Journal of Chromatography, 370 (1986) 139–147
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 19 030

QUANTITATIVE THIN-LAYER CHROMATOGRAPHY IN ACCELERATED STABILITY STUDIES FOR PREDICTION OF INHERENT SENSITIVITY OF DRUGS TOWARD OXYGEN

LASZLO R. TREIBER
Chemical Engineering R & D, Merck Sharp & Dohme Research Laboratories, Rahway, NJ 07065 (U.S.A.)
(Received August 18th, 1986)

SUMMARY

This paper discusses a novel technique for studying the inherent sensitivity of materials toward oxygen and the utility of quantitative thin-layer chromatography, as a tool in such studies. The degradation generally followed first order kinetics up to about 60% decomposition, indicating that the usual kinetic treatment applied to homogeneous systems can be used. The method can also detect degradation products, in many cases adding a considerable diagnostic element to its predictive value.

Among the model compounds tested testosterone was the most stable with *ca.* 95% recovery following a 190-h exposure to air on standard silica gel plates. The half-life time of the other model substances under similar experimental conditions was estimated by means of direct measurements or by extrapolation, and found to range from approximately 300 h to 1 h 10 min with cortisone marking the upper value and cholesta-3,5-diene the lower one.

INTRODUCTION

Accelerated stability studies are one of the main tools used in drug development. They allow making key decisions in terms of selecting conditions for processing, handling, storing, assaying and formulating any given new product, in an early stage of development. The most frequently investigated stress conditions are temperature, air, light, moisture, pH and various additives or excipients.

The oxidative degradation of many compounds on thin-layer plates has been known for a long time. As a matter of fact, assay procedures are occasionally frustrated by it, so that various methods have been developed to protect samples susceptible to oxidation. For vitamin D_1 it was found that samples on silica gel layers wet with an organic solvent are sufficiently stable, but quickly decompose if the sorbent becomes dry. Hence, speedy work and the elimination of the drying steps were recommended to obtain reproducible data. Other methods for stabilizing vitamins A and D and related derivatives included neutralization of the acid surface of silica gel with weakly alkaline excipients (*e.g.* magnesium oxide, triethanolamine), or preparing the vitamin D_3 adduct with cholesterol[2]. For protection of corticosteroids

0021-9673/86/$03.50

exposed to air during prolonged autoradiography on silica gel plates, the inclusion of ascorbic acid into the sorbent layer was recommended[3]. In this case ascorbic acid, itself prone to decomposition on silica gel carrier[4], probably serves as an oxygen scavanger. For the purpose of protecting oxyphenbutazone against oxidation, butylated hydroxytoluene was also recommended[5,6]. The latter serves to complex trace metals that could act as catalysts of oxidation reactions. According to a survey of DeRitter[7], edetic acid is the chelating agent most commonly used for the prevention of oxidative degradation catalyzed by trace metal ions.

Although correlations between the time of exposure to air and the extent of degradation have previously been described[1,6,8], these observations were mostly qualitative aimed at eliminating losses and errors during analytical work, and have never been utilized for the quantitative characterization of the oxidative sensitivity of materials.

EXPERIMENTAL

Pre-coated thin-layer chromatographic (TLC) plates (silica gel 60 F-254 from E. Merck) were first purified for the experiments. They were developed in methanol to their entire length, then reactivated in a drying oven at 110°C for 1 h. The plates were kept in a closed cabinet protected from moisture until used.

The test substances were dissolved in ethyl acetate at a concentration of 2.00 g/l. Aliquots (5 μl) of the stock solutions were applied onto the TLC plates by means of a micropipettor. The first sample applied had the longest exposure time. The time intervals between each subsequent sample constituted a series of exposure times. The final sample used as a reference was applied and dried in a nitrogen atmosphere. Every step of the experiment was conducted at ambient temperature. Up to ten samples were spotted on a standard size (20 × 20 cm) plate. The distance between the samples was 18 mm in accord with the requirements of the Shimadzu CS-920 scanner for automatic scanning. The integral of the reference sample was defined as 100%, and all the recoveries for the other samples were based on that.

Ethyl acetate was used for the development of the chromatogram in all but one case; in the experiment with estradiol, ethyl acetate was replaced with chloroform–dioxane (9:1, v/v). The development of the chromatograms was carried out in equilibrated chambers in the ascending mode. After development, the plates were transferred into a closed chamber for drying in a stream of nitrogen, under complete exclusion of oxygen.

