestershire, UK). Thermal stability studies were performed in a dry air oven (Cintex; Mumbai, India). The pH of the solutions was measured by a pH meter (Mettler-Toledo; Switzerland).

Chromatographic conditions

The method was developed using a Waters Acquity BEH C8 (100×2.1 mm) 1.7-μm particle size column with a mobile phase containing a gradient mixture of solvent A (0.02 Mol/L potassium dihydrogen ortho-phosphate, pH adjusted to 3.4 with ortho-phosphoric acid) and solvent B (a mixture of acetonitrile and methanol in the ratio of 62:38 v/v, respectively). The gradient program [T(min)/%B] was set as 0/30, 3.5/42, 5.0/46, 6.0/49.8, 6.4/54, 6.50/30 and 8/30. The flow rate of the mobile phase was set at 0.3 mL/min. The column temperature was maintained at 30°C and the eluted compounds were monitored at the wavelength of 220 nm. The sample injection volume was 2 μL.

LC–MS conditions

An LC–MS-MS system (Agilent 1100 series liquid chromatograph coupled with Applied Biosystem 4000 Q Trap triple quadrupole mass spectrophotometer with Analyst 1.4 software, MDS SCIEX; Framingham, MA) was used for confirmation of the atomic mass number of unknown compounds formed during forced degradation studies. A Waters Symmetry Shield RP 18, 250×4.6 mm, 5-μm column was used as the stationary phase. Ammonium formate buffer (0.1 Mol/L, pH 3.5) was used as solvent A and acetonitrile and methanol in the ratio of 60:40 v/v was used as solvent B at a flow rate of 1.0 mL/min. The gradient program [T(min)/% solvent B] was set as 0/20, 40/80, 45/20 and 60/20. The analysis was performed in positive electrospray/positive ionization mode. The source voltage was 5,000 V

and source temperature was 450°C. GS1 and GS2 were optimized to 30 and 35 psi, respectively. The curtain gas flow was 20 psi.

Preparation of standard solutions

Diluent was prepared by mixing buffer (0.02 Mol/L KH_2PO_4, pH 3.4), acetonitrile and methanol in the ratio of 50:25:25 v/v/v, respectively. A stock solution of irinotecan HCl (0.4 mg/mL) was prepared by dissolving an appropriate amount of drug in the diluent. Working solutions of 0.32 and 40 μg/mL were prepared from the previously desrcibed stock solution for determination of the related substances and assay determination, respectively. A stock solution of impurities (mixture of imp-A, imp-B, imp-C, imp-D, imp-E, imp-F and imp-G) at 40 μg/mL was also prepared in diluent.

Preparation of system suitability solution

A mixture of irinotecan HCl (160 μg/mL) and all seven impurities (each 0.32 μg/mL) was prepared by dissolving an appropriate amount in diluent.

Preparation of sample solutions

An injection sample (1 mL) equivalent to 20 mg of the drug was dissolved in diluent with sonication for 15 min to give a stock solution containing 800 μg/mL. For determination of related substances, 5 mL of this solution was diluted to 25 mL with diluent to give a solution containing 160 μg/mL. For assay determination, 5 mL of stock solution (800 μg/mL) was diluted to 100 mL with diluent to give a solution containing 40 μg/mL.

Forced degradation studies

Forced degradation studies were performed at a 160 μg/mL concentration of irinotecan HCl on injection to provide an indication of the stability-indicating property and specificity of the proposed method. A peak purity test was carried out for the irinotecan peak by using the PDA detector on stress samples. All the solutions used in forced degradation studies were prepared by dissolving the drug product in a small volume of stressing agents. After degradation, these solutions were diluted with diluent to yield a stated irinotecan HCl concentration of approximately 160 μg/mL. The conditions employed for performing the stress studies are described in the following (14–16).

Acid degradation

An irinotecan sample (1 mL) was transferred into a 25-mL volumetric flask, 5 mL of 0.1 Mol/L HCl was added and the sample was mixed to dissolve the content completely. The flask was placed at 80°C in a water bath for 4 h. After 4 h, the flask was removed and placed on the bench top to attain laboratory temperature. To neutralize the sample, 5 mL of 0.1 Mol/L NaOH was added and the volume was made up with diluent and mixed well. Five milliliters of this solution was diluted to 25 mL with diluent.

Base degradation

An irinotecan sample (1 mL) was transferred into a 25-mL volumetric flask, 2 mL of 0.005 Mol/L NaOH was added and the sample was mixed to dissolve the content completely. The flask was placed at 35°C in a water bath for 5 min. After 5 min, the flask was removed and placed on the bench top to attain laboratory temperature. To neutralize the sample, 5 mL of 0.005 Mol/L HCl was added and the volume was made up with diluent and mixed well. Five milliliters of this solution was diluted to 25 mL with diluent.

Water degradation

An irinotecan sample (1 mL) was transferred into a 25-mL volumetric flask, 5 mL of water was added and the sample was mixed to dissolve the content completely. The flask was placed at 80°C in a water bath for 7 h. After 7 h, the flask was removed and placed on the bench top to attain laboratory temperature and the volume was made up with diluent and mixed well. Five milliliters of this solution was diluted to 25 mL with diluent.

