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LC and LC-MS Study on Stress Decomposition Behavior of Paclitaxel and Establishment of Validated Stability-Indicating Assay Method

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ABSTRACT
Paclitaxel was subjected to different ICH prescribed stress conditions of thermal stress, hydrolysis, oxidation and photolysis. In total, six major degradation products were detected by LC and to elucidate the degradation products of Paclitaxel we develop a rapid and systematic strategy based liquid chromatography–mass spectrometry (LC–MS) profiling and liquid chromatography–tandem mass spectrometry (LC–MS–MS) sub structural technique. For establishment of stability-indicating assay, the reaction solutions in which different degradation products were formed were mixed, and the separation was optimized by varying the LC conditions. An acceptable separation was achieved using a C-18 column and a mobile phase comprising of 65:35 buffer (2 m*M* ammonium acetate, pH 6.9): acetonitrile held isocratic for 26 min followed by a 25 min linear gradient to 20:80 (buffer–acetonitrile) at a flow-rate of 2.0 ml/min. The degradation products appeared at relative retention times (RRT) of 0.14 (side chain methyl ester), 0.16 (baccatin III), 0.43 (10-deacetyl Paclitaxel), 0.52 (Paclitaxel isomer), 0.67 (oxetane ring opened) and 1.33 (7-epi Paclitaxel). The validation studies established a linear response of the drug at concentrations between 50ng/ml and 1000ng/ml. The mean value (±R.S.D.) of correlation coefficient was 0.9998 (±0.01). The mean R.S.D. values for Paclitaxel inter-day precision were 0.24 and 0.90, respectively. The recovery of the drug ranged between 99.42 and 100.58%, when it was spiked to a mixture of solutions in which sufficient degradation was observed.

Keywords: Degradation products, LC-MS, Paclitaxel, Stability-indicating assay.

INTRODUCTION

Paclitaxel, known commonly as taxol, is a member of the taxane family of natural products and is currently used in the treatment of ovarian and breast tumors. Detailed studies were conducted in our laboratory involving HPLC profiles of Paclitaxel and its degradants produced in stress studies. To rapidly facilitate this goal as well as to provide detailed structural information about the degradants, we developed LC–MS and LC–MS–MS strategies based on previous methods for the rapid and systematic elucidation of Paclitaxel degradants in formulation and in bulk. [1-4] Analyses combined optimized HPLC separation conditions on-line with an electrospray MS interface to obtain molecular mass information from the full scan mass spectra (LC–MS) and structural information from the tandem mass spectra (LC–MS–MS). Using these methodologies, structural and sub-structural data for degradants were obtained rapidly and systematically without prior fractionation. In addition, chromatographic resolution of co-eluting or unresolved components was not required to obtain product ion data for structural analysis, due to the mass resolving capability of mass spectrometry.

During the course of drug development, the bulk drug and drug formulation are studied under different stress conditions such as temperature, acid, base, oxidizing conditions, and light. The stressing conditions which may cause drug degradation are utilized to validate the analytical monitoring methods and serve as predictive tools for future formulation and packaging studies. [5] Traditional methods involving process scale-up, isolation, and purification of trace components such as degradants and impurities are expensive and time-consuming. Therefore, the use of methods such as LC–MS profiling which permit the rapid cataloguing and identification of potential degradants are attractive alternatives and serve to decrease the drug product development cycle time. The previously described methodologies and resulting database proved to be extremely useful during the development process of Paclitaxel and were applied to rapidly characterize degradation products of Paclitaxel. In this paper, the profile information obtained during forced degradation studies of Paclitaxel will be discussed. These degradation studies were carried out in acidic, basic, light, as well as exposure to oxidizing (peroxide) conditions to gain an assessment of stability and

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potential degradants which may be encountered during the pharmaceutical development process.

MATERIALS AND METHODS

Materials

Paclitaxel was supplied by Dr. Reddy labs, Hyderabad, India used without further purification. Sodium bicarbonate, ammonium hydroxide and hydrochloric acid (AR grade) were purchased from LOBA Chemie Pvt. Ltd. (Mumbai, India). Hydrogen peroxide was procured from S.D. Fine-Chem Ltd. (Boisar, Maharashtra, India). Acetonitrile (HPLC grade) was purchased from Merck (Mumbai, India). Double distilled Water for analytical purpose was obtained from milli-Q R-O system.

Instrumentation

Precision shaking water baths equipped with MV controller (Lab Tech, India) were used for degradation studies under acidic, alkaline and neutral conditions. Photo degradation was carried out in a photostability chamber (KBF 240,WTB Binder, Tuttlingen, Germany) equipped with a light bank consisting of two UV(OSRAM L73) and four fluorescent (OSRAM L20) lamps and capable of controlling temperature and humidity in the range of ±2°C and ±5% RH, respectively. The light system complied with option 2 prescribed in the ICH guideline Q1B. [6] At any given time, UV energy at the point of placement of samples was 0.6 W/m2 (tested with a calibrated radiometer, model 206, PRC Krochmann GmbH, Berlin, Germany) and visible illumination was 4500 lx (tested using a calibrated lux meter, model ELM 201, Escorp, New Delhi, India). The chamber was set at accelerated condition of 40°C/75% RH during the studies. Other equipments used were an ultrasonic bath (3210, Branson Ultrasonic Corporation, Danbury, CT, USA), precision analytical balance (SE2, SHIMADZU), rotary evaporator (R-114, Buchi Labortechnik, Flawil, Switzerland), aspirator (Eyela A-3S, Tokyo Rikakai Co., Tokyo, Japan) and a freeze dryer (DW8-85, Heto Holten, AllerØd, Denmark).

The HPLC system consisted of a Shimadzu LC- 20AT liquid chromatographic pump, Rheodyne injection port (Rheodyne, Cotati, CA,USA) with a 20 µl sample loop and SPD-M20A Photo diode array(PDA) detector (Shimadzu, Kyoto, Japan). Data collection, integration and calibration were accomplished using LC Solutions chromatography Data system. The chromatographic separation of Paclitaxel was accomplished using 250×4.6mm Waters Xterra MS C18 5µm analytical column. The mobile phase consisted of 65:35 buffer (2 m*M* ammonium acetate, pH 6.9): acetonitrile held isocratic for 26 min followed by a 25 min linear gradient to 20:80 (buffer–acetonitrile) at a flow-rate of 2.0 ml/min. Before use, the mobile phase was filtered by passing it through a 0.22µm filter and the filtrate is degassed by using bath sonicator. The mobile phase was pumped at an isocratic flow of 1 ml/min at room temperature. The PDA detection wave length was set at 228nm. All separations were performed at ambient temperature.

