Journal of Zhejiang University SCIENCE
ISSN 1009-3095
http://www.zju.edu.cn/jzus
E-mail: jzus@zju.edu.cn

Separation and identification of *cis* and *trans* isomers of 2-butene-1,4-diol and lafutidine by HPLC and LC-MS*

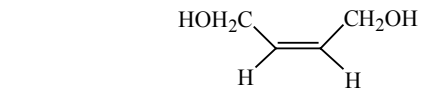
PAN Chun-xiu (潘春秀), XU Xiu-zhu (徐秀珠)†, HE Hong-mei (何红梅),
CAI Xiao-jun (蔡小军), ZHANG Xue-jun (张雪君)
(*Department of Chemistry, Zhejiang University, Hangzhou 310027, China*)
†E-mail: xuxiuzhu@zju.edu.cn
Received Jan. 16, 2004; revision accepted May. 17, 2004

Abstract: The *cis* and *trans* isomers separation of 2-butene-1,4-diol and lafutidine were studied by HPLC on two kinds of chiral columns: (S,S)-Whelk-O 1 and ChiraSpher. The isomers of 2-butene-1,4-diol can be separated on both chiral columns while the isomers of lafutidine can only be resolved on ChiraSpher column. The influence of different type and amount of mobile phase modifier on the isomers separation was extensively studied. The resolution of *cis* and *trans* isomers of 2-butene-1,4-diol was 2.61 on (S,S)-Whelk-O 1 column with hexane-ethanol (97:3, v/v) as the mobile phase. The resolution of lafutidine was 1.89 on ChiraSpher column with hexane-ethanol-THF-diethylamine (92:3:5:0.1, v/v/v/v) as the mobile phase. LC-MS methods were developed to identify the isomer peaks.

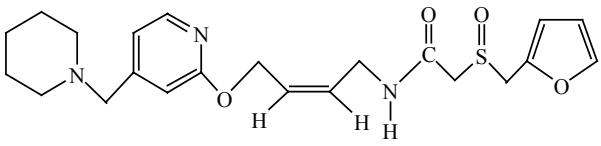
Key words: 2-butene-1,4-diol, Lafutidine, Isomers separation, (S,S)-Whelk-O 1, ChiraSpher, LC-MS
doi:10.1631/jzus.2005.B0074 **Document code:** A **CLC number:** O657.7+2

INTRODUCTION

2-butene-1,4-diol is an important intermediate used in the production of pharmaceuticals, plant-protection agents and pesticides. The predominant geometric isomer of 2-butene-1,4-diol is *cis* isomer (Fig.1a). However, a little *trans* impurity is also present in this intermediate, resulting in a little *trans* isomer impurity in the production, such as lafutidine (Chen and Xu, 2003). Lafutidine ((+/−)-2-(furfurylsulfinyl)-N-[4-[4-(piperidinomethyl)-2-pyridyl]oxy-(Z)-2 butenyl] acetamide, Fig.1b) is a novel anti-ulcer drug possessing an antisecretory effect and gastroprotective activity (Shibata *et al*., 1993; Onodera *et al*., 1995). The function of *trans* isomer of lafutidine might be different from that of *cis* isomer. So the isomers separation of lafutidine and 2-butene-1,4-diol is very important. The authors are not aware of reports of isomers separation of 2-butene-1,4-diol and lafutidine. In the current study, the *cis* and *trans* isomers of 2-butene-1,4-diol and lafutidine were successfully separated by HPLC using two kinds of chiral columns–(S,S)-Whelk-O 1 (Pirkle and Welch, 1992; Shao *et al*., 2003) and ChiraSpher (Krause *et al*., 1999; Schulte *et al*., 2002). The effects of the type and amount of mobile phase modifier on isomers separation were investigated.



(a)



(b)

Fig.1 Structure of *cis*-2-butene-1,4-diol (a) and lafutidine (b)

* Project (No. 03111) supported by the Foundation of Analysis & Measurement of Zhejiang Province, China

EXPERIMENTAL DETAILS

Materials

2-butene-1,4-diol and lafutidine were kindly provided by Zhejiang Province Institute for Drug Control. Hexane, analytical grade, was obtained from Hangzhou Refinery of China Petrochemical Corporation. Tetrahydrofuran (THF, analytical grade) was purchased from Hangzhou Shuanglin Chemical Reagent Factory. All other chemicals were of analytical grade or better and were obtained from various commercial sources.

