Pharmacy Department of Medical Institute of Nanchang University[1]; The Second Hospital of Nanchang[2]; State Key Lab of Food Science and Technology[3], Nanchang University, Nanchang, China

Impurity profile of rifaximin produced in China

LIUCHAO[1], MAIXI[1], WANGCHAO[2], CHUNPENG WAN[3]

Received August 4, 2011, accepted August 22, 2011

Dr. Liuchao, Pharmacy Department of Medical Institute of Nanchang University, Bayi street 603, 330002 Nanchang, Jiangxi province, China
dxy885511@126.com

Pharmazie 67: 283–287 (2012) *doi: 10.1691/ph.2012.1114*

Impurity profiles of rifaximin produced in China were investigated systematically by LCMS methods. Eleven impurities from the raw materials of rifaximin produced in China were detected. We adopted the Diagnostic fragment-ion-based extension strategy (DFIBES) for deducing the structure of unknown impurities. Impurity **1** was the 30-hydroxylated product of rifaximin. Impurity **2** was the 25-deacetyled rifaximin. Impurity 6 was the isomeride of rifaximin. Impurity **9** was rifamycin-O.

1. Introduction

Although there many reports on related substances in raw materials of rifaximin, there are no studies on the impurity profile of rifaximin produced in China. We therefore established a LC-ESI-MS^n method to detect impurities in rifaximin produced in China.

2. Investigations, results and discussion

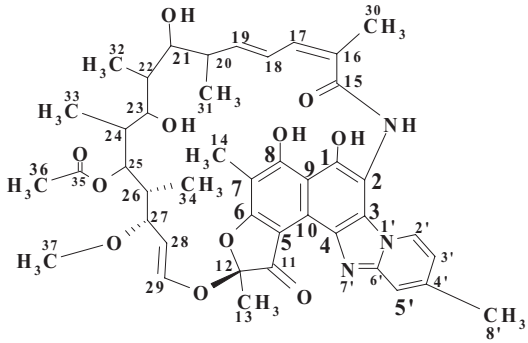
2.1. Impurities reported

Impurities reported for rifaximin are listed in Table 1.

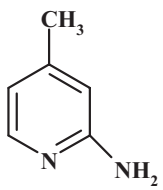
2.2. Mass spectroscopy

A moderate amount of raw rifaximin was weighed accurately. It was dissolved by acetonitrile and was diluted as 3 mg/ml test

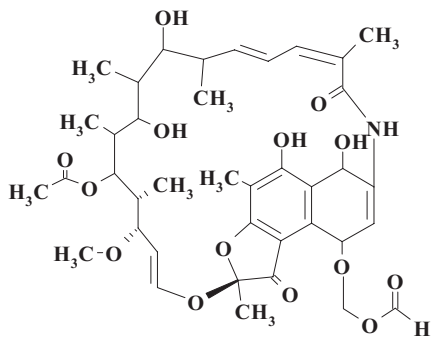
Table 1: Reported impurities in rifaximin



Rifaximin

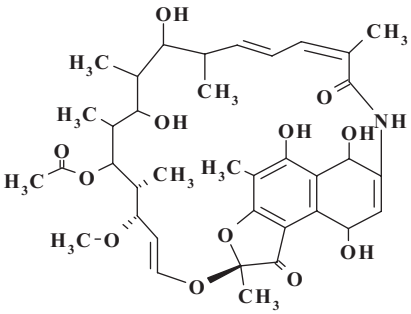


Impurity A

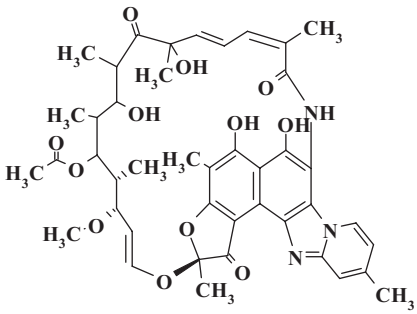


Impurity B (rifamycin B)