The dry plates were quickly transferred into the Shimadzu CS-920 high-speed TLC scanner, the plate compartment of which was continuously purged with nitrogen. The quantitative evaluation of the chromatograms was carried out at the wavelength corresponding to the absorption maximum for the compound being tested.

Plots of the recoveries against the respective exposure times allowed the construction of kinetic curves describing the accelerated oxidative decomposition process.

RESULTS AND DISCUSSION

The model compounds selected cover a wide range of oxidative sensitivity. The

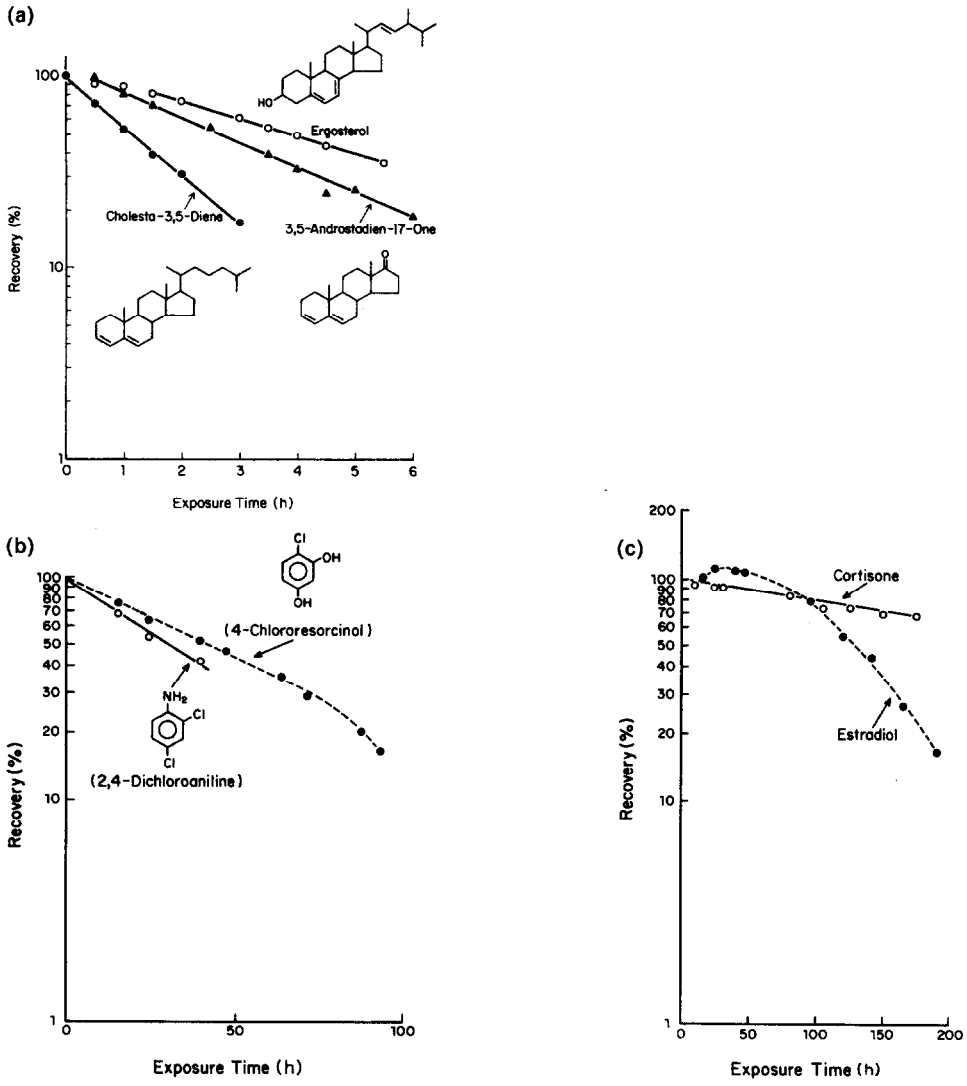

Fig. 1. First order plots of accelerated oxidative degradation as a function of exposure time for (a) cholesta-3,5-diene, 3,5-androstadien-17-one and ergosterol (scanning wavelengths: 235, 235 and 280 nm, respectively); (b) 4-chlororesorcinol and 2,4-dichloroaniline (scanning wavelengths: 280 and 235 nm, respectively); (c) cortisone and estradiol as a function of exposure time, (scanning wavelengths: 245 and 280 nm, respectively).

kinetic plots were constructed from the experimental data as the recoveries on the logarithmic scale against exposure time (Fig. 1). The recovery for testosterone after 190 h of exposure to air was over 95%. Much longer exposure time would be necessary to obtain a meaningful kinetic plot for this particular compound. The other extreme of the series was cholesta-3,5-diene with a half-life time of about 1 h 10 min (Fig. 1a). 3,5-Androstadien-17-one and ergosterol, both structurally related to cho-

lesta-3,5-diene, were more stable with estimated half-life times of 2 h and almost 4 h respectively (Fig. 1a).

