of samples at 205 nm gave interesting qualitative information on the presence of metabolites of ketoprofen. In fact, at 205 nm, the number of metabolites detected was greater than at 255 nm. Such metabolites show a kinetic behavior quite dissimilar from that of ketoprofen. Previous work (5) supports the assumption that area peaks (detected at 205 and 255 nm) having a retention time longer than that of ketoprofen can be referred to hydroxylated metabolites whereas area peaks detectable only at 205 nm can be referred to compounds with modified unsaturated system.

This procedure appears to be useful for studies of separation and characterization of these metabolites. It also has the advantage of rapidity since a technician can process 20–30 samples in 1 day with an automatic sampler and a minimum of active involvement. In conclusion, high sensitivity, specificity, and rapidity, coupled with the ability to separate unchanged ketoprofen from its metabolites, make this procedure particularly suitable for study of the pharmacokinetics of ketoprofen in humans.

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Light Stability of Norethindrone and Ethinyl Estradiol Formulated with FD&C Colorants

E. E. KAMINSKI ˣ, R. M. COHN, J. L. McGUIRE, and J. T. CARSTENSEN *

Received August 18, 1977, from the *Research Laboratories, Ortho Pharmaceutical Corporation, Raritan, NJ 08869.* Accepted for publication August 4, 1978. *Present address: School of Pharmacy, University of Wisconsin, Madison, WI 53706.

Abstract □ In general, light-sensitive tablets exhibit discoloration in the surface layer(s) only. A case is discussed where a quantitative interaction between a drug, ethinyl estradiol (in a combination tablet containing norethindrone and ethinyl estradiol), and a dye (FD&C Red No. 3) occurs, and discoloration exists throughout the tablet. The data suggest that accelerated light studies should be carried further than those dictated by predictive periods so that equilibrium levels can be deduced.

Keyphrases □ Norethindrone—stability in tablets, effect of FD&C dyes □ Ethinyl estradiol—stability in tablets, effect of FD&C dyes □ Dyes, FD&C—effect on stability of norethindrone and ethinyl estradiol in tablets □ Stability—norethindrone and ethinyl estradiol in tablets, effect of FD&C dyes □ Tablets—norethindrone and ethinyl estradiol, effect of FD&C dyes on stability

Drug stability in solution can be affected adversely by the presence of a colorant (1). Since colors are used routinely to identify solid dosage forms, a study was initiated to determine whether such reactions could occur with compressed tablets. Although there are no compendial or regulatory procedures to evaluate the effect of light, previous reports (2) correlated accelerated light studies with ordinary room light exposure.

Reported interactions between light and substances in solid dosage forms usually were confined to the top layer(s) of the tablet (3–5) and occurred when a single component was photosensitive. Photo-induced drug interactions in solid form have not been reported.

This report presents results of studies on the stability of norethindrone and ethinyl estradiol in uncolored compressed tablets as well as in tablets containing selected FD&C colorants when exposed to accelerated light stress.

EXPERIMENTAL

Tablets—All tablets contained 0.5 mg of norethindrone and 0.035 mg of ethinyl estradiol and were prepared with the same excipients by classical granulation techniques. Three formulas were prepared: pink, containing 4.5 μg of erythrosine (FD&C Red No. 3)/tablet; white, containing no colorant; and orange, containing 30 μg of the disodium salt of 1-*p*-sulfophenylazo-2-naphthol-6-sulfonic acid (FD&C Yellow No. 6)/tablet.

The dyes were added in a methanolic solution to the basic excipient system. The uncolored tablets were processed with the same amount of methanol as used in the tablets containing a colorant. All tablets were then dried, screened, lubricated, blended, and compressed in similar fashions.

