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Short communication

HPLC determination of rifampicin and related compounds in pharmaceuticals using monolithic column

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Abstract

A rapid, sensitive and reproducible HPLC method using C18 monolithic column was developed and validated for the analysis of rifampicin (RIF) and its four related compounds, including rifampicin quinone (RQ), rifamycin SV (SV), rifampicin *N*-oxide (RNO) and 3-formylrifamycin SV (3-FR). Chromatographic separation was achieved by using the mixture of methanol–acetonitrile–monopotassium phosphate (0.075 M)–citric acid (1.0 M) (28:30:38:4, v/v) as the mobile phase at a flow rate of 2 mL/min and with UV detection at 254 nm. Calibration curves were obtained in the concentration ranges of 1–40 μg/mL for SV, RNO and 3-FR, 1.5–60 μg/mL for RQ and 5–200 μg/mL for RIF. Limit of quantitation (LOQ) was determined to be 1 μg/mL and the limit of detection (LOD) was 0.2 μg/mL for all studied compounds with a 10 μL injection. The intra-day R.S.Ds. and inter-day R.S.Ds. for the above five compounds were all less than 2.5%. The recoveries of rifampicin from placebo tablets were from 99.7% to 100.5%. The total run time was less than 11 min, as opposed to around 60 min by using C18 particle-packed column. In conclusion, by this developed method, RIF and its related compounds can be determined rapidly with good precision and accuracy in pharmceuticals.

Keywords: Rifampicin; Related compounds; Monolithic column; High performance liquid chromatography

1. Introduction

Rifampicin (RIF) is a semisynthetic macrocyclic antibiotic derived from *Streptomyces mediterranei*. It is categorized as one of the first line antituberculous agents and is also used for the treatment of other infectious diseases [1–4]. However, many impurities can be introduced to the final RIF product during the process of fermentation and synthesis, and can be generated via degradation pathway during storage [5], which include rifampicin quinone (RQ), rifamycin SV (SV), rifampicin *N*-oxide (RNO) and 3-formylrifamycin SV (3-FR). The United States Pharmacopoeia (USP), British Pharmacopoeia (BP) and Chinese Pharmacopoeia (ChP) all establish criteria for the quality control of RIF and its related compounds in pharmaceuticals [6–8]. A number of papers involving HPLC methods for the determination of RIF have also been published [9–12]. But only one paper was searched to carry out the method evaluation of the determination of the related compounds of RIF [5].

Monolithic columns, which are made by sol–gel technology and consist of a single rod of silica-based material, have attracted considerable attention in liquid chromatography as they allow achieving separation faster than the conventional columns [13–15]. The highly porous monolithic rods of silica have revolutionary bimodal pore structures, that is, they can provide unique combination of macropores (2 μm) and mesopores (12 nm). The former allow rapid flow of the mobile phase at low pressure, while the latter create the large uniform surface on which adsorption takes place, thereby enabling high performance chromatographic separation. With the emergence of commercial monolithic columns, the reports concerning practical applications of monolithic column in HPLC separations are increasing and its excellent performance has been confirmed [16–20].

In this paper, a simple, rapid, precise and accurate HPLC method for the determination of RIF and its related compounds in pharmaceuticals was developed using the monolithic column and the separation efficiency and analysis speed of monolithic

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column were compared with those of the conventional particle-packed column.

2. Experimental

2.1. Materials and reagents

RIF, RQ, SV, RNO and 3-FR standards were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The potency of SV is not less than 832 IU/mg and the contents of other four standards are all more than 99.0%. RIF raw material was purchased from Tonglian Group Shenyang Antibiotic Manufacture (Shenyang, China). HPLC grade acetonitrile and methanol were from Concord Tech. Co. (Tianjin, China). Analytical grade monopotassium phosphate and citric acid were from Bodi Chemicals Co. Ltd. (Tianjin, China). Double distilled water was used throughout the experiment.

2.2. Equipment and chromatographic conditions

Chromatographic experiments were performed with a HPLC system equipped with a L-2130 pump, a L-2420 UV–vis detector, a L-2200 autosampler and a HT-130 column heater (Hitachi, Japan). Chromatographic signals were acquired and processed by T-2000L chromatography data system (V 5.23) (Techcomp Limited, Beijing, China). A Chromolith Performance RP-18e (100 mm × 4.6 mm) column equipped with a Chromolith RP-18e guard cartridge (10 mm × 4.6 mm) (Merck, Germany) and an AT. Chrom C18 column (150 mm × 4.6 mm, 5 μm) (Lanzhou Insititute of Chemical Physics, China) were used for separation.

The optimized mobile phase was a mixture of methanol–acetonitrile–monopotassium phosphate (0.075 M)–citric acid (1.0 M) (28:30:38:4, v/v). The flow rate was set at 2 mL/min and the detection wavelength was set at 254 nm. The injection volume was 10 μL.