LC-MS studies were carried out in negative electro spray ionization (ESI) mode. Preparative LC-MS system, used for isolation of one of the degradation products, included a 2767 sample manager, 2996 binary gradient module, control fluidic organizer, micromass ZQ quadrapole detector and data management software MassLynx ver. 4.1 (all from Waters Corporation, Milford, MA, USA).

Conduct of stress studies

The stress studies were carried out under the conditions of dry heat, hydrolysis, oxidation, and photolysis, as defined by ICH. [6] For thermal stress testing, the drug powder was sealed in glass ampoules and heated in dry-bath at 50°C for 60 days and also at 60°C for 15 days. Degradation samples were prepared from a stock solution of Paclitaxel (2 mg/ml in acidified methanol, pH 5.5). Base degradation was performed by incubating 1.0 ml of Paclitaxel stock solution with 0.1 ml of aqueous Na_2CO_3 (0.05 *M*) for 10 min to produce a pH 8 solution, followed by re-establishment of pH 5.5 by addition of 1–2µl of concentrated HCl. Acid degradation was performed by incubating 2.0 ml of Paclitaxel stock solution with 0.8 ml of aqueous HCl (2.4 *M*) for 240 min, followed by the addition of 0.38 ml of concentrated ammonium hydroxide to re-establish a pH 5.5 solution. Oxidative degradation was performed by incubation of 1.0 ml of Paclitaxel stock solution diluted with 3 ml of 30% hydrogen peroxide to a concentration of 0.5 mg/ml. The formation of a white precipitate was observed since Paclitaxel has limited solubility in aqueous solutions.

The photolytic studies were carried out in solid state by spreading a thin layer of drug in a petri-dish and exposing it directly to the combination of UV and florescent light in a photo stability chamber set at accelerated conditions of temperature and humidity. A parallel set was kept in dark under similar conditions.

Separation studies

First, the reaction solutions were individually subjected to LC studies, followed by a mixture of those solutions in which reasonable decomposition was observed. The studies were conducted using a mobile phase composed of water: acetonitrile. The separation was achieved by changing the mobile phase composition as well as the flow rate. The overall objective here was to develop a selective SIAM (stability indicating assay methods) for characterization of the degradation products.

Validation of the method

Validation of the optimized LC method was done with respect to various parameters, as required under ICH guideline Q2 (R1). [7] To establish linearity and range, a stock solution of the drug was prepared at strength of 1mg/ml, which was further diluted to prepare solutions in the drug concentration range of 50-1000ng/ml. The solutions were injected in triplicate into the LC column, keeping the injection volume constant (10µl). Precision of the method was studied by making six injections of three different concentrations, *viz*., 100, 400, and 1000ngml^{-1} on the same day and the values of relative standard deviation (R.S.D.) were calculated to determine intra-day precision. These studies were also repeated on different days to determine interday precision. Accuracy was evaluated by fortifying a mixture of degraded solutions with three known concentrations, *viz*., 50, 200, and 500µg/ml of the drug. The recovery of the added drug was determined. The specificity of the method was established through study of resolution factors of the drug peak from the nearest resolving peak and also among all other peaks. Selectivity was confirmed through peak purity studies using a PDA detector.

Application of the developed method to stability samples

The developed method was used to analyze stability samples of a formulation containing Paclitaxel and RELICOAT (a proprietary polymer of Relisys Medical Devices Ltd.), which

is used to coat the Coronary stents. The formulation was stored under accelerated conditions of temperature and humidity for 3 months before analysis.

Characterization of degradation product(s)

LC-MS studies were carried out to determine *m/z* values of the major degradation products formed under various stress test conditions. The obtained values were compared with the molecular weights of known degradation products of Paclitaxel.

RESULTS AND DISCUSSION

Degradation behavior and Characterization of degradation products

Mass chromatograms in the negative electron spray ionization (ESI) mode for the drug and degradation products and the degradation behavior of the drug in individual stress conditions were outlined below.

Hydrolysis

Base degradation

Due to the high sensitivity of mass spectrometry, implementation of LC–MS profiling methods was found to be particularly advantageous for the rapid characterization of low level degradants. The chromatographic method used in these and other Paclitaxel profiling studies provided good resolution and reproducible relative retention (RRT, relative to Paclitaxel) times using standardized HPLC conditions.

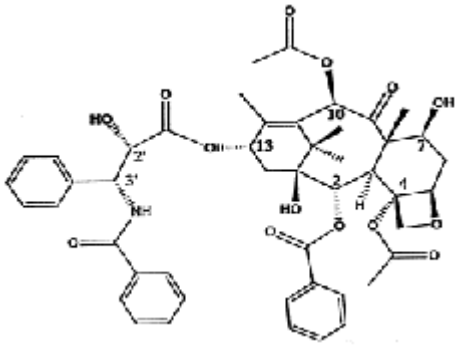


Fig. 1: Structure of Paclitaxel

The structure of Paclitaxel has shown in Fig. 1 illustrates the ester attachment of the Paclitaxel side chain to the tricyclic (baccatin) core as well as the various sites of acetylation on the diterpene core. Fig. 2 illustrates the HPLC–UV chromatogram of bulk Paclitaxel used in these degradation studies. Several low level impurities are observed in bulk Paclitaxel. Since Paclitaxel is a natural product, it is not surprising that trace level impurities are carried through the large scale purification process. As shown in Fig. 3, dissolution of Paclitaxel in basic aqueous/methanolic solutions results in the rapid formation of several degradants. Three early eluting degradants and a single late eluting degradant are formed upon exposure of Paclitaxel to basic conditions. The elution order of the three early eluting degradants indicates they are less lipophilic than Paclitaxel. Based on the structure of Paclitaxel, a favorable chemical process with basic conditions would involve hydrolysis of the ester linkages. In fact, information obtained on-line during LC–MS profile studies of the base induced degradants indicates molecular masses consistent with the hydrolyzed side chain and core.

