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Photoreactivity of biologically active compounds. XVIII. Photostability of ofloxacin in the solid state and in a tablet formulation

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The photostability of ofloxacin in the solid state has been investigated. The change in colour of uncoated and film coated ofloxacin tablets and compressed ofloxacin was studied as a function of irradiance level and total exposure energy. The degradation of ofloxacin in the various preparations was quantified by HPLC and the antimicrobial activity was determined for selected tablets. The structure of two main degradation products from ofloxacin in the solid state has been postulated from LC-MS analysis. Both products have an absorption cut-off below 400 nm and cannot explain the observed change in tablet colour. There was no apparent relationship between the change in colour and the loss of active substance or antibacterial activity for the preparations investigated. The change in colour was easily detectable at rather low exposure levels. Apparently, there was a difference in light sensitivity between the two film-coated tablet batches investigated. The results obtained were partly dependent on the conditions within the radiation chamber (e.g. exposure time and irradiance level), which emphasizes the importance of testing the samples under various conditions unless the results are unequivocal. The tablets were sensitive to visible light although ofloxacin only has a neglectible absorption above 400 nm. The film coated ofloxacin tablets did, however, absorb above 400 nm with a cut-off at approximately 520 nm. A change in tablet coating to include a component that filters visible light in addition to UV radiation might provide a solution to the discolouration problem and prevent batch to batch variations with respect to light sensitivity.

1. Introduction

Ofloxacin belongs to the fluoroquinolone antimicrobial class, which is widely prescribed for a variety of gram-positive and gram-negative infections. Fluoroquinolones absorb energy in the ultra violet (UV) range of the electromagnetic spectrum and they are characterized by photochemical instability (Fasani et al. 1999; Navaratnam and Claridge 2000; Tiefenbacher et al. 1994; Yoshida et al. 1993). Loss of antibacterial activity of fluoroquinolone derivatives has been reported after UV exposure, suggesting this to be the result of the photodegradation of the substances (Ferguson et al. 1988; Matinez et al. 1998). Photodegradation in the solid state can be separated into two parts; the change of external appearance (i.e. change of colour) and the chemical degradation of the drug molecule. Change of appearance is not always directly related to the chemical degradation and may therefore not affect the efficacy of the drug. The patients might, however, feel uneasy to use medications that are discoloured and the compliance is thereby reduced. In other cases the photoinduced changes can lead to the formation of degradation products with reduced acitivity or a highly toxic potential, and a change in physical appearance of the product could be an indication for the preparation to be discarded. Photoinduced changes in the solid state is a surface phenomenon because the radiation will penetrate only fractions of a millimetre into a solid sample. The depth of penetration will be dependent on factors like the porosity of the sample and the prescence of a film coating (Lachman et al. 1960). The use of a general mathematical model to predict the rate of a solid state photoreaction can be of little value because the radiation is not evenly distrubuted throughout the sample (Sande 1996). Changes in reaction rate can be observed as the duration of the irradiation is increased due to alterations of the quantity of light reflected and absorbed at the surface, and possible absorption of certain amounts of energy by the decomposition products formed (Lachman 1962). The molecules on a solid surface, e.g. a tablet have a very restricted mobility which normally leads to alteration in the composition and ratio of products formed compared to a drug solution. Ofloxacin in the solid state and in tablet preparations undergoes a change in colour when exposed to natural daylight. In the present work the photoinduced colour change has been studied in pure ofloxacin (present in a compressed form), and in uncoated and filmcoated ofloxacin tablet formulations and compared to chemical stability and antibacterial activity of the individual preparations.

2. Investigations and results

2.1. Irradiation

The tablets (i.e. pure ofloxacin in a compressed form, uncoated and filmcoated ofloxacin tablets and placebo tablets) were exposed using a xenon lamp according to Option 1 in the ICH Guideline on photostability testing of drugs (ICH 1997). Two batches of identically prepared filmcoated tablets were investigated (batch 1 and 2). The ICH Guideline does not demand a certain irradiance or irradiation time but requires a total energy in the UV and visible range of the spectrum. The irradiance can be set to different levels and the testing time would be adjusted accordingly, varying between 7.1 h at 765 W/m^2 and 21.8 h at 250 W/m^2. In the present study the tablets were therefore tested at different irradiances (250 W/m^2, 550 W/m^2 and 765 W/m^2) until certain illumination energies were reached. To exclude temperature effects the black standard temperature (BST) was set to 35 °C, resulting in a chamber temperature below 30 °C. The results obtained by artificial light were compared to natural daylight in the window sill of a south-west facing window. The samples were presented in glass dishes. In the Suntest the dishes were covered with a thin plastic wrap (Gladpack). The plastic wrap transmits only 85% of the incident light above 310 nm and this is taken into account in the calulation of energy levels. The temperature inside the dishes was detected by a temperature recording strip (sensitivity range 37–65 °C). With one exception, the temperature was below the lower detection limit of the strip in all samples.

