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RESEARCH ARTICLE

Predicting the photostability characteristics of active pharmaceutical ingredients using electron paramagnetic resonance spectroscopy

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Abstract

Objective: The purpose of this study was to determine if electron paramagnetic resonance (EPR) spectroscopy could be used to develop a quick method to predict the longer-term photostability characteristics of active pharmaceutical ingredients (APIs) for use in the early development phase.

Materials and methods: EPR spectroscopy was used to study the photodegradation of nearly fifty different APIs in the solid state.

Results: Free radical formation was detected in all but three of the APIs studied and singlet oxygen formation was also detected in the presence of five of the APIs tested. The extent of free radical formation in each API after 10 minutes exposed to light was used to rank the APIs in terms of their stability characteristics and determine the probable risk of photodegradation of the API during International Conference on Harmonisation (ICH) compliant photostability testing. A correlation was obtained between the extent of free radical formation on exposure to light and the known level of photodegradation products formed during ICH compliant testing.

Conclusions: The EPR methods were shown to have the potential to predict the ICH level of photodegradation for an API in the solid state using only a small amount of sample and after just 10 minutes exposed to light. This testing can be performed in a shorter time frame than ICH compliant testing and can potentially be used early in development to predict the photostability characteristics of an API.

Keywords: Photodegradation, free radicals, singlet oxygen, prediction, pharmaceutical

Introduction

Along with heat, moisture and oxygen, light is an important factor in considering the stability of APIs. The International Conference on Harmonisation (ICH) guidelines state that the intrinsic photostability characteristics of all new APIs and products should be evaluated to demonstrate that, as appropriate, light exposure does not result in unacceptable change[1]. These guidelines have been implemented in Europe, the United States and Japan for over 10 years[2]. Photodegradation is defined as degradation caused by exposure to light and can depend on wavelength, intensity and time of exposure[3], temperature, the presence of excipients, heavy metals and oxygen[4]. Photodegradation can result in loss of potency of the product, toxic degradation products and light-induced side effects after administration. For ICH compliant testing, samples should be exposed to an overall illumination of not less than 1.2 million lux hours and an integrated near UV energy of not less than 200 watt hours/square meter within the spectral range of 320–800 nm[1]. This equates to a number of days of storage in a standard light cabinet. API photostability testing consists of two parts, forced degradation and confirmatory testing[1]. Forced degradation testing allows the overall photostability characteristics of the API to be evaluated for method development purposes

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and degradation pathway elucidation. Confirmatory studies provide the information necessary for handling, packaging and labeling. Samples are analyzed for any changes in physical properties, strength and degradant levels.

To determine whether a molecule is likely to undergo photodegradation, the absorption spectrum of the molecule can be compared to the emission spectrum of the light source or day light for any overlap. A large number of APIs are white and so do not absorb visible light but may absorb UV radiation[5]. It is also possible to predict the photostability of a new API from the molecular features[6] or at least determine the likely degradation products. Functional groups that can undergo photodegradation are well known[7] and the types of photodegradation reactions of APIs have been well described elsewhere[7].

In the solid state, light radiation can be reflected, refracted, scattered, transmitted or absorbed by a sample so normally only a limited portion of a static sample is actually exposed to light. Particle size, surface area, color, crystal structure and polymorphic[8], hydrate and salt forms are all important properties that can affect photostability in the solid state[9]. In the literature, there have been relatively few studies reported of photostability of APIs in the solid state[3], although there are many published studies performed in solution.

Primary photochemical reactions can only occur when a molecule absorbs energy and becomes electronically excited, although the molecule may not necessarily degrade due to competitive dissipative pathways[10,11]. The absorption of light by a ground state molecule results directly in an electronically excited singlet state[7]. The excited singlet state molecule can then undergo a number of processes, including radiationless deactivation back to the ground state, fluorescence, intersystem crossing to a triplet state, energy transfer or a photochemical reaction[7]. From the excited triplet state, a molecule can also undergo radiationless deactivation back to the ground state, phosphorescence, electronic energy transfer to ground state molecular oxygen[12] (resulting in singlet oxygen) or a photochemical reaction. In the presence of oxygen photodegradation mechanisms can therefore be very complicated. Singlet oxygen and the superoxide anion can both be formed which are very reactive species[7,12]. Many photochemical reactions, therefore, have complex mechanisms and understanding can be assisted by EPR spectroscopy[13].