2.3. Preparation of solutions

All solutions were prepared with acetonitrile as solvent.

2.3.1. Standard solutions

The stock solutions of standards were prepared individually at 1 mg/mLand stored at −18 °C, which were stable within 3 days. The calibration solutions were prepared with appropriate aliquots of the stock solutions to give the concentration range of 5–200 μg/mL for RIF, 1.5–60 μg/mL for RQ and 1–40 μg/mL for SV, RNO and 3-FR. The working standard solution for the assay of RIF was prepared at 0.08 mg/mL. The calibration solutions and the working standard solution were prepared daily and used within 3 h.

2.3.2. Test solutions for RIF tablets assay

Rifampicin tablets were self-made in our laboratory. The excipients included low-substituted hydroxypropylcellulose (L-HPC), microcrystalline cellulose (MCC) and magnesium stearate and the labeled content of RIF was 150 mg per tablet. The test solution of tablets for assay was prepared as follows: 10 tablets were weighed to determine the average tablet weight and finely powdered. An accurately weighed powder equivalent to 80 mg RIF was placed in a 100 mL volumetric flask and diluted to volume. The solution was ultrasonicated for 30 s and filtered by 0.45 μm nylon membranes. The filtrate was diluted 10 times and then injected immediately.

2.4. Selectivity studies

To study the selectivity of the method, about 10 mg RIF was exposed to 2 mL HCl (1 M), NaOH (1 M) or 30% H_2O_2 solutions by heating in a 40 °C water bath for 1 h. The solutions exposed to HCl or NaOH were neutralized and all the solutions were filtered through a 0.45 μm filter before injection into the column.

2.5. Method validation

Method validation parameters studied were linearity, precision, accuracy and limit of quantitation (LOQ) and limit of detection (LOD).

Linearity was determined by building three calibration curves. For the construction of each calibration curve, six calibration standard solutions were prepared and each standard solution was injected once. Calibration curves were generated by plotting the peak area versus the concentration of the analyte. The LOQ was defined as the lowest concentration that could be determined with acceptable accuracy and precision. The LOD was determined by diluting solutions of known concentration until the response was three times the noise.

The system precision (injection repeatability) was determined by performing six-repeated injection of the same standard solution. Intra-day precision was determined by injecting six standard solutions on the same day ($n = 6$) and inter-day precision was determined by injecting standard solutions prepared on three different days ($n = 3$). Relative standard deviation (R.S.D.%) of peak area was calculated to represent precision. Accuracy was evaluated by the recovery determination of spiked RIF solutions at the concentration of 60, 80 and 100 μg/mL.

3. Results and discussions

3.1. Optimization of mobile phase composition

The mobile phase composition was optimized on the basis of ChP method. The aqueous phase was composed of monopotassium phosphate (0.075 M) and citric acid (1.0 M), and the organic phase was selected from methanol, acetonitrile or their mixture. Many efforts were made on the adjustment of the ratios of the components of mobile phase. The separation of RQ and SV was most difficult, and 3-FR was very susceptible to the change of organic modifier in the mobile phase. It was found that using methanol or acetonitrile alone as the organic modifier could not provide satisfactory resolution in a reasonable analysis time. An optimized composition of the mobile phase was methanol–acetonitrile–monopotassium phosphate (0.075 M)–citric acid (1.0 M) (28:30:38:4, v/v), which

Table 1
Chromatographic data for the separation of rifampicin and its four related compounds on a monolithic column and a conventional particle-packed column

Compounds	Chromolith RP-18e column				AT. Chrom C18 column			
	t_R	k	N	Rs	t_R	k	N	Rs
RQ	1.95	1.41	3445	6.10[a]	9.23	4.49	3607	7.31[a]
SV	2.32	1.86	3976	2.64[b]	11.21	5.67	4277	2.90[b]
RIF	2.91	2.91	3997	3.39[b]	15.40	8.17	3976	4.77[b]
RNO	3.78	3.67	3941	3.97[b]	22.41	12.34	3731	5.36[b]
3-FR	9.21	10.37	4275	13.08[b]	55.30	31.92	5357	13.94[b]

t_R, retention time; k, retention factor; N, number of theoretical plates; Rs, resolution.
[a] The value refers to the Rs between RQ and RIF.
[b] The value refers to the Rs between two compounds, which are nearest in the chromatogram.

