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Photochemical fluorimetric analysis of phenylbutazone and its degradation products*

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Abstract: A room temperature photochemical spectrofluorimetric (RTPF) method has been developed for the assay of phenylbutazone (PB), and its major degradation products. Fluorescence spectral properties of PB, its degradation products, and their photoproducts are reported, as well as the optimal irradiation times (ranging from 4 to 45 min), which correspond to maximum fluorescence signals of photoproducts. Linear log–log calibration plots were obtained over a 50- to 1000-fold range of concentration, and limits of detection ranged between 1 ng/ml and 1.2 μg/ml. This has been shown to be a convenient technique, in terms of simplicity, short measurement times, sensitivity, and precision.

Keywords: *Photochemical fluorescence; phenylbutazone; degradation products; pharmaceutical analysis.*

Introduction

Phenylbutazone (PB) is a well-known pharmaceutical compound, which belongs to the non-steroidal anti-inflammatory group of drugs. It possesses antiarthritic and analgesic properties, and it is used largely in the treatment of many inflammatory or rheumatic diseases. However, several unpleasant side-effects which are due to PB degradation products occur in the body, and pharmacokinetic studies are needed in order to monitor PB concentrations changes. Therefore, it is very important to be able to determine quantitatively and specifically PB and its major degradation products at low concentration levels. The current official analytical procedures, which are based on titrimetry, potentiometry or colorimetry, are time-consuming, lack sensitivity and selectivity, and moreover do not allow the detection of degradation products [1, 2]. Several other techniques have been proposed for the quantitation of PB including colorimetry for the assay of PB in serum [3, 4], UV spectrometry [5, 6], luminescence spectrometry at 77 K [7–9], NMR spectrometry [10–13], chromatography [5, 14–18] and LC- or GC-mass spectrometry [19, 20].

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Recently, Kovalenko *et al.* [21] have shown that the photodecomposition of PB occurred rapidly in aqueous solution. This finding is interesting from an analytical standpoint, because room temperature photochemical-fluorescence (RTPF) has been demonstrated to be a versatile, selective, and sensitive technique for assaying a variety of photoreactive drugs and other organic compounds leading to fluorescent photoproducts [22–24].

In the present paper, the application of RTPF to the quantitative analysis of PB and its main degradation products is evaluated.

Experimental

Reagents

Phenylbutazone, (4-butyl-4-hydroxy-1,2-diphenyl-3,5-)-pyrazolidinedione (4-OH PB), butyltartronic acid mono-(*N*,*N*'-diphenyl)hydrazide (TADH), butylmalonic acid mono-(*N*,*N*'-diphenyl)hydrazide (MADH), and *N*-caproyl-hydrazobenzene (CHB) were prepared in these laboratories. Solvents used were chloroform and ethanol (analytical grade, Prolabo, Paris).

Apparatus

A Perkin–Elmer model LS-5 and a Kontron model SFM-25 spectrophotofluorimeter were used for the fluorescence measurements. A 200-W HBO Osram mercury arc lamp with an Oriel model 8500 power supply was used for the irradiation of solutions. A MGW Lauda model K4R thermostat was used.

Procedure

Solutions of PB and degradation products were prepared by the dilution of ethanolic (or chloroformic) stock solutions (1×10^{-3} M). All solutions were protected from light and were used within 12 h to avoid decomposition. The sample holder containing a 1-cm square quartz cuvette was placed at about 40 cm from the mercury arc lamp, and the solution irradiated during a fixed period of time at a constant temperature of 295 K. Fluorescence signal vs irradiation time $f(I_F, t_{irr}) = 0$ curves were established at the wavelength of maximum fluorescence of the photoproduct (λ_F^{max}). Log–log calibration curves were obtained using the photoproduct maximum fluorescence intensity values (I_F^{max}) which correspond to the optimal irradiation time (t_{irr}^{opt}). The range of linearity was determined and linear regression analysis of data performed on a programmable calculator.

Results and Discussion

Fluorescence spectral properties

The fluorescence excitation and emission wavelengths and intensities of PB, its degradation products and their photoproducts are presented in Table 1.

