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Journal of Pharmaceutical and Biomedical Analysis
34 (2004) 493–499
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

A HPLC validated assay of paclitaxel's related impurities in pharmaceutical forms containing Cremophor® EL

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

Abstract

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

Keywords: Paclitaxel; Related impurities; Cremophor® EL; HPLC

1. Introduction

Paclitaxel was first isolated from the bark of the Pacific Yew tree, *Taxux brevifolia*, in the 1960s. Thirty years after its original identification paclitaxel was approved by Food and Drug Administration for the treatment of refractary breast cancer, non-small cell lung cancer [1] and more recently for epithelial ovarian cancer [2]. Although numerous studies on the HPLC method for the separation of paclitaxel have been reported there are few methods for the quantitative determination of related impurities and degradation products in paclitaxel finished pharmaceitical forms [3–7]. The major problem met during a HPLC method development is related to the presence of polyoxyl castor oil—the main constituent of paclitaxel's clinical formulation vehicle. For this reason most of the HPLC methods reported include a sample pretreatment that is complicated, time consuming and expensive.

Based on the results already published [6] this paper reports a HPLC method for the quantitative determination of 11 taxanes in the presence of polyoxyl castor oil without liquid–liquid or solid–phase extractions. The method could be used to establish the quality of pharmaceutical forms containing paclitaxel as active ingredients and polyoxyl castor oil as one of the major excipients. The need for such a method is mandatory for Quality Control Departments, as paclitaxel does not exist in any official pharmacopoeia monograph. The method proposed was fully validated in accordance with the provisions of the ICH Guide [8,9]. The investigation of validation characteristics was performed differently, depending on the objective of the determination.

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doi:10.1016/S0731-7085(03)00632-0

2. Experimental

2.1. Materials

The study was conducted with 12 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 of them from Dabur (Ghaziabad, India): baccatin III 93.40% assay, 10-deacetyl-baccatin III 95.00% assay, 10-deacetyl-7-epi-paclitaxel 98.90% assay; two from (NPI, USA): 7-epi-10-deacetyl-baccatin III 96.40% potency and *N*-benzoyl-(2*R*,3*S*)-3-phenylisoserine ethyl ester 99.75% potency 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). The 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 and a Merck Hitachi system have been used. System 1 (Hewlett-Packard 1100) consisted of 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. System 2 (Hewlett-Packard 1100) consisted of 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. System 3 was Merck Hitachi (Darmstadt, Germany) and consisted of a pump L-7100LPG, autosampler L-7250, oven L-7360, detector L-7455, and a degasser L-7615.

The chromatographic separations were carried out using a column from Supelco (Taufkirchen, Germany) type Supelcosil LC-F (stationary phase: pentafluorophenyl), 5 μm, 25 cm × 4.6 mm i.d. supplied with a precolumn Supelguard LC-F (stationary phase: pentafluorophenyl), 2 cm cartridge with a column temperature of 25 °C. Detection was by UV at $\lambda = 227 \pm 2$ nm and the reference at $\lambda = 360 \pm 20$ nm. The data were acquired and processed by use of HP ChemStation for LC software. The ORIGIN software (Micro Cal Inc., version 4.10) was employed for the linear regression analysis.

2.3. Separation studies

Separations were achieved using water and acetonitrile in a gradient elution as follow from 0 to 20 min 70% acetonitrile, from 20 to 60 min the percent of acetonitrile decreased to 60% and it was kept constant at this value until 70 min. The time for column conditioning was 20 min. The injection volume was 10 μl.

2.4. Solutions

2.4.1. Paclitaxel stock solution

The stock solution of paclitaxel reference standard (1.2 mg ml^{-1}) was prepared in acetonitrile. The working standard solution (0.6 mg ml^{-1}) was obtained by dilution of the stock solution in acetonitrile.

2.4.2. Matrix of excipients

A solution of Cremophor® EL, alcohol (absolute) and citric acid (anhydrous) in acetonitrile has been made. The concentration of each excipient is the same as in the finished product.

