

Cleaning validation: determination of residue of ofloxacin

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An HPTLC method was developed for monitoring residue of ofloxacin (antibiotic belonging to fluoroquinolones) on stainless steel surfaces. Six simulated samples at 5 mg/m² were prepared by spreading calculated amount of pharmaceutical (as a solution) on 5 dm² stainless steel surface (0.25 mg of ofloxacin). After evaporation of solvent the residue was swabbed with a cotton band wetted with 25 mM NaOH in ethanol. The cotton band was extracted with the same solvent. After filtration two aliquots from each extract were applied to the prewashed HPTLC silica gel 60 plate together with 6 standards. Sample test solutions and standards were applied to both sides of the plate. The plate was developed in horizontal chamber, sandwich configuration, from both sides at once. Developing solvent was methanol – chloroform – conc. ammonia 10:7:3 v/v/v and developing distance 30 mm. Dry plates were dipped into paraffin – *n*-hexane solution (1:2 v/v) for enhancement of fluorescence. Greenish blue fluorescent spots were visible on dark blue background at 366 nm. By densitometric quantification of fluorescence linear calibration curve was obtained from 2 to 20 ng of ofloxacin (Fig. 1), appropriate for the determination of residue from surfaces of different sizes. Repeatability of chromatography of 10 ng ofloxacin expressed as relative standard deviation was 6.3% (n=6). The mean recovery of residue (n=6) at 5 mg/m² swabbed from 5 dm² was over 80%. Densitometrical limit of detection was 150 pg of ofloxacin. The method can be applied to the routine control of pharmaceutical equipment cleanliness.

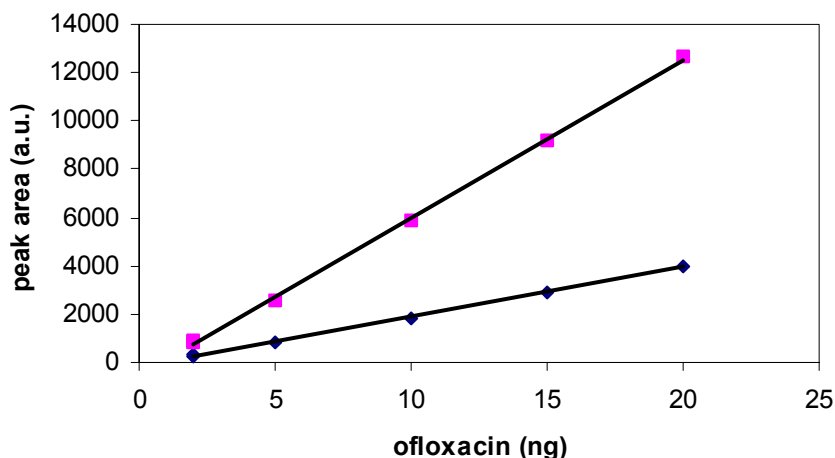


Fig. 1 Densitometric measurement of fluorescence of ofloxacin. Both lines were obtained from the same plate, the upper one after the dipping into paraffin/ *n*-hexane solution.