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Supercritical fluid chromatography of paclitaxel

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Abstract

The analysis of paclitaxel and related compounds was investigated using supercritical fluid chromatography (SFC). The separation of paclitaxel and five related taxanes was compared on two SFC instruments. The typical analysis time was 20 min on system I and 4 min on system II. Based on this, SFC system II was selected for further studies and a separation of paclitaxel from fifteen of its impurities or degradation products was achieved in about 35 min.

1. Introduction

Supercritical fluid chromatography (SFC) is a complimentary technique to high-performance liquid chromatography (HPLC) and gas chromatography (GC). The advantages of SFC include the possibility of analysis of thermally labile compounds and the use of both HPLC and GC type detectors such as UV–Vis and flame-ionization. Commercial instruments for both capillary- and packed-column SFC are available.

There have been reports of the use of SFC in the analysis of pharmaceuticals [1–10]. Wong and Dellafera [1] demonstrated the use of capillary SFC in therapeutic drug monitoring of phenobarbital in serum using a polymethylsiloxane stationary phase and a carbon dioxide mobile phase. Later et al. [2] have reported the analysis of steroids, antibiotics and cannabinoids on polymethylsiloxane capillary columns using a carbon dioxide mobile phase. Crowther and Henion [3] demonstrated the SFC–mass spectrometric analysis of codeine, caffeine, cocaine, phenylbutazone and methocarbamol using packed amino and silica columns and a modified direct liquid-introduction interface. The mobile phase was carbon dioxide modified with methanol. Smith and Sanagi [4] have reported the SFC analysis of barbiturates using a packed column containing a polystyrene–divinylbenzene or octadecylsilane stationary phase with methanol-modified carbon dioxide as mobile phase. Perkin et al. [5] have reported the analysis of veterinary antibiotics (levamisol, furazolidone, chloramphenicol and lincomycin) on a packed column containing an aminopropyl stationary phase also utilizing carbon dioxide with modifier. Jagota and Stewart [6] have reported the SFC analysis of diazepam and chlordiazepoxide and related compounds. The separation was achieved on a cyanopropyl-50 column with a carbon dioxide mobile phase. Jagota and Stewart [7] have also

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reported the separation of non-steroidal anti-inflammatory drugs on cyanopropyl-50 and biphenyl-30 columns with a carbon dioxide mobile phase. Smith et al. [8] reported the separation of ranitidine and its metabolites using packed column supercritical fluid chromatography. Supercritical carbon dioxide modified by a mixture of methanol–methylamine–water was used as the mobile phase. The separations were achieved on a cyanopropyl column. Berger and Wilson [9] presented the separation of antipsychotic drugs using a packed cyanopropyl column with a tertiary mobile phase consisting of carbon dioxide, methanol and isopropylamine. Berger and Wilson also reported [10] the separation of antidepressants using a packed column SFC with a tertiary mobile phase. A LiChrospher cyanopropyl column with a mobile phase consisting of supercritical fluid carbon dioxide with 10% modifier (methanol with 0.5% isopropylamine) was used for the separation.

Paclitaxel is a promising anticancer drug approved for the treatment of ovarian and some other cancers. Its injectable formulation is marketed under the trade name Taxol®. HPLC has been the method of choice for separation of paclitaxel and related compounds. There are several methods published in the literature for the separation of paclitaxel and related taxanes. Most of these methods have long run times. Harvey et al. [11] reported the separation of paclitaxel from related taxanes using reversed-phase HPLC with isocratic elution. Using an ODS-2 microcolumn, they were able to separate paclitaxel from related taxanes in 60 min. Witherup et al. [12] published the separation of paclitaxel and related taxanes on a C_{18} column. The mobile phase was MeOH–H_2O–MeCN (20:48:32) at a flow-rate of 1 ml/min and the total run time was 25 min. Currently at Bristol-Myers Squibb an HPLC method is used for the separation of paclitaxel and its related compounds. The run time is 70 min.

There is only one report on the use of SFC in the analysis of paclitaxel-related compounds. Heaton et al. [13] reported the supercritical fluid chromatography of taxicin I and taxicin II from the English Yew tree. The separations were compared on both capillary and packed columns. It was found that packed columns are better for quantitative analysis of these compounds. A cyano column with a carbon dioxide and methanol gradient was used for the analysis. This work was preliminary in nature and did not satisfy the need of a purity/impurity assay.

