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Long-term physical and chemical stability of a generic paclitaxel infusion under simulated storage and clinical-use conditions

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Abstract

Study objectives: The physical and chemical stability of paclitaxel infusion, prepared from a generic concentrate, was investigated in Freeflex polyolefin bags containing 0.9% sodium chloride or 5% glucose under refrigerated storage and in-use conditions (25°C) to facilitate an outpatient chemotherapy dose-banding scheme, and to avoid wastage when chemotherapy is delayed for clinical reasons.

Methods: Paclitaxel infusions were sampled at predetermined time-intervals and analysed for physical stability (pH, sub-visual particulates, % weight loss, visual appearance) and chemical stability (assay) using a validated stability-indicating high performance liquid chromatography method.

Results: Paclitaxel infusion was chemically stable with variation in assay values within ± 5% until the time the infusion precipitated on prolonged storage at 5°C and 25°C. The stability of paclitaxel infusion prepared from generic concentrate at three concentrations (0.3 mg/mL, 0.75 mg/mL and 1.2 mg/mL) at 5°C in 5% glucose was 28, 16 and 12 days respectively. Physical instability was identified as the limiting factor influencing the stability of the infusion.

Conclusion: The stability of paclitaxel infusion was limited by physical stability and this was dependent on paclitaxel concentration in the infusion, diluent used and the storage temperature.

Keywords

paclitaxel, Freeflex, stability, infusions, compatibility

Introduction

Paclitaxel, a potent chemotherapeutic agent was discovered during a large-scale screening programme conducted by the National Cancer Institute in the 1960s and was initially extracted and isolated from the bark of Western Yew, Taxus brevifolia [1]. Since its discovery, paclitaxel has been indicated in the treatment of a variety of cancers such as advanced ovarian and breast cancer, non-small cell lung cancer and AIDS-related Kaposi's sarcoma [2-4]. In 1979, Schiff and co-workers [5] rekindled interest in the development of paclitaxel by demonstrating its novel mechanism of action. The antitumour activity of paclitaxel is due to its ability to promote polymerisation of tubulin, which results in the formation of stable, dysfunctional microtubules and causes cell death due to disruption of the normal tubule dynamics required for cell division and vital inter-phase processes [6].

During formulation development, a primary consideration essential for developing a stable intravenous formulation involves selecting a formulation vehicle in which the drug has adequate solubility. The major impediment to the aqueous solubility of paclitaxel is its extreme lipophilicity [7-9], which presents difficulties in developing stable parenteral formulations. To circumvent this problem, co-solvents are frequently used in the formulation [10] and commercially available paclitaxel injection is therefore formulated in a 50:50 solvent mixture of Cremophor EL and dehydrated ethanol [11]. However, Cremophor EL causes severe hypersensitivity reactions in patients and premedication with steroids, antihistamines and H_2-receptor antagonists is required [12-15]. Furthermore, this formulation vehicle is incompatible with some plastics and is known to leach diethylhexylphthalate plasticizers from polyvinyl chloride (PVC) infusion bags and administration sets [16-18]. It is therefore recommended that paclitaxel infusion be prepared and stored in glass, polypropylene or polyolefin containers.

In clinical practice, paclitaxel is administered after diluting the drug concentrate for injection with 0.9% sodium chloride or 5% dextrose to achieve a final concentration of 0.3-1.2 mg/mL [19-20]. For an infusion to be safe and therapeutically effective, adequate physical and chemical stability under clinical use conditions is required to facilitate accurate and reproducible dosing of the drug.

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However, physical stability of commercially available paclitaxel infusion after dilution is adequate only for short-term storage. Published reports indicated that paclitaxel infusion exhibited physical instability due to precipitate formation on storage [21], and the extent of precipitation was greater for infusions containing more than 0.6 mg/mL paclitaxel [22]. Moreover, long-term stability is required for the development of dose-banding strategies [23] and for the avoidance of wastage on occasions when treatment is delayed after the infusion has been prepared.

In this study, a generic paclitaxel injection (Mayne Pharma) which was pH balanced using anhydrous citric acid to a pH range of 5-7, to improve stability of the formulation [24] was investigated. The main objective of this work was to determine the physical and chemical stability of paclitaxel infusion at concentrations 0.3 mg/mL, 0.75 mg/mL and 1.2 mg/mL, in 0.9% sodium chloride or 5% glucose under storage at 5°C and 25°C in polyolefin infusion bags. In addition, a limited number of infusions prepared using the innovator product (Taxol) were included for comparison.