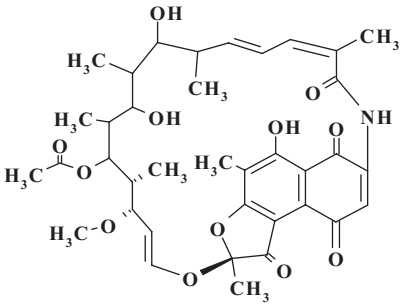
Table 1: (*Continued*)



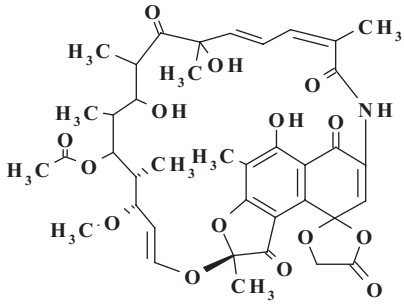
Impurity C (rifamycin SV)



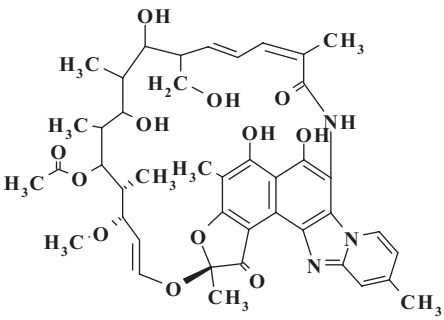
Impurity D (rifamycin Y)



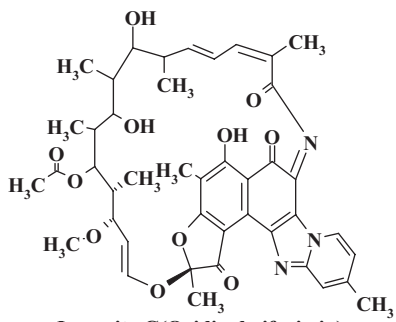
Impurity E (rifamycin S)



ImpurityF (rifamycin O)



Impurity H(Hydroxy-rifaximin)



Impurity G(Oxidised-rifaximin)

Name or code name	Molecular formula	$[M+H]^+$
Rifaximin	$C_{43}H_{51}N_3O_{11}$	786
Impurity A	$C_6H_8N_2$	109
Impurity B (rifamycin B)	$C_{39}H_{51}NO_{14}$	758
Impurity C (rifamycin SV)	$C_{37}H_{49}NO_{12}$	700
Impurity D (rifamycin Y)	$C_{43}H_{49}N_3O_{12}$	800
Impurity E (rifamycin S)	$C_{37}H_{45}NO_{12}$	696
Impurity F (rifamycin O)	$C_{39}H_{45}NO_{15}$	768
Impurity G (oxidised-rifaximin or rifaximin-quinone)	$C_{43}H_{49}N_3O_{11}$	784
Impurity H (hydroxyl-rifaximin)	$C_{43}H_{51}N_3O_{12}$	802

solution. According to the LC-MS conditions given in 3.2, 5 μl of the test solution was injected. MS^1 full scan and MS^2 full scan were carried out. The result was shown at Fig. 1 and Table 2.

2.3. Analysis on the main impurities in rifaximin

*2.3.1. Impurity **1***

$[M+H]^+$ of impurity **1** was 802. Its molecular weight was consistent with the molecular weight of hydroxylated impurity H of rifaximin. It is therefore suggested that impurity **1** was hydroxylated rifaximin. Stradi et al. (2009) also separated an impurity whose molecular weight was 802 from raw material of commercial rifaximin. They had determined its accurate structure by NMR and found it hydroxylated at position 30 of impurity X. But it was not the impurity H in the European Pharmacopeia. According to the MS^2 fragment ions as m/z770,752,734,710,692,532 and 362 of impurity **1**, we confirmed that impurity **1** was the impurity X.