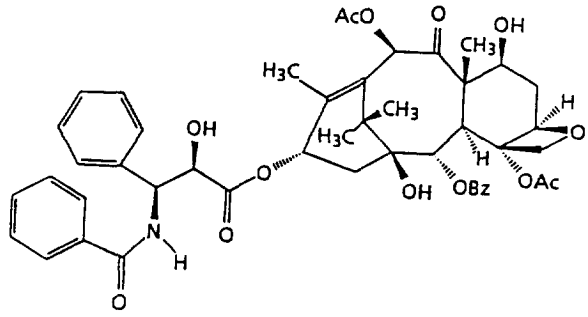

Fig. 1. Structure of paclitaxel.

In this paper the use of SFC for the separation of paclitaxel and sixteen of its impurities/degradants is presented. The separations were carried out on a diol column and the run time was 35 min. The structural formula of paclitaxel is shown in Fig. 1. Table 1 shows the list of impurities/degradants. The best possible separations obtained in our laboratory on two different SFC systems are compared.

Table 1
Impurities/degradants of paclitaxel

Identity
10-Deacetylbaccatin III
Baccatin
Ethyl ester of sidechain
Photodegradant
10-Deacetyltaxol
Impurity F
2-Debenzoyltaxol-2-pentenoate
10-Deacetyl-7-epitaxol
Paclitaxel
7-Epitaxol
Impurity K
7-TES-Baccatin III
7-TES-13-Acetylbaccatin III
7-TES-Taxol
Impurity O
7-TES-2'-MOP-Taxol

2. Experimental

2.1. Reagents

Supercritical fluid chromatography grade unmodified carbon dioxide was purchased from Scott Specialty Gases (Plumsteadville, PA, USA). Paclitaxel and related compounds were obtained from the Chemical Process Technology department, Bristol-Myers Squibb (New Brunswick, NJ, USA). HPLC grade methanol was obtained from Baxter (McGaw Park, IL, USA).

2.2. Instrumentation

Two instrument systems were used.

System I

A supercritical fluid chromatograph (Model 200 A, Suprex Corp., Pittsburgh, PA, USA), equipped with a high-pressure syringe pump, column oven and UV detector was used. The UV detector was a Linear 206 PHD equipped with a high-pressure cell (Linear Instruments Corporation, Bellefonte, PA, USA). A 100-μl Hamilton HPLC syringe (Baxter Healthcare Corporation, McGaw Park, IL, USA) was used for sample introduction.

System II

A second supercritical fluid chromatograph (Model G1205A, Hewlett-Packard Co., Avondale, PA, USA), capable of providing modifier gradient, and equipped with a dual pump system, column oven and UV detector was used. The UV detector was a HP1050 detector equipped with a high-pressure cell. A HP SFC autosampler (Model 7673) was used for the sample introduction.

2.3. Chromatographic conditions for the final SFC method

The following conditions were used: column, LiChrospher diol 5 μm, 250 × 4.6 mm; oven temperature, isothermal at 30°C; flow-rate, 2.0 ml/min; pressure, isobaric at 150 bar; wavelength, 227 nm; mobile phase, carbon dioxide at 150 bar with a methanol gradient. Methanol gradient: 0–3 min: 8% methanol, 3–28 min: ramp to 28% methanol, 28–33.7 min: ramp to 35% methanol, 33.7–37.7 min: 35% methanol.

3. Results and discussion

At first, the separation of paclitaxel and four of its related taxanes (baccatin III, 10-deacetylbaccatin, cephalomannine and epitaxol) was investigated on system I. Different columns (Brownlee RP-18, Deltabond C_{18}, Deltabond Octyl, Deltabond Phenyl, and Deltabond Cyano) with unmodified carbon dioxide as mobile phase were investigated for the separation. Several pressure gradients and oven temperatures were attempted. The separation of paclitaxel and related compounds was achieved by a Deltabond Cyano column in 20 min (Fig. 2). This was the best separation possible on this system. The chromatographic conditions included a pressure gradient (initialize at 150 atm for 5 min, ramp at 5 atm/min to 400 atm; oven temperature, isothermal at 35°C). A UV detector at 227 nm was used.

