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Journal of Pharmaceutical and Biomedical Analysis
34 (2004) 501–507
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Rapid HPLC method for the determination of paclitaxel in pharmaceutical forms without separation

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Received 13 December 2002; received in revised form 10 October 2003; accepted 15 October 2003

Abstract

A HPLC method has been developed for the in-process determination of the paclitaxel from dosage pharmaceutical forms. This method ensures the rapid determination of paclitaxel in the presence of polyoxyl castor oil—the main constituent of paclitaxel's clinical formulation vehicle. The method is simple and rapid and does not require any preliminary treatment of the sample. The method was fully validated.

Keywords: Paclitaxel; Cremophor® EL; HPLC

1. Introduction

The paclitaxel belongs to the taxanes—a structurally and mechanistically unique class of antineoplastic agents—and was the first taxane used in clinical development [1,2]. The low solubility of the paclitaxel in aqueous systems was the major problem in the formulation of this compound [3]. This problem was solved by the development of a concentrated solution in polyoxyl castor oil and dehydrated alcohol. A number of papers have reported methods for the determination of paclitaxel in biological fluids, including capillary electrophoresis [4], liquid-chromatography–mass spectrometry [5,6] and HPLC [7–11]. A critical search of the literature reveals that at this time the HPLC method has emerged as the technique of choice for the separation and determination of paclitaxel. The methods cited are selective but time consuming and for this reason could not be used for the in-process control of formulated paclitaxel. The increase in drug availability has finally led to the need for analytical methods for the in-process quality control of the formulated drug. These methods must be rapid and selective with regard to the polyoxyl castor oil—the main constituent of paclitaxel's clinical formulation vehicle. The analytical methods used for the quality control of formulated paclitaxel require a multi-step sample treatment that is laborious and time consuming. Generally the HPLC methods require a laborious sample preparation including liquid–liquid extraction, solid phase extraction or a combination of both procedures [12].

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doi:10.1016/S0731-7085(03)00628-9

As an official monograph of paclitaxel does not exist in any pharmacopoeia the aim of our study was the development of a selective and sensitive chromatographic method for the rapid in-process determination of paclitaxel in the presence of polyoxyl castor oil. This method could be used for the evaluation of the parameter assay during the quality control of the formulated drug. This paper describes a practical, selective and rapid HPLC method for the in-process determination of paclitaxel without any preliminary separation and has been developed based on a previously published paper [13]. The proposed method has been validated according to the ICH guide [14,15] and has been applied for in-process control and also for bulk and finished product quality control.

2. Experimental

2.1. Materials

The study was conducted with 10 working standards. Six of them were purchased from Indena (Milan, Italy): paclitaxel 97.13% potency, cephalomannine 90.69% potency, paclitaxel C 94.61% potency, 7-epi-cephalomannine 87.57% potency, 7-epi-paclitaxel 92.92% potency, *N*-methylpaclitaxel C 85.85% potency; three from Dabur (Ghaziabad, India): baccatin III 93.40% assay, 10-deacetyl-baccatin III 95.00% assay, 10-deacetyl-7-epi-paclitaxel 98.90% assay and one from Lipomed (Arhsheim, Switzerland) 10-deacetyl-paclitaxel 97.93% assay. The polyoxyl castor oil (Cremophor® EL) was purchased from BASF AG (Ludwigshafen, Germany), alcohol, absolute and citric acid, anhydrous from Merck (Darmstadt, Germany). Water for chromatography was purified using a MilliQ ultra pure water system Biocel A 10, Millipore (Yvelines, France) and the acetonitrile gradient grade was purchased from Aldrich (Taufkirchen, Germany).

2.2. Equipment

Two Hewlett-Packard 1100 (Boeblingen, Germany) chromatographic systems have been employed. System 1 consisted in a quaternary pump G 1311A, a PDA detector G 1315A, an automatic injector G 1313A, a column thermostat G 1316A and an on-line degasser G 1322A. The chromatographic system 2 consisted in a binary pump G 1312A, a PDA detector G 1315A, an automatic injector G 1313A, a column thermostat G 1316A and an on-line degasser G 1322A. The chromatographic separations were carried out using a column purchased from Supelco (Taufkirchen, Germany) type Supelcosil LC-F (stationary phase: pentafluorophenyl), 5 μm, 25 cm × 4.6 mm supplied with a precolumn Supelguard LC-F (stationary phase: pentafluorophenyl), 2 cm cartridge with column temperature steed at 25 °C. The detection was at $\lambda = 227 \pm 2$ nm, reference $\lambda = 360 \pm 20$ nm. The data were collected and processed by means of HP ChemStation for LC software. The ORIGIN program (Micro Cal Inc., version 4.10) was employed for the linear regression analysis.