2,4-Dichloraniline and 4-chlororesorcinol (Fig. 1b) were found to have half-life times of *ca.* 30 and 40 h, respectively. The half-life of cortisone (Fig. 1c) was estimated by extrapolation to be about 300 h. Estradiol shows a kinetic curve (Fig. 1c) quite different from the other examples. This deviation will be discussed in detail later on.

The decomposition products of cholesta-3,5-diene, 3,5-androstadien-17-one and ergosterol are very poorly detectable on the TLC plate at the respective scanning wavelength, suggesting that the probable site of the oxidative attack is the conjugated diene systems. Attempts to isolate and identify a stable oxidative degradation product were unsuccessful. Various methods indicated that the decomposition involves a very complicated cascade of reactions without the accumulation of any well-defined, detectable degradation product. Although under different experimental conditions, nevertheless similar sensitivity to oxidative degradation of other conjugated double-bond systems was reported earlier[9,10].

In contrast to the above examples, the oxidative degradation products of the substituted benzene derivatives, 2,4-dichloroaniline (Figs. 2 and 3) and 4-chlororesorcinol (Figs. 4 and 5) are readily detectable as distinct chromatographic zones. Since the results are plotted as percentage of the area counts based on the 0-time sample as 100%, the apparent recovery can be over 100% for any given fraction that has an absorption coefficient greater than that of the parent compound. This is the most probable explanation for the seemingly increasing recovery of estradiol in the early phase of the kinetic curve. Obviously, a recovery greater than 100% cannot be real (Fig. 1c). A degradation product with a higher absorption coefficient than estradiol, and not separated chromatographically, is the most likely explanation for the anomaly experienced here. Due to its sensitivity toward oxygen it, too, decomposes, giving rise to a number of more stable subsequent degradation products (Figs. 6 and 7), and, of course, further contributing to the distortion of the kinetic curve.

In case of cortisone the site of oxidative degradation appears to be the 1,3-dihydroxyacetone side-chain. Three well defined fractions increase in intensity con-

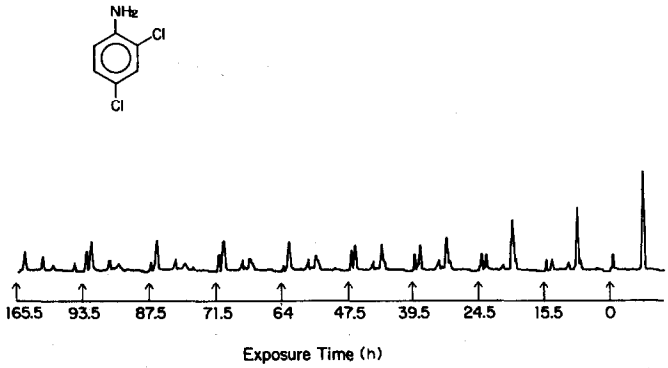

Fig. 2. 2,4-Dichloroaniline: densitometric scan of a series of time samples. Note the poor separation between the parent compound and a third degradation product.

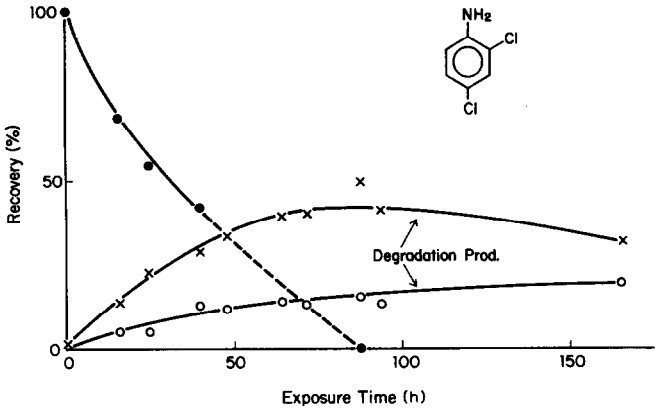

Fig. 3. 2,4-Dichloroaniline: plot of the parent compound and two degradation products. Note that due to poor separation between the parent compound and a third degradation product the data are incomplete.

currently with the decrease of the cortisone concentration (Figs. 8 and 9). The oxidative attack at the α,β-unsaturated keto group would probably lead to derivatives detectable poorly, if at all, at 240 nm. Similar observations were reported in conjunction with stability studies on prednisolone solutions[11].

