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REVIEW

# Aminoglycoside antibiotics — Two decades of their HPLC bioanalysis

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ABSTRACT: Several reviews have been published on high-performance liquid chromatographic (HPLC) methods for the determination of aminoglycoside antibiotics (aminoglycosides) in biological fluids [e.g. Nilsson-Ehle, I. (1983). *J. Liq. Chromat.* **6**: 251]. Of these, the paper by Maitra *et al.* [(1979a). *Clin. Chem.* **25**: 1361.] briefly summarizes the early 2 –3 years of experience on HPLC assaying of amikacin, gentamicin, netilmicin and tobramycin in body fluids. The reviews by Nilsson-Ehle, I. [(1983). *J. Liq. Chromat.* **6**: 251] and by Miner, D. J. [(1985). Antibiotics. In *Therapeutic Drug Monitoring and Toxicology by Liquid Chromatography*, (Wong S. H. Y., ed.), ch.10, p. 269. Marcel Dekker, New York and Basel.] devoted to the monitoring of antibiotics, also evaluated the first 6–8 years of the application of HPLC assays for the aminoglycosides amikacin, gentamicin, netilmicin, sisomicin and tobramycin. This report presents a great majority of the HPLC assay methods published during the last two decades for determining practically a dozen different aminoglycoside antibiotics in body fluids, particularly in the serum or plasma, and in urine.


## INTRODUCTION

Aminoglycoside antibiotics (aminoglycosides) are hydrophilic molecules consisting of an aminated cyclitol associated with an amino sugar. The p$K_a$ values of the amino groups in the drug molecules range between 7.0 and 8.8. In addition to these amino groups, the presence of several hydroxyl groups contributes to the hydrophilicity of the aminoglycosides.

All aminoglycosides are readily soluble in water. In aliphatic alcohols they display a very limited solubility, while in more hydrophobic organic solvents they are practically insoluble. In aqueous solutions at physiological pH value the drug molecules are charged.

Aminoglycoside antibiotics are valuable in the treatment of serious infections caused by gram-negative bacteria. After intramuscular administration they are rapidly absorbed (Miner, 1985). Aminoglycosides are not metabolized, to a small extent they are bound to plasma proteins and are excreted almost entirely unchanged by the kidney.

All aminoglycosides have a narrow therapeutic range and, moreover, their use is limited because of the potential renal and otovestibular toxicity. (While the renal damage is reversible and can be well controlled during treatment, ototoxic side effects can cause permanent loss of the inner ear function.) The small differences between the effective and toxic concentrations call for monitoring of the given aminoglycoside levels, to ensure optimal therapy and to minimalize the risk of toxic side effects, particularly in patients with renal failure (Cabanes *et al.*, 1991).

Nephrotoxicity appears to be related to elevated trough serum concentrations and significant tissue accumulation, as predicted by a two-compartment pharmacokinetic model (D'Souza and Ogilvie, 1982). For these reasons, adjusting the aminoglycoside dosage has been advocated to minimize possible oto- and nephrotoxicity.

Desirable levels of all aminoglycoside antibiotics are in the order of micrograms per millilitre (Table 1). Owing to the relatively short plasma elimination half-lives, which are about 2 h (Miner, 1985), a defined drug regimen should be applied. For the measurement of plasma or serum concentrations of a given aminoglycoside, microbiological, radioenzymatic, immunological, chromatographic, and several other assay methods have been introduced (Anhalt, 1977; Anhalt and Brown, 1978).

### Amikacin

Amikacin, a semi-synthetic derivative of kanamycin (Wong *et al.*, 1982), is widely used against gram-negative bacterial infections, and, like other aminoglycosides, has the potential for nephro- and ototoxicity (Kabra *et al.*,

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**Abbreviations used:** BT, butyl toyopearl; CM, carboxymethylated; HPLC, high-performance liquid chromatography; LLE, liquid/liquid partitioning; SPE, solid-phase extraction.

