Journal of Pharmaceutical & Biomedical Analysis
Vol. 6, Nos 6–8, pp. 875–878, 1988
Printed in Great Britain

0731–7085/88 $3.00 + 0.00
Pergamon Press plc

Stability of flavone-8-acetic acid (LM975) in aqueous solutions by high-performance liquid chromatography*

RICHARD F. BETTERIDGE, CATHERINE P. LAND and ANDREW G. BOSANQUET†

Bath Cancer Research Unit, Royal United Hospital, Combe Park, Bath BA1 3NG, UK

Abstract: A high-performance liquid chromatographic method has been developed to investigate the stability of solutions of flavone-8-acetic acid (LM975) during preparation and storage. LM975 (20 μg ml^{-1} in PBS) was found to be completely stable for 10 days at 80°C as long as light was rigorously excluded. The drug showed no significant adsorption to containers of different materials or to two filtration units tested. Drug degradation did occur however, on exposure to light. In normal laboratory light the $t_{0.95}$ (5% degraded) was 30.3 min, in intense natural light (laboratory window sill) the $t_{0.95}$ was 3.3 min and in intense artificial light (100 W bulb at 10 cm) the $t_{0.95}$ was 13.8 min. NMR and mass spectral analysis of the isolated degradation product implied the formation of the decarboxylated product, 8-methyl flavone. It is suggested that care be taken to exclude light during the preparation, storage and infusion of solutions of flavone-8-acetic acid.

Keywords: *Flavone-8-acetic acid; 8-methyl flavone; reversed phase high performance liquid chromatography; stability; light LM975.*

Introduction

Flavone-8-acetic acid (LM975, NSC 347512, I) is one of a series of flavonoids selected by the National Cancer Institute (NCI) for clinical trial due to its unusual preclinical activity against colon adenocarcinoma 38 in mice [1–3], and the first Phase I trials are being conducted now [4, 5].

The drug has been reported as being stable at 70°C, pH 9.6 for 12 h, but to degrade at pH >10.5 with a $t_{1/2}$ at pH 11.2 of 2.4 h [3]. However no studies have been reported that directly investigate the drug's stability under the conditions that might be expected when the drug is prepared and stored for *in vitro* chemosensitivity assays i.e. at low concentrations in aqueous buffer solutions.

A high-performance liquid chromatographic (HPLC) method has therefore been used to determine the stability during preparation and storage of solutions of LM975.

* Presented at the "International Symposium on Pharmaceutical and Biomedical Analysis", September 1987, Barcelona, Spain.

† To whom correspondence should be addressed.

Materials and Methods

Flavone-8-acetic acid (monosodium salt; Lipha Research, Lyon, France) was supplied by the Cancer Research Campaign Phase I/II Clinical Trials Subcommittee. Methanol (HPLC grade) was obtained from Rathburn Chemicals Ltd. (Walkerburn, Scotland), and orthophosphoric acid (Analar) from BDH Chemicals Ltd. (Poole, UK). LM975 was dissolved at 100 mg ml^{-1} in double distilled water then diluted to 20 mg ml^{-1} with normal saline (150 mM NaCl; Phoenix Pharmaceuticals, Gloucester, UK) and 200 μl aliquots stored frozen in polypropylene tubes (Nunc tubes, Intermed, Denmark). Further dilution to 20 μg ml^{-1} was achieved using Dulbecco's phosphate buffered saline (PBS; Oxoid, Basingstoke, UK). The standard (20 μg ml^{-1}) was made by diluting LM975 in mobile phase and was stored at −20°C. All dilutions were done in the shortest possible time and with rigorous exclusion of light.

A high-performance liquid chromatographic (HPLC) method has been developed using a mobile phase consisting of methanol–10 mM orthophosphoric acid (70:30%, v/v) at a flow rate of 1 ml min^{-1} and a 5-μm Spherisorb ODS1 reversed-phase column. The equipment used has been described in detail previously [6].

