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Improved photostability indicating ion-pair chromatography method for pergolide analysis in tablets and in the presence of cyclodextrins

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Abstract

Pergolide (PG) a semi-synthetic ergot alkaloid derivative used mainly for the treatment of Parkinson's disease is known to be a photosensitive drug substance. The major photodegradation products are PG sulphoxide (SX) and PG sulphone (SN), which are also the main impurities of the bulk drug substance. It is widely metabolized to more than 10 metabolites including SX and SN. In this work an improved photostability indicating ion-pair chromatography method for PG mesilate was developed. The method can be applied in the determination of PG and impurities in aqueous solutions and in tablets for routine analysis. This new method is appropriate for the quantitative determination of PG in the presence of its impurities and photodegradation products and can also be used for PG complexes with cyclodextrins (commonly used as photostabilizing agents). Furthermore it is suitable for the quantitation of its impurities and its thermal or photo-induced decomposition products. Separation was achieved on a ThermoQuest C_{18} BDS column and Sodium octanosulphonate was used as ion-pairing agent. Analysis was performed at 223 nm. Validation parameters included: specificity, linearity, precision and accuracy, limit of quantitation and suitability. The method was found to be specific and linear for PG, as well as for SX and, SN impurities. The recovery was $100.83 \pm 0.46\%$ for PG, $99.86 \pm 0.33\%$ for SX and $99.77 \pm 1.84\%$ for SN. Finally the photodegradation profile of PG mesilate was studied in different initial sample concentration. The obtained result revealed that: PG photolysis is catalyzed by its degradation products and that decrease of initial sample concentration reduces the rate of PG photoinduced degradation.

Keywords: Pergolide; Sulphoxide; Sulphone; Impurities; Ion-pair chromatography; HPLC method; Photostability; Cyclodextrins

1. Introduction

Pergolide (PG) mesilate (Fig. 1a) [1] is a semi-synthetic ergot alkaloid derivative used mainly for the treatment of Parkinson's disease as an alternative to levodopa especially in the late stages of the disease [2,3]. It is a dopamine receptor agonist at D_1, D_2 and D_3 postsynaptic dopamine receptor sites in the nigrostriatal system. It has been further used in acromegaly, glaucoma and disorders associated with hyperprolactinaemia [4].

Pergolide is known to be a photosensitive drug substance [4] and all the necessary precautions are taken during the manufacturing of PG products, e.g. opaque blister packaging. The major photodegradation products are PG sulphoxide (SX) (Fig. 1b) [1] and PG sulphone (SN) (Fig. 1c) [1], which are also the main impurities of the bulk drug substance. PG is widely metabolized to more than 10 metabolites including *N*-despropyl-pergolide, SX and SN [4,5].

It is difficult to trace in literature methods suitable for the quantitation of very low PG concentration and its impurities [6–8]. The analysis proved more difficult and complicated when PG has to be quantified in its formulations, as the only existing references are in the Pharmacopoeias. [1,9]. The method described in European Pharmacopoeia 4.2 [1] is suitable for the determination of impurities in the bulk drug substance however, it proved to be not appropriated for pharmaceutical formulations or its photodegradation products, because of the observed co-elution of SX with excipients. USP monograph (USP26NF21) of PG tablets, on the other hand, refers to a method that can be

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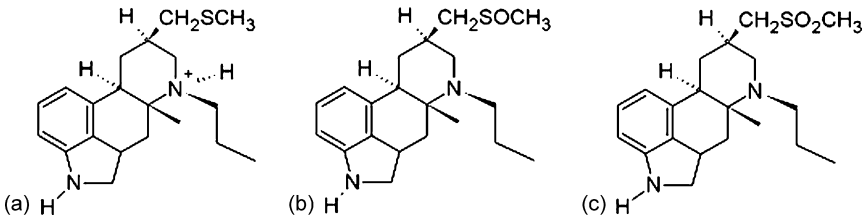

