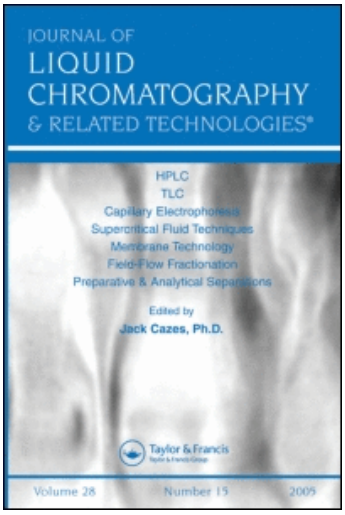

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Recognition of Artifacts Occurring in TLC

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RECOGNITION OF ARTIFACTS OCCURRING IN TLC

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ABSTRACT

The increased production of drugs requires a concomitant assessment of drug purity. Chromatography in general, and thin layer chromatography in particular, play an important role in determination of the impurity profiles of drug candidates. However, in using chromatography to determine impurities, the chemist must be careful, since extraneous zones or spots do not always indicate impurities. They may instead be artifacts, produced in the chromatographic system. In this paper we present a phenomenon related to on-plate decomposition. MK0912 was chosen as a model compound. To overcome the on-plate degradation an inclusion compound was formed with γ-cyclodextrin in the spotting solution, followed by a mobile phase containing hexadecyltrimethylammonium bromide as a micelle generator. This technique proved to be successful for preventing degradation during chromatography.

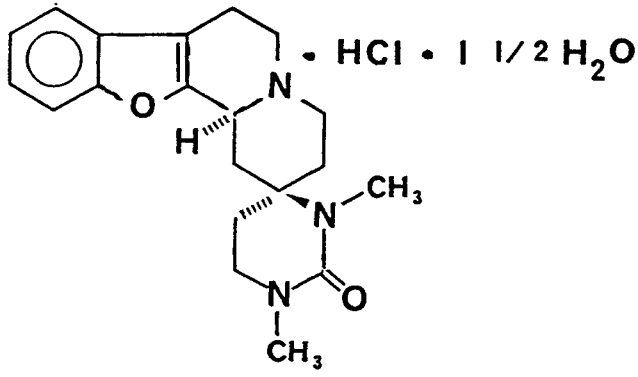
INTRODUCTION

Drug production in the pharmaceutical industry is the result of a joint effort between several fields of expertise such as biotechnology, natural product isolation and organic synthesis [1]. Once a compound is produced by one of these areas, the ensuing scale up from the laboratory scale to industrial production is a tedious process involving many purity assays as well as characterization of the drugs impurity profile. The increased demand for new drugs not only requires accurate assessment of purity but also dictates that it be performed in a time efficient manner. Thin layer chromatography (TLC) fulfills these requirements.

TLC is a powerful tool for monitoring impurity profiles of drugs not only because of its speed but also because of the wide range of detection methods available (as opposed to HPLC methods)[2]. However, when developing a TLC method the interpretation of the chromatogram is not always straight forward. The general tendency among chemists is to designate multiple spots in a TLC separation as impurities. This may not always be the case, since some spots may

be artifacts of the system due to degradation of the sample during development [3]. There are several sources of extraneous zones in chromatography which will be discussed below.

In the present paper we wish to outline an example of extraneous zones obtained in TLC as a result of on-plate degradation. Using γ-cyclodextrin in the spotting solution and micelles as a mobile phase additive we were able to avoid decomposition and to obtain an acurate impurity profile. It should be emphasized that in our laboratory we encountered several cases of such artifacts and we report an example using MK0912 whose structure is presented below.



EXPERIMENTAL

Stationary phase

HPTLC silica-gel plates (10 x 10 cm) with fluorescence indicator at 254 nm were purchased from E. Merck (Darmstadt, Germany).

Mobile phase

The mobile phases used in our experiments consisted of ethyl acetate-hexane-methanol-ammonia (50:35:15:2, v/v) and ethyl acetate-methanol-hexane (40:40:20, v/v) containing different amounts of hexadecyltriethylammonium bromide (HDTAB).

Sample preparation

The analyte was dissolved in methanol or a 2 M methanolic solution of urea containing 8.8×10^{-5} M γ-cyclodextrin. In some cases spots were scraped from the support, extracted from the stationary phase with chloroform and respotted. The spotting procedure was performed with a Linomat III automatic spotter (Camag, Switzerland).

Chromatographic chamber

The chromatography was performed in rectangular chromatographic chambers. The level of mobile phase was 0.5 cm.