Oxidation degradation

An irinotecan sample (1 mL) was transferred into a 25-mL volumetric flask, 5 mL of 6% hydrogen peroxide was added and the sample was mixed to dissolve the content completely. The flask was placed at 80°C in a water bath for 4 h. After 4 h, the flask was removed and placed on the bench top to attain laboratory temperature and the volume was made up with diluent and mixed well. Five milliliters of this solution was diluted to 25 mL with diluent.

Thermal degradation

An irinotecan sample (1 mL) was transferred into a 25-mL volumetric flask and placed in a hot air oven at 80°C for 16 h. After 16 h, the flask was removed and placed on the bench top to attain laboratory temperature and the volume was made up with diluent and mixed well. Five milliliters of this solution was diluted to 25 mL with diluent.

Humidity degradation

A saturated solution of potassium sulfate was prepared and placed in a dry glass desiccator at 25°C, which produced approximately 85–90% of relative humidity. To obtain the effect of humidity on irinotecan, a volumetric flask containing a sample (1 mL) was kept in the aforementioned glass desiccator at 25°C/90% RH, and sample was analyzed after seven days, as described previously.

Photolytic degradation

Susceptibility of the drug product to light was studied (14). Irinotecan HCl injection sample vials for photostability testing were placed in a photostability chamber and exposed to a white florescent lamp with an overall illumination of 1.2 million lux hours and near UV radiation with an overall illumination of 200 watt/m^2/h at 25°C. Following removal from the photostability chamber, the sample was prepared for analysis, as previously described.

Method Validation

The proposed method was validated as per ICH guidelines (15, 16). The following validation characteristics were addressed: specificity, accuracy, precision, limit of detection and quantification, linearity, range and robustness.

System suitability

System suitability was determined before sample analysis from six replicate injections of the standard solution containing 0.32 μg/mL irinotecan HCl. The acceptance criteria were less than 5% relative standard deviation (RSD) for peak areas, USP tailing factor less than 2.0 for irinotecan peak from standard solution. From the system suitability solution, resolution should be at minimum 1.5 between imp-C and imp-A.

Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities and excipients. Placebo interference was evaluated by analyzing the placebo that was prepared as per the test method. No peak due to placebo was detected at the retention times of irinotecan and its impurities. The specificity of the developed LC method for irinotecan was studied in the presence of its impurities and degradation products.

Precision

The precision of the method was verified by repeatability and intermediate precision. Repeatability was checked by injecting six individual preparations of irinotecan HCl sample spiked with its seven impurities (0.2% of each impurity with 160 μg/mL irinotecan HCl). The percent RSD of area for each impurity was calculated. The intermediate precision of the method was also evaluated using different analysts and different instruments and performing the analysis on different days.

Assay method precision was evaluated by carrying out six independent assays of a real sample of irinotecan HCl at the 40 μg/mL level against a qualified reference standard. The intermediate precision of the assay method was evaluated by a different analyst.

Limits of detection and quantification

The limit of detection (LOD) and limit of quantification (LOQ) for irinotecan and its impurities were determined at signal-to-noise ratios of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentrations. A precision study was also performed at the LOQ level by injecting six individual preparations and calculating the %RSD of the area for each analyte.

Linearity

Linearity test solutions for the assay method were prepared from irinotecan HCl stock solutions at six concentration levels from LOQ to 200% of assay analyte concentration (LOQ, 20.8, 31.2, 41.6, 62.4 and 83.2 μg/mL). The peak area versus concentration data was treated by least-squares linear regression analysis. Linearity test solutions for the related substances method were prepared by diluting impurity stock solutions to the required concentrations. The solutions were prepared at six concentration levels from LOQ to 200% of specification level (LOQ, 0.16, 0.24, 0.32, 0.48 and 0.64 μg/mL). Calibration curves were plotted between the responses of peak versus analyte concentrations. The coefficient correlation, slope, y-intercept and percent bias of the calibration curve are reported.

Accuracy

Accuracy of the assay method was evaluated in triplicate using a real sample at four concentration levels at 20.8, 41.6, 62.4 and 83.2 μg/mL. To determine the accuracy of the related substances method, recovery experiments were conducted on a real sample by spiking an impurity blend solution. The study was carried out in triplicate using four concentration levels at LOQ, 0.16, 0.32, 0.48 and 0.64 μg/mL. The percentage recoveries for irinotecan and its impurities were calculated.

Robustness

To determine the robustness of the developed method, experimental conditions were deliberately altered and the resolution between imp-C and imp-A, and system suitability parameters for irinotecan HCl standard were recorded. The variables evaluated in the study were pH of the mobile phase buffer (± 0.2), column temperature ($\pm 5^{\circ}$C), flow rate (± 0.03 mL/min) and percentage of organic in the mobile phase ($\pm$ 5%).

Sample and standard solution stability

The stability of irinotecan in solution was determined by leaving test solutions of sample and reference standards in tightly capped volumetric flasks at room temperature for 48 h and analyzing at 24 h intervals. The percent assay of the results was calculated for the solution stability experiment.