In the majority of cases the degradation curves followed first order kinetics (Fig. 1), which is quite remarkable for a heterogeneous system. The explanation offered at the present time is as follows: when applied on a sorbent in small quantities, two key conditions are met. First, the solid state form is most likely amorphous, since materials in crystalline form generally are more resistant to most stress conditions[12]. Second, distributed on a large surface as a thin film, the material is uniformly exposed to the gas phase so that the diffusion of oxygen through the film is not a rate limiting factor. As a result, although the reaction formally is heterogeneous, this morphology creates conditions characteristic of a homogeneous system. The kinetic curves are indeed quite linear for a wide range of decomposition. Deviation from linearity is not evident until about 60% of the material is decomposed (Fig. 1a and b), except for an initial lag in case of 3,5-androstadien-17-one and ergosterol.

It is interesting to note that the oxygen-sensitive sites in cholesta-3,5-diene and

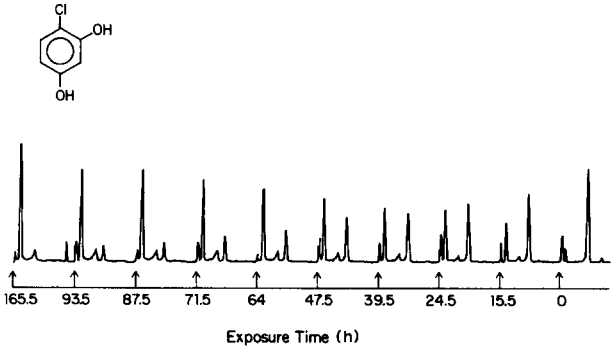

Fig. 4. 4-Chlororesorcinol: densitometric scan of a series of time samples.

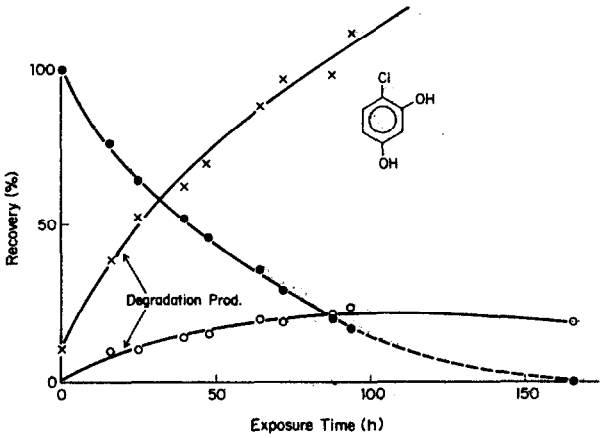

Fig. 5. 4-Chlororesorcinol: plot of the parent compound and two degradation products.

3,5-androstadien-17-one are identical. Yet, the difference in stability is significant. It seems that compounds with identical oxygen-sensitive sites may display greater stability toward oxygen if the molecule contains a polar substituent. This polar moiety can be situated in a location remote from the sensitive site. A possible explanation is that non-polar surfaces adsorb molecular oxygen more readily than polar surfaces thus providing a greater oxygen concentration. A more elaborate interpretation would require studying the three-dimensional structure of the molecules, and their orientation in the film deposited on the adsorbent. Similar observations were made

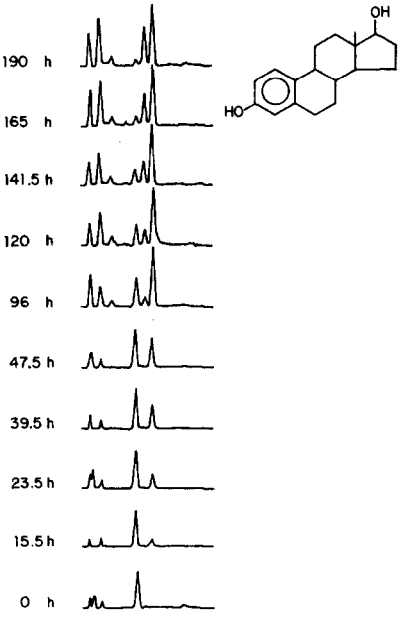

Fig. 6. Estradiol: densitometric scan of a series of time samples. Note the modified arrangement of the scans rendering a better illustration.