The electrospray LC–MS interface utilized in these studies is an extremely soft ionization process and produces primarily pseudo-molecular ions such as $(MH)^+$ or $(M+NH_4)^+$ ions, providing definitive molecular mass information. Due to the lack of either an acidic or basic functional group, taxanes generally produce ammoniated molecular ions $(M+NH_4)^+$ when ammonium ions are present in the mobile phase. The LC–MS mass chromatograms corresponding to the ammoniated molecular ions of Paclitaxel and its base-induced degradants are shown in Fig. 4. The difference between the molecular mass of Paclitaxel and the degradant is indicative of the substructural differences between the compounds. For example, comparison of the molecular mass difference between Paclitaxel (MW 853) and the degradant at 12.2 min (MW 811) demonstrates a molecular mass difference of 42 Da, which is commonly indicative of an acetyl substructure in taxanes. The observed molecular masses of the degradants are consistent with the methyl ester of the Paclitaxel side chain, baccatin III (Paclitaxel core), deacetylated Paclitaxel, and an isomer of Paclitaxel.

When dealing with complex mixtures, the production of only molecular ions is an ideal situation for molecular mass confirmation. Unfortunately, the lack of fragmentation information in the full scan mass spectrum is detrimental from the structure elucidation perspective. However, when electrospray ionization is coupled on-line with tandem mass spectrometer, detailed structural information for each component can be obtained. [8-10]

In these studies, we relied heavily on these unique taxane fragmentation patterns and our previous taxane database to assist in the structural determination of the degradation products. In addition to chromatographic retention characteristics and molecular mass information, structures of degradants were proposed based on the comparison of their fragmentation patterns to those of Paclitaxel and other well characterized taxanes which were used as substructural templates. Interpretation of the structures of unknown degradants proceeded by the association of specific product ions and neutral losses with specific substructures. This MS–MS comparative method is based on the premise that the targeted degradants would be expected to retain much of the original Paclitaxel structure such as the taxane core substructure. Therefore, Paclitaxel-related degradants would be expected to undergo similar fragmentations to Paclitaxel and other taxane standards. Common MS–MS product ions and neutral losses observed in Paclitaxel and the unknown taxanes were evidence for common substructures and differences were indicative of variance in those substructures. Fig. 5A illustrates the MS–MS product ion spectrum of the base induced degradant at *m/z* 829 $(M+NH_4^+)$. Fig. 5B illustrates the product ion spectrum of Paclitaxel at *m/z* 871 $(M+NH_4^+)$. Comparison of the fragmentation template of Paclitaxel to that of the product ion spectrum of the degradant indicates a great deal of similarity. Diagnostic product ions indicative of the Paclitaxel side chain at *m/z* 286, 268, and 240 are present in both spectra indicating the structural difference in the degradant is not located on the side chain. However, a key product ion at *m/z* 569 indicative of the Paclitaxel core is noticeably absent in the product ion spectrum of the degradant. Instead, a product ion at *m/z* 527 is observed. This 42 Da mass difference is due to deacetylation in the degradant. Based on this structural information as well as molecular mass and chromatographic retention information, the degradant was assigned as 10-deacetylpaclitaxel.

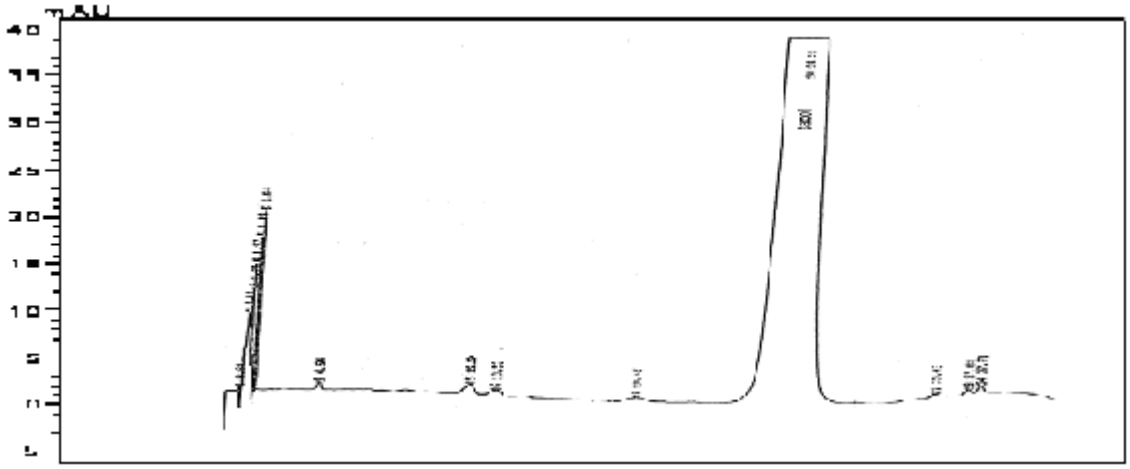


Fig. 2: HPLC chromatogram (228 nm) of Paclitaxel bulk drug lot used in degradation studies