Filmcoated tablets (batch 1) were placed in the window sill of a south-west facing window in a temperature controlled room (20 °C). The tablets were exposed for 194 h. The colour of the tablets was measured at defined time intervals. The indoor light was turned off during the experiment. The exposure period included midsummer; i.e. the time of the year with a maximum period of daylight in Scandinavia. Unfortunately, the weather during the experiment was mostly cloudy and therefore the irradiance was somewhat lowered compared to "worst case" conditions. On a sunny midsummer day the natural irradiance in Oslo would be approximately 524 W/m^2 (290–800 nm) and 51 W/m^2 in the UV (290–400 nm) (Personal information from the Norwegian Radiation Protection Authority). On a cloudy day the total irradiance can typically fluctuate at levels between 10 and 25% of the maximum intensity (Aman and Thoma 2003). In our window sill the average irradiance was estimated to approximately 150 W/m^2 per 24 h resulting in an overall illumiation of approximately 1200 Wh/m^2, corresponding to 4500 kJ/m^2 in the Suntest CPS+.

2.2. Colour measurements

The change in colour as a function of irradiance was detected for the various ofloxacin preparations.

The colour of the film coated tablets changed towards red as a function of light exposure as demonstrated by an increase in a^* value in the $L^*a^*b^*$ colour space (CIELAB).The data for batch 1 are presented in Table 1. The change was apparently a linear function of the light dose up to a total of 5400 kJ/m^2. The results were similar for the two batches tested (data for batch 2 not shown). The change in the red colour component seemed to be independent of the irradiance level. The rate of the red colour change showed a nearly 50% reduction in the presence of a UV-filter (cut -off 406 nm, 50% T at 418 nm) as demonstrated by the change in the slope of the curves (Table 1). Visible light does, however, obviously have the capacity of introducing a colour change to the tablet surface independent of the UV component. Ofloxacin in aqueous solution has an absorption cut-off at 420 nm; the absorption between 400 and 420 nm being almost neglectible. Film coated ofloxacin tablets, however, show an extensive absorption above 400 nm with a cut-off at approximately 520 nm as demonstrated by the reflectance spectrum.

The change in b^* value for the film coated tablets varies between the batches (Tables 2 and 3). In batch 1 a slight change towards yellow is observed at all irradiance levels in the absence of a UV-filter. In batch 2 the change towards yellow is immediately apparent, even by visual detection. At an irradiance level of 550 W/m^2 in the presence of a UV-filter there seems to be a slight change towards blue (batch 1). The change in b^* takes place initially and does apparently not change much above a dose of 1800 kJ/m^2. In both batches the yellowing of the surface by the end of the experiment is tending to be most extensive at the lowest lamp intensity (i.e 250 W/m^2). This seems however, only to be valid at low total energy levels (see below, Table 4).

Tablets exposed in the window sill seem to follow the same pattern as tablets exposed in the Suntest CPS+ at low total energy levels, although the rate for the change in a^* value is apparently somewhat higher compared to the tablets in the Suntest (batch 1) (Table 1). The irradiation dose that is stipulated for the tablets in the window sill is, however, a very rough estimate and is based on an equal distribution of the light intensity throughout 24 h. The uncertainty in this estimate combined with a weather- and time dependent variation in spectral distribution for natural daylight might explain the observed differences. The overall conclusion is that the correlation between the acceler-

Table 1: Change in a^* value in the $L^*a^*b^*$ color space (CIELAB) for film coated ofloxacin tablets (batch 1) as a function of irradiation (kJ/m^2) and irradiance (n = 6)

Dose (kJ/m^2)	Irradiance 250 W/m^2	Irradiance 550 W/m^2	Irradiance 765 W/m^2	Irradiance 550 W/m^2 + UV filter	Window sill
0	0.04 ± 0.47	0.44 ± 0.45	0.37 ± 0.43	0.57 ± 0.24	0.12 ± 0.19
900	0.95 ± 0.48	1.25 ± 0.21	1.03 ± 0.14	1.26 ± 0.19	1.30 ± 0.09
1800	1.40 ± 0.25	1.76 ± 0.10	1.66 ± 0.15	1.40 ± 0.13	2.09 ± 0.09
2700	1.91 ± 0.27	2.14 ± 0.16	2.32 ± 0.19		
3600	2.17 ± 0.25	2.66 ± 0.27	2.60 ± 0.14	2.13 ± 0.17	3.01 ± 0.17
4500	2.42 ± 0.23				3.50 ± 0.26
5400	2.80 ± 0.24	3.16 ± 0.39	3.40 ± 0.23	2.51 ± 0.14	
Reg	0.9755	0.9796	0.9883	0.9780	0.9758
y = ax + b	y = 0.0005x + 0.39	y = 0.0005x + 0.72	y = 0.0006x + 0.55	y = 0.0003x + 0.77	y = 0.0007x + 0.51

The irradiation dose stipulated for the tablets in the window sill is a very rough estimate and is based on an equal distribution of the light intensity throughout 24 h. Reg is the linear regression coefficient for y = ax + b (± min/max values)

Table 2: Change in b^* value in the $L^*a^*b^*$ color space (CIELAB) for film coated ofloxacin tablets (batch 1) as a function of irradiation (kJ/m^2) and irradiance (n = 6)