The excitation and emission maxima of PB occur at 283 and 450 nm respectively in ethanol. These values are very close to the literature values (290 and 460 nm, respectively) [7]. The irradiation of a 1×10^{-4} M ethanolic solution of PB generates a new emission band with a maximum at about 340 nm (λ_{ex} = 298 nm) (Fig. 1). The intensity of this band, which has been shifted to a wavelength 110 nm less than that of

Table 1
Fluorescence properties of phenylbutazone, its degradation products and their photoproducts

Compound*	Non-irradiated		Photoproduct			
	λ_{ex}, nm	λ_{em}, nm	λ_{ex}, nm	λ_{em}, nm	t_{irr}	I_F†
PB	283	450	243, 298†	340	30	7.3
PB§	NF‖	NF‖	338	390	4	5.8
4-OH PB	334	370¶	290	345	6	5.8
MADH	329	363	305	370	1	4.6
TADH	300	328	292	365	3	3.7
CHB	337	372**	290	360	2	1.8

*Concentration: 1×10^{-4} M and solvent = ethanol, unless otherwise noted.
†I_F: Fluorescence emission maximum intensity of the photoproduct normalized to the fluorescence intensity (1.0) of the non-irradiated corresponding compound.
‡Wavelength of maximum excitation is underlined.
§Solvent: chloroform; concentration = 1×10^{-3} M.
‖NF: Not fluorescent.
¶Excitation-dependent band; e.g. for λ_{ex} = 300 nm, λ_{em} = 330 nm.
**Excitation- and concentration-dependent band; e.g. for λ_{ex} = 319 nm, λ_{em} = 350 nm.

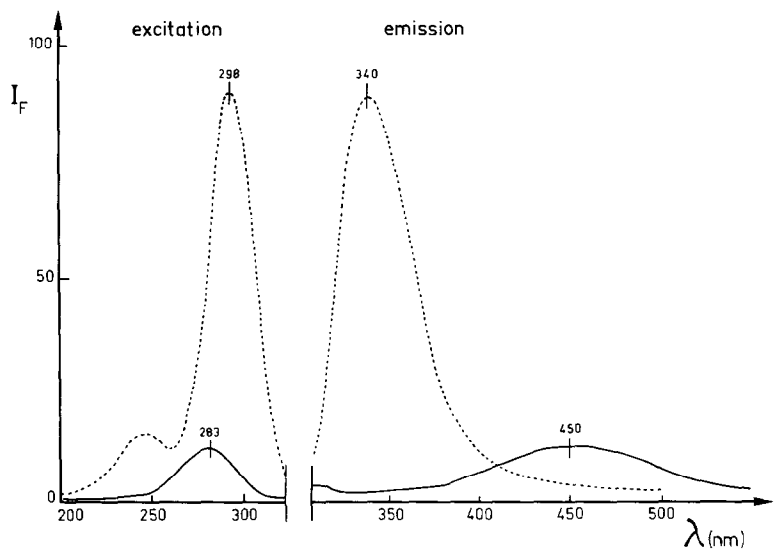

Figure 1
Effect of UV irradiation on the excitation and emission fluorescence spectra of PB (1×10^{-4} M) in ethanol. ———: before irradiation. ------: after 30 min irradiation.

non-irradiated PB, is about seven times greater than that of PB after a 30-min irradiation time. This is attributed to the formation of a photoproduct that fluoresces much more strongly than PB itself. This photoproduct is not stable, as the 340-nm band disappears when the solution is kept in the dark for 12 h. Phenylbutazone is barely fluorescent in chloroform, but its irradiation generates an emission band at 390 nm (λ_{ex} = 338 nm), which is also due to the formation of a fluorescent photoproduct. The large difference of emission wavelengths in both solvents may be due either to different photoproducts formed in the two solvents, or to a difference of polarity of its ground and excited states.

In the latter case, the shift would indicate that the singlet excited state of the photoproduct is less polar than its ground state, as already observed for other nitrogen heterocycles [25].

In the case of 4-OH PB, the emission band shows a maximum at 370 nm (λ_{ex} = 334 nm), but its position depends on the excitation wavelength (Table 1). When a 1×10^{-4} M ethanolic solution of 4-OH PB is irradiated, a more intense emission band occurs at 345 nm (λ_{ex} = 290 nm).