Fig. 1. Structure of PG mesilate and its impurities: (a) pergolide mesilate {[(*6aR,9R,10aR*)-9-[(*methylsulphanyl*) *methyl*]-*7-propyl-4,6,6a,7,8,9,10,10a-octahydroindolo* [*4,3-fg*] *quinoline monomethane sulphonate*]}; (b) pergolide sulphoxide {(*6aR,9R,10aR*)-9-[(*methylsulphinyl*) *methyl*]-*7-propyl-4,6,6a,7,8,9,10,10a-octahydroindolo* [*4,3-fg*] *quinoline*}; (c) pergolide sulphone {(*6aR,9R,10aR*)-9-[(*methylsulphonyl*) *methyl*]-*7-propyl-4,6,6a,7,8,9,10,10a-octahydroindolo [4,3-fg] quinoline*}.

applied only for impurities determination in tablets [6]. Despite its usefulness, this method was not efficient for PG analysis in the presence of its photodegradation products.

All the above prompted us to develop a new chromatographic method for the routine PG analysis in the presence of impurities, in new formulations (generic pergolide tablets) as well as in the presence of photodegradation products. Quantitation in the presence of cyclodextrins (CD) very often used as photostabilizing excipients [10–12] was also achieved. Furthermore, the photodegradation profiles of PG in different initial concentrations were studied.

2. Experimental

2.1. Chemicals

Pure PG mesilate (M_W, 410.5898) and Working Standard, its impurities (pergolide sulphoxide and pergolide sulphone) and its tablet formulation, containing PG mesilate equivalent to 0.05, 0.25 and 1 mg PG (base), used throughout this study were provided by: Pharmathen S.A. (Attika, Greece). Sodium Octanesulphonate ($C_8H_{17}NaO_3S$) and other chemicals were of analytical grade and were purchased from PAI Panreac Quimica SA. Water used was deionized and filtered through Milli-Q Plus water purifying system, Millipore (Bedford, USA). All other solvents used were of HPLC grade.

2.2. Instrumentation

Liquid chromatography experiments were carried out using Shimadzu 2010C apparatus equipped with double window UV Detector, auto-sampler, column oven, pumps and degasser.

X-ray diffraction experiment using powdered PG, βCD and PG:βCD complexes was conducted on a Philips PW1710 (Bragg-Brentano geometry) with Cu Kα filtered from Ni absorbing Kβ.

The photodegradation experiments were performed with a Suntest CPS Accelerated Exposure Xenon Burner Machine, Atlas Material Testing Technology LLC (Chicago, Illinois, USA), which simulates the solar irradiation in wavelength distribution. Irradiation intensity was 765 J s^{-1} m^{-2}.

2.3. Chromatographic conditions

The mobile phase used consisted of 70:30 (v/v) aqueous phase: acetonitrile. For the preparation of the aqueous phase, 2 g of sodium octanesulphonate were dissolved in 1000 ml water (9.2×10^{-3} M) containing 2 ml of glacial acetic acid (pH 3.2). Separation was achieved on ThermoQuest C18 BDS silica column, 250 mm × 2.1 mm i.d., 5 μm. Column temperature was kept at 40 °C, flow rate was adjusted to 0.3 ml/min and the injection volume was 10 μl. The detection wavelength was set at 223 nm. A sample chromatogram is presented in Fig. 2.

2.4. Sample preparation

The solvent used for most of the preparations was 1:1 mixture of MeOH:HCl 0.01N (preparation solvent). All prepared solutions were stored under light protection.

2.4.1. Preparation of stock standard solutions

For the preparation of the PG stock standard solution accurately weighted 52.4 mg of PG mesilate was dissolved in 100.0 ml of preparation solvent giving a stock standard solution of 400 μg/ml equivalent to PG base. Furthermore stock standard solutions were prepared at 200 μg/ml for both the impurities SX and SN by separately weighting and dissolving 10 mg of each impurity in 50 ml of preparation solvent.

