NMR METHOD OF ASSAY FOR GENTAMICIN

J. K. Kwakye,
B.Pharm, M Sc, Ph D, MPSG
Department of Pharmaceutical Chemistry
University of Science and Technology
Kumasi, Ghana

ABSTRACT

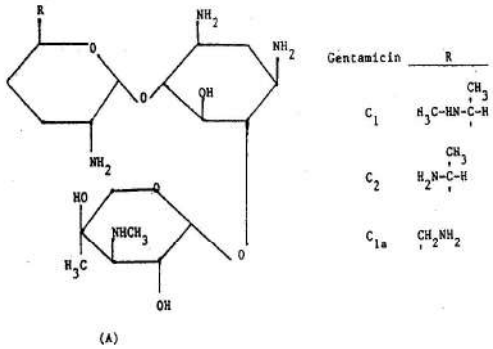
Nuclear Magnetic Resonance (NMR) has been used successfully to assay Gentamicin dosage forms. The method gives 100.5% accuracy with a variation of 2.3% for pure samples. It is a fast method of analysis. An assay which normally takes 14 or 18 hours by microbiological or ion exchange chromatographic methods respectively can now be performed in 30 minutes by the use of NMR.

KEYWORDS: Total gentamicins, NMR, Variation, Integration, Maleic Acid

INTRODUCTION

With the increasing short time demands of analytical results and the influx of new sophisticated formulations, it has become necessary to design fast and accurate analytical methods to meet the demands. The possibility to utilize NMR to quantify pharmaceutical preparations has been investigated and a number of commercial preparations have been assayed successfully [1, 2].

Gentamicin (A) which is a broad spectrum, basic deoxystreptamine containing antibiotic produced by Micromonospora species, is a mixture of three major components designated C_1, C_2 and C_{1a} [3]. Assay procedures for the determination of the total Gentamicins and the individual components are based on microbiological assay and chromatographic techniques [4, 5, 6]. NMR has been adopted only as a limit test to monitor the proportions of the main components [5, 7]. It is based on the N-methyl ratio of the peak at δ 2.75 to the peak a δ 2.95 (See Fig. 1). Therefore it does not give the actual amount of gentamicin present. This paper reports the successful NMR methods of assay for the total gentamicin.



(A)

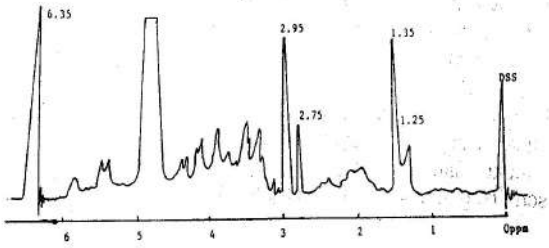


Figure 1: PNR Spectrum of a mixture of Genitamicin sulphate and maleic acid in D_20

The method involves the addition of maleic acid (internal standard) to the test sample and subsequent extraction with D_2O (Solvent). The appropriate analytical peaks (See Table 1) are integrated after the NMR spectrum has been recorded. The weight (Wt) of the Gentamicin in the sample is then calculated from the equation given below:

$$W_t = \frac{E_T \, I_T \, W_s}{E_s \, T_s}$$

Where E_T and E_s are NMR "equivalent weights" of the total Gentamicin and the internal standard (Maleic acid) respectively; I_T and I_s are the average integrals of the test and standard analytical peaks and W_s is the weight of the standard (Maleic acid) taken.

Table 1: Assignment of the Analytical Peaks

Compound	Analytical group	Resonance Position (δ) ppm	NMR Equivalent Weight
Gentamicin (Test sample)	N-CH_3	2.95	154.33
Maleic acid (Internal standard)	CH=CH	6.35	58.04

The assay procedure was applied to (1) Pure gentamicin and (2) three commercial gentamicin samples.

EXPERIMENTAL

APPARATUS

NMR Spectra were recorded on a Perkin Elmer R12B instrument operating at 60MHz with a probe temperature of 37°± 1°C and integration accuracy of 99.88 + 0.03%.

Assay of Pure Gentamicin

Gentamicin (various weights - See Table 3) and maleic acid (Ca 40mg) were dissolved in D_2O (0.5 ml). The NMR spectrum was recorded and the appropriate analytical signals (Table 1) integrated several times.

Assay of Commercial Preparations

1. Gentamicin sulphate powder

Gentamicin sulphate (Ca 90mg) and maleic acid (Ca 50 mg) were dissolved in D_2O (0.5 ml). The NMR spectrum was recorded and the analytical signals integrated five times.

2. Gentamicin injection.

2 ml of each ampoule were pipetted into small beakers and left in an oven at a temperature of 40°C and a pressure below 5 mmHg to remove solvent. The residue together with maleic acid (Ca 40mg) was then dissloved in D_2O (0.5 ml) and the spectrum recorded with the analytical signals integrated five times.

RESULTS

The results for both the pure gentamicin and the commercial products are shown in Tables 2 and 3 respectively.

Table 2: Total Gentamicin Assay using pure samples.