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**Table 1. Therapeutic and toxic concentrations of some aminoglycosides[a]**

| Aminoglycoside | Therapeutic levels (μg/mL) | Toxic concentrations (μg/mL) Peak | Trough |
|---|---|---|---|
| Amikacin | 8–16 | >35 | >10 |
| Gentamicin | 4–8 | >12 | >2 |
| Kanamycin | 8–16 | >35 | >10 |
| Tobramycin | 4–8 | >12 | >2 |

[a] Adapted from Maitra *et al.* (1979a).

1984). Since, compared with most other aminoglycosides, the amikacin molecule has fewer functional groups susceptible to attacks from the enzymatic system of the microorganism, this antibiotic is often used clinically in treating infections resistant to gentamicin or tobramycin (Wong *et al.*, 1982).

## Gentamicin

Gentamicin is a product of *Micromonospora purpurea.* Of the five components isolated, three are major gentamicin fractions, $C_1$, $C_{1a}$ and $C_2$ (Fig. 1), present in roughly equal amounts, and two are minor components, designated $C_{2a}$ and $C_{2b}$, which represent approximately 4% of the gentamicin composition (Getek *et al.*, 1983).

Figure 2 illustrates a typical HPLC record of gentamicin $C_1$, $C_{1a}$ and $C_2$, as observed on analysing a serum sample of a healthy volunteer (Šoltés *et al.*, 1983). However, the individual fractions of gentamicin should be quantitated only when the aim is a detailed pharmaco- or toxicokinetic study of each drug component (Bäck *et al.*, 1979). For most pharmacokinetic studies, as well as for drug monitoring purposes, the determination of any one component level is fully satisfactory.

## Kanamycin

The general term kanamycin includes at least three known aminoglycosides, namely kanamycin A, kanamycin B and kanamycin C (Mays *et al.*, 1976).

## Neomycin

There are three neomycins, i.e. neomycin A, neomycin B and neomycin C.

## Netilmicin

Netilmicin is a semi-synthetic aminoglycoside antibiotic prepared from sisomicin (Dionisotti *et al.*, 1988). It has a broad spectrum of activity against both gram-positive and gram-negative bacteria, including strains resistant to other aminoglycosides. Some clinical data suggest that netilmicin has less nephro- and ototoxicity than other aminoglycosides. However, to ensure optimal therapeutic efficacy with minimal incidence of side-effects, netilmicin monitoring should be mandatory. This is particularly important in life-threatening infections in patients with impaired renal function, or when long-term therapy is required.

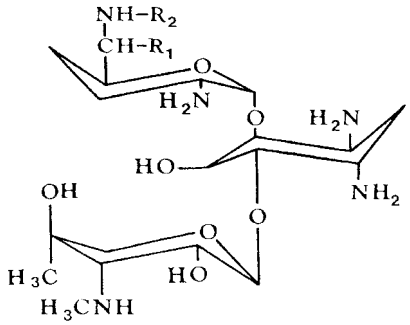


**Figure 1.** Chemical structure of gentamicin. Gentamicin $C_1$: $R_1=R_2=CH_3$; gentamicin $C_{1a}$: $R_1=R_2=H$; gentamicin $C_2$: $R_1=CH_3$, $R_2=H$.

## Sisomicin

By its chemical structure, sisomicin is a dehydro analogue of gentamicin $C_{1a}$ (Tawa *et al.*, 1988).

## Spectinomycin

Spectinomycin is a broad-spectrum aminocyclitol antibiotic isolated from the fermentation broth of *Streptomyces spectabilis* (Myers and Rindler, 1979).

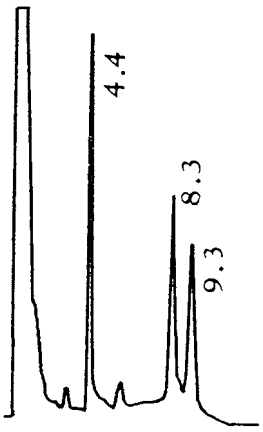


**Figure 2.** Chromatogram of a serum sample containing gentamicin $C_1$ (4.4), $C_{1a}$ (8.3) and $C_2$ (9.3 min). (For the HPLC conditions see Šoltés *et al.*, 1983.)