3. Results and discussion

The validation of the HPLC method for the determination of the content of related substances/degradation products was carried out on synthetic mixtures containing 11 impurities (cephalomannine, 7-epi-cephalomannine, paclitaxel C, 7-epi-paclitaxel, *N*-methyl-paclitaxel C, baccatin III, 10-deacetyl-paclitaxel, 10-deacetyl-baccatin III, 10-deacetyl-7-epi-paclitaxel, 7-epi-10-deacetyl-baccatin III, *N*-benzoyl-(2*R*,3*S*)-3-phenylisoserine ethyl ester), together with adequate quantities of paclitaxel and excipients matrix. The solutions for testing were prepared differently, in accordance with the validation parameters studied and the objective of the demonstration. The ratio between paclitaxel and excipients was always according to the composition of finished products and the addition of

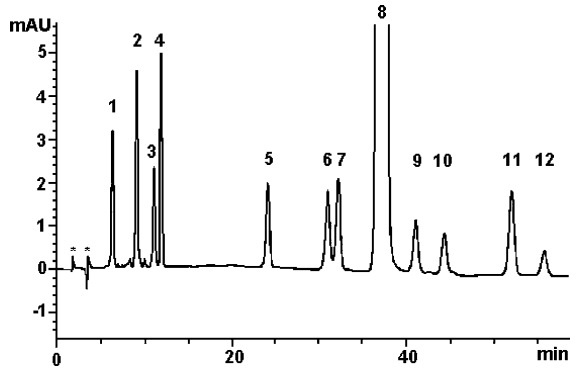

Fig. 1. Typical chromatogram for paclitaxel and related substances/degradation products: (*) solvent; (1) 10-deacetyl-bacatin III; (2) 7-epi-10-deacetyl-bacatin III; (3) bacatin III; (4) *N*-benzoyl-(2*R*,3*S*)-3-phenylisoserine ethyl ester; (5) 10-deacetyl-paclitaxel; (6) cephalomanine; (7) 10-deacetyl-7-epi-paclitaxel; (8) paclitaxel; (9) paclitaxel C; (10) 7-epi-cephalomanine; (11) 7-epi-paclitaxel; (12) *N*-methyl-paclitaxel C.

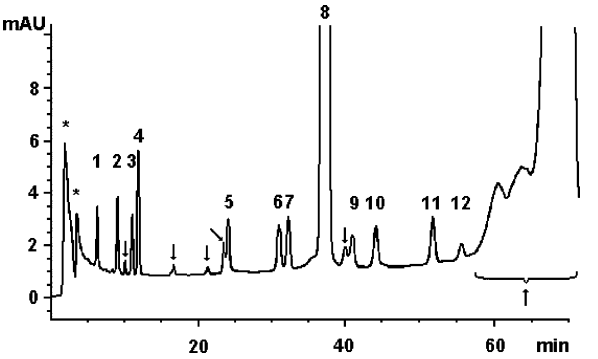

Fig. 2. Typical chromatogram for paclitaxel, related substances/degradation products and Cremophor® EL: (*) solvent; (→) Cremophor® EL; (1) 10-deacetyl-bacatin III; (2) 7-epi-10-deacetyl-bacatin III; (3) bacatin III; (4) *N*-benzoyl-(2*R*,3*S*)-3-phenylisoserine ethyl ester; (5) 10-deacetyl-paclitaxel; (6) cephalomanine; (7) 10-deacetyl-7-epi-paclitaxel; (8) paclitaxel; (9) paclitaxel C; (10) 7-epi-cephalomanine; (11) 7-epi-paclitaxel; (12) *N*-methyl-paclitaxel C.

related substances/degradation products matched the impurity level imposed by ICH rules.