The stability of irinotecan and its impurities in solution for the related substances method was determined by leaving a spiked sample solution in a tightly capped volumetric flask at room temperature for 48 h and measuring the amounts of seven impurities at 24 h intervals for 48 h.

Results and Discussion

Development and optimization of the stability-indicating method

The primary objective of the chromatographic method was to separate the critical, closely eluting pairs of imp-C and imp-A, and imp-A and imp-G and to elute irinotecan as a symmetrical peak. The blend, containing 160 μg/mL of irinotecan HCl and 0.32 μg/mL of each seven impurities, prepared in diluent, was used for separation. All the impurities of irinotecan HCl were subjected to separation by reversed-phase UPLC on a Waters Acquity BEH C8, 100 × 2.1 mm, 1.7-μm column with pH 3.0, 0.02 Mol/L potassium dihydrogen ortho-phosphate buffer as a solvent A and pH 3.0, 0.02 Mol/L potassium dihydrogen ortho-

phosphate buffer–acetonitrile in a 20:80 ratio as solvent B. Two compounds, imp-A and imp-G, were merged together, and imp-B was co-eluted with irinotecan. With the addition of 20% acetonitrile in the place of buffer in solvent B, three compounds, imp-B, imp-A and imp-G, were separated, but resolution was less than 1.5. To increase the resolution, the composition of solvent B was modified to acetonitrile–methanol in a 62:38 ratio. Resolution between pairs of imp-A and imp-G, and imp-B and irinotecan was more than 2.0, but resolution between imp-C and imp-A decreased to 1.5. To increase

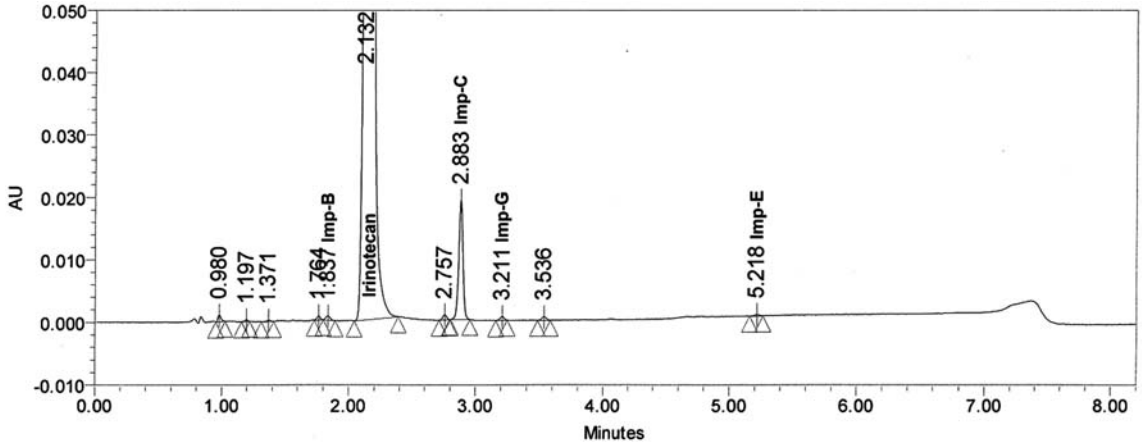

Figure 2. Typical chromatogram of acid degradation sample.