Dose (kJ/m^2)	Irradiance 250 W/m^2	Irradiance 550 W/m^2	Irradiance 765 W/m^2	Irradiance 550 W/m^2 + UV filter	Window Sill
0	0.01 ± 0.75	−0.57 ± 0.86	−0.49 ± 0.57	−0.66 ± 0.50	−0.24 ± 0.19
900	0.85 ± 1.42	0.57 ± 1.50	1.06 ± 1.04	−0.83 ± 0.76	−0.003 ± 1.00
1800	1.91 ± 1.02	0.63 ± 1.14	1.42 ± 0.92	−0.19 ± 1.09	0.63 ± 0.90
2700	2.02 ± 0.91	0.93 ± 1.36	1.53 ± 1.21	−0.07 ± 0.99	
3600	2.02 ± 0.85	0.58 ± 1.71	1.78 ± 1.03	−0.50 ± 0.54	0.45 ± 0.62
4500	1.93 ± 1.01				0.10 ± 0.76
5400	1.92 ± 0.42	0.72 ± 1.58	1.56 ± 1.05	−0.19 ± 1.01	

The irradiation dose stipulated for the tablets in the window sill is a very rough estimate and is based on an equal distribution of the light intensity throughout 24 h. (± min/max values)

Table 3: Change in b^* value in the $L^*a^*b^*$ color space (CIELAB) for film coated ofloxacin tablets (batch 2) as a function of irradiation (kJ/m^2) and irradiance (n = 3)

Dose (kJ/m^2)	Irradiance 250 W/m^2	Irradiance 550 W/m^2	Irradiance 765 W/m^2
0	0.29 ± 0.19	0.37 ± 0.23	0.72 ± 0.50
900	2.83 ± 0.93	3.09 ± 0.34	3.68 ± 1.28
1800	4.02 ± 0.80	3.32 ± 0.34	4.47 ± 1.66
2700	4.65 ± 1.03	4.14 ± 0.47	4.08 ± 1.30
3600	5.18 ± 0.84	3.98 ± 0.41	4.48 ± 1.62
5400	5.80 ± 1.23	4.44 ± 0.28	4.90 ± 2.21

(± min/max values)

ated studies and the real-time studies seems to be acceptable for these tablets.

The total colour change (ΔE) at the UV-dose (200 Wh/m^2) and endpoint (1.2 mill lux h) as recommended by the ICH Guideline is presented in Table 4. The observed change in colour per unit time becomes less towards the end of the exposure, and particularly in batch 1 the colour change seems to go towards a saturation level. This is not unexpected as the colour change is a surface phenomenon. The total colour change (batch 1) observed in the prescence of a UV-filter when exposed to the recommended UV dose (200 Wh/m^2) is approximately half the value of the change observed for unprotected tablets. However, by the time the endpoint is reached the colour change is at comparable levels in the protected and unprotected tablets (Table 4). The total change in colour is tentatively lower at a medium irradiance level.

The observed changes in a^* and b^* values and in total colour for the uncoated preparations exposed at 765 W/m^2 to a level of 1.2 mill lux h are presented in Table 5. The total change in colour shows a three to four-fold increase for uncoated tablets compared to the film-coated preparations under the same conditions (Table 4). Pure, compressed ofloxacin showes even a more extensive change in colour than the uncoated tablets. It is interesting to observe that the change in a^* value (i.e. towards red) is in the same range in the film-coated and uncoated tablets, e.g. Δa determined to 5.2 ± 0.3 (batch 1), 5.9 ± 0.6 (batch 2) and 4.9 ± 0.2 (uncoated tablets) at the end of exposure. The change in b^* value (i.e. towards yellow) does, however, show an extensive difference between the two formulations. In film-coated tablets Δb^* is 1.1 ± 0.8 (batch 1) and 5.6 ± 2.3 (batch 2) while the corresponding change in uncoated tablets is 20.8 ± 1.2 at the endpoint.

The ratio between Δa^* and Δb^* remains approximately constant for all the uncoated preparations containing ofloxacin, both in the abscence and in the prescence of a UV-filter (Table 5). In the prescence of a UV-filter the change in Δa^*, Δb^* and ΔE at the endpoint is reduced to approximately 1/3 of the change that is observed in the unprotected sample. This is quite different from the film-coated tablets where the total color change at the endpoint (ΔE) apparently was independent of the presence of a UV-filter (Table 4).

Film-coatings containing TiO_2 have a cut-off around 360 nm (Reed 2004). This should indicate that such film coatings would offer little protection for tablets containing compounds absorbing above 360 nm. The major part of the absorption spectrum of ofloxacin in solution is below 360 nm, and it is therefore expected that the film-coating would photostabilize the formulation as observed in this study. It is however, apparent that the film-coating will not fully protect the tablets towards light. The UV-filter experiments demonstrate that radiation > 400 nm (i.e. radiation that is not filtered by the coating) can induce a change in colour. This is consistent with the reflectance spectrum of solid ofloxacin and of film-coated tablets, showing that the solid samples absorbs far into the visible part of the spectrum. The film-coating seems to protect specifically against the reaction(s) that induce(s) a yellowing of the tablets (i.e. a change in b^* value), while it has

Table 4: The total color change ($\Delta E = \Delta E^*ab$) of film coated ofloxacin tablets (batch 1 and 2) at the UV-dose and endpoint as recommended by the ICH Guideline (batch 1: n = 6; batch 2: n = 3), and % ofloxacin decomposed at the endpoint (negative numbers indicate an apparent higher concentration in the irradiated tablets).