Materials and Methods

Paclitaxel (6 mg/mL, batch numbers: (B) N036850, P036869, P026862) was supplied by Mayne Pharma Plc. Freeflex infusion bags containing 250 mL, 0.9% sodium chloride, (batch number: SL 7201 08, S1720606) and 5% glucose, (batch number: SD 7206 01) were purchased from Fresenius Kabi Ltd. Taxol 6 mg/mL injection (batch number: 5B00028) was obtained from Bristol-Myers Squibb Ltd. All other chemicals and reagents used were either analytical grade or high performance liquid chromatography (HPLC) grade, as appropriate.

Preparation of drug infusion

Paclitaxel infusion was prepared under EU class A conditions, following GMP procedures by aseptic addition of paclitaxel (6 mg/mL) concentrate to the infusion bag containing 0.9% sodium chloride or 5% glucose to achieve the required nominal concentration of 0.1 mg/mL, 0.3 mg/mL, 0.75 mg/mL and 1.2 mg/mL. These concentrations were selected to represent paclitaxel doses normally used in clinical practice. After adding the drug concentrate, the infusion bag was gently agitated to promote adequate mixing. Triplicate infusion bags of each concentration/diluent combination were placed in blue polythene overwraps to protect from light and incubated in a pharmaceutical refrigerator (5°C) or an incubator (25°C) to simulate storage and clinical use conditions. Immediately after preparation (t = 0) and at appropriate time intervals (see results section), samples were withdrawn, equilibrated to room temperature and analysed for physical stability (visible and sub-visual particulates, pH, % weight loss) and chemical stability (assay).

Determination of chemical stability

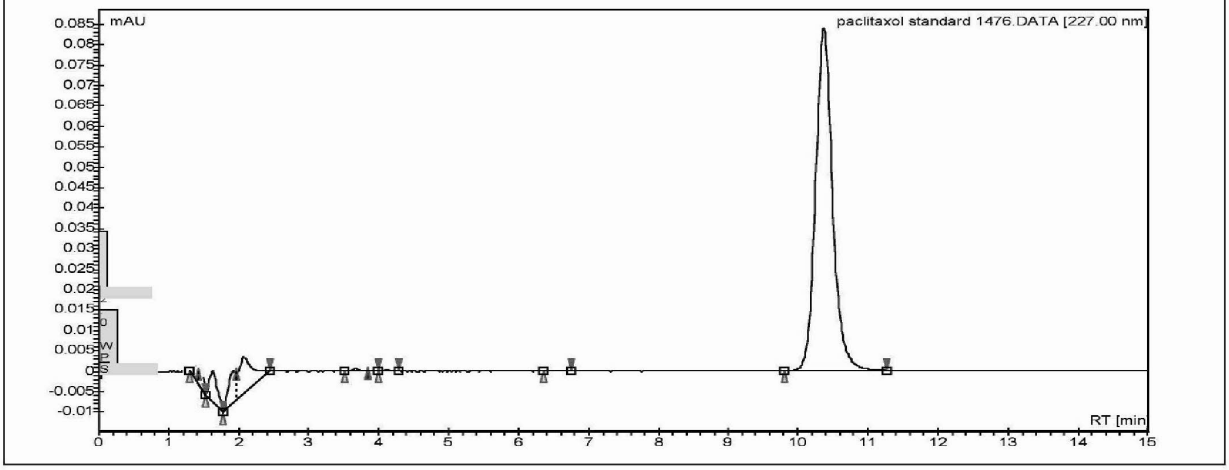
Paclitaxel was analysed using a validated stability-indicating reverse phase HPLC method. The HPLC system consisted of a quaternary gradient pump (Jasco PU-2089 plus) an in-line degasser, autosampler (Jasco AS-2057 plus), photodiode array detector (Jasco MD-2010 plus).