2.4.2. Preparation of working standard solutions

Sets of working standards were prepared by appropriate dilutions of the stock standard solutions for PG at 0.06, 0.3, 0.6, 0.72,

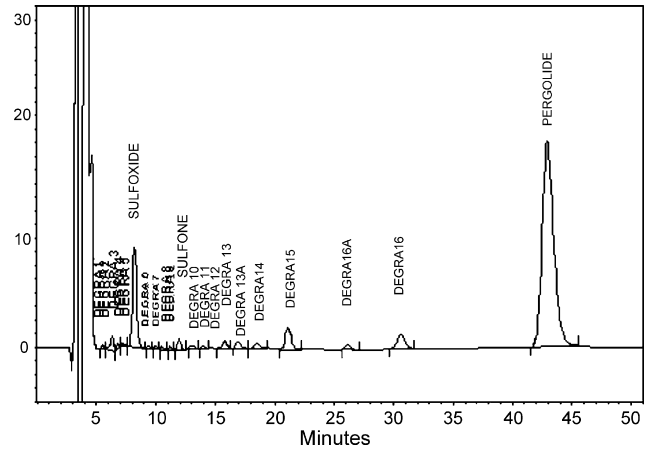

Fig. 2. Chromatogram showing successful resolution between all peaks of impurities and degradation products, after 90 min of irradiation.

4.0, 12, 20, 80 μg/ml; for SX at 0.2, 0.6, 1.8, 3.6, 12, 20 μg/ml and for SN at 0.04, 0.24, 0.8, 20 μg/ml.

In order to evaluate the quality features of HPLC method three (0.02 mg/ml) PG solutions containing both the SX and SN impurities at three different concentration levels were prepared: (a) 0.36 μg/ml SX and 0.024 μg/ml SN; (b) 0.6 μg/ml SX and 0.04 μg/ml SN; (c) 0.72 μg/ml SX and 0.048 μg/ml SN. Furthermore a sequence of five PG (0.02 mg/ml) solutions were prepared, containing SX at 0.72, 0.60, 0.48, 0.36, 0.30 μg/ml and SN at 0.048, 0.040, 0.032, 0.024, 0.020 μg/ml, respectively. Another solution was prepared containing 0.15 μg/ml PG and both impurities at 0.02 μg/ml. Additionally a 0.02 mg/ml PG solution from tablets was prepared spiked with both SX and SN at 0.02 μg/ml.

2.4.3. Preparation of sample solutions

Tablets of three different PG strengths 0.05, 0.25 and 1 mg were used for the preparation of the sample stock solutions. Ten tablets of each deferent strength were ground and an amount equivalent to 0.1, 0.5 or 1 mg PG, respectively, was accurately weighed and dissolved using the preparation solvent in final volume of 5, 25 or 50 ml, respectively. The final concentration of the sample stock solution was 200 μg/ml in all samples. Appropriate dilutions were performed to prepare the required working sample solutions.

2.5. Preparation and characterization of complexes with cyclodextrins

Aqueous solution of 320 μg/ml (7.79×10^{-4} M) PG was combined with appropriate quantities of accurately weighed βCD in order to obtain four solutions with molar ratios 1:1, 1:2, 1:5 and 1:20. The solutions were shaken in a constant temperature water bath at 30 ± 1 °C, until equilibrium was reached (4 days). The equilibrium was evidenced by continuous monitoring of their UV spectra as the absorption of PG was affected by the extent of complexation.