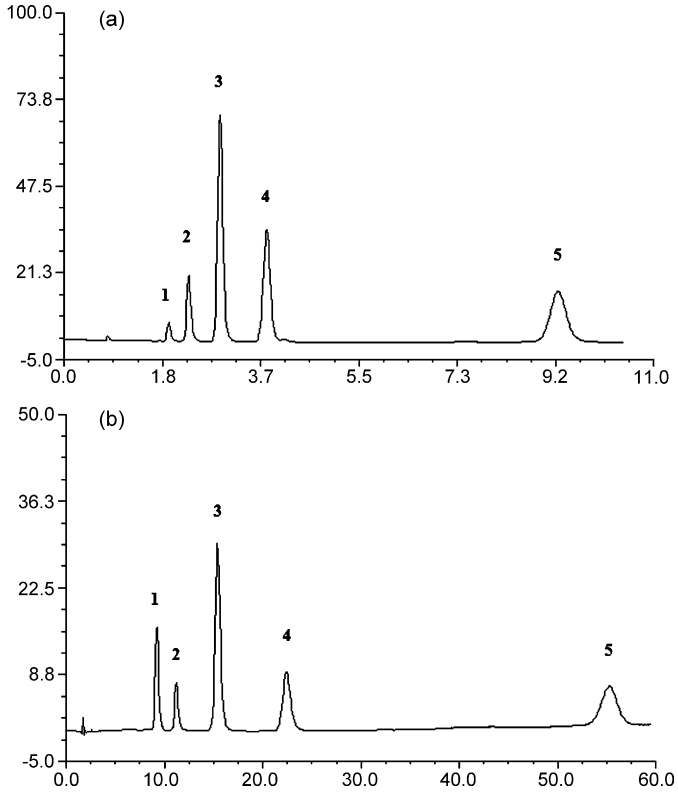

Fig. 1. Typical chromatograms of rifampicin and its four related compounds on a monolithic column (a) and a conventional column (b) with methanol–acetonitrile–monopotassium phosphate (0.075 M)–citric acid (1.0 M) (28:30:38:4, v/v) as mobile phase. Peaks—1, RQ; 2, SV; 3, RIF; 4, RNO; 5, 3-FR.

provided the resolution value of 2.64 (Table 1) between RQ and SV with a total analysis time less than 11 min.

3.2. Chromatography

Complete separation of RIF and its four related compounds on the monolithic column can be seen from Fig. 1a. Relevant chromatographic data obtained are reported in Table 1. These results show that the developed method meets the separation and system suitability requirements claimed in USP, BP and ChP for the analysis of RIF and its related compounds, except for the elution sequence of SV and RIF different from that required by ChP. For comparison, the chromatographic data (Table 1) was also obtained on a conventional C18 particle-packed column using the same mobile phase, with chromatogram shown in Fig. 1b. Although the length of monolithic column was shorter than that of the conventional column (10–15 cm) and the flow rate was higher (2.0 mL/min versus 1.0 mL/min), separation efficiency was comparable for both the columns judged from the number of theoretical plates (N) and resolution (Rs). Furthermore, the total analysis time was reduced significantly, almost six times shorter for monolithic column (10 min) than that for the conventional column (60 min). Compared to a Zorbax Eclipse C8 column (250 mm × 4.6 mm, 5 μm) reported previously [5], the analysis time of the monolithic C18 column could also be saved up to two times.

3.3. Selectivity results

The chromatogram of the standards of RIF and its related compounds (Fig. 1a) indicated the adequate resolution of all the studied compounds. Stress tests of RIF were studied further to assess the selectivity of the method. Fig. 2 shows the chromatograms of RIF solutions that had been exposed to HCl (1 M), NaOH (1 M) and 30% H_2O_2 solutions. RQ and 3-FR were always generated in all stress test solutions while RNO was not detected in the sample solution exposed to NaOH (1 M). That SV was not detected in all stress test solutions, suggesting that it was not easily generated during degradation process but was more possible via introduction during fermentation or synthesis. An unknown impurity appeared at about 2.14 min, which was significant in chromatograms of sample solutions exposed to NaOH and H_2O_2. It could not be resolved completely from RQ and is needed to be ascertained in future. In any case, RIF was separated well from other impurities.

3.4. Validation results

The data of three calibration curves of RIF and its related compounds are listed in Table 2. The linearity was obtained in the range of 1–40 μg/mL for SV, RNO and 3-FR, 1.5–60 μg/mL for RQ and 5–200 μg/mL for RIF with high correlation coefficients (R^2), from 0.9997 to 0.9999. The R.S.D.% values of the slope and the intercept were less than 3.3%.

The system precision (injection repeatability) is a measure of the method variability that can be expected for a given analyst performing the analysis. The R.S.D.% values for six injections

Table 2
Linear regression data for analysis of rifampicin and its related compounds

Compounds	Concentration range (μg/mL)	Slope ± S.D.	Intercept ± S.D.	R^2
RIF	5–200	11,082 ± 207	−230.3 ± 6.4	0.9998
RQ	1.5–60	10,450 ± 110	−236.9 ± 6.1	0.9998
SV	1–40	6,908 ± 90	216.6 ± 7.1	0.9997
RNO	1–40	7,660 ± 161	−246.7 ± 5.8	0.9998
3-FR	1–40	10,588 ± 64	−281.8 ± 8.4	0.9999

of RQ, SV, RIF, RNO and 3-FR at the concentration of 30, 20, 60, 20, 20 μg/mL were 0.4, 1.6, 0.7, 1.0 and 0.7, respectively. The data of intra-day and inter-day precision determined by the standard solutions at the same concentration as above can be seen from Table 3. The R.S.D.% values were all less than 2.5%.