*2.3.2. Impurity **2***

$[M+H]^+$ of impurity **2** was 744 which was 42Da different from the hydrogen ion of rifaximin. From its MS^2 fragment ions' analysis, we deduced that impurity **2** was 25-deacetyled rifaximin.

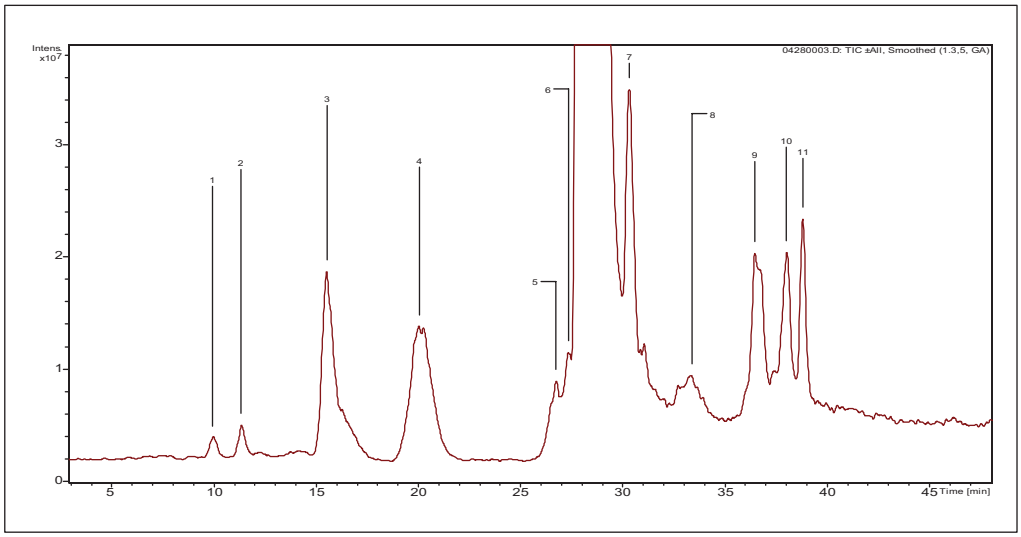


Fig. 1: LC-TIC chromatogram of raw material of rifaximin produced in China

Table 2: Chromatographic and mass spectral informations of main impurities of raw material of rifaximin produced in China

Code of peak	RT (min)	MS^1 $[M+H]^+$(m/z)	Key fragment ions of MS^2
1	10.0	802	770,752,734,710,692,532,362
2	11.3	744	712,694
3	15.4	784	752,725,692,661,638,612,540,512,49354,347
4	20.0	784	752,692,510,491,408,345,317,289
5	26.9	800	782,767,750,690,362,362,311,
6	27.3	786	754,736,694
7	30.4	800	768,750,732,708,690,672,470,362
8	33.3	784	766,734,692,674,646,583,512,483,454,345
9	36.4	768	750,709,658,623,596,564,538,496,474,426,374
10	38.0	784	752,734,692,674,649,594,540,511,454,388,345
11	38.8	784	752,734,692,674,639,564,511,482,454,345

2.3.3. Impurities whose $[M+H]^+$ was 784

$[M+H]^+$ of impurities **3**, **4**, **8**, **10** and **11** was 784. Their molecular weight was consistent with the molecular weight of rifaximin-quinone which suggested that these impurites were oxidation products of rifaxmin. Besides, they were isomerides.

*2.3.4. Impurity **6***

$[M+H]^+$ of impurity **6** was 786. Its molecular weight was consistent with the molecular weight of rifaximin. So they were isomerides. But the structure of impurity **6** needs be further studied.

2.3.5. Impurities whose $[M+H]^+$ was 800

$[M+H]^+$ of impurity **5** and **7** was 800. Its molecular weight was consistent with the molecular weight of rifamycin Y. But the structure of impurities **5** and **7** need be further studied.

*2.3.6. Impurity **9***

$[M+H]^+$ of impurity **9** was 768. Its molecular weight was consistent with the molecular weight of rifamycin O. From its MS^2 fragment ions' analysis, we deduced that impurity **9** was rifamycin O.

