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Short communication

Evaluation of a silica-based monolithic column in the HPLC analysis of taxanes

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Abstract

The Merck KGaA Chromolith RP-18 column is based on a unique sorbent material consisting of a monolithic rod which has a high internal area and porosity, allowing quality separations in a minimum of time. The use of such a silica-based monolithic column has been applied to one of our most challenging HPLC separations, that is the impurity profiling of an analog of Taxol®. Different types of conventional HPLC columns with silica particle-based packings, as well as the Chromolith RP-18 column, have been investigated with a combination of mobile phases to achieve the separation of all the impurities of a synthetic taxane currently under development. The performance of the Merck KGaA Chromolith column was found to compare quite favorably to a conventional silica particle-based column.

Keywords: HPLC; Pharmaceutical analysis; Monolithic column; Silica-based monolithic column; Taxol; Taxanes

1. Introduction

Analogs of Taxol® (paclitaxel) destined to be used as potential new anticancer drugs are currently under development at Bristol-Myers Squibb. Synthesis of taxanes is typically associated with a large number of impurities, which makes their analysis and impurity profiling particularly challenging. As many as 36 individual impurities have been found in some samples of synthetic taxanes. Many of these impurities are originating from the starting material, which is isolated from a natural source and is used as a precursor in the synthesis of taxanes [1,2].

In support of an investigative new drug application with the USFDA, a HPLC method was developed and validated for the determination of purity and the impurity profiling of BMS-275183-01, a Taxol® analog. This paper will demonstrate the challenges involved in developing a HPLC method for synthetic taxanes. A variety of columns, including Merck KGaA monolithic Chromolith RP-18 column, with a combination of different mobile phases and gradients were investigated to achieve the separation of the numerous impurities formed during synthesis.

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Monolithic liquid chromatography columns consist of one piece of either an organic polymer or silica with flow-through pores [3]. The use of monolithic columns has been reported in various analytical separations by HPLC and capillary electrochromatography [4–11]. While several of these applications involve the separation of proteins and biopolymers on monolithic polymer columns, fewer studies have been conducted on monolithic silica columns. The Chromolith RP-18 is a new column technology based on silica monolithic rods with high porosity, which is claimed to permit high quality separations using higher flow rates [12,13]. In this study, the performance of the Merck KGaA monolithic silica reverse-phase column in the impurity profiling of a Taxol® analog will be compared with a traditional silica particle-based column.

2. Experimental section

A 1100 system from Agilent Technologies (Palo Alto, CA) with UV detection was used for the HPLC separations. A 1100 HPLC system in combination with a variable-wavelength detector and Finnigan Matt TSQ 7000 mass spectrometer (San Jose, CA) was used for the LC–MS experiments. A variety of silica-based HPLC columns investigated to achieve the impurity profiling of BMS-275183-01 are listed in Table 1. All the solvents used were high-quality HPLC grade solvents. The mobile phase flow rate was set at 1.0 ml/min to avoid high back pressure with silica particle-based columns. 10 μl injections of BMS-275183-01 at 0.5 mg/ml were performed and the column temperature was maintained at 30 °C or ambient temperature. Samples of BMS-275183-01 were obtained from the Process Research and Development group at the Bristol-Myers Squibb Pharmaceutical Research Institute laboratories (Candiac, QC, Canada).

3. Results and discussion

Results from the investigation of a wide variety of conventional silica particle-based columns clearly indicate an insufficient separation of the more polar impurities on hydrophobic column packings. Both the Zorbax SB-phenyl and the YMC ODS-A failed to separate the polar impurities. Even less hydrophobic columns such as the Waters Symmetry RP8 with a lower carbon load and the YMC ODS-AL with residual silanols for mixed-mode separations did not achieve a complete separation of the many impurities involved in analyzing the synthetic taxanes. The Supelco LC-F column with a pentafluorophenyl endcapped stationary phase, the selectivity of which was expected to be quite different from traditional reversed phase columns for taxanes, was not

Table 1
Silica particle-based HPLC columns investigated in the development of a purity assay and impurity profiling method for BMS-275183-01, an analog of Taxol®