### Streptomycin

Streptomycin was the first aminoglycoside antibiotic to be used in chemotherapy (Kurosawa *et al.*, 1985; Kubo *et al.*, 1986; Kubo *et al.*, 1987).

### Tobramycin

Tobramycin is used in the treatment of serious gram-negative infections (Haughey *et al.*, 1980). It has a narrow therapeutic range and can cause severe nephro- and ototoxicity at serum concentrations exceeding 12 μg/mL (Kabra *et al.*, 1983; Table 1).

### The early history of gentamicin HPLC bioanalysis

In 1977, three American teams, i.e. Anhalt (1977), Maitra *et al.* (1977) and Peng *et al.* (1977a), published the first accounts on HPLC bioanalysis of gentamicin. Two of these methods (Anhalt, 1977; Maitra *et al.*, 1977) allow us to separate and determine the serum concentration of the three major fractions of gentamicin, namely $C_1$, $C_{1a}$ and $C_2$. The third method (Peng *et al.*, 1977a) results in the separation of fraction $C_1$ from the couple $C_{1a}$ and $C_2$, which are eluted as a single peak ($C_{1a} + C_2$).

Detection of gentamicin molecules, which do not bear any marked chromophoric functional group, was accomplished by chemical derivatization. The derivatization agent used was either *o*-phthalaldehyde (Anhalt, 1977; Maitra *et al.*, 1977) or dansyl chloride (Peng *et al.*, 1977a; Chiou *et al.*, 1978). While the first agent reacts with free primary amino groups of gentamicin practically immediately even at laboratory temperature, on using dansyl chloride an increased reaction temperature (75°C/5 min) is required.

All three teams paid particular attention to prechromatographic treatment of the biological sample, i.e. serum (Anhalt, 1977; Maitra *et al.*, 1977) or plasma (Peng *et al.*, 1977a). The problem lies in the separation of the highly polar water soluble compound from the aqueous, protein containing matrix.

In their first step, Peng *et al.* (1977a) precipitated the plasma proteins by adding acetonitrile — an organic water-miscible solvent. In the next step, they admixed a water-immiscible organic liquid — dichloromethane — to the water-acetonitrile solution. This resulted in a phase separation, with gentamicin being partitioned to the upper aqueous layer. On applying this procedure, the gentamicin molecules, or those of other aminoglycosides determined, are transferred into the aqueous phase at high and constant recovery.

A qualitatively different technique for the isolation of polar, charged gentamicin molecules from serum samples was introduced by their extraction using solid adsorbents, e.g. microparticulate silica gel (Maitra *et al.*, 1977) or the ion-exchanger of carboxymethylated (CM) Sephadex (Anhalt, 1977). Maitra's team (Maitra *et al.*, 1977) first trapped the gentamicin molecules on a silica gel minicolumn bed. In the next step, they on-column derivatized these molecules with *o*-phthalaldehyde, and the derivatized gentamicin was eluted from the column with ethanol. The ethanolic eluate was injected onto the HPLC column, and the separation of derivatized gentamicin molecules was performed in the reversed-phase mode.

Anhalt (1977) exploited the ion-exchange technique. The entrapped gentamicin was displaced from the CM-Sephadex with alkaline sodium sulphate solution. An aliquot of this sample was then directly injected onto the HPLC column operating in the reversed-phase ion-pairing mode. Detection of gentamicin molecules was provided by post-column derivatization with the *o*-phthalaldehyde solution.

Despite the fact that the publication of these first three bioanalytical methods of gentamicin HPLC determination (Anhalt, 1977; Maitra *et al.*, 1977; Peng *et al.*, 1977a) is dating back over two decades, the majority of the later developed analytical procedures is based on these pioneer designs. This is understandable in the light of the: (a) high polarity, charge of water soluble molecules of the aminoglycosides determined, and (b) presence of primary and secondary amino groups as well as -OH group(s) allowing chemical derivatization. Substantial variables of the HPLC methods discussed are given in Appendix 1.

