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Research paper

Photostability of dithranol

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Abstract

The influence of light on the antipsoriatic drug dithranol was investigated. A Suntest CPS with xenon lamp and liquid cooling was used as light source for the test. Solutions of dithranol in different organic solvents and in therapeutical concentrations in excipients for the preparation of topical formulations were tested under defined conditions. The extent and rate of photodegradation was determined and compared with the degradation of light-protected solutions. The drug content in the solutions was measured by HPLC. Degradation products were characterised and identified by diode array technique and HPLC–mass spectrometry coupling. The results showed a strong dependency of the photodegradation on the excipient or solvent used.

Keywords: Dithranol; Anthralin; Stability; Photostability; Topical use

1. Introduction

The investigation of the influence of light on the stability of drugs has gained more and more importance in recent years. For a variety of drug substances a considerable instability under the influence of light has been proved. This also concerns topically used drugs such as corticosteroids and antimycotics [1,2].

Although the semisolid topical formulations are protected by their packaging in light-resistant tubes against the influence of light during storage, a light-induced degradation may occur during production or after application to the skin affecting the effectiveness of the drug product. As dithranol is a very unstable drug substance, it seemed necessary to investigate its degradation under the influence of light.

Concerning the photostability of dithranol, there are so far no experimental results, although its instability in dermatics is generally known. Indications for that can be found in a monograph of the Deutscher Arzneimittel-Codex, where storage protected from light is required [3]. Other results mark dithranol under irradiation as a sensitizing substance in photoreactions [4]. Dithranol is transformed into its degradation product danthron by singlet oxygen. Under basic conditions the degradation products dithranol dimer and later dithranol brown appear out of dithranol under the influence of oxygen superoxide. There is no information about the extent of photoinstability of dithranol in dermatic excipients or in solvents.

The aim of this investigation on photostability was to determine the rate and extent of degradation in solution under the influence of light and to investigate the influence of different dermatic excipients on the photodegradation of the drug substance.

The drug substance was solved in the respective solvent, irradiated over a defined period of time and the amount of degraded drug substance was determined by means of HPLC. The manufacture with liquid excipients for dermatics was done in a therapeutic concentration of 0.1% dithranol. At this concentration the drug substance is completely soluble at room temperature in all excipients used. The irradiation was also carried out under defined condi-

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tions at room temperature. The rate of degradation of dithranol was determined depending on the excipient.

2. Materials and methods

2.1. Irradiation of dithranol

2.1.1. Apparatus

Suntest CPS with 1800 W xenon lamp and adjustable irradiation energy up to max. 765 $W \cdot m^{-2}$ (Heraeus, Hanau); UV special filter (UV edge at about 290 nm) (Heraeus); quartz glass cuvette 10 mm, quartz glass cuvette 2 mm (Hellma, Müllheim); ultrasonic bath Sonorex RK 510 (Bandelin, Berlin).

2.1.2. Materials

All excipients used were of pharmacopoeial quality, if described. Dithranol (Synopharm, Barsbüttel); medium-chain triglycerides (Hüls, Witten); macrogol 400 (Hoechst, Frankfurt); liquid paraffin (DEA, Hamburg); methanol p.a., acetone p.a. (Merck, Darmstadt); tetrahydrofuran p.a. (Fluka, Neu-Ulm).

2.1.3. Manufacturing of the test solutions

The drug substance was dissolved in a concentration of 10 mg·100 ml^{-1} in the organic solvents by ultrasonification and transferred into a quartz glass cuvette. The solutions of the drug substance in liquid excipients were prepared in a concentration of 0.1% in the same way.

2.1.4. Procedure of the irradiation tests

The irradiation of dithranol in solvents was done in 1cm quartz glass cuvettes in the Suntest CPS. The irradiation energy was kept at 720 $W \cdot m^{-2}$. By water cooling the heating of the samples over a temperature of 25°C was excluded. A reference sample under the protection of aluminium foil was examined under the same conditions. Over a period of 3 h samples were taken and the extent of degradation was examined by HPLC. Examination of the solutions of dithranol in dermatic excipients was done in 2-mm quartz glass cuvettes. All other conditions remained the same as for the tests with dithranol in solvents.

2.2. Determination of dithranol by HPLC

2.2.1. Apparatus

HPLC system. High pressure pump units M 6000 A, system controller M 720, automatic injection system WISP 710B, integrator data module M 730, photodiode array detector M 990 with PC evaluation unit, and multiwavelength detector M 490 (Millipore/Waters, Eschborn).

Chromatographic system. Column LiChrocart 125-4, LiChrospher RP Select B (5 μm), pre-column Hibar with LiChrospher 100 RP 18 (5 μm) (Merck).