Irradiance	UV-dose ΔE (batch 1)	Endpoint ΔE (batch 1)	Dark control ΔE (batch 1)	UV-dose ΔE (batch 2)	Endpoint ΔE (batch 2)	Dark control ΔE (batch 2)	% decomposed (batch 1)	% decomposed (batch 2)
250 W/m^2	5.44 ± 1.78	6.40 ± 1.07	0.94 ± 0.64	8.35 ± 1.76	10.06 ± 3.19	1.98 ± 0.25	−1.1 ± 0.2	−1.0 ± 0.2
550 W/m^2	4.23 ± 1.02	4.94 ± 0.99	1.26 ± 0.72	6.56 ± 0.34	7.71 ± 0.24	1.45 ± 1.04	5.6 ± 0.8	10.5 ± 6.2
765 W/m^2	5.45 ± 1.00	6.06 ± 0.90	1.20 ± 1.03	6.47 ± 1.73	8.67 ± 2.18	1.76 ± 0.37	−3.8 ± 0.5	−4.6 ± 0.6
550 W/m^2 + UV filter	2.70 ± 0.14	4.62 ± 0.59	1.16 ± 0.48				−4.3 ± 0.6	

(± min/max values)

Table 5: Change in a^* and b^* values and in total color change ($\Delta E = \Delta E^*ab$) for various uncoated ofloxacin preparations exposed at 765 W/m² (n = 6)

Sample	Endpoint Δa^*	Endpoint Δb^*	Endpoint ΔE	Dark control Δa^*	Dark control Δb^*	Dark control ΔE
Compressed ofloxacin	6.34 ± 0.68	25.24 ± 1.67	27.08 ± 2.12	0.20 ± 0.04	0.06 ± 0.01	1.74 ± 1.42
Uncoated ofloxacin tablets	4.88 ± 0.23	20.80 ± 1.19	21.93 ± 1.18	− 0.01 ± 0.02	0.01 ± 0.20	0.73 ± 0.76
Placebo tablets	0.14 ± 0.01	−1.24 ± 0.13	1.29 ± 0.08	0.02 ± 0.01	0.38 ± 0.13	1.53 ± 0.18
Compressed ofloxacin + UV-filter*)	2.10 ± 0.36	9.72 ± 1.16	10.88 ± 2.37	− 0.03 ± 0.05	− 0.04 ± 0.16	0.97 ± 0.35

Placebo = tablet without ofloxacin; * The tablets are only exposed to the recommended UV-dose (200 Wh/m²) (± min/max values)

minor effect on the reaction(s) responsible for the development of a red colour at the tablet surface. This indicates that the reddening of the tablet surface is mainly caused by visible light. The situation is somewhat different for compressed, pure ofloxacin where the presence of a UV-filter seems to reduce the reddening and the yellowing to the same extent (Table 5). The excipients in the tablets might therefore have an influence on the process(es) that lead(s) to the change in colour.

2.3. Degradation of ofloxacin

The amount of ofloxacin in the tablets was determined by HPLC equipped with a diode-array detector. The reproducibility of the HPLC method was within 5.6% (n = 8). The detection limit was 25 nmol/l. Quantitation of unexposed 10 mg ofloxacin tablets (n = 9) resulted in an average ofloxacin content of 10.03 mg (±1.02, ± max/min values). Interference from the main photodegradation products formed by forced degradation of ofloxacin in the solid state and in aqueous solution could not be detected. There is no apparent decrease in the ofloxacin content in tablets exposed at 250 W/m² or 765 W/m². The fact that the ofloxacin content seemed to be slightly higher in the exposed tablets compared to the dark control could not be explained. However, at an irradiance level of 550 W/m² a degradation was observed in the tablets from both the batches. It should be mentioned that the two batches were exposed in two separate, but identical Suntest CPS+ test chambers, and that the individual series (e.g. exposure and

