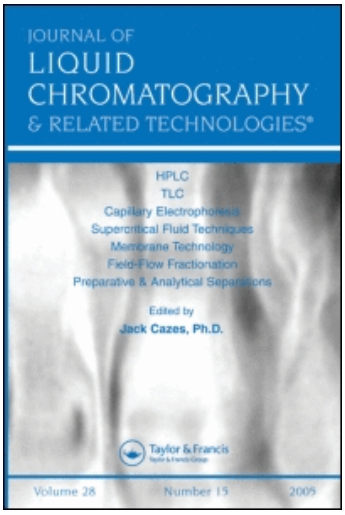

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

DETERMINATION OF AMIKACIN IN BIOLOGICAL TISSUES BY HPLC

Benjamín Santos[a]; Maria Luisa Sayalero[a]; Aránzazu Zarzuelo[a]; José M. Lanao[a]
[a] Department of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, University of Salamanca, Salamanca, Spain

Online publication date: 05 July 2002

To cite this Article Santos, Benjamín , Sayalero, Maria Luisa , Zarzuelo, Aránzazu and Lanao, José M.(2002) 'DETERMINATION OF AMIKACIN IN BIOLOGICAL TISSUES BY HPLC', Journal of Liquid Chromatography & Related Technologies, 25: 2, 287 — 297
To link to this Article: DOI: 10.1081/JLC-100108746
URL: http://dx.doi.org/10.1081/JLC-100108746

J. LIQ. CHROM. & REL. TECHNOL., 25(2), 287–297 (2002)

DETERMINATION OF AMIKACIN IN BIOLOGICAL TISSUES BY HPLC

Benjamín Santos,* Maria Luisa Sayalero, Aránzazu Zarzuelo, and José M. Lanao

Department of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, University of Salamanca, 37007 Salamanca, Spain

ABSTRACT

A rapid, specific, and reliable technique has been developed for the determination of Amikacin in biological tissues by high performance liquid chromatography. The technique involves the use of reverse phase liquid chromatography employing methanol, water (69 : 21, v/v), and tripotassium EDTA (2.2 g/L) as the mobile phase and emission and excitation wavelengths of 360 nm and 435 nm, respectively; the range of concentrations used being 0.25–25 μg/mL.

The techniques afford excellent linearity, accuracy, and within- and between-day precision for the concentration range studied, allowing it to be used in daily practice for the determination of Amikacin at the laboratory.

*Corresponding author. E-mail: bsantos@usal.es

INTRODUCTION

The aminoglycoside antibiotics are a family of drugs widely used in the outpatient setting and, especially, in hospitals (1–3).

Although they provide more efficient treatment, (4) the new dosage schedules of the aminoglycoside antibiotics have not managed to solve the main limitation of this group of drugs, such as their potential toxicity for the ear and, especially, the kidney (5).

The use of biocompatible and biodegradable vectors (6,7) that selectively modify tissue drug distribution, improving therapeutic efficiency, and reducing toxicity, is an excellent alternative in antibiotic therapy (8).

A possible alternative in the field of vector systems is that of microspheres made of biocompatible polymers (9,10).

For the determination of Amikacin by HPLC with fluorescence detection, it is necessary to perform a derivatization reaction using o-phthaladehyde (11). This was thought to be more promising, because the fluorescent product is detectable with lower levels of antibiotic.

The aim of the present work, was to set up and validate (12–16) a reverse-phase chromatographic technique for the quantification of Amikacin in biological samples following subcutaneous administration of a microsphere formulation, a technique that can be applied to the quantitative and qualitative analysis of the active principle in studies aimed at the development of new administration routes of the drug and of aminoglycoside antibiotics in general.

EXPERIMENTAL

HPLC Instrumentation

The chromatographic equipment comprised a Shimadzu pump (mod. LC-10AD), a Shimadzu system controller (mod. SCL-10A), and a Shimadzu auto-injector (mod. SIL-10AXL) coupled to a Shimadzu fluorescence detector (mod. RF-10AXL). Data collection was accomplished with a Shimadzu chromatography data system Class VP version 4.0.

Reverse phase Kromasil 100 C-18 columns (15×0.4 cm) (Tecnokroma, Barcelona, Spain) of 5 µm particle size were used. The mobile phase was composed of a mixture (69 : 31 v/v) of methanol, water and 2.2 g of EDTA tripotassium salt (Sigma, Steinhein, Germany) (17). This mobile phase was prepared daily, filtered in a Supelco vacuum system (mod. 5-8068) with a 0.45 µm nylon filter (Whatman, Malstone, U.K.), and degassed in a P-Selecta ultrasound bath (mod. M-515). Flow rate during the assays was 1.5 mL/min and λ_{exc} was

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

360 nm and λ_{emi} 435 nm. The process was carried out in a temperature-controlled bath at 37°C (18).