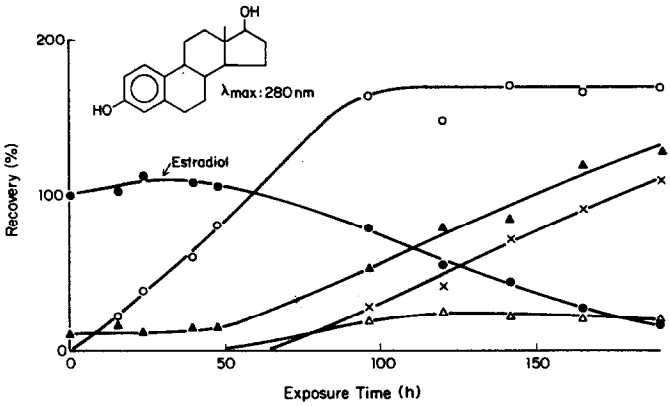

Fig. 7. Estradiol: plot of the parent compound and four degradation products. R_F values: (▲) 0.00, (△) 0.11, (●) 0.34, (×) 0.44, (○) 0.52.

on other classes of compounds currently under investigation. Experimental details will be made available in future publications; however, the examples presented here already demonstrate a relationship between chemical structure and oxidative stability. Future studies will include investigations on how to utilize the above finding in drugs design and formulation.

It should be pointed out that this method is primarily recommended for the rapid evaluation of inherent sensitivity to oxygen, and its use will probably be limited when batch-to-batch variations of stability are studied. Cholesta-3,5-diene, 3,5-androstadien-17-one and ergosterol are known to be sensitive to oxygen in crystalline bulk form. Although the information available from manufacturers is only qualitative, the correlation with the data presented here is convincing; these three compounds were the least stable in this test. Long-time stability studies on the crystalline bulk materials will be necessary to obtain quantitative correlation.

Several of the model compounds investigated are considered practically stable, when the well-developed crystals of the pure materials are exposed to air. However, the comparison of the model compounds is still valid in terms of their inherent sen-

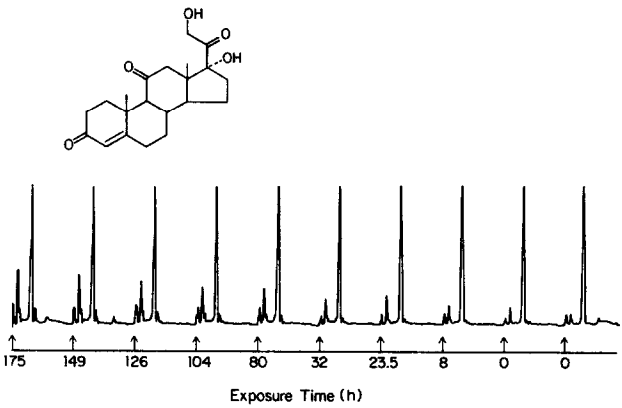

Fig. 8. Cortisone: densitometric scan of a series of time samples.

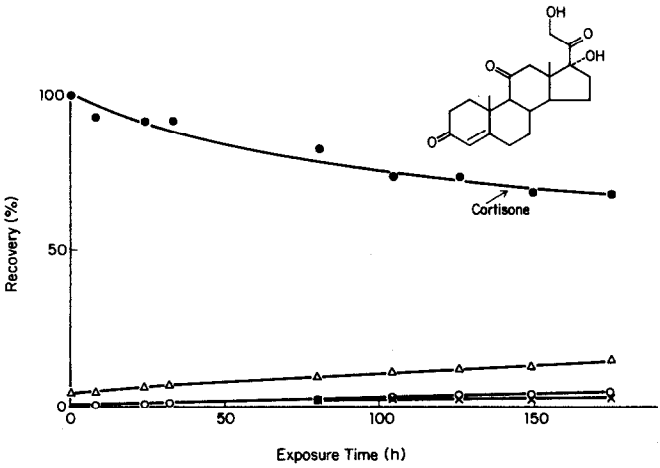

Fig. 9. Cortisone: plot of the parent compound and three degradation products. R_F values: (△) 0.00, (×) 0.05, (●) 0.28, (○) 0.37.