## HPLC METHODS FOR BIOANALYSIS OF AMINOGLYCOSIDES

Compared with some other bioanalytical assays (e.g. microbiological), the proper HPLC method usually has adequate selectivity for the specific determination of a given aminoglycoside antibiotic (Anhalt and Brown, 1978; Marples and Oates, 1982). When the aminoglycoside is a multicomponent mixture, such as that of gentamicin, this method can also provide information on the content of each individual drug fraction. Since, during treatment the patient's blood levels of a given aminoglycoside are in the order of μg/mL, the emphasis does not lie primarily on the sensitivity of the HPLC method used. Rather a fast, simple, reliable method for monitoring the levels of the drug in small samples, preferably of plasma, is required. The HPLC methods suitable for clinical laboratories must be unequivocally free of interference from other drug(s) administered simultaneously with the given aminoglycoside.

It should be noted, however, that essentially one and the same HPLC method may be equally suitable both for clinical practice and for pharmacological research purposes. Yet, different conditions of a research oriented situation studying the kinetics of an aminoglycoside

antibiotic in the organism, and of monitoring the drug therapeutic levels in patients, may require the involvement of several HPLC methods for the determination of individual representatives of this rather broad group of remedies (see Appendixes 1 and 2).

The following sections present an outline of the main issues which should be preconsidered before the use of any one of the existing HPLC methods, or before the development of a novel HPLC assay for the determination of aminoglycoside(s).

## Body fluid

Blood and urine are the body fluids most frequently drawn to determine the concentrations of the given aminoglycoside. Although assay results for plasma or serum are usually identical, plasma has an advantage, since blood can be centrifuged immediately after sampling and, moreover, a smaller volume of blood is required. However, when the microparticulate silica gel is used for treatment, serum is the sample of choice (Maitra *et al.*, 1977).

## Direct on-column injection of untreated body fluid

Successful repetitive determinations of a given aminoglycoside antibiotic by direct injection of the body fluid onto the analytical HPLC column, depend foremost on: (a) the compatibility of the injected sample with the mobile, as well as the stationary phase used, and (b) the actual level of the given aminoglycoside(s) in the examined body fluid. The latter decisively limits the efficacy of the direct injection approach, since only a minute volume of the biological sample can usually be introduced into the HPLC equipment.

In the papers reviewed, the direct on-column injection of untreated body fluids was used only in the urine analysis (Maloney and Awni, 1990). However, with serum or plasma, the technique of on-line sample preparation has been employed by injecting a large volume of body fluid into the HPLC equipment operating in the column-switching arrangement (Essers, 1984; Tamai *et al.*, 1986; Matsunaga *et al.*, 1988). For example, the on-line serum treating on a butyl toyopearl (BT) 650-M precolumn packing, along with column-switching, was used for the determination of gentamicin (Tamai *et al.*, 1986). This somewhat curious HPLC method, however, did not reach a broad application in practice.

## Internal standard

On working with a modern microprocessor-controlled HPLC apparatus, and on applying automatic prechromatographic sample treatment, the use of an internal standard is not necessary. Yet, in several of the assays listed in Appendix 1, only the application of the internal standard warrants the required precision of the HPLC bioanalytical method in determining the given aminoglycoside.

The most frequently used approach is to employ another aminoglycoside as the internal standard. This approach is well-substantiated since substances with similar chemical structure can be expected to behave similarly both in the prechromatographic and chromatographic steps. Nevertheless, the procedure reported for synthesizing 1-*N*-acetylgentamicin $C_1$ as an appropriate internal standard (Anhalt and Brown, 1978) is to be recommended.

In conclusion, on using a single detector setting over the whole course of the HPLC run, the choice of the optimal ratio between the amount of the internal standard used and the concentration of the aminoglycoside determined, is rather important.