Analytical Methods—Single tablets were disintegrated and then dissolved in a homogenizer with methanol as the solvent. The solution containing the dissolved steroids was filtered and divided into two

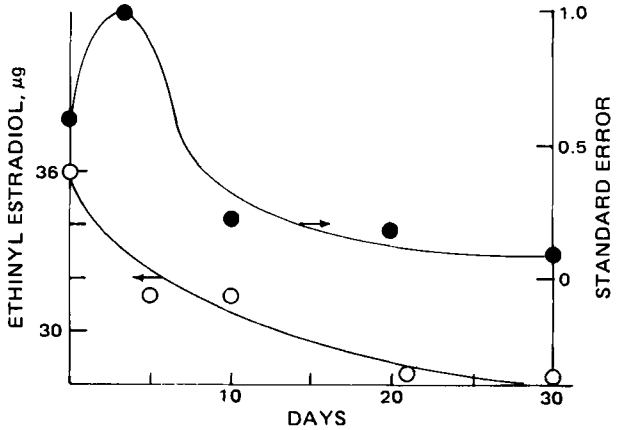

Figure 1—*Ethinyl estradiol assays as a function of time (left scale, open circles) and standard error of the assay mean as a function of time (right scale, closed circles).*

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Table I—Norethindrone Assays after Storage in Light Cabinet [a]

Formula	Initial Assay	*SEM*	5-Day Assay	*SEM*	10-Day Assay	*SEM*	21-Day Assay	*SEM*	30-Day Assay	*SEM*
White:										
Exposed	504	4.6	506	3.5	491	4.6	488	3.2	474	2.6
Control			511	4.8	501	5.4	500	5.2	507	3.8
Pink:										
Exposed	500	4.4	503	3.7	494	3.3	489	2.4	484	2.8
Control			511	6.8	507	4.6	509	3.6	495	2.1
Orange:										
Exposed	499	5.3	509	6.2	489	7.3	495	5.2	495	5.0
Control			513	6.6	500	5.6	532	3.3	506	2.6

[a] Assay and standard error of the mean are expressed in micrograms per tablet. The standard deviation may be calculated by multiplying the standard error of the mean $\times \sqrt{10}$, where 10 is the number of tablets analyzed.

Table II—Ethinyl Estradiol Assays after Storage in Light Cabinet [a]

Formula	Initial Assay	*SEM*	5-Day Assay	*SEM*	10-Day Assay	*SEM*	21-Day Assay	*SEM*	30-Day Assay	*SEM*
White:										
Exposed	35.7	0.24	36.1	0.24	35.0	0.44	34.2	0.21	34.3	0.22
Control			36.2	0.33	34.5	0.54	35.1	0.25	35.8	0.56
Pink:										
Exposed	36.0	0.60	31.3	1.01	31.3	0.21	28.4	0.18	28.4	0.09
Control			35.4	0.74	35.5	0.67	35.9	0.29	34.9	0.26
Orange:										
Exposed	35.1	0.11	35.0	0.34	34.6	0.22	34.6	0.36	34.9	0.36
Control			35.3	0.28	34.6	0.53	37.0	0.38	36.3	0.37

[a] Assay and standard error of the mean are expressed in micrograms per tablet. The standard deviation may be calculated by multiplying the standard error of the mean $\times \sqrt{10}$, where 10 is the number of tablets analyzed.

Table III—Regression Values for Assays: Slope (*b*), Intercept (*a*), and Correlation Coefficient (γ)

	Norethindrone						Ethinyl Estradiol					
	Exposed			Control			Exposed			Control		
Formula	*a*	*b*	γ	*a*	*b*	γ	*a*	*b*	γ	*a*	*b*	γ
White	506	−1.01	−0.95	505	−0.04	−0.13	35.9	−0.06	−0.89	35.5	−0.005	−0.100
Pink	502	−0.61	−0.95	507	−0.21	−0.39	34.0	−0.22	−0.88	35.8	−0.021	−0.595
Orange	501	−0.25	−0.41	504	. 0.43	0.39	35.0	−0.008	−0.43	34.9	0.059	0.737

streams. The first stream was mixed with isonicotinic acid-hydrolyzed reagent, which produced a chromophore absorbing in the 380-nm region (6). The second stream was mixed with 90% sulfuric acid, which produced a chromophore in the presence of ethinyl estradiol. The acid-induced fluorescence determination can be traced to the work of Khoury and Cali (7).