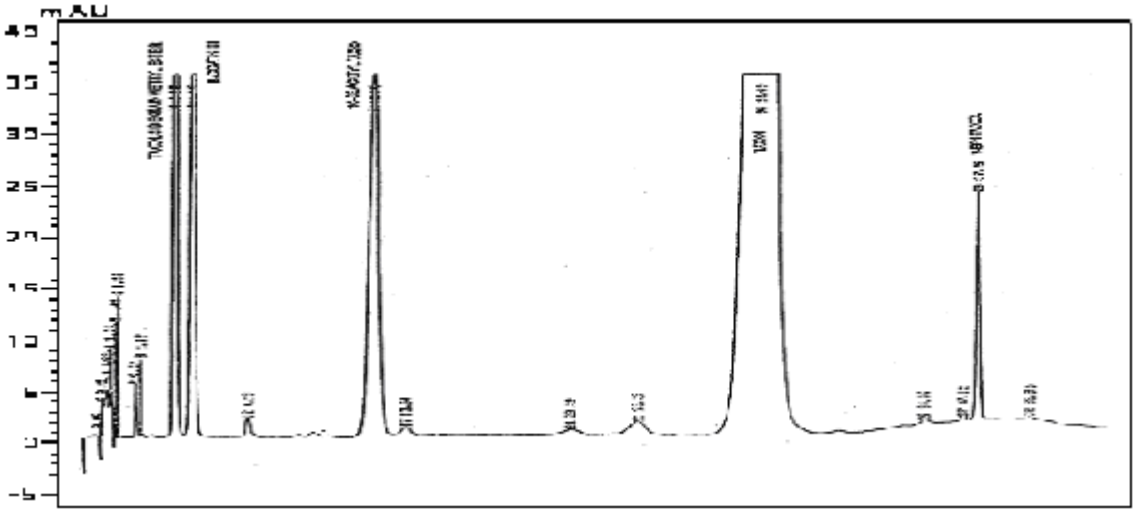


Fig. 3: HPLC chromatogram (228 nm) of base-induced degradants of Paclitaxel

During the development of Paclitaxel and our studies involving the identification of taxanes related to Paclitaxel in natural and process related extracts, we noticed a consistent pattern of two isobaric components producing two distinct chromatographic peaks in the mass chromatograms of *Taxus* extracts. This pattern was the result of epimerization of Paclitaxel analogs at the 7-position. The later eluting isomer had the 7-β-configuration and their identities, in several cases, were confirmed using synthesized or isolated standards. The formation of these epimers was also found to be pH dependent. At neutral pH and above, epimerization was favored. With these previous insights, the late eluting Paclitaxel isomer observed in the base degradation study, can be assigned as 7-epipaclitaxel. This same diagnostic comparative process was used to identify the other base-induced degradants as the methyl ester of the side chain and baccatin III. Formation of the methyl ester of the side chain resulted from esterification with methanol present in the sample diluent. Fig. 6 summarizes the base degradation pathway determined for Paclitaxel.

Acid degradation

Fig. 7 illustrates the HPLC-UV chromatogram of bulk Paclitaxel incubated in an aqueous/methanolic solution of HCl for 240 min. The two primary degradation products elute prior to Paclitaxel, indicating their polar characteristics versus Paclitaxel. The earlier eluting degradant at 13.0 min has the same relative retention time and molecular mass as 10-deacetylpaclitaxel. Apparently, the acetate group at the 10-position is fairly labile as evidenced by the presence of 10-deacetylpaclitaxel in bulk paclitaxel, base-degraded paclitaxel and acid degraded paclitaxel. The molecular mass of the later eluting, 19.1 min degradant was determined to be 871 Da. This molecular mass difference corresponds to an 18 Da increase relative to Paclitaxel. Examination of the product ion spectrum of this degradant shown in Fig. 8 indicates the diagnostic product ions indicative of the Paclitaxel side chain at *m/z* 286, 268, and 240 are present indicating the structural difference in the degradant is not located on the side chain. Product ions characteristic of the core were observed at *m/z* 587 and *m/z* 527, indicative of the 18 Da increase.

In addition, the typical core ions observed for Paclitaxel at *m/z* 569 and *m/z* 509 are also present resulting from loss of water. Based on the structure of Paclitaxel, a chemical process with acidic conditions would involve the addition of water (18 Da) to the Paclitaxel core, possibly opening of the oxetane ring. This information coupled with preliminary NMR data led to the proposal of an oxetane ring opened product which also suggested migration of the C-4 acetyl group to the oxetane oxygen. Based on the minimal degradation observed with the relatively harsh acidic conditions, Paclitaxel appears to be quite stable in an acidic environment.

