Journal of Chromatography, 151 (1978) 133–146

CHROM. 10,543

GAS–LIQUID CHROMATOGRAPHIC METHOD FOR THE ASSAY OF AMINOGLYCOSIDE ANTIBIOTICS IN SERUM

JAMES W. MAYHEW and SHERWOOD L. GORBACH

Infectious Disease Service, New England Medical Center Hospital, and Tufts University School of Medicine, 171 Harrison Avenue, Boston, Mass. 02111 (U.S.A.)

(First received May 17th, 1977; revised manuscript received August 9th, 1977)

SUMMARY

A gas–liquid chromatographic (GLC) method is presented for the rapid analysis of gentamicin, tobramycin, netilmicin, and amikacin from human serum. This procedure may have application to all aminoglycoside drugs. The three isomers of gentamicin are resolved as two bands, while tobramycin, netilmicin, and amikacin appear in this system as single bands. Normal serum constituents do not interfere with chromatograms. Thus far, no assay interference has been found in cases where other drugs and antibiotics were administered concurrently with aminoglycoside therapy. Dose-response data demonstrating linear recovery are included for all four aminoglycosides as well as a comparison of the GLC method with the microbiological method for the assay of gentamicin and amikacin.

Quantitation is based upon the relative response of the antibiotics to a fixed amount of the internal standards, either kanamycin A or paromomycin B. These standards are clearly resolved as symmetrical peaks from the antibiotics of assay interest. Isothermal chromatographic analysis time is less than 8 min, while total assay time per single serum specimen is approximately 50 min. Preparation of serum includes: precipitation, evaporative drying of the supernatant, a two-stage derivatization (N-trimethylsilylimidazole, N-heptafluorobutyrylimidazole), and a single hexane extraction with a water wash. The methodology described may be applied to the analysis of other compounds (*e.g.*, saccharides, amino-saccharides, amino acids, etc.) which do not readily partition into an organic phase.

INTRODUCTION

Aminoglycoside antibiotics have become the agents of choice for a variety of serious infections involving Gram-negative bacilli. All compounds in this group have a close toxic–therapeutic ratio. Thus, the dose recommended to ensure biologic activity may prove toxic in a substantial portion of patients.

Current methods to measure serum levels of aminoglycoside include the microbiological assay, enzymatic assay, and radioimmunoassay. All of these techni-

ques have certain limitations which make them less than ideal for routine laboratory use in a general hospital.

The microbiological assay is the most commonly used, but may require 4–24 h of incubation[1–3] and can be quite inaccurate[4]. The enzymatic assay[5] is an excellent alternative, but the instability of enzymes[6] as well as the cost of a scintillation counter may present difficulties. The radioimmunoassay is rapid (3–4 h), very sensitive and accurate, and is not susceptible to interference from concurrent antimicrobials. The disadvantages are the reluctance of clinical laboratories to use radioactive materials, and the requirement for specific antibody for each aminoglycoside.

We have attempted to develop an aminoglycoside assay utilizing gas–liquid chromatography (GLC). This system offers the potential advantages of rapid performance (less than one hour for determination); accuracy, based on internal standards; readily available reagents which do not require radiolabeling, stabilization of enzymes or growth of microorganisms; and use of equipment which is reasonably priced and is versatile enough to be used for other chemical determinations in a laboratory.

METHODS

Serum preparation

Human serum containing an aminoglycoside drug was accurately delivered in 0.1-ml aliquots, into 10 × 75 mm disposable culture tubes (Fig. 1). 0.25 ml of a 2-μg/ml aqueous stock solution of kanamycin A was added as internal standard to sera containing gentamicin, tobramycin, or netilmicin. For standardization of amikacin, 0.25 ml of a 12-μg/ml aqueous stock of paromomycin B was added. The solutions were placed in a sandbath maintained at 81°–83°, allowed to equilibrate (3–5

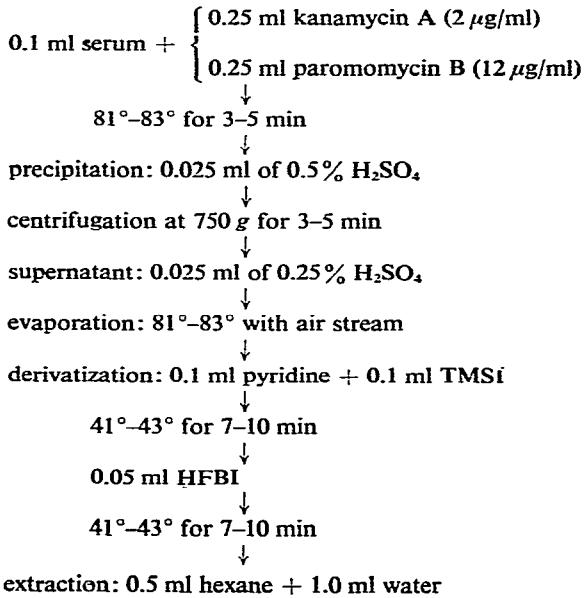

Fig. 1. Preparation technique for GLC analysis of serum aminoglycoside levels.

min), and precipitated by the addition of 0.025 ml of 0.5% H_2SO_4. The milky, finely granular specimens were immediately centrifuged at 750 *g* for 3–5 min. The clear supernatants were transferred to clean 10 × 75 mm tubes and supplemented with 0.025 ml of 0.25% H_2SO_4 (pH 4.5–5.0). Evaporation of the acidified supernatant required approximately 5 min in the 81°–83° sandbath under a vigorous air stream. At apparent dryness the tubes were sealed with cork stoppers (size no. 1).