Detection

Detection was performed under short and long wave UV light at 254 and 366 nm respectively, using a high intensity portable lamp (Spectronics Corporation, New York, U.S.A).

RESULTS AND DISCUSSION

The on-plate degradation was studied with several analytes. MK0912 was used as a model compound and was chromatographed on a HPTLC silica-gel plate using a mobile phase of ethyl acetate-hexane-methanol-ammonia (50:35:15:2, v/v). The analyte solution was applied at the origin of the TLC plate as a band. The plates were visualized under short wave and long wave UV. A photograph of the chromatogram under short wave UV is presented in Fig. 1a. A major spot was observed at an R_f of 0.43. By inspecting the same chromatogram under long wave UV,

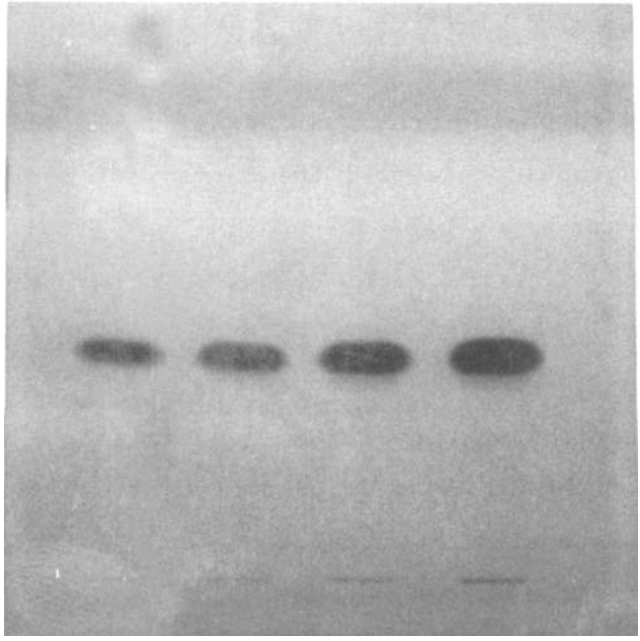


Fig.1. Separation of MK0912 on silica-gel HPTLC. a - visualized under short wave UV, b - visulaized under long wave UV. Mobile phase: ethyl acetate-hexane-methanol-ammonia (50:35:15:2, v/v). The amount spotted increases from left to right (1.25, 2.5, 5.0 and 7.5 μg).

additional fluorescent spots were encountered. The intensity of these spots increased after heating the plate at 120°C for ten minutes. The extraneous spots were located at R_fs of 0.0, 0.54 and 0.59 (Fig. 1b)

The occurrence of multiple spots in TLC has been thoroughly investigated. There are several sources of

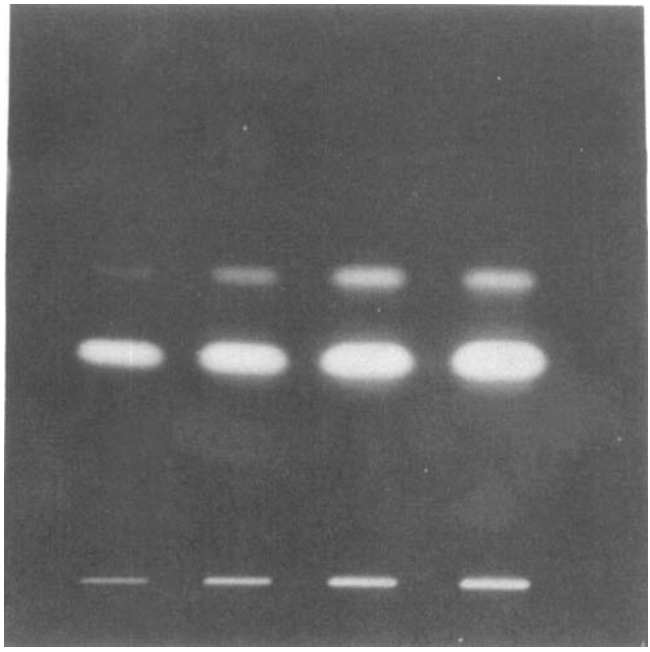


Fig. 1B

multiple spots in TLC: chemical reaction [4], impurities present in the sample solution [5], discontinuities in the stationary [6] and mobile phases [7], formation of charged species and/or complexes [8], and equilibrium between species [9].