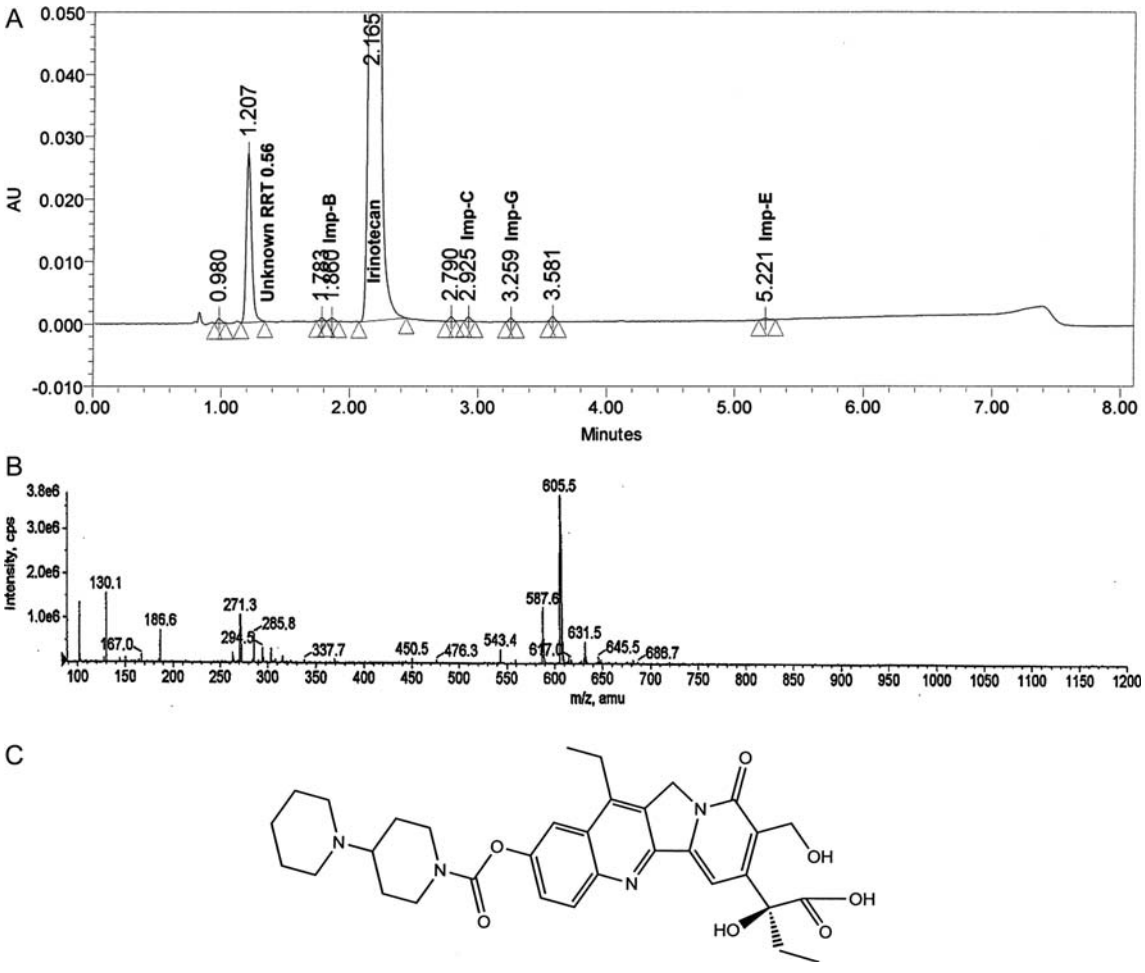

Figure 3. Typical chromatogram of base degradation sample (A); mass spectrum of degradant (RRT 0.56) (B); proposed structure of degradant (RRT 0.56) (C).