EPR spectroscopy is a very sensitive technique for the detection of unpaired electrons. Free radical formation on exposure to light can, therefore, be detected after short exposures allowing fast analysis. In addition, only a small amount of sample is required compared to more formal ICH compliant photostability testing. EPR spectroscopy has previously been used to study the photodegradation and phototoxicity of Nifedipine, used in the treatment of hypertension, in solution and to identify the radical intermediates[14,15]. Chignell et al. have also extensively used EPR spectroscopy and spin trapping techniques to investigate the reactive radical intermediates involved in the photodegradation and phototoxicity of a range of active molecules in solution and published over 20 papers in the area, including studies of Amiodarone[16], an anti-arrhythmic drug, and benoxaprofen[17], an anti-inflammatory drug. The photodegradation of additional non-steroidal anti-inflammatory drugs[18] and anti-malarial drugs[19,20] has also been studied in solution by EPR spectroscopy. In the solid state, the photodegradation of Nifedipine[21] and more recently, simvastatin and lovastatin have been previously investigated by EPR spectroscopy[22], but solid-state studies in this area are limited. Singlet oxygen sensitized by an API can also be detected by EPR spectroscopy, by photolysing an aerated solution of the API in presence of a hindered amine e.g., 2,2,6,6-tetramethylpiperidine[23,24]. The amine then reacts with singlet oxygen to form Tempo, a stable nitroxide radical species that can be detected by EPR spectroscopy.

EPR has previously been used to predict the photostability of polymers[25,26] and the effect of UV radiation on the color of leather in the textile industry[27] but has not to our knowledge been used to predict photodegradation in the pharmaceutical industry. The use of EPR combined with chemometric techniques to measure the extent of degradation of an API has, however, been demonstrated in terms of oxidative degradation[28]. In this work, the photodegradation of a range of APIs has been studied *in situ* in an EPR spectrometer both in the solid state (to monitor free radical formation) and in solution (to determine whether each API has the potential to generate singlet oxygen). For the APIs that were shown to degrade only via a free radical route, the extent of radical formation in the solid state after exposure to light for a fixed period in the EPR spectrometer was then used to predict the longer-term photostability characteristics of the API.

Methods

API samples

The APIs used in this study were all in development at AstraZeneca, as detailed in Table 1. The numerical identification of each API was assigned purely in the order in which the samples were tested. The majority were basic compounds in either the free base or salt forms. A number of APIs were tested as both the free base and as a salt form. A smaller number were acidic compounds containing carboxylic acid groups. The APIs tested contained a wide range of other functional groups including amines, amides, alcohols, phenols, aldehydes, ketones, ethers, cyclopropane, sulphoethers, sulphonamides, nitrile and phosphate groups and heterocyclic groups such as pyrroles, thiophenes, azoles, thiazoles, triazoles,

Table 1. Properties of the APIs tested.

API No.	Form			Common functional groups																		Therapeutic area
	Free base	Free Acid	Salt	Amine	Amide	Alcohol	Aldehyde/ ketone	Ether	Cyclopropane	Sulphother	Sul- phonoamide	Nitrile	Carboxylic Acid	Phosphate	Pyrrole	Azole	Pyrolli- dine	Piperidine	Pipera- zine	Pyridine	Diazine	
1			X					X							X		X				X	Oncology
2			X	X	X											X					X	Oncology
3	X			X				X										X			X	Oncology
4			X	X	X													X			X	Oncology
5	X			X							X					X				X	X	Oncology
6	X				X				X		X	X				X				X	X	Inflammation
7			X	X	X			X						X		X					X	Oncology
8	X				X				X										X		X	Oncology
9	X					X				X												Oncology
10	X											X				X						Oncology
11	X				X					X		X										Oncology
12	X			X	X	X										X						Oncology
13	X				X													X				Oncology
14			X			X					X		X						X			Cardiovascular
15	X				X		X	X										X	X			Oncology
16			X	X	X			X						X		X			X			Oncology
17	X							X				X					X			X		Gastrointestinal
18		X				X																Inhalation
19	X					X		X		X	X										X	Cardiovascular
20			X		X							X								X		Inflammation
21			X	X	X	X																Inflammation
22	X							X				X									X	Inhalation
23	X				X				X										X		X	Inflammation
24			X		X				X										X		X	Inflammation
25	X						X	X			X								X		X	Inflammation
26	X						X				X								X	X	X	Inflammation
27		X		X	X								X			X						Cardiovascular
28	X				X		X														X	Cardiovascular
29	X				X		X									X					X	Oncology
30			X	X	X	X	X															Inhalation
31	X			X	X	X	X									X						Inhalation
32			X	X	X											X					X	Inhalation