In our study of MK0912, artifacts were detected presumably due to stationary phase catalyzed chemical reactions. Decomposition occurring during a

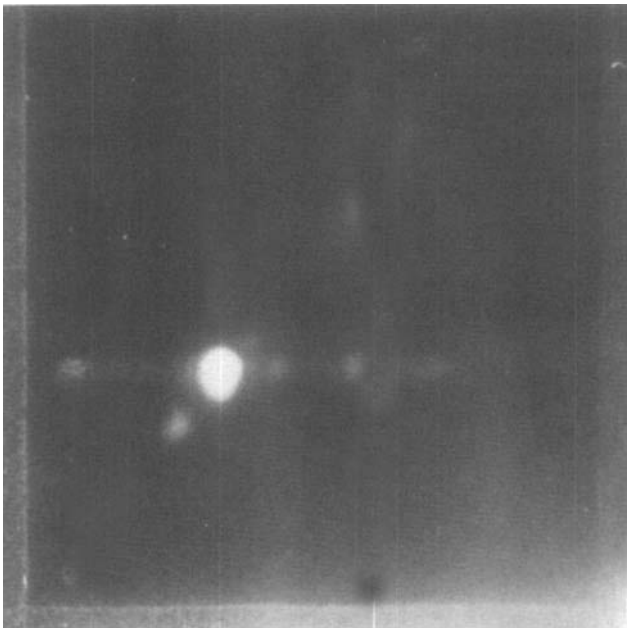


Fig.2. Two-dimensional TLC showing a - slow kinetics of decomposition and b - fast kinetics of decomposition

separation can be evidenced by applying the same interaction in two directions. A slight improvement in resolution (increased by a factor of squared root of 2) [10] may be observed due to the increased path length traveled by the solute. A diagonal pattern of impurities should be obtained if no decomposition is occurring during the chromatographic process. Conversely, if on-plate decomposition occurs, off-

Fig. 2A

diagonal spots can be observed. If the kinetics of decomposition is fast, then well defined spots can be seen off diagonal. Conversely, if the kinetics of decomposition is slow then streaking will occur on the plate [1]. Fig. 2a and 2b illustrate these phenomena.

In order to probe the system for on-plate decomposition, a two-dimensional TLC was performed

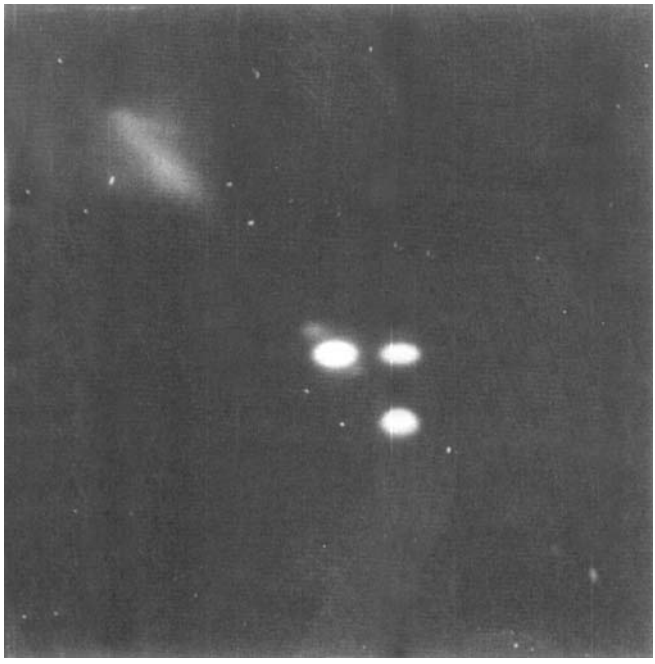


Fig.3. Two dimensinal TLC of MK0912. Conditions same as in Fig.1.

and the result is shown in Fig. 3. The appearance of an off-diagonal spot indicates that MK0912 undergoes decomposition.

A stability study was performed in order to establish whether the decomposition occurs in the sample solution or under chromatographic conditions (in the mobile phase or on the stationary phase). First, UV spectra of the compound dissolved in