In order to obtain the complex in solid state the above-prepared solutions of 1:2 and 1:5 drug:CD ratio were freeze dried. The obtained white powders were used for the powder X-ray diffraction experiment. The diffraction diagrams showed that the reflection pattern of 1:2 and 1:5 PG:βCD complexes was dramatically different from those of the parent βCD and PG. Fig. 3 illustrates the diagrams of βCD and PG:βCD

2.6. Photodegradation study

PG solutions of 0.03, 0.05, 0.08, 0.3 and 0.5 mg/ml were prepared in HCl 0.01N to be used for the photodegradation experiment. Aliquots (3 ml) were transferred into a 1 cm path length cuvette (quartz) and were exposed to Xenon lamp irradiation. Photodegradation experiments were performed according to ICH, side by side with a validated quinine chemical actinometric system, in order to ensure the stability of the radiation intensity and the repeatability of the results [13]. Samples were stirred throughout the exposure time. At predetermined time intervals 200 μl aliquots of the irradiated solution were withdrawn from the cuvette and immediately diluted as appropriate with mobile phase. The resulting solutions were analyzed by the newly developed LC method. The extent of photodegradation was calculated by monitoring the remaining quantity of PG in predetermined time intervals of irradiation.

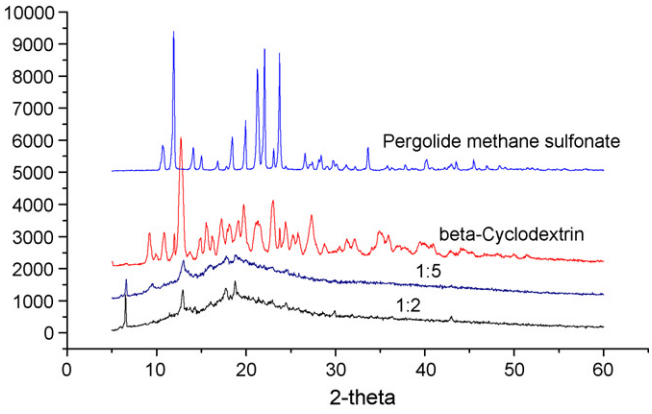

Fig. 3. Powder X-ray diffraction diagrams showing the changes after complexation of pergolide with cyclodextrins.

Samples wrapped in aluminum foil were used as dark controls. No concentration changes were observed due to possible thermal reactions.

3. Results and discussion

3.1. Development of photostability indicating HPLC method

The method described in USP26NF21 under the monograph of PG mesilate tablets for chromatographic purity test failed to provide acceptable results, due to significant interferences between SX and the excipients of the generic PG tablets used. As a result the method could not be used for either the quantitation of SX or PG photostability studies, when the generic tablets were tested. Furthermore, as the test concentration provided by the USP method is too low for the determination by means of UV–vis or PDA detector, a higher test concentration was needed. The limitation remained that PG is formulated in tablets of very low strength containing 1, 0.25 and 0.05 mg in total weight of 300 mg. Thus the highest concentration that could be achieved for PG was 0.02 mg/ml and was set as test concentration.

The detection wavelength was set at 223 nm due to optimal absorption when compared to the other maximum at 280 nm ($\varepsilon_{223} \approx 5\varepsilon_{280}$). A longer column with same packing material and smaller internal diameter was also selected to improve signal response. The gradient scheme described in EP (ion-pair agent containing mobile phase), as well as numerous mobile phase and gradient rate modifications, were unsuccessful resulting in long equilibration times and drifting baselines. Therefore, a generally more simple and reproducible isocratic elution approach was implemented accepting that the total analysis time would increase.

The outcome of the EP method assay test was in general satisfactory, although the interference of SX with excipients was not

totally eliminated. However, the ratio of organic modifier in the mobile phase was further optimized in order to achieve acceptable resolution for SX and a reasonable run time. The best results were acquired with a proportion of aqueous phase:acetonitrile 70:30. The resulting separation is depicted in Fig. 2. Using these conditions both the separation of SX and a reasonable run time were accomplished.

3.2. Method validation

Throughout the validation experiments the test concentration for PG was set at 0.02 mg/ml, accordingly the test concentrations for SX and SN were set at 3% and 0.2% of PG test concentration, thus 0.6 and 0.04 μg/ml, respectively. These values were selected according to in house stability testing data:

(a) *Range*: The range for individual known impurities has been set for SX and SN 3–120% and 50–120% of their test concentration, respectively.

