J. Pharm. Pharmac., 1978, **30**, 378

COMMUNICATIONS

Comparison of three methods of evaluating the composition of gentamicin sulphate

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Gentamicin consists of a mixture of closely related antibiotic substances produced by *Micromonospora purpurea*. The porportions of the antibiotic components in the mixture can vary and these have been defined for the three major components: gentamicins C_1, C_2 and C_{1A} intended for clinical use (Code of Federal Regulations, 1976). Four methods are available for evaluating sample composition of gentamicin. That of the Food and Drug Administration is to separate the major components by descending paper chromatography; the components are extracted and assayed biologically by a plate diffusion assay in terms of a gentamicin standard which is a mixture of the three components (Code of Federal Regulations, 1976). Potencies of the three components in microgram equivalents are corrected on the basis of numerical 'factors' relating the activities of the components to the gentamicin standard. The content of each component is expressed as a percentage of the total content in microgram equivalents. A quantitative densitometric estimation of the three components separated by thin-layer chromatography has been proposed (Wilson, Richard & Hughes, 1973). The same biological response 'factors' are used to obtain the biological activity from the weight of each of the three gentamicin components determined by measuring the spots revealed by reaction with ninhydrin. Alternatively, gentamicin complex can be separated by ion-exchange chromatography and the components quantitated by measuring the optical rotation of the eluate (Thomas & Tappin, 1974). Four major gentamicin C components (C_1, C_{1A}, $C_{2(i)}$, $C_{2(ii)}$) and three minor components were demonstrated in samples of gentamicin sulphate by this method. The monograph for gentamicin sulphate (British Pharmacopoeia, 1973) specifies ^1H nuclear magnetic resonance (nmr) spectrometry to monitor the proportions of the main components. The method depends on the measurement of the peak height signals for the *N*-methyl and *C*-methyl groups present in all three main components and of those present in C_1 and C_2 only; by defining these two peak height ratios the composition of gentamicin is specified within acceptable limits.

This report compares the analysis of gentamicin sulphate by the following three methods: chromatography followed by microbiological assay (Code of Federal Regulations, 1976), nmr spectrometry (British Pharmacopoeia, 1973), and ion-exchange chromatography (Thomas & Tappin, 1974). The results of the bioautographic assays were supplied by the manufacturer, the Schering Corporation; the nmr spectrometry results were the overall means obtained in a recent collaborative study (Calam, Gilbert & others, 1978). The results of the ion-exchange chromatography are expressed as a percentage for each of the four gentamicin

Table 1. *Analysis of samples of gentamicin sulphate by bioautographic assay, as per cent microgram equivalents relative to the gentamicin master standard (Code of Federal Regulations, 1976): by ion-exchange chromatography as per cent optical activity of gentamicin C components, Thomas & Tappin, 1974), and by nmr spectrometry as per cent gentamicin C_1 calculated from N-methyl ratio of peak at δ 2·75 to peak at δ 2·95 (British Pharmacopoeia, 1973) and per cent standard error ($P = 0.95$) obtained with three replicates for each of the three methods.*

Method sample	Bioautographic assay			Ion-exchange chromatography				Nmr spec.
	C_1	C_2	C_{1A}	C_1	$C_{2(i)}$	$C_{2(ii)}$	C_{1A}	C_1
1	29·8	36·7	33·9	33·85	31·11	6·56	28·46	35·1
2	34·6	40·2	25·2	35·68	33·16	6·51	24·64	34·4
3	34·6	40·2	25·2	35·23	33·48	6·44	24·84	34·5
4	36·3	30·4	33·4	34·55	30·29	5·91	29·21	33·5
5	ND	ND	ND	35·88	36·05	6·42	21·66	35·2
6	34·9	31·1	34·0	32·26	29·49	7·03	31·20	32·6
7	39·5	24·5	36·0	37·88	25·83	5·05	31·23	39·0
8	32·6	34·3	33·1	33·72	30·71	5·72	29·82	34·9
9	33·0	32·0	35·0	36·27	29·93	6·16	27·63	35·4
10	37·1	34·8	28·0	43·85	30·29	6·22	19·63	41·2
11	24·7	36·4	38·9	32·66	36·00	6·02	25·31	30·6
% s.e.	±5·61	±4·99	±4·95	±5·32	±4·30	±7·50	±4·62	±1·38

C components. The three minor components, which contribute between 1·0 to 7·0% of the total optical activity recorded, possess little or no biological activity, therefore their contribution has been omitted when calculating the proportions of the gentamicin C components present in the samples.