Mobile phase. Acetonitrile Lichrosolv (Merck), distilled water, manufactured by Muldestor (Wagner and Munz, München); acetic acid p.a. (Fluka). Ratio of mobile phase acetonitrile/water/acetic acid was 58:37:5. Filter: 0.22 μm cellulose acetate (Sartorius, Göttingen).

Sample preparation. Ultrasonic bath Sonorex RK 510 (Bandelin, Berlin); 0.22 μm PTFE filter (Sartorius); dithranol (Aldrich, Steinheim); 2-chlorthioxanthone (Aldrich); methanol p.a. (Merck); tetrahydrofuran p.a. (Fluka).

The amount of the test solutions corresponding to 2 mg of dithranol was spiked with 25 ml of a solution of 1.5 mg·100 ml^{-1} of 2-chlorthioxanthone (internal standard) in tetrahydrofuran and homogenised in an ultrasonic bath. The samples were transferred into a 50-ml volumetric flask and filled up to the mark with methanol. The solutions were filtered through a 0.22-μm PTFE-filter and transferred into HPLC vials. The concentration of dithranol in the solutions was determined by HPLC.

HPLC determination. The samples were analysed in a concentration of 4 mg·100 ml^{-1} (injection volume 25 μl) on a LiChrocart 125-4, LiChrospher RP Select B (5 μm) column. The flow rate was 1.1 $ml \cdot min^{-1}$. The wavelength for the detection of dithranol and its degradation products was 394 nm. The retention time for dithranol was 5.9 min, for the internal standard 2-chlorthioxanthone 8.2 min, for the degradation products danthron 5.1 min and dithranol dimer 13.1 min.

Quantitative evaluation. The method for the quantitative evaluation of the chromatogram was the internal standard method. The necessary peak areas for substance and standard were determined by automatic integration. Samples and standards were quantified by three determinations. For a calibration curve concentrations of the substance were used between 1 mg·100 ml^{-1} and 5 mg·100 ml^{-1} (5 points). The concentration for the internal standard in the injected solution was 0.75 mg·100 ml^{-1}.

Data of the HPLC determination. The analytical method for the quantification of dithranol and its separation from degradation products (danthron, dithranol dimer) is described in the dithranol monograph of the DAC, 1986 [3]. Revalidation data for the adapted method [5] are as follows. Capacity factor (k'): 4.2 (dithranol); selectivity (α): 1.22 (dithranol/degradation product: danthron); resolution (R): 1.76 (dithranol/degradation product: danthron); recovery: 98.1–101.7% (spiked samples of dithranol in different semi-solid matrices); precision (s_{rel}): 1.11% (repetition precision, $n = 6$); linearity (correlation coefficient): 0.9998.

2.3. Mass spectrometric determinations

2.3.1. Apparatus

Low pressure gradient pump system HP 1050, UV-VIS detector HP 1050 V, integrator HP 3396, HP 5989A MS engine with particle beam interface (Hewlett-Packard, Waldbronn).

2.3.2. Materials

Column EcoCart 125-3 LiChrospher 60 RP Select B (5 μm); acetonitrile Lichrosolv (Merck); distilled water, manufactured by Muldestor (Wagner and Munz); acetic acid p.a. (Fluka); 0.22 μm cellulose acetate filter, 0.22 μm PTFE filter (Sartorius); dithranol (Aldrich); methanol p.a. (Merck); tetrahydrofuran p.a. (Fluka).

2.3.3. Experimental conditions

HPLC. Column EcoCart 125-3 LiChrospher 60 RP Select B (5 μm); flow rate 0.5 ml/min; injection volume 20 μl; Solvent A: acetonitrile 58T/water 37T/acetic acid 5T; Solvent B: acetonitrile 90T/water 10T; gradient: 0–6 min 100% solvent A, 6–12 min 60% solvent A/40% solvent B, 12–20 min 100% solvent A; detection wavelength 394 nm; interface: helium pressure 55 psi, temperature 60°C; mass spectrometer: 50–500 amu.

2.3.4. Procedure for the mass determinations

The experimental conditions for the HPLC separation of the drug substance and its degradation products had to be adapted for the different flow rates and column of the HPLC–mass spectrometry. For the separation of the drug substance at acceptable analytical times a gradient system was chosen. The concentration of the sample solutions were the same as for the quantitative HPLC determinations. After separation of the substances by HPLC the single peaks were transferred into the mass spectrometer by a particle beam interface. The masses occurring in the mass spectrometer were registered in a total ion chromatogram, and the characteristic masses for the different peaks were classified.

3. Results

3.1. Photostability of dithranol in organic solvents

The photodegradation of dithranol in solution in solvents of different polarity was examined for the determination of possible differences in the rate and way of degradation.