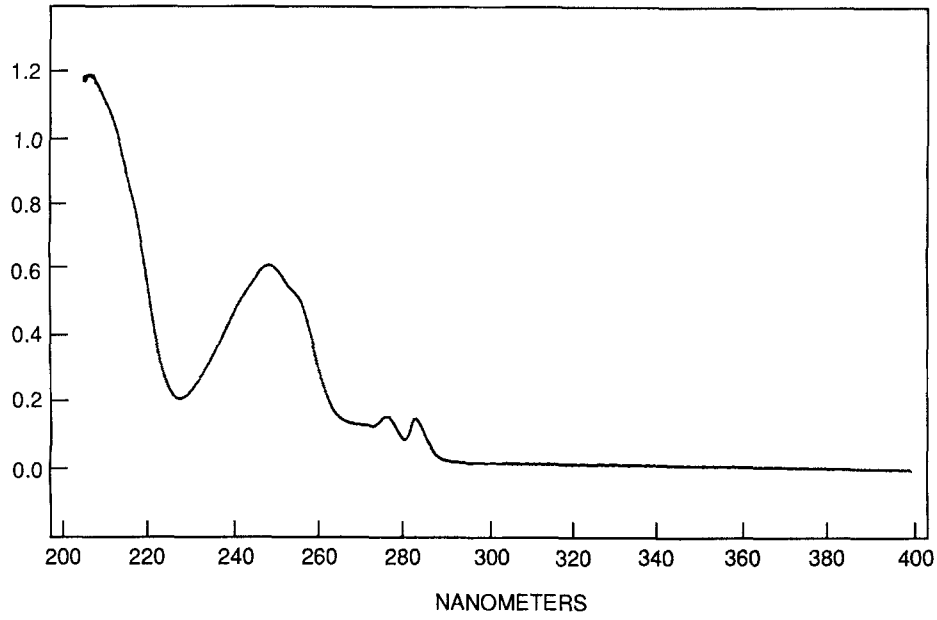


Fig.4. UV spectra of MK0912 over a one hour time interval.

methanol were recorded at ten minute intervals for one hour. Figure 4 depicts the spectra taken during the one hour period. The spectra were perfectly superimposable suggesting no decomposition had occurred in the time frame examined.

The role of the stationary phase in the decomposition of MK0912 was then investigated. Each spot was scraped from the stationary phase and

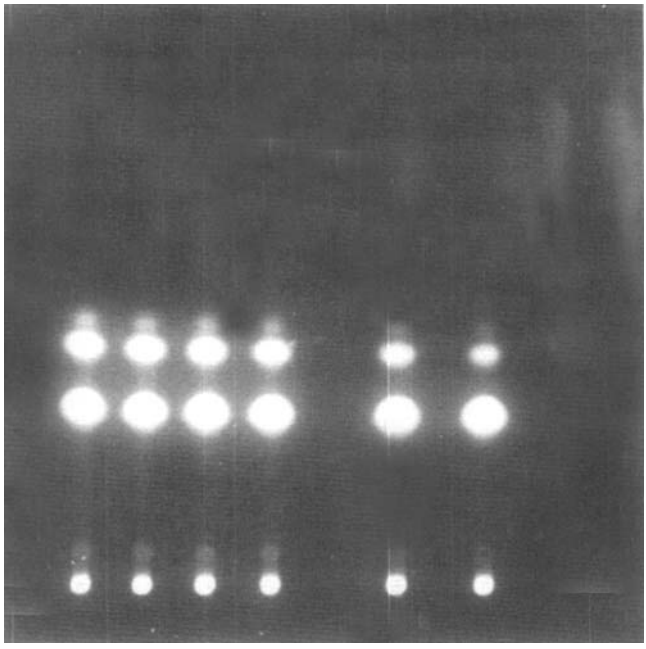


Fig.5. The influence of stationary phase on decomposition of MK0912.

independently subjected to two-dimensional TLC. It is interesting to note that the two dimensional TLC of each exhibited an off diagonal pattern implying on-plate decomposition. In addition several spots all of equal amounts were spotted on a plate at ten minute intervals. The time elapsed between spotting of the first and last spot was 60 minutes. Figure 5 shows the results of this study.