Table 1. Continued

API No.	Form			Common functional groups																	Therapeutic area
	Free base	Free Acid	Salt	Amine	Amide	Alcohol	Aldehyde/ ketone	Ether	Cyclopropane	Sulphother	Sul- phonoamide	Nitrile	Carboxylic Acid	Phosphate	Pyrrole	Azole	Pyrolli- dine	Piperidine	Pipera- zine	Pyridine	Diazine	
33			X	X			X													X		Neuroscience
34			X	X	X							X				X		X				Infection
35	X			X												X					X	Oncology
36			X	X	X														X			Neuroscience
37	X				X					X										X		Neuroscience
38	X			X	X		X												X			Neuroscience
39	X			X	X											X		X		X		Neuroscience
40		X			X		X					X			X	X		X				Infection
41		X			X		X					X			X							Infection
42	X			X			X			X								X		X		Neuroscience
43	X			X	X		X												X	X		Oncology
44	X			X			X				X					X				X		Oncology
45	X			X	X		X														X	Neuroscience
46	X			X					X										X			Neuroscience
47			X			X	X		X										X			Neuroscience

oxadiazoles, pyridine, diazines, thiazepine, azetidine, thiazolidines, dioxolanes, piperidine, piperazine and morpholines.

EPR analysis

The EPR spectra were recorded at room temperature on a *cw*-Bruker EMX X-band spectrometer operating at 100 kHz field modulation and equipped with an ER 4104OR optical transmission cavity. A LOT Oriel 300 W Xenon arc lamp, fitted with a liquid IR filter, a <310 nm cut off filter and a focusing assembly, was used to shine light down a quartz rod (3 mm diameter × 25 cm length) into the EPR spectrometer sample cavity. The irradiance of the light source was measured at the end of the quartz rod, as experienced by the samples, as approximately 44 kLux and 17 Wm^{-2}.

Solid-state radical formation analysis

Approximately, 5 mg of the API under test was accurately weighed and clamped between the slides of an EPR tissue cell (WG-807-Q) to allow maximum surface area for exposure to the irradiation. An initial EPR spectrum of the non-degraded sample was recorded. The EPR spectrum of the API was then recorded during exposure to light over a 10-min period. The extent of radical formation during the period exposed to light was calculated by measuring the peak area of the free radical response in the 10-min light exposed spectrum and subtracting the peak area of any response in the initial spectrum for each API and reported per mole of API exposed to light.

Singlet oxygen formation analysis

One hundred microliters of 2,2,6,6-tetramethyl piperidine (Sigma Aldrich) was diluted to 100 mL with methanol to prepare the hindered amine solution. Approximately, 10 mg of each API was then accurately weighed and dissolved in 10 mL of the hindered amine solution, aerated and transferred into an EPR flat cell (ER 160FC-Q) for analysis. The samples were then exposed to light for up to 60 min *in situ* in the EPR spectrometer and EPR spectra were recorded every 6 min to monitor for formation of the Tempo radical. A schematic of this indirect EPR method for the detection of singlet oxygen generation is shown in Figure 1.

Qualitative data analysis

The results for the extent of free radical formation on exposure to light were used to rank the APIs tested in terms of their photostability characteristics and the singlet oxygen formation results were used to highlight any APIs that had the potential to degrade via this additional mechanism of photodegradation.

Quantitative data analysis

A set of ten APIs, currently or recently in development, for which the level of photodegradation products formed during ICH compliant photostability testing, was known and that were shown not to generate singlet oxygen formation on exposure to light were used as a calibration set. The extent of free radical formation in these 10 APIs was correlated to the known level of photodegradation products formed after ICH compliant testing, from which the level of photodegradation in other APIs can be predicted.