Test Conditions—All samples were subjected to accelerated conditions by placing them in a light cabinet containing both incandescent and fluorescent bulbs operated at an approximate light intensity of 1000 footcandles. The selection of the light source was designed to approximate the spectral energy curve of sunlight.

Two samples of each formula were exposed: A, in an open dish; and B, in a light-transparent container. Two samples (controls) were stored in the light cabinet in light-protecting containers; C, in a folding box; and D, in a foil overwrap. Five units of each sample were tested at the intervals shown in Tables I and II.

RESULTS AND DISCUSSION

Table I summarizes the accelerated light stability data for norethindrone and Table II summarizes the data for ethinyl estradiol from the three formulations. In no case were there significant differences ($p = 0.05$) between means or variances from Groups A and B. Therefore, the data for these two groups were pooled to give one mean and standard error for the exposed group. The same held true for Groups C and D, which were combined and listed as the control group. In several cases, however, there were significant differences between the exposed group and the control. Each number in the table represents an average of 10 assays.

The linear regression parameters for the data are given in Table III. With the white and pink tablets, there was a slight but significant regression in the data of the exposed samples for norethindrone. Rank testing of the data and the correlation coefficient are obvious indicators of this regression. This correlation was not demonstrated with significance for the orange tablets. There was no indication of light sensitivity regarding norethindrone (in fact, the colored tablets appeared to be better than the uncolored control), so the interaction in the system probably occurred between the dye and ethinyl estradiol only. The largest loss for norethindrone amounted to only 6%.

With ethinyl estradiol, the white formula showed slight but significant regression (loss), but the pink formula showed substantial losses. This latter result is shown graphically in Fig. 1. The orange formula was significantly more stable than the white, and the yellow colorant seemed to stabilize the ethinyl estradiol while the red dye seemed to interact with it.

Erythrosine is a fluorescein derivative and is thus in a class of compounds that are known sensitizing agents for photooxygenation and photorearrangement reactions (8, 9). The disodium salt of 1-*p*-sulfophenylazo-2-naphthol-6-sulfonic acid is, however, an azo dye and not considered a sensitizer for such reactions. Thus, based on the experimental results obtained from the 30-day accelerated light stability study, it appears that erythrosine can induce photochemical changes in ethinyl estradiol-containing tablets. Removal of this dye or its replacement with a nonsensitizing dye results in tablets with superior ethinyl estradiol light stability.

The 21% loss of ethinyl estradiol (mol. wt. 296) was on the order of 8 μg, *i.e.*, 27 nmoles, whereas the amount of red dye (mol. wt. 880) present (4.5 μg) was only 5 nmoles, which suggests a possible stoichiometric reaction. In the tablets containing the red dye, the entire tablet discolored, as opposed to the conventional type of light sensitivity that leads to a surface fading only (3–5).

The data in Table II seem to imply a trend: the standard error at the onset is small (and comparable to that derived from content uniformity data) but increases with time as the reaction comes to completion. This increase is by no means demonstrated with statistical confidence, but the chemical implication that the rate of reaction has either not started or has come to an end is not contradicted by the data.

Accelerated light studies should be carried further than those dictated by predictive periods so that "equilibrium" levels can be deduced.

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Quantitative Structure–Activity Relationships and Carminative Activity II: Steric Considerations

BRIAN K. EVANS *, KENNETH C. JAMES ˣ, and DAVID K. LUSCOMBE

Received January 10, 1978, from the *Welsh School of Pharmacy, University of Wales Institute of Science and Technology, Cardiff, CF1 3NU, United Kingdom.* Accepted for publication August 9, 1978.

*Present address: Department of Pharmacy, University Hospital of Wales, Cardiff, United Kingdom.

Abstract □ The unexplained variation in the relationships between carminative activities and octanol–water distribution coefficients of various classes of compounds was explained. The steric substituent constant (E_s) value, van der Waals volume, and molecular connectivity were introduced into a previously derived correlation. Each parameter brought about an improvement, and molecular connectivity was the most successful. Correlations containing molecular connectivity terms explained the excess variation. The results were in agreement with a mechanism in which carminative activity depended on the availability of the oxygen atom in the functional group of the molecule and was reduced when the substituent attached to the oxygen hindered the interaction between the oxygen atom and the receptor.