3.1. Validation of the method

3.1.1. Specificity and selectivity

The specificity/selectivity of the analytical procedure was confirmed by the analysis of synthetic solutions containing 100% of the normal working concentration of the paclitaxel, of related substances/degradation products and excipients. The HPLC results presented in Figs. 1 and 2 show a good chromatographic separation of known related substances from paclitaxel and excipients matrix. The parameters relevant for the chromatographic

Table 1
Parameters of the chromatographic separation evaluated on a simulated sample (active ingredient, related substances/degradation (0.1% reporting thresholds) products and matrix excipients)

Compound	Retention time	Relative retention time	Symmetry factor	Resolution
10-Deacetyl-baccatin III	6.26	0.17	1.25	–
7-Epi-10-deacetyl-baccatin III	8.99	0.24	1.11	7.84
Matrix excipients	9.96	0.27	0.94	2.74
Baccatin III	10.96	0.29	1.15	2.62
N-Benzoyl-(2*R*,3*S*)-3-phenyl ethyl ester	11.78	0.32	1.07	1.92
Matrix excipients	16.63	0.45	0.85	11.17
Matrix excipients	21.24	0.57	0.85	9.02
Matrix excipients	23.49	0.63	0.00	3.31
10-Deacetyl-paclitaxel	24.10	0.65	1.07	0.82
Cephalomannine	30.99	0.83	1.10	8.73
10-Deacetyll-7-epi-paclitaxel	32.16	0.86	1.17	1.43
Matrix excipients	35.86	0.96	0.00	3.40
Paclitaxel	37.28	1.00	1.16	1.29
Matrix excipients	39.98	1.07	1.10	2.51
Paclitaxel C	40.96	1.10	1.30	0.86
7-Epi-cephalomannine	44.17	1.19	1.13	3.14
7-Epi-paclitaxel	51.86	1. 39	1.12	7.36
N-Methyl-paclitaxel C	55.65	1.49	1.16	2.50

Paclitaxel 100%, impurity reported level 0.5%.

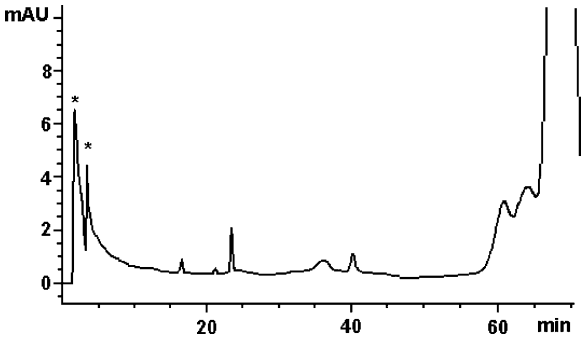

Fig. 3. Chromatogram for Cremophor® EL: (*) solvent.

separation are summarized in Table 1. The separation of related taxanes in a sample containing Cremophor® EL was more difficult because this excipient shows multiple chromatographic peaks (Fig. 4). The peaks of 10-deacetyl-paclitaxel and paclitaxel C are close to several strong peaks of Cremophor® EL and the resolution between the excipient peak and the related substance peak is lower than 1 (0.82 and 0.84, respectively). Chromatographic separation becomes discriminatory only after the substraction of excipients' matrix chromatogram (Fig. 3) from the chromatogram of the simulated sample (Fig. 2), when the resolution increases to 20.64 for 10-deacetyl-paclitaxel and to 3.59 for paclitaxel C (Fig. 4). The separated peaks corresponding to the typical chromatogram presented in Fig. 4 have adequate purity factors (ranging from 0.9081 to 1.000). Taking into account the features