HPLC column	Description	Evaluation of performance
Zorbax SB-phenyl, dimension: 250 × 4.6 mm, 5 μm particle size, Catalog # 880975-912	Stable bond disopropyl phenethyl stationary phase	Insufficient separation of polar compounds
YMC ODS-A, dimension: 250 × 4.6 mm, 5 μm particle size, Catalog #AA12S052546WT	Endcapped C18 separation phase	Insufficient separation of polar compounds
Waters symmetry RP-8, dimension: 150 × 4.6 mm, 3.5 μm particle size, Catalog # 094269	Endcapped C8 stationary phase	Incomplete separation of some impurities
YMC ODS-AL, dimension: 250 × 4.6 mm, 5 μm particle size, Catalog #AL12S052546WT	C18 separation phase with residual silanol groups	Incomplete separation of the impurities
Supelco LC-F, dimension: 250 × 4.6 mm, 3μm particle size, Catalog # 59158	Pentafluorophenyl endcapped stationary phase	Inadequate separation for impurity profiling
YMC ODS-AQ, dimension: 250 × 4.6 mm, 3 μm particle size, Catalog #AQ12S032546WT	C18 stationary phase with hydrophilic endcapping	Best resolution achieved of all the impurities

much more successful in completely resolving all the impurities from BMS-275183-01. In the past, pentafluorophenyl columns have been successfully applied to the assay of Taxol® by HPLC [14]. As a matter of fact, only the YMC ODS-AQ provides sufficient selectivity to achieve complete separation of all the impurities of BMS-275183-01. The combination of a hydrophobic high carbon load and a relatively hydrophilic surface for endcapping silanol groups appear to provide the unique selectivity for the impurity profiling of this Taxol® \tf="DM8"\char178}}*analog*.

The gradient elution and separation of BMS-275183-01 and its impurities on a YMC ODS-AQ column, 4.6 × 250 mm, were optimized. The optimized HPLC conditions with the YMC ODS-AQ column are presented in Fig. 1a. Ultraviolet detection was performed at 228 nm where the absorption spectrum of BMS-275183-01 is maximum. Adjustments of the mobile phase pH from 2 to 6.5 with either trifluoroacetic acid or ammonium acetate, did not have a significant impact on the separation efficiency and selectivity. In fact, the baseline tends to drift more than if only a water:acetonitrile gradient is being used. The optimized method was validated for precision, linearity, limits of sensitivity and specificity. Results from the validation are presented in Table 2.

The impurity profiling of BMS-275183-01 was challenged on the Merck KGaA monolithic Chromolith RP-18 column using an acetonitrile:water gradient. The gradient elution had to be modified since the retention time of BMS-275183-01 diminished considerably on the monolithic column. This had obvious consequences on the column selectivity for BMS-275183-01 and the resolution of impurities. It was necessary to reduce the amount of acetonitrile in the mobile phase by 20–25% to obtain a similar retention time than on the YMC ODS-AQ column. Fig. 1 presents the best impurity profiling of BMS-275183-01 achieved on a Chromolith RP-18 column versus the results obtained on a YMC ODS-AQ column. Impurities representing at least 0.03 area percent are reported, while impurities below 0.03% are not considered. Using the separation parameters of Fig. 1b, the plate height, a measure of the column efficiency, is about 2.5 times lower on the Chromolith than on the YMC ODS-AQ column. A plate number superior to 165 000 plates per column was obtained for BMS-275183-01 on the 100 mm monolithic column, using acetone as a void volume marker. In comparison the YMC ODS-AQ column, 250 mm-in-length, provided slightly less than 100 000 plates per column in the validated conditions.

As established by LC–MS, the impurity profiling of BMS-275183-01 is similar on both the silica particle-based YMC ODS-AQ and the Chromolith RP-18 monolithic columns. The elution order of some of the impurities has changed on the Chromolith column, but the most significant difference is a 10-methyl carbonate analog, which is not separated from the taxane BMS-275183-01 on the monolithic column. BMS-275183-01 and 10-methyl carbonate impurity are difficult to separate by HPLC, especially when considering the similarity in the structure of both compounds (Fig. 2). However, a USP resolution superior to 2.0 between BMS-275183-01 and the 10-methyl carbonate impurity was obtained with the YMC ODS-AQ column. Despite modifications in the gradient and the separation conditions, we were unable to separate BMS-275183-01 and its 10-methyl carbonate counterpart on the Chromolith column.