High-performance Liquid Chromatography (HPLC)

Isomers separations were performed using Waters 2690 Separations Module equipped with a Waters 996 Photodiode Array Detector and Waters Millennium^{32} System (Waters Co., Milford, MA, USA). (S,S)-Whelk-O 1 (Fig.2a) and ChiraSpher (Fig.2b) chiral columns were gifts from Prof. Dr. Kinkel (Georg-Simon-Ohm University of Applied Sciences, Nürnberg, Germany). The mobile phases were composed of n-hexane and polar modifier(s) and they were filtered through a 0.45 μm membrane filter (Millipore) and degassed by sonication before use. The sample solution was prepared by dissolving an appropriate amount of 2-butene-1,4-diol or lafutidine in ethanol and was filtered through a 0.2 μm membrane filter (Millipore) before being injected into the HPLC. The chromatographic parameters, retention factor (k'), separation factor (α) and resolution (Rs) were calculated automatically with Waters Millennium^{32} System. The dead time (t_0) of the column was determined with 1,3,5-tri-*tert*-butyl benzene as a non-retained compound.

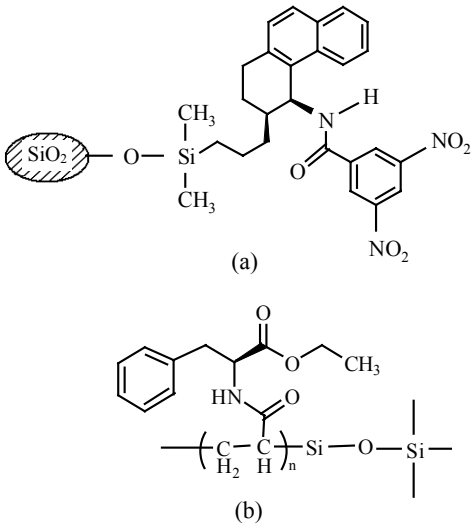


Fig.2 Structure of stationary phase. (a) (S,S)-Whelk-O 1; (b) ChiraSpher

Liquid Chromatography-Mass Spectrometry (LC-MS)

LC-MS analyses were performed in the positive ion mode using an Agilent 1100 Series LC system (Agilent Co., USA) interfaced to an Esquine 3000 plus MS detector (Bruker Daltonics Inc., Germany). The isomers of 2-butene-1,4-diol or lafutidine were separated using the same chromatographic conditions with Water HPLC system. The electrospray needle was held at 4000 V. The temperature of ion source was 100 °C for 2-butene-1,4-diol and 200 °C for lafutidine.

RESULTS AND DISCUSSION

Isomers separation of 2-butene-1,4-diol

At first, the isomers separation of 2-butene-1,4-diol was tested on an C_{18} and a silica gel columns, but the isomers could not be resolved on these two columns since the polarity of 2-butene-1,4-diol was so strong that the solute was not retained on the ODS column while it could not be eluted from the silica gel column. Then separation was attempted on two kinds of chiral columns, (S,S)-Whelk-O 1 and ChiraSpher. The initial separations were conducted with ethanol, *iso*-propanol or *n*-butanol as the alcoholic mobile phase modifier. The results revealed that the solute could not be eluted from these two columns when *iso*-propanol or *n*-butanol was used as the mobile phase modifier. So ethanol was selected as the polar modifier in the mobile phase.

Table 1 shows the influence of ethanol concentration in mobile phase on the isomers separation of 2-butene-1,4-diol on (S,S)-Whelk-O 1 column. As seen in Table 1, when ethanol concentration in mobile phase was 10%, the isomers could not be resolved due to the weak retention of the solute. However, as the ethanol concentration decreased, the retention of solute (k'), separation factor (α) and resolution (Rs) were

all steadily increased, suggesting that the polar interaction (mainly hydrogen-bonding interaction) between solute and stationary phase was not only the primary factor for solute retention but also playing some roles in isomeric recognition. The influence of ethanol concentration in mobile phase on the isomers separation of 2-butene-1,4-diol on ChiraShper column is shown in Table 2. In order to improve the peak shape and resolution, a basic additive, diethylamine was added in the mobile phase. As can be seen in Table 2, with decrease of ethanol concentration in mobile phase, the retention factor (k') and resolution (Rs) were increased steadily while the separation factor (α) was essentially unchanged. The results indicated that for the isomers separation on ChiraSpher column, the polar interaction between solute and stationary phase was also a predominant factor for solute retention but contributed little to the isomeric recognition. The improvement of resolution was due to increase of solute retention time. Comparison of Table 1 and Table 2 revealed that the isomers of 2-butene-1,4-diol obtained much better separation on the (S,S)-Whelk-O 1 column. Fig.3a shows the chromatogram of isomers separation of 2-butene-1,4-diol on (S,S)-Whelk-O 1 column. Since the ultraviolet (UV) absorbance of 2-butene-1,4-diol was weak and the amount of *trans* isomer impurity very small (~3%), the UV spectrum detected by PDA detector was insufficient for identifying the isomer. Then an LC-MS method was developed to identify the small *trans* isomer peak. The mass spectra of *cis* and *trans* isomer peaks are presented in Figs.3b and 3c respectively. We can see that the mass spectra of *trans* isomer peak are identical with that of *cis* isomer peak and that m/z 110.9 is the mass charge ratio of molecular ion of 2-butene-1,4-diol plus sodium ion (Na^+).