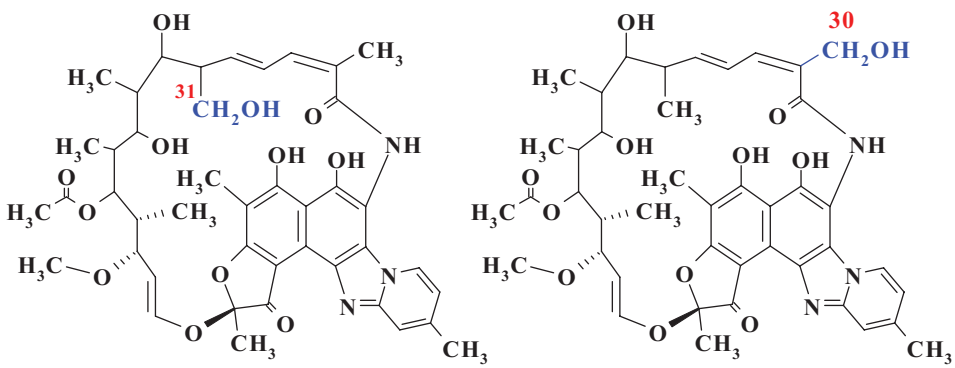


Fig. 2: Structures of impurity H and impurity X

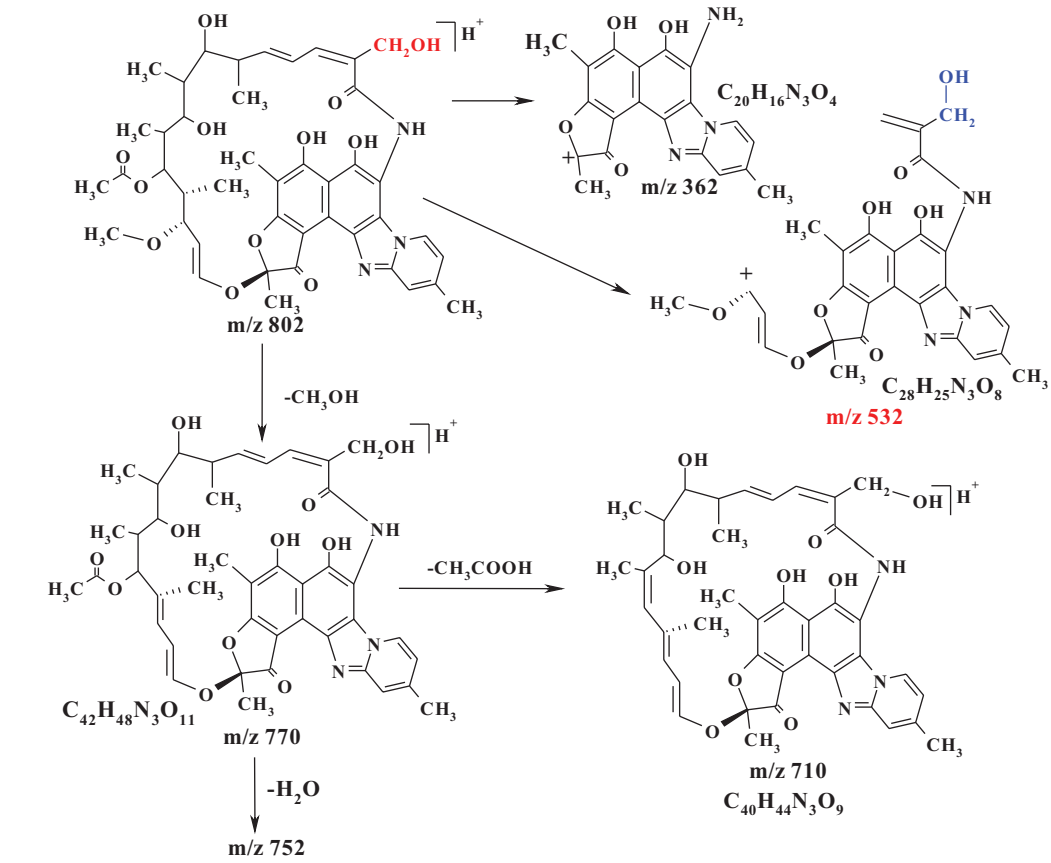


Fig. 3: Splitting way figure of mass spectrum of impurity **1**

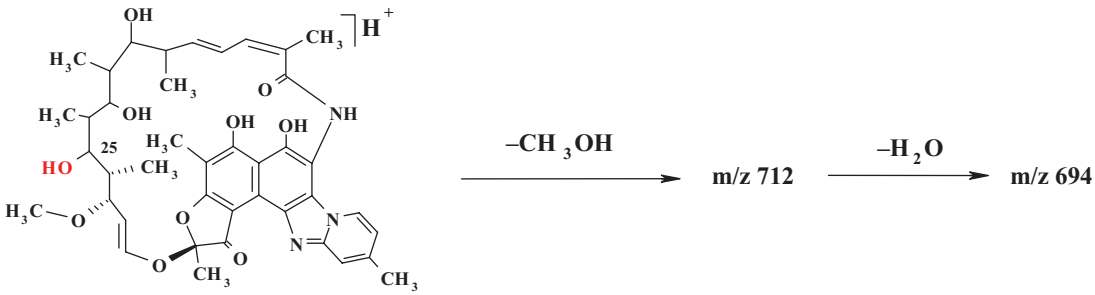


Fig. 4: Impurity **2**

3. Discussion

We established a LC-MS^n method to determine related substances in the raw materials of rifaximin produced in China and detected eleven impurities. We adopted the Diagnostic fragment-ion-based extension strategy (DFIBES) for deducing the structure of unknown impurities. In particular, there were many amounts of oxidation products in the raw materials of rifaximin produced in China. So we found out that the rifaximin could be easily oxidized and therefore recommend that rifaximin should be stored under nitrogen.

Among these related substances, we found hitherto unknown compounds. So far, there is no literature on the related substances in the raw materials of rifaximin produced in China. Only by the MS^2 or MS^n fragment ions' analysis, we could not describe all the related substances accurately, which was an disfigurement of the LC-MS method.

Table 3: Chromatographic conditions of HPLC gradient elution method

Time (min)	%B	Flow rate (mL/min)
0.00	48.0	1
13.00	48.0	1
14.00	41.0	1
23.00	41.0	1
24.00	60.0	1
33.00	60.0	1
34.00	67.0	1
60.00	67.0	1
61.00	48.0	1

4. Experimental

4.1. Materials and methods

All reagents and spectroscopic solvents pure grade employed for LC/MS analyses were provided by nanchang university. The raw material of rifaximin was prepared by our lab.

4.2. LC-ESI-MS^n

An Agilent 1100 LC /MSD Trap apparatus was used. The column was Symmetry-Shield RPC$_{18}$(Waters USA, 4.6 mm × 150 mm i.d., 5 µm particle size). The flow rate was 1 ml/min. The column temperature was 35 °C. The injection quantity was 20 µl. The post-column split ratio is 4:1. The mobile phase was employed as the gradient elution method. The conditions