2.3. Separation studies

First of all, the separation of the paclitaxel from Cremophor® EL was optimized and then a mixture of the 10 taxanes (paclitaxel and nine related taxanes) and Cremophor® EL was analyzed.

Separations were achieved using water and acetonitrile in a gradient elution according to the following program: from minute 0 to 18 min, 40% acetonitrile; from 18 to 20 min, 45% acetonitrile; and 20–35 min 100% acetonitrile. The injection volume was 10 μl and the mobile phase flow rate was kept constant at 1 ml min^{-1}.

2.4. Stock solutions

The paclitaxel stock solution was prepared by dissolving 15 mg of this compound in acetonitrile in a 25 ml volumetric flask. The concentration of paclitaxel is 0.6 mg ml^{-1}. The working standard solution (0.3 mg ml^{-1}) was obtained by the dilution of the stock solution in acetonitrile.

3. Results and discussion

The composition of the gradient was optimised until a good separation of paclitaxel from Cremophor® EL (Fig. 1), the other related taxanes from each other and both paclitaxel and Cremophor® EL (Figs. 2 and 3) has been achieved. It was found that the proposed

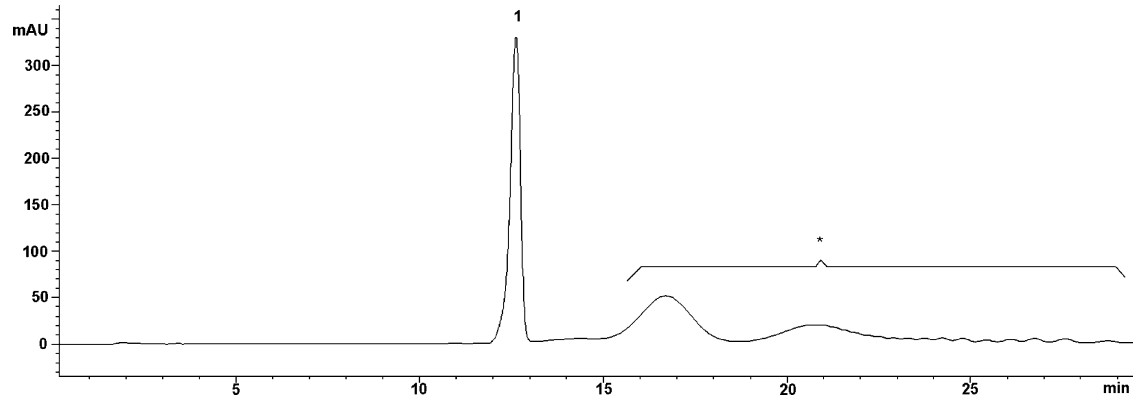

Fig. 1. Typical chromatogram for a solution of paclitaxel in Cremophor® EL: (1) paclitaxel (*) Cremophor® EL.

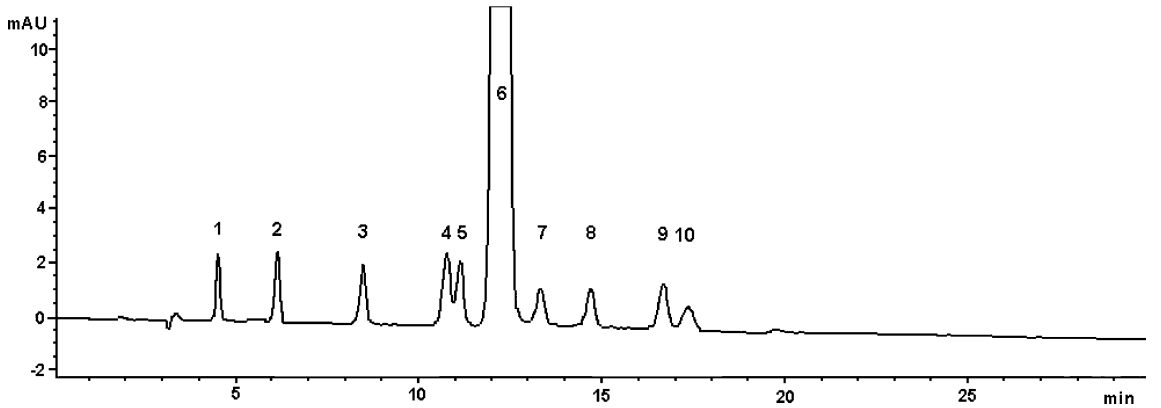

Fig. 2. Typical chromatogram for a mixture of 10 taxanes: (1) 10-deacetyl-bacatin III, (2) bacatin III, (3) 10-deacetyl-paclitaxel, (4) cephalomanine, (5) 10-deacetyl-7-epi-paclitaxel, (6) paclitaxel, (7) paclitaxel C, (8) 7-epi-cephalomanine, (9) 7-epi-paclitaxel, (10) *N*-methyl-paclitaxel C.