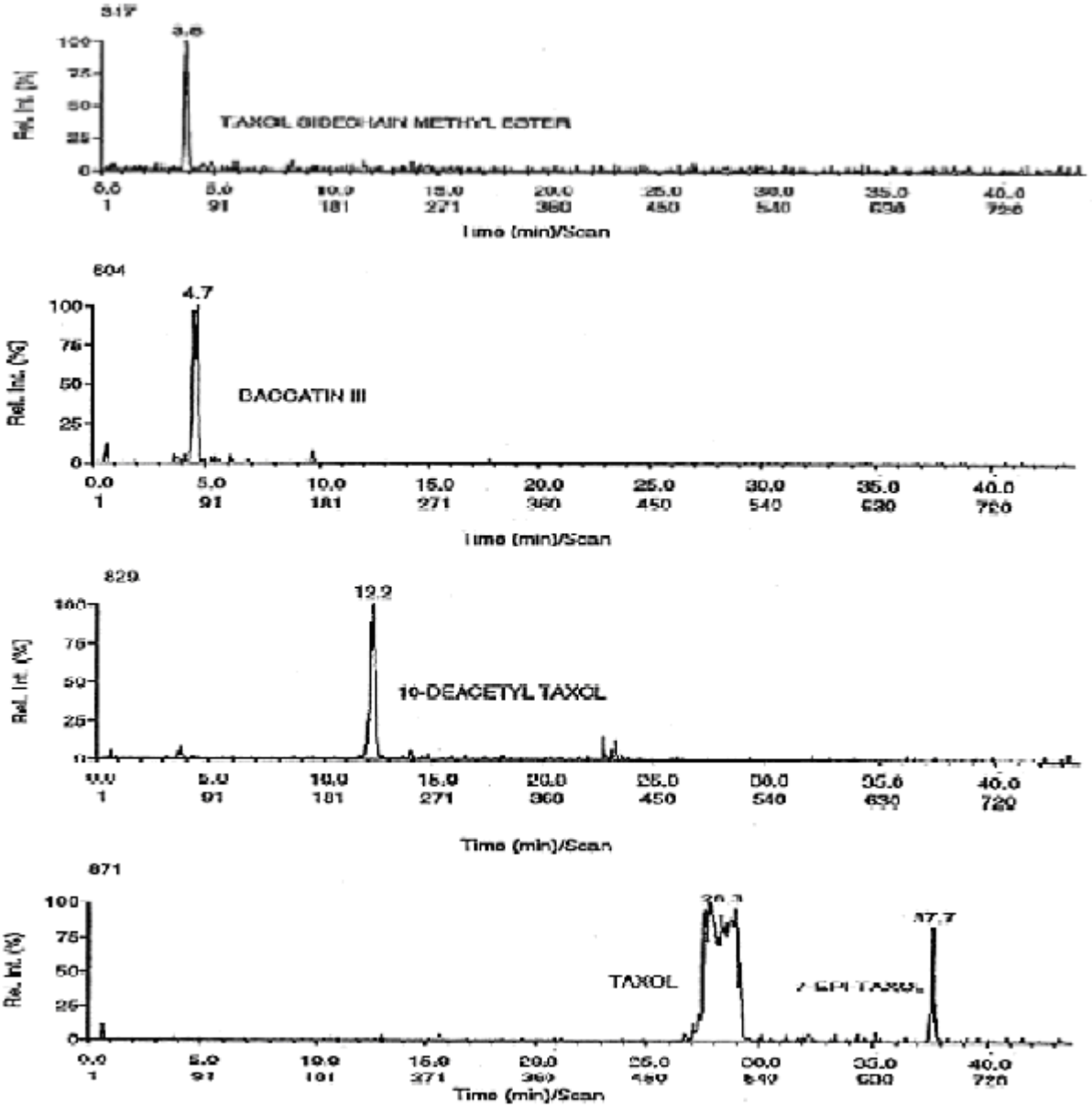
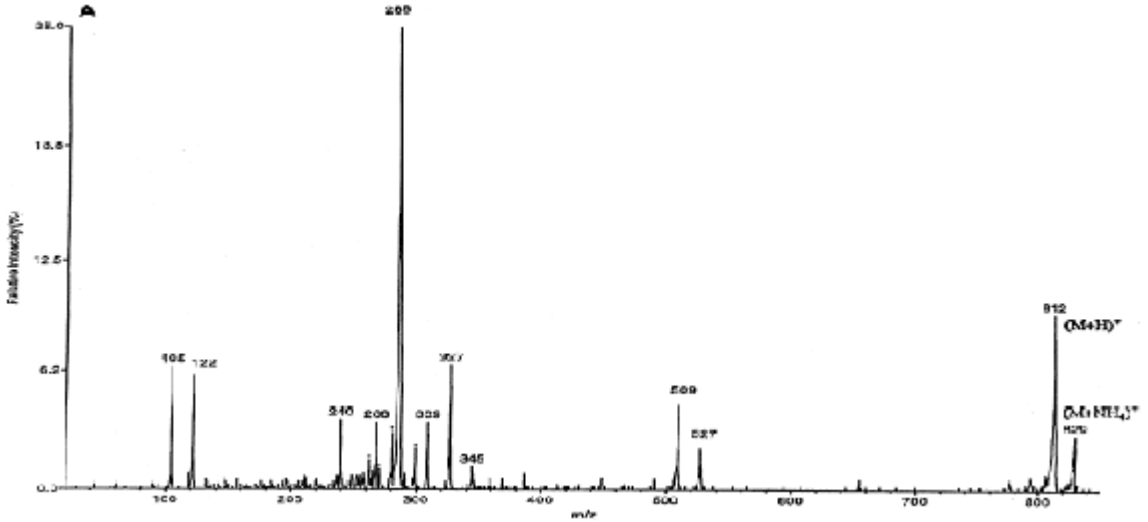


Fig. 4: LC–MS mass chromatograms (extracted ion current profiles) of paclitaxel treated with sodium carbonate for 10 min, at the *m*/*z* ratio 1 of their $(M+NH_4)^+$ ions, in the degraded sample shown in Fig. 3.



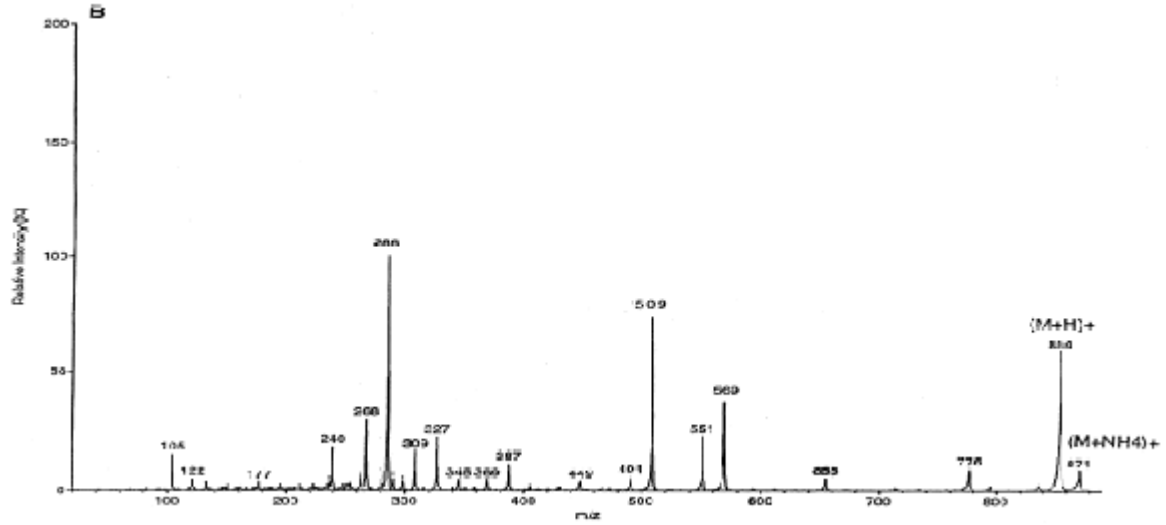


Fig. 5: (A) Product ion spectrum of the ion at *m*/*z* 829 $(M+NH_4)^+$ of the base-induced degradant. Product ions, neutral losses and their correspondence to specific substructures are indicated. (B) Product ion spectrum of the *m*/*z* 871 $(M+NH_4)^+$ ion of Paclitaxel used as template for structural analysis of related taxanes.