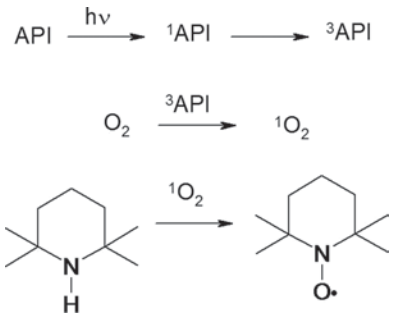

Figure 1. Schematic of indirect EPR method for the detection of singlet oxygen generation.

Results

The API types tested contain various functional groups, so the free radicals formed in each API on exposure to light are different. The aim of this work was to develop a method to predict the extent of degradation over the longer term based on the amount of radicals formed during a short exposure to light. The fact that the type of radical formed from each API cannot be published is not thought detrimental to the study. The issue of different radical types potentially having different lifetimes themselves and affecting the quantitative results was addressed as much as possible by optimizing the analysis time. Sufficient exposure to light was required such that significant and differential free radical formation occurred, but over a short period of time such that the signal did not start to decay as the lifetime of the radicals was reached. This was determined as a 10-min light exposure time during the method development phase of this study. Examples of the typical spectra recorded are shown in Figure 2, thought to be dominated by carbon and oxygen based radicals. Free radical formation was detected in the majority of the APIs tested, as shown in Figure 3. Only three of the APIs showed no detectable free radical formation on exposure to light.

Five of the APIs tested also showed significant singlet oxygen formation when dissolved in methanol and exposed to light for up to 60 min, as indicated in Figure 3. Examples of the spectra obtained due to generation of Tempo, confirming singlet oxygen formation, are shown in Figure 4. These five APIs also showed significant free radical formation on exposure to light. Although the structures of these five APIs cannot be published here, no common functional group could be identified in the APIs that may result in singlet oxygen formation. The

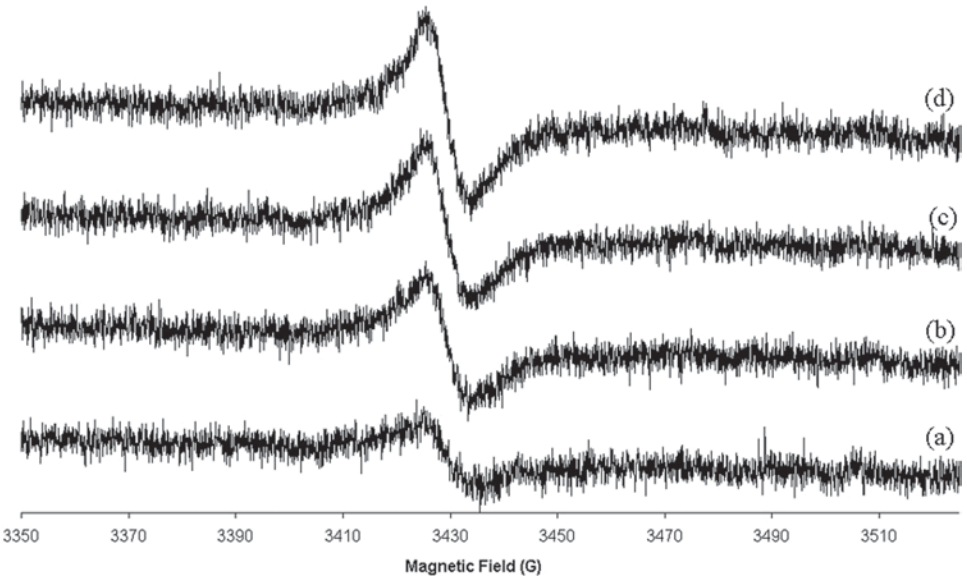

Figure 2. An example of EPR spectra obtained showing free radical formation in API 13 on exposure to light (A) initial (B) 10 min (C) 20 min (D) 30 min.

remaining majority of the APIs showed no significant singlet oxygen formation on exposure to light under the conditions used.