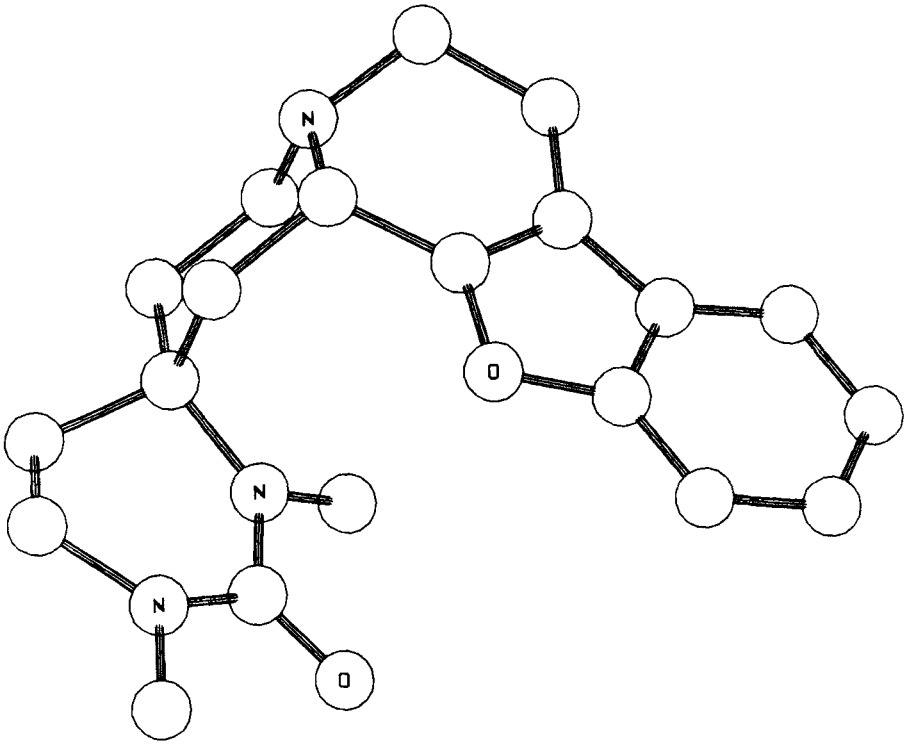


Fig.6. Stereochemical view of MK0912.

It is clear that the intensity of the leading spots (R_fs 0.54 and 0.59) are less in the latter run than in the initial run. The above results indicate that the extraneous spots were generated on the plate. This suggests the stationary phase is responsible for the compound's degradation during the spotting procedure.

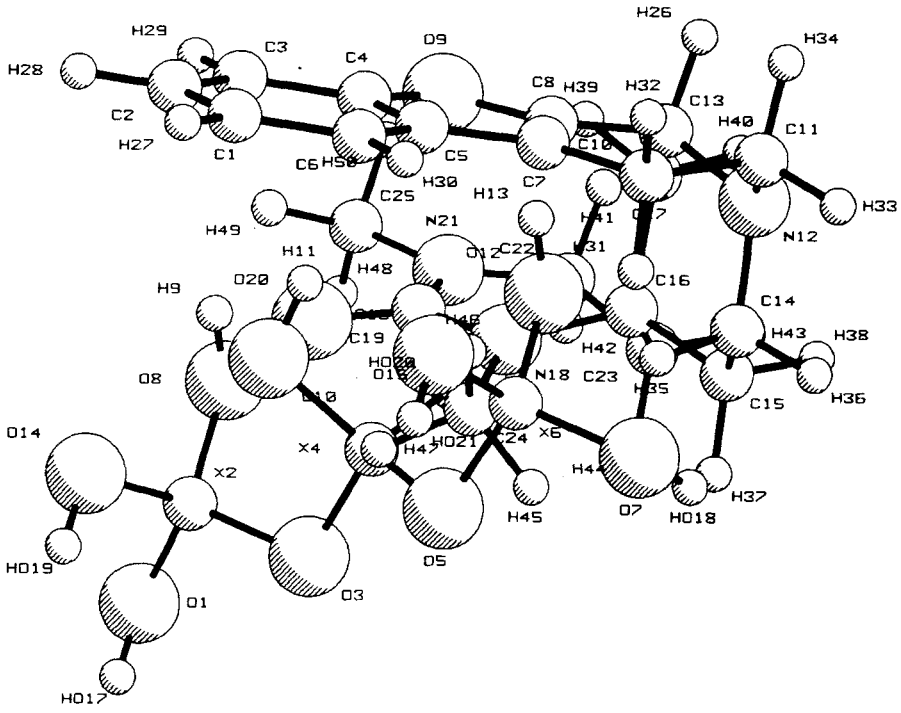


Fig.7. Stereochemical view of the interaction between MK0912 and silica-gel. (where X = silicon atom).

The stereochemical view of MK0912 presented in Figure 6 shows that the structure is bent in a "U" shape whith the carbodiamide and aromatic portion of the molecule being almost parallel to each other. The carbodiamide portion of the molecule will interact with the silica-gel backbone through hydrogen bonding, leaving the aromatic portion

completely exposed to air (Figure 7). In this configuration the aromatic portion of the molecule is especially susceptible to oxidation and the fluorrescent spots detected in the chromatogram under long wave UV (see Figure 1b) could concievably be oxidative degradates. Since oxidation would increase conjugation in this case, the fluorescent properties of the degradates are not surprising.