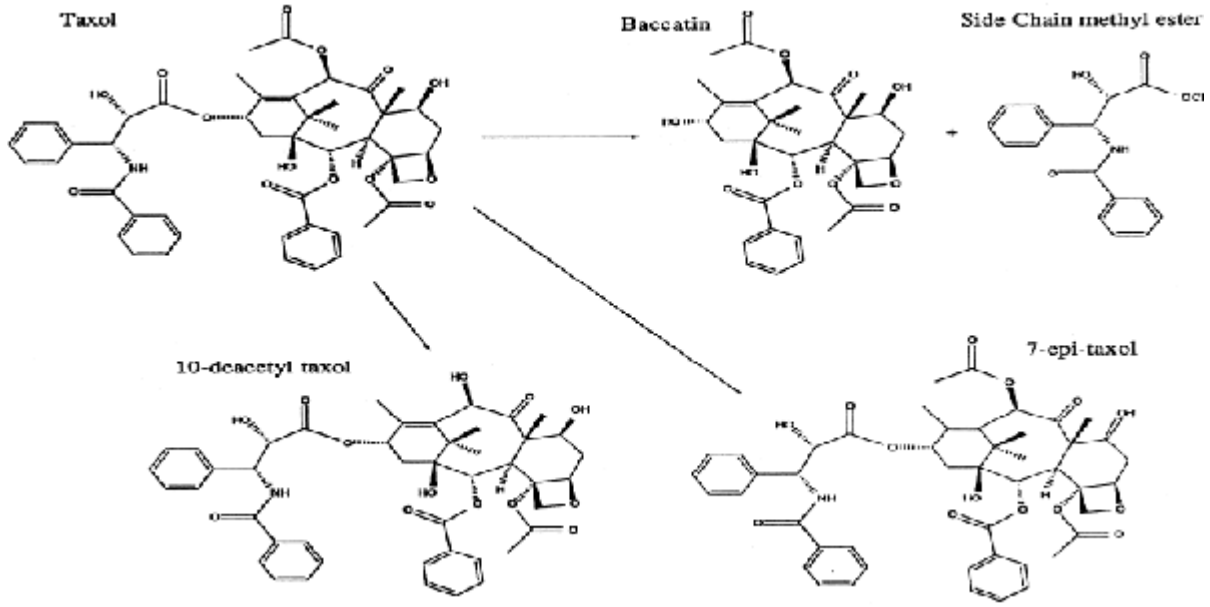


Fig. 6: Base-induced degradation pathway of Paclitaxel determined by LC–MS profiling

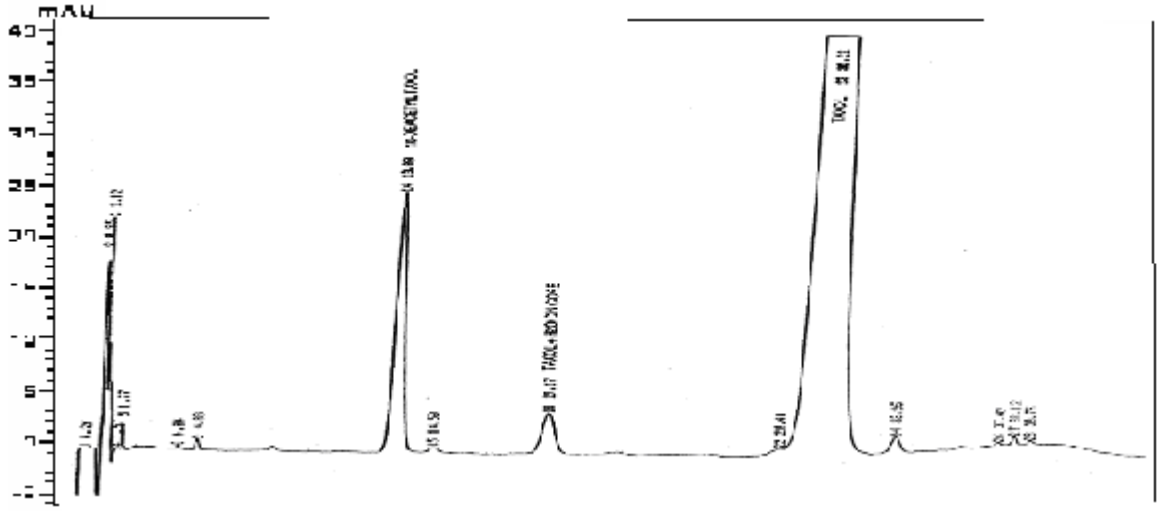


Fig. 7: HPLC chromatogram (228 nm) of the acid induced degradants of paclitaxel. Separation conditions described in Fig. 2

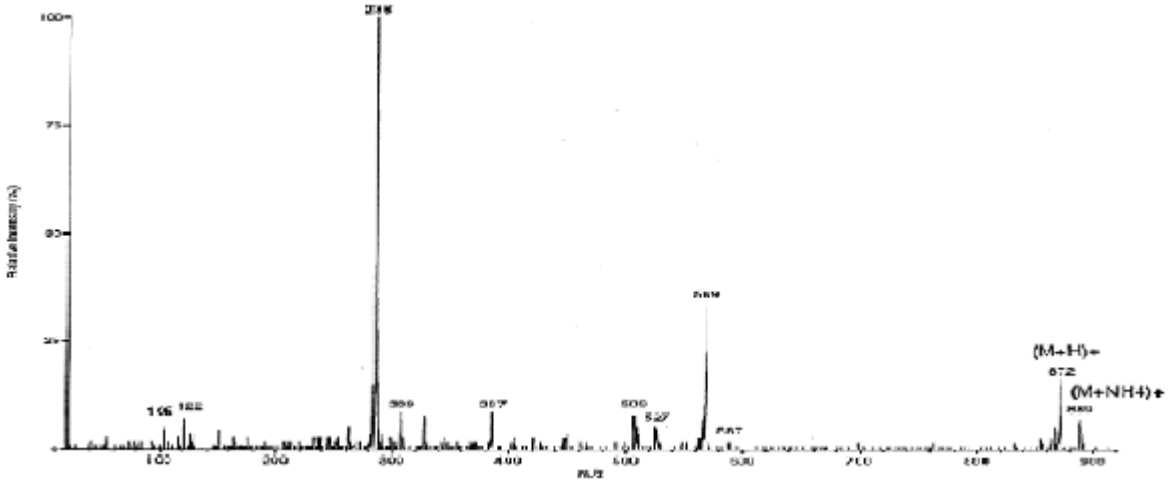


Fig. 8: Product ion spectrum of the acid degradant of Paclitaxel at *m/z* 889 $(M+NH_4)^+$