Keyphrases □ Carminative activities—various oxygen-containing compounds, related to solubility, steric considerations □ Solubility—various oxygen-containing compounds, related to carminative activities, steric considerations □ Structure–activity relationships—carminative activities of various oxygen-containing compounds related to solubility, steric considerations

Recently, the carminative activities of 34 alcohols, esters, ethers, phenols, and carbonyl compounds were described (1). Carminative activity was expressed as the ability to produce a 50% inhibition (ID_{50}) of a standard response to carbachol in the guinea pig isolated ileum preparation. Regression analysis revealed a significant rectilinear relationship between log $1/ID_{50}$ and the logarithms of the octanol–water distribution coefficients (log P), but the correlation equation explained only 50% of the variation.

However, each chemical class of compounds, in isolation, produced more precise correlations, indicating the influence of factors other than solubility on carminative activity. Electronic distributions were unimportant, and it was suggested that the variation was due to the oxygen atom being shielded by the group attached to it. Consequently, the bulkier the group, the lower would be the carminative activity. Therefore, the hypothesis was tested by introducing steric parameters into the correlation equation between log P and log $1/ID_{50}$.

EXPERIMENTAL

The ID_{50} results and octanol–water distribution coefficients were reported previously (1). The compounds examined and other data are listed in Table I. All compounds consist of an oxygen joined to two chemical groups, one of which may be hydrogen. In choosing the groups to which to assign steric parameters, the decision was unambiguous with four of the five ethers since they each had two identical groups linked to oxygen. The vinyl group was chosen for the fifth compound, ethyl vinyl ether, since it was the smaller group. Similarly, values for hydrogen were given to all of the alcohols and phenols. The smaller groups were chosen because it was considered that interaction between oxygen and the receptor site would take place along the path of least resistance.

In each compound, the groups are located on opposite sides of the oxygen atom so that it is unlikely that both groups would interfere at once. Therefore, the probability of interaction through the bulkier group would be low. No logical way of allocating steric parameters to the carbonyl compounds, in accord with these assignments, was possible, so this class of compounds was omitted from the correlations. It was suggested previously (1) that the carbonyl oxygen of the esters, and not the ether oxygen, was responsible for carminative activity. Parameters for $CH_3C=$ were assigned, therefore, to the esters (since they are all acetates).

RESULTS AND DISCUSSION

Taft (2) assigned substituent constants (E_s), which are a measure of steric effects on reaction rates, to a range of substituent groups. The best correlation of log $1/ID_{50}$ against log P, reported previously (1), and the E_s value is:

$$\log 1/ID_{50} = 0.128 + \underset{8.8}{0.521(0.059)} \log P + \underset{4.7}{0.360(0.077)} E_s \quad \text{(Eq. 1)}$$

$$n = 22 \quad r = 0.915 \quad s = 0.220 \quad F_{2,19} = 49\alpha(0.001) = 10.2$$

where n represents the number of results considered (reduced from 26 to 22 because an E_s value for $CH_3C=$ was not available), r is the correlation coefficient, and s is the standard deviation. The figures in parenthesis following the coefficient in log P and E_s represent their standard errors ($p = 0.05$), and the figures below the coefficients are the ratios of the coefficients to their standard errors. Comparison of these values with t values obtained from statistical tables indicated that both physical parameters are related to log $1/ID_{50}$ ($p < 0.001$).

Equation 1 explains 84% of the variation and is a significant improvement on the corresponding equation containing log P alone. The relationship is confirmed by the figures shown below the equation, which are the results of analysis of variance of the observed results about the regression, followed by an F test.

Kier and coworkers (3, 4) developed a range of parameters, collectively termed molecular connectivity (χ), related to the topological structure of organic groups. The simplest term, the zero-order connectivity, is designated ${}^0\chi^v$ and is calculated from:

$${}^0\chi^v = \sum (\delta_i)^{-1/2} \quad \text{(Eq. 2)}$$

where δ_i is a number assigned to each nonhydrogen atom and reflects the

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