The separations were compared on SFC system II. Ideally the same conditions as used in system I should be usable in the system II. This was not possible since the restrictor mechanism is different for system II. There are more parameters on system II which can optimized compared to system I. In system II the flow-rate of the mobile phase can be controlled independently of the pressure and temperature. While on system I no gradient of the organic modifier can be run, this can be done on system II. After investigating several gradients and temperatures it was found that a pressure gradient with a methanol-modified carbon dioxide gave the best results. The oven temperature was kept isothermal at 35°C. A baseline separation of all major peaks was obtained in 5 min. Fig. 3 shows the separation. Table 2 compares the results from the two systems. The capacity factors shown in Table 2 are used for comparison purposes only since it was a gradient run. However, these parameters can be used as system

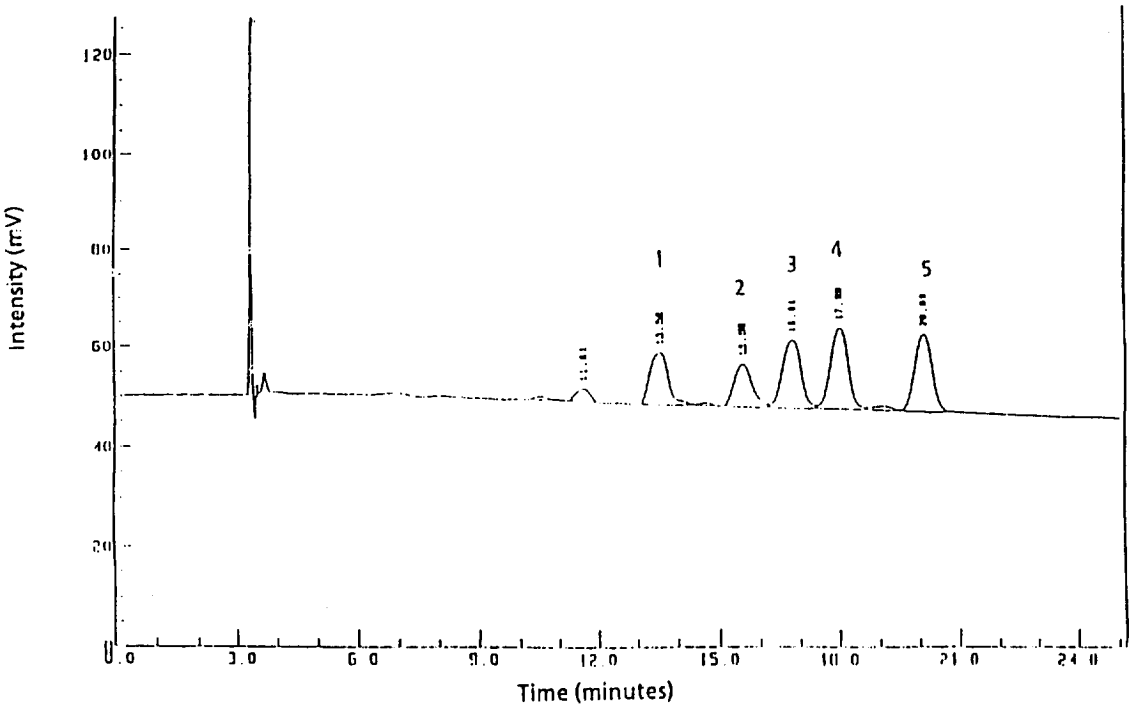

Fig. 2. Separation of baccatin (1), 10-deacetylbaccatin III (2), cephalomannine (3), 7-epitaxol (4) and paclitaxel (5) on system I.

suitability criteria for day-to-day analyses. Comparison of instruments was made for this application only.

Once it was found from the initial experiments that system II gave better results for paclitaxel separations, a method was developed to separate paclitaxel from 16 of its impurities and degradation products. Initially, separations were carried out on the Cyano column. It was found that it was not possible to separate all 16 compounds on this column. Based on this observation, a more polar Diol column was chosen (LiChrospher Diol 5 μm, 250 × 4.6 mm) and separation of paclitaxel from its impurities and degradants was achieved in 35 min. Chromatographic conditions are described in the Experimental sec-

Table 2
Comparison of chromatographic performance characteristics on two supercritical fluid chromatographs

Compound	SFC system I					SFC system II				
	Retention time (min)	Capacity factor (k')	Resolution (R_s)	Assymetry factor (T_f)	No. of theoretical plates	Retention time (min)	Capacity factor (k')	Resolution (R_s)	Assymetry factor (T_f)	No. of theoretical plates
Baccatin III	13.5	3.0	2.3	1.3	1940	1.9	3.1	3.0	1.1	4620
10-Deacetyl baccatin	15.5	3.6	1.4	1.5	3250	2.4	4.2	1.8	1.0	2540
Cephalomannine	16.8	3.9	1.5	1.3	3780	2.9	5.1	1.9	1.5	1410
7-Epitaxol	17.9	4.3	2.5	1.1	5690	3.5	8.1	2.2	1.3	2030
Paclitaxel	20.0	4.9	–	1.3	5400	4.2	9.4	–	1.2	2580

$R = \frac{2(t_2 - t_1)}{W_a + W_b}$; T_f calculated at 10% peak height.