Methanol, acetone and tetrahydrofuran were used as solvents to prepare solutions with a concentration of 10 mg·100 ml^{-1} of dithranol. These solutions were irradiated in 1-cm quartz glass cuvettes for up to 3 h. To distinguish the degradation of the drug substance in solution from the light induced degradation samples of the solution were kept under the same conditions under light protection. The samples were irradiated in a Suntest CPS with a xenon lamp. The produced xenon light is adapted to natural sunlight in a wide range. Deviations are corrected by the use of an IR reflector and special UV filters.

With the solution of dithranol in methanol, a considerable degradation can be observed even without the influence of light. The content of dithranol decreased by 79.5% within 3 h in a light-protected solution. Under irradiation the solution in methanol showed an initial acceleration of the degradation. After 30 min in the irradiated solution 40.5% of the dithranol had degraded, under light protection only 24.5%. However, after 3 h the remaining content of the drug substance in both solutions was the same (Fig. 1). In methanol only a little amount of danthron was found. The major degradation product was dithranol dimer.

The irradiation of dithranol in tetrahydrofuran proved to be much more stable than the solution in methanol (Fig. 2). The light-protected solution showed a decrease by only 1.7%. Under irradiation the decrease in content was accelerated to 6.7% after 3 h. This acceleration could be observed for the first time after 1 h. As in the solution in methanol, the major degradation product in tetrahydrofuran was the dimer structure. Only little danthron could be found in the solutions.

In acetone a higher photodegradation of dithranol occurred than in methanol or tetrahydrofuran (Fig. 3). The drug substance was stable under light protection for the test period of 3 h. No degradation could be determined in the solution. Under the influence of light, after 15 min a strong degradation could already be observed. The amount of degraded dithranol was 43.5%. After 3 h the content of drug substance decreased by 63.9%. In acetone as solvent the pathway of the degradation reaction is contrary to methanol and tetrahydrofuran. Here the main degradation product is danthron. The appearance of danthron can be noticed as early as after a few minutes by a strong yellow colour of the solution. The occurrence of a new peak in the chromatogram of the light-exposed acetone solution is also noteworthy. Because of the shorter retention times it seems to be a more hydrophilic degradation product, being only existent in the acetone solution under the influence of light.

3.2. Photostability of dithranol in dermatic excipients

For the investigations on the photostability of dithranol in excipients for topical formulations, liquid macrogol 400 as a hydrophilic excipient and medium-chain triglycerides and liquid paraffin as lipophilic excipients were used. In these

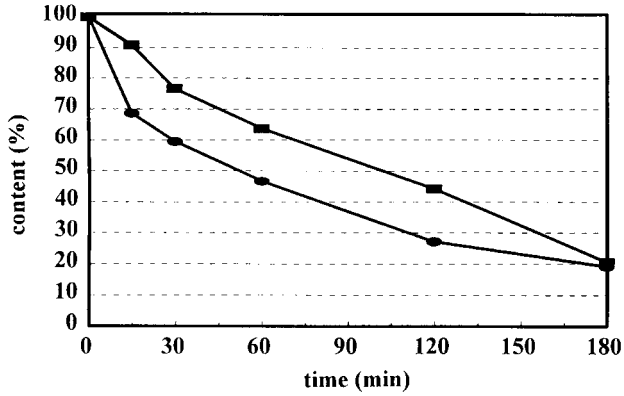

Fig. 1. Degradation of dithranol in methanol with and without the influence of light. ■, Dithranol 10 mg/100 ml light-protected; ●, dithranol 10 mg/100 ml light-exposed.

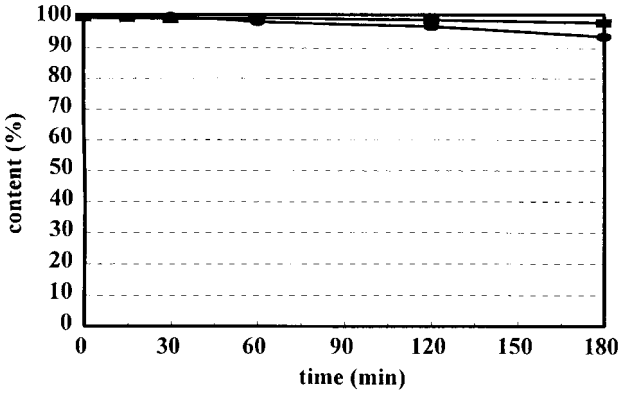

Fig. 2. Degradation of dithranol in tetrahydrofuran with and without the influence of light. ■, Dithranol 10 mg/100 ml light-protected; ●, dithranol 10 mg/100 ml light-exposed.