The monolithic column offers a very low resistance to flow. This is attributed to the high porosity of the monolithic silica stationary phase. A back pressure of less than 500 psi has been monitored on a 100 mm Chromolith column with a flow rate of 1.0 ml/min of a 20:80 acetonitrile:-water mobile phase. This low resistance to flow provides the opportunity of using much higher flow rates with the Chromolith RP18 column. Increasing the mobile phase flow rate from 1 to 4 ml/min really increased the speed of the assay and impurity profiling of BMS-275183-01, and consequently, reduced the run time by about 30%. However, the resolution of the early eluting impurities of BMS-275183-01 was starting to be more seriously affected at a mobile phase flow rate of 4 ml/min. Unless flow programming is used to allow the separation of the early eluting impurities, higher flow rates than 4 ml/min should be avoided

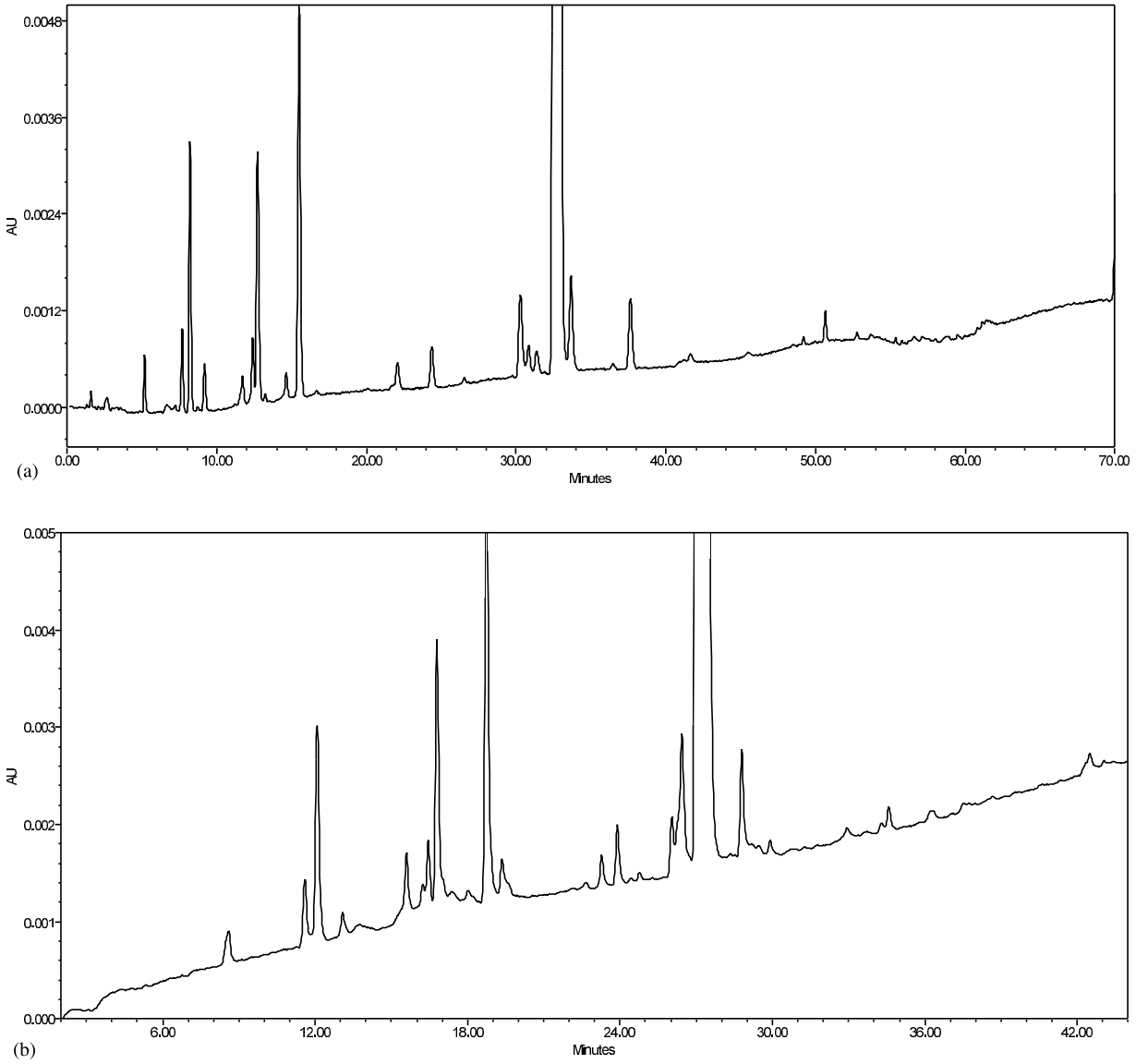

Fig. 1. Optimized impurity profiling of BMS-275183-01. (a) HPLC purity of BMS-275183-01 (RT = 32.8 min) at 96.65 area percent, with 17 reported impurities. YMC ODS-AQ 4.6 × 250 mm column with mobile phases A and B, 100% acetonitrile and 40:60 acetonitrile:water, respectively. Gradient: 10–40%A in 40 min, then to 90%A in 15 min, hold 20 min at 1 ml/min. UV detection at 228 nm. (b) HPLC purity of BMS-275183-01 (RT = 27.2 min) at 96.82 area percent, with 16 reported impurities. Chromolith RP18 4.6 × 100 mm column with mobile phases A and B, 100% acetonitrile and water, respectively. Gradient: 20–60%A in 30 min, then to 90%A in 15 min, hold 5 min at 1 ml/min. UV detection at 228 nm.