Chemicals and Reagents

Amikacin sulphate (Sigma-Aldrich Chemie, Steinhein, Germany), methanol (Merck Darmstad, Germany), o-phthaldialdehyde (Sigma-Aldrich Chemie, Steinhein, Germany), sodium carbonate (Panreac, Barcelona), potassium di-hydrogen phosphate. (Panreac, Barcelona), sodium hydroxide (Panreac, Barcelona), 2-propanol (Panreac, Barcelona), boric acid (Panreac, Barcelona), 2-mercaptoethanol (Sigma-Aldrich Chemie, Steinhein, Germany).

Sample Preparation

Prior to their analytical determination by HPLC, the biological samples were conditioned in a process comprising four essential phases (deproteinisation, removal of impurities, the derivatization reaction, and extraction of the derivatized compound), as described in Fig. 1.

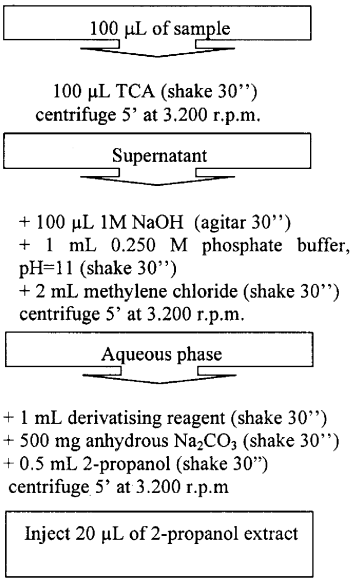

Figure 1. Protocol used for conditioning biological samples for the determination of Amikacin by HPLC.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

The derivatization reaction is the main reaction in the analysis of Amikacin by HPLC since the aminoglycosides do not absorb UV radiation and neither do they emit fluorescent radiation, such that in this technique a pre-column reaction was employed, using o-phthaldialdehyde as the derivatizing reagent. The derivatizing solution was prepared fresh daily and was made up by dissolving 20 mg of o-phthaldialdehyde in a solution made of 2 mL of mercaptoethanol, 10 mL of ethanol, and 100 mL of borate buffer, pH = 10.4 (19).

Once derivatized, the samples were stored on ice until their determination.

Selectivity

To study the selectivity of the method, (15) a sample of plasma was prepared with the components used to manufacture the microspheres: *d,l*-lactide-co-glycolide (0.1%), cottonseed oil (0.01%), and soybean lecithin (0.01%).

Sensibility

The sensibility of technique was evaluated using quantification limited (Q.L.) and detection limited (D.L.) from the following equations (16).

$$\text{Q.L.} = \frac{a + (3^* s_a)}{b^* \sqrt{n}}$$

$$\text{D.L.} = \frac{a + (10^* s_a)}{b^* \sqrt{n}}$$

where a is the ordinate at the origin and b is the slope of the calibration straight line, s_a is the standard deviation of ordinate at the origin of the calibration straight lines and n is the number of points, for concentrations ranging from 0.25–5 μg/mL.

Quantification

The concentration of Amikacin in the problem samples was determined from the following equation:

$$x = \frac{(response - a)}{b}$$

where x is the concentration of Amikacin in μg/mL, a is the ordinate at the origin of the calibration straight line, b is the slope of the calibration straight line, and *response* is the area of the chromatographic peak (15).

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

RESULTS AND DISCUSSION

Selectivity

Fig. 2 shows a chromatogram of Amikacin (25 μg/mL) in plasma as compared with a plasma blank containing (*d,l*-lactide-co-glycolide, cottonseed oil, soybean lecithin).

The good selectivity and specificity of the analytical technique used for Amikacin determination can be seen (tr = 7.50), no overlapping of the chromatographic peaks or interferences with any other compounds possibly present in the samples analyzed being observed.

Linearity

Fig. 3 shows the chromatograms of the calibration straight line of concentrations between 2.5 and 25 μg/mL.