HPLC method and validation

HPLC parameters

Mobile phase composition: 50% of 20mM ammonium acetate (pH 5), 40% Acetonitrile and 10% Methanol
Column: C_8 (Phenomenex), 5 µm (250 x 4.6 mm)
Flow rate: 1.5 mL/min
Injection volume: 20 µL
Detection: 227 nm (0.02 AUFS)

Figure 1: HPLC chromatogram of paclitaxel (60 µg/mL)



Calibration plot

A typical chromatogram obtained by injecting a solution of paclitaxel (60 μg/mL) is shown in Figure 1. A six-point calibration plot of paclitaxel over the range 15 to 90 μg mL^{-1} was constructed with triplicate injections at each concentration. The data was subjected to linear least-square regression analysis and the plot was found to be linear over the range tested with a regression coefficient of 0.9997. Inter- day and intra- day precision was 1.6% and 1.4% respectively.

Stability-indicating ability of LC assay

A study was performed to determine whether paclitaxel could be resolved from any degradation products, and confirm that the assay was stability-indicating. In addition, spectral purity of paclitaxel peak was assessed using Chrompass software. Table 1 reveals the effect of forcibly degrading paclitaxel by alkaline, acidic or oxidative conditions or by heating at 55°C. Paclitaxel (60 μg/mL) stored in the refrigerator was used as a control sample for comparison purposes. The peak retention time of paclitaxel control sample was approximately 11.4 minutes with a drug concentration of 61.65 μg/mL. On exposure to oxidative degradation, elevated temperature of 55°C or acid hydrolysis, paclitaxel retention time was approximately 11.4 minutes in each case with a drug concentration of 57.89 μg/mL, 61.09 μg/mL and 30.53 μg/mL, respectively. However, a cloudy gel was observed in paclitaxel solution subjected to alkaline hydrolysis. The dispersion was filtered using a 0.2 μm filter and the filtrate analysed for drug content by HPLC. Results indicated absence of drug peak implying that the paclitaxel had completely precipitated under alkaline conditions and had been removed by the filtration process. Under other stress conditions there was a distinct separation between the drug and degradation product peak. In all cases, the spectral purity of the paclitaxel peak (by photodiode-array detector) was greater than 98%.

Determination of physical stability

Sub-visual particulates

Sub-visual particle counts of infusions at 10 μm and 25 μm particulate diameters were conducted at predetermined time intervals using a calibrated particle counter (Model LS-200, Particle Measuring Systems). A fixed volume of sample was withdrawn using a computer controlled syringe sampler and passed through Liquilaz sensor at a controlled flow rate and any particles present in solution counted.

Table 1: Effect of oxidative degradation, alkaline hydrolysis, acid hydrolysis and elevated temperature (55°C) on paclitaxel LC assay and on peak purity of the paclitaxel peak

Treatment	Retention time (minutes)	Quantity (μg/mL)	Peak purity (%)
Control	11.37	61.65	100.53
Oxidative degradation	11.36	57.89	98.55
Alkaline hydrolysis	-	-	-
Exposed to 55°C	11.35	61.09	98.67
Acid hydrolysis	11.36	29.06	98.09

Visual appearance

This was assessed against both black and white backgrounds under standard laboratory lighting and the infusions were examined for any change in colour, clarity or for the presence of particulate matter. Any form of turbidity, visible particles or fibres was classed as "precipitation".

pH measurement

A combinational glass electrode and a Hanna pH302 meter (Hanna Instruments, Scientific Laboratory Supplies Ltd) was initially calibrated using standard reference solutions of pH 4 and pH 7 and was used to measure the pH of infusions at specific time intervals for the duration of the study. Addition of paclitaxel formulation excipients ethanol and Cremophor EL to the reference solutions did not influence the accuracy of pH measured.

Weight change

The infusion bags were weighed before and after sampling on a calibrated analytical balance (AND, AD-1131) and the % weight increase or decrease on incubation calculated.

Results

Stability of paclitaxel 0.3 mg/mL, 0.75 mg/mL and 1.2 mg/mL infusion in Freeflex infusion bags

It was envisaged that the stability results obtained would enable an appropriate shelf-life to be assigned for various pre-filled paclitaxel infusions used in dose-banding schemes. The chemical stability data for generic paclitaxel infusion at concentrations 0.3 mg/mL , 0.75 mg/mL and 1.2 mg/mL, in 0.9% sodium chloride or 5% glucose and stored at 5°C or 25°C in Freeflex infusion bags for varying periods of time are shown in Table 2. The infusion was considered to be chemically stable if variations in drug concentration did not exceed ± 5% of the initial concentration. The initial concentration of paclitaxel at time zero was assigned 100% and subsequent assay values were expressed as a percentage of the initial concentration. In each case, data reported are the means and relative standard deviation for 3 infusions.