to obtain an acceptable impurity profile for BMS-275183-01.

The Chromolith column is currently available in only two dimensions, 50 and 100 mm, but the low back pressure of the Chromolith column offers the possibility of coupling two columns together to obtain higher plate counts and improve the chromatographic resolution. Unfortunately, two

Table 2
Validation summary of a HPLC method using a YMC ODS-AQ column for the purity assay and impurity profiling of a taxane, BMS-275183-01

Validation parameter	Results
Linearity	
Concentration range	25–600 μg/ml
Slope	1.07×10^4
Y-intercept	0.07% of target concentration
Coefficient of correlation	1.000
Precision	
Target concentration	500 μg/ml
Repeatability of six consecutive injections	0.4% R.S.D.
Sensitivity	
Detection limit	0.62 μg/ml
Minimum quantifiable limit	2.06 μg/ml

100-mm Chromolith columns coupled together still failed to achieve the resolution of BMS-275183-01 and its 10-methyl carbonate counterpart.

In conclusion, the Merck KGaA Chromolith column, which is based on a sorbent material consisting of silica monolithic rods, offers several advantages even in challenging HPLC separations such as the impurity profiling of Taxol® analogs. The high porosity of the monolith stationary phase offers a low resistance to flow and allows rapid mass transfer, which is advantageous for the development of rapid HPLC assays. The performance of the Chromolith column was found comparable to that of a traditional silica particle-based YMC ODS-AQ column in the separation of a Taxol® analog from its impurities. Only one