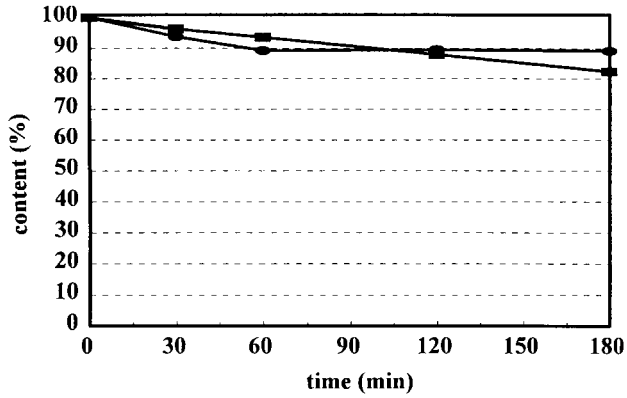

Fig. 4. Degradation of dithranol in macrogol 400 with and without the influence of light. ■, Dithranol 0.1% light-protected; ●, dithranol 0.1% light-exposed.

bases the drug substance was incorporated in a concentration of 0.1%. In this concentration dithranol is soluble in all three bases at room temperature. The solutions were irradiated for 3 h by the Suntest CPS. The thickness of the layer was 2 mm so as to be comparable to an application on the skin. A part of the formulation was kept under the same condition, but light-protected to compare the degradation without the influence of light.

In the macrogol solution the drug content decreased by 6.5% within 1 h and 17.7% within 3 h without the influence of light. In the irradiated solution the decrease was stronger in the beginning, up to 10.7% after 1 h. After 1 h no more accelerating influence of light could be observed (Fig. 4). In macrogol, danthron and dithranol dimer appear as degradation products.

The degradation of the drug substance in medium-chain triglycerides was considerably slower than in macrogol. Within 3 h no degradation of dithranol could be observed. In the irradiated solution the decrease of the drug content was 10.1% after 3 h (Fig. 5). This was almost the same value as for the macrogol solution. The main degradation product in medium-chain triglycerides was the dimer structure of dithranol.

For the drug substance solved in liquid paraffin a good stability, comparable to medium-chain triglycerides, was found. No degradation of dithranol occurred within 3 h. However, under irradiation the degradation in liquid paraffin was the fastest of all tested excipients. The degradation after 30 min was already at 16.2%. After 3 h the drug content decreased by 67.6% (Fig. 6). While in medium-chain triglycerides and macrogol 400 the usual degradation products occurred, in liquid paraffin danthron was the major degradation product as it could also be observed in acetone. An additional more hydrophilic degradation product, which occurred in acetone under the influence of light, could not be found in liquid paraffin.

3.3. Mass spectroscopic investigations

For the identification of the degradation products appearing under the degradation of dithranol, mass spectroscopic investigations were carried out. These were done after a preceding separation of the substances by HPLC. For this a coupling of HPLC and mass spectrometry by a particle beam interface was used. This combination allows a selective evaluation of the mass chromatograms of the substances separated by HPLC. For the investigations the samples were separated by HPLC after irradiation, and

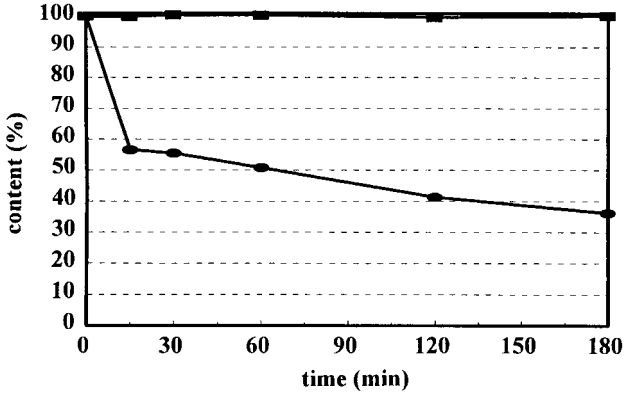

Fig. 3. Degradation of dithranol in acetone with and without the influence of light. ■, Dithranol 10 mg/100 ml light-protected; ●, dithranol 10 mg/100 ml light-exposed.

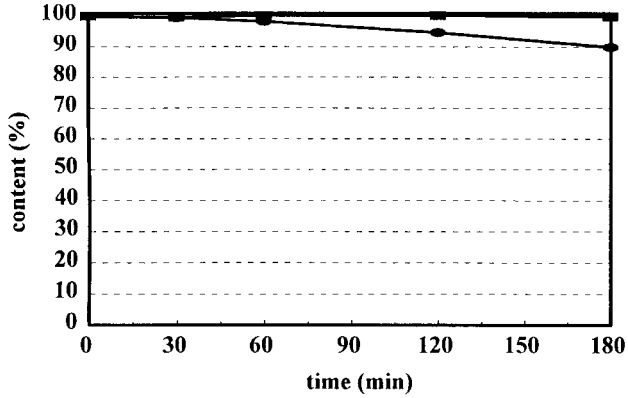

Fig. 5. Degradation of dithranol in medium-chain triglycerides with and without the influence of light. ■, Dithranol 0.1% light-protected; ●, dithranol 0.1% light-exposed.