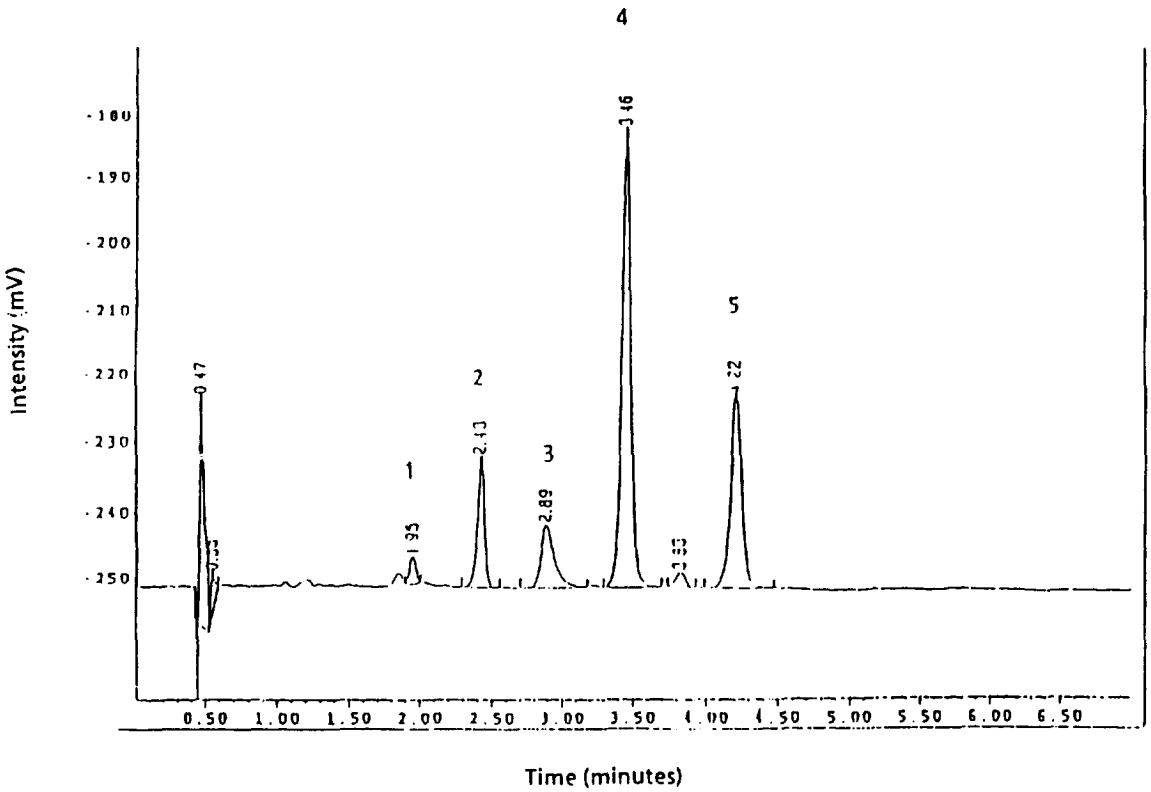

Fig. 3. Separation of baccatin (1), 10-deacetylbaccatin III (2), cephalomannine (3), 7-epitaxol (4) and paclitaxel (5) on system II.

tion. All compounds were optimally separated as shown in Fig. 4. The presence of an unknown impurity at a retention time of 7.05 min was also observed. This could originate from an impurity in the standard used in the experiment. The conditions described above were used as the final SFC method.