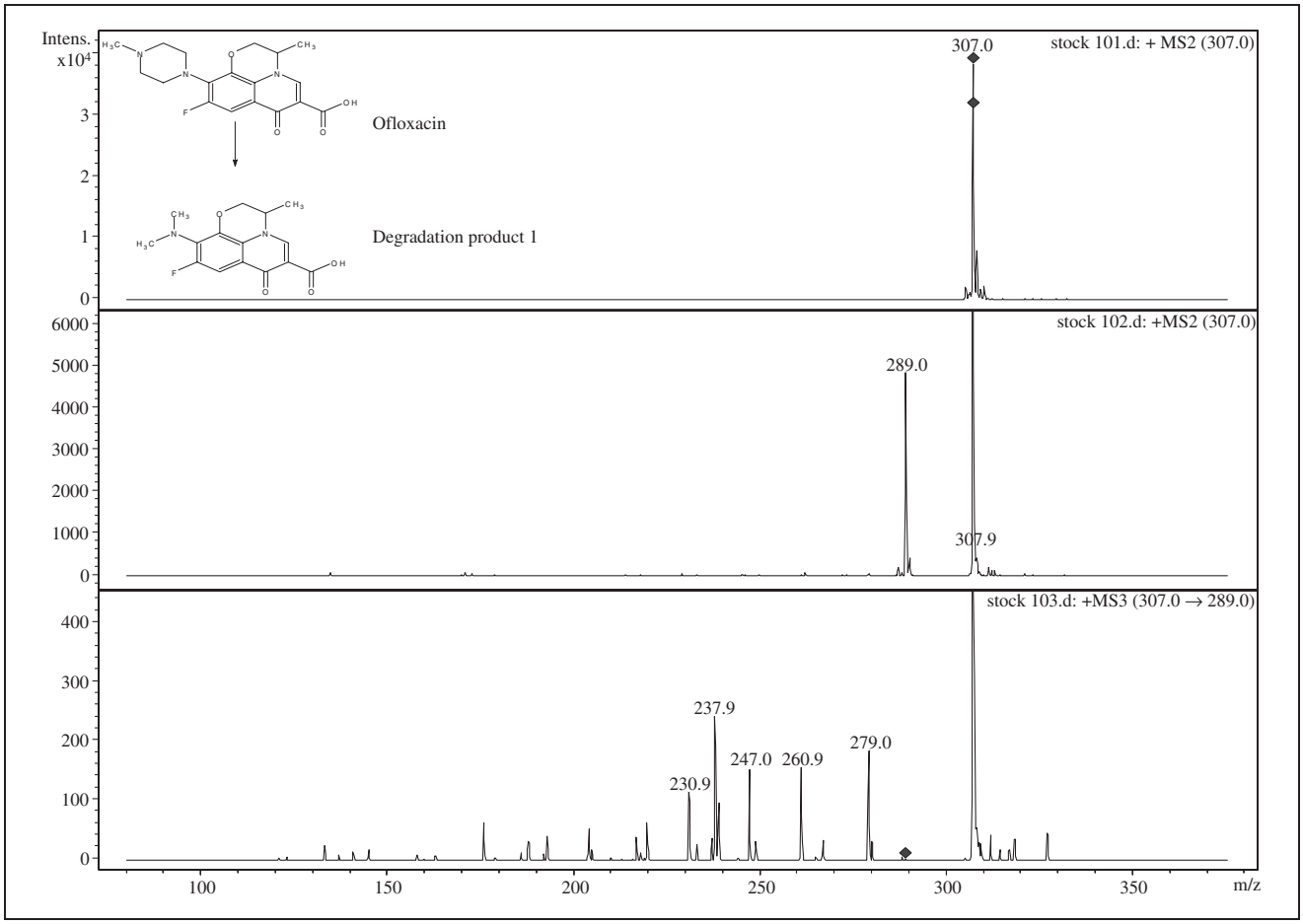

Fig. 1: Postulated structure for the degradation product with a chromatographic retention time of 9.6 min together with LC-MS data. Upper section (ms^1): determination of m/z value for the main component of the peak at 9.6 min, middle section (ms^2): fragmentation of m/z 307, lower section (ms^3): fragmentation of m/z 289 (i.e. formed by loss of water from m/z 307)