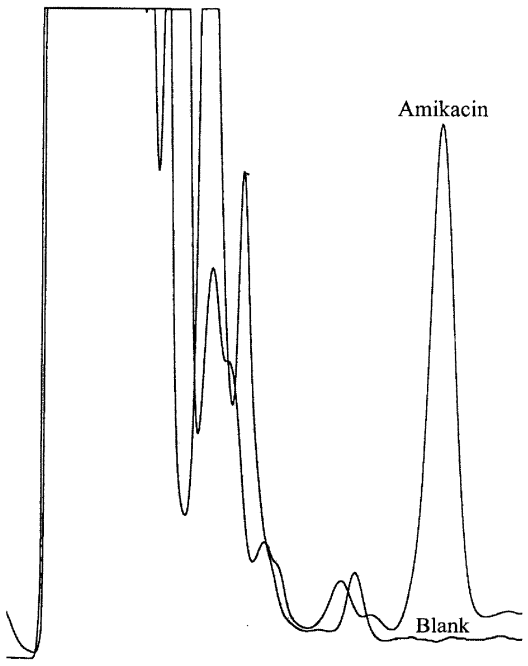

Figure 2. Chromatogram of a blank and of a sample of Amikacin in plasma.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

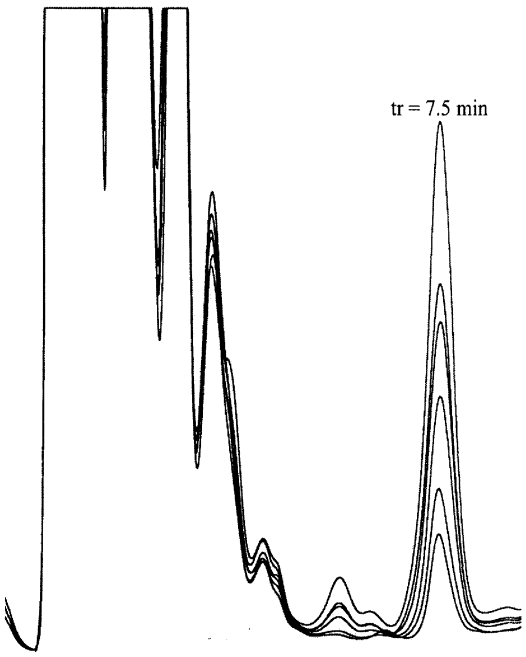

Figure 3. Chromatograms corresponding to the calibration range of Amikacin in plasma (2.5, 5, 10, 15, 20, and 25 μg/mL).

Over the ranges of 0.25–5 μg/mL and 2.5–25 μg/mL for Amikacin, respectively (anticipated concentration range in biological fluids), a linear fit was used with satisfactory results (13). The data were fitted to a line by the equation $y = a + bx$, where $y =$ response (area), $b =$ slope, $a =$ intercepts, and x is the concentration of Amikacin in μg/mL of the standard samples (Fig. 4). Claims of linearity are supported by regression data that include the correlation coefficient (r) and r-squared value (determination coefficient) (14). Table 1 shows the parameters of the equations obtained in the regression study of each concentration in an intra-day (5 times) follow-up.

Analysis of the response factor (fr) served to check the good linearity of calibration. Table 2 shows the C.V.% of the response factor in an intra-day study. As may be seen, the variation coefficient of that factor is lower than 6% for both concentration ranges.

Comparison of the variances observed, reveals that the linearity is correct for a very high level of significance and that the slope of the straight line is significantly different from zero in both cases (Tables 3 and 4).

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

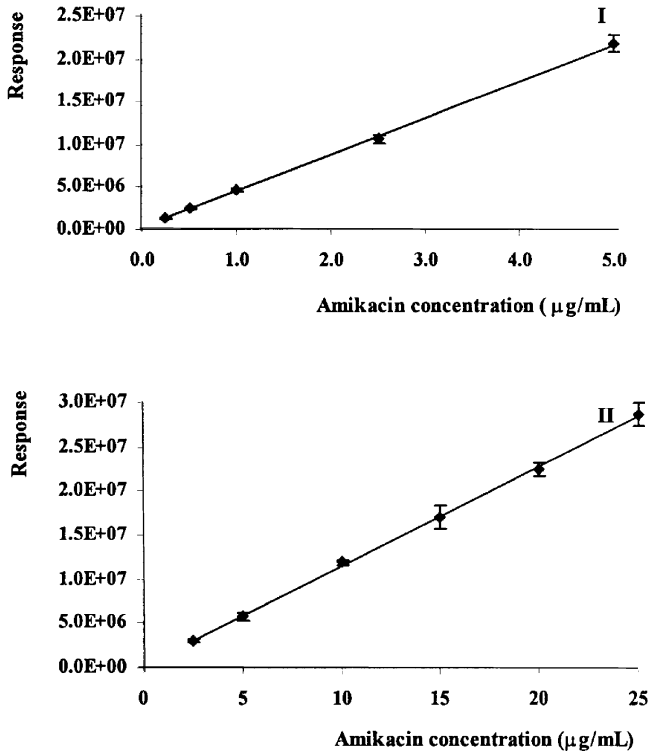

Figure 4. Calibration straight line of Amikacin in plasma for concentrations ranging from 0.25–5 μg/mL (I) and 2.5–25 μg/mL (II), respectively.