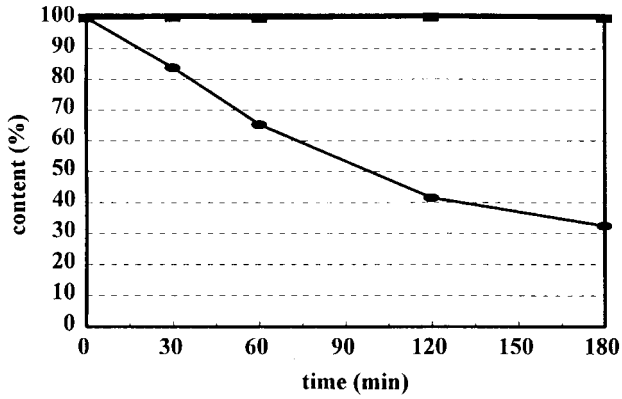

Fig. 6. Degradation of dithranol in liquid paraffin with and without the influence of light. ■, Dithranol 0.1% light-protected; ●, dithranol 0.1% light-exposed.

the mass chromatograms for the different peaks were classified.

Investigating a solution of dithranol with its degradation products by HPLC–mass spectrometry, three different peaks can be characterised in a total ion chromatogram (Fig. 7). These are the peak of dithranol (8.1 min), and the peaks of the degradation products danthron (6.9 min) and dithranol dimer (13.4 min).

Characteristic for the mass spectrum of dithranol is the strong signal of the mass 226 which corresponds to the ionised fragment of the molecule. Furthermore, characteristic fragments of masses 152 and 198 can be observed (Fig. 8).

The mass spectrum of danthron (Fig. 9) shows as a major component the mass of the molecular ion of 240. Compared to the dithranol molecule ion, this is an effect of the oxidation reaction with the entry of an oxygen molecule and the exit of two hydrogen molecules. Further characteristic masses in the danthron spectrum can be registered at 212 and 184.

The mass spectrum of the dimer structure (Fig. 10), the second degradation product of dithranol, is characterised by showing the same masses registered in the spectrum of dithranol. No molecular ion with a theoretical mass of 450 could be found in the spectrum. No fragment in the spectrum showed a mass of more than the mass of the dithranol molecule. Only in the lower range of the spectrum are there differences with the mass of 115, typical for the dithranol spectrum, not occurring in the spectrum of the dimer.

The mass spectroscopic investigations did not indicate any further degradation products under the influence of light. The characteristic degradation products could be found also after irradiation. Only danthron and dithranol dimer could be observed in the spectrum. The extent of formation of the substances, nevertheless, was different. In acetone and paraffin the major part of dithranol degraded to

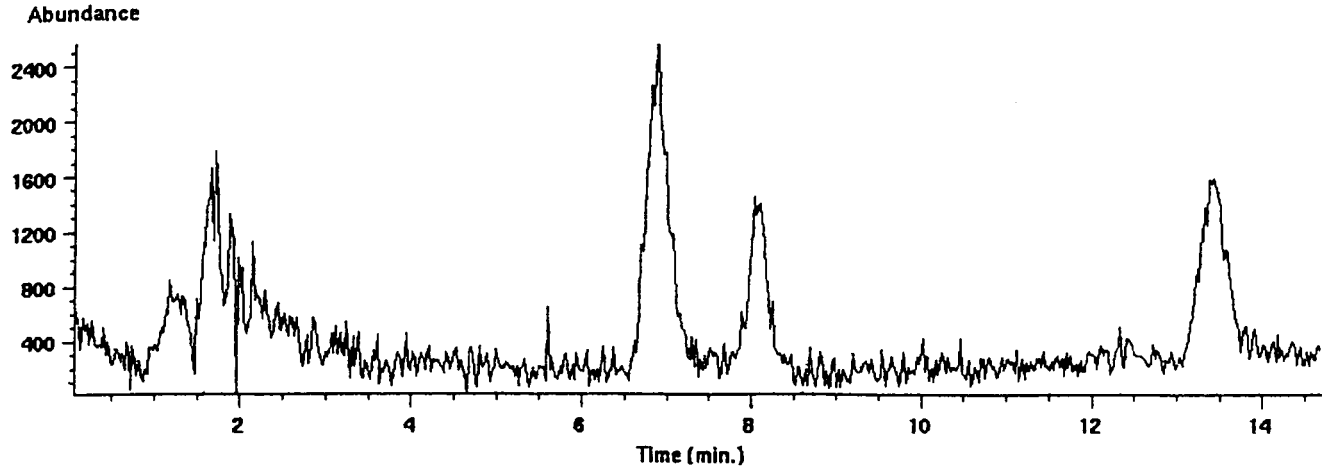

Fig. 7. Total ion chromatogram of dithranol and its degradation products. Danthron, 6.9 min; dithranol, 8.1 min; dithranol dimer, 13.4 min.