Derivatization and extraction

To the dry residues, 0.1 ml of silylation grade pyridine and 0.1 ml of TMSI (N-trimethylsilylimidazole; Pierce, Rockford, Ill., U.S.A.) were added. The tubes were resealed and incubated at 41°–43° for 7–10 min. The reaction mixtures were then supplemented with 0.05 ml of HFBI (N-heptafluorobutyrylimidazole; Pierce), and reincubated at 41°–43° for 7–10 min. Reagent-grade hexanes (0.5 ml) and 1.0 ml of distilled water were added, the tubes were recorked, and the phases mixed by repeated inversion. Routinely 2.0 μl of the organic phase were injected into the chromatograph.

Chromatographic conditions and instrumentation

A Shimadzu GC4BMPF gas chromatograph equipped with an electron capture detector (ECD) (^{63}Ni, 10 mC) was used. The detector was operated at a pulse width of 8 μsec, a frequency of 10 kHz, and a pulse voltage of 50 V. The electrometer input sensitivity was 10 MΩ. The output range was 20 mV for gentamicin, tobramycin, and netilmicin, and 40 or 80 mV for amikacin. Gentamicin, tobramycin and netilmicin analyses were performed using silanized pyrex columns (2 m × 3 mm I.D.) packed with 3% OV-101 coated onto 80–100 mesh Chromosorb W AW DMCS (Applied Science Labs., State College, Pa., U.S.A.). Nitrogen (column head pressure 2.8 kg/cm^2; flow-rate, 60 ml/min) was the carrier. The detector and injector were kept at 287° with the oven at 272°. Amikacin was chromatographed on 1% OV-17 with nitrogen carrier (column head pressure, 3.45 kg/cm^2; flow, 80 ml/min) at detector, injector and oven temperatures of 277°, 277°, and 262°. New columns were flow-conditioned for 2–3 days prior to attachment to the ECD. Derivatized aminoglycoside (equivalent to 60 μg per ml serum) was periodically injected during this time. The columns retained sufficient efficiency and resolution capabilities for one year of continuous operation. A Shimadzu R-201 strip-chart recorder was operated at 2.5 mm/min. Peak areas were measured by a Shimadzu ITG-2A electronic digital integrator (threshold, 200 μV; count delay, 60 sec; minimum detectable slope, 50 μV/min.).

Standard serum antibiotic solutions

Aqueous stock solutions (200 μg/ml) were prepared from standard powders of gentamicin, tobramycin, and netilmicin. For each drug, 0.5 ml of the stock was added to 4.5 ml of normal human serum obtained from the hospital blood bank. The final concentration was 20 μg/ml which was two-fold serially diluted in serum down to 0.625 μg/ml. Serum containing amikacin was prepared in a similar fashion, but was diluted to larger concentrations, reflecting the higher therapeutic serum levels. The serum was stored at —20° (non-frostfree freezer). No change in chromatographic response was noted over 2–3 months of frozen storage.

Internal standard stock solutions

Aqueous stock solutions (200 μg/ml) were prepared from standard powder of kanamycin A and paromycin B. No significant changes in chromatographic response were noted during 2–3 months of 5° storage. Working internal standards (kanamycin A, 2 μg/ml; paromomycin B, 12 μg/ml) were carefully prepared by dilution of these stocks.

Serum aminoglycoside levels by microbiological assay

Serum levels of gentamicin and amikacin were measured by the agar well microbiological method[1,2].

Stability of the gentamicin–kanamycin A ratio

Sera containing known concentrations of gentamicin were supplemented with kanamycin A, derivatized, extracted, and promptly chromatographed. These preparations (0.5 ml hexane over approximately 1.2 ml aqueous) were stored at −20° and reinjected at 24, 48, and 120 h. The areas of the two derivatized drugs were measured to monitor the stability of their ratio.

Blind comparison of serum levels by microbiological and GLC technique

Dilutions of gentamicin in serum were prepared at 1, 5, 10, and 20 μg per ml serum (10 separate aliquots for each concentration). Twenty-one dilutions of amikacin in serum were prepared over the range 0–64 μg per ml serum. These specimens were encoded, frozen at −20°, and distributed for blind analysis by GLC and microbiological technique.

