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Short communication

Direct determination of ciprofloxacin in admixtures with metronidazol and ampicillin by NMR

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Abstract

Methods to analyse mixtures of pharmaceutical compounds are often based on chromatographic separations coupled to UV detectors. These procedures typically have to verify with other methods that the specificity is sufficient because structural information is sometimes too limited. NMR methods can be particularly useful in the analysis of admixtures of antibiotics such as ciprofloxacin because of the inherent high selectivity. This can even be extended by the measurement of diffusion coefficients that help to identify the relevant signal for quantification. Taking advantage of the resolving power of NMR an analytical procedure to directly measure ciprofloxacin without separation steps is presented. The diffusion coefficients of the three components were measured. Tests of precision (repeatability) and recovery of ciprofloxacin without any separation steps in the presence of metronidazol and ampicillin were conducted.

Keywords: Ciprofloxacin; Metronidazol; Ampicillin; NMR

1. Introduction

In the treatment of severe infections, admixtures of antibiotics are used as parenteral infusions. Because of incompatibility problems it is desirable to have an analytical procedure that is able to analyse one component specifically in the presence of other active compounds and solvent systems. Methods to analyse mixtures of ciprofloxacin and metronidazol were developed based on chromatography and derivative spectroscopy [1,2]. Nagaralli et al. [3] used a spectrophotometric method for the determination of a mixture of amoxycillin, ciprofloxacin and piroxicam. A different principle was used by Chadha et al. [4] who measured the in vitro compatibility of ciprofloxacin mixtures with amoxicillin/clavulanic acid using a calorimetric method. Typically, other methods have to verify that the specificity is sufficient because structural information is limited for these methods. This drawback is inherently avoided with NMR based methods which have the power to give all the necessary structural information to prove the identity of the selected signals. Shamsipur et al. [5] used NMR for monitoring ampicillin and its related substances. NMR methods are particularly useful in the analysis of multi-component systems because of the high selectivity of modern instruments [6]. Stability studies benefit especially from the high resolution capacity [7]. This can even be extended by the measurement of diffusion coefficients that will help to identify the relevant signal for quantification. Taking advantage of the latter resolving power of NMR an analytical procedure to measure ciprofloxacin (Fig. 1) in the presence of metronidazol and ampicillin is presented.

2. Materials and methods

The ^{1}H NMR spectra were measured at 25 °C with a 700 MHz Bruker Avance spectrometer. The internal Bruker frequency reference was used. 512 scans were acquired for each proton spectrum. A standard ^{1}H–^{13}C-HSQC two-dimensional experiment was conducted with 0.5 mM ciprofloxacin in sodium phosphate buffer (PB: 50 mM, pH = 6.7, H_2O:D_2O = 90:10), 60 scans and a total

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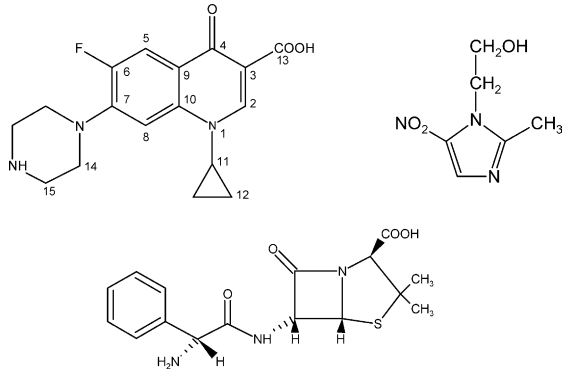

Fig. 1. Chemical formulas of ciprofloxacin, metronidazol and ampicillin.

measurement time of 4 h. A ciprofloxacin stock solution in H_2O was prepared from ciprofloxacin–HCl–H_2O. Starting from this stock solution samples of ciprofloxacin in PB were prepared to obtain a calibration curve (triplicate samples for each concentration) with final concentrations between 0.05 and 0.5 mM ciprofloxacin. The metronidazol (10 mM) and ampicillin (20 mM) stock solutions were made directly with PB. The mixtures were made in PB with final concentrations of ciprofloxacin of 0.1, 0.2 and 0.5 mM. The concentrations of metronidazol and ampicillin were held constant at 0.5 mM. The precision and recovery was tested by six determinations of each mixture. Water suppression was done by using the watergate sequence [8]. The ^{1}H-1D diffusion experiments used the Bruker standard bipolar gradient pulse program with water suppression [9]. The samples for measuring the diffusion coefficients contained all compounds at a concentration of 0.5 mM. A diffusion delay of 50 ms was applied. The rest water signal of a D_2O sample was used for calibration ($D = 1.9 \times 10^{-9}$ m^2/s at 25 °C, [10]). The values of the diffusion coefficients are averages of three measurements.