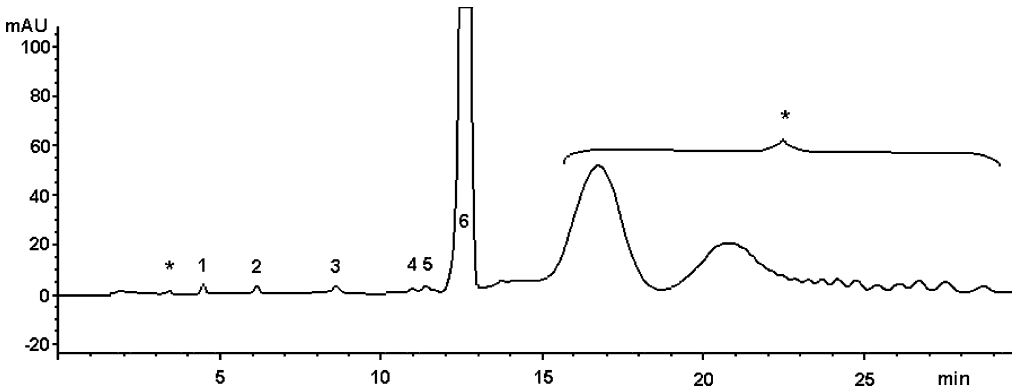

Fig. 3. Typical chromatogram for a mixture of 10 taxanes and Cremophor® EL: (*) Cremophor® EL, (1) 10-deacetyl-bacatin III, (2) bacatin III, (3) 10-deacetyl-paclitaxel, (4) cephalomanine, (5) 10-deacetyl-7-epi-paclitaxel, (6) paclitaxel.

Table 1
Parameters of chromatographic separation

Compound	Chromatographic separation	
	Relative retention time (RRT)	Resolution
10-Deacetyl baccatin III	0.365	–
Baccatin III	0.495	7.0
10-Deacetyl-paclitaxel	0.682	7.0
Cephalomannine	0.874	6.0
10-Deacetyl-7-epi-paclitaxel	0.909	0.9[a]
Paclitaxel	1.000	3.0
Paclitaxel C	1.088	2.0
7-Epi-cephalomannine	1.199	3.0
7-Epi-paclitaxel	1.362	4.0
N-Methyl-paclitaxel	1.418	1.0

[a] *Note*: The method is proposed only for purposes of paclitaxel assay, so the discriminatory separation of 10-deacetyl-7-epi-paclitaxel and cephalomanine is not a mandatory requirement in this case.

composition of the gradient determines a sufficient resolution between paclitaxel and Cremophor® EL and ensures a rapid elution of paclitaxel with a very good efficiency.

The results obtained, expressed in terms of the relative retention time and resolution, are presented in Table 1. The separation of the peaks of cephalomannine and 10-deacetyl-7-epi-paclitaxel is considered sufficient because it allows a distinct and reproducible integration of the two related compounds. The method is proposed only for purposes of paclitaxel assay, so the discriminatory separation of the two impurities is not a mandatory requirement in this case.

3.1. Validation of the method

3.1.1. Specificity and selectivity

The specificity/selectivity of the analytical method was confirmed by the analysis of solutions containing 100% of the normal working concentration of paclitaxel, a known added quantity of related substances/degradation products (in accordance with the limit accepted for each related substance/degradation product [14]) and a known added quantity of excipients (in accordance with qualitative and quantitative composition of the pharmaceutical product tested). The ability to separate all the compounds (related substances, degradation products, excipients) from paclitaxel in the sample was demonstrated by assessing the resolution between the peaks corresponding to various substances, particularly for the compounds with the closest elution relative to paclitaxel (10-deacetyl-7-epi-paclitaxel and paclitaxel C).

Table 2
Results for repeatability

No. of analysis	Retention time (min)	Assay (% of declared)
1	13.071	99.91
2	13.158	98.76
3	13.163	98.69
4	13.034	98.47
5	13.063	99.85
6	13.142	98.51

Mean (M): 99.03%. Standard deviation (S): 0.66. R.S.D.: 0.67%. Confidence interval 95% ($P = 0.05$): 0.69.