A standard curve for the LM975 external standard was prepared by plotting peak area against concentration at 100, 40, 20, 10, 4 and 2 μg ml^{-1}. Regression analysis of calibration data indicated a linear response over this concentration range ($r^2 = 0.999$), and the intercept value was not significantly different from zero (0.27 ± 0.67 μg ml^{-1}). For six replicate injections at each of the above concentrations the relative standard deviation calculated for peak area were 0.96, 1.04, 0.76, 2.14, 1.86 and 2.65% respectively.

Solutions of LM975 were studied under various conditions of temperature [9°C, room temperature (RT; 20–25°C), 37°, 50°, 80°C], light intensity (intense natural, intense artificial and normal laboratory light), and container material (glass, siliconised glass, polypropylene, polyethylene, and polystyrene). Intense natural light was provided by a laboratory windowsill, intense artificial light by a 100 W tungsten bulb 10 cm from the solution and normal laboratory light by simply leaving the solution on the laboratory bench.

Filter units (Gelman, Ann Arbor, Michigan, USA) with membranes made of polysulfone and polytetrafluoroethylene were tested for drug adsorption without any pre-wetting or washing. A solution of LM975 (20 μg ml^{-1} in PBS) was filtered so that 1 ml was collected in ~5 s. The drug concentration in the filtered solution was compared with that of the unfiltered control.

Results and Discussion

The chromatogram in Fig. 1 shows the rapid degradation of LM975 (20 μg ml^{-1}, PBS, glass, RT) in intense natural light (sunny laboratory windowsill). With the HPLC conditions used in this work, retention times of 5.0 min and 13.8 min were obtained for LM975 and a degradation product respectively. The long retention time of the previously unknown degradation product, suggested the formation of a more hydrophobic molecule. This would seem to preclude the possible flavonoid ring opening reaction [7].

During the degradation of LM975 at 20 μg ml^{-1} fine cream needle crystals formed. Increasing the LM975 concentration in the experiment to 1 mg ml^{-1} (15 mg total) allowed the isolation of the crystals by filtration onto a glass sinter and drying over P_2O_5 (3 mg collected). Analysis of this sample and the original LM975 by NMR revealed that

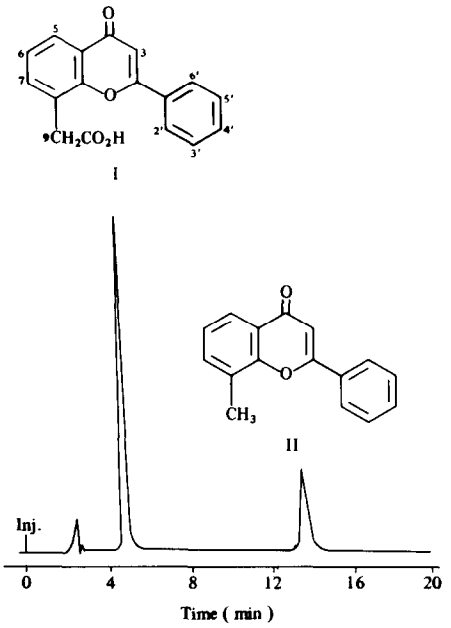

Figure 1
Structures and typical chromatogram of LM975 (I) and its proposed decarboxylated degradation product, 8 methyl-flavone (II), after irradiation with intense natural light for 25 min (20 μg ml^{-1}, RT, PBS).

the methylene CH_2 (δ3.61 ppm, C9), in the LM975 spectrum was not present in the spectrum of the degradation product. Also in the degradation product spectrum there was a singlet at δ2.60 ppm that integrated for 3 protons which was not present in the parent compound. All other peaks found were consistent with the flavonoid ring structure. Chemical ionization mass spectra of the drug and degradation product yielded M+1 molecular ions at 281 and 237 respectively — a loss of 44 mass units. Both the NMR and the mass spectra results support the formation of the decarboxylated degradation product, 8-methyl flavone (II).