(b) *Specificity*: The method is specific for the estimation of impurities in the presence of the degradation products of PG (Fig. 2). To assess method specificity, a working sample solution, all PG, SX and SN working standard solutions, PG solutions spiked with SX and/or SN and a placebo sample were used. Furthermore to assess the stability indicating properties of the proposed method, aliquots of the photodegradation solution exposed for 120 min to xenon lamp irradiation were also analyzed. The chromatograms (Fig. 2) showed that peaks corresponding to photodegradation products did not interfere with PG, SX or SN peaks and had good resolution from each other.

(c) *Linearity of response*: Six different PG solutions containing 3–120% and 50–120% of SX and SN test concentration respectively were injected in triplicate and results are presented in Table 1. The relatively high intercepts can be attributed to the fact that the working concentration range is close to the quantification limit of the method. This is also confirmed by the high standard deviations reported.

Table 1
Parameters of the linear regressions (by the least squares method)

Compound (μg/ml)	Slope[a] × 10^2	*y*-Intercept[b]	r^2
SX (0.02–0.72)	4275.79 ± 26.5	1401.98 ± 911.3	0.9996
SN (0.02–0.048)	383.63 ± 750.4	1040.00 ± 4.00	0.9990
PG (0.15–0.72)	5568.56 ± 46.4	1483.65 ± 964.4	0.9991

[a] In peak area response units/amount.
[b] In peak area response units.

(d) *Limit of quantitation of impurities*: The quantitation limits were found to be: for PG 0.75% of its test concentration equivalent to 0.15 μg/ml, for SX 3% of its test concentration equivalent to 0.02 μg/ml and for SN 50% of its test concentration equivalent to 0.02 μg/ml. Moreover, the method has been proved to be accurate and precise for the selected concentrations.
- *QL Accuracy*: The average recovery at the quantification level was found 97.73 ± 1.95% and 99.68 ± 0.18% for SX and for SN, respectively.
- *QL Precision*: The calculated %R.S.D. ($n = 10$) for the peaks of SX was found 2.4 and for SN 1.5.

(e) *Accuracy*: The % recoveries and their overall average values were calculated for PG, SX and SN at three different concentration levels. The results are presented in Table 2.

(f) *Precision of the system*: Intra assay precision measurements ($n = 10$) provided %R.S.D. values of 0.76, 0.68, 5.3 for PG,

Table 2
Accuracy as % average recoveries of SX, SN and PG calculated within and across three concentration levels (μg/ml) and Precision of the method obtained at each concentration level of accuracy tests

	Accuracy ($n = 3$)			Precision ($n = 10$)		
	Low	Medium	High	Low	Medium	High
SX						
Nominal (μg/ml)	0.360	0.600	0.720	0.360	0.600	0.720
Calculated (μg/ml)	0.360	0.600	0.717	0.360	0.600	0.717
R.S.D. (%)	0.525	0.426	0.071	0.424	0.441	0.081
Recovery (%)	100.08	99.98	99.54			
Mean across levels (R.S.D., %)	99.87 (0.286)					
SN						
Nominal (μg/ml)	0.024	0.040	0.048	0.024	0.040	0.048
Calculated (μg/ml)	0.024	0.039	0.049	0.024	0.039	0.049
R.S.D. (%)	1.227	1.483	0.507	<0.001	1.468	<0.001
Recovery (%)	99.65	97.29	102.36			
Mean across levels (R.S.D., %)	99.77 (2.54)					
PG						
Nominal (μg/ml)	0.300	0.600	0.720	0.300	0.600	0.720
Calculated (μg/ml)	0.302	0.605	0.727	0.302	0.605	0.727
R.S.D. (%)	0.302	0.303	0.789	0.302	0.304	0.787
Recovery (%)	100.73	100.84	100.92			
Mean across levels (R.S.D., %)	100.83 (0.093)					