The comparative examination of the chromatograms in Figs. 2 and 3 reveals the fact that the presence of impurities that are eluted after paclitaxel, with a RRT > 1, is masked by the excipient matrix (Cremophor® EL). Due to this interference the method was not proposed for the control of purity in the finished products. It was maintained only as a dosage method because this method has the advantage of a shorter time of analysis. The proposed method was considered adequate for paclitaxel assay because the peak of paclitaxel is well separated from the compounds that are eluted before and after it (even in the presence of excipients), and the purity factor (995.889) of the peak calculated for 44 spectra obtained during the time of elution of the peak was considered adequate.

3.1.2. Precision

Precision has been estimated by: repeatability and intermediate precision. Repeatability, also defined as 'intra-assay' precision, has been evaluated by six consecutive measurements performed on simulated solutions at a concentration of 100% of the normal analytical working value. Intermediate precision has been determined by evaluating the repeatability of the investigated method (analytical procedure) if reproduced in the same laboratory, but under different operational conditions: different investigator, different column (different batch, same manufacturer), analysis carried out on another day.

The results obtained for repeatability studies are presented in Table 2 and for intermediate precision in Table 3. Method precision has a relative standard de-

Table 3
Results for intermediate precision

No. of analysis	Retention time (min)		Assay (% of declared)	
	Analysis 1[a]	Analysis 2[b]	Analysis 1[a]	Analysis 2[b]
1	13.071	13.041	99.91	100.36
2	13.158	13.002	98.76	100.08
3	13.163	12.967	98.69	100.37
4	13.034	12.875	98.47	100.00
5	13.063	12.830	99.85	100.29
6	13.142	12.861	98.51	100.07

Mean (M): 99.61%. Standard deviation (S): 0.76. R.S.D.: 0.76%. Confidence interval 95% ($P = 0.05$): 0.51.

[a] Results obtained in the first day of testing.

[b] Results obtained in the second day of testing.

viation (R.S.D.) below 1–0.67% for repeatability and 0.76% for intermediate precision—which comply with the acceptance criteria proposed (R.S.D.: not more than 2.0%).

3.1.3. Accuracy

Accuracy was determined on the range of 80–120% of the analytical working concentration of paclitaxel by calculating the recovery. Mixtures of paclitaxel and excipients corresponding to three concentration levels of the drug, namely 80, 100 and 120% of paclitaxel analytical working concentration have been analyzed. Each concentration level was prepared three times.

Method accuracy, determined in the interval 80–120% of the working concentration of the paclitaxel, evaluated by the parameter "recovery" was within the proposed limits (100 ± 2%), with results ranging from 98.2 to 100.5% and a R.S.D. of 0.83%.

3.1.4. Linearity and range

The linearity of the method used for paclitaxel assay was evaluated on the calibration curve of the peak area (y, mAu s) versus the concentration of analyte (x, mg ml^{-1}). Each sample was prepared in duplicate. The equation of linear regression obtained for different concentrations in the range of 80–120% of the normal analytical working concentration for five concentrations of the specified domain is: $y = -237.77 + 22281.06x$ and the correlation coefficient is 0.9990. The intercept is very small and the correlation coefficient close to unity. The values obtained show a good linearity and the fit of Beer's law. The detection limit was established by assessing the signal-to-noise ratio level in a proportion of 3:1 and it was found to be 0.072 μg ml^{-1}. The quantitation limit was 0.240 μg ml^{-1} and corresponds to a signal-to-noise ratio of 10:1.

Table 4
Parameters altered deliberately for the ruggedness test

Time (min)	Acetonitrile (%)	Flow (ml min^{-1})
Gradient 1		
0	40	1.0
7	45	1.0
18	45	1.0
20	100	1.0
35–40	100	2.0
Gradient 2		
0	40	1.0
18	45	1.0
20	100	1.0
35–40	100	2.0
Gradient 3		
0	40	1.0
18	40	1.0
20	100	1.0
35–40	100	2.0

3.1.5. Ruggedness

The ruggedness of the procedure was checked after the following parameters have been altered deliberately: composition of the mobile phase (Table 4); mobile phase flow rate (for the stage of interest, time between 0 and 18 min), in the variants: 0.9 and 1.1 ml min^{-1}, which represent ±10% of the proposed flow (1 ml min^{-1}); temperature, which was programmed in the variants 23, 25 °C (proposed for the assay method) and 27 °C. For each case the influence of changes on the performance of the chromatographic system in the area of interest, namely in the elution interval of 10-deacetyl-7-epi-paclitaxel and paclitaxel C, which is before and after the peak of the analyte of interest (paclitaxel) have been evaluated.