## Prechromatographic sample treatment

Most of the HPLC methods listed in Appendix 1 require approximately 0.1–1.0 mL of plasma or serum. Since direct on-column injection of such a voluminous and complex sample is commonly not feasible, the compound to be examined has to be transferred from the biological matrix, into a small volume of an appropriate liquid which can be introduced onto the HPLC analytical column.

In pharmacokinetics, maximalization of the yield and purity of the analyte is an essential requirement for its trace analysis, while for monitoring high levels of the given aminoglycoside the efficiency of the extraction is not so critical.

The three main approaches for the preparation of analytical samples from the original body fluids are: (a) protein precipitation, (b) solid-phase extraction, and (c) solvent extraction, used individually or in combination.

## Protein precipitation

In the majority of methods for the determination of aminoglycosides in plasma or serum, acetonitrile is applied as the protein precipitating agent. Since aminoglycosides are not extensively bound to any particular plasma protein before the addition of acetonitrile, the drug release from binding sites is simply accomplished by alkalization and dilution of the biological sample. Compared with other precipitating agents, e.g. $CH_3OH$, $HClO_4$ (Kubo *et al.*, 1985), acetonitrile has the great advantage that the precipitated mass of proteins adheres excellently to the test tube wall. This allows us to transfer the supernatant readily and quantitatively into another

vessel. Such a protein free sample can then, ultimately, be directly injected onto the HPLC column operating in the reversed-phase ion-pairing mode. On using this method, an aliquot of the resulting supernatant liquid is injected onto a guard precolumn to protect the performance of the HPLC analytical column. In combination with the post-column derivatization, the whole procedure appears to be highly effective.

## Solid-phase extraction; liquid/solid-phase partitioning (SPE)

As is also evident from Appendix 1, aminoglycosides from body fluids are often isolated by applying the SPE technique.

This technique is particularly suitable for isolating polar analytes, such as aminoglycosides, and has also proven advantageous when the volume of the analysed body fluid is large. One of the favourable properties of solid-phase extraction is the usually high and well-reproducible recovery (Anhalt, 1977; Anhalt and Brown, 1978). Selective isolation of aminoglycoside(s) can be affected primarily by the selection of the adsorbent material (e.g. bare silica gel, cation exchanger), and secondarily by including a suitable sorbent washing step, by which the majority of trapped endogenous material is flushed out.

In the case of working with bare silica as the adsorber, however, the trapped aminoglycoside(s) cannot be simply eluted from the minicolumn adsorbent bed; but after on-column derivatization of the analyte(s) with *o*-phthalaldehyde the derivatized molecules of aminoglycoside(s) are easily elutable with ethanol. This sample is then directly injectable into the HPLC equipment operating in the reversed-phase separation mode.

A small drawback of the ion-exchanging procedure is that the trapped aminoglycosides have to be eluted by using an alkaline solution, of which only a small aliquot can usually be directly introduced into the HPLC equipment. On the other hand, this is the very procedure which yields samples containing really very few interfering substances (Anhalt, 1977).

## Solvent extraction; liquid/liquid partitioning (LLE)

The technique of "classical" solvent extraction of aminoglycosides from a body fluid with an organic extractant is excluded on principle, because of drug insolubility in any water-immiscible liquid. If the LLE technique application is mentioned in Appendix 1, it usually concerns the extraction of the derivatized aminoglycoside molecules (Bäck *et al.*, 1979). An aliquot of the appropriate organic extractant (e.g. ethylacetate) can be analysed as to the content of the drug derivative also by its direct on-column injection (Peng *et al.*, 1977a,b). A more complex procedure, however, consists of the evaporation of the organic solvent, dissolution of the dry residue in the HPLC mobile-phase, and the subsequent analysis of the sample to determine its aminoglycoside content (Larsen *et al.*, 1980).