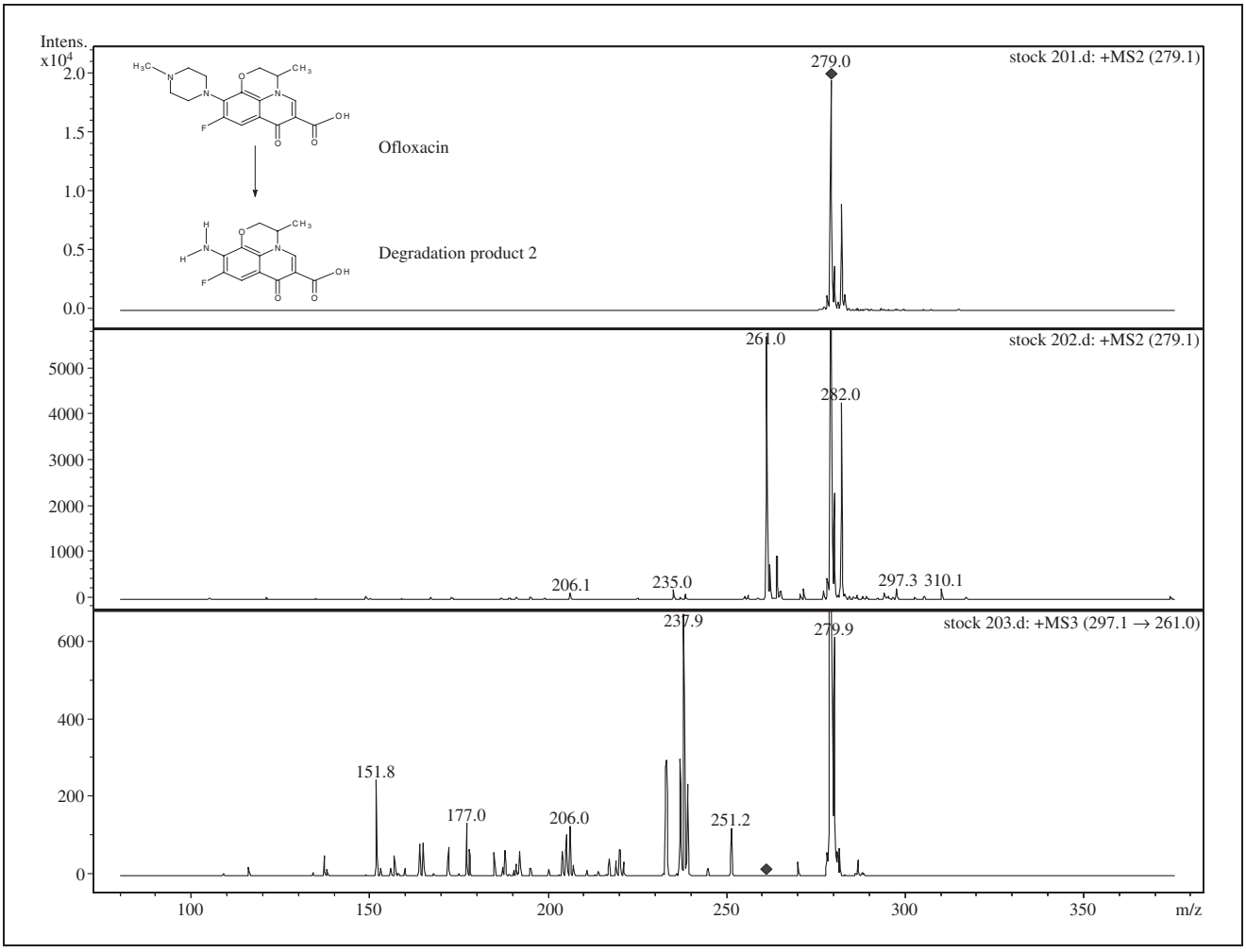

Fig. 2: Postulated structure for the degradation product with a chromatographic retention time of 20.8 min together with LC-MS data. Upper section (ms^1): determination of m/z value for the main component of the peak at 20.8 min, middle section (ms^2): fragmentation of m/z 279, lower section (ms^3): fragmentation of m/z 261 (i.e. formed by loss of water from m/z 279)

analysis of batch 1 vs exposure and analysis of batch 2) were separated in time by several months. It is also interesting to observe that the colour change is slightly less at this irradiance level compared to the tablets exposed at the other irradiance levels. One explanation for the increase in degradation could be that the colour formed at the surface has a protective (filter) effect on the deeper layers of the tablet, thereby limiting the degradation of ofloxacin in severely coloured tablets. The combination of irradiance level and exposure time obtained at 550 W/m^2 seems to be unfavourable for this particular formulation. A decrease in ofloxacin content of 3.1% ($\pm$0.2, n = 6) was observed for film coated tablets stored in the window sill for 194 h.

Two main degradation products could be detected after exposure (1.2 mill lux h) of compressed ofloxacin. They had retention times (t_r) of 9.6 min and 20.8 min respectively, in the chromatographic system used (ofloxacin $t_r = 4.1$ min). Further investigation of these compounds by application of LC-MS (electron spray ionization (ESI)) indicated m/Z = 307 (i.e. molecular mass 306) for the substance with $t_r = 9.6$ min. The UV-spectrum of the HPLC peak in the actual mobile phase showed absorption maxima at 254, 276 and 328 nm. A postulated structure for this degradation product together with LC-MS data is presented in Fig. 1. The LC-MS analysis of the peak at t_r 20.8 mins indicated m/Z = 279 (i.e. molecular mass 278). The UV-spectrum of this HPLC peak showed an absorption maximum at 287 nm with a shoulder at 317 nm. A postulated structure for this degradation product together with LC-MS data is presented in Fig. 2.

2.4. Antibacterial activity

Three ofloxacin tablets subjected to irradiation and three unexposed tablets as controls were tested for antibacterial activity. *Escherichia coli* ATCC 25922 was used as indicator strain in a minimum inhibitory concentration (MIC) assay. As shown in Table 6, no difference was observed between the MIC of the irradiated and the unexposed tablets, i.e. MIC = 0.22 nmol/l.

Table 6: Antibacterial activity of irradiated and unexposed ofloxacin tablets as assessed by MIC determination using Escherichia coli ATCC 25922 as indicator strain

Ofloxacin conc	Irradiated tablets (tablet no)			Unexposed tablets (tablet no)		
	1	2	3	1	2	3
0.44 nmol/l	Neg	Neg	Neg	Neg	Neg	Neg
0.22 nmol/l	Neg	Neg	Neg	Neg	Neg	Neg
0.11 nmol/l	Pos	Pos	Pos	Pos	Pos	Pos

Neg = no growth; Pos = growth of *E. coli*.

3. Discussion

A colour tolerance limit should specify how far a batch can differ from the standard and still be acceptable for use. The major problem with the setting of colour tolerances is that the tolerance should agree with a visual evaluation of acceptability; a parameter that may vary with the colour of the product. How large a colour difference can be before seeing a visual difference depends on the colour and the direction of the colour difference. Two dark blues may have a CIELAB colour difference of 0.3 and have a perceptible colour difference. If the colour difference is in hue, a value of 0.3 may even be unacceptable. On the other hand, two bright yellows may have a ΔE of 1.5 or even 2 and still appear to be equal, especially if the difference is in lightness. Therefore, the colour difference detected instrumentally does not have to be zero in order to visually determine two products to be equal (Marcus 1998). The tolerance limit must be set individually for each product and should take into account the results from visual examination of the preparations. In the present work a total colour change as observed for the film coated tablets by the endpoint exposure (1.2 mill lux h) is easily detectable by the human eye and may therefore lead to discarding of the tablets. By combining the visual examination and the instrumentally detected values a tolerance limit of $\Delta E = 2$ seems reasonable for these film coated tablets which means that they all fail the test, even at the level of the recommended UV dose (200 Wh/m^2). The colour tolerance limit does however, have to be set at a level that is acceptable to both parties (i.e. customer and supplier). The limit should prevent a reduction in patient compliance caused by discolouration of the product but it should secure the process capability of the supplier so that the preparation can be delivered within a reasonable price, assumed that the change in colour does not lead to degradation of the active principle or a change in biopharmaceutical parameters. There is apparently a difference in light sensitivity between the two tablet batches investigated in the present study. Possible explanations are a difference in moisture content, a difference in crystal modification of ofloxacin or a difference in thickness and/or homogeneity of the film coating. For most of the investigated samples there is no apparent relationship between the observed change in colour and a degradation of the active principle.