Quantitation

Determinations of aminoglycoside concentrations in human sera were based on the relative response of each drug to a fixed amount of the appropriate internal standard. The calculation of serum gentamicin (Gm) levels with kanamycin A (KmA) as the internal standard is used for illustrative purposes.

$$F_{Gm} = \frac{\text{area Gm}}{\text{area KmA}} \cdot \frac{5\ \mu\text{g KmA per ml serum}}{\mu\text{g Gm per ml serum}} \tag{1}$$

$$\mu\text{g Gm per ml serum} = \frac{\text{area Gm}}{\text{area KmA}} \cdot \frac{5\ \mu\text{g KmA per ml serum}}{F_{Gm}} \tag{2}$$

Eqn. 1 can be used to calculate the concentration response factor of gentamicin relative to kanamycin A, F_{Gm}, while eqn. 2 incorporates this factor into direct calculation of the serum levels. Table I lists the area ratios and F values obtained from increasing concentrations of gentamicin (0.6–20 μg Gm per ml serum). The F_{Gm} values were not constant over this range of known gentamicin concentrations.

To correct for the observed changes in F_{Gm}, a modification of this calculation method was attempted. Experimental area ratios were compared to the area ratios of the known standards (0.6–20 μg/ml). Gentamicin was calculated using the mean of

TABLE I

CHROMATOGRAPHIC RESPONSE TO STANDARD CONCENTRATIONS IN HUMAN SERUM OF GENTAMICIN

Abbreviations: Gm, gentamicin; KmA, kanamycin A; concentration response factor $F_{Gm} = \frac{\text{area Gm}}{\text{area KmA}} \cdot \frac{5\ \mu\text{g KmA}}{\mu\text{g Gm}}$.

$\left(\frac{\mu g\ Gm}{ml\ serum}\right)$	$\left(\frac{area\ Gm}{area\ KmA^{*}}\right)^{**}$	F_{Gm}
0.63	0.052 ± 0.004	0.435
1.25	0.129 ± 0.005	0.516
2.50	0.290 ± 0.007	0.580
5.00	0.716 ± 0.040	0.716
10.00	1.359 ± 0.049	0.680
20.00	2.475 ± 0.132	0.619

* 5 μg KmA per ml serum.

** Mean of 11 separate trials ± 1 SEM; area measurement by electronic digital integrator.

the F values obtained from the next lowest and next highest area ratios of the known standards. Thus, using Table I, an experimental area ratio (Gm/KmA) of 0.200 would be calculated using an F_{Gm} value of 0.548 [= (0.516 + 0.580)/2].

RESULTS

Retention times

The relative retention times of derivatized gentamicin (three primary isomers), tobramycin, and netilmicin were measured relative to kanamycin A (Table II). The absolute retention time of kanamycin A was between 6.0 and 6.3 min. Gentamicin isomers C_{1A} and C_2 are not resolved in this system and occur in one chromatographic band. Gentamicin isomer C_1 is cleanly resolved from both kanamycin A and the combined C_{1A}–C_2 band. Since the proportion of each isomer may vary among antibiotic lots, quantitation of gentamicin was based on a sum of the peak areas —the combined C_{1A}–C_2 peak plus the single C_1 peak. Fig. 2 is a chromatogram which illustrates the relationships between the bands derived from sera supplemented with each of the three authentic gentamicin isomers and kanamycin A.

TABLE II

RETENTION TIMES FOR DERIVATIZED GENTAMICIN, TOBRAMYCIN, AND NETILMICIN RELATIVE TO KANAMYCIN A (RRT_{KmA})

Kanamycin A absolute retention time, 6.0–6.3 min. RRT_{KmA} may vary ±0.005.

Aminoglycoside	RRT_{KmA}
Gentamicin C_1	0.601
Gentamicin C_{1A}	0.484
Gentamicin C_2	0.487
Tobramycin	0.613
Netilmicin	0.627

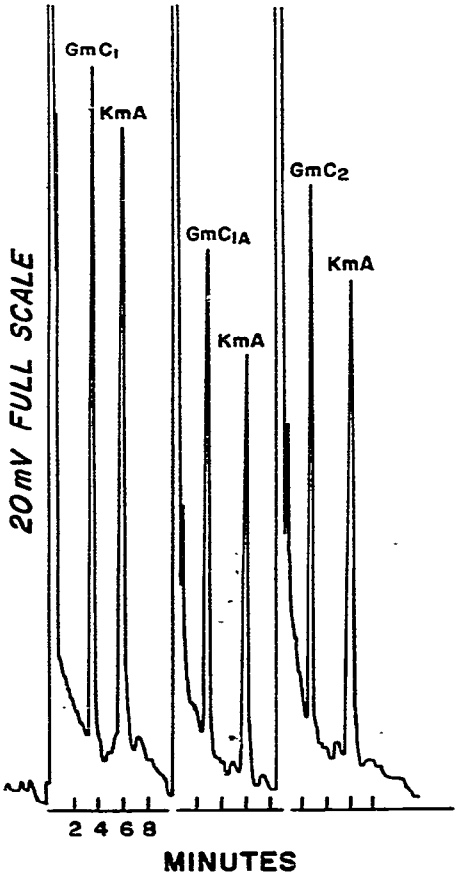

Fig. 2. Gentamicin: chromatographic recovery of isomers C_1, C_{1A}, and C_2 from serum.