For the 10 APIs in the calibration data set (APIs 1–10), the correlation between the level of photodegradation products formed during conventional ICH compliant testing and the extent of free radical formation detected after 10 min exposed to light is shown in Figure 5. A correlation coefficient (R^2) of 0.89 was obtained.

Discussion

Free radical formation was detected in the majority of the APIs tested in this study. Although the structures of the APIs and so the radicals cannot be discussed here, the EPR spectra were in general thought to be dominated by carbon and oxygen based radicals. For one specific API, hyperfine splitting was observed consistent with coupling to a nitrogen nucleus. Due to the nature of the singlet oxygen formation test, the results obtained were not quantitative but they do suggest that this second mechanism of photodegradation is likely to occur for five of the APIs. ICH compliant photostability testing has since been performed for two of the five APIs showing significant singlet oxygen formation on exposure to light. The results obtained were significantly higher than would have been predicted from a free radical mechanism only. This suggests that although the singlet oxygen test is performed in methanol the results can be correlated back to the solid state.

The results obtained allow all the APIs tested to be ranked in terms of the extent of free radical formation detected on exposure to light for 10 min as shown in Figure 3. Due to the possibility of a second method of photodegradation for the APIs that showed evidence of singlet oxygen formation, the risk of photodegradation for these APIs should be considered as high.

For the 10 APIs in the calibration data set a good correlation between the level of photodegradation products formed during conventional ICH compliant testing and the extent of free radical formation detected after 10 min exposed to light was obtained. Considering the variables involved in the experiment and the differences between the two methods of exposure to light and the techniques for sample analysis being compared (EPR spectroscopy and conventional ICH compliant testing), this correlation demonstrates the potential of the EPR methods. As the dataset of APIs tested by both methods grows over time, the model will be continually developed to allow improved quantitative predictions based on the EPR data.

Conclusions

It has been demonstrated that EPR spectroscopy can be used to determine the photostability characteristics of an API in the solid state. In addition, mechanistic information can be obtained in terms of whether the degradation occurs via a free radical or singlet oxygen route or both. The extent of free radical formation on exposure to light for only 10 min can be used to rank the APIs tested in terms of their photostability characteristics and potentially to predict the level of photodegradation products that would be formed during ICH compliant photostability testing. The EPR methods involved are very quick and only use a small quantity of the API, so could be usefully performed during the early development phase of a new API.