Table 3
The inter-day precision study for the evaluation of the system suitability at the test concentration (0.02 mg/ml)

Limits[a]	Area (<5%)	R_t (<1%)	Asymmetry 10% (0.8–1.5)	Theoretical plates[b]	Capacity factor
PG					
Value	11497564	43.7	1.29	33442	15.31
R.S.D. (%)	1.08	0.10	0.82	1.10	0.10
SX					
Value	284628	8.21	1.05	13955	2.91
R.S.D. (%)	1.01	0.06	1.05	0.57	0.08
SN					
Value	14370	11.40	0.99	30727	4.43
R.S.D. (%)	1.31	0.15	8.90	6.68	0.18

[a] Specified requirements for system suitability.
[b] Minimum theoretical plates 28,000 for PG, 12,000 for SX and 22,000 for SN.

SX and SN, respectively. Inter-day assay precision measurements ($n = 10$) provided %R.S.D. values of 0.25, 0.81 and 1.28 for PG, SX and SN, respectively.

(g) *Precision of the method* was evaluated using the data obtained at each concentration level as reported above [section (e) *Accuracy*]. Results are summarized in Table 2.

(h) *System suitability*: The inter-day precision study was used to evaluate the suitability of the system at the test concentration of 0.02 mg/ml. Parameters for PG, SX and SN peaks were calculated and the results are presented in Table 3. All values are well within the specified requirements.

(i) *Robustness*: The robustness of the method was assessed after changing parameters such as detection wavelength, flow rate, column temperature and percentage of organic modifier of the mobile phase. Six replicate injections of the solution, containing 20, 0.6 and 0.04 μg/ml of PG, SX and SN, respectively, were performed for every change of parameter. The suitability of the system was evaluated in order to determine how these changes affect a measurable parameter.

Concluding from the results, the system is robust as all values remain well-within the specified requirements, even after reduction or increase of either the detection wavelength or the flow rate or the column temperature.

3.3. Effect of complexes in UV spectra

The UV spectra of PG mesilate 0.32 and 0.16 mg/ml solutions were recorded and the differences in absorbance at both λ_{max} 279 and 221 nm to those of the complexes were compared. The detected hyperchromic shifts at both λ_{max} were: from 0.509 and 0.644 of free PG to 0.517 and 0.664 of 1:10 CD complexes. It is clear that the formation of complexes affects the spectrum of PG. Therefore, UV spectrophotometry is not applicable for quantitation of PG:CD complexes due to resulting analytical inaccuracies [14,15] which were completely eliminated when the proposed HPLC method was used.

3.4. Pergolide photodegradation plots

Photodegradation experiments performed with samples of different initial concentrations (C_0) revealed that increase of C_0 enhances PG photodegratation. This is clearly demonstrated in Fig. 4.