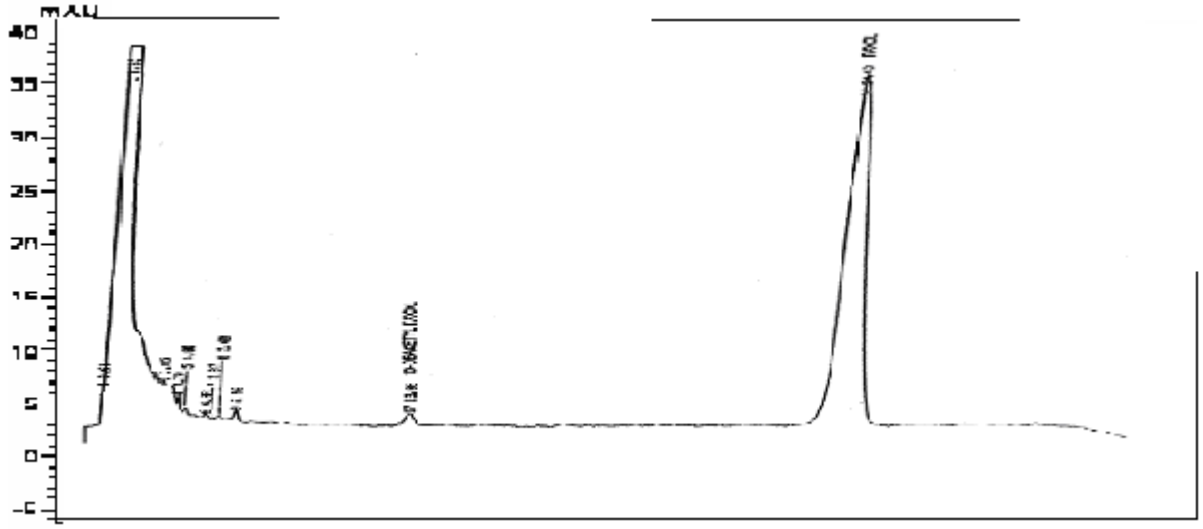


Fig. 9: HPLC chromatogram (228 nm) of the degradation caused by incubation of paclitaxel with aqueous hydrogen peroxide for 15 min. Separation conditions described in Fig. 2.

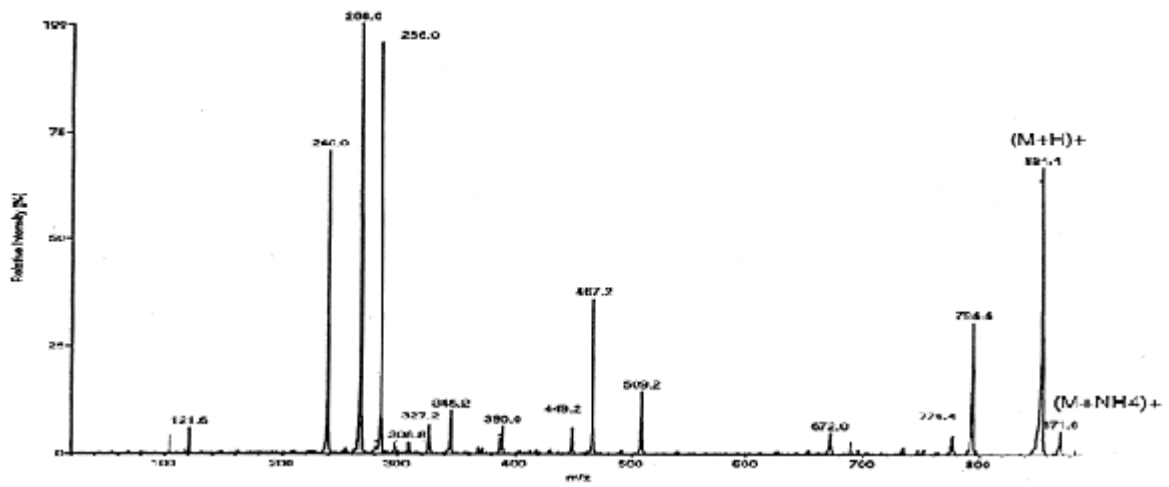


Fig. 10: Product ion spectrum of the photo degradant Paclitaxel isomer, *t* 14.8 min at *m/z* 871 $(M+NH_4)^+$

Oxidation

Fig. 9 illustrates the HPLC-UV chromatogram of bulk Paclitaxel incubated in aqueous solution of hydrogen peroxide. Only a single minor degradant was observed eluting prior to Paclitaxel. This degradant at 13.0 min has the same relative retention time and molecular mass as 10-deacetylpaclitaxel previously observed in the acid and base degraded samples. The lower UV response is due to the

protocol used for dissolution and incubation of Paclitaxel in an aqueous solution of hydrogen peroxide and resulted in less Paclitaxel injected on-column compared to the acid and base degradation studies. Based on the minimal degradation observed with these oxidizing conditions, Paclitaxel also appears to be relatively stable in an oxidizing environment.

Table 1: Paclitaxel degradation data

Retention time	Relative retention time	Molecular weight	Name	Type of stress applied
3.9	0.14	313	Side chain methyl ester	Base
4.6	0.16	586	Baccatin III	Base
9.9	0.35	853	Paclitaxel isomer	Light
12.2	0.43	811	10-deacetyl paclitaxel	Lightbase/acid/H2O2
14.8	0.52	853	Paclitaxel isomer (C3-C11 bridge)	Light
19.2	0.67	871	Oxetane ring opened	Acid
28.6	**1**	**853**	**Paclitaxel**	**-**
37.8	1.33	853	7-epi paclitaxel	Base

Table 2: Linearity data obtained on three different days

Regression parameters	Day 1	Day 2	Day 3	Mean±S.D. (%R.S.D.)
Slope	35,515	34,895	35,187	35,199 ± 310.17 (0.88)
Intercept	120,315	109,940	112,675	114,310± 5377.27 (4.70)
*R*2	0.9999	0.9997	0.9998	0.9998 ± 0.0001 (0.01)