3. Results and discussion

Solutions of ciprofloxacin in PB were measured in the range from 0.05 to 0.5 mM representing the lower limits of the presented NMR measurements with reasonable accuracy. Due to the inherent high dynamic response of NMR methods this concentration range can be extended to much higher values. By recording a ^{1}H–^{13}C-HSQC spectrum the proton at 3.416 ppm was assigned to C15 of the piperazine ring of ciprofloxacin (Fig. 1). Earlier results were measured in organic solvents or at extreme pH values because the solubility is low under physiological conditions [11,12]. The carbon resonances of the piperazine ring of ciprofloxacin in PB at neutral pH are the following: C15 (43.7 ppm) and C14 (41.8 ppm). Therefore, depending on the solvent system the piperazine ring protons of ciprofloxacin differ in a range of 0.5 ppm, and the carbon resonances in a range of 6 ppm.

For quantification the integrals of the proton resonance at 3.416 ppm (ciprofloxacin) were taken. A calibration curve

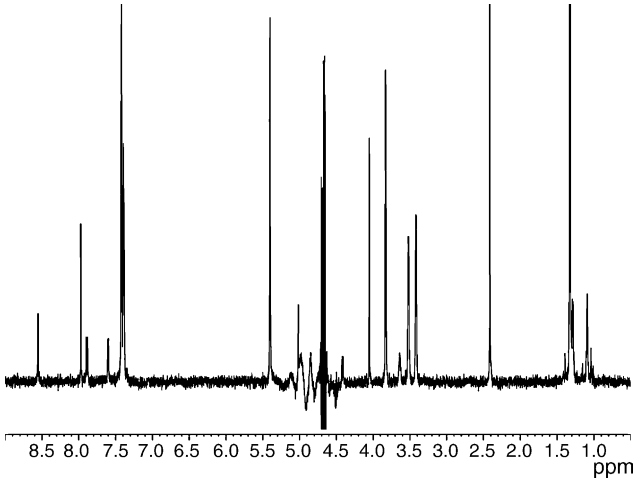

Fig. 2. Proton spectrum of 0.2 mM ciprofloxacin, 0.5 mM metronidazol and 0.5 mM ampicillin in phosphate buffer.

was obtained with a correlation coefficient of $r^2 = 0.997$ using linear regression.

For the analysis of mixtures, the concentrations of the two other components metronidazol and ampicillin (Fig. 1) were fixed at 0.5 mM. A proton spectrum of the mixture of 0.2 mM ciprofloxacin, 0.5 mM metronidazol and 0.5 mM ampicillin is shown in Fig. 2.

To prove the assignment of the signals the diffusion coefficients were measured. A curve fit of the decreased peak intensity with an increased gradient strength yields the diffusion coefficient for the whole molecule if chemical exchange and nuclear Overhauser effects can be neglected (Fig. 3). Under these conditions, all resonances of the same molecule will show the same diffusion coefficient.

The experimental error (three determinations, relative deviation from the average value) for the diffusion coefficients is below ±10%. In dilute solutions the formation of oligomers is normally reduced and the measured values approach the diffusion coefficient at infinite dilution. Due