There are many ways to avoid on-plate decomposition. If the compound undergoes oxidation during the spotting procedure the use of an antioxidant such as BHT can overcome the problem [1,11]. However, in our study, BHT proved to be ineffective. Another way to overcome this phenomenon is to use a compound which can interact with the analyte to form an inclusion compound. Cyclodextrins are known to form inclusion complexes with different analytes [12]. The use of cyclodextrins as a protecting agent against oxidation has been reported by several authors [13,14]. In our experiments we used γ-cyclodextrin as a host molecule in the inclusion process for MK0912. Generally speaking, cyclodextrins can be envisioned as a basket with a hydrophobic interior and a hydrophilic rim [15]. The inclusion of a guest molecule will take place in the

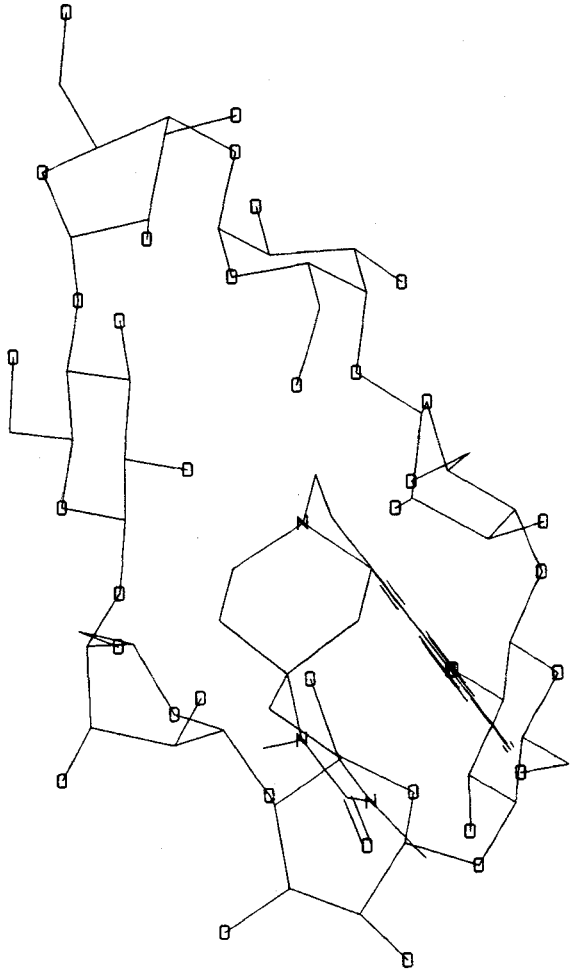


Fig.8. Stereochemical view of the complex γ-cyclodextrin-MK0912.

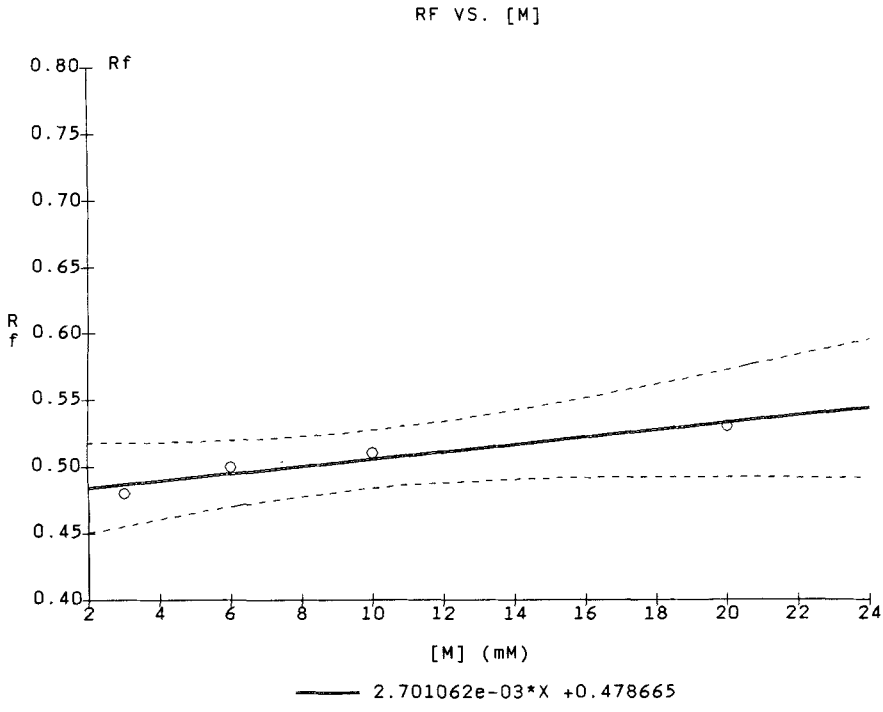


Fig.9. Plot of R_f vs. micelle concentration. For chromatographic conditions see text.

following manner: the guest will associate with the interior of the basket, while the hydrophilic portion will interact with the secondary hydroxyl groups located on the rim of the cyclodextrin basket. A view of the complex γ-cyclodextrin-MK0912 is presented in Figure 8. The host molecule can protect the analyte from oxidation before development.