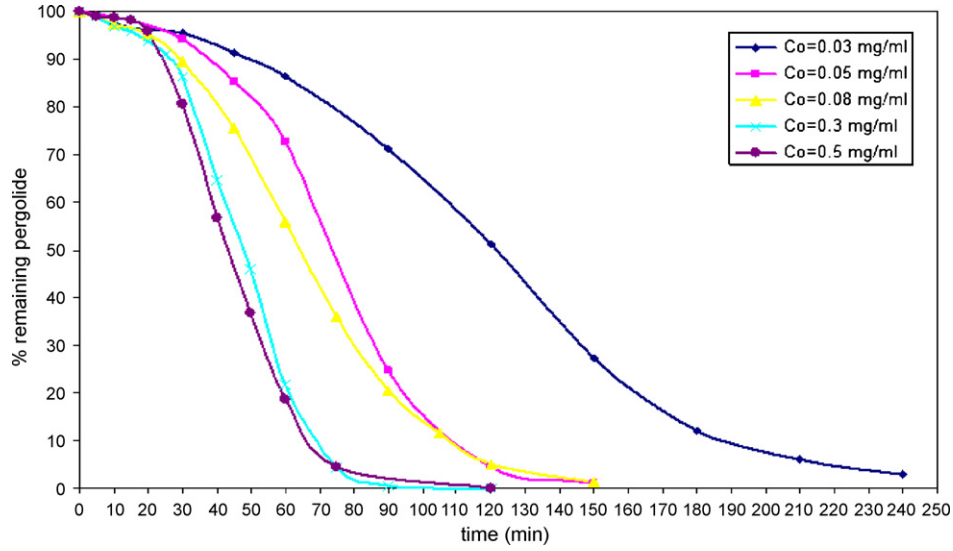

Fig. 4. Remaining pergolide (%) vs. time during photodegradation experiment in different initial sample concentration C_0 at lamp power 765 J s^{-1} m^{-2}.

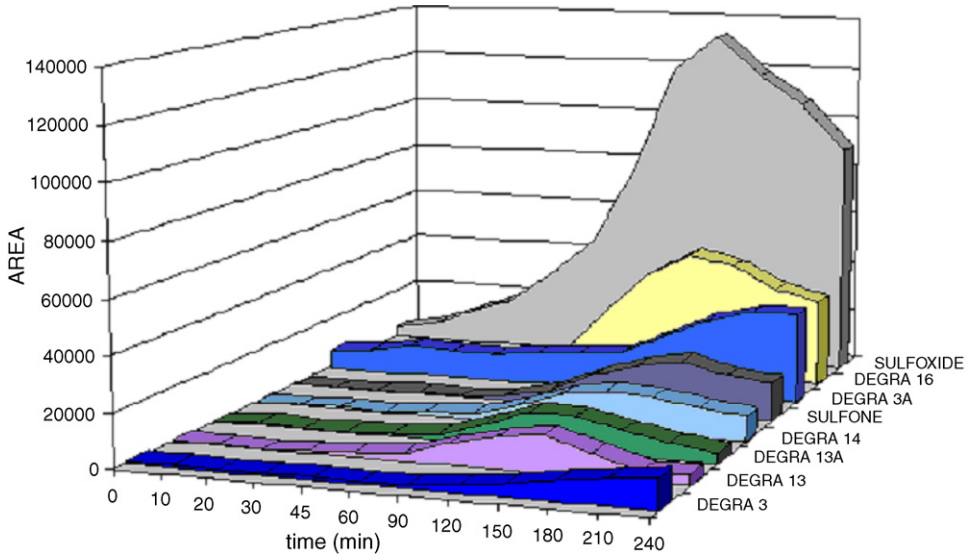

Fig. 5. The concentration of PG degradation products plotted vs. irradiation time.

The drug decay was monitored for more than four half-life times (Fig. 5) at all tested concentrations. The sigmoidal shape of the plots (Fig. 4) revealed that the photodegradation of PG begins with very slow rates, followed by a steep acceleration, probably catalyzed by the presence photodegradation products. After that the rate remains relatively high, yet constant simulating zero-order kinetics. Finally, when PG was reduced to approximately 20% of its initial concentration the profile of the degradation reaction changes to first-order kinetics.

4. Conclusions

A new ion-pair HPLC method has been developed and validated. The assay was found to be specific and suitable for the quantitative determination of PG in the presence of its impurities and photodegradation products. The method exhibited good selectivity and specificity and was also efficient for the determination of its impurities and photodegradation products. It can also be applied in the determination of PG and impurities in aqueous solutions and in tablets for routine analysis and for photostability studies of PG.

Finally the photodegradation profile of PG mesilate was studied in different initial sample concentration. The obtained result revealed that:

- PG photolysis is catalyzed by its degradation products;
- Decrease of initial sample concentration reduces the rate of PG photoinduced degradation.

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