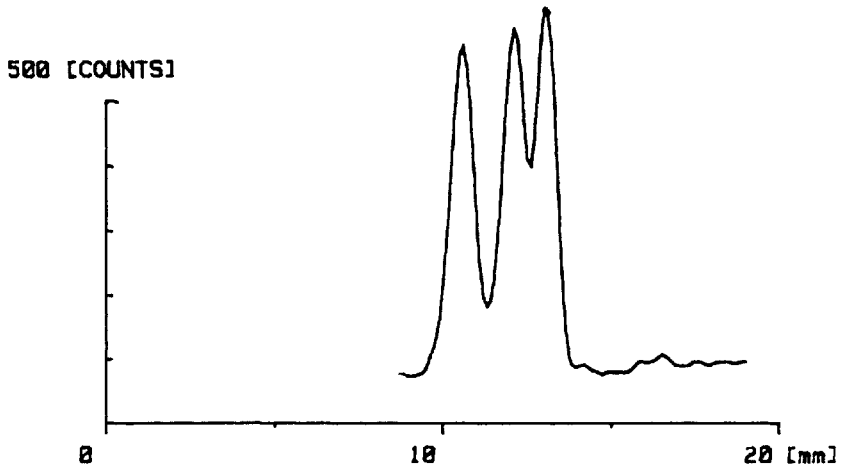

Figure 2 Separation of gentamicin components C_{1a}, C_2/C_{2a}, and C_1. (From Ref. 38.)

methanol–25% ammonia–chloroform (10:10:2) was used as the mobile phase. The spots were derivatized with ninhydrin. Quantification was linear over the range 500–3000 ng/μL.

The separation of nebramycin components (apramycin, carbamoyl kanamycin B, and carbamoyl tobramycin) by silica gel TLC using the mobile phase acetone–ethanol–25% ammonia (1:1:1) was studied (43). Spots were detected by charring followed by scanning at 450 nm. The effect of ammonia content on the R_f values of nebramycin components was investigated. The procedure can be used to control fermentation processes.

A simple and reliable method was developed for the estimation of gentamicin as the bulk drug and gentamicin from plasma and urine (44). The aminoglycoside components gentamicin C_1, C_2, and C_{1a} separated on silica gel plates using chloroform–methanol–20% ammonium hydroxide (2.4:2.2:1.5) as the mobile phase were reacted with NBD-Cl to yield highly fluorescent derivatives. They were quantified by in situ fluorodensitometry. The same components of gentamicin were determined in chicken meat by Vega et al. (45). The homogenized sample was extracted with acetonitile–4% aqueous KCl (9:1), defatted with hexane, and purified on an RP-18 column. The solvent was 20% aqueous solution of K_2HPO_4. Derivatization was done with fluorescamine or ninhydrin. The plates were scanned at 366 nm after excitation in the fluorescence mode for fluorescamine derivatives and at 510 nm in the absorbance mode for ninhydrin derivatives.

Netlimicin in serum was isolated by solid-phase extraction (SPE) on C-18 cartridges and then analyzed by HPTLC followed by derivatization with a mixture of diphenylboronic anhydride and salicylaldehyde (46). SPE on copolymeric bonded silica (with C_8 and sulfonic groups) was used for the isolation of hygromycin B from serum and plasma (47). Sample eluates were spotted on silica gel TLC plates with concentrating zones and developed with acetone–ethanol–99.9% ammonium hydroxide (1:1:1). Hygromycin B was visualized by derivatization with fluorescamine. Hygromycin added to bovine plasma was detectable at 25 ppb and added to swine serum at 50 ppb. Neomycin and gentamycin from aqueous solutions could be estimated in a similar way at a level of 50 ppb. Gentamicin, neomycin, spectinomycin, hygromycin B, and streptomycin were separated with this TLC system.

Similar chromatographic conditions were used for determination of hygramycin B isolated from biological fluids (48). Derivatization with fluorescamine was replaced by spraying with 0.2 M citrate buffer and UV detection at 365 nm. Hygramycin was isolated, as previously, on copolymeric resin. Then further purification was done using affinity chromatography. Recently, a thorough review on aminoglycosides was published that included many analytical methods among them thin-layer chromatography (5).