sitivity toward oxygen. A similar objective was previously achieved electrochemically by measuring the standard oxidation potential of an appropriate half-cell[13]. However, this method determines instability in solution. The method described here is much more widely applicable and technically simpler than the electrochemical procedure. Its potential importance lies in the possibility of predicting the behavior of materials under various conditions of manufacturing, storage and formulation. For instance, the method could be used to study the effects of various excipients and formulation methods. Silica gel is occasionally used as additive[2,4,7,12]. If the formulation calls for depositing a compound on silica gel from a volatile solvent, this method could predict possible problems associated with oxidative degradation. Of course, test conditions could be modified as to accommodate virtually any additive considered. Silica gel was selected here only for practical reasons: after exposure, the chromatogram could be developed on the same plate, and in addition to the predictive value of the method, the detection of the degradation products allowed the demonstration of certain diagnostic values. Obviously, silica gel as a carrier for the exposure can be replaced by any other excipient candidate (magnesium oxide, starch, cellulose, etc.), while the chromatography can be performed on any sorbent found suitable for the purpose. TLC plates with pre-adsorbent layers are commercially available. The pre-adsorbent could be designed according to the needs of the screening program. This laboratory is already involved with preliminary experiments including modified silica gel used as carrier for exposure, and TLC as well as high-performance liquid chromatography are employed for the evaluation of the stressed materials.

This method can also serve the evaluation of potential problems associated with manufacturing operations. If a compound is found to be unstable in this test, crystallization conditions will have to be such that the amount of amorphous material formed on the crystal surface, *e.g.*, due to drying of mother liquor residues, be reduced to a minimum. Crystal imperfection can also occur in presence of certain contaminants the effects of which could also be predicted by means of this test.

Finally, appropriate test results might call for processing and storage all together in an inert atmosphere.

The advantages of this method are as follows. (1) Small amounts (< 2 mg) of the material are needed per experiment. Even precious chemicals can be tested with minimum quantities sacrificed. (2) The degradation reaction is truly accelarated: even at ambient temperature, the results are obtained within a very short time. (3) As a consequence of (1) and (2), predictive, and to some extent, diagnostic information can be generated in a very early phase of product development. (4) The simplicity and speed of the technique allows its use in screening for excipients and dosage forms.

These and other related topics are currently under investigation, and will be reported in due course.

ACKNOWLEDGEMENTS

Valuable comments and suggestions obtained from Drs. R. G. Bergstrom, J. Brenner, G. V. Downing, D. W. Fink and E. S. Inamine are gratefully acknowledged.

REFERENCES

1 H. R. Bolliger and A. König, *Z. Anal. Chem.*, 214 (1965) 1.
2 E. Knobloch, V. Janata, M. Auskova, K. Mnoucek, O. Matousova and M. Likarova, *Cesk. Farm.*, 20 (1971) 244.
3 S. Frgacić and Z. Knicwald, *J. Chromatogr.*, 94 (1974) 291.
4 E. DeRitter, L. Magid, M. Osadca and S. H. Rubin, *J. Pharm. Sci.*, 59 (1970) 229.
5 *British Pharmacopoeia 1980*, Vol. I, H.M. Stationery Office, London, 1980, p. 321.
6 H. Fabre, A. Ramiaramanana, M. D. Blanchin and B. Mandrou, *Analyst (London)*, 110 (1985) 1289.
7 E. DeRitter, *J. Pharm. Sci.*, 71 (1982) 1073.
8 K. Macek, *J. Chromatogr.*, 33 (1968) 332.
9 T. E. Eble and E. R. Garrett, *J. Am. Pharm. Assoc., Sci. Ed.*, 43 (1954) 536.
10 J. E. Tingstad and E. R. Garrett, *J. Am. Pharm. Assoc., Sci. Ed.*, 43 (1960) 352.
11 Ch. Knopp, *Sci. Pharm.*, 52 (1984) 247.
12 D. C. Monkhouse, *Drug Dev. Ind. Pharm.*, 10 (1984) 1373.
13 P. J. Stewart and I. G. Tucker, *Aust. J. Hosp. Pharm.*, 15 (1985) 111.