The ruggedness of the assay method, as evaluated in the area of interest by the main parameters of the chromatographic system, is demonstrated by the results obtained, which are listed in Tables 5–7. Method ruggedness, checked after deliberate alterations of mobile phase composition, flow and temperature shows that the changes of the operational parameters do not lead to essential changes of the performance of the

Table 5
Influence of changes in mobile phase composition on the performance of the chromatographic system

Compound	Relative retention time (RRT)			Number of plates (*N*)			Resolution (*R*)		
	Gradient 1	Gradient 2	Gradient 3	Gradient 1	Gradient 2	Gradient 3	Gradient 1	Gradient 2	Gradient 3
10-Deacetyl-7-epi-paclitaxel	0.924	0.906	0.902	10186	9435	5236	–	–	–
Paclitaxel	1.000	1.000	1.000	16132	11353	8518	2.23	2.60	2.56
Paclitaxel C	1.085	1.082	1.087	13589	10936	11144	1.93	2.15	2.51

Table 6
Influence of changes in mobile phase flow on the performance of the chromatographic system

Compound	Relative retention time (RRT)			Number of plates (*N*)			Resolution (*R*)		
	0.9 ml/min	1.0 ml/min	1.1 ml/min	0.9 ml/min	1.0 ml/min	1.1 ml/min	0.9 ml/min	1.0 ml/min	1.1 ml/min
10-Deacetyl-7-epi-paclitaxel	0.906	0.903	0.902	10359	9435	6264	–	–	–
Paclitaxel	1.000	1.000	1.000	12549	11353	10869	2.62	2.60	2.34
Paclitaxel C	1.082	1.085	1.087	11855	10936	10292	2.18	2.15	2.13

chromatographic system (resolution, retention time for the peak of interest).

3.1.6. Stability of the solution

The chemical stability of paclitaxel in the reference solution has been studied for a period of 11 days of storage at 2–8 °C.

The data concerning the paclitaxel stability in the reference solution are presented in the diagram of the dispersion of experimental stability data relative to the theoretical value (Fig. 4). Pacliatxel is stable in a solution stored for 6 days at 2–8 °C.

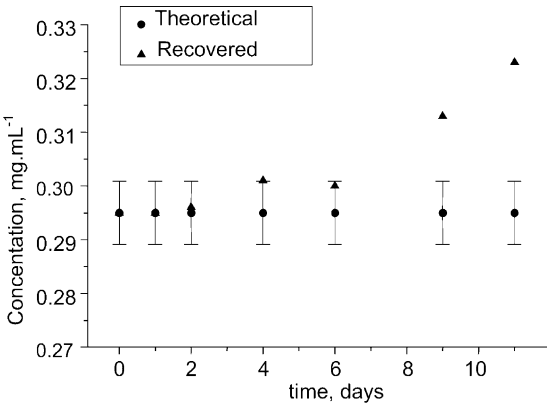

Fig. 4. The stability of the paclitaxel's solution.

3.1.7. Application

The method has been applied on real samples of SINDAXEL® (manufactured by SINDAN S.R.L. Pharmaceutical Co., Romania) for the determination of paclitaxel during the formulation. The recovery

Table 7
Influence of temperature changes on the performance of the chromatographic system

Compound	Relative retention time (RRT)			Number of plates (*N*)			Resolution (*R*)		
	23 °C	25 °C	27 °C	23 °C	25 °C	27 °C	23 °C	25 °C	27 °C
10-Deacetyl-7-epi-paclitaxel	0.899	0.901	0.900	6455	7015	6857	–	–	–
Paclitaxel	1.000	1.000	1.000	11223	11421	10917	2.43	2.45	2.45
Paclitaxel C	1.085	1.085	1.090	10908	14742	13607	2.15	2.34	2.39

obtained was between the range $100 \pm 2.12\%$, while the acceptance criteria was $100 \pm 5.00\%$.

4. Conclusion

The suitability of the HPLC method for the determination of paclitaxel in the presence of Cremophor® EL was proved. The HPLC method has adequate selectivity, good linearity, sensitivity, precision, accuracy and ruggedness.

The validation report confirms the fact that the proposed HPLC method can be used as a method for the in-process determination of paclitaxel and also for the determination of paclitaxel in finished products.

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