Accuracy was determined only for RIF. The recovery values of RIF from the placebo tablets were 99.9%, 100.5% and 99.7% at the concentration of 60, 80 and 100 μg/mL, respectively ($n = 3$).

LOQ and LOD of each compound were determined to be 1 μg/mL and 0.2 μg/mL for a 10 μL injection, respectively.

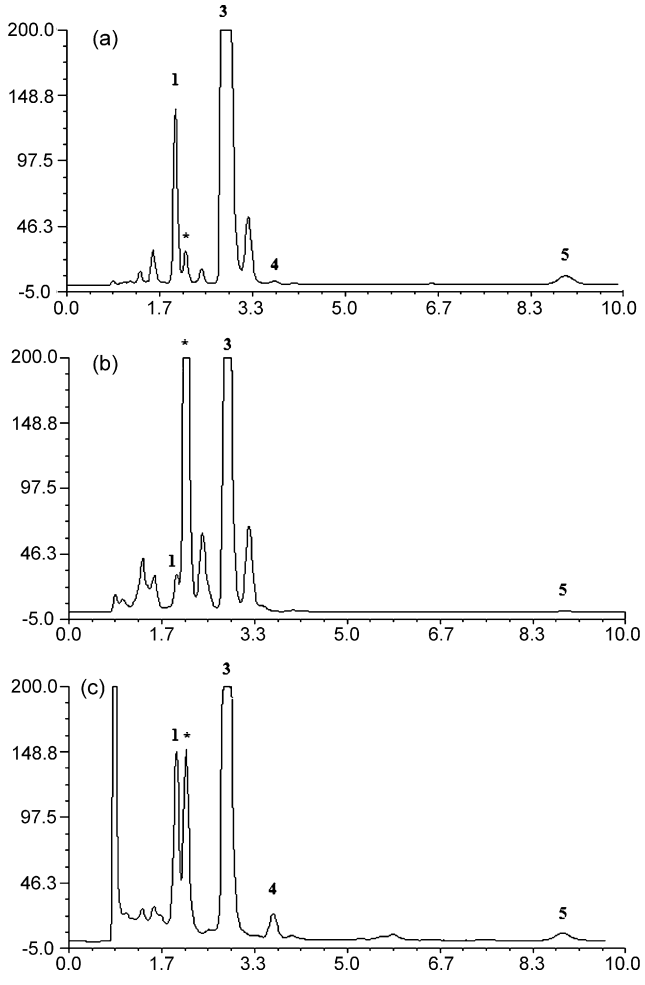

Fig. 2. Chromatograms of standard solutions of rifampicin after degradation by mol/L HCl (a), mol/L NaOH (b) and 30% H_2O_2 (c). The chromatographic conditions and number notations in the chromatogram were the same as in Fig. 1. The peak marked with asterisk represents an unknown impurity.

Table 3
Intra-day and inter-day precision of rifampicin and its related compounds

Compounds	Concentration (μg/mL)	Intra-day R.S.D.% ($n = 6$)	Inter-day R.S.D.% ($n = 3$)
RIF	60	1.1	1.2
RQ	30	2.0	2.4
SV	20	0.6	0.8
RNO	20	0.7	1.2
3-FR	20	0.6	1.0

Table 4
Rifampicin content of drug samples ($n = 3$)

Samples	Specification	Content[a]	R.S.D.%
Solution tablet for eye drops	5 mg/tablet	91.8	0.3
Self-made tablet	150 mg/tablet	99.8	0.8
Crude drug	>93.0%	96.9	0.6

[a] The content of solution tablet for eye drops and self-made tablets was expressed as a percentage of the amount declared on the preparations; the content of crude drug was calculated with reference to the dried substance.

3.5. Analysis of RIF in pharmaceuticals

The applicability of the method to the determination of RIF was examined by analyzing one batch of crude drug, self-made tablet and commercially available eye drops, respectively. The assay results are listed in Table 4. The contents of the RIF were all within the recommended limits of ChP, but the tolerance for related compounds was exceeded in eye drops.

4. Conclusions

A HPLC method for the determination of RIF and its related compounds was developed and validated on a monolithic C18 column. The analysis time was much shorter while the separation efficiency remained equivalent to that on a conventional C18 particle-packed column. The most significant advantages of this method are its simplicity and rapidness, thus it is very suitable to routine quality control analysis of RIF in crude drug and pharmaceuticals.

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