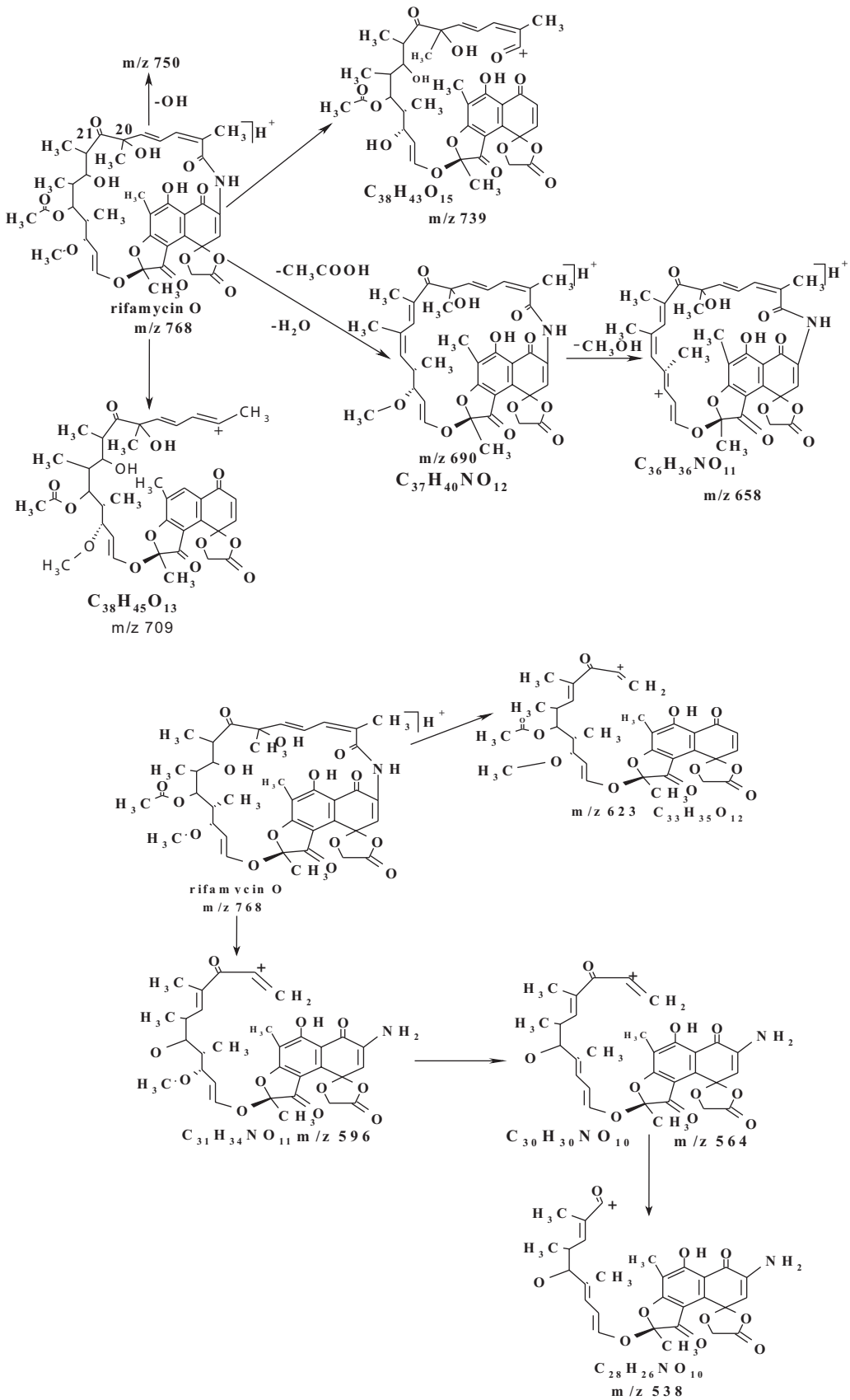


Fig. 5: Splitting way figure of mass spectrum of impurity **9**

were as follows: Mobile phase A: deionized water, B: acetonitrile: methanol (30:20).

The ion source was an ESI source. The temperature of the ion source was 350 °C. The pressure of the atomizer chamber was 275.8 kPa. The flow rate of atomization gas was 2 L/min. The flow rate of dry gas was 8 L/min. The ion voltage was 1.5 KV. The range of ion scan was 100∼900 m/z. The scanning mode was selected-ion monitoring (SIM).

Reference

Stradi R, Nava D, Nebuloni M, Pastura B, Pini E (2009) Structural elucidation of the Rifaximin Ph. Eur. Impurity H. J Pharm Biomed Anal 51: 858–865.