The retention time of amikacin relative to paromomycin B was 1.73 ($RRT_{PmB} = 1.73$) on 1% OV-17. The absolute retention time of paromomycin B was usually in the range 2.9–3.1 min. Among other aminoglycosides, the RRT_{KmA} for kanamycin B was 0.714. The antibiotic preparation designated "kanamycin base" responds in this system with the retention characteristics of kanamycin A ($RRT_{KmA} = 1.0$, $RRT_{KmB} = 1.408$).

Chromatographic tracings

Figs. 3, 4 and 5 demonstrate dose-response chromatograms for derivatized gentamicin, tobramycin, and netilmicin, respectively, on 3% OV-101 (0.6–20 μg per ml serum). Fig. 6 illustrates derivatized amikacin on 1% OV-17 (5.0–60 μg per ml serum). A new run can be initiated safely each 8–8.5 min. For gentamicin, tobramycin, and netilmicin, the optimum calculated range of drug quantities presented to the detector is 0.26 ng (0.63 μg per ml serum) to 8.0 ng (20 μg per ml serum).

Quantitation

Tables I, III, IV and V list the area ratios and concentration response factors (F) obtained from dose-response studies of gentamicin, tobramycin, netilmicin and

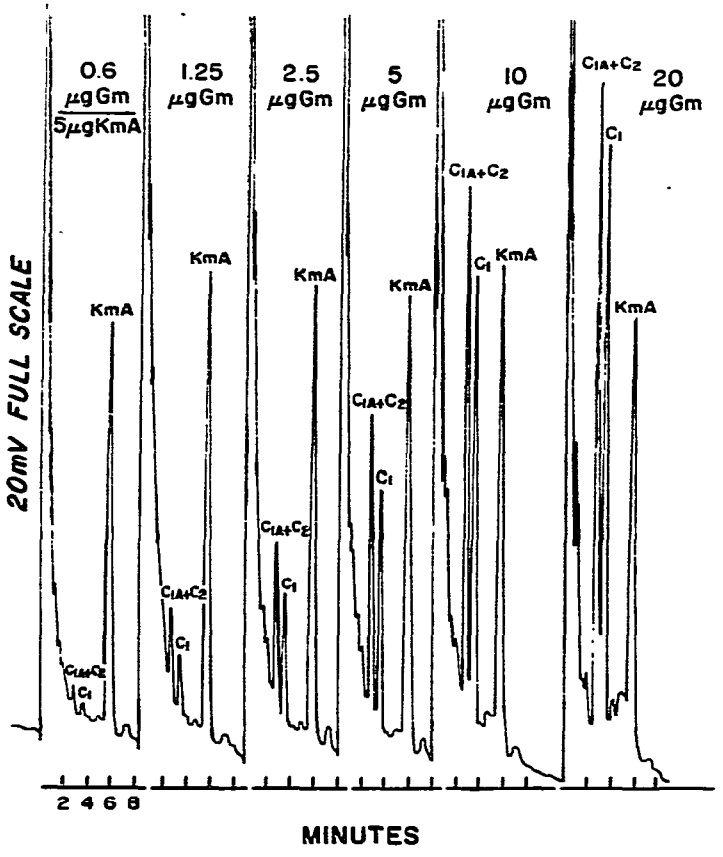

Fig. 3. Gentamicin: chromatographic dose-response from serum.

amikacin in human serum. In general, a two-fold increase in drug concentration is correlated with an approximate doubling of area ratios.

Concentration response factor (F_{Gm}) varied from 0.580 to 0.716 over the range 2.5–20 μg/ml (Table I). The concentration response factors calculated from 0.63 μg/ml (0.435) and 1.25 μg/ml (0.516) may indicate a substantial decrease in unit response to gentamicin at these low serum levels. Since F_{Gm} values were variable, the calculation techniques described in Methods were applied.

Table III indicates that F_{Tm} may be firm over the range from 1.25 to 20 μg/ml. A possible decrease in unit response is again noted at 0.63 μg/ml. At 10 μg/ml, the F_{Tm} of 0.945 is clearly outside the trend of the other values.

Serum recovery data for netilmicin (Table IV) suggests a unit detector response approximately half that found with gentamicin. The F_{Nm} values from 2.5 to 10 μg/ml are very consistent. Curiously, F_{Nm} at 0.63 μg/ml (0.299) is in line with the other three consistent values, while F_{Nm} at 1.25 μg/ml (0.224) suggests a decrease in unit response. An increase in response is observed at 20 μg/ml (0.377).