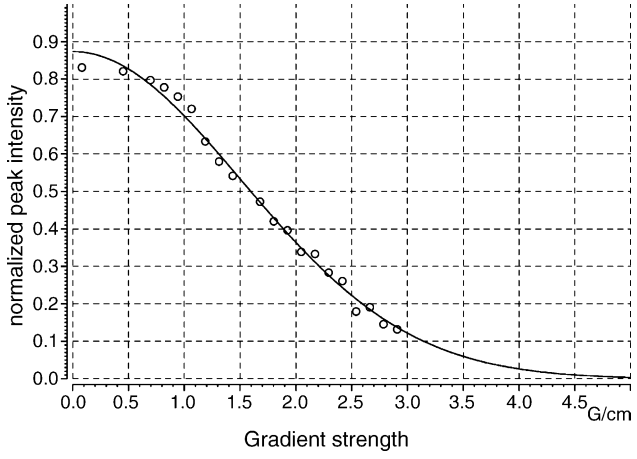

Fig. 3. Decreasing peak intensity of the ciprofloxacin proton at 3.416 ppm with increasing gradient strength.

Table 1
Precision and recovery of ciprofloxacin in the presence of metronidazol and ampicillin

Nominal concentration of ciprofloxacin (mM)	Nominal concentration of metronidazol and ampicillin (mM)	Rel. S.D.[a] (%)	Recovery (%)
0.1	0.5	5.11	103.8
0.2	0.5	3.88	103.6
0.5	0.5	1.87	101.5

[a] Standard deviation.

to the low molecular weight of metronidazol the average value of the diffusion coefficient is higher than for the other two components: $D_{(\text{metronidazol, 3.83 ppm})} = 1.09 \times 10^{-9}$ m^2/s, $D_{(\text{ampicillin, 1.33 ppm})} = 4.63 \times 10^{-10}$ m^2/s and $D_{(\text{ciprofloxacin, 3.416 ppm})} = 5.63 \times 10^{-10}$ m^2/s. As a subscript, the ppm values of well resolved signals are indicated. This facilitates the quantification of the ciprofloxacin proton signals which can be identified by having the same diffusion coefficient. The measurement time for each of the diffusion experiments was 15 min. With this type of measurement the presence of, e.g. dimers can be identified simultaneously. The repeatability precision of the system for the determination of ciprofloxacin in the presence of metronidazol and ampicillin is shown in Table 1. It was tested by six repeated analyses at three different ciprofloxacin concentrations. The recovery of ciprofloxacin in mixtures of all three compounds are listed in Table 1. In conclusion, the two quality parameters (repeatability and recovery) justify the use of NMR in the analysis of ciprofloxacin solutions in the presence of other antibiotics. It is clear that the approach utilizing the different diffusion coefficients will aid the search for quantifiable signals.

References

[1] E. Vega, N. Solá, J. Pharm. Biomed. Anal. 25 (2001) 523–530.
[2] E. Vega, V. Dabbene, M. Nassetta, N. Solá, J. Pharm. Biomed. Anal. 21 (1999) 1003–1009.
[3] B.S. Nagaralli, J. Seetharamappa, M.B. Melwanki, J. Pharm. Biomed. Anal. 29 (2002) 859–864.
[4] R. Chadha, N. Kashid, D.V.S. Jain, J. Pharm. Biomed. Anal. 36 (2004) 295–307.
[5] M. Shamsipur, Z. Talebpour, H.R. Bijanzadeh, S. Tabatabaei, J. Pharm. Biomed. Anal. 30 (2002) 1075–1085.
[6] U. Holzgrabe, B.W.K. Diehl, I. Wawer, J. Pharm. Biomed. Anal. 17 (1998) 557–616.
[7] S.O. Thoppil, P.D. Amin, J. Pharm. Biomed. Anal. 22 (2000) 699–703.
[8] M. Liu, X. Mao, C. He, H. Huang, J.K. Nicholson, J.C. Lindon, J. Magn. Reson. 132 (1998) 125–129.
[9] D. Wu, A. Chen, C.S. Johnson, J. Magn. Reson. A 115 (1995) 260–264.
[10] L.G. Longsworth, J. Phys. Chem. 64 (1960) 1914–1917.
[11] A. Zieba, A. Maslankiewicz, J. Sitkowski, Magn. Reson. Chem. 41 (2004) 903–904.
[12] S.C. Wallis, L.R. Gahan, B.G. Charles, T.W. Hambley, P.A. Duckworth, J. Inorg. Biochem. 62 (1996) 1–16.