Once an optimal separation was achieved for all the compounds, validation studies were done at these conditions. The final SFC method was found to be linear from 10 μg/ml to 10 mg/ml for paclitaxel. Eight impurities/degradants were selected (based on retention times and availability) as model compounds for recovery studies. A linear response was observed for all eight compounds over the range 10–60 μg/ml ($r > 0.99$, $n = 6$). Recovery studies were performed by spiking a sample of paclitaxel (10 mg/ml) with 10–60 μg/ml (corresponding to 0.1% to 0.6% of paclitaxel) of impurities/degradants ($n = 6$). Recoveries of 95–105% were obtained for all eight compounds. Typical recovery data are shown in Tables 3–5. Reproducibility of the method was determined by multiple injections of individual impurities at a concentration of 20 μg/ml (corre-

Table 3
Recovery of paclitaxel spiked with baccatin

Concentration added (μg/ml)	Concentration found (μg/ml)	Recovery (%)
0	39.96	–
10.42	49.25	97.8
20.84	60.56	99.6
31.26	73.37	103.0
41.68	83.28	102.0
52.10	91.88	99.8
62.52	103.26	100.8
Mean		100.5
S.D.		1.9
R.S.D. (%)		1.9

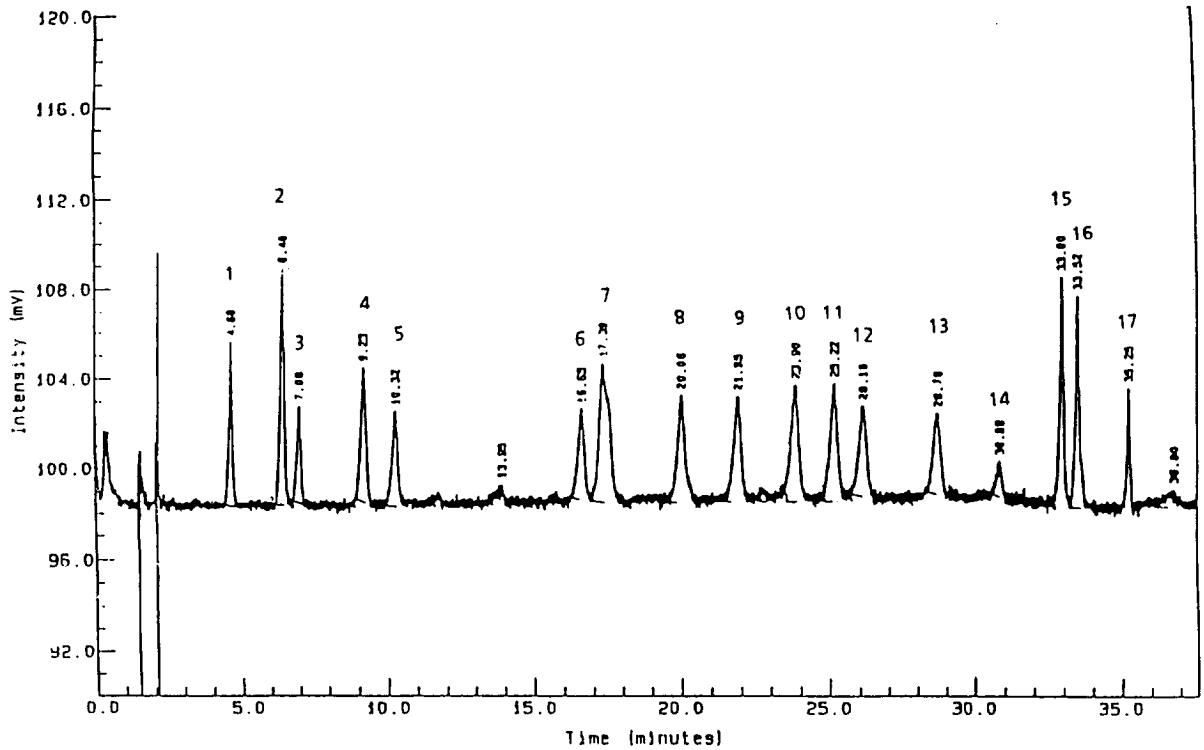

Fig. 4. Separation of paclitaxel and sixteen of its impurities/degradants on system II. 7-TES-13-acetylbaccatin III (1), ethyl ester of side chain (2), unknown (3), 7-TES-2′-MOP-taxol (4), 7-TES-baccatin III (5), 7-TES-taxol (6), baccatin (7), 7-epitaxol (8), 2-debenzoyltaxol-2-pentenoate (9), 10-deacetyl-7-epitaxol (10), paclitaxel (11), 10-deacetylbaccatin III (12), impurity O (13), photodegradant (14), 10-deacetyltaxol (15), impurity F (16), and impurity K (17).