Amikacin (Table V), tested over its elevated range of expected therapeutic

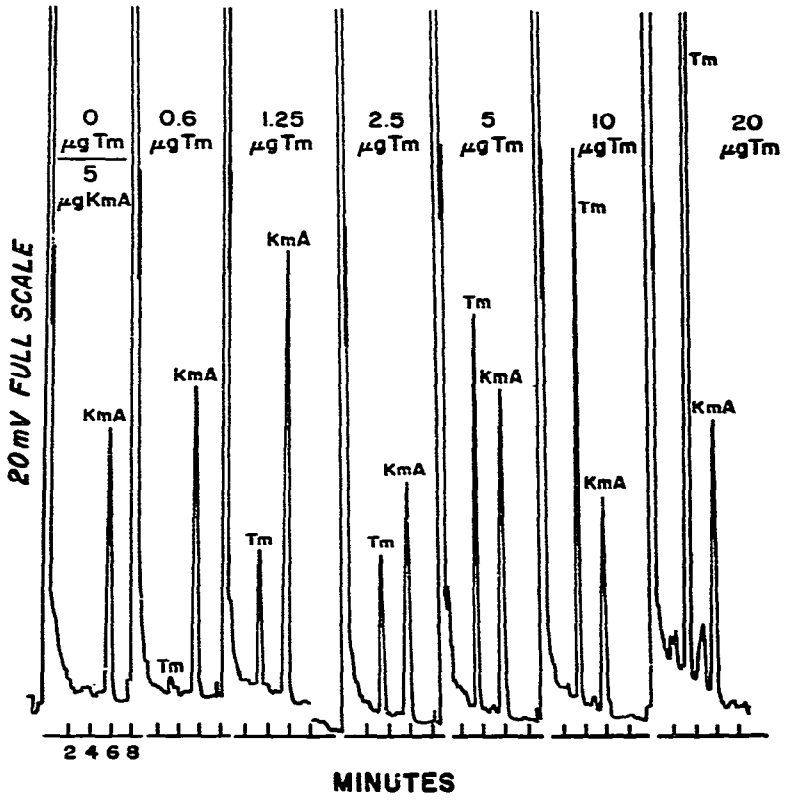

Fig. 4. Tobramycin: chromatographic dose-response from serum.

serum concentrations, appears to have the highest, as well as the most consistent, concentration response factors.

Stability of derivatized drug

The stability of derivatized aminoglycoside ratios was examined (Table IV) to determine if specimens could be prepared at one time and reliably analyzed later. In this experiment, the ratios (Gm/KmA) were reasonably stable up to 48 h, with decomposition seen at 120 h. This 120-h decomposition may indicate a faster hydrolysis of gentamicin ethers and amides relative to those of kanamycin A.

Comparison between assay systems

Blind comparison studies relating the chromatographic and microbiological serum gentamicin assay systems and establishing 95% confidence limits are summarized in Table VII. Both techniques provide good estimates of the actual values. For the levels 5, 10 and 20 μg Gm per ml serum, GLC values tend to underestimate, while the microbiological test slightly overestimates the actual values. The standard errors over this range are somewhat lower with the chromatographic technique.

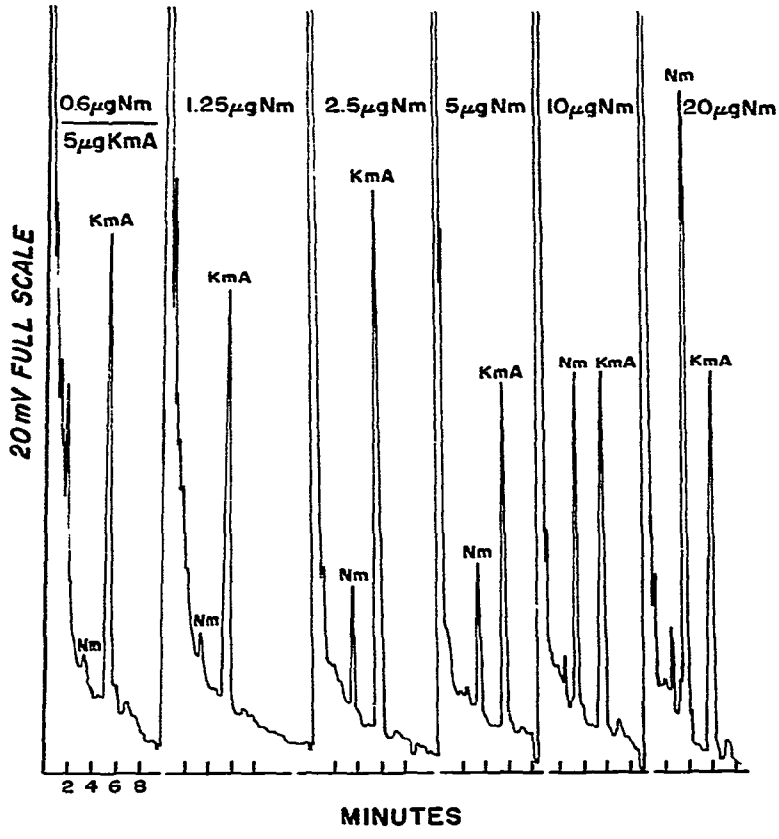

Fig. 5. Netilmicin: chromatographic dose-response from serum.