Table 1 The influence of ethanol concentration in mobile phase on the isomers separation of 2-butene-1,4-diol on (S,S)-Whelk-O 1 column

Hexane-ethanol (v/v)	k_1'	α	Rs
90:10	0.42	1.00	–
95:5	1.11	1.12	1.17
97:3	2.21	1.20	2.61

k_1': the retention factor of the *cis* isomer of 2-butene-1,4-diol; Chromatographic conditions: flow rate, 1.0 ml/min; temperature, 25 °C; detection wavelength, 202.8 nm

Table 2 The influence of ethanol concentration in mobile phase on the isomers separation of 2-butene-1,4-diol on ChiraSpher column

Hexane-ethanol-diethylamine (v/v/v)	k_1'	α	Rs
80:20:0.1	1.89	1.16	1.11
90:10:0.1	5.31	1.18	1.21
95:5:0.1	7.85	1.17	1.48

k_1': the retention factor of the *cis* isomers of 2-butene-1,4-diol; Chromatographic conditions: flow rate, 1.0 ml/min; temperature, 25 °C; detection wavelength, 202.8 nm

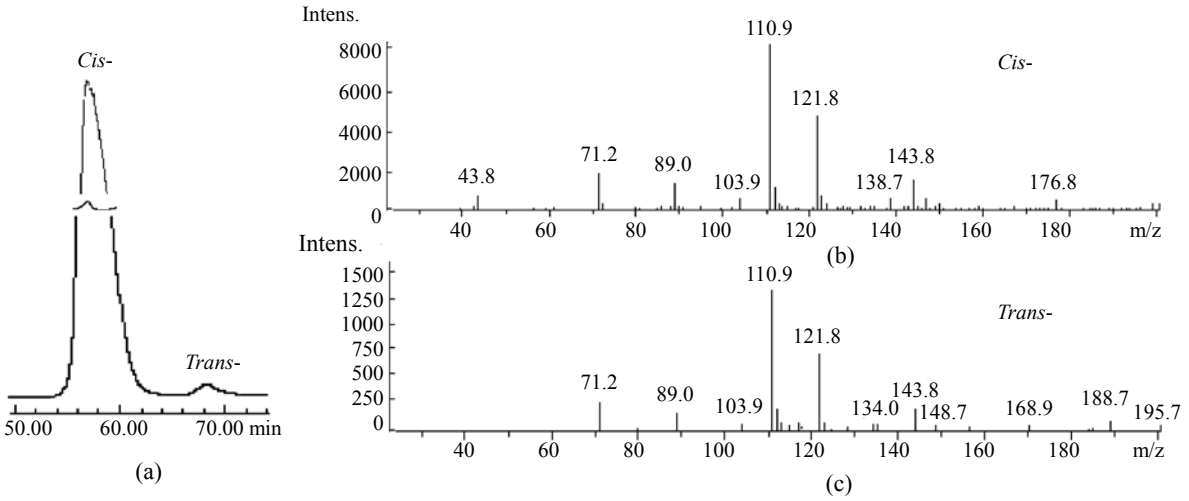


Fig.3 The isomers separation chromatogram and isomers mass spectra of 2-butene-1,4-diol

Chromatographic conditions: column, (S,S)-Whelk-O 1; mobile phase, hexane-ethanol (97:3, v/v); flow rate, 1.0 ml/min; temperature, 25 °C; detection wavelength, 202.8 nm. (a) The chromatogram of isomers separation of 2-butene-1,4-diol; (b) The mass spectrum of *cis* 2-butene-1,4-diol; (c) The mass spectrum of *trans* 2-butene-1,4-diol