The structure of two major degradation products has been postulated (Figs. 1 and 2). The fluorine has apparently been conserved in the degradation process and the degradation seems to have taken part in the N-methylpiperazine moiety, indicated by the loss of one nitrogen atom. This is consistent with previous reports on aqueous photolysis of ofloxacin and levofloxacin (one of the enantiomers of ofloxacin) (Fasani et al. 1999; Yoshida et al. 1993). Product 1 (HPLC $t_r = 9.6$ min, molecular weight 306) has not previously been reported among the aqueous photolysis products while product 2 (HPLC $t_r = 20.8$ min, molecular weight 278) has been identified as one of the main photolysis products from levofloxacin in aqueous solution (Yoshida et al. 1993). Ionization of the pseudo molecular ion of product 1 at 307 m/z (Fig. 1, upper section) in the ion trap (ms^2) demonstrates fragmentation to m/z 289 and loss of one molecule of water (Fig. 1, middle section). Further fragmentation (ms^3) of m/z 289 results in major peaks at m/z 279, 261 (loss of CHO), 247, 238 and 231 respectively (Fig. 1, lower section). Ionization of the pseudo molecular ion at m/z 279 (Fig. 2, upper section) of the second degradation product demonstrates fragmentation (ms^2) to m/z 261 and 235 and loss of one molecule of water and CO_2 respectively (Fig. 2, middle section). Further fragmentation (ms^3) of m/z 261 results in major peaks at m/z 251, 238, 206, 177 and 152 respectively (Fig. 2, lower section). The absorption characteristics of degradation products 1 and 2 cannot explain the observed change in colour towards red and yellow of the tablets. Both products have an absorption cut-off below 400 nm.

No difference in antimicrobial activity could be observed between exposed and non-exposed tablets under the experimental conditions selected. The combined results from quantitation and antibacterial screening indicate that the tablets do not have to be discarded although a slight change in surface colour might be observed under in use conditions. It should, however, be emphasized that before such a recommendation can be made, an extended stability study including a justification of impurity limits based on the ICH Drug Product Impurity Guidelines (ICH Q3A, Q3B) must be carried out. This is of major importance as photodecomposition of fluoroquinolone antimicrobials previously has been reported to result in an increased cytotoxicity and possibly also phototoxic and photocarcinogenic reactions (Martinez et al. 1998). A general problem for giving recommendations from results based on accelerated photostability studies is to make a correlation between in use conditions and the results obtained according to the ICH Guideline on phototstability testing. The recommended UV exposure (200 $Wh\,m^{-1}$) roughly corresponds to 1 to 2 days close to a sunny window while the end criterion of 1.2 mill lux h exceeds this minimum requirement by a factor of approximately 2.5 under the present experimental conditions (Tønnesen and Baertschi 2004). This should be taken into account in the evaluation of the results, particularly when one or more reactions are specifically related to the incident UV radiation.

As mentioned above, a change in colour that is easily detectable and thereby might be unacceptable to the patient is obtained at rather low exposure levels. Based on the findings in this study, a change in tablet coating to include a component that filters visible light in addition to UV radiation might provide a solution to the problem and prevent batch to batch variations with respect to light sensitivity. This would be of importance in order to increase the compliance, especially on the hospital wards. The results obtained are partly dependent on the conditions within the radiation chamber (e.g. exposure time and irradiance level). This emphasizes the importance of testing the samples under various conditions unless the results are unequivocal. For practical reasons the lag time between exposure and quantitative analysis could not be standardized in the present study. This might influence the results as dark reactions initiated by a photochemical process can proceed slowly and might only be detected after a certain lag-time.

4. Experimental

4.1. Materials

Ofloxacin, triethylamine and ammonium acetate were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). HPLC-grade acetonitrile was purchased from Merck, Darmstadt, Germany. The water was ultra-purified water, Milli-Q_{PLUS} (Millipore, Molsheim, France). Carmellosum monohydricum (carboxymethyl cellulose), solani amylum and magnesium stearate were purchased from Norsk Medisinaldepot, Oslo, Norway. Film coated ofloxacin tablets were purchased from the Hospital pharmacy, St. Olavs Hospital, Trondheim, Norway. They consisted of ofloxacin 200 mg, lactose monohydrate, maizenna, hydroxypropyl cellulose, carboxymethyl cellulose,

magnesium stearate (total weight 300 mg) with a film coating consisting of ethylhydroxypropyl cellulose, macrogol 8000, talcum and titanium dioxide (E171). Temperature recording strip 37–65 °C (WWR International).

