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Development of a sensitive and rapid method for rifampicin impurity analysis using supercritical fluid chromatography

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ABSTRACT

A novel simple, fast and efficient supercritical fluid chromatography (SFC) method was developed and compared with RPLC method for the separation and determination of impurities in rifampicin. The separation was performed using a packed diol column and a mobile phase B (modifier) consisting of methanol with 0.1% ammonium formate (w/v) and 2% water (v/v). Overall satisfactory resolutions and peak shapes for rifampicin quinone (RQ), rifampicin (RF), rifamycin SV (RSV), rifampicin N-oxide (RNO) and 3-formylrifamycinSV (3-FR) were obtained by optimization of the chromatography system. With gradient elution of mobile phase, all of the impurities and the active were separated within 4 min. Taking full advantage of features of SFC (such as particular selectivity, non-sloping baseline in gradient elution, and without injection solvent effects), the method was successfully used for determination of impurities in rifampicin, with more impurity peaks detected, better resolution achieved and much less analysis time needed compared with conventional reversed-phase liquid chromatography (RPLC) methods.

1. Introduction

Nowadays, impurity profiling of pharmaceuticals is receiving critical attention from analysts, since the presence of impurities in active pharmaceutical ingredients (APIs) may influence the efficacy and safety of the pharmaceutical products. In pharmacopoeias and ICH guidelines [1], HPLC methods, especially RPLC methods, are the most generally used tools for impurity control of most APIs. However, because the number of impurities and the structure of every impurity of a pharmaceutical ingredient cannot be known, some impurity peaks may be overlooked due to overlong retention times or co-elution with other peaks. A feasible and reliable approach to check specificity is to develop a secondary method to separate peaks of interest using a different separation mechanism [2].

Rifampicin (RF), a semisynthetic macrocyclic antibiotic, is widely used in the treatment of tuberculosis and staphylococcal infection [3]. The chemical structures of RF and its potential related substances rifampicin quinone (RQ), rifamycin SV (RSV), rifampicin N-oxide (RNO) and 3-formylrifamycinSV (3-FR) are shown in Fig. 1. As an optimal method for separation, RPLC has been used in the analysis of rifampicin and its impurities [4–7].

A novel SFC method was developed, different from the conventional RPLC method enabling fast and efficient analysis of impurities in rifampicin. Since SFC differs significantly in chromatographic selectivity by providing a different retention mechanism from RPLC [8,9], the new method maximizes the probability of revealing all peaks in rifampicin bulk sample. The analysis of the impurities was accomplished in approximately 4 min. Utilizing supercritical liquid CO_2 as the primary mobile phase with a low viscosity, which results in fast diffusion of the compounds in the mobile phase, SFC is considered to be an approach to separate both non-polar [10] and polar compounds [11]. In addition, SFC minimizes the consumption of mobile-phase solvents (e.g., methanol), thereby generating less waste for disposal and significantly reducing the cost of analysis relative to conventional RPLC methods. Particularly, in this paper rifampicin sample dissolved in organic solvents can be injected in SFC without additional dilution steps to exchange solvents for RP-compatible diluents, then the higher sample concentration enabled improvement in accuracy and sensitivity when analyzing trace amount of impurities. The novel method for rifampicin impurity analysis represents a “green” alternative to RPLC, for better sep-

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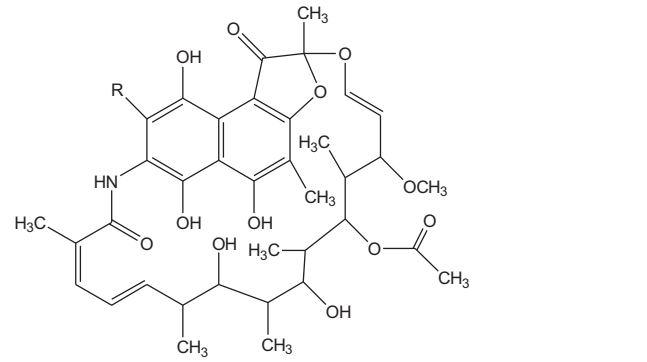
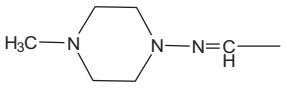
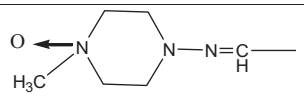
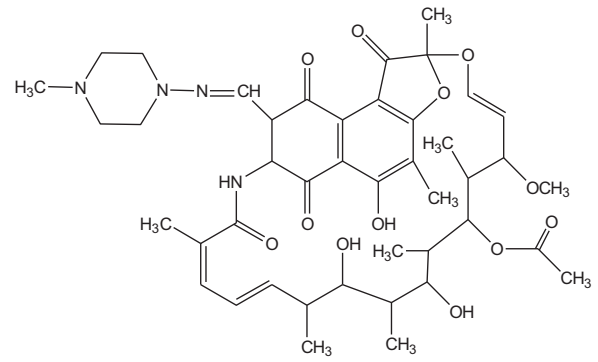
0731-7085/



Compound	R
Rifampicin (RF)	
3-formylrifamycin SV (3-FR)	
Rifamycin SV (RSV)	H—
Rifampicin N-oxide (RNO)	



Rifampicin quinone (RQ)

Fig. 1. Chemical structures of RQ, RF, RSV, RNO and 3-FR.

aration efficiency, ability to detect more impurity peaks and a substantial savings in time by above 10-fold.