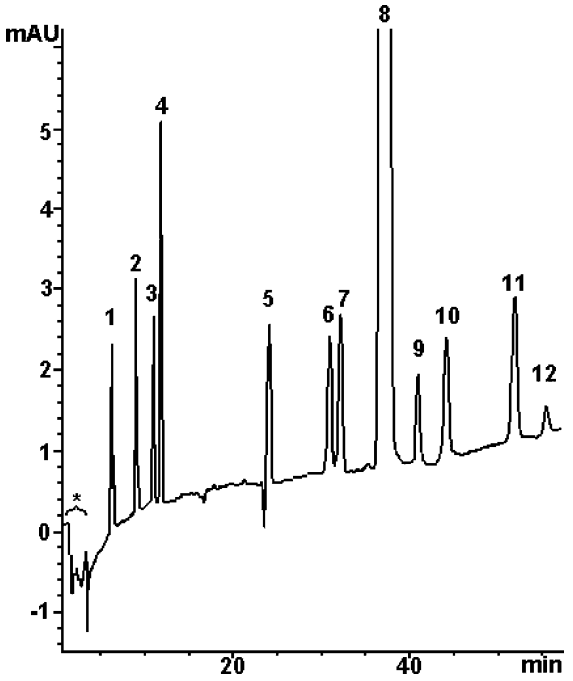

Fig. 4. Chromatogram obtained by subtraction of the excipients matrix chromatogram (Fig. 3) from the chromatogram of the simulated sample (Fig. 2): (*) solvent; (1) 10-deacetyl-bacatin III; (2) 7-epi-10-deacetyl-bacatin III; (3) bacatin III; (4) *N*-benzoyl-(2*R*,3*S*)-3-phenylisoserine ethyl ester; (5) 10-deacetyl-paclitaxel; (6) cephalomanine; (7) 10-deacetyl-7-epi-paclitaxel; (8) paclitaxel; (9) paclitaxel C; (10) 7-epi-cephalomanine; (11) 7-epi-paclitaxel; (12) *N*-methyl-paclitaxel C.

Table 2
Detection limit/quantitation limit

Compound	Detection limit		Quantitation limit	
	$\mu g\,ml^{-1}$	Identification thresholds (%)	$\mu g\,ml^{-1}$	Qualification thresholds (%)
10-Deacetyl-baccatin III	0.087	0.014	0.291	0.048
7-Epi-10-deacetyl-baccatin III	0.153	0.025	0.306	0.051
Baccatin III	0.131	0.022	0.262	0.044
N-Benzoyl-(2*R*,3*S*)-3-phenylisoserine ethyl ester	0.091	0.015	0.152	0.025
10-Deacetyl-paclitaxel	0.280	0.047	0.560	0.093
Cephalomannine	0.123	0.020	0.491	0.082
10-Deacetyl-7-epi-paclitaxel	0.155	0.026	0.621	0.103
Paclitaxel C	0.373	0.062	1.118	0.186
7-Epi-cephalomannine	0.172	0.029	0.516	0.086
7-Epi-paclitaxel	0.154	0.026	0.617	0.103
N-Methyl-paclitaxel C	0.599	0.100	1.498	0.250

Identification thresholds < 0.5%; qualification thresholds < 0.5% [10].

Table 3
Results of linearity studies

Related substance	Potency (%)	Slope	Intercept	S.D.	r	ARF	RRF
10-Deacetyl-baccatin III	95.00	16.16	1.88	0.63 ($n = 10$)	0.9994	0.0619	0.7515
7-Epi-10-deacetyl-baccatin III	96.40	16.14	0.36	0.61 ($n = 10$)	0.9995	0.0619	0.7406
Baccatin III	93.40	14.22	−0.26	0.39 ($n = 10$)	0.9997	0.0703	0.6724
N-Benzoyl-(2*R*,3*S*)-3-phenylisoserine ethyl ester	99.75	27.82	0.08	0.67 ($n = 11$)	0.9998	0.0359	1.2318
10-Deacetyl-paclitaxel	97.93	19.76	0.12	0.73 ($n = 9$)	0.9994	0.0506	0.89124
Cephalomannine	90.69	21.24	−0.07	0.60 ($n = 9$)	0.9991	0.0471	1.0344
10-Deacetyl-7-epi-paclitaxel	98.90	22.68	−0.16	0.70 ($n = 9$)	0.9996	0.0441	1.01291
Paclitaxel C	94.61	11.68	0.02	0.41 ($n = 7$)	0.9993	0.0856	0.5452
7-Epi-cephalomannine	87.57	20.21	0.67	0.72 ($n = 8$)	0.9985	0.0495	1.01938
7-Epi-paclitaxel	92.92	24.37	0.33	0.71 ($n = 9$)	0.9996	0.0410	1.15844
N-Methyl-paclitaxel C	85.85	9.03	−0.83	0.54 ($n = 7$)	0.9979	0.1107	0.4645
Paclitaxel	97.13	21.99	1.11	0.72 ($n = 10$)	0.9996	–	1.0000

