Asian Journal of Chemistry; Vol. 25, No. 6 (2013), 3523-3524

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.13812

NOTE

Quantitative Analysis of Etimicin by ^{1}H NMR Spectroscopy

SHUYU LIU[1,*], SHANGCHEN YAO[2] and CHANGQIN HU[2]

[1]Chemistry and Chemical Engineering Institute of Shanghai University of Engineering Science, No. 333 Longteng Road, Songjiang District, Shanghai 201620, P.R. China
[2]National Institutes for Food and Drug Control, No. 333 Longteng Road, Songjiang District, Shanghai 201620, P.R. China

*Corresponding author: E-mail: shuyuliu1219@yahoo.com.cn

(*Received*: 31 March 2012; *Accepted*: 29 December 2012) AJC-12636

A simple and rapid quantitative NMR assay was developed to measure the etimicin. The response linearity was ensured by linear determination factors R^2, which was 0.9989. The quantitation of the etimicin was reproducible and the method relative standard deviation is 0.573 %. The relative errors, measured against the labeled amount of etimicin, ranged from 0.47-0.68 %.

Key Words: Etimicin, Quantitative, Nuclear magnetic resonance.

Etimicin is mainly used as the sulphate, is a new semi-synthetic, water soluble aminoglycoside antibiotic (Fig. 1)[1]. As can be seen, neither etimicin nor its related substances contain a significant UV absorbing chromophore. No liquid chromatographic method has been described to analyze etimicin sulphate as a drug substance and to determine possible impurities. Microbiological assay is not able to distinguish between the main components and the impurities present in the drug[2]. The quantitative nuclear magnetic resonance (qNMR) method, which does not require consideration of impurities or a reference standard of known content, but only uses a common chemical substance of known content for comparison, can determine the absolute contents of a drug. Furthermore, this method is rapid, convenient, accurate, and highly specific and does not require an absorption coefficient[3]. Therefore, the aim of the current work was to develop an ^{1}H NMR method for quantification of etimicin.

^{1}H NMR measurements were made on a Bruker 400 spectrometer operating at 400 MHz.

The reference standard of etimicin sulphate was from the National Institute for Control of Pharmaceutical and Biological Products (NICPBP), China. The internal standard of maleic acid (purity 99.0 %) was purchased from Sigma, USA. Heavy water was from Cambridge Isotope Laboratories Inc, UK.

Methods: The five samples were prepared by mixing the etimicin sulphate with maleic acid in different weight ratios. Then the samples were dissolved in 0.5 mL D_2O. The concentrations of etimicin and maleic acid in the samples are listed in Table-1.

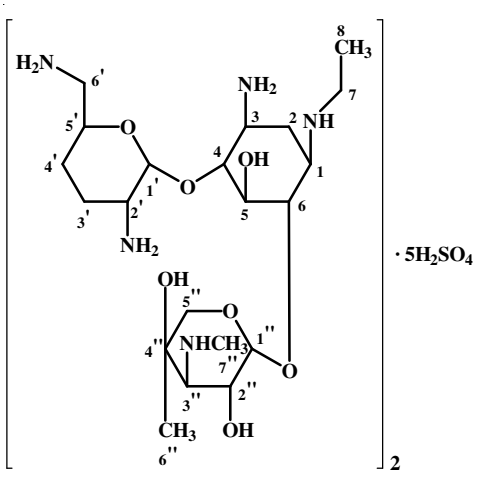

Fig. 1. Chemical structures of etimicin sulphate

TABLE-1
CONCENTRATIONS OF ETIMICIN SULPHATE AND MALEIC ACID IN THE SAMPLES (mg mL^{-1})

Sample No.	Etimicin sulphate	Maleic acid
1	7.7	3.74
2	12.32	3.34
3	17.98	3.04
4	24.18	3.24
5	32.78	2.26

The solvent was D_2O. The experiments were carried out with the following parameters optimized for quantitative NMR: P1 of 12.86 μs, irradiation frequency of 400.13 MHz, delay of

20 s and 16 scans. Phase and baseline corrections were done manually.

For quantitative methods, the internal standard method is used widely at present. The purity of the analyte P_x can be calculated as follows[4]:

$$P_x = \frac{I_x}{I_{Std}} \times \frac{N_{Std}}{N_x} \times \frac{M_x}{M_{Std}} \times \frac{m_{Std}}{m} \times P_{Std} \quad (1)$$

where M_x and M_{Std} are the molar masses of the analyte and the standard, respectively, m is the weighed mass of the investigated sample, M_{Std} and P_{Std} are the weighed mass and the purity of the standard and N_{Std} and I_{Std} correspond to the number of spins and the integrated signal area of a (typical) NMR line of the standard.