Statistical analysis of the two assay systems for amikacin showed that the precision of GLC was not significantly different from that of the microbiological technique (Table VIII). The means of percent deviations from actual amikacin concentrations were 11.2% by the GLC estimate and 14.2% by the microbiological estimate.

DISCUSSION

Effective techniques have been developed in isomer separation and potency determinations from standard powders, preparations, and ointments for at least five aminoglycosides[8]. These analyses generally include: silylation, lyophilization, flame ionization detection, and some forms of internal and external standardization. The internal standards (*e.g.*, trilaurin) are not good homologues of aminoglycosides in terms of physical and reactive characteristics. In general, these procedures are excellent for potency studies in milligram ranges, but are inappropriate for quantitation of aminoglycoside antibiotics in serum.

The analytical system for aminoglycosides described in the report appears to combine speed and relative simplicity with acceptable accuracy and precision. A

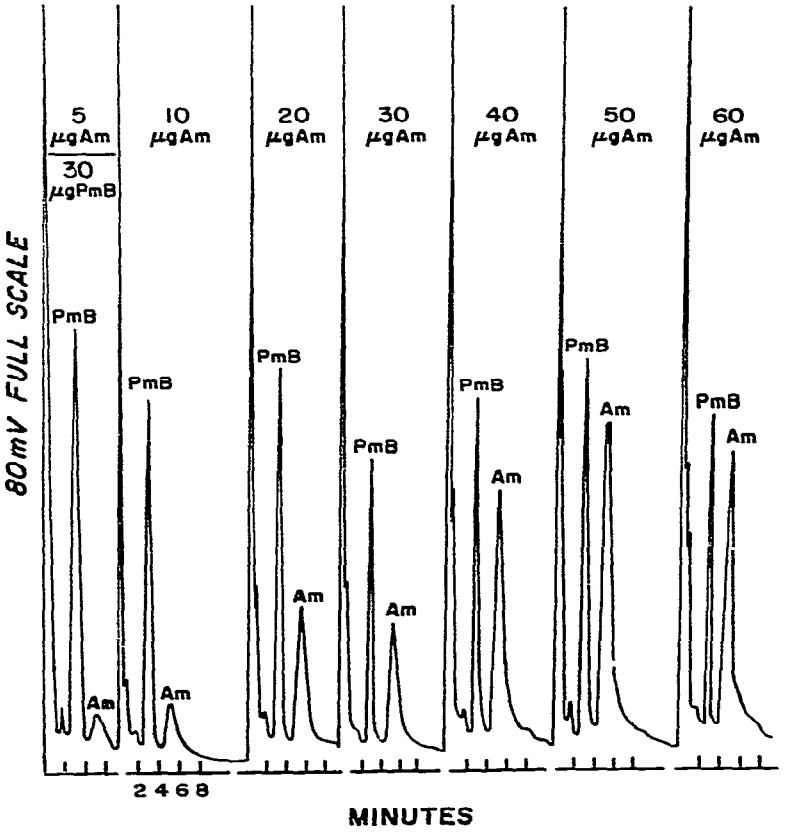

Fig. 6. Amikacin: chromatographic dose-response from serum.

TABLE III

CHROMATOGRAPHIC RESPONSE TO STANDARD CONCENTRATIONS OF TOBRAMICIN IN HUMAN SERUM

Abbreviations: Tm, tobramycin; KmA, kanamycin A; concentration response factor $F_{Tm} = \frac{\text{area Tm}}{\text{area KmA}} \cdot \frac{5\,\mu\text{g KmA}}{\mu\text{g Tm}}$.

$\left(\frac{\mu g\ Tm}{ml\ serum}\right)$	$\left(\frac{area\ Tm}{area\ KmA^*}\right)$**	F_{Tm}
0.6	0.064	0.533
1.25	0.203	0.812
2.5	0.432	0.864
5.0	0.812	0.812
10.0	1.890	0.945
20.0	2.996	0.749

* 5 μg KmA per ml serum.

** Mean of three separate trials; area measurement by electronic digital integrator.

TABLE IV

CHROMATOGRAPHIC RESPONSE TO STANDARD CONCENTRATIONS OF NETILMICIN IN HUMAN SERUM

Abbreviations: Nm, netilmicin; KmA, kanamycin A; concentration response factor $F_{Nm} = \frac{\text{area Nm}}{\text{area KmA}} \cdot \frac{5\ \mu\text{g KmA}}{\mu\text{g Nm}}$.