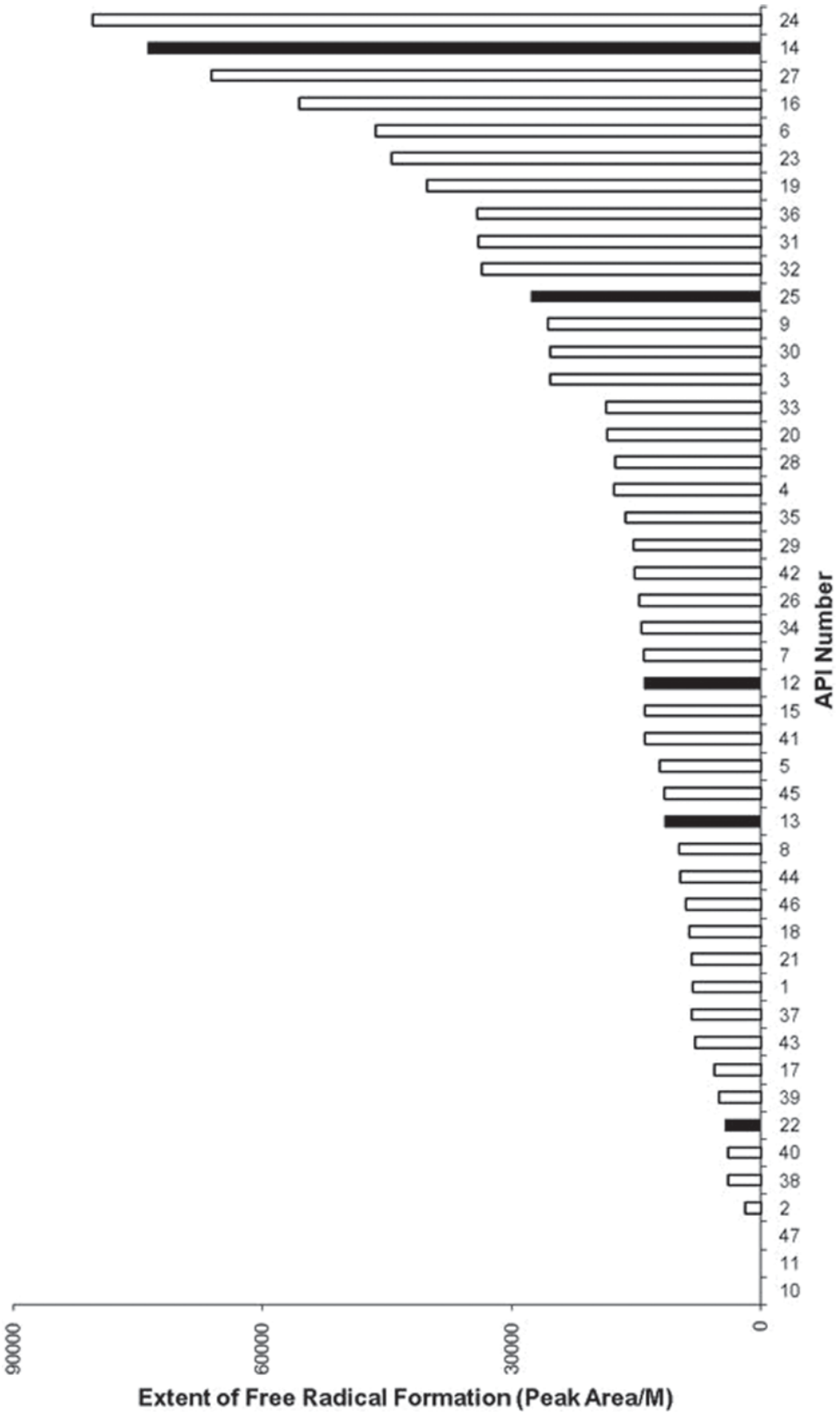

Figure 3. Ranking of all the APIs tested in terms of extent of free radical formation, unshaded = no evidence of singlet oxygen formation, shaded = evidence of singlet oxygen formation.

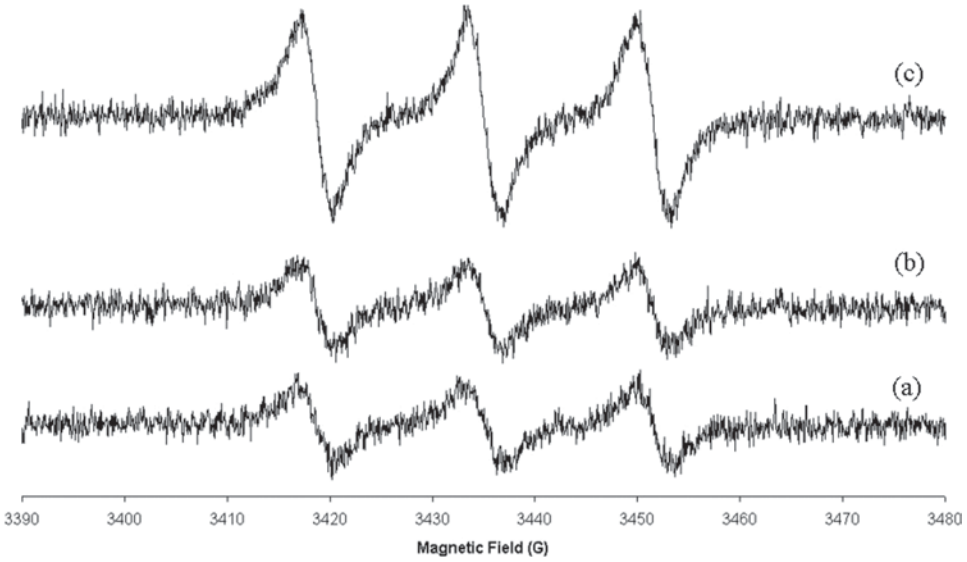

Figure 4. An example of EPR spectra showing singlet oxygen formation in a solution of API 13 in methanol on exposure to light (A) initial (B) 30 min (C) 60 min.

