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Determination of bleomycin in bioadhesive formulations by gradient-elution HPLC

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Bleomycin is a mixture of antineoplastic antibiotics employed in the treatment of various squamous cell carcinomas, including those of the cervix and the skin, and in the treatment of Hodgkin's disease and other lymphomas It has been formulated for localised administration to the cervical canal as double-layered sticks (Iwata et al 1987) or as a compressed disk (Machida et al 1979). The aim of the present study was to develop a rapid HPLC assay for the determination of bleomycin in bioadhesive dosage forms based on those reported previously (Woolfson et al 1995).

Formulations were prepared using methods discussed previously (Woolfson et al 1998). Analysis was performed by reverse-phase high performance liquid chromatography (HPLC) on a Waters Alliance system with photodiode array detection. The column used was a Waters NovaPak (3.9mm x 150mm; 5μm d.p) at 30°C. The mobile phase consisted of HPLC water and acetonitrile, each containing trifluoroacetic acid (TFA) (0.1%). Gradient elution was performed at 1.0 ml min^{-1} (Table 1).

Time (mins)	0	5	10	11	12	15
%A (Acetonitrile/TFA)	10	20	50	50	10	10
%B (H_2O/TFA)	90	80	50	50	90	90

Table 1. Experimental conditions for gradient elution HPLC analysis of bleomycin.

Wet formulations were spiked at two levels (1.5 and 0.15 units g^{-1}) with bleomycin sulphate USP. The purpose of the lower level sample was to characterise the performance of the method close to the expected limit of detection. A calibration curve was constructed by spiking HPLC water with bleomycin covering the range 0-3.0 units g^{-1}. Samples (n = 4) were prepared for analysis by 100-fold dilution with HPLC water. 5μL of the resulting solutions were injected and a chromatogram extracted at 290nm from the diode array data. Areas from the two major bleomycin peaks (USP, 1985) were compared to standards and mean recoveries calculated (Table 2). Limits of detection were determined from signal to noise ratios (6:1) on the sample spiked at 0.15 units g^{-1} (Table 2).

	Peak 1	**Peak 2**
Retention times (min)	3.28	3.89
r^2	0.994	0.999
% recovery from gels (1.5 units g^{-1})	91 ± 6.5	86 ± 6.4
% recovery from gels (0.15 units g^{-1})	162 ± 21	148 ± 39
Limits of detection (units/g)	0.3	0.45

Table 2. Recoveries and limits of detection for bleomycin USP in bioadhesive formulations.

Determination of peak areas corresponded to the requirements for bleomycin analysis (USP, 1985). The results obtained indicate that the method employed may be used to determine reproducibly therapeutically relevant levels of bleomycin with a limit of detection of 0.3 units g^{-1}. Chromatograms obtained from standard samples and formulations are readily comparable, indicating both the full and efficient extraction of bleomycin from the dosage form. Further, the use of a gradient elution method ensures that all the relevant peaks may be determined within 10 minutes, compared to the 30 minutes of the established method of analysis (USP, 1985).

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