## Chemical derivatization

Chemical derivatization of aminoglycoside antibiotics is inevitable for their sensitive detection with a photo-/fluoro-HPLC-detector. Of the functional groups characteristic for virtually all aminoglycosides, the $-NH_2$ group is mostly involved in derivatization reactions. Further reactive groups, i.e. -NH- and -OH, are also exploited for derivatization reactions. On using inappropriate reaction conditions, however, more than one single derivative may arise (Tawa *et al.*, 1988).

Under alkaline conditions, *o*-phthalaldehyde reacts rapidly with primary amines to produce a very well-detectable fluorescent product (Anhalt, 1977). A disadvantage of fluorescent pre-column labelling of aminoglycosides with *o*-phthalaldehyde is the low stability of the derivative, and that despite storage of the samples at low temperature and in dark conditions (Bäck *et al.*, 1979). The fluorescence of the derivatized product was found to decrease with time (Maitra *et al.*, 1979c). This calls for a post-column derivatization arrangement. Such a design results in highly productive on-line determinations, an advantage overriding the drawback of a higher consumption of the derivatization agent, and of the need for a further (HPLC) pump (Anhalt, 1977).

Of the other derivatization agents for fluorescence labelling, dansyl chloride (Peng *et al.*, 1977a, 1977b; Chiou *et al.*, 1978), ninhydrin (Kubo *et al.*, 1987), or sodium $\beta$-naphthoquinone-4-sulphonate (Kubo *et al.*, 1986) have been used. Derivatization of aminoglycoside molecules for UV-detection was performed by 1-fluoro-2,4-dinitrobenzene (Barends *et al.*, 1980; Barends and Zwaan, 1981; Wong *et al.*, 1982; Barends *et al.*, 1983; Dionisotti *et al.*, 1988), 2,4,6-trinitrobenzene-1-sulphonic acid (Kabra *et al.*, 1983; 1984) and benzene sulphonyl chloride (Larsen *et al.*, 1980).

## HPLC separation modes: stationary and mobile phases

Undoubtedly, reversed-phase HPLC is the dominating separation mode of aminoglycosides. Normal-phase HPLC of neither native nor of derivatized aminoglycosides on silica gel column packings is of any practical value.

Bonded $C_{18}$-silica gels in combination with reversed-phase ion-pairing conditions seem to be the optimal selection. Buffered aqueous mobile phases with an appropriate ion-pairing agent warrant adequate HPLC

resolution and reduce the peak tailing of native or derivatized aminoglycoside molecules (Anhalt, 1977; Anhalt and Brown, 1978; Rumble and Roberts, 1987).

### Detection principles

Fluorescence and UV-light absorbance of the derivatized aminoglycosides are prevailing in the specific and sensitive detection of these compounds in HPLC (see Appendix 1). Fluorescence is, however, more specific than the UV-light absorption as not all absorbing compounds fluoresce. Moreover, the appropriate selection of both excitation and emission wavelengths of the HPLC spectrofluorometer permits easy differentiation of the co-eluted compounds.

Owing to its extra high selectivity, the spectrofluorometric detector has been gaining in favour for aminoglycosides monitoring, while, because of its very high sensitivity, the filter fluorometer is preferred in pharmacokinetic studies of this group of drugs.

## CONCLUDING REMARKS

The paper reviews HPLC methods for the determination of aminoglycoside antibiotics in body fluids, covering the literature of about twenty years (Appendix 1), i.e. from 1977 with papers by Maitra *et al.* (1977), Anhalt (1977) and Peng *et al.* (1977a,b), up to 1996, e.g. Stead and Richards (1996).

For some representatives of this group of drugs only one method has been published, while for other aminoglycosides, e.g. gentamicin, a series of HPLC methods are available. The existence of a number of methods for the determination of a given drug, such as gentamicin, and the continuous development of new approaches, is accounted for by the advances in the HPLC technique itself on the one hand, and by the effort to broaden the understanding of the dynamics and kinetics of the given aminoglycoside in the organism on the other (Ďurišová *et al.*, 1995).