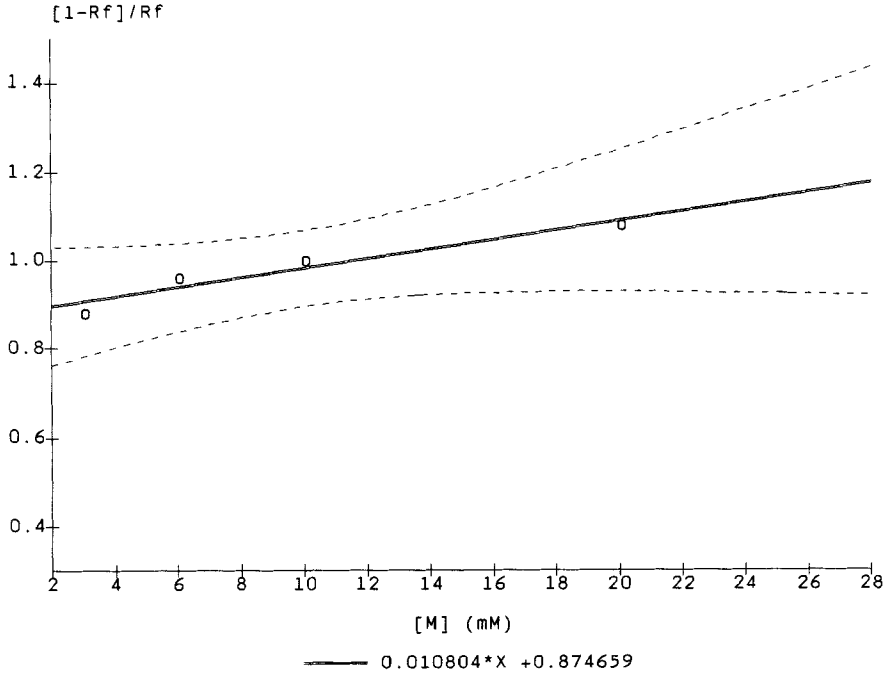


Fig.10. Plot of $[1-R_f]/R_f$ vs. micelle concentration. For chromatographic conditions see text.

Once chromatography has begun, the compound is displaced from the cyclodextrin cavity and travels along with the mobile phase. In order to protect it from degradation during chromatography we selected HTAB as a mobile phase additive. At concentration above 2 mM [16] the additive forms micelles in the mobile phase of ethyl acetate-methanol-hexane