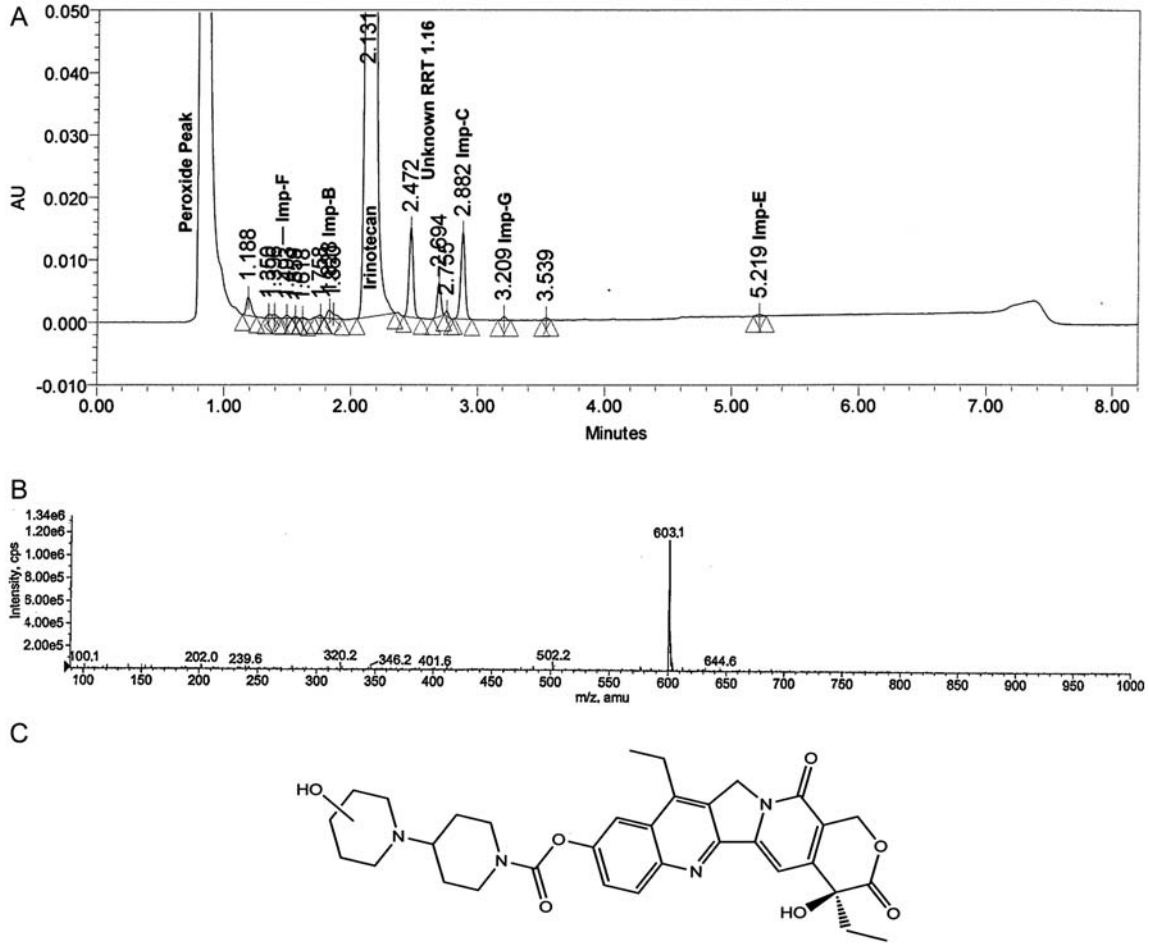

Figure 4. Typical chromatogram of oxidative degradation sample (A); mass spectrum of degradant (RRT 1.16) (B); proposed structure of degradant (RRT 1.16) (C).

the resolution between imp-C and imp-A, the buffer pH of solvent A was optimized and satisfactory resolution was obtained at the buffer pH of 3.4.

Attempts were made with gradient elution with solvents A and B using different Acquity BEH C18, 100 × 2.1 mm, 1.7-μm particles and Acquity UPLC™ HSS T3 (100 × 2.1 mm) 1.8 μm, using different buffer pH (4.0 and 6.5) conditions. However, with all of these conditions, separation of impurities was not satisfactory.

The use of buffer prepared by adjusting the pH of 0.02 Mol/L potassium dihydrogen orthophosphate to 3.4 with orthophosphoric acid as solvent A and the mixture of acetonitrile and methanol in the ratio of 62:38, v/v, as solvent B with gradient elution of time(min)/% solvent B: 0/30, 3.5/42, 5.0/46, 6.0/49.8, 6.4/54, 6.50/30 and 8/30 at a flow rate of 0.3 mL/min enabled the separation of all pair compounds and eluted irinotecan as a symmetrical peak. Interference from excipients was also checked, and no interference was observed.

Forced degradation behavior

UPLC and LC–MS studies of samples obtained during forced degradation studies of irinotecan under different conditions suggested the degradation behavior described in the following.

Acid degradation

The drug was found to be slightly unstable under 0.1 Mol/L HCl at 80°C for 4 h. The major impurity in the study was found to be imp-C (0.98%) and 0.04% as a maximum unknown degradant at a relative retention time (RRT) of approximately 0.46, with total impurities of approximately 1.22% (Figure 2).

Base degradation

The drug was found to be unstable under 0.005 Mol/L NaOH at 35°C for 5 min. The major degradant in the study was found to be an unknown degradant (1.72%) at an RRT of approximately 0.56, with total impurities of approximately 1.95% (Figure 3A). The ESI mass spectrum of this peak (RRT 0.56) showed $[M + H]^+$ at m/z 605.5 (Figure 3B). Based on the structure of the drug irinotecan, the most probable structure of the proposed degradant is shown in Figure 3C.

Water degradation

Because the drug did not undergo any significant degradation under the conditions of water at 80°C for 7 h, the drug seems to be stable under these conditions.