In ethanol MADH exhibits fluorescence excitation and emission maxima at 329 and 363 nm, respectively. A 1-min irradiation generates a red-shifted emission band with a maximum at 370 nm (λ_{ex} = 305 nm) which is five times more intense than that of non-irradiated MADH. This band is also attributed to the formation of a fluorescent photoproduct.

It is found TADH is weakly fluorescent; after irradiation, an emission occurs at 365 nm (λ_{ex} = 292 nm). For a 1×10^{-4} M ethanolic solution, the intensity of this band is enhanced about four times after a 3-min irradiation time.

The fluorescence spectrum of CHB is concentration-dependent, and the position of the emission band varies with the excitation wavelength, which may indicate the presence of two singlet excited state species. The irradiation of a 1×10^{-4} M ethanolic solution of CHB generates an emission band at 360 nm (λ_{ex} = 290 nm), which is very similar to that of irradiated TADH. This band is probably due to the formation of a fluorescent photoproduct.

Effect of irradiation time on the fluorescence intensity

The fluorescence intensity vs irradiation time $f(I_F, t_{irr}) = 0$ curves for PB and its degradation products were determined at the wavelength of maximum fluorescence of the photoproduct (Fig. 2). All curves show similar behaviour, i.e. a rapid increase of fluorescence signal, which reaches a maximum at the optimal irradiation time (t_{irr}^{opt}), a plateau and then a slight decrease in intensity. The t_{irr}^{opt} values depend on the solvent; in the case of PB, they are 4 min in chloroform and 30 min in ethanol. The values also depend on the analyte and ranged from 1 min for MADH to 30 min for PB in ethanol. In

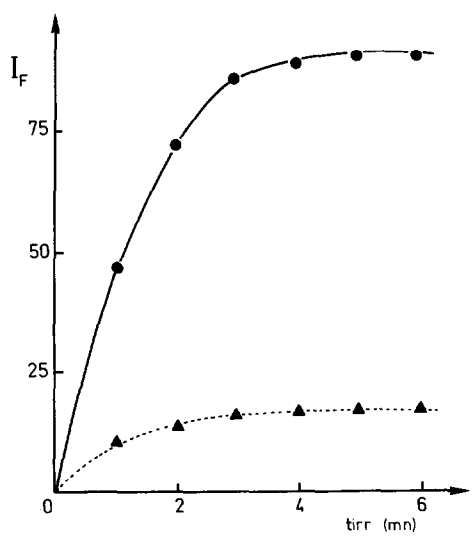

Figure 2
Effect of irradiation time on the PB fluorescence intensity in chloroform; ●——●: 1×10^{-3} M PB. ▲---▲: 1×10^{-4} M PB.

Table 2
Statistical treatment of the photochemical–fluorimetric calibration curves of phenylbutazone and its degradation products

Compound	t_{irr}* (min)	LDR†	Slope‡	Correlation coefficient‡	LOD§ (μg/ml)
PB	45	200	0.50	0.988	0.001
PB‖	4	50	0.85	0.997	1.2
MADH	4	200	0.67	0.992	0.1
TADH	15	400	0.52	0.981	0.008
CHB	15	1000	0.60	0.991	0.024

*t_{irr}: Irradiation time used for determining the fluorescence intensity of the photoproduct.
†LDR: Linear dynamic range, corresponding to the ratio of upper concentration of linearity (within 5%) to the detection limit.
‡Slopes and correlation coefficients calculated by least squares treatment of experimental data on a programmable calculator.
§LOD: Limit of detection, defined as the concentration of the solution giving a signal-to-noise (S/N) ratio of 3.
‖Solvent: Chloroform.

contrast, the profiles of the curves and the t_{irr}^{opt} values were almost independent of the initial concentration of the compound under study (Fig. 2).

Analytical figures of merit

The log–log RTPF calibration curves characteristics and the limit of detection (LOD) of PB and its degradation products are summarized in Table 2. Photoproduct maximum fluorescence intensities (I_F^{max}) were used. The linear dynamic ranges (LDR) spanned a 50- to 1000-fold range of concentration. Slopes of the log–log calibration curves were lower than unity for most compounds, indicating non-linearity of the non-logarithmic calibration plots. The correlation coefficients values were larger than 0.98 for PB and its degradation products, showing that the precision of the RTPF analytical curves is good. The LOD values (S/N = 3) are very low, ranging from 1 ng/ml for PB in ethanol to 0.1 μg/ml for MADH. The LOD value of PB is much lower than those of other methods, which range from 0.05 to 50 μg/ml [3–10, 15–19]. The LOD value of MADH (0.1 μg/ml) is lower than that of 0.2 μg/ml reported by Fabre *et al.* [15].