In prechromatographic treatment of samples, the process of SPE on a cation exchanger can be considered the procedure of choice, warranting the required result. Concerning the separation mode, the reversed-phase ion-pairing HPLC appears to remain the prevailing principle. For detection, the post-column tracing with the use of *o*-phthalaldehyde seems to be the most appropriate way. However, current innovations in other bioanalytically applicable methods, particularly advances in capillary electrophoresis, have also exerted their impact on the determination of aminoglycosides (Oguri and Miki, 1996).

### Acknowledgement

The GAS 1021 grant from the Grant Agency for Sciences, Bratislava, Slovak Republic, is gratefully acknowledged.

**APPENDIX 1**

| AMINOGLYCOSIDE<br>Body fluid | Internal standard | Sample treatment procedure(s) | Derivatization | Detection | References |
|---|---|---|---|---|---|
| AMIKACIN | | | | | |
| Plasma | | PP, SPE | Pre-col. | UV | Wong *et al.*, 1982 |
| Plasma | | UF | Pre-col. | UV | Papp *et al.*, 1992 |
| Plasma | Tobramycin | SPE | Post-col. | FL | Wichert *et al.*, 1991 |
| Serum | | PP, SPE | Pre-col. | UV | Wong *et al.*, 1982 |
| Serum | | SPE | Pre-col. | FL | Maitra *et al.*, 1978 |
| Serum | Kanamycin | PP, SPE | Pre-col. | UV | Kabra *et al.*, 1984 |
| Serum | Kanamycin | SPE | Pre-col. | UV | Barends *et al.*, 1983 |
| Serum | Neamine | Col. sw. | Pre-col. | FL | Essers, 1984 |
| Serum | Tobramycin | SPE | Post-col. | FL | Anhalt and Brown, 1978 |
| Urine | | | Pre-col. | UV | Papp *et al.*, 1992 |
| ASTROMICIN | | | | | |
| Serum | Netilmicin | SPE | Post-col. | FL | Kawamoto *et al.*, 1984 |
| DIBEKACIN | | | | | |
| Serum | | PP | Post-col. | FL | Kubo *et al.*, 1985 |
| Serum | Tobramycin | Col. sw. | Pre-col. | FL | Essers, 1984 |
| GENTAMICIN | | | | | |
| Plasma | | PP, LLE | Pre-col. | FL | D'Souza and Ogilvie, 1982 |
| Plasma | | PP, LLE | Pre-col. | FL | Peng *et al.*, 1977a |
| Plasma | | SPE | Pre-col. | FL | Stead and Richards, 1996 |
| Serum | | Col. sw. | Post-col. | FL | Tamai *et al.*, 1986 |