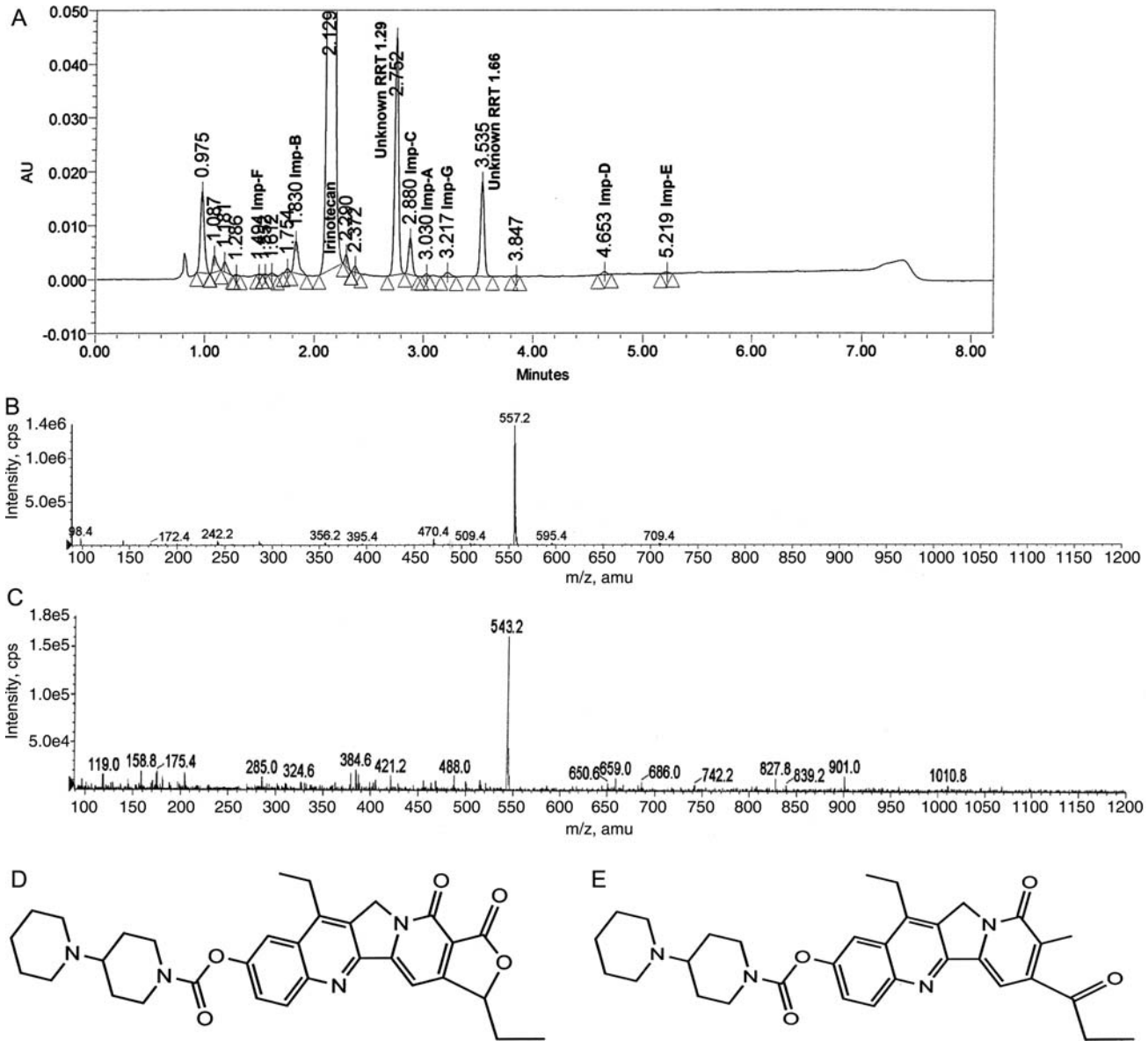

Figure 5. Typical chromatogram of photolytic degradation sample (A); mass spectrum of degradant (RRT 1.29) (B); mass spectrum of degradant (RRT 1.66) (C); proposed structure of degradant (RRT 1.29) (D); proposed structure of degradant (RRT 1.66) (E).

Oxidation degradation

The drug was found to be more labile to 6% hydrogen peroxide at 80°C for 4 h conditions. The major impurity in the study was found to be imp-C (0.73%) and 0.73% as a maximum unknown degradant at an RRT of approximately 1.16, with total impurities of approximately 2.22% (Figure 4A). The ESI mass spectrum of this peak (RRT 1.16) showed $[M + H]^+$ at m/z 603.1 (Figure 4B); the probable structure is given in Figure 4C.

Thermal degradation

Upon subjecting the irinotecan sample to dry heat at 105°C in a hot air oven for 16 h, no significant degradation was observed.

Humidity degradation

The irinotecan sample was quite stable under the humid conditions that were employed during the study. The sample showed no major degradation to humidity conditions.

Photolytic degradation

Irinotecan was found to be highly susceptible to light exposure. The major degradants observed in the sample exposed to both UV and visible light were found to be unknown degradants at RRTs of 0.46 (0.76%), 1.29 (2.34%) and 1.66 (0.98%). The major known impurities in the study were found to be imp-C (0.37%) and imp-B (0.40%), with total impurities of approximately 5.78% (Figure 5A). The ESI mass spectrum of the unknown peaks at RRT 1.29 and RRT 1.66 showed $[M + H]^+$

at m/z 557.2 and 543.2, respectively (Figures 5B and 5C); the probable structures of these degradants are given in Figures 5D and 5E.

Method Validation

System suitability

System suitability was checked for the conformance of suitability and reproducibility of chromatographic system for analysis. The system suitability was evaluated on the basis of retention time, %RSD of peak area and USP tailing factor for the irinotecan peak from standard solution, and resolution between imp-C and imp-A from the system suitability solution. All critical parameters tested met the acceptance criteria (Table I).

Specificity

No interferences were observed due to placebo and sample diluent at the retention times of irinotecan and its impurities. All forced degradation samples were analyzed using a PDA detector to ensure the homogeneity and purity of the irinotecan peak. All known impurities and unknown degradation products were well separated under all the forced degradation conditions employed, and the purity angle was found to be less than the purity threshold for the irinotecan peak. Apart from the peak homogeneity, the PDA spectrum for all related impurities and irinotecan were compared against their standard spectrums. Identification for the impurities and irinotecan was performed by comparing their PDA spectrum, purity plots and RRTs along with those of the standard, and all were found to match. The mass balance (% assay + % sum of all degradants + % sum of all impurities) results were calculated for all degradation samples and found to be more than 97% (Table II).

Table I
System Suitability Test Results

Parameters	Specification	Observed values	
		Precision	Intermediate precision
Retention time (Mean ± %RSD, $n = 6$)	≥1.9 and ≤2.4 min ± 1.0	2.141 ± 0.1	2.176 ± 0.1
Resolution*	≥1.5	2.1	2.0
Area (%RSD, $n = 6$)	≤5.0%	1.3	1.3
USP tailing	≤2.0	1.1	1.1

*Resolution between imp-C and imp-A.