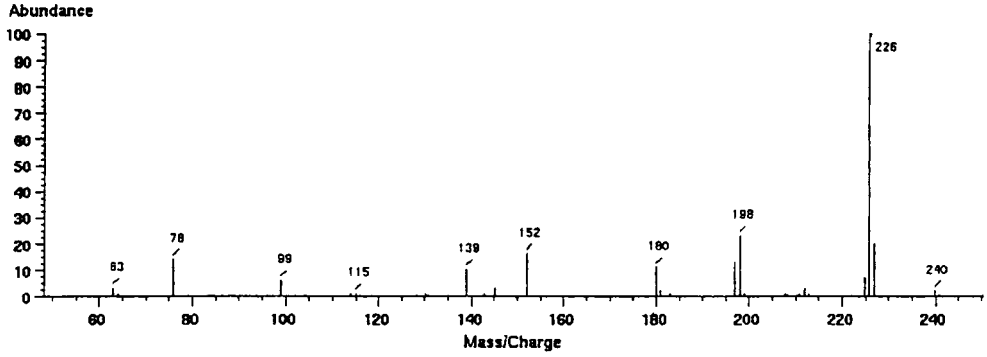

Fig. 8. Mass spectrum of dithranol.

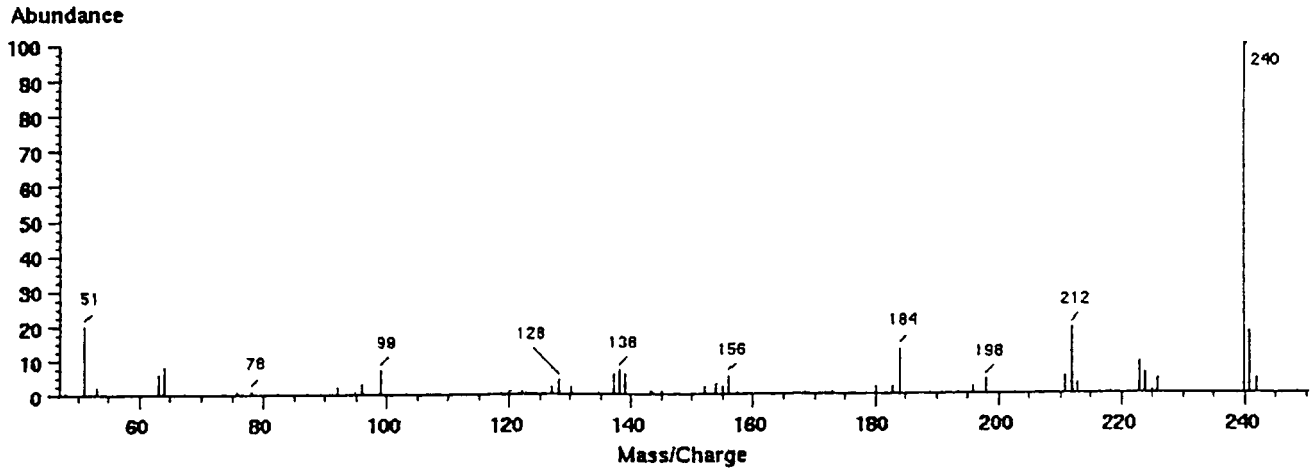

Fig. 9. Mass spectrum of danthron.

danthron. In methanol, tetrahydrofuran and medium-chain triglycerides, the main degradation product was dithranol dimer, whereas only little danthron was found. In macrogol both degradation pathways could be observed.

The additional degradation peak in acetone, which could be found in the HPLC chromatogram (Fig. 11), could not be identified with the HPLC–mass spectrometry coupling. The UV spectrum of the peak obtained by diode array technique had maxima similar to the spectrum of dithranol.

4. Discussion

Investigations on the photostability of dithranol showed a considerable dependency of the degradation on the influence of light. The extent and the degradation products are strongly dependent on the solvent or excipient used (Fig. 12).

In acetone the strongest degradation occurred. Here the influence of light is the decisive factor for the accelerated degradation, because the drug substance is stable under light protection during the same period of time. In methanol as a solvent there is only a little dependency of the degradation rate on the influence of light. The degradation is so fast that the acceleration by irradiation is only significant in the beginning. Tetrahydrofuran is a solvent in which dithranol is most stable under the influence of light. Here only a little acceleration of the degradation could be found under irradiation. The polarity of the solvent seems to have only a minor effect on the degradation mechanism because the dipole moments of methanol (1.7 debye) and tetrahydrofuran (1.63 debye) show no great difference. Also the dielectricity constants of the solvents (methanol 32.6, tetrahydrofuran 7.4, acetone 20.7) do not show any direct correlation to the photodegradation. A protective influence of the absorption of the solvent can also be excluded, because the absorption of methanol and tetrahydrofuran starts only below 290 nm. The light irradiated through the sample does not reach below this range. Only acetone shows little absorption up to 350 nm. In this range a protective influence of the solvent itself may be possible. However, in acetone the photodegradation is accelerated. A possible

