Pakistan Journal of Pharmaceutical Sciences 21
4(1) 21-26 (1991)

SPECTROPHOTOMETRIC ASSAY OF FORMYLMETHYLFLAVIN AND PHOTOPRODUCT-LGMICHROME IN ORGANIC SOLVENTS

IQBAL AHMAD and QAZI FASIHULIAH
Department of Pharmaceutical Chemistry, University of Karachi,
Karachi-75270, Pakistan

ABSTRACT

A spectrophotometric method has been developed for the assay of formylmethylflavin and its major photoproduct, lumichrome, in organic solvents. Absorption measurements are made at the respective maxima of the two compounds and the concentrations are determined by a two-component assay. The reproducibility of the method lies within ± 5 percent. The method is specific and can be conveniently employed for photodegradation studies of formyimethylfavin in organic solvents.

Introduction

Smith and Metzler (1963) isolated formylmethylflavin as a major intermediate of the anaerobic photodegradation of riboflavin in aqueous solution. Formylmethylflavin is photolysed to lumichrome and lumiflavin in aqueous solution (McBride and Metzler, 1967; Treadwell *et al.,* 1968; Heelis *et at,* 1980) and to lumichrome in organic solvents (Fasihullah, 1988). The uv and visible absorption characteristics of these compounds in aqueous and organic solvents have recently been reported (Ahmad and Fasihullah, 1990; Ahmad and Rapson, 1990). The present work is based on the development of a spectrophotometric method for the assay of photolysed solutions of formylmethylflavin in organic solvents.

Materials and Methods

Formylmethylflavin (FMF) was prepared by the method of Fall and Petering (1956). Lumichrome (LC) was obtained from Sigma Chemical Co. and recrystallised from glacial acetic acid. All solvents were analytical grade or of the purest form available from B.D.H/Merck, and were redistilled before use.

A 10^{-4}M solution of FMF, in the organic solvent was placed in a 100 ml volumetric flask (pyrex) and irradiated with Philips HPLN 125 W high pressure mercury-vapour fluorescent lamp (emission at 405, 436 and 545 nm) fixed at a distance of 30 cm in a radiation chamber. Samples were withdrawn at appropriate intervals for absorption measurements using a Shimadzu UV-visible recording spectrophotometer (Model UV-240). The base line was automatically corrected by the built-in memory at the initializing period. The concentrations of FMF and LC were calculated by a two-component assay (Chatten, 1969; Fell, 1986).

Results and Discussion

Choice of wavelengths:

It is important to choose appropriate analytical wavelengths for maximum accuracy in the analysis of mixtures. Wavelengths showing high sensitivity of measured absorbance and minimum interference from instrumental factors are ideal for analytical work (Jaffe and Orchin, 1962). Since the absorption characteristics of FMF and LC vary with a change is solvent (Fasihullah, 1988), it is not possible to use a particular set of analytical wavelengths for the assay of these compounds. The absorption spectra of FMF and LC in ethanol are shown in Figure-1. The two compounds exhibit similar spectra in other organic solvents. Thus the respective absorption maxima of FMF and LC in organic solvents (Table-1) could be used as the analytical wavelengths to achieve the best possible results.

Table-1

Molar absorptivities of formylmethylflavin and lumichrome used in the two-component spectrophotometric assay*

Compound	Solvent	Molar Absorptivity x 10^{-4} SD, M^{-1} cm^{-1}	
		nm (ε)	nm (ε)
Formyl-methylflavin	Water, pH 2.0 (KCl/HCl buffer)	385 (1.638±0.004)	
	Acetonitrile	411 (1.153±0.005)	380 (0.741±0.006)
	Methanol	444 (1.124±0.004)	384 (0.490±0.007)
	Ethanol	446 (1.122±0.003)	384 (0.462±0.007)
	1-Propanol	447 (1.078±0.003)	385 (0.440±0.006)
	1-Butanol	447 (1.086±0.004)	384 (0.449±0.006)
	Dichloroethane	446 (1.205±0.005)	381 (0.485±0.005)
	Chloroform	447 (1.228±0.005)	384 (0.474±0.007)
Lumichrome	Water, pH 4.5 (acetate buffer)	356 (1.080 0.004)	
	Acetonitrile	441 (0.025±0.001)	380 (0.746±0.004)
	Methanol	444 (0.028±0.002)	384 (0.773±0.005)
	Ethanol	446 (0.023±0.001)	384 (0.783±0.003)
	1-Propanol	447 (0.010±0.001)	385 (0.718±0.003)
	1-Butanol	447 (0.010±0.001)	385 (0.714±0.004)
	Dichloroethane	446 (0.023±0.001)	380 (0.510±0.005)
	Chloroform	447 (0.013±0.001)	384 (0.574±0.004)

* Values expressed as mean ± SD, n = 3-5.