The results of the analysis of different batches of gentamicin sulphate are shown in Table 1. Four artificial mixtures were prepared on a weight basis from the individual gentamicin C sulphates (C_1, C_{1A} and C_2), these were homogeneous when examined by paper chromatography; analysis of the mixtures is shown in Table 2. Per cent standard error of three replicate determinations made using each method (Table 1) indicate that nmr spectrometry was the most reproducible method; the other two methods, which are more complex, have similar precisions. Compared with the bioautographic assay, the percent variations in the estimates of gentamicin C_1 content in the different batches were (+10·45 to −5·75) by ion-exchange chomatography and (+13·41 to −3·29) by nmr spectrometry. With such a limited number of results it is not clear which method is the most accurate. The content of gentamicin C_1 appears to have been underestimated in samples 10 and 11 by the bioautographic assay. All eleven samples complied with the specifications of the British Pharmacopoeia including the requirements for nuclear magnetic resonance. The percent variations obtained for estimates of gentamicin C_1 in the artificial mixtures were (+1·6 to −6·75) by ion-exchange chromatography and (+14·4 to 3·08) by nmr spectrometry. This suggests that other components could be contributing to the peak at δ2·75, thus overestimating the content of gentamicin C_1 (e.g. samples B and D).

Table 2. *Analysis of artificial mixtures of gentamicin C sulphate components (% w/w): by ion-exchange chromatography as per cent optical activity of gentamicin C components (Thomas & Tappin, 1974); and by nmr spectrometry as per cent gentamicin C_1 calculated from N-methyl ratio of peak at δ 2·75 to peak at δ 2·95 (British Pharmacopoeia, 1973).*

	Mixture w/w			Ion-exchange chromatography				Nmr spec.
Sample	C_1	C_2	C_{1A}	C_1	C_2(I)	C_2(II)	C_{1A}	C_1
A	51·9	48·1	0·0	46·58	41·82	11·59	0·00	50·30
B	19·8	20·9	59·3	19·66	13·88	3·77	62·68	22·33
C	15·0	10·0	75·0	15·36	8·36	2·12	74·14	18·90
D	30·0	70·0	0·0	29·04	55·94	15·04	0·00	31·40

The main disadvantage of bioautographic assay is the time required, up to 18 h. A procedure which is based on the use of biological 'factors' may work satisfactorily in the laboratory in which these factors have been determined experimentally, but such 'factors' may not be constant in different laboratories. Such a method can give no more than an approximate indication of the composition of a sample of gentamicin. The ion-exchange chromatographic method takes 14 h per sample; at present the comparative composition only can be obtained and this is based on the assumption that the optical rotation is the same for each component. This method could be readily adapted to determine the eluted components by post-column derivatization with either fluorimetric or colorimetric reagents (fluorescamine, *o*-phthalaldehyde or ninhydrin). If the three minor components were considered undesirable in samples of gentamicin sulphate, ion-exchange chromatography would provide a convenient method of quantifying the amount present. Nmr spectrometry has been shown to be a precise and reproducible method in several laboratories (Calam & others, 1978), it also has the advantages of speed and simplicity. The *N*-methyl ratio of the peaks at δ 2·75 to that at δ 2·95 can be used to estimate the gentamicin C_1 content of a sample.

Ion-exchange chromatography provides a detailed profile of the composition of gentamicin, which is valuable for the manufacturers and users of bulk gentamicin sulphate. Nmr spectrometry is the method of choice for checking that a sample of gentamicin complies with a defined composition, especially when examined in conjunction with the other tests in the pharmacopoeial monograph.

November 7, 1977

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