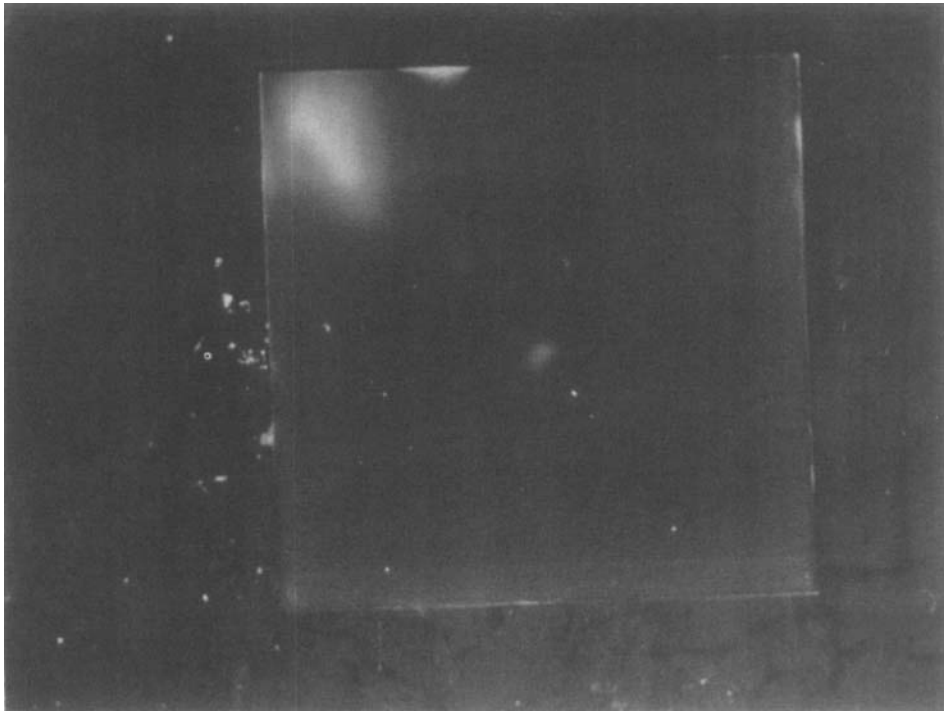


Fig.11. Two-dimensional TLC of MK0912 using γ-cyclodextrin in the spotting solution and HDTAB in the mobile phase. For chromatographic conditions see text.

(40:40:20, v/v). The formation of micelles is a dynamic process in which the compound is partitioned between micelles located in the mobile phase and stationary phase. Plotting R_f vs. micelle concentration (Figure 9) a positive slope was obtained showing that the partitioning phenomena

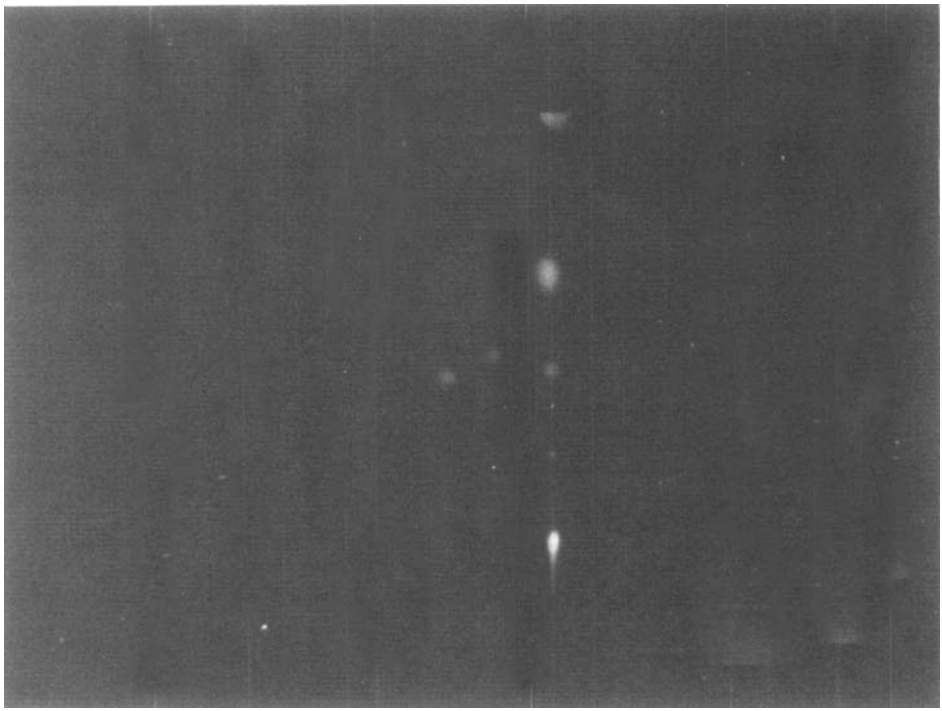


Fig.12. TLC separation of MK0912 using γ-cyclodextrin in the spotting solvent and HDTAB in the mobile phase. Left channel: MK0912; right channel: crude sample. For chromatographic conditions see text

occurs. A positive slope was also obtained from a plot of $[1-R_f]/R_f$ vs. micelle concentration (Figure 10). From the ratio of slope and Y intercept the partition coefficient of MK0912 for each micelle concentration can be obtained [17]. The values obtained also support the partitioning process (data

not shown). In addition, the two-dimensional TLC using the above conditions, shows no off diagonal spots, indicating that decomposition has been eliminated (Figure 11).

The new method was investigated further by spotting a crude synthesis sample of MK0912. A mobile phase of ethyl acetate-methanol-hexane (40:40:10, v/v) containing 20 mM HTAB was used. The results are shown in Figure 12. A good separation of the impurities was obtained.

CONCLUSION

The results presented outline the importance of correct assessment of impurity profiles. As shown, some impurities may have their origin in the chromatographic system. In our experiments we demonstrated that MK0912 undergoes decomposition during the spotting procedure. By using γ-cyclodextrin in the spotting procedure coupled with micelles in the mobile phase the phenomenon was eliminated.

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