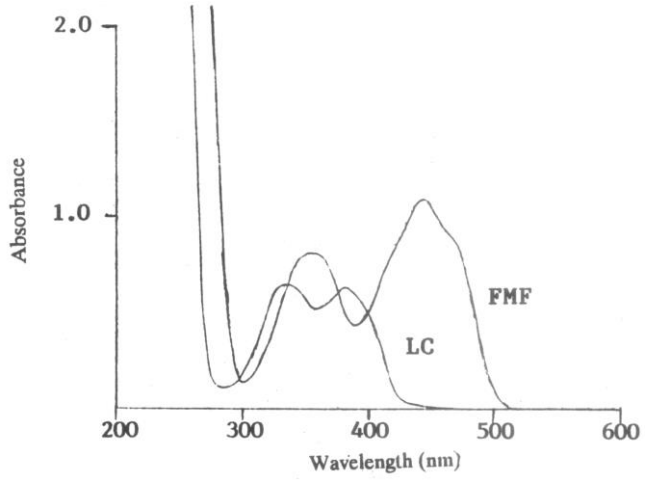

Figure 1. Absorption spectra of formylmethylflavin and lumichrome in ethanol.

Validity of Beer's law:

The simultaneous determination of two or three-components in a mixture rests on the assumption that the substances concerned contribute additively to the total absorbance at the analytical wavelengths. This assumption should be tested with mixtures of the material and the validity of Beer's law relation should be confirmed prior to the analysis (Willard *et al.,* 1974). This was achieved for FMF and LC, alone and in mixtures, at the appropriate wavelengths in all the solvents. The molar absorptivities (Table-1) used for the calculation of concentrations of FMF and LC represent the means of determinations at least 3-5 different concentrations over the range likely to be found in the reaction mixtures. Very few values determined in organic solvents are available in the literature (e.g., lumichrome; Koziol, 1966) for comparison of these data.

Reproducibility of method :

Typical results of the determination of molar concentrations of FMF and LC at various time intervals for photolysis reactions in ethanol and choroform are reported in Table-2. In each case the total molar balance (FMF + LC) is in good agreement with the initial concentration of FMF (10^{-4}M) and the values lie within ± 5% indicating the reliability of the analytical data. This magnitude of error may be expected in view of the presence of minor unknown photoproducts (Treadwell *et al,* 1968) and the compositional variations during the reaction in a particular solvent. As the concentration of these products increases with the progress of the reaction, the molar balance is also increased upto 5% depending upon the solvent used. However, the method is specific and may be conveniently employed for photodegradation studies of FMF in organic solvents.

Table-2

Photolysis of 10^{-4}M formylmethylflavin solution in ethanol and chloroform. Concentrations of FMF and LC ($Mx10^{5}$)

Time	Ethanol			Chloroform		
mins.	FMF	LC	Total	FMF	LC	Total
0	10.00	0.00	10.00	10.00	0.00	10.00
30	7.34	2.71	10.05	9.17	0.84	10.01
60	5.85	4.26	10.11	8.13	1.93	10.06
90	5.06	5.37	10.43	7.57	2.58	10.15
120	4.74	5.72	10.46	6.75	3.46	10.21
150	4.61	5.89	10.50	6.33	3.97	10.30
180	4.50	6.03	10.53	5.98	4.38	10.36

References

Ahmad, I. and Fasihullah, Q. (1990). Effect of solvent on uv and visible spectra of formylmethylflavin and photoproducts, *Pak. J. Pharm. Sci.* **3**: 7-11.

Ahmad, I. and Rapson, H.D.C. (1990). Multicomponent spectrophotometric assay of riboflavin and photoproducts, *J. Pharm. Biomed. Anal.* **8**: 217-223.

Chatten, L.G. (1969). Pharmaceutical Chemistry, Vol. 2, Marcel Dekker, New York, pp. 13-27.

Fall, H.H. and Petering, H.G. (1956). Metabolic Inhibitors, 1. 6,7-Dimethyl-9-(2'-hydroxyethyl)-isoalloxazine and derivatives, *J. Am. Chem. Soc.* **78**: 377-381.

Fasihullah, Q. (1988). Photolysis of flavins in aqueous and organic solvents, Ph.D. Thesis, University of Karachi, Karachi.

Fell, A.F. (1986). In "Clarke's Isolation and Identification of Drugs", 2nd ed. (Moffat, A.C., Ed.), The Pharmaceutical Press, London, pp. 227-228.

Heelis, P.F., Phillips, G.O., Ahmad, I. and Rapson, H.D.C. (1980). The photodegradation of formylmethylflavin-A steady state and laser flash photolysis study, *Photobiochem. Photobiophys.* **1**: 125-130.

Jaffe, H.H. and Orchin, M. (1962). Theory and Applications of Ultraviolet Spectroscopy, John Wiley, New York, pp. 557-560.

Koziol, J. (1966). Studies on flavins in organic solvents, 1. Spectral characteristics of riboflavin, riboflavin tetrabutyrate, and lumichrome, *Photochem. Photobiol.* **5**: 41-54.

McBride, M.M. and Metzler, D.E. (1967). Photochemical degradation of flavines, III. Hydroxyetbyl and formylmethyl analogues of riboflavin, *Photochem. Photobiol.**6:*** 113-123.

Smith, E.C. and Metzler, D.E. (1963). The photochemical degradation of riboflavin, *J. Am. Chem. Soc.* **85**: 3285-3788.

Treadwell, G.E., Cairns, W.L. and Metzler, D.E. (1968). Photochemical degradation of flavins, V. Chromatographic studies of the products of photolysis of riboflavin, *J. Chromamatogr*. 35: 376-388.

Willard, H.H., Merritt, L.L. Jr. and Dean, LA. (1974). Instrumental Methods of Analysis, 5th ed., von Nostrand, New York, Chap. 4.