Selecting monitor signals for quantification: The monitor signal was chosen as the one that was best separated from the other signals for etimicin sulphate in the experiment. The structure of etimicin sulphate is given in Fig. 1. The H1' (δ = 5.05, doublet) and H1" (δ = 5.79, doublet) were selected from the etimicin peaks for quantization (Fig. 2).

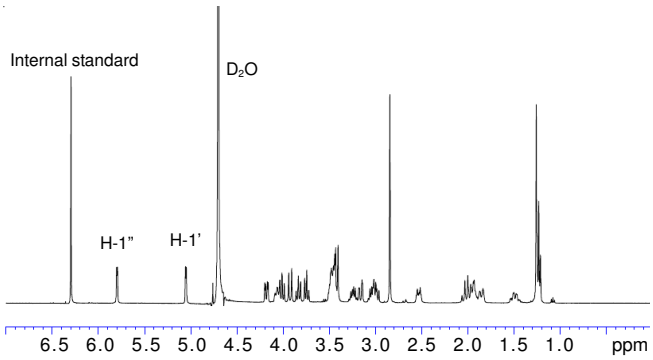

Fig. 2. ^{1}H NMR spectrum of etimicin, with internal standard (δ = 6.31, singlet) and solvent (δ = 4.70) peaks

Selection of an internal standard: The internal standard was chosen as which signal peaks were best separated from the signal peaks of etimicin in the ^{1}H NMR spectrum. As shown in Fig. 2, the chemical shift of maleic acid is 6.31 and its signal were best separated from the signals of etimicin. Moreover, we considered that maleic acid had the characteristics of an internal standard in terms of solubility, acidity, stability, moisture content, dehydration, complexation and reactivity[5].

Selecting NMR parameters: We accumulated ^{1}H NMR spectra using different delay times (dl) in experiments where the etimicin sulphate constituted the sample and maleic acid was the internal standard. For delay times of 20 and 30 s, the peak areas for maleic acid were 6.756 and 6.718, respectively and the calculated purities of the etimicin sample were 58.6 and 58.9 %. When dl was longer than 20 s, the peak area of the internal standard became invariant. We therefore used 20 s as the delay time in subsequent experiments.

Quantitative analysis results

Linearity: The linearity was checked by plotting the ratio of peak area of etimicin to maleic acid against the content of etimicin to maleic acid (Fig. 3). The response linearity was ensured by linear determination factors R^2, which was 0.9989.

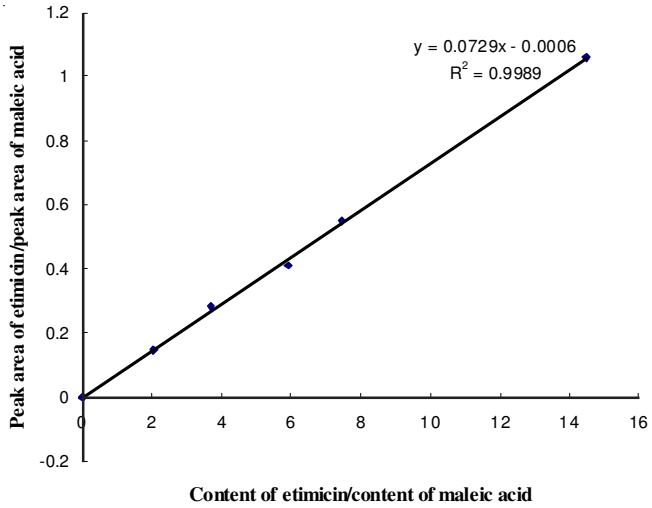

Fig. 3. Calibration straights of the etimicin

Precision: The repeatability was measured by five experiments carried out on a sample containing 17.98 mg of etimicin using the aforementioned procedure (Table-1). The relative standard deviation was 0.573 % and therefore it ensured the precision of the measurements.

Precision: The accuracy was validated by comparison of the labeled amount of etimicin with the purity of etimicin measured by NMR. The purity of etimicin was calculated using the eqn. 1. The relative errors, measured against the labeled amount of etimicin, ranged from 0.47-0.68 %, which indicated a good accuracy of the measurements.

REFERENCES

1. J. Liu, X.-L. Hu, Y.-Q. Shen, J. Fan and M. Xhao, *Chin. J. Pharm.*, **31**, 358 (2000).
2. L.L. Xi, G.F. Wu and Y. Zhua, *J. Chromatogr. A*, **1115**, 202 (2006).
3. U. Holzgrabe, B.W.K. Diehl and I. Wawer, *J. Pharm. Biomed. Anal.*, **37**, 557 (1998).
4. F. Malz and H. Jancke, *J. Pharm. Biomed. Anal.*, **38**, 813 (2005).
5. G.F. Pauli, B.U. Jaki and D.C. Lankin, *J. Nat. Prod.*, **68**, 133 (2005).