Table 1. Regression Linearity Fit by Equation ($y = a + bx$)

Straight Line	a	Confident Interval (95%)	b	r	r^2
0.25–5 μg/mL	267957	-182039–717953	4294631	0.995	0.991
2.5–25 μg/mL	251544	-295200–798288	1115656	0.997	0.993

a = intercepts, b = slope, r = correlation coefficient, r^2 = determination coefficient.

Sensibility

2.00E-01 and 6.01E-02 are the quantification and detection limits, respectively. The samples with concentrations between the above limits can be detected but not adequately quantified.

Table 2. Study of Relative Factor of Response (fr) in an Intra-Day Study

Concentration	x	σ_{n-1}	% C.V.
0.25–5 μg/mL	4723570.21	466031.00	9.98
2.5–25 μg/mL	1143489.23	63314.95	5.54

x = mean value, σ_{n-1} = standard deviation, % C.V. = variation coefficient.

Table 3. Analysis of Variance for the 0.25–5 μg/mL Concentration Range

Source of Variation	SS	Degrees of Freedom	MS	DF	Probability
Regression	1.42E+15	1	1.43E+15	2538.38	10E-16
Residual	1.29E+13	23	5.61E+11		
Total	1.44E+15	24			

Table 4. Analysis of Variance for the 2.5–25 μg/mL Concentration Range

Source of Variation	SS	Degrees of Freedom	MS	DF	Probability
Regression	2.37E+15	1	2.36E+15	4022.03	10E-16
Residual	1.65E+13	28	5.88E+11		
Total	2.38E+15	29			

Accuracy

The technique was exact in measuring the response by areas for the straight line corresponding to low concentrations and, also, for high ones. In the first case, mean recovery was 99.18% ± 11.7, with a C.V. (%) of 11.89. The real and observed values did not show statistically significant differences when subjected to Student's *t*-test: t_{exp} (2.11) < t_{tab} (2.75), $P > 0.05$ and 29 degrees of freedom. In the second case, mean recovery was 99.28 ± 6.05, with a C.V. (%) of 6.10. Likewise, the real and observed values did not show statistically significant differences: t_{exp} (0.94) < t_{tab} (2.75), $P > 0.05$ and 29 degrees of freedom.

Precision

To study the precision of this analytical method, standard solutions at the same concentrations as those described above were used. Repeatability or intra-day precision studies were carried out by analyzing the samples five times on the

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Table 5. Results of Study of Precision of a 0.25–5 and 2.5–25 μg/mL Concentration Range of Amikacin in the Intra-Day Study

Conc. (μg/mL)	Mean Area	C.V. (%)	Conc. (μg/mL)	Mean Area	C.V. (%)
0.25	1001372	8.65	2.5	2968880	8.25
0.50	2493932	9.91	5	5731720	8.02
1	4707806	9.97	10	11945443	2.52
2.5	10672134	9.42	15	17056447	7.49
5	21871501	6.01	20	22440559	3.49
			25	28643532	4.52

Table 6. Results of Study of Precision of a 0.25–5 and 2.5–25 μg/mL Concentration Range of Amikacin in the Inter-Day Study

Conc. (μg/mL)	Mean Area	C.V. (%)	Conc. (μg/mL)	Mean Area	C.V (%).
0.25	2271701	8.64	2.5	3191215	8.25
0.50	2978321	12.42	5	5987620	9.34
1	5054075	4.24	10	11961913	4.52
2.5	10828164	11.66	15	17050056	8.22
5	22479004	10.75	20	25148629	5.70
			25	28113028	7.42

same day, and reproducibility studies were performed by analyzing five samples on five different days (inter-day precision). The mean values of the results obtained and their variation coefficients are shown in Tables 5 and 6.

In both the intra-day and inter-day precision studies, the CVs of the measurements by areas for both straight lines were in all cases lower than 15%, pointing to the good repeatability and reproducibility of the analytical technique.