**APPENDIX 1. Continued**

| Body fluid | Internal standard | Sample treatment procedure(s) | Derivatization | Detection | References |
|---|---|---|---|---|---|
| AMINOGLYCOSIDE | | | | | |
| Serum | | PP, LLE | Pre-col. | FL | Bäck *et al.*, 1979 |
| Serum | | PP | Post-col. | FL | Kubo *et al.*, 1982 |
| Serum | | PP | Pre-col. | UV | Barends *et al.*, 1980 |
| Serum | | SPE | Pre-col. | FL | Maitra *et al.*, 1977<br>Šoltés *et al.*, 1983<br>Rumble and Roberts, 1987 |
| Serum | | SPE | Post-col. | FL | Anhalt, 1977 |
| Serum | 1-*N*-Acetylgentamicin $C_1$ | SPE | Post-col. | FL | Anhalt and Brown, 1978 |
| Serum | Netilmicin | PP, LLE | Pre-col. | UV | Larsen *et al.*, 1980 |
| Serum | Tobramycin | Col. sw. | Pre-col. | FL | Essers, 1984 |
| Serum | Tobramycin | PP | Pre-col. | UV | Barends and Zwaan, 1981 |
| Serum | Tobramycin | SPE | Pre-col. | FL | Marples and Oates, 1982 |
| Urine | | SPE, LLE | Pre-col. | FL | D'Souza and Ogilvie, 1982 |
| ISEPAMICIN | | | | | |
| Plasma | Dibekacin | SPE | Post-col. | FL | Maloney and Awni, 1990 |
| Urine | Dibekacin | | Post-col. | FL | Maloney and Awni, 1990 |
| KANAMYCIN | | | | | |
| Serum | | PP | Post-col. | FL | Kubo *et al.*, 1985 |
| MICRONOMICIN | | | | | |
| Serum | Sisomicin | SPE | Post-col. | FL | Kawamoto *et al.*, 1984 |
| NETILMICIN | | | | | |
| Plasma | | PP, LLE | Pre-col. | FL | Peng *et al.*, 1977b |
| Plasma | Gentamicin | PP, LLE | Pre-col. | FL | Santos *et al.*, 1995 |
| Serum | | Col. sw. | Pre-col. | FL | Essers, 1984 |
| Serum | | PP, LLE | Pre-col. | FL | Bäck *et al.*, 1979 |
| Serum | Astromicin | SPE | Post-col. | FL | Kawamoto *et al.*, 1984 |
| Serum | Gentamicin $C_{1a}$ | PP | Pre-col. | UV | Dionisotti *et al.*, 1988 |
| Serum | Tobramycin | SPE | Pre-col. | FL | Marples and Oates, 1982 |
| SISOMICIN | | | | | |
| Plasma | | SPE, LLE | Pre-col. | FL | Tawa *et al.*, 1988 |
| Serum | | Col. sw. | Pre-col. | FL | Matsunaga *et al.*, 1988 |
| Serum | Tobramycin | Col. sw. | Pre-col. | FL | Essers, 1984 |
| Serum | Tobramycin | PP | Pre-col. | UV | Barends and Zwaan, 1981 |
| Serum | Tobramycin | SPE | Post-col. | FL | Kawamoto *et al.*, 1984 |
| STREPTOMYCIN | | | | | |
| Serum | | PP | Post-col. | FL | Kubo *et al.*, 1986<br>Kubo *et al.*, 1987 |
| Serum | Dihydrostreptomycin | SPE | | UV | Kurosawa *et al.*, 1985 |
| TOBRAMYCIN | | | | | |
| Serum | | PP, LLE | Pre-col. | FL | Bäck *et al.*, 1979 |
| Serum | | PP | Post-col. | FL | Stobberingh *et al.*, 1982 |
| Serum | | SPE | Pre-col. | FL | Maitra *et al.*, 1979b |
| Serum | Amikacin | SPE | Post-col. | FL | Anhalt and Brown, 1978 |
| Serum | Gentamicin $C_{1a}$ | Col. sw. | Pre-col. | FL | Essers, 1984 |
| Serum | Gentamicin $C_2$ | PP, LLE | Pre-col. | FL | Haughey *et al.*, 1980 |
| Serum | Netilmicin | SPE | Pre-col. | FL | Marples and Oates, 1982 |
| Serum | Sisomicin | PP | Post-col. | FL | Kubo *et al.*, 1984 |
| Serum | Sisomicin | PP, SPE | Pre-col. | UV | Kabra *et al.*, 1983 |
| Urine | Gentamicin $C_2$ | LLE | Pre-col. | FL | Haughey *et al.*, 1980 |

Nonstandard abbreviations: Col. sw. = column switching, FL/UV = fluoro-/UV-detection, PP = protein precipitation, Pre-/Post-col. = pre-/post-column, UF = ultrafiltration.

## APPENDIX 2

Apramycin
  Bekanamycin
  Butirosin
Dactimicin
Dihydrostreptomycin A and dihydrostreptomycin B
  Fortimicin A and fortimicin B
  Framycetin
Kanamycin A, kanamycin B = kanendomicin, and kanamycin C
  Lividomycin
Neomycin A, neomycin B and neomycin C
  Paromomycin
Ribostamycin
  Sagamicin = Gentamicin $C_{2b}$
  Spectinomycin
Verdamicin
  Xylostasine

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