Table 4
Recovery of paclitaxel spiked with 7-epitaxol

Concentration added (μg/ml)	Concentration found (μg/ml)	Recovery (%)
0	4.0	–
9.80	13.86	100.4
19.60	24.00	101.7
29.40	33.10	99.1
39.20	43.77	101.3
49.00	53.50	100.9
58.80	62.95	100.2
Mean		100.6
S.D.		0.9
R.S.D. (%)		0.9

Table 5
Recovery of paclitaxel spiked with 7-TES-TAXOL

Concentration added (μg/ml)	Concentration found (μg/ml)	Recovery (%)
0	7.79	–
10.01	17.65	99.2
20.02	27.29	98.1
30.03	37.69	99.7
40.04	47.51	99.4
50.05	56.39	97.5
60.06	64.98	95.8
Mean		98.3
S.D.		1.5
R.S.D. (%)		1.5

Table 6
Precision for multiple injections of impurities/degradants of paclitaxel at 20 μg/ml

Impurities/ Degradants	Area of impurities/degradants of paclitaxel										
	INJ 1	INJ 2	INJ 3	INJ 4	INJ 5	INJ 6	INJ 7	INJ 8	Mean	S.D.	R.S.D. (%)
7-TES-13-Acetylbaccatin III	27 516	26 272	27 863	28 158	26 988	27 303	27 695	26 922	27 340	602	2.2
7-TES-2′-MOP-Taxol	37 197	35 909	36 999	35 308	35 031	34 880	37 631	35 112	36 008	1106	3.1
7-TES-Baccatin III	28 293	28 610	28 952	27 617	26 774	28 319	27 716	27 281	27 945	726	2.6
7-TES-Taxol	36 590	35 867	34 323	35 626	36 176	35 059	36 815	34 758	35 652	884	2.5
Baccatin	31 652	29 575	30 310	30 651	30 271	31 652	29 691	29 681	30 432	832	2.7
7-Epitaxol	38 778	36 865	38 306	36 558	38 392	38 006	37 404	36 021	37 541	986	2.6
Impurity O	36 110	34 863	34 321	34 352	36 009	34 935	35 159	34 543	35 037	695	2.0
10-Deacetyltaxol	35 928	34 153	35 489	35 769	36 898	37 084	36 371	35 048	35 843	968	2.7

Table 7
Comparison of relative retention behavior of paclitaxel impurities/degradants on HPLC and SFC systems

HPLC[a] system		SFC system[b]	
Compound	k'	Compound	k'
10-Deacetylbaccatin III	1.3	7-TES-13-Acetylbaccatin III	2.09
Baccatin	3.2	Ethyl ester of sidechain	3.30
Ethyl ester of sidechain	3.4	7-TES-2′-MOP-Taxol	5.16
Photodegradant	7.8	7-TES-Baccatin III	5.85
10-Deacetyltaxol	9.0	7-TES-Taxol	10.10
Impurity F	11.1	Baccatin	10.59
2-Debenzoyltaxol-2-pentenoate	15.9	7-Epitaxol	12.32
10-Deacetyl-7-epitaxol	19.0	2-Debenzoyltaxol-2-pentenoate	13.60
Paclitaxel	20.3	10-Deacetyl-7-epitaxol	14.87
7-Epitaxol	28.2	Paclitaxel	15.77
Impurity K	30.7	10-Deacetylbaccatin III	16.39
7-TES-Baccatin III	40.7	Impurity O	18.29
7-TES-13-Acetylbaccatin III	44.0	Photodegradant	19.59
7-TES-Taxol	44.4	10-Deacetyltaxol	20.99
Impurity O	44.8	Impurity F	21.35
7-TES-2′-MOP-Taxol	49.0	Impurity K	22.52

[a] Water–acetonitrile gradient; flow-rate, 1 ml/min; detector set at 227 nm (inhouse method).
[b] See chromatographic conditions, Experimental section.

sponding to 0.2% in a paclitaxel sample). A precision of ≤3.1% (R.S.D.) was obtained for all compounds (Table 6).

A comparison of the relative behavior of impurities/degradants of paclitaxel using optimal HPLC conditions versus optimal SFC conditions is shown in Table 7. It can be observed that the SFC method gives results comparable to those obtained with HPLC but in half the run time. The table also shows that the elution order is different for SFC compared with HPLC which may be due to the different retention mechanisms of the two techniques. Capacity factors (k') are used here only for comparison purposes since the HPLC and SFC separations are obtained under gradient conditions.

In summary the development of a rapid SFC method for the separation of paclitaxel and related compounds is described. The method can be used for impurity/purity profiling and can easily be adopted for stability-indicating purposes.

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