n: number of determinations; S.D.: standard deviation; *r*: correlation coefficient; ARF: (slope of regression line of the related substance)$^{-1}$ (μg ml^{-1} (mAU s)$^{-1}$) + RRF = (slope of regression line of the related substance) × (slope of regression line of paclitaxel)$^{-1}$ × (potency paclitaxel) × (potency related substance)$^{-1}$.

described above, the HPLC method is considered selective and specific for the separation of the taxanes existing in a pharmaceutical formulation containing Cremophor® EL as excipient.

3.2. Detection limit and quantification limit

The detection limit was established by estimating the signal-to-noise ratio level in a proportion of 3:1 for each signal. The quantitation limit was established by assessing the signal-to-noise ratio level in a proportion of 10:1 for each signal. Table 2 presents the results for each related substance, expressed in μg ml^{-1}.

3.3. Linearity

The linearity of the method for the assay of a related substance has been demonstrated in the specific interval from the quantitation limit to the 120% of the qualification thresholds [10] of related substance. Test solutions for each related substance, corresponding to minimum 6 and maximum 11 concentration levels within the recommended range have been prepared in duplicate. Based on linearity investigation, the determination of the regression slope for each case, the absolute response factors (ARF) for each related substance and the relative response factors (RRF)

Table 4
Critical comparison between the ARF, RRF and method using standards

Compound	Real concentration of the sample (%)	Assay (percent relative to paclitaxel)		
		Calculation using a standard	Calculation using ARF	Calculation using RRF
10-Deacetyl-baccatin III	0.500	0.477	0.493	0.265
7-Epi-10-deacetyl-baccatin III	0.500	0.487	0.483	0.256
Baccatin III	0.500	0.499	0.486	0.206
N-Benzoyl-(2*R*,3*S*)-3-phenylisoserine ethyl ester	0.500	0.502	0.493	0.751
10-Deacetyl-paclitaxel	0.500	0.502	0.492	0.384
Cephalomannine	0.580	0.601	0.592	0.576
10-Deacetyl-7-epi-paclitaxel	0.500	0.503	0.492	0.501
Paclitaxel C	0.500	0.544	0.535	0.151
7-Epi-cephalomannine	0.300	0.313	0.312	0.285
7-Epi-paclitaxel	0.550	0.557	0.550	0.686
N-Methyl-paclitaxel C	0.500	0.524	0.497	0.092

Table 5
Accuracy and precision of the method

Related substance	Recovery (%)	Precission	
		Intra-day	Inter-day
10-Deacetyl-baccatin III	101.13 ± 1.95	100.47 ± 1.25	101.05 ± 1.13
7-Epi-10-deacetyl-baccatin III	99.77 ± 1.53	99.57 ± 0.47	100.25 ± 0.86
Baccatin III	98.82 ± 1.81	99.02 ±1.33	98.26 ± 1.21
N-Benzoyl-(2*R*,3*S*)-3-phenylisoserine ethyl ester	99.36 ± 1.34	100.15 ± 0.68	99.67 ± 0.59
10-Deacetyl-paclitaxel	99.97 ± 1.04	99.43 ± 1.92	101.42 ±1.57
Cephalomannine	102.55 ± 1.43	100.89 ± 1.37	99.32 ± 1.20
10-Deacetyl-7-epi-paclitaxel	99.08 ± 1.36	98.47 ± 1.43	99.43 ± 1.13
Paclitaxel C	98.95 ± 1.85	99.53 ± 1.21	99.60 ± 1.38
7-Epi-cephalomannine	103.65 ± 1.16	101.58 ± 1.53	100.28 ± 1.79
7-Epi-paclitaxel	100.98 ± 0.90	99.81 ± 0.27	99.24 ± 0.63
N-Methyl-paclitaxel C	100.34 ± 1.64	99.62 ± 1.74	99.04 ± 1.54