Paclitaxel infusion was examined visually at predetermined time intervals for the presence of particulates or turbidity (indicating paclitaxel precipitation) and any change in the colour of the infusion. Results indicated that the infusion exhibited precipitation on prolonged storage at 5°C and 25°C and this was the stability limiting factor of the infusion. Visual observation indicated that the

extent of precipitation increased on storage and resulted in turbidity for paclitaxel (0.3 mg/mL) infusion and the formation of white aggregated precipitate for the more concentrated paclitaxel (1.2 mg/mL) infusion.

The data in Table 2 indicate that tendency to precipitation was related to both concentration of the infusion and to storage temperature, with reduced physical stability at the higher paclitaxel concentrations and at room temperature storage. Prior to precipitation, the LC assay values obtained for all infusions were within the applied limits (± 5%). In addition, there was no evidence of any degradation products in the LC chromatograms. The maximum shelf-life was therefore assigned to the maximum sampling time at which the infusions (in triplicate) remained visibly clear with no evidence of precipitation.

Data for physical stability (other than visual appearance) are summarised in Table 3. This table shows that up to the sample times corresponding to the assigned shelf-life, there were no significant changes in pH or weight for any of the infusions studied. Most of

Table 2: Chemical stability for infusions of paclitaxel (Mayne) at concentrations of 0.3 mg/mL , 0.75 mg/mL and 1.2 mg/mL in 0.9% sodium chloride or 5% glucose at 5°C or 25°C

Diluent	Storage time (d)	Mean Assay (RSD, n = 3) as % initial concentration remaining for each infusion concentration/ storage temperature combination					
		0.3 mg/mL		0.75 mg/mL		1.2 mg/mL	
		5°C	25°C	5°C	25°C	5°C	25°C
G5	2		99.75(0.9)		102.29(0.7)		100.99(0.6)
N/S	2		98.21(0.9)		99.16(0.9)		99.48(0.8)
G5	3	101.73(0.8)		100.64(2.0)		101.28 (1.2)	
N/S	3	99.28(0.6)		98.55(0.77)		100.39 (0.6)	
G5	4		99.17(0.3)		100.62(1.1)		99.23(0.6)
N/S	4		97.89(0.4)		99.87(1.1)		97.42(0.5)
G5	6	100.64(0.5)	101.13(0.2)	100.57(0.3)	*	100.48 (1.5)	*
N/S	6	99.92(1.6)	98.84(0.2)	100.29(0.4)	98.34(0.88)	100.15 (1.3)	*
G5	8		98.97(0.9)		*		
N/S	8		*		*		
G5	9	100.65(0.5)		101.43(0.3)		99.13 (0.1)	
N/S	9	98.98(0.7)		100.87(0.6)		97.7 (0.2)	
G5	10		*				
N/S	10		*				
G5	12	98.90(0.7)		101.58(0.8)		99.5 (0.6)	
N/S	12	96.99(0.6)		100.78(0.4)		98.22 (0.7)	
G5	14	100.66(0.8)		101.49(1.9)		*	
N/S	14	97.74(1.9)		101.04(0.3)		*	
G5	16	98.28(0.8)		102.71(0.4)			
N/S	16	96.9(1.0)		102.33(0.7)			
G5	18	101.83(0.5)		103.03(1.0)			
N/S	18	99.81(0.8)		102.86(0.4)			
G5	20	99.66(0.6)		99.61(0.3)			
N/S	20	98.62(0.7)		99.34(0.8)			
G5	23	100.13(1.2)		*			
N/S	23	99.41(1.3)		*			
G5	25	100.75(0.2)					
N/S	25	99.37(0.5)					
G5	28	101.16(0.6)					
N/S	28	99.28(0.6)					

* Visible precipitation in infusion (G5 = 5% glucose, N/S = 0.9% sodium chloride)

the sub-visual particulate counts for particle sizes >10 μm diameter obtained at the time corresponding to the assigned shelf-life would be considered high for an intravenous infusion. However, it would not be appropriate to apply pharmacopoeial limits to an infusion for which the marketing authorisation requires administration via a 0.22 μm in line filter.