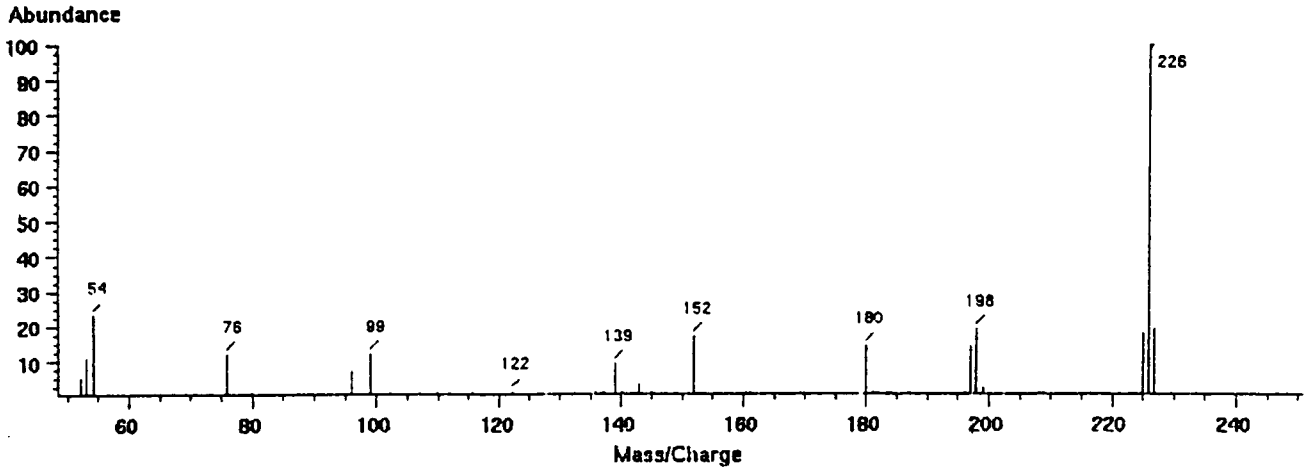

Fig. 10. Mass spectrum of dithranol dimer.

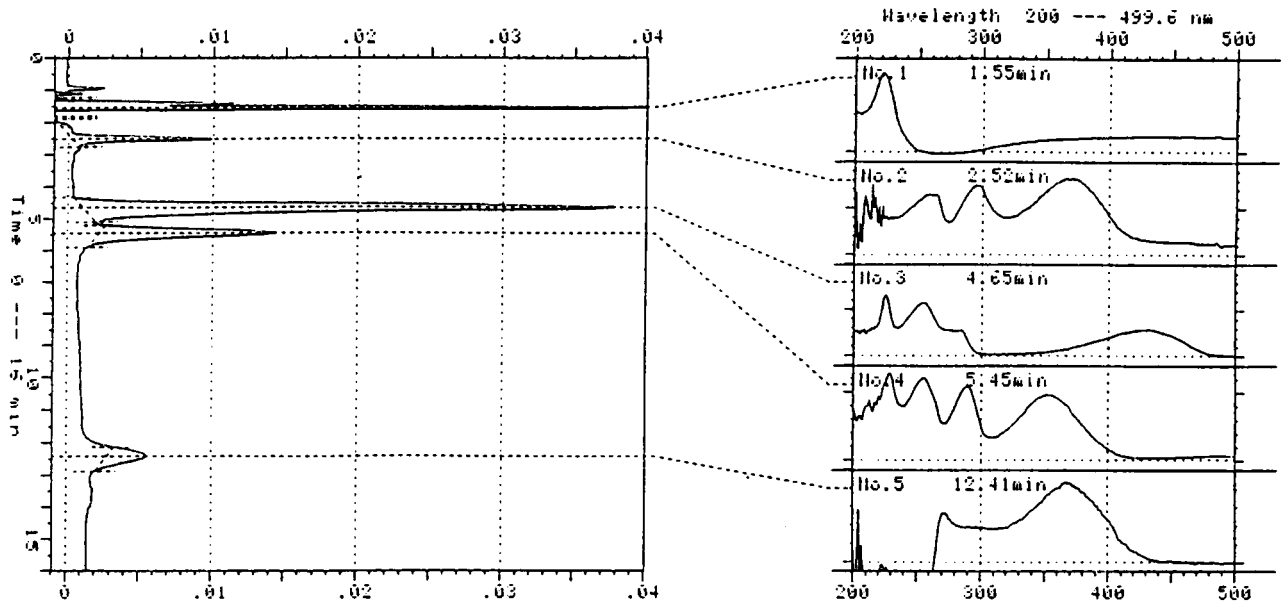

Fig. 11. Photodiode array chromatogram of dithranol in acetone after light exposure. Photoproduct, 2.52 min; danthron, 4.65 min; dithranol, 5.45 min; dithranol dimer, 12.41 min.

reason for this may be the fact that acetone itself is known as a photosensitiser in photochemical reactions [6] and can initiate such a reaction.