$\left(\frac{\mu g\ Nm}{ml\ serum}\right)$	$\left(\frac{area\ Nm}{area\ KmA^*}\right)$**	F_{Nm}
0.063	0.036 ± 0.003	0.299
1.25	0.056 ± 0.005	0.224
2.5	0.154 ± 0.013	0.308
5.0	0.310 ± 0.008	0.310
10.0	0.603 ± 0.031	0.302
20.0	1.507 ± 0.057	0.377

* 5 μg KmA per ml serum.

** Mean of 5 separate trials ±1 SEM; area measurement by electronic digital integrator.

TABLE V

CHROMATOGRAPHIC RESPONSE TO STANDARD CONCENTRATIONS OF AMIKACIN IN HUMAN SERUM

Abbreviations: Am, amikacin; PmB, paromomycin B; concentration response factor $F_{Am} = \frac{\text{area Am}}{\text{area PmB}} \cdot \frac{30\ \mu\text{g PmB}}{\mu\text{g Am}}$.

$\left(\frac{\mu g\ Am}{ml\ serum}\right)$	$\left(\frac{area\ Am}{area\ PmB^*}\right)$**	F_{Am}
0.5	0.144	0.864
10	0 307	0.921
20	0.699	1.049
30	0.942	0.942
40	1.532	1.150
50	1.783	1.070
60	2.246	1.123

* 30 μg PmB per ml serum.

** Mean of 3 separate trials; area measurement by electronic digital integrator.

TABLE VI

STABILITY OF GENTAMICIN/KANAMYCIN A AREA RATIOS DURING STORAGE OF DERIVATIZED SPECIMENS

Derivatized preparations stored at −20° (0.5 ml hexane and approximately 1.2 ml aqueous). Ratios are the mean of two separate trials.

μg Gm per ml serum	*Area gentamicin/area kanamycin A*			
	0 h	*24 h*	*48 h*	*120 h*
0.63	0.057	0.064	0.061	0.057
1.25	0.122	0.122	0.144	0.113
2.5	0.260	0.268	0.255	0.154
5.0	0.774	0.768	0.811	0.633
10.0	1.199	1.253	1.315	1.104

TABLE VII

BLIND COMPARISON OF GLC AND MICROBIOLOGICAL ASSAYS FOR SERUM GENTAMICIN LEVELS

μg Gm per ml serum (actual)	*μg Gm per ml serum (estimated)*	
	*GLC assay**	*Microbiological assay**
1	1.11 ± 0.076	0.90 ± 0.049
5	4.72 ± 0.265	5.56 ± 0.356
10	9.55 ± 0.503	11.11 ± 0.964
20	19.19 ± 0.639	20.91 ± 0.716

* Mean of 10 separate trials ±1 SEM.

TABLE VIII

COMPARISON OF GLC AND MICROBIOLOGICAL ASSAYS FOR SERUM AMIKACIN LEVELS

Actual concn. by supplementing serum with known quantities of drug (μg/ml)	*Concn. estimated*	
	GLC (μg/ml)	*Microbiological (μg/ml)*
0	0	0
0	0	0
4	2.8	5.2
6	5.2	5.8
8	7.5	8.4
8	8.6	8.6
12	10.3	15.0
15	13.8	13.0
16	16.5	18.0
20	16.9	22.0
24	24.6	25.0
24	22.7	20.0
30	23.4	30.0
32	34.3	45.0
40	41.7	30.0
43	40.9	55.0
48	55.8	50.0
51	41.6	45.0
53	40.2	34.0
64	44.8	50.0
64	64.6	65.0
Number of trials, *N*	21	
Degrees of freedom, *df*	20	
Variance	36.996	50.048
Variance ratio, *F*	1.353	

single serum specimen may be quantitated within 50 min. Since the chromatographic bands of the derivatized drugs are directly visualized, the emergence of new or unique peaks relative to the internal standard may indicate modification of the drug by host- or microbe-mediated reactions. The technique should be applicable to all aminoglycoside drugs with variations only in choice of internal standard and chromatographic conditions, *i.e.* liquid phase, temperature, carrier rate. The conditions described here

for amikacin can be applied to butirosin A and B, paromomycin A, streptomycin, and neomycin. These antibiotics are primarily in the 450–600 molecular weight range. In addition, the procedures may be used for the broad range of saccharides, aminosaccharides, and other compounds which do not readily partition into an organic phase. A combination of silylation and acylation for catecholamines has been described[9].

No interfering chromatographic bands have been observed in normal serum when compared to serum containing authentic aminoglycoside. Studies now in progress with serum from patients indicate to date no interference from simultaneously administered cephalosporins, penicillins, chloramphenicol, or clindamycin. A variety of other drugs also were being administered to these patients.