4.2. Methods

4.2.1. Preparation of tablets

Uncoated tablets were prepared by direct compression of a mixture of ofloxacin (71%), carboxy methylcellulose (23%), lactose (2.5%), maizenna (3%) and magnesium stearate (0.5%) by use of a manuel single punch tabletting machine. The tablets had a diameter of 8 mm, and a thickness of 1–2 mm. The hardness was less than for the film coated tablets but the mechanical strength was sufficient for handling the tablets throughout the experiment. Placebo tablets were prepared according to the formulae above except that carboxymethyl cellulose was used as a substitute for ofloxacin. Pure ofloxacin was also compressed to tablets by use of the same equipment and under equal compression force. The film coated tablets were commercially produced and the details of the production conditions are not known.

4.2.2. Irradiation of the samples

4.2.2.1. Artificial daylight

Irradiation was performed in a SUNTEST CPS+ (Atlas, Gelnhausen, Germany). The light source was a xenon lamp (1.5 kW) equipped with a 6 mm special glass filter, transmitting light corresponding to exposure behind window-glass (cut-off approximately 310 nm). The cabinet was equipped with a SunCool^{TM} device (Atlas) which maintains a constant chamber temperature (30 °C). The intensity was measured by using a XenoCal Sensor (Atlas) with a spectral range 300–800 nm. Dark controls were added to evaluate temperature effects. The samples were exposed according to the ICH Guideline (9). The black standard temperature was set to 35 °C.

4.2.2.2. Natural daylight

Calibration of incident light was performed by use of a XenoCal Sensor (Atlas) with a spectral range of 300–800 nm.

4.2.3. Colour measurements and recording of reflectance spectra

The colour of the tablets was determined by use of a Minolta CM-3500 spectrophotometer. The tablet colour was measured by used of the $L^*a^*b^*$ color space (CIELAB). The color was determined at 3 different points of the tablet surface and each point was measured 3 times. Six tablets were used in each study. The reflectance spectra of the tablets were recorded by the same instrument.

4.2.4. HPLC

4.2.4.1. Preparation of standards and samples

A stock solution (1 mM) of ofloxacin was prepared in ultra-purified water containing 1 ml NaOH (0.01 M) per 100 ml. External standards (0.1–100 μM) were prepared from the stock solution after dilution by the HPLC mobile phase. The samples were prepared by carefully powdering each tablet in a pre-sterilized mortar. An accurate amount (200 mg) of the resulting powder was dissolved in 100 ml sterile saline. The samples were transferred to an Incubator Shaker (New Brunswick, Scientific co.inc. Edison, New Jersey, USA) and shaken at 220 rpm for 1 h at 37 °C. Each sample was then divided into 2 × 50 ml samples and centrifuged (Eppendorf Centrifuge 5810 R, Eppendorf AG, Hamburg, Germany) at 533 × g for 10 min. The clear supernatant was further divided into smaller volumes; each sample stored in a 200 μl sterile Nunc vial or a sterile test tube at −80 °C prior to microbiological testing or quantitative analysis.

4.2.4.2. Quantitative analysis

Ofloxacin was quantified using an HPLC method modified according to Maraschiello et al. (2001). Briefly, the HPLC system (HP 1100 series) consisted of a Hewlett Packard 1100 gradient pump equipped with an automatic injector, a 1100 Hewlett Packard diode-array absorption detector (295 nm) and a personal computer using Chem Station Software from Hewlett Packard. Aliquots (20 μl) of the samples were injected on a 3.5 μm Symmetry C-18 reversed phase column (46 × 100 mm), Part. No. WAT066220, connected to a C-18 guard column (5 μm). The mobile phase consisted of triethylamine (4.5 ml), double-distilled water (830 ml) and acetonitrile (140 ml). The pH was adjusted to 2.3 with 85% H_3PO_4 before addition of acetonitrile. The mobile phase was degassed by filtering through a 0.5 μm filter (Millipore, Bedford, Mass.). The flow rate was 1 ml/min. The retention time for ofloxacine was 4.2 min. The absorption spectra of standards and samples were identical with a characteristic peak at 295 nm. The retention times for the two main degradation products were 9.6 and 20.8 min respectively. Each analysis was performed in dublicate or triplicate.

4.2.5. LC-MS

Selected samples were analyzed by the same column as described above (*4.2.4.2*) on an LC-MS system (Agilent 1100) using the same mobile phase with the exception that triethylamine was replaced with 3% ammoniumacetat (50 mM). The LC-MS system consisted of a quaternary pump with an online degasser, an automatic liquid sampler, a thermostated column compartment and a single quadropol mass selective detector (g1946 SL with electrospray ion-source) using the scan mode. Ionization was obtained by electron spray (ESI).

4.2.6. Assay for antibacterial activity

MIC testing was performed by a macrodilution broth method as described elsewhere (NCCLS 2000). Ofloxacin concentration after powdering the tablets was determined by HPLC. Ofloxacin was diluted in Mueller-Hinton broth (Difco Laboratories, Detroit, USA) to achieve ofloxacin concentrations corresponding to 0.44, 0.22 and 0.11 nmol/l, respectively. To each tube was added *Escherichia coli* ATCC 25922 to a final concentration of 5×10^5 CFU/mL. The tubes were incubated at 35 °C for 20 h in ambient air before being read.

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