All the results are recovery (%) ± standard deviation.

respectively have been calculated. The results are summarized in Table 3. Based on the critical comparison of the results obtained by the ARF, RRF and the method using standards, that employing ARF has been adopted (Table 4). This method has the advantage that it does not require taxanes standards for quantitative determination of related/degradation products.

3.4. Accuracy

Method accuracy was determined by calculating the recovery of each related substance of interest from synthetic samples corresponding to three concentration levels, each being tested three times and corresponding to a domain ranging from the qualification thresholds of related substance at 120% of the proposed specification. The values obtained for accuracy are presented in Table 5.

3.5. Precision

The precision was evaluated by repeatability and intermediate precision. For repeatability, six measurements are carried out for each related substance of interest, using solutions prepared at a concentration of 100% of the qualification thresholds. For the evaluation of intermediate precision, the determinations have been carried out on another day, by a different investigator and with a different column. The results presented in Table 5 show a good precision of the method.

3.6. Ruggedness

The ruggedness of the method was checked after the following parameters were altered deliberately: composition of the mobile phase, mobile phase flow rate ($1 \pm 10\%$ ml min^{-1}), temperature ($25 \pm 2\,^{\circ}$C), chromatographic column (two columns have been used) and equipment (three HPLC systems). The parameters of chromatographic separation (retention time, relative retention time (RRT), resolution, number of plates) are not significantly different by variations of the operational parameters.

3.7. Stability of the solution

The chemical stability of solutions containing paclitaxel, related impurities/degradation products and excipients in acetonitrile has been studied for a period of 48 h of storage at 25 °C. The solution is stable for 24 h at 25 °C.

4. Conclusion

The HPLC method proposed for the assay of related substances/degradation products of paclitaxel in the products SINDAXEL®-concentrate for intravenous infusion is adequate for the proposed objective, because method specificity/selectivity ensures that exact results are obtained, given the fact that each related substance of the 11 investigated is discriminated from

other known related substances, active ingredient and excipients, particularly Cremophor® EL, compound which has multiple chromatographic peaks. The critical area of separation is represented by the related substances 10-deacetyl-paclitaxel and paclitaxel C which are separated from the peaks of Cremophor® EL only after the extraction of the excipient matrix chromatogram from the chromatogram of the sample. In these conditions the resolution between the peaks of interest is higher than 1 and the spectral purity of the separated peaks is satisfactory.

The linearity was confirmed for each related substance. The absolute response factors calculated for each related substance allow the quantitative evaluation of each related substance without using reference standards (related substances) for current analyses.

The accuracy of the method was in compliance with the proposed limits and the precision of the method was satisfactory. The ruggedness of the method shows that the performance of the chromatographic system is not significantly influenced by variations of the operational parameters inside an accepted domain.

Because the method for the assay of related substances proposed for current control does not use reference standards of related substances, the most important criterion for the assessment of the performances of the chromatographic system for the parameter "ruggedness" is the relative retention time, as the signals attributed to related substances are identified according to it. The analysis of the data presented has lead to the conclusion that the RRT is inside a deviation domain of ±0.02% relative to the time declared in the method, in each newly created experimental situation. The validation report confirms the fact that the HPLC method studied can be proposed as a method for the purity evaluation of products SINDAXEL®-concentrate for intravenous infusion.

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