Figure 2 shows the graphical representation of the decay of LM975 with time under various lighting conditions. In intense artificial light the $t_{0.95}$ (5% degradation) was 13.8 min and in intense natural light the degradation was even faster with $t_{0.95}$ being 3.3 min. In normal laboratory light the decay of LM975 was slower but still appreciable with a $t_{0.95}$ of 30.3 min. In the dark no decomposition product was observed even at a temperature of 80°C for 10 days.

In the dark the container material had no effect on the stability of the drug in solution (20 μg ml^{-1}, PBS). However in the light the drug showed greater stability in polyethylene and polypropylene. This may have been due to their opaque nature — these plastics reducing the transmission of light to the solution.

Minimal adsorption of LM975 to filters made of polysulfone and polytetrafluoroethylene was found with the filtered sample being 99.3 ± 1.2% (mean ± S.D.; $N = 3$) and 98.6 ± 1.2% respectively of the unfiltered control.

In conclusion, flavone-8-acetic acid (LM975), although stable in PBS (20 μg ml^{-1}) at temperatures up to 80°C, was found to be unstable if light was not rigorously excluded from the solution. Care must be taken, therefore, to exclude light during the preparation and storage of such solutions. The formation of crystals or a precipitate during standing would suggest that degradation has taken place.

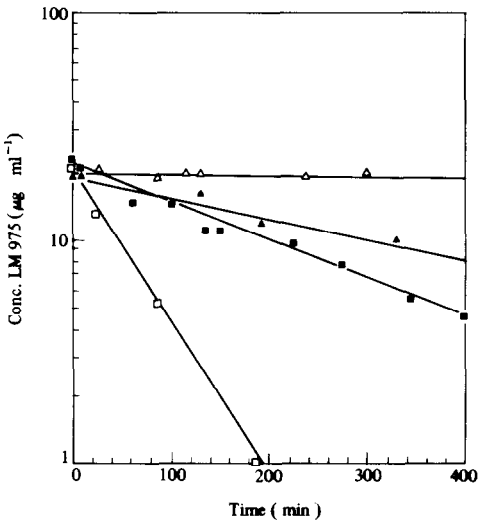

Figure 2
Decay of LM975 with time under various lighting conditions (20 μg ml^{-1}, RT, PBS). Symbols: □———□, intense natural light; ■———■, intense artificial light; ▲———▲, normal laboratory lighting; △———△, dark.

Schedules chosen for phase I study of LM975 in man include 6 and 24 h infusions [4, 5]. We strongly suggest that for long term infusions of LM975 a cover be used to rigorously exclude light from the drug solution.

Acknowledgements — We would like to thank for their help Dr D. E. V. Wilman for the NMR spectra and Dr A. J. Floyd for the mass spectra.
This work was funded by the Bath Cancer Research Unit and the Cancer Research Campaign.

References

[1] J. Plowman, V. L. Narayanan, D. Dykes, E. Szarvasi, P. Briet, O. C. Yoder and K. D. Paull, *Cancer Treat. Rep.* **70**, 631–635 (1986).
[2] M. C. Bibby, J. A. Double, R. M. Phillips and P. M. Loadman, *Br. J. Cancer* **55**, 159–163 (1987).
[3] D. S. Zaharko, C. K. Griesaber, J. Plowman and J. C. Cradock, *Cancer Treat. Rep.* **70**, 1415–1421 (1986).
[4] C. Bradley, S. B. Kaye, D. J. Kerr, A. Setanoians, J. Cassidy, L. Adams, E. M. Rankin, T. Young, G. Forrest and M. Soukop, *Br. J. Cancer* **56**, 215 (1987).
[5] P. J. O'Dwyer, D. Shoemaker, D. S. Zaharko, C. Grieshaber, J. Plowman, T. Corbett, F. Valeriote, S. A. King, J. Craddock, D. F. Hoth and B. Leyland-Jones, *Cancer Chemother. Pharmacol.* **19**, 6–10 (1987).
[6] A. G. Bosanquet and E. D. Gilby, *J. Chromatogr.* **232**, 345–354 (1982).
[7] B. Hausteen, *Biochem. Pharmacol.* **32**, 1141–1148 (1983).

[*Received for review 9 October 1987; revised manuscript received 30 October 1987*]