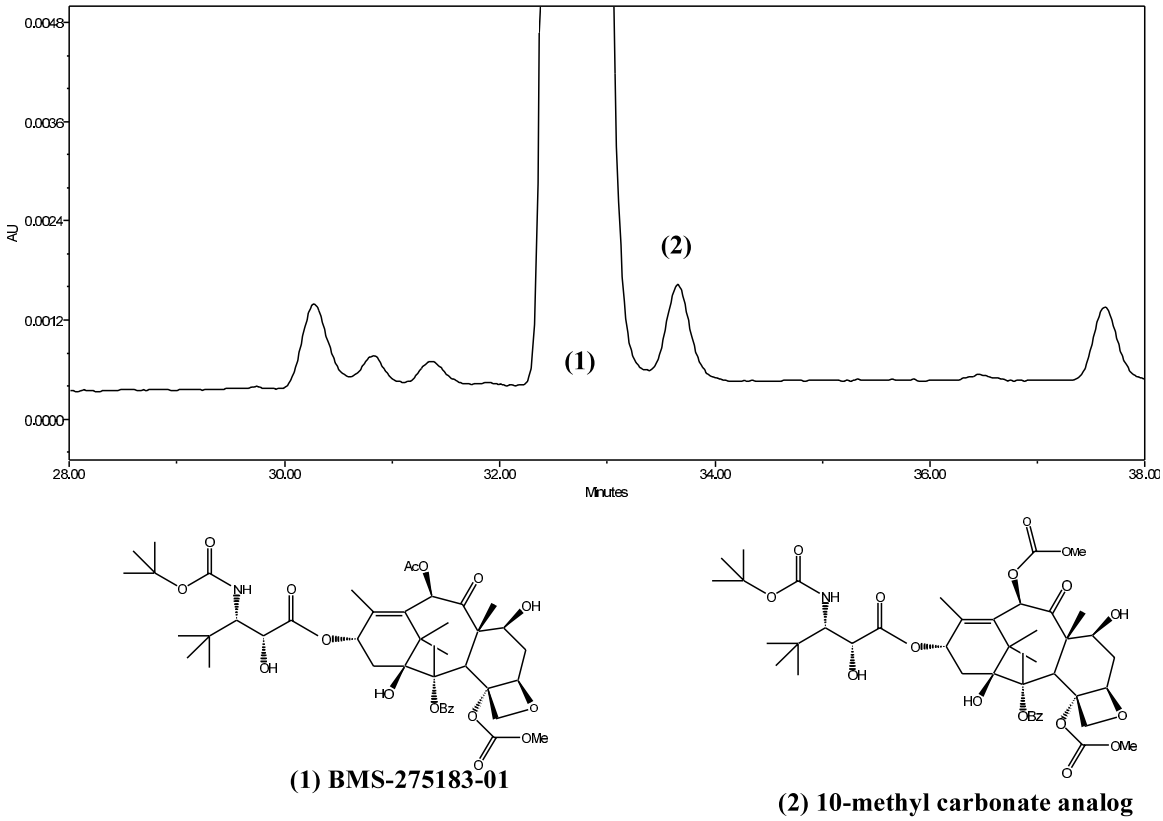

Fig. 2. Resolution of BMS-275183-01 and its 10-methyl carbonate analog on the YMC ODS-AQ column.

impurity, the structure of which is very similar to its parent compound BMS-275183-01, could not be separated on the Chromolith column. As compared with silica particle-based columns, the Chromolith RP-18 provides excellent column efficiency.

References

[1] J.N. Denis, A.E. Greene, D. Ghénard, F. Guéritte-Voegelein, L. Mangatal, P. Potier, J. Am. Chem. Soc. 110 (1988) 5917–5919.
[2] V. Farina, S. Huang, Tetrahedron Lett. 33 (1992) 3979–3982.
[3] N. Tanaka, H. Kobayashi, K. Nakanishi, H. Minakuchi, N. Ishizuka, Anal. Chem. 73 (2001) 420A–429A.
[4] S. Xie, F. Svec, J.M.J. Frechet, J. Chromatogr. A 775 (1997) 65–72.
[5] S.F. Xie, R.W. Allington, F. Svec, J.M.J. Frechet, J. Chromatogr. A 865 (1999) 169–174.
[6] F. Svec, E.C. Peters, D. Sykora, J.M.J. Frechet, J. Chromatogr. A 887 (2000) 3–29.
[7] M. Laemmerhofer, F. Svec, J.M.J. Frechet, W. Lindner, Anal. Chem. 72 (2000) 4623–4628.
[8] K. Branovic, A. Buchacher, M. Barut, A. Strancar, D. Josic, J. Chromatogr. A 903 (2000) 21–32.
[9] R.N. Wu, H.F. Zou, M.L. Ye, Z.D. Lei, J.Y. Ni, Electrophoresis 22 (2001) 544–551.
[10] R. Plumb, G. Dear, D. Mallett, J. Ayrton, Rapid Comm. Mass Spectrom. 15 (2001) 986–993.
[11] T. Jiang, J. Jiskra, H.A. Claessens, C.A. Cramers, J. Chromatogr. A 923 (2001) 215–227.
[12] F. Rabel, K. Cabrera, D. Lubda, Am. Lab. 32 (24) (2000) 20–22.
[13] K. Cabrera, D. Lubda, K. Sinz, C. Schäfer, Am. Lab. 33 (4) (2001) 40–41.
[14] S.L. Richheimer, D.M. Tinnermeier, D.W. Timmons, Anal. Chem. 64 (1992) 2323–2326.