Wt of Maleic Acid (mg)	Total Wt of Gentamicin (Mg) W_t	Average Integrals (cm) I_s	Average Integrals (cm) I_T	W_t of Gentamicin Obtained (W_o mg)	$\frac{W_o \times 100}{W_t}$ %
40.8	45.4	5.06	2.11	45.2	99.6
40.2	52.7	4.98	2.47	53.1	100.7
40.7	35.9	4.98	1.63	35.4	98.6
41.1	45.6	5.12	2.11	45.0	98.8
39.8	60.1	4.87	2.90	63.1	105.0
40.3	30.5	4.96	1.43	30.9	101.3
40.2	104.1	4.96	4.74	102.1	98.0
40.1	36.0	4.93	1.70	36.8	102.3

Mean 100.5%

Coeff. of variation 2.3%

Table 3: Summary of Total Gentamicins Assay Results for Commercial Products.

Commercial Sample and Batch Number	Labelled Strength	Mean Potency found
Gentamicin sulphate Powder No. 60901	58.3% w/w	57.8% w/w
Gentamicin Injection No. 11OE	80.10mg/2ml	78.10mg/2ml
Gentamicin sulphate Powder No. 61101	58.3% w/w	57.6% w/w

DISCUSSION

Integration of the signal at δ 2.95 ppm (see Fig.1) gives an area indicative of the total gentamicins since the resonance is due to the N-CH_3 of the Garosamine moiety which is common to all three components. Similarly, the signal at δ 1.35 is due to the C-CH_3 of he Garosamine ring and hence represents the total Gentamicins. However, integration of the latter signal was not feasible because of signal overlap. Thus the N-CH_3 signal at δ 2.95 and the maleic acid olefinic proton signal at δ 6.35 were utilised for the analysis. The equivalent weight of the total gentamicins (E_T) were obtained as shown below:

$$E_T = \frac{\text{Total M.Wt. of the Gentamicins}}{\text{Total No. of protons giving the signal at } \delta\ 2.95}$$

$$= \frac{477 + 463 + 499}{9} = 154.33$$

The ratios of the individual components were approximated to be 33⅓% each.

The method gives very accurate results but the standard deviation is rather high. Though quantitative NMR analysis usually gives higher standard deviations as compared with other instrumental methods of analysis such as UV, the standard deviation obtained for this work and other work done on the same instrument is slightly higher than normal [1]. This might be attributed to instrumental factors. The slightly lower potencies found for the commercial products as compared with the labelled strength is a true reflection of the age of the samples.

The method is fast and simple. For a single analysis, a maximum of 30 minutes is required including sample preparation and all necessary calculations. This is a clear advantage over the microbiological and ion exchange chromatographic assay methods which normally takes about 18 hours and 14 hours respectively.

One other principal advantage of the NMR quantitative analytical method relative to most other spectroscopic techniques is the absence in NMR of quantities analogous to the absorption coefficients or extinction coefficients found in other types of spectra. The intensity of an NMR signal for a given nuclear isotope is proportional to the number of nuclei contributing to the signal but is independent of the chemical nature of a given isotopic nucleus. Thus in principle a proton NMR analysis for

Gentamicin can be based on a standard signal from maleic acid or any other convenient proton-containing molecule. This is a very distinct advantage for it often means in practice that the compound being analysed in a mixture need not be available in pure form for use as standard. Therefore, the problem of searching for reference samples of the same chemical nature is absent with the use of NMR.

Another advantage of the NMR is the possible revelation of the presence of impurities in significant concentration in the NMR spectrum. It is often possible to identify the impurities, and NMR quantitative analysis is then a useful check on the quality of the sample.

The project was extended to the assay of the individual components, viz. C_1, C_2 and C_{1a}, in the total Gentamicin. This was however, not successful. The failure was discovered to be due to interference from other minor components in the Gentamicin mixture.

CONCLUSION

The total gentamicin has been assayed successfully by NMR quantitative method of analysis. Under proper conditions of integration and measurement of integrals, the method gives 100.5% accuracy with 2.3% variation. The assay can be completed in about 30 minutes. The microbiological assay method takes up to 18 hours, and the ion-exchange chromatographic method takes 14 hours per sample. In view of the accuracy and speed of the method and provided careful consideration is given to instrumental factors the method can be adopted for routine analysis of the drug.

ACKNOWLEDGEMENT

The author would like to express his gratitude to Nicholars Laboratories, U.K. for their generous supply of gentamicin samples used for the project. He would also like to thank Professor R. T. Parfitt, University of Bath through whom the samples were obtained.

REFERENCES

1. KWAKYE, J. K. [1985]. Use of NMR for Quantitative Analysis of Pharmaceuticals, Talanta, 32(11), 1069.

2. KASLER, F. [1973]. Quantitative Analysis by NMR Spectroscopy, Academic Press, London and New York.

3. ODEN, E. H., STANDER, H. and WEINSTEIN, M. J. [1963]. 'Antimicrobial Agents and Chemotherapy', J. C. Sylvester, ed., (Am. Soc. for Microbiol. 1964) pp. 8-13.

4. F. D. A. WASHINGTON D. C. - Federal Register 36(119), 19 June, 1971; 11811-11814.

5. British Pharmacopoea [1973]. Addendum 1975, p. 22.

6. THOMAS, A. H. and TAPPIN, S. D. [1974.] Separation of Gentamicin Complex by ion-exchange column chromatography, J. Chromatog, 97, 280.

7. CALAM, D. H., GILBERT, J. N. T., LIGHTBOWN, J. W., POWELL, J. M. and THOMAS, A. H. [1978]. Control of the composition of Gentamicin sulphate by proton magnetic resonance spectrometry. J. Pharm. Pharmacol, 30(4), 220.

8. THOMAS, A. H. [1978]. Comparison of three methods of evaluating the composition of gentamicin sulphate. J. Pharm. Pharmac. 30, 378.