Discussion

The shelf-life of paclitaxel infusions was limited by physical stability in terms of visible precipitation of drug from the infusion. Chemical stability was maintained until, and probably beyond, the time at which precipitation was observed. Particulate formation in paclitaxel infusion could be due to drug degradation, "seeding" by particulate matter in the diluent, components being leached from the infusion bag into the solution forming an insoluble precipitate or, more probably, from diluting the paclitaxel concentrate with the infusion fluid to produce an unstable formulation. Additional studies, not reported here, confirmed that the precipitate was, in each case, paclitaxel.

Sub-visual particulate monitoring data are not fully reported here, but typically an increase in the sub-visual particulate count at 10 μm was observed in the sample taken prior to the day on which visible precipitation was first evident. This technique may be a useful predictor of physical stability, if only at a qualitative level.

Previously, Xu et al [25] reported that the stability of paclitaxel 0.1 mg/mL and 1 mg/mL in 5% dextrose or 0.9% sodium chloride injection was maintained for at least 3 days at 4, 22 or 32°C. However, further storage at these temperatures resulted in precipitation which, as with this study, was the primary factor limiting stability. In this study, the results presented in Tables 2 and 3 indicate that paclitaxel infusion at concentrations 0.3 mg/mL, 0.75 mg/mL and 1.2 mg/mL stored at 5°C were physically and chemically stable for up to 28, 20 and 12 days in 5% glucose and 28, 20 and 12 days in 0.9% sodium chloride respectively. Stability of the infusions decreased at 25°C and at concentrations 0.3 mg/mL, 0.75 mg/mL and 1.2 mg/mL infusions were stable for 8, 4 and 4 days in 5% glucose and 6, 6 and 4 days in 0.9% sodium chloride, respectively. There was a negligible change in pH and the variation over 28-day study period was less than 0.6 pH units in all cases. Moisture diffusion across the infusion bag was minimal with less than 1% weight loss in all containers. Moisture loss from the bag would not therefore exert any significant effect on the paclitaxel assay value following either refrigerated or room temperature (25°C) storage.

The findings of this study confirm that the shelf-life of paclitaxel infu-

Table 3: Summary of physical stability data for paclitaxel (Mayne) infusion of various concentrations diluted in 5% glucose or 0.9% sodium chloride, under storage at 5°C or 25°C

Diluent	Storage Temp. °C	Paclitaxel Conc. (mg/mL)	Assigned Shelf-life[a] (days)	Sub-visual Particulates (count/mL)[b] 10 μm	25 μm	pH range[c]	Weight loss (%)[b]
5% glucose	5	0.3	28	120	0.44	4.1-3.5	<0.6
0.9% sodium chloride	5	0.3	28	75	0.22	4.0-3.4	<0.2
5% glucose	25	0.3	8	49	0.50	4.1-3.8	<0.6
0.9% sodium chloride	25	0.3	6	107	0.22	3.8-3.9	<0.2
5% glucose	5	0.75	20	99	0	3.9-3.6	<0.1
0.9% sodium chloride	5	0.75	20	194	1.11	3.9-3.5	<0.2
5% glucose	25	0.75	4	50	0	3.9-3.7	<0.2
0.9% sodium chloride	25	0.75	6	52	0.11	3.9-3.5	<0.1
5% glucose	5	1.2	12	78	0.33	4.0-3.7	<0.2
0.9% sodium chloride	5	1.2	12	118	0.44	3.8-3.5	<0.5
5% glucose	25	1.2	4	83	0.22	4.0-3.9	<0.1
0.9% sodium chloride	25	1.2	4	76	0.33	3.8-3.5	<0.3

Reported data are means for 3 infusions (n = 3)

[a] Assigned to the longest storage time for which infusions were within assay specification and did not show precipitation (see Table 2)

[b] Values obtained at the sample time corresponding to assigned shelf-life as defined in footnote [a], above.

[c] Range of values obtained over period from time = 0 to the assigned shelf-life defined in footnote [a], above.

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