Precision

The %RSD of assay of irinotecan during the assay method repeatability study was 0.1% and the %RSD for the areas of imp-A, imp-B, imp-C, imp-D, imp-E, imp-F and imp-G in the related substances method repeatability study was within 1.7%. The %RSD of assay results obtained in the intermediate precision study was within 0.5% and the %RSD for the peak areas of imp-A, imp-B, imp-C, imp-D, imp-E, imp-F and imp-G were well within 2.0%, confirming the precision of the method. The %RSD values are presented in Table III.

LOD and LOQ

The LOD, LOQ and precision at LOQ values for irinotecan and its seven impurities are reported in Table III.

Table II
Summary of Forced Degradation Results*

Stress Condition	% Impurity								% Degradation	% Assay of active substance	Mass balance (%)
	Imp-A	Imp-B	Imp-C	Imp-D	Imp-E	Imp-F	Imp-G	MUSI†			
Sample unstressed	ND	0.03	0.04	ND	0.02	ND	0.03	0.08	0.36	99.3	99.7
Acid hydrolysis	ND	0.04	0.98	ND	0.01	ND	0.03	0.04	1.22	97.7	98.9
Base hydrolysis	ND	0.03	0.04	ND	0.02	ND	0.03	1.72	1.95	96.4	98.4
Oxidation degradation	ND	0.04	0.73	ND	0.02	0.02	0.03	0.73	2.22	95.9	98.1
Thermal Degradation	ND	0.03	0.05	ND	0.02	ND	0.03	0.18	0.46	98.4	98.9
Water Degradation	ND	0.02	0.04	ND	0.02	ND	0.03	0.18	0.38	97.6	98.0
Photolytic degradation	0.02	0.40	0.37	0.04	0.02	0.01	0.06	2.34	5.78	91.4	97.2
Humidity Degradation	ND	0.03	0.04	ND	0.02	ND	0.03	0.18	0.38	99.0	99.4

*Note: ND = not detected.
†MUSI = Maximum unspecified impurity.

Table III
Linearity and Precision Data

Parameter	Irinotecan	Imp-A	Imp-B	Imp-C	Imp-D	Imp-E	Imp-F	Imp-G
LOD (μg/mL)	0.021	0.020	0.023	0.025	0.024	0.021	0.025	0.023
LOQ (μg/mL)	0.063	0.062	0.069	0.076	0.072	0.065	0.076	0.069
Correlation coefficient	0.9999	0.9998	0.9996	0.9997	0.9996	0.9995	0.9996	0.9996
Intercept	5920.7	−69.0	−379.5	−258.9	−524.2	−253.3	−182.2	−237.0
Slope	29225.9	43448.6	42328.3	43457.9	44371.2	42295.3	26594.6	25455.7
Bias at 100% response	0.5	−0.5	−2.6	−1.6	−3.3	−1.8	−1.9	−3.0
Precision (%RSD)	0.1	0.5	0.8	1.7	0.7	0.7	0.8	0.7
Intermediate precision (%RSD)	0.5	1.0	2.0	1.7	1.0	1.7	1.1	1.9
Precision at LOQ (%RSD)	4.1	2.2	2.6	4.4	2.2	2.9	3.4	3.7

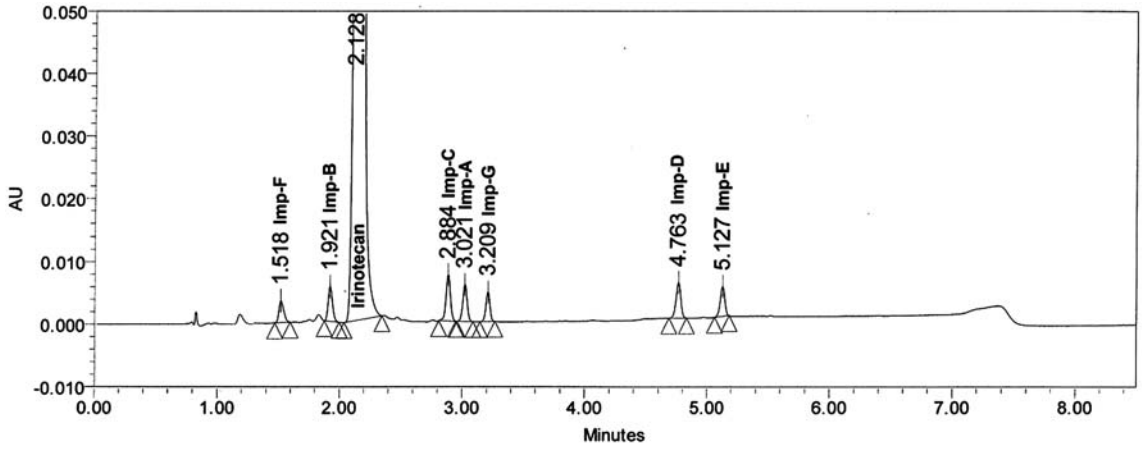

Figure 6. Typical chromatogram of irinotecan HCl sample spiked with all seven impurities.