For pharmaceutical use, the results of the irradiation in excipients for the preparation of topical formulations are of special importance. It could be shown that in macrogol, where the drug substance is already unstable without irradiation, the influence of light plays only a subordinate role for the degradation reaction. Also in methanol, no additional acceleration of the degradation is found.

In medium-chain triglycerides, in which the drug substance shows much better stability than in macrogol, there is only little acceleration of the degradation under the influence of light. In a paraffin base, which offers a high stability for dithranol under the exclusion of light, a distinct instability exists under the influence of light. Because paraffins are preferably used as a base for dithranol formulations in therapy, this has to be considered during application. Low drug concentrations used for long-term treatment on the patient's skin are especially affected. As with the solvents there was no protection due to the absorption of the base itself. An influence of traces of impurities in the excipients, as this is possible for paraffin [7], cannot generally be excluded.

The accumulation of degradation products under irradiation is partly different from the accumulation under light protection. In paraffin under the exclusion of light only dithranol dimer and dithranol brown are formed as degradation products. If the drug substance is irradiated in paraffin the oxidation product is almost entirely danthron. This leads to the conclusion that under irradiation the temperature-controlled pathway of degradation has only little meaning for the total degradation. The acceleration of the degradation

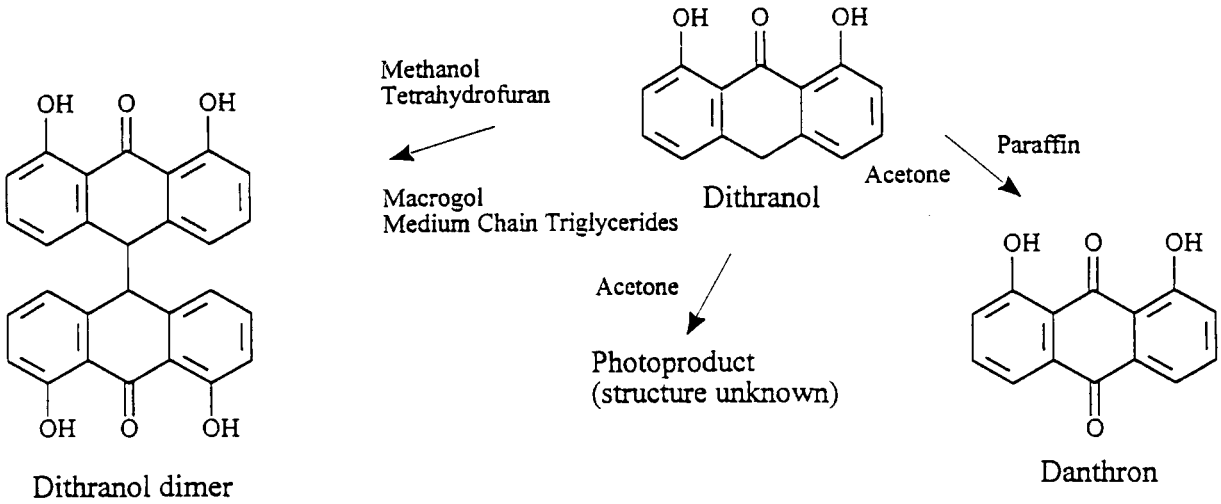

Fig. 12. Main degradation products of dithranol depending on solvent and excipient.

can be mainly attributed to the degradation pathway induced by light energy. A similar observation can be made for acetone as a solvent. Here an additional hydrophilic degradation product is formed besides the usual degradation products which occur only in acetone. In medium-chain triglycerides and methanol only a small amount of the total degradation is formed as danthron. Also, in both solutions only little additional degradation of dithranol under the influence of light was found. Here the pathway preferring the photodegradation to danthron is hardly followed. Further investigations have to show the mechanisms of the dependency of the degradation reactions on the excipients and solvents used.

The mass spectroscopic investigations on the degradation products allow on the basis of the characterisation of the mass spectra an identification of dithranol and danthron. For the dimer structure this cannot be achieved. The structure of the molecule is so unstable that ionisation leads to an immediate cleavage of the dimer in the mass spectrometer. The degradation product formed in acetone could not be characterised by mass spectrometry. The concentration of this structure was not high enough, and further degradation too fast, to give a characteristic mass spectrum.

With the basic information gained from this investigation a further study on rate, extent and mechanisms of photodegradation on the skin should be performed in the future.

Acknowledgements

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