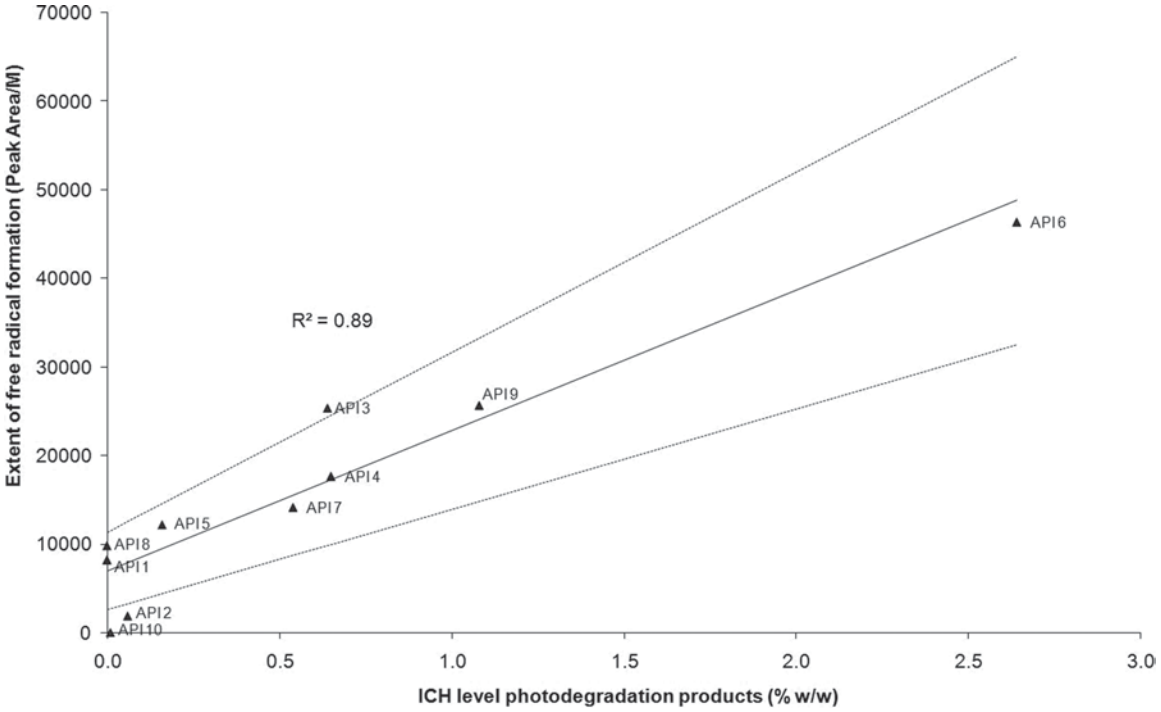

Figure 5. Quantitative results showing 95% confidence limits.

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Declaration of interest

The authors report no conflicts of interest.

References

1. ICH Q1B, (1997). Photostability testing of new drug substances and products, Fed. Reg. 62, 27115–27122.
2. Tonnesen HH. (2004). Photostability testing of drugs and drug formulations–why and how? In: Tonnesen HH, ed. Photostability of Drugs and Drug Formulations. Boca Ranton: CRC Press LLC, pp. 1–7.
3. Glass BD, Novak, Cs, Brown ME. (2004). The thermal and photostability of solid pharmaceuticals. J Therm Anal Calorim, 77:1013–1036.
4. Nema S, Washkuhn RJ, Beussink DR. (1995). Photostability testing: An overview. Pharm Technol, 19:170–185.
5. Moore DE. (2004). Photophysical and photochemical aspects of drug stability, in: Tonnesen HH, ed. Photostability of Drugs and Drug Formulations. Boca Ranton: CRC Press LLC, pp. 9–40.
6. Ioele G, De Luca M, Oliverio F, Ragno G. (2009). Prediction of photosensitivity of 1,4-dihydropyridine antihypertensives by quantitative structure-property relationship. Talanta, 79:1418–1424.