Conclusion

It is concluded from this study that room temperature photochemical-fluorimetry is a sensitive, precise, simple and rapid method for the determination of phenylbutazone and its degradation products. Because of the differences in fluorescence emission wavelengths and optimal irradiation times between the photoproducts of PB and its metabolites, RTPF appears to be a rather selective technique. The results suggest the need for further work to confirm the applicability of RTPF to the determination of degradation products in the presence of PB, and to the detection of these substances in physiological liquids.

References

[1] British Pharmacopoeia 1988, p. 986, The Pharmaceutical Press, London (1988).
[2] United States Pharmacopeia, XXI, 826, United States Pharmacopeial Convention, Rockville, MD (1980).

[3] M. E. El-Kommos, *Analyst* **108**, 1144–1147 (1983).
[4] K. P. R. Chowdary and N. Aparajitha, *Indian Drugs* **24**, 109–110 (1986).
[5] H. Fabre and B. Mandrou, *J. Pharm. Sci.* **70**, 460–461 (1981).
[6] M. A. Korany, A. M. Wahbi, M. A. ElSayed and S. Mandour, *Anal. Lett.* **17B**, 1373–1389 (1984).
[7] N. Strojny and J. A. De Silva, *J. Chromatogr. Sci.* **13**, 583–588 (1975).
[8] J. N. Miller, D. L. Phillips, D. T. Burns and J. W. Bridges, *Talanta* **25**, 46–49 (1978).
[9] I. Khasawneh, J. Erkhoff, D. Siegel, A. Jurgensen, E. Inman and J. D. Winefordner, *Microchem. J.* **31**, 281–287 (1985).
[10] R. Stromberg, *J. Pharm. Sci.* **73**, 1653–1654 (1984).
[11] J. K. Kwayke, *Talanta* **32**, 1069–1071 (1985).
[12] P. Goya, C. Ochoa, I. Rozas, A. Alemany and M. L. Jimeno, *Magn. Res. Chem.* **24**, 444–450 (1986).
[13] F. Matsui, D. L. Robertson, M. A. Poirie and E. G. Lovering, *J. Pharm. Sci.* **69**, 469–471 (1980).
[14] C. Sarbu, *J. Chromatogr.* **367**, 286–288 (1986).
[15] H. Fabre, B. Mandrou and H. Eddine, *J. Pharm. Sci.* **71**, 120–122 (1982).
[16] T. Marunaka, T. Shibata, Y. Minami and Y. Umeno, *J. Chromatogr. Biomed. Appl.* **183**, 331–338 (1980).
[17] E. M. Chan and S. C. Chan, *J. Anal. Toxicol.* **8**, 173–176 (1984).
[18] M. E. Sharp, *J. Anal. Toxicol.* **11**, 8–11 (1987).
[19] T. R. Covey, E. D. Lee and J. D. Henion, *Anal. Chem.* **58**, 2453–2460 (1986).
[20] L. Boniforti, M. G. Quaglia and M. Terraciano, *Ann. Ist. Super. Sanita* **23**, 97–104 (1987).
[21] N. P. Kovalenko, Yu. A. Ershov and S. F. Orlova, *J. Photochem.* **31**, 289–296 (1985).
[22] J. W. Birks and R. W. Frei, *Trends Anal. Chem.* **1**, 361–367 (1982).
[23] M. Tsuchiya, E. Torres, J. J. Aaron and J. D. Winefordner, *Anal. Lett.* **17**, 1831–1841 (1984).
[24] J. Fidanza and J. J. Aaron, *J. Pharm. Biomed. Anal.* **5**, 619–623 (1987).
[25] J. J. Aaron, M. D. Gaye, C. Parkanyi, N. S. Choo and L. Von Szentpaly, *J. Mol. Struct.* **156**, 119–135 (1987).

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