Table IV
Evaluation of Accuracy Study

Amount spiked*	% Recovery†							
	Irinotecan	Imp-A	Imp-B	Imp-C	Imp-D	Imp-E	Imp-F	Imp-G
LOQ	102.5 ± 2.3	103.4 ± 2.6	99.2 ± 3.1	104.1 ± 3.7	101.6 ± 2.5	102.8 ± 3.1	101.1 ± 3.3	103.5 ± 3.7
50%	101.8 ± 0.2	99.0 ± 1.0	96.4 ± 2.0	103.8 ± 1.4	99.8 ± 1.0	100.7 ± 0.5	100.1 ± 1.8	102.2 ± 1.6
100%	101.0 ± 0.1	99.7 ± 0.3	96.8 ± 0.2	101.9 ± 2.6	100.1 ± 0.7	100.9 ± 0.3	100.1 ± 0.9	101.5 ± 0.7
150%	101.9 ± 0.1	99.3 ± 0.3	97.8 ± 0.2	101.8 ± 0.5	99.5 ± 0.3	99.9 ± 0.5	99.2 ± 1.2	102.3 ± 0.9
200%	100.9 ± 0.1	100.0 ± 0.3	99.5 ± 0.4	102.8 ± 0.5	100.2 ± 0.2	101.0 ± 0.4	100.6 ± 0.7	100.0 ± 0.5

*Amount of seven impurities spiked individually with respect to 0.2% specification level.
†Mean ± %RSD for three determinations.

Linearity

The linearity calibration plots for the assay method were obtained over the tested calibration range, i.e., 0.063 to 83.2 µg/mL and the obtained correlation coefficient was greater than 0.999. This result shows that an excellent correlation existed between the peak area and concentration of the analyte.

Linear calibration plot for the related substances method was obtained over the calibration range tested, i.e., LOQ to 0.4% for impurities. The obtained correlation coefficient was greater than 0.999 (Table III). This result shows that an excellent correlation existed between the peak area and the concentrations of imp-A, imp-B, imp-C, imp-D, imp-E, imp-F and imp-G.

Accuracy

The percentage recovery of irinotecan from injection ranged from 100.9 to 102.5%. The percentage recovery of impurities in irinotecan sample varied from 96.4 to 104.1%. The LC chromatogram of the spiked sample at 0.2% level of all seven impurities in an irinotecan injection sample is shown in Figure 6. The percent recovery values for irinotecan and its impurities are presented in Table IV.

Robustness

In all of the deliberately varied chromatographic conditions (flow rate, column temperature, pH of mobile phase buffer and composition of organic solvent), all analytes were adequately resolved and the elution order remained unchanged. The resolution between the critical pair of imp-C and imp-A was greater than 1.9, the tailing factor for the irinotecan peak from the standard solution was less than 1.1 and RSD for peak areas was less than 1.8% (Table V).

Sample and standard solution stability

The assay (%) of irinotecan during the solution stability experiment was within ± 2%. The variability in the estimation of all seven irinotecan impurities was within ± 10% during the solution stability experiment when performed using the related substances method. The results from the related substances solution stability experiment confirmed that standard solution and sample solutions were stable up to 48 and 24 h, respectively. The assay solution stability experiment exhibited stability of the standard and sample solutions up to 48 h.

Conclusions

The rapid gradient RP-UPLC method developed for the quantitative analysis of irinotecan HCl and related substances in pharmaceutical dosage forms is precise, accurate, linear, robust and specific. Satisfactory results were obtained from the validation of the method. The method is stability-indicating and can be used for routine analysis of production samples and to check the stability of irinotecan HCl injections.

Table V
Robustness Results of UPLC Method

Variation in chromatographic condition	Observed system suitability parameters			
	Retention time $\geq$ 1.9 and $\leq$ 2.4 min $\pm$ 1.0*	Resolution† $\geq$ 1.5	USP Tailing $\leq$ 2.0	% RSD $\leq$ 5.0 ($n = 6$)
Column temperature 25°C	2.233 $\pm$ 0.1	1.9	1.1	1.4
Column temperature 35°C	2.125 $\pm$ 0.1	2.1	1.1	1.2
Flow rate 0.27 mL/min	2.370 $\pm$ 0.1	2.2	1.1	1.5
Flow rate 0.33 mL/min	2.016 $\pm$ 0.0	1.9	1.1	1.8
Acetonitrile 95%	2.224 $\pm$ 0.1	1.9	1.1	1.5
Acetonitrile 105%	2.102 $\pm$ 0.1	2.3	1.1	0.7
Methanol 95%	2.122 $\pm$ 0.5	2.1	1.1	1.3
Methanol 105%	2.218 $\pm$ 0.0	1.9	1.1	1.8
Mobile phase buffer pH 3.2	2.113 $\pm$ 0.1	2.1	1.1	1.6
Mobile phase buffer pH 3.6	2.172 $\pm$ 0.1	2.0	1.1	1.2

*Mean $\pm$ % RSD, $n = 6$.
†Resolution between Imp-C and Imp-A.

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