2. Materials and methods

2.1. Instrumentation

The SFC method was conducted using Waters Acquity UPC^2 system (Acquity Ultra Performance Convergence Chromatography^{TM}) equipped with a binary solvent delivery pump, an autosampler, a column oven, a PDA detector, and a backpressure regulator (BPR).

A Shimadzu HPLC device that consisted of a LC-20AT pump, an SIL-20AVP autosampler, and an SPDM10AVP DAD detector was used for RPLC method.

Columns for SFC were: (1) ACQUITY UPC^2 BEH (ethylene bridged hybrid silica) (1.7 μm, 3.0 × 100 mm), (2) ACQUITY UPC^2 Torus 2-PIC (2-picolylamine bonded) (1.7 μm, 3.0 × 100 mm), (3) ACQUITY UPC^2 Torus Diol (high-density diol bonded) (1.7 μm, 3.0 × 100 mm), and (4) ACQUITY UPC^2 Torus 1-AA (1-Aminoanthracene bonded) (1.7 μm, 3.0 × 100 mm). Chromatographic column for RPLC was a Shiseido capcell DD C8 (5 μm, 150 mm × 4.6 mm).

2.2. Materials

LC grade methanol, acetonitrile, formic acid and triethylamine were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Other reagents were of analytical reagent grade and obtained from Nanjing Chemical Co. (Nanjing, China). The reference substances of RQ, RF, RSV, RNO and 3-FR were obtained from National Institute for Food and Drug Control (Beijing, China). Rifampicin bulk sample was obtained from the Zhejiang Xinchang Pharmaceutical (Xinchang, China).

2.3. Methods

For optimization of SFC analysis, the strategy is: selection of the stationary phase–adjustment of the mobile phase–tuning of gradient program–fine adjustment of other experimental parameters such as column temperature and back pressure. For the initial screening, methanol was used as the modifier in an initial gradient elution profile (T = 0 min, CO_2-Modifier 88:12(v/v); T = 4 min, 65:35(v/v), and last 1 min; Flow rate: 2.0 mL min^{-1}, Oven temp: 50 °C) in order to compare the separation efficiency of different columns and mobile phases. The 5 pure standards were dissolved in acetonitrile to obtain a solution containing 0.1 mg/mL of each compound and injected for optimization. The injected volume was 2 μL and the detection wavelength was set at 254 nm. Spectra were recorded in the range of 200–800 nm. Concentration of rifampicin in test solution was about 10 mg/mL for sample analysis.

For RPLC analysis, the mobile phase consisted of a solution (containing 0.1%phosphoric acid, 1.9 g/L of sodium perchlorate 5.9 g/L of citric acid and 20.9 g/L of potassium dihydrogen phosphate) and acetonitrile (65:35, v/v) as described in European Pharmacopoeia (EP) [12] and British Pharmacopoeia (BP) [13] which is similar to that in the United States Pharmacopeia (USP) [14]. The flow rate was set at 1.5 mL min^{-1} and a constant column temperature was maintained at 30 °C. The injected volume was 20 μL. 20 mg of rifampicin sample was dissolved in 10 mL of acetonitrile to produce stock solution, and the solution was further diluted (10-fold) with the buffer described in EP 8.0 immediately prior to injection into the chromatography.

Peak tracking in both analyses was performed by the correlation of spectra as reference [15]. Three-dimensional (3D) spectro-chromatographic data produced by SFC-DAD were compared with that produced by RPLC-DAD to develop two-dimensional chromatographic spectral correlative maps, and simultaneous identification of the chromatographic peaks can be obtained rapidly. Solutions of each reference substance were injected to ensure accurate peak identification through comparison of retention time when unresolved peaks co-eluted.

3. Results and discussion

3.1. Selection of stationary phase

Possessing different selectivity, four available SFC columns packed with different stationary phases were chosen to study the retention and separation of RQ, RF, RSV, RNO and 3-FR. That was hybrid silica stationary phase unbounded (ACQUITY UPC^2 BEH), 2-picolylamine(ACQUITY UPC^2 Torus 2-PIC), high-density diol (ACQUITY UPC^2 Torus Diol) and 1-aminoanthracene (ACQUITY UPC^2 Torus 1-AA).