Quantitative ^{1}H NMR Spectroscopy: Determination of alcohol content in indinavir sulphate

M. I. A. Gonçalves*
Departamento de Farmácia – Faculdade de Ciências Farmacêuticas – USP; Faculdades Integradas São Paulo e Faculdades Oswaldo Cruz, São Paulo, SP, Brazil.
miathisp@usp.br

T. J. Andreolli
Departamento de Farmácia – Faculdade de Ciências Farmacêuticas – USP, São Paulo, Sp, Brazil

C. J. Valduga
Lab. de Metabolismo de Lípedes – INCOR – Faculdade de Medicina – USP, São Paulo, SP, Brazil

Keywords: indinavir; ethanol; ^{1}H NMR

Abstract: *Indinavir sulfate is an inhibitor of the human immunodeficiency virus (HIV) protease. The HIV protease is vital to the replication of HIV-1 virus. Indinavir competitively inhibits this enzyme, preventing the critical cleavage of the precursor of the necessary polyproteins. The sulfate salt ethanolate is its pharmaceutical form. According to USP, the standard method to quantify the percentage of ethanol present in the pharmaceutical form is made by employing gas chromatography. In this work we quantified ethanol by ^{1}H NMR and compared it with gas chromatography. The difference between the percentages of ethanol obtained by the two methods employed was small, suggesting that ^{1}H NMR could be used as a new method to quantify ethanol in the pharmaceutical form of indinavir sulfate. The main advantage of this method is that the amount of samples required to analysis is smaller and the time of analysis can be shorter.*

Indinavir sulfate is an inhibitor of the human immunodeficiency virus (HIV) protease. The HIV protease is vital to the replication of HIV-1 virus. Indinavir competitively inhibits the enzyme, preventing the critical cleavage of the precursor of the necessary polyproteins. The sulfate salt ethanolate is rather soluble in water and methanol in its pharmaceutical form ($C_{36}H_{47}N_5O_4 \bullet H_2SO_4$).

According to USP[1], the standard method to quantify the percentage of ethanol present in the pharmaceutical form of indinavir sulfate is gas chromatography (GC). In this work, we quantified ethanol by ^{1}H NMR and compared it with GC. In ^{1}H NMR spectroscopy the signal area is normally proportional to the number of nuclei contributing to the signal, provided that saturation is avoided. It is therefore possible to use ^{1}H NMR for quantitative determinations in chemistry. The integrals are usually used and are proportional to molar ratios.

The quantification of the ethanol present in the pharmaceutical form of indinavir sulfate by GC[2] was performed according to the following conditions: Gas chromatograph (HP 6890 chromatograph, equipped with methylsilicon column, 30m of length and 0,32 mm I.D.) equipped with a flame ionization detector. The initial oven temperature was 35 ^{o}C for 2 min, reaching 150 ^{o}C at 20 ^{o}C/min and remaining at this temperature for 2 min. The injector temperature was 200 ^{o}C and the carrier gas flow (N_2) was 225 Kpa/min.

Samples were accurately weighed (about 0.5 g) and dissolved into a 10 mL volumetric flask with 10 mL of 1 % n-butanol in water. For the standard preparation, 5.0 g of ethanol was

accurately weighed and put into a 100 mL volumetric flask until reaching a volume with 1% of n-butanol in water. 0.5 μL of the sample and standard preparations were injected into the chromatograph. Chromatograms were recorded and the peak responses were measured. Peaks were identified according to the retention time, and the percentage of ethanol was calculated using the following formula:

$$\% \text{ ethanol} = \frac{\text{Response ratio}_{\text{sample preparation}}}{\text{Response ratio}_{\text{standard preparation}}} * \frac{\text{Weight}_{\text{standard}}}{\text{Weight}_{\text{sample}}} * 100$$

The NMR spectra of indinavir were taken in a Bruker DPX-300 instrument (301.1 MHz for ^{1}H). Samples were dissolved in D_2O (deuterowater). Free induction decays (FIDs) were acquired at 25 ^{o}C by using a spectral width of 10 ppm for ^{1}H.

The main resonances were assigned (δ in ppm): 8.71 (1H, s), 8.63 (1H, d, *J* = 5.6 Hz), 8.46 (1H, d, *J* = 8.0 Hz), 7.91 (1H, dd, *J* = 8.0 and 6.0 Hz), 7.36-7.00 (9H, m), 4.14-4.03 (1H, m), 4.02-3.80 (4H, m), 3.61 (1H, d, *J* = 12 Hz), 3.25-3.07 (2H, m), 3.05-2.81 (5-6H, m), 2.80-2.58 (5H, m), 1.82 (1H, m), 1.60-1.43 (1H, m), 1.14 (1H, s). The values obtained from ethanol were (δ in ppm): 3.5 (2H, q, *J* = 7.1 Hz), 1.03 (3H, t, *J* = 7.1 Hz).

The indinavir sulfate spectrum shows a triplet signaled at 1.0 ppm from CH_3 of ethanol. The ethanol/indinavir sulfate ratio by weight was calculated using the following equation:

$$G_{et} / G_{ind} = F_{et} / F_{ind} * N_{ind} / N_{et} * M_{et} / M_{ind}$$

Whereas G_{et} and G_{ind} are the parts by weight of the components, ethanol and indinavir, as well as and F_{et} and F_{ind}, are the areas of the signal of CH_3 (ethanol) and CH_3 (indinavir). N_{ind} and N_{et} are the number of nuclei causing the signal, and M_{et} and M_{ind} are the molecular weight of the two components, 46 and 711.88, respectively. Table 1 shows the analysis of ethanol by GC and ^{1}H NMR in percentage values performed for 32 samples of indinavir sulfate.The difference between the percentages of ethanol obtained by the two methods employed was small, suggesting that ^{1}H NMR could be used as a new method to quantify ethanol in the pharmaceutical form of indinavir sulfate. The main advantage of this method is that the amount of samples required for analysis is smaller and the time of analysis can be shorter.

Table 1. Percentage of ethanol obtained by GC and ^{1}H NMR for different samples of the pharmaceutical form of indinavir sulfate.

Sample	% GC	% ^{1}H NMR	Diffe	Sample	% GC	% ^{1}H NMR	difference
1	2.96	3.45	0.49	17	3.98	4.53	0.55
2	2.97	3.45	0.48	18	4.3	5.05	0.75
3	4.08	4.37	0.29	19	4.14	4.81	0.67
4	4.21	4.37	0.16	20	3.65	4.31	0.66
5	3.87	5.11	1.24	21	3.15	4.29	1.14
6	4.15	4.62	0.47	22	3.56	4.78	1.22
7	4.08	4.64	0.56	23	3.6	4.71	1.11
8	4.01	4.87	0.86	24	4.23	5.19	0.96
9	3.74	4.51	0.77	25	4.23	5.59	1.36
10	3.59	4.66	1.07	26	4.42	5.84	1.42
11	4.28	5.20	0.92	27	4.59	5.51	0.92
12	4.24	5.01	0.77	28	4.96	6.53	1.57
13	4.15	5.22	1.07	29	4.72	5.78	1.06
14	4.11	5.40	1.29	30	4.45	5.22	0.77
15	3.99	4.57	0.58	31	4.02	5.04	1.02
16	3.84	4.53	0.69	32	3.78	4.99	1.21

References

1. THE UNITED STATES PHARMACOPEIA, 25th ed., United States of America, 2002, p.1980.

2. S. Braun, H.O. Kalinowski, S. Berger, 100 AND MORE BASIC NMR EXPERIMENTS- A PRACTICAL COURSE, Germany, 1996, p.231.