7. Albini A, Fasani E. (1998). Photochemistry of drugs: An overview of practical problems. Spec Publ– R Soc Chem, 225:1–73.
8. Nyqvist H, Wadsten T. (1986). Preformulation of solid dosage forms: Light stability testing of polymorphs as a part of a preformulation program. Acta Pharm Technol, 32:130–132.
9. Tonnesen HH. (2004). Formulation approaches for improving solubility and its impact on drug photostability. In: Tonnesen HH, ed. Photostability of Drugs and Drug Formulations. Boca Ranton: CRC Press LLC, pp. 351–371.
10. Thatcher S R, Mansfield RK, Miller RB, Davis CW, Baertschi SW. (2001). Pharmaceutical photostability. A technical guide and practical interpretation of the ICH guideline and its application to pharmaceutical stability–part 1. Pharm Technol, 25:50–62.
11. Allen JM, Allen SK. (2007). Basic principles of drug photostability testing. Drugs Pharm Sci, 163:79–86.
12. Moore DE. (1987). Principles and practice of drug photodegradation studies. j Pharm Biomed Anal, 5:441–453.
13. Moore DE, (1987). Photochemistry in medicine. Techniques for analysis of photodegradation pathways of medicinal compounds. Trends Analyt Chem, 6:234–238.
14. Stasko A, Brezová V, Biskupic S, Ondrias K, Misík V. (1994). Reactive radical intermediates formed from illuminated nifedipine. Free Radic Biol Med, 17:545–556.
15. Stasko A, Misik V, Omelka L. (1994). Irradiated nifedipine–A nitroso spin trap. Magn Reson Chem, 32:370–372.
16. Li AS, Chignell CF. (1987). Spectroscopic studies of cutaneous photosensitizing agents–IX. A spin trapping study of the photolysis of amiodarone and desethylamiodarone. Photochem Photobiol, 45:191–197.
17. Reszka K, Chignell CF. (1983). Spectroscopic studies of cutaneous photosensitizing agents–IV. The photolysis of benoxaprofen, an anti-inflammatory drug with phototoxic properties. Photochem Photobiol, 38:281–291.
18. Moore DE, Chappuis PP. (1988). A comparative study of the photochemistry of the non-steroidal anti-inflammatory drugs, naproxen, benoxaprofen and indomethacin. Photochem Photobiol, 47:173–180.
19. Motten AG, Martínez LJ, Holt N, Sik RH, Reszka K, Chignell CF et al. (1999). Photophysical studies on antimalarial drugs. Photochem Photobiol, 69:282–287.
20. De Faria DLA, Santos PS. (1991). Primaquine diphosphate and its cation radical: A spectrochemical investigation. Spectrochim Acta A Mol Biomol Spectrosc 47A: 1653–1660.
21. Buttafava A, Faucitano A, Fasani E, Albini A, Ricci A. (2002). EPR evidence of a triplet biradical in the photolysis of nifedipine. Res Chem Intermed, 28:231–237.
22. Mielcarek J, Naskrent M, Grobelny P. (2009). Photochemical properties of simvastatin and lovastatin induced by radiation. J Therm Anal Calorim, 96:301–305.
23. Lion Y, Delmelle M, van de Vorst A. (1976). New method of detecting singlet oxygen production. Nature, 263:442–443.
24. Lion Y, Gandin E, Van de Vorst A. (1980). On the production of nitroxide radicals by singlet oxygen reaction: An EPR study. Photochem Photobiol, 31:305–309.
25. Gerlock JL, Bauer DR, Briggs LM. (1986). Electron spin resonance studies to predict rates of coating photodegradation. Org Coatings, 8:365–382.
26. Binns MR, Lukey CA, Hill DJT, O'Donnell JH, Pomery PJ. (1992). A new technique for the study of reactive species generated during the initial stages of polymer photodegradation. Polym Bull, 27:421–424.
27. Pizzi A, Simon C, George B, Perrin D, Triboulot MC. (2004). Tannin antioxidant characteristics in leather versus leather light stability: Models. J Appl Polym Sci, 91:1030–1040.
28. Williams HE, Loades VC, Claybourn M, Murphy DM. (2006). Electron paramagnetic resonance spectroscopy studies of oxidative degradation of an active pharmaceutical ingredient and quantitative analysis of the organic radical intermediates using partial least squares regression. Anal Chem, 78:604–608.