Isomers separation of lafutidine

The isomers separation of lafutidine was also attempted on (S,S)-Whelk-O 1 and ChiraSpher columns. However, in contrast to 2-butene-1,4-diol, the *cis* and *trans* isomers of lafutidine can only be resolved on ChiraSpher column. The influence of ethanol concentration in mobile phase on the isomers separation of lafutidine is shown in Table 3 showing that as the ethanol concentration decreased from 10% to 5%, the solute retention factor increased strikingly; while the separation factor and resolution improved only a little. As the ethanol concentration further decreased to 3%, the solute could not be eluted from the column in 100 min. In order to shorten the solute retention time and improve the resolution, THF, an organic solvent with excellent solubility and weak polarity was added in the mobile phase. The influence of THF concentration on the isomers separation is also shown in Table 3. We can see that after THF was added in the mobile phase and as its concentration increased from 3% to 5%, the solute retention factor decreased dramatically and the resolution improved a lot, but, no further improvement was observed as the THF concentration increased further. The effect of diethylamine concentration in mobile phase was also studied. The results are also listed in Table 3. As shown by the data, there is essentially no influence on the isomers separation with increase of diethylamine concentration. As can be seen from the data in Table 3, different type and amount of mobile phase modifier has great influence on the solute retention (k') and resolution (Rs), but, the separation factor (α) is essentially unchanged, suggesting that, for ChiraSpher column, the attractive interactions (hydrogen-bonding, dipole-dipole and π-π interaction) between solute and stationary phase is a predominant factor for solute retention but they are not important for isomeric recognition. According to the above results, 92:3:5:0.1 of hexane-ethanol-THF-diethylamine (v/v/v/v) was selected as the optimum composition of mobile phase. The chromatogram of isomers separation of lafutidine under this optimum condition is presented in panel A of Fig.4. An LC-MS method was also developed to identify the lafutidine isomers peaks. As shown in Figs.4b and 4c, the mass spectra of *cis* and *trans* isomers peaks are identical and m/z 432.0 is the mass charge ratio of lafutidine molecular ion.

Table 3 The influence of different polar mobile phase modifiers concentration on the isomers separation of lafutidine on ChiraSpher column

Hexane-ethanol-THF-diethylamine (v/v/v/v)	k_1'	α	Rs
90:10:0:0.1	3.95	1.11	1.09
95:5:0:0.1	10.00	1.16	1.22
97:3:0:0.1*	–	–	–
94:3:3:0.1	10.21	1.16	1.56
92:3:5:0.1	7.83	1.17	1.89
90:3:7:0.1	7.23	1.13	1.75
90:3:7:0.2	6.66	1.16	1.51
90:3:7:0.5	6.12	1.16	1.62

k_1': the retention factor of the *cis* isomers of lafutidine; Chromatographic conditions: column, ChiraSpher; flow rate, 1.0 ml/min; temperature, 25 °C; detection wavelength, 285.0 nm; *Solutes were not eluted in 10 min

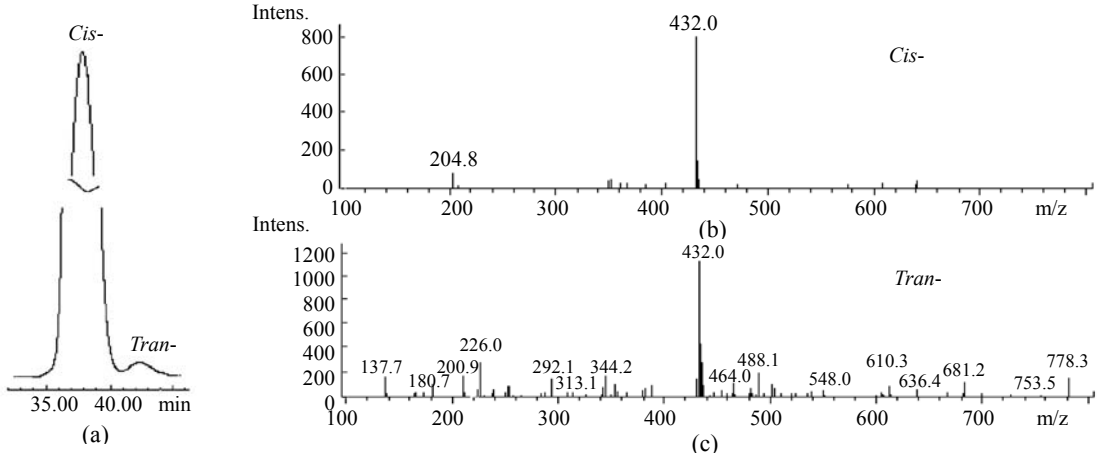


Fig.4 The isomers separation chromatogram and isomers mass spectra of lafutidine

Chromatographic conditions: column, ChiraSpher; mobile phase, hexane-ethanol-THF-diethylamine (92:3:5:0.1, v/v/v/v); for other conditions (Table 3). (a) The chromatogram of isomers separation of lafutidine; (b) The mass spectrum of *cis* lafutidine; (c) the mass spectrum of *trans* lafutidine