These are several points of the assay procedure worthy of expanded discussion. Optimal results are achieved when preparation temperatures do not exceed 85°. The formation of silyl ethers (TMSI reaction, specific for hydroxyl groups) does occur more rapidly at higher temperatures (*e.g.* 75°) but a compromise has been chosen between time and temperature to generate reproducible chromatograms under the mildest of preparation conditions. The formation of heptafluorobutyrylamides (HFBI reaction) above 70° has resulted in the appearance of multiple chromatographic bands. Our most reliable results with both silylation and acylation have been found at derivatization temperatures from 40° to 55°. Reproducible derivatizations of hydroxyl and amino groups with HFBI alone, as well as acylation attempts with trifluoroacetylimidazole and trifluoroacetic anhydride, have been unsatisfactory.

Derivatized gentamicin is stable for at least 48 h at −20°. Preliminary data indicate derivatized tobramycin and amikacin to be similarly stable. However, derivatized netilmicin appears to hydrolyze rapidly within 24 h. Drying of the hexane phase and resolubilization results in almost complete loss of chromatographic response.

In the protein precipitation step, the fluid should become opaque, white, and finely granular in order to produce a clear supernatant. The addition of 0.25% H_2SO_4 to that supernatant is vital for consistent chromatography. This may suggest that formation of heptafluorobutyrylamides is favored when amino groups of the trimethylsilylated antibiotic are charged. Supernatants which have not been thoroughly dried appear cloudy after the TMSI step and result in a cloudy final hexane phase. Chromatographic results from these specimens are extremely unreliable.

Since kanamycin A and paromomycin B are members of the same class of compounds as the four aminoglycosides discussed, they serve as nearly ideal internal standards. These markers share similar characteristics such as molecular weight and configuration, reactive hydroxyl and amino groups, stability, solubility, and interaction with chromatographic systems. Stock solutions of the standards are easily handled on a routine basis.

Certain lots of kanamycin A, however, may contain a trace band which elutes at $RRT_{KmA} = 0.61$. This band does not conform to authentic kanamycin B ($RRT_{KmA} = 0.71$), but does occur in the region of gentamicin C_1, tobramycin, and netilmicin. The maximum relative area of this band is less than 0.2 μg aminoglycoside per ml serum. Therefore, it is necessary to check internal standard lots by addition to normal serum without other aminoglycosides. An analagous band has not been observed from paromomycin B.

Extremely low absolute integration values for the internal standards can lead

to substantial quantitative error. If the value is less than 50% of the area expected, the specimen should be redone.

In general, the relative sensitivity of ECD operation and preparation techniques are designed to favor linear reproducible response over therapeutic aminoglycoside ranges. The minimum detectable limit may in fact be a hundred-fold lower than the 0.6 μg aminoglycoside per ml serum level.

CONCLUSION

Serum levels of gentamicin, tobramycin, netilmicin, and amikacin can be reliably assayed using gas–liquid chromatography and electron capture detection. The internal standards used are kanamycin A and paromomycin B. Specimen preparation includes: precipitation of the serum–internal standard mixture; evaporation of the resulting supernatant; two-stage derivatization with TMSI and HFBI; and extraction into hexane with water wash. Linear, reproducible dose–response curves of physiological levels are presented. Gentamicin and amikacin serum levels by GLC compare well to those determined by the microbiological method. Interfering peaks were not detected in the sera of patients and normal individuals.

ACKNOWLEDGEMENTS

The authors wish to thank: Dr. John G. Bartlett for his extensive preliminary efforts to initiate these studies; Dr. Thomas Louie for early work on analytical techniques; and Ms. Elizabeth Kling for the antimicrobial assays. Generous support has been provided by the Packard, Schering, Bristol and Lilly Corporations. Drs. Everett (Packard), Kerschner (Schering), Volcano (Bristol), Koch (Lilly), and Machamer (Parke-Davis) have variously contributed both pure antibiotic isomers and valuable suggestions and counsel.

REFERENCES

1 L. D. Sabath, J. I. Casey, P. A. Ruch, L. L. Stumpf and M. Finland, *J. Lab. Clin. Med.*, 78 (1971) 457.
2 D. V. Alcid and S. J. Seligman, *Antimicrob. Ag. Chemother.*, 3 (1973) 559.
3 E. Warren, R. J. Snyder and J. A. Washington, Jr., *Antimicrob Ag. Chemother.*, 1 (1972) 46.
4 D. S. Reeves, *Postgraduate Med. J.*, 50 (suppl. 7) (1974) 20.
5 R. K. Holmes and J. P. Sanford, *J. Infect. Dis.*, 129 (1974) 519.
6 R. C. Tilton, J. R. Murphy and E. Mallett, *New Engl. J. Med.*, 287 (1972) 1100.
7 J. E. Lewis, J. C. Nelson and H. A. Elder, *Nature (London)*, 239 (1972) 214.
8 K. Tsuji and J. H. Robertson, *Methods Enzymol.*, 43 (1975) 213.
9 M. G. Horning, A. M. Miss, E. A. Boucher and E. C. Horning, *Anal. Lett.*, 1 (1968) 311.