Table 3: Reproducibility and precision data obtained during intra-day (*n* = 6) and inter-day (*n* = 3) studies

Actual concentration ($ngml^{-1}$)	Intra-day measured concentration ($ngml^{-1}$)±S.D.; %R.S.D.	Inter-day measured concentration ($ngml^{-1}$)±S.D.; %R.S.D.
100	98.864 ± 0.357; 0.361	98.581 ± 0.684; 0.699
400	403.256 ± 1.151; 0.285	403.917 ± 3.999; 0.990
1000	1001.849 ± 0.731; 0.073	992.644 ± 9.971; 1.004

Table 4: Recovery studies (*n*=3)

Actual concentration ($ngml^{-1}$)	Measured concentration ($ngml^{-1}$)±S.D.; %R.S.D.	Recovery (%)
50	49.71 ± 0.274; 0.552	99.42
200	203.00 ± 1.615; 0.795	100.58
500	501.18 ± 1.361; 0.271	100.12

Photolysis

A number of light induced degradants were observed. From a stability perspective, the largest and most interesting degradant corresponded to the 14.8 min peak which had a molecular mass identical to Paclitaxel (MW 853). Other lower level photodegradants observed in this profile included a second early eluting Paclitaxel isomer at 9.9min and three isomers of Paclitaxel minus a core carbonyl at retention times of 27.8, 30.2, and 36.4min. The product ion spectra of each of the photodegradants observed indicated a modification of the Paclitaxel core. The MS–MS product ion spectrum of the 14.8 min Paclitaxel isomer is shown in Fig. 10. Examination of the product ion spectrum of this primary photo degradant indicates the same diagnostic product ions indicative of the Paclitaxel side chain at *m/z* 286, 268, and 240 are present. In addition, the product ion at *m/z* 509, indicative of the deacetylated core, is also present. The absence of the product ion at *m/z* 569 indicates facile loss of a single molecule of acetic acid is favored for this Paclitaxel isomer. This is also consistent with the observation of a new product ion at *m/z* 794 corresponding to loss of acetic acid from the protonated molecule. In addition, a new product ion at *m/z*467 is observed and possibly resulting from consecutive loss of ketene (CH_2CO) from the *m/z* 509 product ion. The changes in the product ion spectrum of this photo degradant relative to product ion spectrum of Paclitaxel suggested a structural difference (rearrangement) near acetate on the core of this particular photo degradant. Small amounts of this photo degradant were isolated and characterized by proton NMR which indicated the C3–C11 bridge shown in Fig. 10. [11]

A summary of all degradation products, including major photolysis products, observed in the in these LC–MS profile studies are tabulated in Table 1. Based on the light induced degradation profile, Paclitaxel appears to be susceptible to a variety of photolytic reactions. As a result, this database serves as a valuable library to reference any new degradants observed in long-term storage stability samples or new process impurities.

Thermal stress

The exposure of the solid drug to 50°C for 60 days and 60°C for 15 days did not result in significant decomposition. It indicated that Paclitaxel was stable to dry heat.

Validation of the stability-indicating method

The data obtained from linearity studies are given in Table 2. The response of Paclitaxel was strictly linear in the concentration range between 100 and 10000$ngml^{-1}$. The mean (±%R.S.D.) values of slope, intercept and correlation coefficient were 35,199 (±0.88), 114,310 (±4.70) and 0.9998 (±0.01), respectively. The %R.S.D. values for intra-day and inter-day precision studies (Table 3) were <0.5% and <1.1%, respectively, confirming that the method was sufficiently precise. Good separation was achieved even when the procedure was repeated by a different person, thus confirming the reproducibility of the method. As shown from the data in Table 4, good recoveries were made at the added concentration of 50, 200 and 500 $ngml^{-1}$, with the mean recovery being 100.04%.

Applicability of the developed method to stability samples

The developed method was found to apply even to real stability samples, which was verified through successful analyses of single formulation containing Paclitaxel, RELICOAT and Release-T (Drug Eluting Coronary Stent) containing Paclitaxel stored at accelerated conditions of temperature (40°C) and humidity (75% RH) for 3 months. Although minor decomposition was observed, the degradation products were still discernible and well separated. In general, degradation was more in the formulation than Release-T. Indirectly, it was also established that the developed method could even be used for formulations containing Paclitaxel along with polymers, without interference from the latter due to lack of UV absorbance at the wavelength of analysis (228 nm).

It was possible in this study to develop a stability-indicating LC assay method for Paclitaxel by subjecting the drug and formulation to ICH recommended stress conditions. The drug and degradation products got well separated. The high sensitivity of mass spectrometry is particularly advantageous for application to samples which contain trace impurities and degradants, a situation frequently encountered in pharmaceutical discovery and development research. A

strategy involving the use of LC–MS profiling and LC–MS–MS substructural analysis has been shown to be capable of providing a highly sensitive and specific method for rapidly obtaining molecular mass and structural information about low level impurities and degradants.

Degradation products were elucidated on the basis of their chromatographic relative retention times using standardized HPLC conditions, molecular mass information obtained from the full scan mass spectrum acquired during LC–MS profiling, and the product ion spectrum acquired during LC–MS–MS substructure analysis studies. Degradation products formed upon exposure to basic conditions included baccatin III, Paclitaxel side chain methyl ester, 10-deacetylpaclitaxel, and 7-epipaclitaxel. Degradation products formed upon exposure to acidic conditions included 10-deacetylpaclitaxel and the oxetane ring opened product. Treatment with hydrogen peroxide produced only 10-deacetylpaclitaxel. Exposure to high intensity light produced a number of degradants. The most abundant photo degradant of Paclitaxel corresponded to an isomer which contains a C3–C11 bridge. Other lower level photodegradants observed in this profile included a second early eluting Paclitaxel isomer and three isomers of Paclitaxel minus a core carbonyl. Based on these studies, an LC–MS degradant database, including information on molecular structures, chromatographic behavior, and molecular mass and substructure-specific MS–MS product ions of the components has been developed.

Using this methodology, detailed structural information is typically obtained for potential degradants in less than one day. Most importantly, these predictive studies will provide a foundation for future work involving the analysis of new Paclitaxel degradation products.

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