CONCLUSION

In conclusion, using two kinds of chiral columns, the *cis* and *trans* isomers of 2-butene-1,4-diol and lafutidine were successfully separated by HPLC. The methods have potential applications in the determination of *trans* isomer impurity in *cis*-2-butene-1,4-diol and lafutidine. Furthermore, since ChiraSpher column is characterized by its high stability and high loading capacity (Boonen *et al*., 1997), this column can be used for semi-preparative separation of lafutidine isomers and therefore this method could be useful for further pharmacological investigation of the individual isomer of lafutidine.

References

Boonen, G., Beck, M., Häberlein, H., 1997. Contribution to the quantitative and enantioselective determination of kavapyrones by high-performance liquid chromatography on ChiraSpher NT material. *Journal of Chromatography B*, **702**:240.

Chen, W.Q., Xu, D.Y., 2003. Lafutidine, a novel antiulcer drug. *Chinese J. New Drugs Clin. Rem*., **4**:247-250 (in Chinese).

Krause, K., Girod, M., Chankvetadze, B., Blaschke, G., 1999. Enantioseparations in normal- and reversed-phase nano-high-performance liquid chromatography and capillary electrochromatography using polyacrylamide and polysaccharide derivatives as chiral stationary phases. *J. Chromatogr. A*, **837**:51-63.

Onodera, S., Shibata, M., Tanaka, M., Inaba, N., Yamamura, T., Ohnishi, H., 1995. Gastroprotective activity of FRG-8813, a novel histamine H_2-receptor antagonist, in rats. *Jpn. J. Pharmacol*., **68**:161-173.

Pirkle, W.H., Welch, C.J., 1992, An improved chiral stationary phase for the chromatographic-separation of underivatized naproxen enantiomers. *J. Liq. Chromatogr*., **15**:1947-1955.

Schulte, M., Devent, R., Grosser, R., 2002. Enantioseparation of Gantofiban precursors on chiral stationary phases of the poly-(N-acryloyl amino acid derivative)-type. *Journal of Pharmaceutical and Biomedical Analysis*, **27**:627-637.

Shao, B.H., Xu, X.Z., Lu, J.D., Cai, X.J., Zou, L., Fu, X.Y., 2003. Comparison of enantioseparation and chiral recognition mechanism of racemic naproxen esters on (S,S)-whelk-O 1 and CDMPC chiral columns. *Acta Chim. Sinica*, **61**:1635-1640.

Shibata, M., Yamamura, T., Inaba, N., Onodera, S., Chiba, Y., Ohnishi, H., 1993. Gastric antisecretory effect of FRG-8813, a new histamine H_2-receptor antagonist, in rats and dogs. *Eur J. Pharmacol*., **235**:245-253.

Note:

JZUS sincerely apologizes for the defective "**Fig.2 The possible mechanism of reduction of α-diketones"** published in "*Zhang et al. /J Zhejiang Univ SCI 2004 5(10):1175-1179*" and will publish the corrected version of Fig.2 in this issue (2005, Vol. 6B, No.1, p.78):

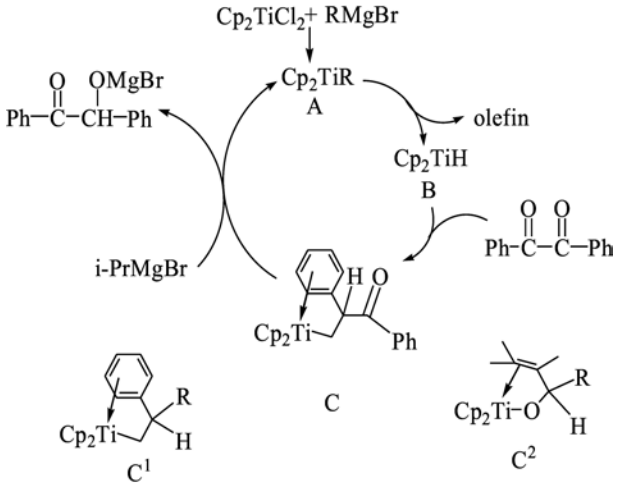


Fig.2 The possible mechanism of reduction of α-diketones