CONCLUSIONS

A method for the quantitation of Amikacin in biological samples has been developed and validated. Our results reveal the excellent linearity, precision, and accuracy of the analytical technique, allowing it to be used as a standard method for the quantitation of Amikacin, both in clinical practice and in research. Also, only a small sample volume is required, which is important when studies with this type of sample are to be performed.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

With small variations, the technique could be used for the quantitative and qualitative determination of other aminoglycoside antibiotics.

REFERENCES

1. Zembower, T.R.; Noskin, G.A.; Posteninick, M.J.; Nguyen, C.; Peterson, L.R. The Utility of Aminoglycosides in an Era of Emerging Drug Resistance. Int. J. Antimicrob. Agents. **1998**, *10*, 95–105.
2. Barriere, S.L. Aminoglycosides: A Reassement of their Therapeutic Role. Clin. Pharm. **1998**, *7*, 385.
3. Levin, S. Antibiotics of Choice in Suspected Serious Sepsis. J. Antimicrob. Chemother. Suppl A **1981**, *8*, 133.
4. Rello, J.; Triginer, C. Dosis Única Diaria de Aminoglucósidos. Rev. Esp. Quimioter. **1997**, *10*, 195–202.
5. Schentag, J.J.; Cerra, F.B.; Plant, M.E. Clinical And Pharmacokinetic Characteristics of Aminoglycosides Nephrotoxicity in 201 Critically Ill Patients. Antimicrob. Agents. Chemother. **1982**, *21*, 721.
6. Lim, F. *Biomedical Applications of Microencapsulation*; CRC Press, Inc.: Boca Raton, Florida, **1984**.
7. Rembaum, A; Toke, Z.A *Microspheres: Medical and Biological Applications*; CRC Press: Boca Raton, FL, **1988**.
8. Chandrashekar, G.; Udupa, N. Biodegradable Injectable Implant Systems for Long Term Drug Delivery Using Poly (Lactic-Co-Glycolic) Acid Copolymers. J. Pharm. Pharmacol. **1996**, *48*, 669.
9. Arshary, R. Microspheres for Biomedical Applications: Preparation of Reactive and Labelled Microspheres. Biomaterials. **1993**, *14*, 5.
10. Reza, U.L. Biodegradable Poly(Lactic Acid) and Poly(Lactide-Co-Glycolide) Polymers in Sustained Drug Delivery. Drug Devel. Ind. Pham. **1990**, *16* (16), 2353.
11. Wichert, B.; Schreier, H.; Derendorf, H. Sensitive Liquid Chromatography Assay for the Determination of Amikacin in Human Plasma. J. Pharm. Biomed. Anal. **1991**, *9* (3), 251–254.
12. Egan, H. Methods of Analysis: An Analysis of Methods. J. Assoc. Anal. Chem. **1977**, *60*, 260–267.
13. Garcel, J.; Mariné A.; Codony, R. Evaluación y Control de Calidad de los Métodos de Análisis Químico IV: Parámetros que Caracterizan al Método Analítico: Criterios de Fiabilidad (Exactitud, Precisión, Sensibilidad y Selectividad) y Criterios de Practicabilidad. C.I.F. **1988**, *7* (2ª ep.), 182–189.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

14. Jenke, D.R. Chromatographic Meted Validation: A Review of Current Practices and Procedures. General Concept And Guidelines. J. Liq. Chrom. Rel. Technol. **1996**, *19* (5), 719–736.
15. Lang, J.R.; Bolton, S.A. A Comprehensive Method Validation Strategy for Bioanalytical Applications in the Pharmaceutical Industry 1. Experimental Considerations. J. Pharm. Biomed. Anal. **1991**, *9* (5), 361–375.
16. Pérez Cuadrado, J.A.; Pujol Forn. *Validación de Métodos Analíticos.* Asociación Española de Farmacéuticos de la Industria, Barcelona, **2001**.
17. Pierini, N. Analisi HPLC Degli Isomeri Di Posizione Della Amikacin e Loro Determinazione in Specialita Medicinali. Boll. Chim. Farm. **1984**, *123*, 169–174.
18. Shyamay, K.; Maitra, S.K.; Yoshikawa, T.T.; Steyn, C.M.; Guze, L.B.; Shotz, M.C. Amikacin Assay is Serum by High–Performance Liquid Chromatography. Antimicrob Agents Chemother. **1978**, *14*, 880–885.
19. Santos, M.; García, E.; López, F.G.; Lanao, J.M.; Dominguez–Gil. A. Determination in Plasma by HPLC. J. Pharm. Biomed. Anal. **1995**, *13*, 1059–1062.

Received July 26, 2001
Accepted August 31, 2001
Manuscript 5621

Request Permission or Order Reprints Instantly!